HPLC METHODS FOR PHARMACEUTICAL ANALYSIS

Volumes 2-4

George Lunn

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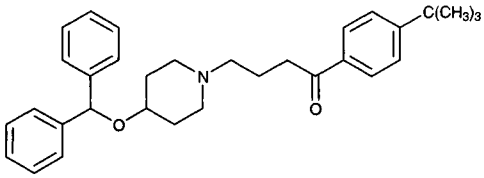
Ebastine



Molecular formula: $C_{32}H_{39}NO_2$
Molecular weight: 469.67
CAS Registry No.: 90729-43-4
Merck Index: 3534
Lednicer No.: 4 48

SAMPLE
Matrix: blood
Sample preparation: SPE (no details)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Sup RS C18 (Prolabo)
Mobile phase: MeCN:200 mM pH 3.5 acetate buffer:triethylamine 80:20:0.025
Detector: UV 254

CHROMATOGRAM
Retention time: 17
Internal standard: terfenadine (8)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: carebastine, metabolites

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Van Rooij,J.; Schoemaker,H.C.; Bruno,R.; Reinhoudt,J.F.; Breimer,D.D.; Cohen,A.F. Cimetidine does not influence the metabolism of the H_1-receptor antagonist ebastine to its active metabolite carebastine, *Br.J.Clin.Pharmacol.*, **1993**, *35*, 661–663.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Add 3 mL MeCN to microsomal incubation, centrifuge at 800 g for 10 min, evaporate an aliquot of the supernatant to dryness in a centrifugal concentrator. Dissolve the residue in 200 μL MeOH and inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Inertsil ODS-3 (GL Sciences, Tokyo, Japan)
Mobile phase: Gradient. A was MeCN. B was 12 mM pH 4.5 ammonium acetate. A:B 35:65 for 3 min, to 85:15 over 25 min
Column temperature: 40
Flow rate: 1
Detector: Radioactivity, Radiomatic Flo-One/Beta A-515 detector (Packard Instrumental Co., Meriden, CT), column effluent mixed with Ultima Flo-M scintillant pumped at 2 mL/min

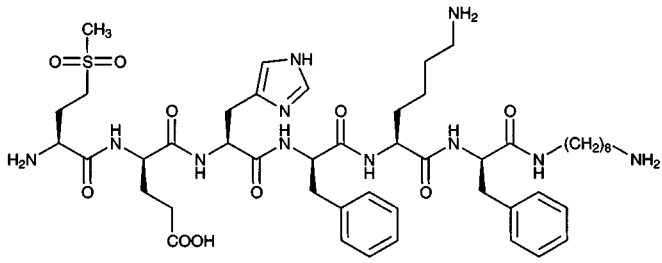
OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver

REFERENCE
Hashizume,T.; Mise,M.; Terauchi,Y.; O,L.; Fujii,T.; Miyazaki,H.; Inaba,T. N-Dealkylation and hydroxylation of ebastine by human liver cytochrome P450, *Drug Metab.Dispos.*, **1998**, *26*, 566–571.

Ebiratide

Molecular formula: $C_{48}H_{73}N_{11}O_{10}S$
Molecular weight: 996.24
CAS Registry No.: 105250-86-0



SAMPLE

Matrix: blood

Sample preparation: Condition a 100 mg C18 SPE cartridge (Varian) with 2 mL MEOH and 2 mL water. 400 μL Plasma + 800 μL water, mix, add to the SPE cartridge, wash with 1 mL water, elute with 1 mL 5 mM HCl in MeOH:water 30:70. Evaporate the eluate to dryness in a freeze dryer, reconstitute with 100 μL 100 mM pH 9.0 borate buffer, add 100 μL 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in MeCN, mix, heat at 50° for 30 min, inject a 100 μL aliquot. (Synthesis of 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (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 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ tlc plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei.)

HPLC VARIABLES

Column: 150 × 4.6 5C18 (Vydac)

Mobile phase: Gradient. A MeCN:MeOH:50 mM pH 6.0 imidazole nitrate 26.7:13.3:60. B MeCN:MeOH:50 mM pH 6.0 imidazole nitrate 40:20:40. A:B from 100:0 to 55:45 over 15 min, to 30:70 over 45 min, re-equilibrate at initial conditions for 15 min.

Flow rate: 1

Injection volume: 100

Detector: Chemiluminescence. The column effluent mixed with the reagent pumped at 1.2 mL/min and the mixture flowed through a coil at 30° to the detector. (Reagent was 100 mM hydrogen peroxide containing 0.5 mM bis[4-nitro-2-(3,6,9-trioxadecyloxycarbonyl)phenyl]oxalate (Wako, Richmond VA).)

CHROMATOGRAM
Retention time: 33
Limit of detection: 250 fmole

KEY WORDS
derivatization; rat; plasma; SPE; fluorescence detection is more sensitive

REFERENCE
Hamachi,Y.; Nakashima,K.; Akiyama,S. High performance liquid chromatography with peroxyoxalate chemiluminescence detection of synthetic peptide, ebiratide, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2377–2387.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 100 μM solution in 100 mM pH 9.0 borate buffer. 200 μL Solution + 200 μL 30 mM DBD-F in MeCN, mix, heat at 50° for 30 min, inject an aliquot. (DBD-F is 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole. It can be purchased from Tokyo Kasei Kogyo or synthesized as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene form EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ tlc plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413).)

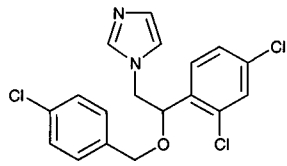
HPLC VARIABLES
Column: 150 × 4.6 5C18 protein and peptide (Vydac)
Mobile phase: MeCN:50 mM Na_2HPO_4 40:60 adjusted to pH 7.0 with 20% phosphoric acid
Flow rate: 1
Injection volume: 10
Detector: F ex 440 em 580 or UV 220

CHROMATOGRAM
Retention time: 10
Limit of detection: 0.25 pmole (F)

KEY WORDS
derivatization

REFERENCE
Hamachi,Y.; Tsujiyama,T.; Nakashima,K.; Akiyama,S. High-performance liquid chromatography with fluorescence detection of ebiratide using 4-(N,N-dimethylamino-sulphonyl)-7-fluoro-2,1,3-benzoxadiazole as a fluorgenic reagent, *Biomed.Chromatogr.*, **1995**, *9*, 216–220.

Econazole



Molecular formula: $C_{18}H_{15}Cl_3N_2O$
Molecular weight: 381.69
CAS Registry No.: 27220-47-9, 68797-31-9 (nitrate)
Merck Index: 3550
Lednicer No.: 2 249

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

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

CHROMATOGRAM
Retention time: 20.137

KEY WORDS
whole blood

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

SAMPLE
Matrix: formulations
Sample preparation: Powders. Extract a sample equivalent to about 10 mg econazole twice with 20 mL portions of MeOH with magnetic stirring, filter extracts, combine, dilute to 50 mL with MeOH. Remove a 2.5 mL aliquot and add it to 2 mL 200 μg/mL miconazole in MeOH, dilute to 10 mL with MeOH, inject a 10 μL aliquot. Creams. Condition a Baker diol SPE cartridge with 6 mL dichloromethane. Add a sample equivalent to 10 mg econazole to 30 mL dichloromethane, sonicate for 2 min, make up to 50 mL with dichloromethane, filter, add 2 mL of the filtrate to the SPE cartridge. Wash with two 3 mL portions of n-hexane:dichloromethane 4:1, aspirate to dryness, elute with three 1 mL portions of MeOH:100 mM triethylamine adjusted to pH 7.0 with acetic acid 4:1. Combine the eluates and dilute them to 5 mL with MeOH.

Remove a 2.5 mL aliquot and add it to 1 mL 200 μg/mL miconazole in MeOH, dilute to 5 mL with MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Nova-Pak RP-18
Mobile phase: MeOH:THF:100 mM triethylamine adjusted to pH 7.0 with acetic acid 70:12:18
Flow rate: 0.8
Injection volume: 10
Detector: UV 230

CHROMATOGRAM
Retention time: 2.5
Internal standard: miconazole (3.5)

KEY WORDS
creams; powders; SPE

REFERENCE
Cavrini,V.; Di Pietra,A.M.; Gatti,R. Analysis of miconazole and econazole in pharmaceutical formulations by derivative UV spectroscopy and liquid chromatography (HPLC), *J.Pharm.Biomed.Anal.*, **1989**, *7*, 1535–1543.

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

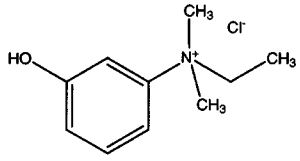
CHROMATOGRAM
Retention time: 14
Internal standard: ketoconazole (7)

OTHER SUBSTANCES
Simultaneous: clotrimazole, ketoconazole, bifonazole, tioconazole, isoconazole, miconazole, fenticonazole

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.

Edrophonium chloride



Molecular formula: $C_{10}H_{16}ClNO$
Molecular weight: 201.70
CAS Registry No.: 116-38-1
Merck Index: 3562

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 100 μL 500 ng/mL neostigmine in water + 500 μL 100 mM picric acid in 100 mM NaOH (pH adjusted to 7) + 400 μL 100 mM NaH_2PO_4 + 12 mL water saturated dichloromethane, shake vigorously for 5 min, centrifuge at 2000 g for 10 min. Remove 10 mL of the organic phase and add it to 200 μL 1 mM tetrabutylammonium hydrogen sulfate, shake vigorously for 30 s, centrifuge at 2000 g for 2 min, discard most of the organic layer, centrifuge at 2000 g for 1 min, inject a 50 μL aliquot of the aqueous layer. (Store glassware in 100 mM tetramethylammonium chloride solution and wash 5 times with water before use.)

HPLC VARIABLES

Guard column: 50 × 3.2 30-40 μm Perisorb RP-2 (Merck)
Column: 150 × 4.6 5 μm Ultrasphere octyl
Mobile phase: MeCN:water 20:80 containing 10 mM sodium heptanesulfonate, 10 mM NaH_2PO_4, and 2.5 mM tetramethylammonium chloride, pH adjusted to 3 with concentrated sulfuric acid
Flow rate: 2
Injection volume: 50
Detector: UV 214

CHROMATOGRAM

Retention time: 3.5
Internal standard: neostigmine (5)
Limit of quantitation: 5 ng/mL

KEY WORDS

serum; pharmacokinetics

REFERENCE

De Ruyter,M.G.M.; Cronnelly,R.; Castagnoli,N.,Jr. Reversed-phase, ion-pair liquid chromatography of quaternary ammonium compounds: determination of pyridostigmine, neostigmine and edrophionium in biological fluids, *J.Chromatogr.*, **1980**, *183*, 193–201.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 50 × 4.6 5 μm Suplex pKb-100 (Supelco)
Mobile phase: MeCN:buffer 30:70 (Buffer was 10 mM pH 7.0 sodium phosphate containing 5 mM sodium dodecyl sulfate.)
Column temperature: 35
Flow rate: 2
Detector: UV 210

CHROMATOGRAM

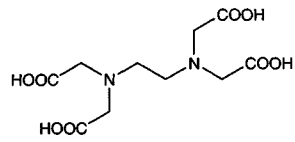
Retention time: 1.6

OTHER SUBSTANCES

Simultaneous: 4'-methylphenazone, neostigmine bromide

REFERENCE
Supelco Catalog, **1994**, p. 771.

EDTA



Molecular formula: $C_{10}H_{16}N_2O_8$
Molecular weight: 292.25
CAS Registry No.: 60-00-4, 150-38-9 (tri Na salt), 64-02-8 (tetra Na salt), 6381-92-6 (di Na salt dihydrate), 139-33-3 (di Na salt), 25102-12-9 (di K salt dihydrate), 2001- 94-7 (di K salt), 58167-76-3 (di K salt monohydrate), 23411-34-9 (Ca di Na salt hydrate), 62-33-9 (Ca di Na salt)
Merck Index: 3559

SAMPLE
Matrix: cell suspensions
Sample preparation: Centrifuge cell suspension at 14000 rpm for 2 min, dilute if necessary, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 LC18 (Supelco)
Mobile phase: MeCN:buffer 0.5:99.5 (Buffer was 1.5 mL/L acetic acid containing 1 g/L ammonium acetate, 0.5 g/L 1,2-diaminopropane-N,N,N',N'-tetraacetic acid, 0.5 mL/L ammonium hydroxide, and 0.1 mL/L triethylamine.) (After 2.5 min wash column with MeCN:water 60:40 containing 0.5 g/L 1,2-diaminopropane-N,N,N',N'-tetraacetic acid, and 0.1 mL/L triethylamine for 5.5 min, re-equilibrate for 4 min.)
Flow rate: 1
Injection volume: 30
Detector: UV 360 (as ferric complex)

KEY WORDS
edetic acid measured as ferric complex

REFERENCE
Lauff,J.J.; Steele,D.B.; Coogan,L.A.; Breitfeller,J.M. Degradation of the ferric chelate of EDTA by a pure culture of an *Agrobacterium sp.*, *Appl.Environ.Microbiol.*, **1990**, *56*, 3346–3353.

SAMPLE
Matrix: fertilizer
Sample preparation: Prepare an aqueous solution, filter (paper), filter (0.2 μm), inject an aliquot.

HPLC VARIABLES
Guard column: Ion Pac AG7 (Dionex)
Column: 10 μm Ion Pac AS7 (Dionex)
Mobile phase: 70 mM nitric acid
Flow rate: 0.5
Injection volume: 50
Detector: UV 330 following post-column reaction with 1 g/L $Fe(NO_3)_3.9H_2O$ in 2% perchloric acid

CHROMATOGRAM
Retention time: 5

KEY WORDS
post-column reaction

REFERENCE
Vande Gucht,I. Determination of chelating agents in fertilizers by ion chromatography, *J.Chromatogr.A*, **1994**, *671*, 359–365.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with water to give an EDTA concentration of 0.01%, mix with an equal volume of 0.02% cupric nitrate, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 10 μm Adsorbosphere C18
Column: 150 × 4.5 5 μm Resolve C18 (Waters)
Mobile phase: MeCN:buffer:water 20:20:80 (Buffer was 195 mL water and 6 mL 1 M tetrabutylammonium hydroxide in MeOH adjusted to pH 6.5 ± 0.1 with 1 M phosphoric acid.)
Flow rate: 1.5
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 8.5

OTHER SUBSTANCES
Simultaneous: sorbic acid
Noninterfering: benzalkonium chloride, hydroxyethyl cellulose, Miranol 2MCA, phenylmercuric nitrate, polyvinyl alcohol, propylene glycol, thimerosal

KEY WORDS
ophthalmic solutions; derivatization; complexation

REFERENCE
Hall,L.; Takahashi,L. Quantitative determination of disodium edetate in ophthalmic and contact lens care solutions by reversed-phase high-performance liquid chromatography, *J.Pharm.Sci.*, **1988**, *77*, 247–250.

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

HPLC VARIABLES
Column: 250 × 4.1 10 μm Anion/R (Alltech)
Mobile phase: MeCN:buffer 25:75 (Buffer was 11 mM nitric acid containing 2 mM cupric nitrate, pH adjusted to 3.0 with 10 M NaOH.)
Flow rate: 1
Injection volume: 20
Detector: UV 250

CHROMATOGRAM
Retention time: 8

KEY WORDS
stability-indicating; rugged; ophthalmic solution; complexation; copper complexes

REFERENCE
Kord,A.S.; Tumanova,I.; Matier,W.L. A novel HPLC method for determination of EDTA in a cataract inhibiting ophthalmic drug, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 575–580.

SAMPLE
Matrix: formulations
Sample preparation: Dilute ophthalmic cleanser 1:25 with 100 μM ferric chloride hexahydrate in water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 3 μm Adsorbosphere HS C18
Mobile phase: 50 mM Tetrabutylammonium hydrogen sulfate in water
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 5.2
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Noninterfering: degradation products

KEY WORDS
derivatization; complexation; ophthalmic cleanser; stability-indicating

REFERENCE
Tran,G.; Chen,C.; Miller,R.B. HPLC method for the determination of EDTA in an ophthalmic cleanser, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1499–1508.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 10 μM solution of the ligand with a 100 μM solution of lutetium chloride, pass through a 70 × 20 column of 70-130 μm AG 50W-X8 cation-exchange resin (sodium form, Bio-Rad), inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Prodigy 5 ODS-2 (Phenomenex)
Mobile phase: Gradient. A was 1 mM potassium sulfate containing 3 mM tetrapropylammonium bromide. B was MeCN:3 mM potassium sulfate 4:96. A:B 100:0 for 4 min, to 0:100 over 3 min.
Flow rate: 1
Injection volume: 50
Detector: F ex 360 em 500 following post-column reaction. The column effluent mixed with the reagent pumped at 0.3 mL/min and the mixture flowed through a 5.1 m × 0.5 mm ID knitted PTFE coil. The effluent from the coil mixed with 1 M NaOH pumped at 0.3 mL/min and the mixture flowed to the detector. (Reagent was 1 mM trans-1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid (CDTA) in 1 mM 8-hydroxyquinoline-5-sulfonic acid, adjusted to pH 2.8 with acetic acid.)

CHROMATOGRAM
Retention time: 7.5
Limit of detection: 25 nM

OTHER SUBSTANCES
Simultaneous: trans-1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid (CDTA), diethylenetriaminepentaacetic acid (DTPA), ethylene glycol-bis(β-aminoethylether) N,N,N',N'-tetraacetic acid (EGTA), nitrilotriacetic acid (NTA)

KEY WORDS
derivatization; complexation; post-column reaction; use a metal-free system

REFERENCE
Ye,L.; Lucy,C.A. Ion chromatographic determination of chelating ligands based on the postcolumn formation of ternary fluorescent complexes, *J.Chromatogr.A*, **1996**, *739*, 307–315.

SAMPLE
Matrix: water
Sample preparation: Filter (0.2 μm cellulose nitrate) river water, pass the filtrate through a 10 × 5 column filled with SPE 7090 sulfonic acid material (Baker), collect 2-6 mL, evaporate to dryness in an oven at 90°, add 1 mL buffer, add 20 μL iron solution, heat at 90° for 3 h,

cool, add 40 μL 50 mM tetrabutylammonium bromide in buffer, inject a 200 μL aliquot. (Buffer was 15 mM formic acid containing 5 mM sodium formate and 1 mM tetrabutylammonium bromide. The iron solution was 1 mM ferric nitrate in 10 mM nitric acid.)

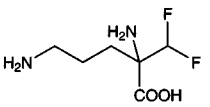
HPLC VARIABLES
Guard column: 4 × 4 Lichrocart (Merck)
Column: 250 × 4 Lichrocart RP-18 (Merck)
Mobile phase: MeCN:buffer 8:92 (Buffer was 15 mM formic acid containing 5 mM sodium formate, pH 3.3.)
Flow rate: 1
Injection volume: 200
Detector: UV 258

CHROMATOGRAM
Retention time: 7.6
Limit of detection: 3 nM

KEY WORDS
protect from light; river water; derivatization; complexation; SPE

REFERENCE
Nowack,B.; Kari,F.G.; Hilger,S.U.; Sigg,L. Determination of dissolved and adsorbed EDTA species in water and sediments by HPLC, *Anal.Chem.*, **1996**, *68*, 561–566.

Eflornithine



Molecular formula: $C_6H_{12}F_2N_2O_2$
Molecular weight: 182.17
CAS Registry No.: 67037-37-0, 68278-23-9 (HCl), 96020-91-6 (HCl monohydrate)
Merck Index: 3564
Lednicer No.: 4 2

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 20 μL 250 μg/mL 4-amino-3-hydroxybutyric acid in water + 400 μL MeOH, centrifuge at 800 g for 20 min. Remove the supernatant and add it to 200 μL 20 mM pH 7.5 phosphate buffer, mix an aliquot of this mixture with an equal volume of reagent, inject. (Reagent was 10 mg o-phthalaldehyde in 1 mL EtOH, add 100 μL 2-mercaptoethanol, add 10 mL 100 mM pH 7.5 phosphate buffer, store in the dark, freshly prepare every 3 days.)

HPLC VARIABLES
Guard column: 37-50 μm Bondapak C18/corasil
Column: 5 μm C18 Radial-Pak (Waters)
Mobile phase: Gradient. A was MeOH:isopropanol:100 mM pH 7.5 phosphate buffer 5:3:92. B was MeOH:MeCN:isopropanol:water 80:5:5:10. A:B 80:20 for 3 min, to 50:50 over 3 min, maintain at 50:50 for 5 min, re-equilibrate at initial conditions for 7 min.
Flow rate: 0.2 for 1 min then 1.5
Detector: F ex 335 em 418

CHROMATOGRAM
Retention time: 19
Internal standard: 4-amino-3-hydroxybutyric acid (13)
Limit of detection: 250 ng/mL
Limit of quantitation: 500 ng/mL

KEY WORDS
plasma; derivatization

REFERENCE
Smithers,J. A precolumn derivatization high-performance liquid chromatographic (HPLC) procedure for the quantitation of difluoromethylornithine in plasma, *Pharm.Res.*, **1988**, *5*, 684–686.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 100 μL 1 mg/mL norvaline in water + 2 mL ice-cold 80% EtOH, vortex for 30 s, centrifuge at 3000 rpm for 5 min, remove the supernatant, extract the residue twice more with 2 mL portions of ice-cold 80% EtOH. Combine the supernatants and dry them under vacuum at 50°, dissolve in 2 mL water and 2 mL buffer. 100 μL Mixture + 100 μL 6 mg/mL dansyl chloride in acetone, let stand at room temperature in the dark for 4 h, add 800 μL water adjusted to pH 8.5 with NaOH, vortex for 15 s, centrifuge at 3000 rpm, inject a 25-50 μL aliquot. (Buffer was 500 mM sodium bicarbonate adjusted to pH 8.5 with 1 M NaOH.)

HPLC VARIABLES
Guard column: 15 × 4.6 5 μm C8 (Rainin)
Column: 150 × 4.6 5 μm C8 (Rainin)
Mobile phase: Gradient. A was THF:10 mM sodium acetate adjusted to pH 4.18 with 1 M acetic acid 5:95. B was MeCN:THF 90:10. A:B from 100:0 to 63.3:36.7 over 19 min, to 0:100 over 2 min, re-equilibrate at 100:0 for 7 min.
Flow rate: 1.5 for 21 min, 2.0 for 2 min, 1.75 for 3 min, 1.5 for 2 min
Injection volume: 25-50
Detector: UV 330

CHROMATOGRAM
Retention time: 14.73
Internal standard: norvaline (17.66)
Limit of detection: 10000 ng/mL

KEY WORDS
serum; derivatization; pharmacokinetics

REFERENCE
Cohen,J.L.; Ko,R.J.; Lo,A.T.L.; Shields,M.D.; Gilman,T.M. High-pressure liquid chromatographic analysis of eflornithine in serum, *J.Pharm.Sci.*, **1989**, *78*, 114–116.

SAMPLE
Matrix: solutions

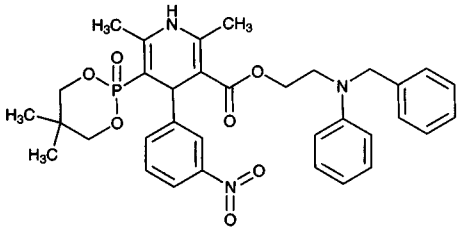
HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere ion pair
Mobile phase: Buffer prepared from 0.92 g L-proline and 1 g $CuSO_4.5H_2O$ in 1 L water, pH adjusted to 5.5 with 1 mL 5 M NaOH.
Column temperature: 30
Flow rate: 0.5
Detector: F ex 340 em 455 following post-column derivatization. The column effluent and the reagent pumped at 0.35 mL/min were mixed and allowed to flow through a 3 m × 0.3 mm i.d. PTFE reaction coil at 50° to the detector. (Reagent was 800 mg o-phthalaldehyde in 10 mL EtOH + 500 mM pH 10.5 potassium borate buffer, filter (0.45 μm), add 3 mL Brij 35, add 2.5 mL 2-mercaptoethanol, store in the dark.)

CHROMATOGRAM
Retention time: 6.4 (+), 13.9 (-)

KEY WORDS
post-column reaction

REFERENCE
Wagner,J.; Gaget,C.; Heintzelmann,B.; Wolf,E. Resolution of the enantiomers of various α-substituted ornithine and lysine analogs by high-performance liquid chromatography with chiral eluant and by gas chromatography on Chirasil-Val, *Anal.Biochem.*, **1987**, *164*, 102–116.

Efonidipine



Molecular formula: $C_{34}H_{38}N_3O_7P$
Molecular weight: 631.67
CAS Registry No.: 111011-76-8 (HCl ethanol)
Merck Index: 3566

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 100 ng/mL IS in MeOH + 40 μL 10 mM diethylamine, extract twice with 5 mL portions of ether. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 70 μL aliquot.

HPLC VARIABLES

Column: 50 × 4.6 2.5 μm Inertsil ODS
Mobile phase: MeCN:50 mM ammonium acetate 55:45
Column temperature: 40
Flow rate: 1
Injection volume: 70
Detector: MS, Hewlett-Packard 1090L and 5988A, ion source 276°, probe stem 95-106°, probe tip 165-175°, m/z 631

CHROMATOGRAM

Retention time: 4.4
Internal standard: heptadeutero-efonidipine (m/z 638)
Limit of detection: 0.5 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Nakabeppu,H.; Asada,M.; Oda,T.; Shinozaki,Y.; Yajima,T. Plasma and urinary metabolites of efonidipine hydrochloride in man, *Xenobiotica*, **1996**, *26*, 229–239.

SAMPLE

Matrix: urine
Sample preparation: Condition a 1 mL 100 mg Bond Elut C18 SPE cartridge with 2 mL MeCN and 2 mL water. 5 mL Urine + 10 μL 5 μg/mL IS in MeOH, mix, add to the SPE cartridge, wash with 3 mL water, wash with 2 mL MeCN:water 30:70, wash with 1 mL MeCN:water 45:55, elute with 2 mL MeCN:water 70:30. Add 500 μL 1 M NaOH and 5 mL diethyl ether to the eluate, shake, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 50 μL n-hexane:EtOH 2:1, inject a 15 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 LiChrospher Si60
Column: 250 × 4.6 Nucleosil 50-5
Mobile phase: n-Hexane:MeOH:chloroform 120:15:5
Column temperature: 40
Flow rate: 1
Injection volume: 15
Detector: UV 254

CHROMATOGRAM

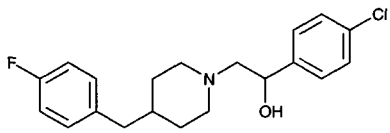
Retention time: 11.5
Internal standard: (±)-2-[benzyl(phenyl)amino]ethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-5-(4,4,6,6-tetramethyl-2-oxo-1,3,2-dioxaphosphorinan-2-yl)-3-pyridinecarboxylate hydrochloride (P00-851743) (10)

Limit of detection: 1 ng/mL

KEY WORDS
normal phase; pharmacokinetics; SPE

REFERENCE
Nakabeppu,H.; Asada,M.; Oda,T.; Shinozaki,Y.; Yajima,T. Plasma and urinary metabolites of efonidipine hydrochloride in man, *Xenobiotica*, **1996**, *26*, 229–239.

Eliprodil



Molecular formula: $C_{20}H_{23}ClFNO$
Molecular weight: 347.86
CAS Registry No.: 119431-25-3, 136634-88-3 (HCl)

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 200 µL Plasma + 200 µL pH 6.5 buffer + 20 µL 200 U/mL β-glucuronidase (from E. coli, Sanofi-Pasteur), mix, heat at 37° for 24 h, make up to 1 mL with water, add 20 µL 1.25 µg/mL IS, add 1 mL pH 12 buffer, vortex, add 6 mL n-hexane, shake at 40 rpm for 10 min, centrifuge at 15° at 900 g for 5 min, centrifuge at -20° at 900 g for 8 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 70°, add 200 µL 0.1% (S)-(+)-1-(1-naphthyl)ethyl isocyanate in MeCN under a stream of nitrogen, vortex for 30 s, heat at 70° for 40 min, evaporate to dryness under a stream of nitrogen, add 250 µL MeCN:pH 4.5 buffer 40:60, vortex, inject a 150 µL aliquot on to column A and elute to waste with mobile phase A, after 5 min elute the contents of column A on to column B with mobile phase B, after 2.5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. (Backflush column A with MeCN:water 70:30, MeCN, MeOH, and THF, then re-equilibrate with MeCN:water 50:50 at 2 mL/min.) Urine. 100 µL Urine + 100 µL pH 6.5 buffer + 50 µL 200 U/mL β-glucuronidase (from E. coli, Sanofi-Pasteur), mix, heat at 37° for 24 h, make up to 1 mL with water, add 20 µL 20 µg/mL IS, add 1 mL pH 12 buffer, vortex, add 6 mL n-hexane, shake at 40 rpm for 10 min, centrifuge at 15° at 900 g for 5 min, centrifuge at -20° at 900 g for 8 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 70°, add 200 µL 0.1% (S)-(+)-1-(1-naphthyl)ethyl isocyanate in MeCN under a stream of nitrogen, vortex for 30 s, heat at 70° for 40 min, evaporate to dryness under a stream of nitrogen, add 250 µL MeCN:pH 4.5 buffer 40:60, vortex, inject a 50 µL aliquot on to column A and elute to waste with mobile phase A, after 5 min elute the contents of column A on to column B with mobile phase B, after 2.5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. (Backflush column A with MeCN:water 70:30, MeCN, MeOH, and THF, then re-equilibrate with MeCN:water 50:50 at 2 mL/min.) (Prepare pH 6.5 buffer by adding 20 mL 136.08 mg/mL KH_2PO_4 to 780 mL water, adjusting pH to 6.5 with 1 M KOH, and making up to 1 L with water. Prepare pH 12 buffer by dissolving 26.5 g sodium carbonate and 21 g sodium bicarbonate in 800 mL water, adjust pH to 12 with about 30 mL 30% NaOH, make up to 1 L with water. Prepare pH 4.5 buffer by dissolving 6.8 g KH_2PO_4 in 1 L water.)

HPLC VARIABLES
Column: A 20 × 4.6 5 µm Supelguard LC8; B 20 × 4.6 40 µm Pelliguard LC8 (Supelco) + 150 × 4.6 5 µm Hypersil C8 BDS
Mobile phase: A MeCN:water 50:50; B MeCN:MeOH:buffer 56:2:42 (Prepare buffer by dissolving 6.8 g KH_2PO_4 and 3.4 mL orthophosphoric acid in 4 L water, pH 2.6.)
Flow rate: A 2; B 1.2
Injection volume: 50-150
Detector: F ex 275 em 336

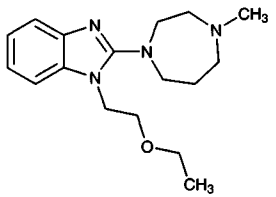
CHROMATOGRAM
Retention time: 16 (S-(+)), 17 (R-(-))
Internal standard: (+)-α-(3,4-dichlorophenyl)-4[(4-fluorophenyl)methyl]piperidine-1-ethanol hydrochloride (SL83.0601-10, Synthélabo Recherche, Bagneux, France) (19, 21)

Limit of quantitation: 0.75 ng/mL (plasma), 50 ng/mL (urine)

KEY WORDS
derivatization; plasma; column-switching; pharmacokinetics; chiral

REFERENCE
Malavasi,B.; Ripamonti,M.; Rouchouse,A.; Ascalone,V. Stereoselective determination of unchanged and glucuroconjugated eliprodil, a new anti-ischaemic drug, in human plasma and urine by precolumn derivatization and column-switching high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.A*, **1996**, *729*, 323–333.

Emedastine



Molecular formula: $C_{17}H_{26}N_4O$
Molecular weight: 302.42
CAS Registry No.: 87233-61-2, 87233-62-3 (fumarate)
Merck Index: 3597

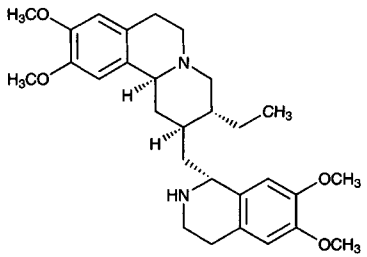
SAMPLE
Matrix: perfusate

HPLC VARIABLES
Column: 150 × 5 Inertsil ODS
Mobile phase: MeCN:buffer 50:50 (Buffer was 25 mM pH 2.4 phosphate buffer containing 0.25% sodium lauryl sulfate.)
Flow rate: 1.4
Detector: UV 280

KEY WORDS
rabbit; pharmacokinetics

REFERENCE
Harada,S.; Takahashi,Y.; Nakagawa,H. Transdermal administration of emedastine, *Biol.Pharm.Bull.*, **1993**, *16*, 884–888.

Emetine



Molecular formula: $C_{29}H_{40}N_2O_4$
Molecular weight: 480.65
CAS Registry No.: 483-18-1, 316-42-7 (2HCl)
Merck Index: 3600

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

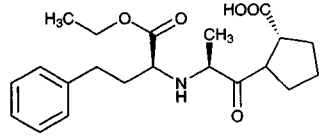
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85: 15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.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

CHROMATOGRAM
Retention time: 9.385

KEY WORDS
whole blood

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

Enalapril



Molecular formula: $C_{20}H_{28}N_2O_5$
Molecular weight: 376.45
CAS Registry No.: 75847-73-3, 76095-16-4 (maleate)
Merck Index: 3605
Lednicer No.: 4 58, 81-84

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

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85: 15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 211.1

CHROMATOGRAM
Retention time: 3.432

KEY WORDS
whole blood

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

SAMPLE
Matrix: formulations
Sample preparation: Add MeOH:100 mM pH 4.5 phosphate buffer 20:80 to powdered capsules or tablets so as to give an enalapril concentration of ca. 46 μg/mL, stir for 15 min, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.5 5 μm Hypersil ODS
Mobile phase: MeCN:MeOH:20 mM pH 2.5 sodium heptanesulfonate 35.15:1.85:63
Flow rate: 1
Injection volume: 20
Detector: UV 215

CHROMATOGRAM
Retention time: 10.0

KEY WORDS
capsules; tablets

REFERENCE
Bonazzi,D.; Gotti,R.; Andrisano,V.; Cavrini,V. Analysis of ACE inhibitors in pharmaceutical dosage forms by derivative UV spectroscopy and liquid chromatography (HPLC), *J.Pharm.Biomed.Anal.*, **1997**, *16*, 431–438.

SAMPLE
Matrix: solutions

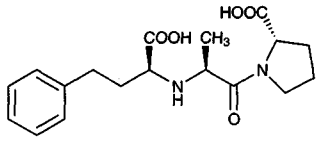
HPLC VARIABLES
Column: 250 × 4 10 μm LiChrosorb RP-18
Mobile phase: MeCN:buffer 30:70 (Buffer was 67 mM KH_2PO_4 adjusted to pH 2.4 with phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 211

CHROMATOGRAM
Retention time: 8.58

OTHER SUBSTANCES
Simultaneous: benazepril, cilazapril

REFERENCE
Gumieniczek,A.; Przyborowski,L. Determination of benazepril and cilazapril in pharmaceuticals by high performance liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2135–2142.

Enalaprilat



Molecular formula: $C_{18}H_{24}N_2O_5.2H_2O$
Molecular weight: 384.43
CAS Registry No.: 76420-72-9, 84680-54-6 (dihydrate)
Merck Index: 3606

SAMPLE

Matrix: blood
Sample preparation: Condition a 500 mg Bond Elut SCX SPE cartridge with 3 mL MeOH and 12 mL 1% acetic acid. Add 1 mL plasma to the SPE cartridge, wash with 3 mL water, wash with 3 mL MeCN, dry under vacuum, elute with 2 mL 500 mM HCl in MeCN. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute with 100 μL 50 mM triethylamine in MeCN, vortex for 1 min, add 50 μL 60 mM ethyl chloroformate in MeCN, vortex for 1 min, add 200 μL 3 mM L-leucine-(4-methyl-7-coumarinylamide) in MeOH, vortex for 1 min, let stand for 4 min, evaporate to dryness under a stream of nitrogen, reconstitute with 200 μL MeCN: water 50:50, inject a 5-15 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Axxiom octyl (Richard Scientific, Novato)
Mobile phase: Gradient. A was 1 L water containing 2 mL 85% phosphoric acid. B was 1 L MeCN containing 2 mL 85% phosphoric acid. A:B 52:48 for 15 min, to 10:90 over 15 min.
Injection volume: 5-15
Detector: F ex 330 em 390

CHROMATOGRAM

Retention time: 38
Limit of detection: 20 ng/mL

KEY WORDS

plasma; derivatization; SPE

REFERENCE

Lévai,F.; Liu,C.-M.; Tse,M.M.; Lin,E.T. Pre-column fluorescence derivatization using leucine-coumarnylamide for HPLC determination of mono- and dicarboxylic acids in plasma, *Acta Physiol.Hung.*, **1995**, *83*, 39–46.

SAMPLE

Matrix: formulations
Sample preparation: Formed from the hydrolysis of enalapril with NaOH.

HPLC VARIABLES

Column: 5 μm 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

perfusate buffer; 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: formulations
Sample preparation: Dissolve tablets in MeCN:1 mM pH 2 KH_2PO_4 50:50, centrifuge, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb C8
Mobile phase: MeCN:buffer 35:65 (Buffer was 1 mM KH_2PO_4 adjusted to pH 2 with phosphoric acid.)
Column temperature: 40
Flow rate: 2.5
Injection volume: 50
Detector: UV 215

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: degradation products, enalapril, 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 C_8 column, *J.Chromatogr.A*, **1995**, *707*, 245–254.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 μBondapak 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: Condition a Sep-Pak C18 SPE cartridge with 10 mL MeOH, 10 mL water, and 20 mL 100 mM HCl. 1 mL Urine + 10 μL 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 μL 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 μL MeOH:chloroform 10:90, vortex for 30 s. Put this solution in an another tube, evaporate to dryness, reconstitute with 100 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:THF:15 mM pH 2.9 KH_2PO_4 6:1:1:92
Column temperature: 40
Flow rate: 1.5
Injection volume: 10
Detector: UV 206

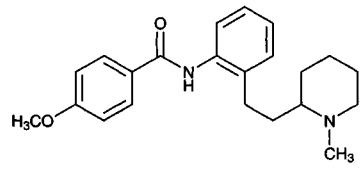
CHROMATOGRAM
Retention time: 10.5
Internal standard: enalaprilat

OTHER SUBSTANCES
Extracted: lisinopril

KEY WORDS
SPE; enalaprilat is IS

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.

Encainide



Molecular formula: $C_{22}H_{28}N_2O_2$
Molecular weight: 352.48
CAS Registry No.: 66778-36-7, 37612-13-8, 66794-74-9 (HCl)
Merck Index: 3609
Lednicer No.: 3 56

SAMPLE
Matrix: blood
Sample preparation: 2 mL Serum or plasma + 100 μL 10 μg/mL trimethobenzamide in MeOH + 1 mL buffer + 10 mL n-butyl chloride:isopropanol 95:5, shake for 10 min, centrifuge at 500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of air at 40°, reconstitute the residue in 200 μL chloroform and 200 μL 25 mM HCl, vortex for 10 s, centrifuge at 500 g for 3-5 min, inject a 50-60 μL aliquot of the upper aqueous layer. (Buffer was 630 mL of a solution containing 1 M boric acid and 1 M KCl + 370 mL 1 M sodium carbonate, adjust pH to 9.0.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm cyanopropyldimethylsilyl (PCN) (Supelco)
Mobile phase: MeCN:0.06% phosphoric acid 15:85 containing 0.01% octylamine
Flow rate: 2 (After analysis wash out system with MeCN:water 20:80.)
Injection volume: 50-60
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Internal standard: trimethobenzamide (5.5)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: dipyridamole, oxazepam
Noninterfering: amiodarone, caffeine, chloral hydrate, chlordiazepoxide, diazepam, ethosuximide, flecainide, lidocaine, methadone, mexiletine, nicotine, phenobarbital, phenytoin, primidone, procainamide, propranolol, quinidine, tocainide, tricyclic antidepressants

KEY WORDS
plasma; serum

REFERENCE

Dasgupta,A.; Rosenzweig,I.B.; Turgeon,J.; Raisys,V.A. Encainide and metabolites analysis in serum or plasma using a reversed-phase high-performance liquid chromatographic technique, *J.Chromatogr.*, **1990**, *526*, 260–265.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 μL 5 μg/mL 4-methylpropranolol in water + 500 μL buffer + 5 mL dichloromethane:diethyl ether 70:30, shake vigorously for 5 min, centrifuge at 2000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot. (Buffer was pH 10.5 prepared from 200 mM sodium carbonate and 200 mM sodium bicarbonate.)

HPLC VARIABLES

Guard column: 10 mm long 5 μm Resolve (Waters)
Column: 150 × 4.6 5 μm Resolve (Waters)
Mobile phase: MeOH:water:methanesulfonic acid:triethylamine 400:4:0.02:0.02
Flow rate: 1
Injection volume: 100
Detector: UV 270

CHROMATOGRAM

Retention time: 7.0
Internal standard: 4-methylpropranolol (3.6)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Simultaneous: amiodarone, propranolol, sotalol
Noninterfering: acebutolol, caffeine, disopyramide, hydroquinidine, nadoxolol, quinidine, theobromine, theophylline

KEY WORDS

plasma

REFERENCE

Poirier,J.-M.; Lebot,M.; Cheymol,G. Analysis of encainide and its three major metabolites in plasma by column liquid chromatography, *J.Chromatogr.*, **1990**, *534*, 223–227.

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: 1.7
Internal standard: protriptyline (4)

OTHER SUBSTANCES

Extracted: acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, chlorpromazine, desipramine, dextromethorphan, diazepam, diphenhydramine, doxepin, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, ibuprofen, imipramine, maprotiline, methadone, methaqualone, midazolam, norchlorimipramine, nordiazepam, nordoxepin, norfluoxetine, nortriptyline, norverapamil, promazine, propafenone, propoxyphene, propranolol, protriptyline, temazepam, trazodone, trimipramine, verapamil

Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin

Interfering: lidocaine, mexiletine, pentazocine, quinidine

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, urine

Sample preparation: 1 mL Plasma or urine + 200 μL 1 (plasma) or 50 (urine) μL/mL ethaverine hydrochloride in water + 100 (plasma) or 500 (urine) μL 500 mM pH 8.5 TRIS-HCl buffer + 2 g NaCl + 5 mL butyl chloride:isopropanol 95:5, vortex for 15 s, centrifuge at 3000 g for 10 min, repeat the extraction. Combine the organic layers and evaporate them to dryness in a vortex-evaporator at 45°, reconstitute the residue in 50 μL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax Sil

Mobile phase: EtOH:water:methanesulfonic acid 500:25:0.5 (plasma) or 500:10:0.25 (urine)

Flow rate: 1.2

Detector: UV 270

CHROMATOGRAM

Retention time: 11.6 (plasma), 13.4 (urine)

Internal standard: ethaverine (6.9 (plasma), 7.6 (urine))

Limit of detection: 1 ng (plasma)

Limit of quantitation: 2.5 ng (plasma)

OTHER SUBSTANCES

Extracted: metabolites

Simultaneous: quinidine

Noninterfering: acetaminophen, alprazolam, aspirin, cephalexin, chloral hydrate, diazepam, digoxin, dipyridamole, docusate, flurazepam, furosemide, hydrochlorothiazide, ibuprofen, isosorbide dinitrate, lidocaine, lorazepam, meclizine, mexiletine, nifedipine, norfloxacin, oxazepam, ranitidine, tocainide, triamterene, triazolam

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Turgeon,J.; Funck-Brentano,C.; Gray,H.T.; Roden,D.M. Improved high-performance liquid chromatographic assay for encainide and its metabolites in human body fluids, *J.Chromatogr.*, **1989**, *490*, 165–174.

SAMPLE
Matrix: bulk
Sample preparation: 0.01-5 μg Encainide + 50 μL MeCN:N,N-diisopropylethylamine 90:10 + 50 μL (-)-menthyl chloroformate:MeCN 10:90, heat at 60° for 2 h, evaporate to dryness, reconstitute in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere silica
Mobile phase: Hexane:ethyl acetate:triethylamine 85:15:1
Flow rate: 1
Injection volume: 500
Detector: UV 261

CHROMATOGRAM
Retention time: 20 (+), 21 (-)
Limit of detection: 10 ng

KEY WORDS
chiral; derivatization; normal phase

REFERENCE
Prakash,C.; Jajoo,H.K.; Blair,I.A.; Mayol,R.F. Resolution of enantiomers of the antiarrhythmic drug encainide and its major metabolites by chiral derivatization and high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *493*, 325–335.

SAMPLE
Matrix: solutions

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

CHROMATOGRAM
Retention time: 9.42 (A), 4.91 (B)

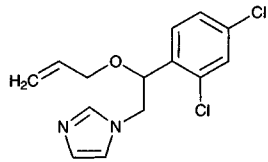
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, 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, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol,

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

KEY WORDS
also details of plasma extraction

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

Enilconazole



Molecular formula: $C_{14}H_{14}Cl_2N_2O$
Molecular weight: 297.18
CAS Registry No.: 35554-44-0
Merck Index: 3622

SAMPLE
Matrix: food
Sample preparation: Condition a 3 mL 500 mg Sep-Pak Vac Silica SPE cartridge with 10 mL MeOH and 10 mL ethyl acetate. Condition a 3 mL 300 mg Bond Elut PRS cartridge with 10 mL water and 10 mL MeOH. Place the Sep Pak Vac Silica cartridge on top of the Bond Elut PRS cartridge. Citrus fruit. Slice and homogenize citrus fruit with a mixer. 5 g Aliquot of sample + 20 g anhydrous sodium sulfate + 1.5 g anhydrous sodium hydrogen phosphate + 30 mL ethyl acetate, blend at high-speed, centrifuge at 3100 rpm for 8 min, remove the supernatant. Re-extract with 20 mL ethyl acetate, combine the supernatants. Add the crude extract to the double SPE cartridge, allow to pass at ca. 1 mL/min, wash with 5 mL ethyl acetate, remove the Sep-Pak Vac Silica cartridge. Wash the second SPE cartridge with 10 mL MeOH and 10 mL 100 mM NaCl, elute with 10 mL mobile phase, inject a 20 μL aliquot. Banana. 10 g Homogenized sample + 40 g anhydrous sodium sulfate + 50 mL ethyl acetate, extract as described above. Re-extract with 30 mL ethyl acetate, combine the supernatants. Add the crude extract to the double SPE cartridge, allow to pass at ca. 1 mL/min, wash with 5 mL ethyl acetate, remove the Sep-Pak Vac Silica cartridge. Wash the second SPE cartridge with 10 mL MeOH and 10 mL 100 mM NaCl, elute with 10 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm TSKgel ODS-80Ts (TOSOH, Japan)
Mobile phase: MeCN:MeOH:water 40:30:30 containing 10 mM sodium 1-tridecanesulfonate, adjusted to pH 2.5 with phosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 225

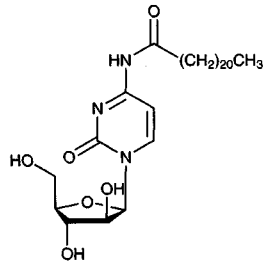
CHROMATOGRAM
Retention time: 17
Limit of detection: 100 ng/g (citrus fruit), 50 ng/g (banana)

OTHER SUBSTANCES
Extracted: thiabendazole

KEY WORDS
citrus fruit; banana; SPE

REFERENCE
Ito,Y.; Ikai,Y.; Oka,H.; Hayakawa,J.; Kagami,T. Application of ion-exchange cartridge clean-up in food analysis. I. Simultaneous determination of thiabendazole and imazalil in citrus fruit and banana using high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.A*, **1998**, *810*, 81–87.

Enocitabine



Molecular formula: $C_{31}H_{55}N_3O_6$
Molecular weight: 565.79
CAS Registry No.: 55726-47-1
Merck Index: 3624

SAMPLE
Matrix: blood, bone marrow fluid, CSF, urine
Sample preparation: Plasma, CSF. 1 mL Plasma or CSF + 2 mL THF, mix, keep at 0° for 1 h, centrifuge at 1000 g for 15 min, filter (0.45 μm) the supernatant, inject a 10 μL aliquot. Blood, bone marrow fluid. 1 mL Blood or bone marrow fluid + 1 mL water + 3 mL THF, mix, keep at 0° for 1 h, centrifuge at 1000 g for 15 min, filter (0.45 μm) the supernatant, inject a 10 μL aliquot. Urine. Centrifuge urine at 12000 g for 15 min, filter, inject a 10 μL aliquot.

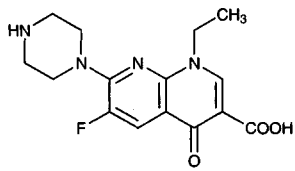
HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeOH:water 95:5
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Limit of detection: 200 ng/mL

KEY WORDS
plasma; whole blood; pharmacokinetics

REFERENCE
Ueda,T.; Nakamura,T.; Ando,S.; Kagawa,D.; Sasada,M.; Uchino,H.; Johno,I.; Akiyama,Y. Pharmacokinetics of N^4-behenoyl-1-β-D-arabinofuranosylcytosine in patients with acute leukemia, *Cancer Res.*, **1983**, *43*, 3412–3416.

Enoxacin



Molecular formula: $C_{15}H_{17}FN_4O_3$
Molecular weight: 320.32
CAS Registry No.: 74011-58-8
Merck Index: 3625

SAMPLE
Matrix: blood
Sample preparation: Filter 1 mL plasma using a micropartition system (MPS-1, Amicon, MA) while centrifuging at 2000 g for 20 min at 10°, inject an aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb ODS-2 endcapped
Mobile phase: MeCN:buffer 13:87 containing 5 mM tetrabutylammonium sulfate, adjusted to pH 2.5 with 1 M NaOH (Buffer was 100 mM citric acid containing 200 mM ammonium perchlorate.)
Column temperature: 37
Flow rate: 1
Detector: UV 271

CHROMATOGRAM
Retention time: 8.01
Internal standard: ofloxacin (10.54)

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Zlotos,G.; Bücker,A.; Kinzig-Schippers,M.; Sorgel,F.; Holzgrabe,U. Plasma protein binding of gyrase inhibitors, *J.Pharm.Sci.*, **1998**, *87*, 215–220.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Plasma + 1 mL 100 mM pH 7.0 K_2HPO_4 adjusted to pH 7.0 with 85% orthophosphoric acid + 100 μL 300 μg/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 μL MeOH:50 mM NaOH 2:1, vortex, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm 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: 6.2
Internal standard: nalidixic acid (5.0)

OTHER SUBSTANCES
Extracted: fenbufen, felbinac
Interfering: ofloxacin, norfloxacin

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: 500 μL Serum + 50 μL 80 μg/mL ciprofloxacin in water + 500 μL 7% perchloric acid, vortex for 10 s, centrifuge at >700 g for 10 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 8 μBondapak C18 Radial-PAK

Mobile phase: MeOH:18 mM KH_2PO_4 containing 0.13 mM heptanesulfonic acid:concentrated phosphoric acid 30:70:0.1
Flow rate: 3
Injection volume: 20
Detector: UV 268

CHROMATOGRAM
Retention time: 4.6
Internal standard: ciprofloxacin
Limit of detection: 200 ng/mL

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: 100 µL Plasma + 100 µL saturated sodium bicarbonate + 50 µL 20 µg/mL ciprofloxacin in saturated sodium bicarbonate + 5 mL chloroform:isopropanol 90:10, 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 KH_2PO_4 + 5.94 g $Na_2HPO_4.2H_2O$ + 1.49 g tetrabutylammonium hydrogen sulfate per L.)
Flow rate: 1.3
Injection volume: 25
Detector: UV 280

CHROMATOGRAM
Retention time: 4.5
Internal standard: ciprofloxacin
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Simultaneous: theophylline, norfloxacin

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: 150 µL Plasma + 75 µL 10% trichloroacetic acid + 600 µL chloroform, vortex for 5 min, centrifuge at 13800 g for 10 min. Remove 500 µL of the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 150 µL mobile phase, inject a 50 µL aliquot.

HPLC VARIABLES
Column: 80 × 4.6 5 µm Zorbax C8
Mobile phase: MeOH:0.01% trifluoroacetic acid 25:75
Flow rate: 1.2

Injection volume: 50
Detector: F ex 280 em 418

CHROMATOGRAM
Retention time: 5.8
Internal standard: enoxacin

OTHER SUBSTANCES
Extracted: norfloxacin

KEY WORDS
plasma; rat; enoxacin is IS

REFERENCE
Hussain,M.S.; Chukwumaeze-Obiajunwa,V.; Micetich,R.G. Sensitive high-performance liquid chromatographic assay for norfloxacin utilizing fluorescence detection, *J.Chromatogr.B*, **1995**, *663*, 379–384.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 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 μL 60 mM KH_2PO_4 adjusted to pH 2.6 with orthophosphoric acid, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 150 × 3 7 μm Separon SGX C18 (Tessek, Prague)
Mobile phase: THF:buffer 5.5:94.5 (Buffer was 60 mM KH_2PO_4 adjusted to pH 2.6 with orthophosphoric acid:triethylamine 97:3 containing 50 μg/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: 5.0
Internal standard: enoxacin

OTHER SUBSTANCES
Extracted: ofloxacin

KEY WORDS
plasma; enoxacin is IS

REFERENCE
Macek,J.; Ptácek,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, CSF, tissue
Sample preparation: Plasma, CSF. 100 μL Plasma or CSF, add 1.0 mL 100 mM phosphate buffer and 1 μg IS in MeOH. Extract with 5 mL chloroform (Caution! Chloroform is a carcinogen!) containing 1.0% ethyl chloroformate by shaking with a reciprocal shaker for 10 min. Remove 4 mL organic phase, dry it with rotary evaporator at 40°, reconstitute the residue in 100 μL mobile phase and inject a 20 μL aliquot. Tissue. Homogenize brain sample in 100 mM pH 7.0 phosphate buffer 1:4. Mix 1 mL homogenate with IS, extract with 5 mL dichloromethane by shaking with a reciprocal shaker for 10 min. Remove 4 mL organic phase and back-extract with 4 mL 1 mM sodium hydroxide, extract the aqueous phase as described above for the plasma, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 6 Nucleosil 5C18

Mobile phase: MeOH:5 mM sodium lauryl sulfate 60:40, adjusted to pH 2.5 with phosphoric acid
Column temperature: 50
Flow rate: 1.2
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Internal standard: ciprofloxacin
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: felbinac

KEY WORDS
plasma; brain; rat; derivatization

REFERENCE
Ohtani,H.; Noma,S.; Kawakami,J.; Sawada,Y.; Iga,T. Lack of potentiation with felbinac patch on the convulsive toxicity of enoxacin in rats, *Biol.Pharm.Bull.*, **1996**, *19*, 995–997.

SAMPLE
Matrix: blood, nasal secretions, saliva, sweat, tears, urine
Sample preparation: Plasma, saliva. 100 μL Plasma or saliva + 25 μM MeCN:perchloric acid 80:20, centrifuge, inject a 10 μL aliquot of the supernatant. Urine. Dilute urine 1:20 or 1:200 with 100 mM pH 4.5 acetate buffer, inject a 10 μL aliquot. Nasal secretions. Collect nasal secretions on 25 mg cotton for 20 min, add 300 μL isotonic NaCl, after 1 h squeeze, centrifuge the solution, inject a 50 μL aliquot of the supernatant. Tears, sweat. Inject tears and sweat directly.

HPLC VARIABLES
Column: 5 μm Spherisorb ODS II C18
Mobile phase: MeCN:buffer 12:88 to 14:86 (Buffer was 100 mM citric acid containing 40 mM ammonium perchlorate and 5 mM tetrabutylammonium hydroxide.)
Flow rate: 1-2
Injection volume: 10-50
Detector: UV 340

CHROMATOGRAM
Limit of quantitation: 78 ng/mL (nasal secretions), 1.95 μg/mL (urine), 39 ng/mL (plasma, saliva, tears, sweat)

OTHER SUBSTANCES
Simultaneous: metabolites, oxoenoxacin

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Jaehde,U.; Sörgel,F.; Naber,K.G.; Zürcher,J.; Schunack,W. Distribution kinetics of enoxacin and its metabolite oxoenoxacin on excretory fluids of healthy volunteers, *Antimicrob.Agents Chemother.*, **1995**, *39*, 2092–2097.

SAMPLE
Matrix: blood, saliva
Sample preparation: 500 μL Plasma or saliva + 50 μL 50 ng/mL difloxacin, vortex briefly, add 500 μL 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 μL mobile phase, inject a 50-200 μL aliquot.

HPLC VARIABLES
Guard column: μBondapak C18 Guard-Pak
Column: 300 × 3.9 10 μm μBondapak 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: 5.2
Internal standard: difloxacin (8.8)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: ciprofloxacin, theophylline
Simultaneous: 1,7-dimethylxanthine
Noninterfering: 1,3-dimethyluric acid, hypoxanthine, 1-methyluric acid, 1-methylxanthine, 3-methylxanthine, 7-methylxanthine, theobromine
Interfering: caffeine

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: Plasma. Mix 100 μL plasma with 900 μL 100 mM phosphate buffer, 100 μL 10 μg/mL IS and 5 mL chloroform:ethyl chloroformate 99:1, shake for 10 min, centrifuge at 1620 g for 5 min, evaporate the organic phase under reduced pressure, dissolve the residue in 100 μL MeOH:50 mM NaOH 2:1, inject a 20 μL aliquot. Tissue. Homogenate the cerebrum sample with 4 volumes of 100 mM phosphate buffer. Mix 1 mL homogenate with 100 μL 10 μg/mL IS and 5 mL dichloromethane, shake for 10 min, centrifuge at 1620 g for 5 min. Mix 4 mL 1 mM NaOH with 4 mL organic phase, shake for 10 min, centrifuge it at 1620 g for 5 min, collect 3 mL aqueous phase and treat in a manner similar to that for the plasma samples, except for the IS addition. Inject a 20 μL aliquot. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil 5 C 18
Mobile phase: MeOH:5 mM sodium dodecylsulfate adjusted to pH 2.5 with phosphoric acid
Flow rate: 0.8
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Internal standard: ciprofloxacin

OTHER SUBSTANCES
Extracted: foscarnet

KEY WORDS
plasma; brain; mouse; pharmacokinetics; derivatization

REFERENCE
Matsuo,H.; Ryu,M.; Nagata,A.; Uchida,T.; Kawakami,J.-I.; Yamamoto,K.; Iga,T.; Sawada,Y. Neurotoxicodynamics of the interaction between ciprofloxacin and foscarnet in mice, *Antimicrob.Agents Chemother.*, **1998**, *42*, 691–694.

SAMPLE

Matrix: blood, tissue

Sample preparation: Blood. Briefly vortex 500 μL plasma, 20 μL 8 μg/mL pefloxacin, and 500 μL pH 7.4 phosphate buffer, add 5 mL dichloromethane:isopropanol 80:20, vortex for 30 s, shake gently for 15 min on an electric shaker, centrifuge at 1500 g for 10 min. Separate the lower layer using phase-separating filter paper (Whatman 1 PS), evaporate the organic phase under nitrogen at 25°, dissolve the residue in 100 μL mobile phase, inject a 20μL aliquot. Tissue. Pulverize prostatic tissue under liquid nitrogen, weigh a 200 mg aliquot, add 20 μL pefloxacin, vortex briefly, incubate at 4° for 2 h, add 500 μL pH 7.4 phosphate buffer and 5 mL dichloromethane:isopropanol 80:20, vortex for 30 s, shake gently for 15 min on an electric shaker, centrifuge at 1500 g for 10 min. Separate the lower layer using phase-separating filter paper (Whatman 1 PS), evaporate the organic phase under nitrogen at 25°, dissolve the residue in 100 μL mobile phase, inject a 20μL aliquot. (The pH 7.4 phosphate buffer was 28.2 g K_2HPO_4 and 5.17 g KH_2PO_4 in 1 L water.)

HPLC VARIABLES

Guard column: 4 × 4 5 μm LiChrospher 100 RP-18

Column: 250 × 4 5 μm Nucleosil C18

Mobile phase: MeCN:pH 2.1 buffer 20.9:79.1 (Prepare the mobile phase by dissolving 18.1 g citric acid and 4.1 g ammonium perchlorate in about 300 mL distilled water, add 209 mL MeCN, dilute to 1 L with water, and add 3 mL tetrabutylammonium hydroxide. Filter through a 0.45 μm HV Millipore filter.)

Flow rate: 0.9

Injection volume: 20

Detector: UV 340

CHROMATOGRAM

Retention time: 5.2

Internal standard: pefloxacin (6.8)

Limit of detection: 10 ng/mL (plasma), 25 ng/mL (tissue)

Limit of quantitation: 20 ng/mL (plasma), 50 ng/mL (tissue)

OTHER SUBSTANCES

Extracted: 4-oxo-enoxacin

Noninterfering: amikacin, ciprofloxacin, fosfomycin, ofloxacin, rifampicin, roxithromycin, tobramycin, vancomycin

KEY WORDS

pharmacokinetics; plasma; prostatic tissue; prostate

REFERENCE

Hamel,B.; Audran,M.; Costa,P.; Bressolle,F. Reversed-phase high-performance liquid chromatographic determination of enoxacin and 4-oxo-enoxacin in human plasma and prostatic tissue. Application to a pharmacokinetic study, *J.Chromatogr.A*, **1998**, *812*, 369–379.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. Condition a Sep-Pak C18 SPE cartridge with 1 mL 4% phosphoric acid in MeOH and 10 mL water. Add 1 mL plasma to the SPE cartridge, wash with 6 mL water, elute with 1 mL 4% phosphoric acid in MeOH, wash out with 1 mL water. Combine the eluates and make up to 2 mL with water, inject a 50 μL aliquot. Urine. 100 μL urine + 900 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: μBondapak C18/Corasil

Column: 300 × 3.9 μBondapak C18

Mobile phase: MeCN:MeOH:water 5:30:70 containing 1.74 g K_2HPO_4 and 20 mg sodium heptanesulfonate, pH adjusted to 3 with phosphoric acid

Flow rate: 2

Injection volume: 10-50

Detector: F ex 285 em 440

OTHER SUBSTANCES
Extracted: norfloxacin

KEY WORDS
plasma; SPE; this procedure can be used for enoxacin (see Infection 1986; 14 (Suppl. 3); S203)

REFERENCE
Gutzler,F.; de Vries,J.X. Bestimmung von Norfloxacin in Plasma und Urin durch Hochdruckflüssigkeitschromatographie, *Fortschr.Antimikr.Antineoplast.Chemother.*, **1984**, *3*, 673–677.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 500 µL Plasma + 50 µL 20 µg/mL difloxacin + 200 µL MeCN: 60% perchloric acid 80:20, vortex, centrifuge for 5 min, inject a 75 µL aliquot of the supernatant. Urine. 200 µL Urine + 500 µL water + 200 µL 200 µg/mL difloxacin + 200 µL pH 7.4 phosphate buffer. Pass through an Analytichem C18 SPE cartridge, inject a 10 µL aliquot of the eluate.

HPLC VARIABLES
Column: 100 × 4.6 5 µm RAC II Partisil ODS 3
Mobile phase: MeCN:buffer 15:85 (Buffer was 100 mM citrate containing 650 µL/L 20% tetrabutylammonium hydroxide and 450 mg/L ammonium perchlorate.)
Flow rate: 1
Injection volume: 10-75
Detector: UV 340

CHROMATOGRAM
Internal standard: difloxacin
Limit of quantitation: 833 ng/mL (urine), 25 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Grasela,T.H.,Jr.; Schentag,J.J.; Sedman,A.J.; Wilton,J.H.; Thomas,D.J.; Schultz,R.W.; Lebsack,M.E.; Kinkel,A.W. Inhibition of enoxacin absorption by antacids or ranitidine, *Antimicrob.Agents Chemother.*, **1989**, *33*, 615–617.

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

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

Injection volume: 10-30
Detector: UV 268.9

CHROMATOGRAM
Retention time: 7.672

KEY WORDS
whole blood

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

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 25:75 containing 200 mM KCl
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 265

REFERENCE
Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE
Matrix: solutions
Sample preparation: Filter (0.45 μm) a solution in MeCN:water 10:90, inject an aliquot of the filtrate.

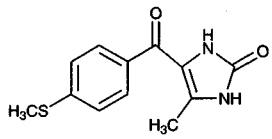
HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: MeCN:buffer 7:93 (Buffer was 25 mM phosphoric acid adjusted to pH 3.89 with 100 mM tetrabutylammonium hydroxide.)
Flow rate: 1
Injection volume: 10
Detector: UV 280

CHROMATOGRAM
Retention time: 7.8

OTHER SUBSTANCES
Simultaneous: ciprofloxacin, 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.

Enoximone



Molecular formula: $C_{12}H_{12}N_2O_2S$
Molecular weight: 248.31
CAS Registry No.: 77671-31-9
Merck Index: 3627

SAMPLE

Matrix: blood
Sample preparation: 2 mL Plasma + 100 µL 4.16 µg/mL IS in MeOH + 3 mL MeCN, vortex, add 2 mL 100 mM pH 7.5 sodium phosphate buffer, centrifuge at 900 g for 20 min. Remove 6 mL of the supernatant and add it to 9 mL ethyl acetate, shake on a reciprocating shaker for 20 min, centrifuge. Remove 10 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 50-55°, reconstitute the residue in 200 µL MeOH, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 6 µm Zorbax C8
Mobile phase: MeOH:water 60:40
Flow rate: 1
Injection volume: 20
Detector: UV 313

CHROMATOGRAM

Retention time: 6.0
Internal standard: 1,3-diethyl-1,3-dihydro-4-(4-methoxybenzoyl)-5-methyl-2H-imidazol-2-one (MDL 17,043) (11.5)
Limit of quantitation: 25 ng/mL

KEY WORDS

plasma; dog; pharmacokinetics

REFERENCE

Chan,K.Y.; Lang,J.F.; Okerholm,R.A. Quantitative determination of cardiotonic agent MDL 17,043 in plasma by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *272*, 396–400.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Plasma + 100 µL 6 µg/mL ID in MeOH + 3 mL MeCN, vortex, centrifuge at 900 g for 20 min. Remove the supernatant and add it 9 mL ethyl acetate, shake for 20 min, centrifuge for 5 min. Remove 10 mL of the upper organic layer and evaporate it to dryness under a stream of nitrogen at 50-55°, reconstitute the residue in 100 µL MeOH, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 6 µm Zorbax CN
Mobile phase: MeOH:water 45:55
Flow rate: 1
Injection volume: 20
Detector: UV 313

CHROMATOGRAM

Retention time: 6.8
Internal standard: 1-ethyl-4-methyl-5-[p-methoxybenzoyl]-4-imidazolin-2-one (MDL 18,763) (10.5)
Limit of quantitation: 62.5 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

Noninterfering: chlorthalidone, clonidine, digitalis, digoxin, furosemide, hydralazine, hydrochlorothiazide, isosorbide dinitrate, levothyroxine (synthroid), methyldopa, nitroglycerin, phenazopyridine, sulfamethoxazole (gantanol), tolazamide (tolinase), zomepirac

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Chan,K.Y.; Ohlweiler,D.F.; Lang,J.F.; Okerholm,R.A. Simultaneous analysis of a new cardiotonic agent, MDL 17,043, and its major sulfoxide metabolite in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *306*, 249–256.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 100 μL 3 μg/mL + 1.5 mL MeCN, vortex for 15 s, centrifuge at 3000 g for 10 min. Add the supernatant to 5 mL ethyl acetate, rotate at 20 rpm for 20 min, centrifuge at 3000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40-45°, reconstitute the residue in 120 μL MeOH:water 20:80, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. MeCN:water 86:14 for 3 min, to 10:90 (step gradient), maintain at 10:90 for 6 min, re-equilibrate at initial conditions for 10 min.
Flow rate: 1
Injection volume: 20
Detector: UV 365

CHROMATOGRAM
Retention time: 8.25
Internal standard: MDL 82249 (7.75)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: acenocoumarol, allopurinol, amikacin, amiloride, amiodarone, aspirin, bromazepam, captopril, cefoperazone, ceftriaxone, chlorazepate, clonidine, digitoxin, dihydralazine, diltiazem, dipyridamole, doxycycline, flumequine, furosemide, gentamicin, heparin, hydrochlorothiazide, isosorbide dinitrate, lidocaine, lorazepam, meperidine, mezlocillin, minocycline, nalidixic acid, netilmicin, nifedipine, nitrazepam, nitroglycerin, penicillin G, propafenone, protamine, sodium nitroprusside, spironolactone, ticarcillin

KEY WORDS
serum; pharmacokinetics

REFERENCE
Tarral,E.; Jehl,F.; Gallion,C.; Monteil,H. Dosage de l'enoximone et de son principal métabolite dans le sérum et l'urine par chromatographie liquide à haute performance [Determination of enoximone and its principle metabolite in serum and urine using high performance liquid chromatography], *Therapie*, **1990**, *45*, 1–6.

SAMPLE
Matrix: blood, dialysate
Sample preparation: The dialyzer had two 300 mm path length blocks in series. The membrane was Cuprophan C-type (Technicon) with a cut-off of 10000 Daltons. Dialyze 600 μL serum against 10 mM ammonium phosphate buffer, pump the buffer continuously through the dialyzer and through a trace-enrichment column packed with 20 mg 5 μm Hypersil ODS at 2.5 mL/min, after 3 min flush the contents of the trace-enrichment column onto the analytical column with the mobile phase, after 3 min remove the trace-enrichment column from the circuit, monitor the effluent from the analytical column.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Spherisorb ODS II

Mobile phase: Gradient. A was buffer:water 4:96. B was MeCN:buffer:water 70:4:26. A:B 85:15 for 2.5 min, to 40:60 over 4 min, return to initial conditions over 0.5 min, re-equilibrate for 4 min. (Buffer was 38.5 g ammonium acetate in 800 mL water, adjust the pH to 5.0 with glacial acetic acid, make up to 1 L with water.)
Flow rate: 2
Detector: UV 335

CHROMATOGRAM
Retention time: 3
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; column-switching; trace enrichment; pharmacokinetics

REFERENCE
Cooper,J.D.; Turnell,D.C. Automatic preparation of human serum samples for analysis of the drug enoximone and its sulphoxide metabolite using high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *380*, 109–116.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 1 mL water + 100 μL 6 μg/mL IS in MeOH, add to a Bond Elut C18 SPE cartridge, wash with 18 mL water, elute with 3 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot. Urine. 300 μL Urine + 700 μL 1 M pH 9.0 Tris-HCl buffer + 1 mL water + 100 μL 6 μg/mL IS in MeOH, add to a Bond Elut C18 SPE cartridge, wash with 18 mL water, elute with 3 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

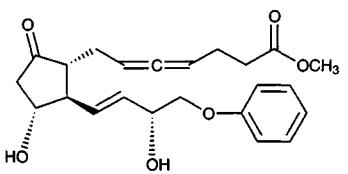
HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax CN
Mobile phase: MeOH:50 mM pH 4.6 phosphate buffer 40:60
Flow rate: 1
Injection volume: 20
Detector: UV 340

CHROMATOGRAM
Internal standard: MDL 18,763
Limit of quantitation: 500 ng/mL (urine), 5 ng/mL (plasma)

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Morita,S.; Sawai,Y.; Heeg,J.F.; Koike,Y. Pharmacokinetics of enoximone after various intravenous administrations to healthy volunteers, *J.Pharm.Sci.*, **1995**, *84*, 152–157.

Enprostil



Molecular formula: $C_{23}H_{28}O_6$
Molecular weight: 400.47
CAS Registry No.: 73121-56-9
Merck Index: 3629
Lednicer No.: 4 10

SAMPLE
Matrix: blood
Sample preparation: Prepare a SPE cartridge by adding 150 mg 40 μm Bondesil phenyl (Analytichem) to a 5 mL column. Condition SPE cartridge with 2 mL MeOH, 2 mL water, and 1 mL 20 mM pH 3.0 sodium acetate buffer. 2 mL Plasma + IS (500-1000 cpm), filter (20 μm), add to SPE cartridge, wash with 300 μL pH 3 buffer, wash with 1 mL MeOH:water 40:60, wash with 300 μL 0.3% acetic acid, wash with 1 mL water, elute with 1.5 mL MeOH:water 60:40. Evaporate the eluate under a stream of nitrogen, reconstitute in 125 μL 1 mg/mL 2-bromoacetyl-6-methoxynaphthalene in MeCN and 100 μL 34.6 mg/mL 18-crown-6 in MeCN, add about 2 mg anhydrous sodium sulfate, add about 2 mg potassium carbonate, shake gently for 1 h, evaporate under nitrogen, dissolve the residue in 250 μL dichloromethane. Inject a 200 μL aliquot onto a 100 × 4.6 5 μm Spheri-5 silica column, elute to waste with dichloromethane:MeCN 20:70, elute a 1 min fraction containing the analyte onto a 220 × 4.6 5 μm Spheri-5 silica column, elute this column with the same mobile phase, collect a fraction containing the analyte. Evaporate the fraction to dryness and reconstitute it in 1 mL dichloromethane, add it to an AASP silica SPE cartridge (Analytichem), elute the contents of the cartridge onto column A with mobile phase A for 1 min, elute column A to waste with mobile phase A, elute a 1 min fraction containing the analyte and mix it with water pumped at 2 mL/min, the combined effluent flows onto column B. At the end of this time elute column B with mobile phase B onto column C, elute a 1 min fraction containing the analyte and mix it with water pumped at 0.15 mL/min, store the diluted column effluent in a 600 μL sample loop. Flush the contents of the sample loop onto column D with mobile phase C for 8 min, elute the contents of column D with mobile phase D onto column E, monitor the effluent from column E.

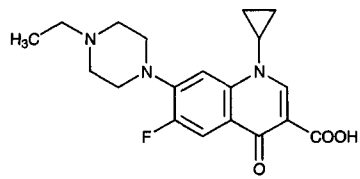
HPLC VARIABLES
Column: A 250 × 4.6 7 μm Chemcosorb 7CN (Dychrom); B 250 × 4.6 5 μm Spheri-5 C18; C 250 × 1 5 μm Hypersil C18; D 30 × 1 5 μm Hypersil C18; E 150 × 1 3 μm Hypersil C18
Mobile phase: A MeOH:water:acetic acid 40:60:0.1; B MeOH:water:acetic acid 50:50:0.1; C water; D MeOH:water 40:60
Flow rate: A 1; B 0.05; C 0.1; D 0.05
Injection volume: 200
Detector: F ex 325 em 450 (Corrion S40-450 and LL-400 filters), laser fluorescence, specially constructed apparatus

CHROMATOGRAM
Retention time: 20 (of the corresponding acid)
Internal standard: tritiated enprostil acid
Limit of quantitation: 0.005 ng/mL

KEY WORDS
plasma; SPE; derivatization; column-switching; normal phase; reversed phase; heart cut; microbore; laser fluorescence

REFERENCE
Kiang,C.H.; Nolan,T.; Huang,B.L.; Lee,C.P. Determination of femtomole/milliliter concentrations of enprostil acid in human plasma using high-performance liquid chromatography-laser-induced fluorescence detection, *J.Chromatogr.*, **1991**, *567*, 195–212.

Enrofloxacin



Molecular formula: $C_{19}H_{22}FN_3O_3$
Molecular weight: 359.40
CAS Registry No.: 93106-60-6
Merck Index: 3630
Lednicer No.: 5 125

SAMPLE
Matrix: blood

SAMPLE
Matrix: blood
Sample preparation: Prepare a SPE cartridge by adding 150 mg 40 μm Bondesil phenyl (Analytichem) to a 5 mL column. Condition SPE cartridge with 2 mL MeOH, 2 mL water, and 1 mL 20 mM pH 3.0 sodium acetate buffer. 2 mL Plasma + IS (500-1000 cpm), filter (20 μm), add to SPE cartridge, wash with 300 μL pH 3 buffer, wash with 1 mL MeOH:water 40:60, wash with 300 μL 0.3% acetic acid, wash with 1 mL water, elute with 1.5 mL MeOH:water 60:40. Evaporate the eluate under a stream of nitrogen, reconstitute in 125 μL 1 mg/mL 2-bromoacetyl-6-methoxynaphthalene in MeCN and 100 μL 34.6 mg/mL 18-crown-6 in MeCN, add about 2 mg anhydrous sodium sulfate, add about 2 mg potassium carbonate, shake gently for 1 h, evaporate under nitrogen, dissolve the residue in 250 μL dichloromethane. Inject a 200 μL aliquot onto a 100 × 4.6 5 μm Spheri-5 silica column, elute to waste with dichloromethane:MeCN 20:70, elute a 1 min fraction containing the analyte onto a 220 × 4.6 5 μm Spheri-5 silica column, elute this column with the same mobile phase, collect a fraction containing the analyte. Evaporate the fraction to dryness and reconstitute it in 1 mL dichloromethane, add it to an AASP silica SPE cartridge (Analytichem), elute the contents of the cartridge onto column A with mobile phase A for 1 min, elute column A to waste with mobile phase A, elute a 1 min fraction containing the analyte and mix it with water pumped at 2 mL/min, the combined effluent flows onto column B. At the end of this time elute column B with mobile phase B onto column C, elute a 1 min fraction containing the analyte and mix it with water pumped at 0.15 mL/min, store the diluted column effluent in a 600 μL sample loop. Flush the contents of the sample loop onto column D with mobile phase C for 8 min, elute the contents of column D with mobile phase D onto column E, monitor the effluent from column E.

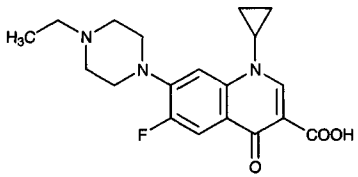
HPLC VARIABLES
Column: A 250 × 4.6 7 μm Chemcosorb 7CN (Dychrom); B 250 × 4.6 5 μm Spheri-5 C18; C 250 × 1 5 μm Hypersil C18; D 30 × 1 5 μm Hypersil C18; E 150 × 1 3 μm Hypersil C18
Mobile phase: A MeOH:water:acetic acid 40:60:0.1; B MeOH:water:acetic acid 50:50:0.1; C water; D MeOH:water 40:60
Flow rate: A 1; B 0.05; C 0.1; D 0.05
Injection volume: 200
Detector: F ex 325 em 450 (Corrion S40-450 and LL-400 filters), laser fluorescence, specially constructed apparatus

CHROMATOGRAM
Retention time: 20 (of the corresponding acid)
Internal standard: tritiated enprostil acid
Limit of quantitation: 0.005 ng/mL

KEY WORDS
plasma; SPE; derivatization; column-switching; normal phase; reversed phase; heart cut; microbore; laser fluorescence

REFERENCE
Kiang,C.H.; Nolan,T.; Huang,B.L.; Lee,C.P. Determination of femtomole/milliliter concentrations of enprostil acid in human plasma using high-performance liquid chromatography-laser-induced fluorescence detection, *J.Chromatogr.*, **1991**, *567*, 195–212.

Enrofloxacin



Molecular formula: $C_{19}H_{22}FN_3O_3$
Molecular weight: 359.40
CAS Registry No.: 93106-60-6
Merck Index: 3630
Lednicer No.: 5 125

SAMPLE
Matrix: blood

Sample preparation: Add 20 μL 10 μg/mL IS in MeOH:0.1% trifluoroacetic acid 15:85 and 5 μL (sic) MeCN to 300 μL plasma. Centrifuge at 600 g for 10 min. Evaporate the supernatant under nitrogen at 40° for 30 min. Reconstitute the residue in 200 μL MeOH:0.1% trifluoroacetic acid 15:85. Inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 12.5 × 4 Zorbax RX-C18
Column: 150 × 4.6 5 μm Zorbax SB-C8
Mobile phase: MeCN:water:trifluoroacetic acid 19:81:0.02
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: UV 279

CHROMATOGRAM
Retention time: 5.6-5.8
Internal standard: norfloxacin (3.8-3.9)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: ciprofloxacin

KEY WORDS
cat; plasma

REFERENCE
Kordick,D.L.; Papich,M.G.; Breitschwerdt,E.B. Efficacy of enrofloxacin or doxycycline for treatment of Bartonella henselae or Bartonella clarridgeiae infection in cats, *Antimicrob.Agents Chemother.*, **1997**, *41*, 2448–2455.

SAMPLE
Matrix: blood
Sample preparation: Filter 1 mL plasma using a micropartition system (MPS-1, Amicon, MA) while centrifuging at 2000 g for 20 min at 10°, inject an aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb ODS-2 endcapped
Mobile phase: MeCN:buffer 20:80 containing 5 mM tetrabutylammonium sulfate, adjusted to pH 2.5 with 1 M NaOH (Buffer was 100 mM citric acid containing 200 mM ammonium perchlorate.)
Column temperature: 37
Flow rate: 1
Detector: UV 279

CHROMATOGRAM
Retention time: 6.54
Internal standard: ciprofloxacin (4.82)

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Zlotos,G.; Bücker,A.; Kinzig-Schippers,M.; Sorgel,F.; Holzgrabe,U. Plasma protein binding of gyrase inhibitors, *J.Pharm.Sci.*, **1998**, *87*, 215–220.

SAMPLE
Matrix: blood, milk
Sample preparation: 500 μL Milk or plasma + 500 μL 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 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 250 × 4.6 3 μm 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: 15.5
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: ciprofloxacin

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, tissue
Sample preparation: Serum. 500 μL Serum + 500 μL 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 μL aliquot of the ultrafiltrate. Tissue. Cut up prostate tissue with a scalpel. Weigh out 100-130 mg tissue, make up to 500 μL 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 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 100 × 4.6 3 μm 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: 10.6
Limit of detection: 4 ng/mL

OTHER SUBSTANCES
Extracted: ciprofloxacin

KEY WORDS
serum; dog; prostate; ultrafiltrate

REFERENCE
Tyczkowska,K.; Hedeen,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: milk
Sample preparation: Condition a 500 mg Bond Elut LRC PRS SPE cartridge with 5 mL MeOH and 5 mL extracting solution 65:35. Add 25 mL extracting solution to 5 mL milk, shake for 15 s, add 4 g anhydrous sodium sulfate, shake for 15 s, centrifuge at 3000 rpm at 5° for 5 min.

Remove the supernatant and repeat the extraction with 25 mL extracting solution as before except do not add any more sodium sulfate, mix mechanically, centrifuge, combine the supernatants, add 25 mL 1% acetic acid, shake for 10-15 s. Freeze for 30 min to facilitate precipitation, centrifuge at 2500 rpm at 5° for 10 min. Add 75 mL to the SPE cartridge, pass the entire sample through the cartridge, then add 2 mL MeOH, wash with 5 mL water, wash with 2 mL MeOH. Elute with 2.5 mL 25% ammonium hydroxide-MeOH. Evaporate to dryness under nitrogen at 55°, dissolve the residue in 2 mL 1% acetic acid, sonicate for 1 min, vortex for 20 s, filter (0.45 μm), inject an aliquot. (Extracting solution was 1% aqueous acetic acid:EtOH 1:99.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil
Mobile phase: MeCN:2% acetic acid 15:85
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: F ex 278 em 450, with a 418 nm cut-off filter

CHROMATOGRAM
Retention time: 4.2
Limit of detection: 0.3 ppb
Limit of quantitation: 5 ppb

OTHER SUBSTANCES
Extracted: ciprofloxacin, difloxacin, sarafloxacin

KEY WORDS
SPE

REFERENCE
Roybal,J.E.; Pfenning,A.P.; Turnipseed,S.B.; Walker,C.C.; Hurlbut,J.A. Determination of four fluoroquinolones in milk by liquid chromatography, *J.AOAC Int.*, **1997**, *80*, 982–987.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 4 μm NovaPak C18
Mobile phase: MeCN:MeOH:buffer:acetic acid 2.5:10:86.5:1 containing 20 mM triethylamine (The pH 2.7 buffer was 0.4% diammonium hydrogen phosphate in water containing 0.4% (?) tetrabutylammonium hydrogen sulfate.)
Flow rate: 1
Detector: UV 279

CHROMATOGRAM
Retention time: 25.8

OTHER SUBSTANCES
Extracted: ciprofloxacin, ofloxacin

REFERENCE
Cester,C.C.; Toutain,P.L. A comprehensive model for enrofloxacin to ciprofloxacin transformation and disposition in dog, *J.Pharm.Sci.*, **1997**, *86*, 1148–1155.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 10 mL 500 mg Bond Elut LRC PRS SPE cartridge with 2 mL MeOH and 2 mL equilibrating solution. 2 g Catfish muscle + 18 mL extracting solution, homogenize for 20 s, centrifuge at 3000 rpm for 5 min, decant the supernatant. Add another 18 mL extracting solution to the pellet and homogenize again, centrifuge at 3000 rpm for 5 min, combine the supernatants. Add 20 mL 1% glacial acetic acid, freeze for 30 min, centrifuge at

2500 rpm at 4° for 10 min. Add the extracts to the SPE cartridge, wash with 2 mL MeOH, 5 mL water, and 2 mL MeOH. Let the SPE cartridge dry for 30 s. Elute with 2 mL MeOH:30% ammonium hydroxide 80:20, dry the eluate under nitrogen at 50°. Reconstitute the residue in 500 μL mobile phase, filter (0.45 μm), inject an aliquot. (The extracting solution was EtOH: water:glacial acetic acid 98:1:1. The equilibrating solution was extracting solution:1% glacial acetic acid 35:20.)

HPLC VARIABLES
Column: 150 × 2 5 μm Inertsil Phenyl
Mobile phase: MeCN:2% formic acid 14:86
Column temperature: 40
Flow rate: 0.35
Injection volume: 50
Detector: MS, Hewlett-Packard 5989, Model 59987A electrospray, nitrogen drying gas 40 mL/min, 260°, nebulizing gas nitrogen, 80 psi, m/z 245

CHROMATOGRAM
Retention time: 5.43-5.76
Limit of detection: 10 ppb
Limit of quantitation: 20 ppb

OTHER SUBSTANCES
Extracted: ciprofloxacin

KEY WORDS
catfish; muscle; SPE

REFERENCE
Turnipseed,S.B.; Walker,C.C.; Roybal,J.E.; Pfenning,A.P.; Hurlbut,J.A. Confirmation of fluoroquinolones in catfish muscle by electrospray liquid chromatography/mass spectrometry, *J.AOAC Int.*, **1998**, *81*, 554–562.

SAMPLE
Matrix: tissue
Sample preparation: Soxhlet extract 20 g finely chopped (20 mesh or finer) tissue with 130 mL dichloromethane:MeOH 90:10 for 15 h at 5 exchanges/hour. If no aqueous layer is present filter through a glass wool plug, wash though with 20 mL and 10 mL dichloromethane. (If an aqueous layer is present add 1 mL 1 M pH 7 phosphate buffer, filter through glass wool plug, separate layers, extract aqueous layer with 20 mL and 10 mL dichloromethane, combine the dichloromethane layers.) Concentrate the dichloromethane extracts nearly to dryness (3-ball Snyder column), add 10 mL hexane, concentrate nearly to dryness (≤ 1 mL). Add the residue to 10 mL 100 mM pH 2 phosphate buffer using three 10 mL portions of hexane (wash original flask with two 10 mL portions of warm dichloromethane (A)), shake vigorously for 2 min, discard hexane layer, wash aqueous layer with 10 mL hexane, wash aqueous layer with dichloromethane (A). Extract the dichloromethane layer with a fresh 10 mL portion of 100 mM pH 2 phosphate buffer. Combine the aqueous layers, adjust pH to 12 with 1.7 mL 5 M NaOH, add 20 mL dichloromethane, shake vigorously for 1 min, centrifuge, separate layers. Extract dichloromethane layer with 10 mL 100 mM pH 12 buffer. Combine the aqueous layers, adjust the pH to 7.0 with 600 μL 30% phosphoric acid, add 30 mL dichloromethane, shake vigorously for 1 min, extract aqueous layer again with 15 mL dichloromethane. Combine dichloromethane layers, filter through a glass wool plug, add 1 mL 5% diethylene glycol in dichloromethane, evaporate to dryness under vacuum at 50°, dissolve the residue in 1 mL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-18 DB
Mobile phase: MeCN:water:triethylamine 20:75:5, adjusted to pH 3.5 with orthophosphoric acid
Flow rate: 1
Injection volume: 10
Detector: F ex 282 em 445 or UV 282

CHROMATOGRAM
Retention time: 5.6

KEY WORDS
chicken; turkey; liver; muscle; skin

REFERENCE
Waggoner,T.B.; Bowman,M.C. Spectrofluorometric determination of BAY Vp 2674 residues in poultry tissues, *J.Assoc.Off.Anal.Chem.*, **1987**, *70*, 813–818.

SAMPLE
Matrix: tissue
Sample preparation: Wash a 500 mg 2.8 mL Bond-Elut SCX cartridge with 1% HOAc in EtOH. Homogenize 2 g muscle tissue in 20 mL 1% HOAc 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 mL 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: 12
Limit of quantitation: < 10 ng/g

OTHER SUBSTANCES
Simultaneous: ciprofloxacin

KEY WORDS
muscle; pig; cow; muscle; 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: tissue
Sample preparation: Condition a 500 mg Bond Elut C18 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

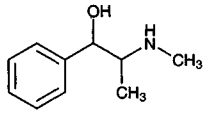
CHROMATOGRAM
Retention time: 15
Limit of detection: 10 ng/g

OTHER SUBSTANCES
Simultaneous: benofloxacin, danofloxacin, ofloxacin

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.

Ephedrine



Molecular formula: $C_{10}H_{15}NO$
Molecular weight: 165.24
CAS Registry No.: 90-81-3 (DL), 134-71-4 (DL HCl), 50-98-6 (L HCl), 134-72-5 (L sulfate), 299-42-3 (-), 50906-05-3 ((-) hemihydrate)
Merck Index: 3645
Lednicer No.: 1 66

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-pak C18 SPE cartridge with EtOH, 5% aqueous bovine serum albumin, and water. 100-500 μL Plasma + 100 ng (l)-norephedrine + 2 mL 500 mM pH 7.0 phosphate buffer, add to the SPE cartridge, wash with 5 mL water, wash with 3 mL EtOH:water 20:80, elute with 8 mL EtOH. Evaporate the eluate to dryness, reconstitute the residue in 100 μL MeCN, add 100 μL 6 mg/mL dansylchloride in MeCN containing 0.03% triethylamine, heat at 50° for 20 min, evaporate to dryness under a stream of nitrogen, reconstitute with 1 mL EtOH:water 90:10, add to an 18 × 6 column containing 80 mg carboxymethyl Sephadex LH-20 (0.95 meq/g), wash with EtOH:water 90:10, elute with 6 mL 50 mM methylamine in EtOH:water 90:10 at 0.1 mL/min. Evaporate the eluate to dryness, reconstitute with 50-100 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: 10 × 4 5 μm Shimpack G-ODS guard column (Shimadzu)
Column: 150 × 6 5 μm Shimpack CLC-ODS (Shimadzu)
Mobile phase: MeOH:0.6% pH 6.5 phosphate buffer 80:30
Flow rate: 1.3
Detector: F ex 316 em 486

CHROMATOGRAM
Retention time: 12.5
Internal standard: (l)-norephedrine (10)

OTHER SUBSTANCES
Extracted: pseudoephedrine

KEY WORDS
plasma; SPE; guinea pig; human; derivatization; pharmacokinetics

REFERENCE
Shao,G.; Wang,D.-S.; Wu,F.; Chen,S.-J.; Luo,X. Separation and determination of (l)-ephedrine and (d)-pseudoephedrine in plasma by high-performance liquid chromatography with fluorescence detection, *J.Liq.Chromatogr.*, **1995**, *18*, 2133–2145.

SAMPLE
Matrix: blood

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

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 259

CHROMATOGRAM

Retention time: 3.95
Limit of detection: <120 ng/mL

KEY WORDS

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

REFERENCE

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

SAMPLE

Matrix: blood, urine

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

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 5.655

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–4163.

SAMPLE

Matrix: bulk
Sample preparation: Prepare a 10 mg/mL solution in 500 mM sodium bicarbonate solutions, extract a 10 mL aliquot twice with 15 mL portions of dichloromethane. Combine the extracts and add 10 μL phenylisothiocyanate, evaporate to dryness under a stream of air, reconstitute with 10 mL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: 70 × 2.1 CO:PELL ODS
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:water:acetic acid 45:54:1
Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 7.5

OTHER SUBSTANCES

Simultaneous: lidocaine, phenylpropanolamine, pseudoephedrine

KEY WORDS

derivatization

REFERENCE

Noggle,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: bulk

Sample preparation: Mix a 1 mg/mL solution in 1 M sodium carbonate with 2 mL 5 mg/mL 8-quinolinesulfonyl chloride in acetone, heat at 65° for 20 min, cool, extract twice with 30 mL portions of chloroform. Combine the extracts and dry them over anhydrous magnesium sulfate, evaporate to dryness under a stream of air, reconstitute, inject an aliquot.

HPLC VARIABLES
Guard column: 70 × 2.1 Co:Pell ODS
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water:acetic acid 40:59:1
Flow rate: 1.5
Detector: UV 254, UV 280

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: amphetamine, methamphetamine, phenmetrazine, phentermine, phenylpropanolamine, pseudoephedrine

KEY WORDS
derivatization

REFERENCE
Noggle,F.T.,Jr.; Clark,C.R. Liquid chromatographic determination of primary and secondary amines as 8-quinolinesulfonyl chloride derivatives, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 687–691.

SAMPLE
Matrix: bulk
Sample preparation: Mix 200 μmole amine with 500 μmole (1S)-(+)-camphor-10-sulfonyl chloride, 10 mL diethyl ether, and 10 mL 1 M NaOH, stir vigorously for 1 h, acidify with concentrated HCl, extract three times with diethyl ether. Combine the organic layers and wash them three times with water, evaporate to dryness, reconstitute with 1 mL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 5 μm Silica 100-RP 18
Mobile phase: MeOH:water 50:50
Column temperature: 40
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 7.16, 8.26 (enantiomers)

OTHER SUBSTANCES
Also analyzed: amphetamine, bamethan, norpseudoephedrine, 1-phenylethylamine

KEY WORDS
derivatization; chiral

REFERENCE
Vogt,C.; Jira,T.; Beyrich,T. HPLC-Trennung racemischer Amine nach Derivatisierung mit (1S)-(+)-Campher-10-sulfonylchlorid, *Pharmazie*, **1990**, *45*, 691.

SAMPLE
Matrix: formulations
Sample preparation: Syrup. Dilute syrup with an equal volume of water. 2 mL Diluted syrup + 2 mL 1% dansyl chloride in acetone + 200 μL 1.5 M sodium carbonate, heat at 45 ± 2° in the dark for 20 min, cool, add 3 mL water, add 500 μL benzene (Caution! Benzene is a carcin-

ogen!), shake. Remove the organic layer and evaporate it to dryness, reconstitute the residue in mobile phase, inject a 10 μL aliquot. Capsules. Sonicate the contents of a capsule in 20 mL water for 10 min, centrifuge at 2000 g for 10 min. 2 mL Supernatant + 2 mL 1% dansyl chloride in acetone + 200 μL 1.5 M sodium carbonate, heat at 45 ± 2° in the dark for 20 min, cool, add 3 mL water, add 500 μL benzene (Caution! Benzene is a carcinogen!), shake. Remove the organic layer and evaporate it to dryness, reconstitute the residue in mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 2.8 10 μm silica gel SI 100 (Merck)
Mobile phase: Diisopropyl ether:isopropanol:concentrated ammonia 48:2:0.3 (Caution! Diisopropyl ether readily forms explosive peroxides!)
Injection volume: 10
Detector: UV 254 or F ex 354 em 476

CHROMATOGRAM
Retention time: 2

OTHER SUBSTANCES
Simultaneous: cephaeline, emetine, codeine (not derivatized, detect at UV 254 only)

KEY WORDS
derivatization; syrup; capsules; normal phase

REFERENCE
Frei,R.W.; Santi,W.; Thomas,M. Liquid chromatography of dansyl derivatives of some alkaloids and the application to the analysis of pharmaceuticals, *J.Chromatogr.*, **1976**, *116*, 365–377.

SAMPLE
Matrix: formulations
Sample preparation: Dilute formulation 1:10. Remove a 1 mL aliquot and add it to 1.5 mL water and 2.5 mL 20 μg/mL ephedrine, inject an 80 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:buffer 35:65 containing 0.05% sodium tetradecyl sulfate (Buffer was 0.83 mM phosphoric acid adjusted to pH 5.0 with triethylamine. Use a 50 × 4.6 5-25 μm LiChroprep Si 60 column before the injector. Wash column with MeCN:83 mM phosphoric acid 40:60 after use.)
Flow rate: 2.5
Injection volume: 80
Detector: UV

CHROMATOGRAM
Retention time: 10
Internal standard: ephedrine

OTHER SUBSTANCES
Simultaneous: ergonovine (ergometrine), oxytocin

KEY WORDS
injections; ephedrine is IS

REFERENCE
Pask-Hughes,R.A.; Corran,P.H.; Calam,D.H. Assay of the combined formulation of ergometrine and oxytocin by high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *214*, 307–315.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablet and add 50 mg to 50 mL MeCN:20 mM pH 3.8 phosphate buffer 3:97, sonicate for 5 min, filter (0.5 μm), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: Supelguard pre-column containing 5 μm Suplex pKb100 (Supelco)
Column: 150 × 4.6 5 μm Suplex pKb100 (Supelco)
Mobile phase: Gradient. MeCN:20 mM pH 3.8 phosphate buffer at 3:97 for 3 min, to 15:85 over 5 min, stay at 15:85 for 4 min, re-equilibrate for 8 min.
Flow rate: 1.5
Injection volume: 20
Detector: UV 220 for 5 min then UV 280

CHROMATOGRAM
Retention time: 2.73
Limit of quantitation: 10 μg/mL

OTHER SUBSTANCES
Simultaneous: methamphetamine, amphetamine, caffeine, 3,4-methylenedioxyamphetamine, N-methyl-3,4-methylenedioxyamphetamine, N-ethyl-3,4-methylenedioxyamphetamine

KEY WORDS
tablets

REFERENCE
Longo,M.; Martines,C.; Rolandi,L.; Cavallaro,A. Simple and fast determination of some phenethylamines in illicit tablets by base-activated reversed phase HPLC, *J.Liq.Chromatogr.*, **1994**, *17*, 649–658.

SAMPLE
Matrix: formulations
Sample preparation: Dilute nasal solution 10-fold with water, filter (0.45 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 125 × 4 5 μm Aluspher RP-select B (Merck)
Mobile phase: Gradient. MeCN:1 mM NaOH from 10:90 to 80:20 over 25 min.
Column temperature: 25
Flow rate: 1.2
Injection volume: 10
Detector: UV 224 or UV 259

CHROMATOGRAM
Retention time: 3
Limit of detection: 5 ng (UV 224)

OTHER SUBSTANCES
Simultaneous: naphazoline, oxymetazoline, xylometazoline

KEY WORDS
nasal solutions

REFERENCE
De Orsi,D.; Gagliardi,L.; Cavazzutti,G.; Mediati,M.G.; Tonelli,D. Simultaneous determination of ephedrine and 2-imidazolines in pharmaceutical formulations by reversed-phase HPLC, *J.Liq.Chromatogr.*, **1995**, *18*, 3233–3242.

SAMPLE
Matrix: formulations
Sample preparation: Dilute syrup with mobile phase to a concentration of 5-100 μg/mL, shake, filter, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm 80 Å Ultrasphere CN
Mobile phase: MeCN:water:EtOH 60:38:2 containing 1 mM perchloric acid

Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: Conductivity, zero suppression 2, range 1 or 10

CHROMATOGRAM
Retention time: 9.4

OTHER SUBSTANCES
Simultaneous: bromhexine, chlorpheniramine, codeine, dextromethorphan, diphenhydramine, 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: solutions
Sample preparation: Mix 40 mL of a 325-375 mM amine solution in THF with 150 mL 10% potassium carbonate, add dropwise 40 mL 275-325 mM reagent in THF, heat at 50° while maintaining at pH 8 or above for 3 h, cool, extract with chloroform. Evaporate the extracts to dryness, reconstitute, inject an aliquot. (Prepare reagent (1-[(4-nitrophenyl)sulfonyl]prolyl chloride) as follows. Mix 40-45 mmoles L-(-)-proline, 40 mL THF, and 200 mL 10% potassium carbonate, add 37-43 mmoles 4-nitrobenzenesulfonyl chloride in 40 mL THF dropwise, heat at 50° and maintain at pH 8 or above for 3 h, cool, acidify to pH 2, extract with chloroform. Extract the organic layers with potassium carbonate in water. Acidify the aqueous layer and extract it with chloroform. Dry the chloroform layer and evaporate it to dryness, recrystallize the resulting 1-[(4-nitrophenyl)sulfonyl]proline from petroleum ether and benzene (Caution! Benzene is a carcinogen!). Stir 15 mmoles 1-[(4-nitrophenyl)sulfonyl]proline in 100 mL benzene and add 75 mmoles thionyl chloride in 50 mL benzene dropwise, heat at 35-40° until the reaction is complete (about 48 h; monitor by IR), evaporate to dryness, recrystallize from n-heptane to give 1-[(4-nitrophenyl)sulfonyl]prolyl chloride (mp 110-110.5°).)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:water 60:40
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Retention time: 5, 6 (enantiomers)

OTHER SUBSTANCES
Simultaneous: pseudoephedrine

KEY WORDS
derivatization; chiral

REFERENCE
Clark,C.R.; Barksdale,J.M. Synthesis and liquid chromatographic evaluation of some chiral derivatizing agents for resolution of amine enantiomers, *Anal.Chem.*, **1984**, *56*, 958–962.

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: 3.68

OTHER SUBSTANCES
Simultaneous: methamphetamine, mescaline, mephentermine, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine, norpipanone, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine, pemoline, benzphetamine, diethylpropion, mazindol, tranylcypromine, caffeine, fenethyline, phendimetrazine, methylphenidate, phenelzine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: phenylephrine, pseudoephedrine, methylephedrine, dimethylamphetamine

REFERENCE
Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

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

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

CHROMATOGRAM
Retention time: 5.49
Limit of detection: 1 fmole (E), 1 nmole (UV)

OTHER SUBSTANCES
Simultaneous: methamphetamine
Also analyzed: acebutolol, alprenolol, flecainide, propranolol

KEY WORDS
derivatization; complexation

REFERENCE

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

SAMPLE

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

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 1.8

OTHER SUBSTANCES

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

REFERENCE

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

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

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 5:1.5:0.5:93
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Simultaneous: phenylpropanolamine, 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: 100 μL 100 μM Solution + 300 μL buffer + 500 μL 1 mM dansyl chloride in acetone, mix, heat at 45° in the dark for 1 h, dilute with MeCN:water 50:50, inject a 20 μL aliquot. (Prepare buffer by adjusting 10 mM sodium bicarbonate to pH 9.0 with NaOH.)

HPLC VARIABLES
Guard column: 30 × 4.6 Spheri-5 RP-18
Column: 250 × 4.6 Inertsil ODS-2
Mobile phase: MeCN:water 70:30 containing 1 mM imidazole, pH adjusted to 7.0 with nitric acid
Flow rate: 1
Injection volume: 20
Detector: Chemiluminescence following post-column reaction. The column effluent mixed with the reagent pumped at 1 mL/min and the mixture flowed through a 300 mm × 0.25 mm ID coil to the detector. (Prepare the reagent by dissolving 112 mg bis(2,4,6-trichlorophenyl) oxalate in 500 mL MeCN, add 8.6 mL 30% hydrogen peroxide, sonicate.), F ex 343 em 530

CHROMATOGRAM
Retention time: 9
Limit of detection: 4 fmole (chemiluminescence), 50 fmole (F)

OTHER SUBSTANCES
Simultaneous: benzylamine, N-isopropylbenzylamine, methamphetamine, N-methylphenethylamine, phenylbutylamine, phenylethylamine, phenylpropanolamine, phenylpropylamine

KEY WORDS
derivatization; post-column reaction; comparison with other derivatization reagents

REFERENCE
Hayakawa,K.; Hasegawa,K.; Imaizumi,N.; Wong,O.S.; Miyazaki,M. Determination of amphetamine-related compounds by high-performance liquid chromatography with chemiluminescence and fluorescence detections, *J.Chromatogr.*, **1989**, *464*, 343–352.

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: 5.5

OTHER SUBSTANCES
Simultaneous: diethylpropion, phenylpropanolamine, amphetamine, methamphetamine, phentermine, fenfluramine
Also analyzed: amitriptyline, chlordiazepoxide, chlorpromazine, desalkylflurazepam, desipramine, desmethyldoxepin, diazepam, doxepin, flurazepam, imipramine, mesoridazine, norchlordiazepoxide, nordiazepam, nortriptyline, oxazepam, prazepam, promazine, thioridazine, thiothixene, trifluoperazine

REFERENCE
Rainin Catalog, C1-94, **1994,** p. 7.24.

SAMPLE
Matrix: solutions

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

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

REFERENCE

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

SAMPLE

Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES

Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: k' 0.74

OTHER SUBSTANCES

Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS

comparison with capillary electrophoresis

REFERENCE

Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

SAMPLE

Matrix: urine
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH, 3 mL MeCN: 10 mM ammonium acetate 40:60 adjusted to pH 3 with acetic acid, and 5 mL water. 5 mL Urine + 5 mL 500 mM ammonium acetate, adjusted to pH 9.5 with ammonia, mix, add to the SPE cartridge, wash with 20 mL 5 mM pH 9.5 ammonium acetate, wash with 0.5 mL water.

Elute with 2 mL MeCN:10 mM ammonium acetate 40:60 adjusted to pH 3 with acetic acid, inject a 50 μL aliquot of the eluate.

HPLC VARIABLES
Column: 150 × 4.6 L-column ODS (Chemical Inspection & Testing Institute, Tokyo)
Mobile phase: Gradient. MeCN:100 mM ammonium acetate 0:100 for 1 min, to 40:60 over 20 min.
Flow rate: 1
Injection volume: 50
Detector: UV 210; MS Shimadzu model QP-1100EX thermospray, vaporizer temperature from 170 to 150° over 20 min. SIM, m/z 166

CHROMATOGRAM
Retention time: 14
Limit of detection: 2–40 ng/mL

OTHER SUBSTANCES
Extracted: 6-acetylmorphine, amphetamine, benzoylecgonine, cocaine, methamphetamine, methylephedrine, morphine, morphine-3-glucuronide, morphine-6-glucuronide

KEY WORDS
SPE

REFERENCE
Tatsuno,M.; Nishikawa,M.; Katagi,M.; Tsuchihashi,H. Simultaneous determination of illicit drugs in human urine by liquid chromatography-mass spectrometry, *J.Anal.Toxicol.*, **1996**, *20*, 281–286.

SAMPLE
Matrix: urine
Sample preparation: 500 μL Urine + N-ethylnordiazepam + chlorpheniramine + 100 μL buffer, centrifuge at 11000 g for 30 s, inject a 500 μL aliquot onto column A with mobile phase A, after 0.6 min backflush column A with mobile phase A to waste for 1.6 min, elute column A with 250 μL mobile phase B, with 200 μL mobile phase C, and with 1.15 mL mobile phase D. Elute column A to waste until drugs start to emerge then elute onto column B. Elute column B to waste until drugs started to emerge, then elute onto column C. When all the drugs have emerged from column B remove it from the circuit, elute column C with mobile phase D, monitor the effluent from column C. Flush column A with 7 mL mobile phase E, with mobile phase D, and mobile phase A. Flush column B with 5 mL mobile phase E then with mobile phase D. (Buffer was 6 M ammonium acetate adjusted to pH 8.0 with 2 M KOH.)

HPLC VARIABLES
Column: A 10 × 2.1 12-20 μm PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 × 3.2 11 μm Aminex A-28 (Bio-Rad); C 25 × 3.2 5 μm C8 (Phenomenex) + 150 × 4.6 5 μm silica (Macherey-Nagel)
Mobile phase: A 0.1% pH 8.0 potassium borate buffer; B 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid; C MeCN:buffer 40:60 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); D MeCN:buffer 33:67 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); E MeCN:buffer 70:30 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)
Column temperature: ambient (column A), 40 (columns B and C)
Flow rate: A 5; B-E 1
Injection volume: 500
Detector: UV 210, UV 235

CHROMATOGRAM
Retention time: k' 2.7
Internal standard: N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)
Limit of detection: 300 ng/mL

OTHER SUBSTANCES

Extracted: methamphetamine, desipramine, nortriptyline, diphenhydramine, methadone, imipramine, flurazepam, amitriptyline, morphine, codeine, hydromorphone, hydrocodone, caffeine, cotinine, benzoylecgonine, secobarbital, oxazepam, phenobarbital, nordiazepam, diazepam, phenylpropanolamine

Interfering: phentermine, amphetamine, phenmetrazine, lidocaine, pentazocine

KEY WORDS

column-switching

REFERENCE

Binder,S.R.; Regalia,M.; Biaggi-McEachern,M.; Mazhar,M. Automated liquid chromatographic analysis of drugs in urine by on-line sample cleanup and isocratic multi-column separation, *J.Chromatogr.*, **1989**, *473*, 325–341.

SAMPLE

Matrix: urine

Sample preparation: 5 mL Urine + 25 μL 1 mg/mL phenylpropanolamine in mobile phase + 100 μL 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 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4 5 μm 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: 8

Internal standard: phenylpropanolamine (11.5)

Limit of detection: 200 ng/mL

OTHER SUBSTANCES

Extracted: norephedrine, norpseudoephedrine, pseudoephedrine, N-methylephedrine, ethylephedrine

Noninterfering: amfepramone, amphetamine, caffeine, chlorphentermine, cocaine, codeine, cropropamide, crotethamide, dimethylamphetamine, etamivan, fencamfamine, heptaminol, leptazol, lidocaine, methoxamine, methylamphetamine, methylphenidate, nicotine, niketamine, meperidine, phendimetrazine, phenmetrazine, pipradol, procaine, prolintane, strychnine

REFERENCE

Imaz,C.; Carreras,D.; Navajas,R.; Rodriguez,C.; Rodriguez,A.F.; Maynar,J.; Cortes,R. Determination of ephedrines in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *631*, 201–205.

SAMPLE

Matrix: urine

Sample preparation: 1 mL Urine + 0.5 mL 1% trichloroacetic acid, centrifuge at 5200 g for 10 min, filter (0.2 μm), inject 20 μL aliquot

HPLC VARIABLES

Column: 250 × 4 Lichrospher 5μm 60 RP-select B

Mobile phase: Gradient. MeCN:50 mM pH 3.2 potassium phosphate buffer from 10:90 to 50:50 over 15 min.

Flow rate: 1.5

Injection volume: 20

Detector: UV 190-370

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Extracted: morphine, phenylpropanolamine, lidocaine, diphenhydramine, nortriptyline, cocaine, benzoylecgonine, norpropoxyphene, nordiazepam
Also analyzed: amitriptyline, amphetamine, meperidine, codeine, (different gradient)

REFERENCE
Li,S.; Gemperline,P.J.; Briley,K.; Kazmierczak,S. Identification and quantitation of drugs of abuse in urine using the generalized rank annihilation method of curve resolution, *J.Chromatogr.B*, **1994**, *655*, 213–223.

SAMPLE
Matrix: urine
Sample preparation: 1 mL Urine + 10 mg β-glucuronidase/arylsulfatase (Helix pomatia, Sigma), heat at 37° overnight, add an equal volume of buffer, centrifuge at 2000 g for 5 min, inject an aliquot of the supernatant onto column A with mobile phase A and elute to waste. After 2.5 min backflush the contents of column A onto column B with mobile phase B, monitor the effluent from column B. Re-equilibrate both columns for 12.5 min before the next injection. (Buffer was 200 mM boric acid adjusted to pH 9.5 with 5 M NaOH.)

HPLC VARIABLES
Column: A 10 × 4.6 5 μm Spherisorb cyanopropyl; B 250 × 4.6 Capcell Pak C18 UG-120 (Shiseido)
Mobile phase: A water; B Gradient. MeCN:buffer from 3:97 to 30:70 over 30 min, to 40:60 over 8 min (Buffer was 3.4 mL/L phosphoric acid adjusted to pH 3.0 with 5 M NaOH.)
Flow rate: A 1.25; B 1
Injection volume: 100
Detector: UV 220

CHROMATOGRAM
Retention time: 8.3
Limit of detection: 250 ng/mL

OTHER SUBSTANCES
Extracted: acebutolol, alprenolol, amphetamine, atenolol, bopindolol, codeine, labetalol, metoprolol, morphine, nadolol, oxprenolol, pindolol, propranolol, timolol

KEY WORDS
column-switching

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

SAMPLE
Matrix: urine
Sample preparation: Inject 15 μL urine, inject a mixture of 5 μL 20 mM 9-fluorenylmethyl chloroformate in MeCN and 45 μL water, and inject 10 μL buffer on to column A and elute to waste with mobile phase A. After 2.8 min backflush the contents of column A on to column B with mobile phase B and start the gradient, monitor the effluent from column B. At the end of the run condition column A with 1 mL mobile phase A. (Buffer was 4% sodium bicarbonate adjusted to pH 10 with 10% NaOH.)

HPLC VARIABLES
Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 125 × 4 5 μm LiChrospher 100 PR-C18
Mobile phase: A water; B Gradient. MeCN:water from 40:60 to 70:30 over 15 min. to 100:0 over 5 min.
Flow rate: A 0.35; B 1
Injection volume: 15

Detector: F ex 264 em 313

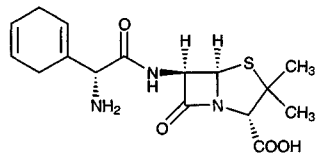
CHROMATOGRAM
Retention time: 11.8
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Extracted: amphetamine, methamphetamine, norephedrine, 3-phenylpropylamine, pseudoephedrine

KEY WORDS
column-switching; derivatization; on-column derivatization

REFERENCE
Herráez-Hernández,R.; Campíns-Falcó,P.; Sevillano-Cabeza,A. Determination of amphetamine and related compounds in urine using on-line derivatization in octadecyl silica columns with 9-fluorenylmethyl chloroformate and liquid chromatography, *J.Chromatogr.B*, **1996**, *679*, 69–78.

Epicillin



Molecular formula: $C_{16}H_{21}N_3O_4S$
Molecular weight: 351.43
CAS Registry No.: 26774-90-3
Merck Index: 3651

SAMPLE
Matrix: perfusate
Sample preparation: Vortex perfusate, centrifuge at 11600 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Guard column: 20 × 2 5 μm Hypersil ODS
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:buffer 35:65 (Buffer was 50 mM KH_2PO_4 containing 0.1% triethylamine adjusted to pH 3 with orthophosphoric acid.)
Flow rate: 1
Injection volume: 100
Detector: UV 220

CHROMATOGRAM
Retention time: 6.0
Limit of detection: 20 ng/mL
Limit of quantitation: 100 ng/mL

REFERENCE
Erah,P.O.; Barrett,D.A.; Shaw,P.N. Reversed-phase high-performance liquid chromatographic assay methods for the analysis of a range of penicillins in in vitro permeation studies, *J.Chromatogr.B*, **1998**, *705*, 63–69.

Epidermal growth factor

Molecular formula: indeterminate
Molecular weight: 6201
CAS Registry No.: 9010-53-1
Merck Index: 3569

SAMPLE
Matrix: blood
Sample preparation: Filter (0.2 μm polysulfone) plasma, inject a 200 μL aliquot on to column A and elute to waste with mobile phase A, after 8 min elute column A to waste with mobile phase B, after 4 min elute the contents of column A on to column B with mobile phase B, after 6 min remove column A from the circuit, elute column B with mobile phase C (start the gradient), monitor the effluent from column B. Re-equilibrate column A with mobile phase A for 17.6 min.

HPLC VARIABLES
Column: A 24 × 4 immunoaffinity (column preparation details in paper); B 20 × 4.6 polysulfoethyl aspartamide (The Nest Group, Southborough, MA)
Mobile phase: A 0.9% NaCl containing 10 mM potassium phosphate and 20 ppm sodium azide, pH 7.4 (Caution! Sodium azide is carcinogenic and should not be discharged to the plumbing system!); B MeOH:100 mM pH 2.50 glycine/HCl buffer 50:50; C Gradient. A was MeCN:5 mM pH 3.0 potassium phosphate buffer 25:75. B was MeCN:600 mM KCl containing 5 mM potassium phosphate, pH 3.0 25:75. A:B 80:20 for 1 min, to 75:25 over 5 min, to 65:35 over 1 min, to 40:60 over 7.5 min, return to initial conditions over 0.1 min, re-equilibrate for 3 min.
Flow rate: A 0.6; B 0.6; C 1
Injection volume: 200
Detector: UV 220 or immunoassay

CHROMATOGRAM
Retention time: 25 (hEGF 1-47), 28 (hEGF 1-48), 32 (hEGF 1-53)

KEY WORDS
plasma; column-switching

REFERENCE
Kagel,J.R.; Rossi,D.T.; Nordblom,G.D.; Dudeck,R.C.; Barksdale,C.M.; Kuo,B.-S.; Wright,D.S. Considerations in the development of a sensitive HPLC assay for human epidermal growth factors in human plasma, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1205–1213.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 150 × 4.6 5 μm TSKgel C18 (TOSOH)
Mobile phase: MeCN:10 mM pH 6.0 diethylenetriamine phosphate 22:78
Flow rate: 0.5
Detector: UV 214

CHROMATOGRAM
Retention time: 26.72 (α-form)

OTHER SUBSTANCES
Simultaneous: deamidated epidermal growth factor (β-form)

REFERENCE
Son,K.; Kwon,C. Stabilization of human epidermal growth factor (hEGF) in aqueous formulations, *Pharm.Res.*, **1995**, *12*, 451–454.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in 200 mM pH 5.6 ammonium acetate, concentrate 10-fold under vacuum, inject a 200-400 μL aliquot

HPLC VARIABLES
Guard column: Bio-Sil ODS-10 (Bio-Rad)
Column: two 300 × 3.9 μBondapak C18 columns in series

Mobile phase: MeCN:buffer 26:74 (Buffer was 50 mM acetic acid adjusted to pH 5.6 with triethylamine.)
Column temperature: 40
Flow rate: 0.5
Detector: UV 280

CHROMATOGRAM
Retention time: 58 (α-form), 65 (β-form)

KEY WORDS
mouse

REFERENCE
Matrisian,L.M.; Larsen,B.R.; Finch,J.S.; Magun,B.E. Further purification of epidermal growth factor by high-performance liquid chromatography, *Anal.Biochem.*, **1982**, *125*, 339–351.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb S5 ODS2 fully end-capped
Mobile phase: Gradient. A was 155 mM NaCl containing 0.2% pentadecafluorooctanoic acid. B was MeCN:isopropanol 50:50 containing 0.2% pentadecafluorooctanoic acid. A:B from 65:35 to 50:50 over 50 min.
Column temperature: 45
Flow rate: 1
Detector: UV 280 or F ex 254 em 340

CHROMATOGRAM
Retention time: 26 (α-form), 28 (β-form)

KEY WORDS
mouse; full discussion of factors affecting selectivity

REFERENCE
Smith,J.A.; O'Hare,M.J. Reversed-phase high-performance liquid chromatography of mouse epidermal growth factor and its congeners: mobile phase optimization with ion-pairing additives, *J.Chromatogr.*, **1984**, *299*, 13–28.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm ODS Hypersil C18
Mobile phase: Gradient. A was 155 mM NaCl adjusted to pH 2.1 with HCl. B was MeCN. A:B 100:0 for 5 min, to 90:10 over 5 min, to 52.5:47.5 over 50 min.
Column temperature: 45
Flow rate: 1
Detector: UV 280

CHROMATOGRAM
Retention time: 35

KEY WORDS
mouse

REFERENCE
Smith,J.A.; Ham,J.; Winslow,D.P.; O'Hare,M.J.; Rudland,P.S. The use of high-performance liquid chromatography in the isolation and characterisation of mouse and rat epidermal growth factors and examination of apparent heterogeneity, *J.Chromatogr.*, **1984**, *305*, 295–308.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: Two 300 × 4.6 10 μm μBondapak C18 columns in series
Mobile phase: MeCN:10 mM pH 7.0 diethylenetriamine phosphate 25:75
Flow rate: 0.5
Detector: UV 280

CHROMATOGRAM
Retention time: 35 (α-form), 42 (β-form), 54 (gamma-form)

KEY WORDS
mouse

REFERENCE
DiAugustine,R.P.; Walker,M.P.; Klapper,D.G.; Grove,R.I.; Willis,W.D.; Harvan,D.J.; Hernandez,O. β-epidermal growth factor is the des-asparaginyl form of the polypeptide, *J.Biol.Chem.*, **1985**, *260*, 2807–2811.

SAMPLE
Matrix: solutions
Sample preparation: Filter (Amicon YM-2, 2000 cut-off), inject an aliquot.

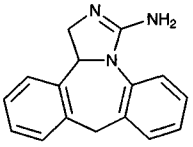
HPLC VARIABLES
Column: 250 × 4.6 Vydac 218TPS
Mobile phase: Gradient. MeCN:buffer 18:82 for 20 min, to 66:34 over 20 min, maintain at 66:34 for 10 min. (Buffer was 10 mM pH 7.2 sodium phosphate buffer.)
Detector: UV 280

CHROMATOGRAM
Retention time: 28

KEY WORDS
human

REFERENCE
Engler,D.A.; Matsunami,R.K.; Campion,S.R.; Stringer,C.D.; Stevens,A.; Niyogi,S.K. Cloning of authentic human epidermal growth factor as a bacterial secretory protein and its initial structure-function analysis by site-directed mutagenesis, *J.Biol.Chem.*, **1988**, *263*, 12384–12390.

Epinastine



Molecular formula: $C_{16}H_{15}N_3$
Molecular weight: 249.32
CAS Registry No.: 80012-43-7 (HCl)
Merck Index: 3655

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL 600 ng/mL diphenidol in water and 600 μL 100 mM sodium carbonate to 100 μL plasma. Add 5 mL dichloromethane, shake on a reciprocal shaker for 10 min, centrifuge at 1000 g for 8 min, dry 4 mL of the lower organic phase in a rotary evaporator at 45°, dissolve the residue in 50 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Cosmosil 5C18-MS (Nacalai Tesque, Japan)

Mobile phase: MeOH:buffer 36:64 (Buffer was 0.3% triethylamine adjusted to pH 4.5 with phosphoric acid.)
Flow rate: 1.3
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 8.1
Internal standard: diphenidol (14.8)
Limit of detection: 4 ng/mL
Limit of quantitation: 10 ng/mL

KEY WORDS
plasma

REFERENCE
Ohtani,H.; Kotaki,H.; Sawada,Y.; Iga,T. Quantitative determination of epinastine in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *683*, 281–284.

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood. Dilute 1 mL plasma with 100 μL 1 M pH 9 phosphate buffer and 100 μL water, add 8 mL chloroform and extract. (Caution! Chloroform is a carcinogen!) Evaporate the organic layer, dissolve the residue in 400 μL mobile phase, inject a 200 μL aliquot. Tissue. Homogenize the brain with 2-fold the weight of water. Dilute 1.5 mL brain homogenate with 500 μL 1 M pH 9 phosphate buffer, add 8 mL chloroform, extract. Evaporate the organic layer and dissolve the residue in 400-500 μL mobile phase. Inject 200 μL aliquot.

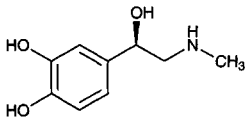
HPLC VARIABLES
Column: 150 × 4.6 Intersil PH
Mobile phase: MeCN:0.018% TFA 15:85
Column temperature: 40
Flow rate: 0.7
Injection volume: 200
Detector: UV 220

CHROMATOGRAM
Limit of quantitation: 5 ng/mL (plasma), 15 ng/mL (brain)

KEY WORDS
brain; cat; mouse; pharmacokinetics; plasma; rat

REFERENCE
Kato,M.; Nishida,A.; Aga,Y.; Kita,J.; Kudo,Y.; Narita,H.; Endo,T. Pharmacokinetic and pharmacodynamic evaluation of central effect of the novel antiallergic agent betotastine besilate, *Arzneimittelforschung*, **1997**, *47*, 1116–1124.

Epinephrine



Molecular formula: $C_9H_{13}NO_3$
Molecular weight: 183.21
CAS Registry No.: 51-43-4 (-), 51-42-3 ((-) bitartrate), 329-65-7 (racemic)
Merck Index: 3656
Lednicer No.: 1 95

SAMPLE
Matrix: blood

Mobile phase: MeOH:buffer 36:64 (Buffer was 0.3% triethylamine adjusted to pH 4.5 with phosphoric acid.)
Flow rate: 1.3
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 8.1
Internal standard: diphenidol (14.8)
Limit of detection: 4 ng/mL
Limit of quantitation: 10 ng/mL

KEY WORDS
plasma

REFERENCE
Ohtani,H.; Kotaki,H.; Sawada,Y.; Iga,T. Quantitative determination of epinastine in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *683*, 281–284.

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood. Dilute 1 mL plasma with 100 μL 1 M pH 9 phosphate buffer and 100 μL water, add 8 mL chloroform and extract. (Caution! Chloroform is a carcinogen!) Evaporate the organic layer, dissolve the residue in 400 μL mobile phase, inject a 200 μL aliquot. Tissue. Homogenize the brain with 2-fold the weight of water. Dilute 1.5 mL brain homogenate with 500 μL 1 M pH 9 phosphate buffer, add 8 mL chloroform, extract. Evaporate the organic layer and dissolve the residue in 400-500 μL mobile phase. Inject 200 μL aliquot.

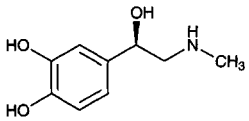
HPLC VARIABLES
Column: 150 × 4.6 Intersil PH
Mobile phase: MeCN:0.018% TFA 15:85
Column temperature: 40
Flow rate: 0.7
Injection volume: 200
Detector: UV 220

CHROMATOGRAM
Limit of quantitation: 5 ng/mL (plasma), 15 ng/mL (brain)

KEY WORDS
brain; cat; mouse; pharmacokinetics; plasma; rat

REFERENCE
Kato,M.; Nishida,A.; Aga,Y.; Kita,J.; Kudo,Y.; Narita,H.; Endo,T. Pharmacokinetic and pharmacodynamic evaluation of central effect of the novel antiallergic agent betotastine besilate, *Arzneimittelforschung*, **1997**, *47*, 1116–1124.

Epinephrine



Molecular formula: $C_9H_{13}NO_3$
Molecular weight: 183.21
CAS Registry No.: 51-43-4 (-), 51-42-3 ((-) bitartrate), 329-65-7 (racemic)
Merck Index: 3656
Lednicer No.: 1 95

SAMPLE
Matrix: blood

Sample preparation: Plasma. Prepare a SPE column by adding 500 μL of a 20% suspension of 19-40 μm Toyopak SP (strong cation-exchange sulfopropyl resin, Na^+ (Toyo Soda)) in water to a 35 × 6 column, wash with two 1 mL portions of 2 M LiOH, wash with two 5 mL portions of water, wash with two 1 mL portions of EtOH:12 M HCl 90:10, wash with two 5 mL portions of water, wash with three 1 mL portions of buffer. 500 μL Plasma + 25 μL 10 nM isoproterenol + 500 μL buffer, mix, add to the SPE column, wash with two 5 mL portions of water, wash with 1 mL MeCN:water 50:50, elute with 300 μL 600 μM potassium ferricyanide in 600 mM KCl:MeCN 50:50, add 50 μL reagent to the eluate, heat at 37° for 40 min, cool in ice-water, inject a 100 μL aliquot. Urine. 10 μL Urine + 1 mL MeCN:500 mM KCl 60:40 + 10 μL 500 nM isoproterenol + 10 μL 75 mM potassium hexacyanoferrate(III) + 100 μL reagent, heat at 37° for 40 min, inject a 100 μL aliquot (J. Chromatogr. 1986, 380, 229). (Prepare buffer by mixing 8 volumes 250 mM LiOH in 200 mM phosphoric acid with 1 volume 200 mM phosphoric acid, pH 5.8. Prepare reagent by dissolving 212 mg 1,2-diphenylethylenediamine in 10 mL 100 mM HCl, pH 6.7.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm TSK-gel ODS-120T (Toyo Soda)
Mobile phase: MeCN:MeOH:50 mM pH 7.0 Tris-HCl buffer 50:10:40 (Wash with MeCN:MeOH: water 50:10:40 for 15 min at the end of each day.)
Flow rate: 1
Injection volume: 100
Detector: F ex 345 em 485 (plasma), F ex 350 em 480 (urine)

CHROMATOGRAM

Retention time: 5
Internal standard: isoproterenol (8)
Limit of detection: 7 pM

OTHER SUBSTANCES

Extracted: dopamine, norepinephrine

KEY WORDS

derivatization; plasma; SPE

REFERENCE

Mitsui,A.; Nohta,H.; Ohkura,Y. High-performance liquid chromatography of plasma catecholamines using 1,2-diphenylethylenediamine as precolumn fluorescence derivatization reagent, *J.Chromatogr.*, **1985**, *344*, 61–70.

SAMPLE

Matrix: blood
Sample preparation: Prepare a 20 × 5 polypropylene column packed with CM-Sephadex preswollen in water, wash with 5 mL 2 M HCl, wash with 10 mL water, wash with 10 mL 100 mM pH 7 phosphate buffer. 1 mL Plasma + 30 μL 80 ng/mL N-methyldopamine, apply to column, wash with 5.5 mL water (A), elute with 3 mL 0.5 M perchloric acid. Collect eluate, add 2 mL 1.5 M pH 9.3 Tris buffer containing 60 mM EDTA, add 20 mg alumina, vortex for 2 min, discard supernatant, add 2 mL water to alumina, mix, centrifuge at 3000 g for 3 min, repeat water wash, remove as much water as possible, elute catecholamines from alumina with 100 μL 100 mM acetic acid with vortexing for 2 min, centrifuge, inject 25 μL aliquot of supernatant. (Wash water A contains levodopa, carbidopa, DOPAC, and O-methyldopa.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:MeOH:25 mM sodium acetate 4:4:92 containing 0.2 mM 1-octanesulfonic acid and 0.3 mM disodium EDTA, pH was adjusted to pH 3 with acetic acid
Flow rate: 0.9
Injection volume: 10
Detector: E, ESA Coulochem 5100 A, 5010 A analytical cell, first electrode +0.25 V, second electrode -0.30 V

CHROMATOGRAM

Retention time: 9

Internal standard: N-methyldopamine (16)
Limit of detection: 0.5 ng/mL

OTHER SUBSTANCES
Simultaneous: norepinephrine, dopamine

KEY WORDS
plasma

REFERENCE
Betto,P.; Ricciarello,G.; Giambenedetti,M.; Lucarelli,C.; Ruggeri,S.; Stocchi,F. Improved high-performance liquid chromatographic analysis with double detection system for L-dopa, its metabolites and carbidopa in plasma of parkinsonian patients under L-dopa therapy, *J.Chromatogr.*, **1988**, *459*, 341–349.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 250 μL 1 ng/mL α-methylnorepinephrine + 1 mL buffer + 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol, shake for 2 min, centrifuge at 20° at 1000 g for 5 min, freeze in acetone/dry ice. Remove the organic phase and add it to 2 mL 1-octanol and 200 μL 80 mM acetic acid, shake, centrifuge at 20° at 1000 g for 5 min, freeze in acetone/dry ice. Discard the organic phase, thaw the aqueous phase and add it to 1 mL 10 mM HCl, 1 mL buffer, and 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol, shake for 2 min, centrifuge at 20° at 1000 g for 5 min, freeze in acetone/dry ice. Remove the organic phase and add it to 2 mL 2 M pH 8.6 ammonia/ammonium chloride buffer containing 13.4 mM EDTA, shake, freeze in dry ice/acetone. Remove the organic layer and add it to 2 mL 1-octanol and 150 μL 80 mM acetic acid, shake, centrifuge at 20° at 1000 g for 5 min, freeze in dry ice/acetone, discard the organic layer. Thaw the aqueous layer and add it to 250 μL MeCN, 50 μL 1.75 M pH 7.05 bicine, and 100 μL 100 mM 1,2-diphenylethylenediamine in 100 mM HCl, add 20 μL 20 mM potassium ferricyanide in water, heat at 37° in the dark for 1 h, keep at 20° in the dark, inject a 100 μL aliquot. (Buffer was 2 M pH 8.6 ammonia/ammonium chloride buffer containing 8.9 mM diphenylborate-ethanolamine complex and 13.4 mM EDTA. Stir buffer with 45 g/L activated alumina for 2 h before use. Wash 1-octanol with 80 mM acetic acid. Recrystallize 1,2-diphenylethylenediamine from toluene:light petroleum (bp 60-80°) 10:90, dry overnight at 60°.)

HPLC VARIABLES
Column: 100 × 4.6 3 μm Cp MicroSpher C18 (Chrompack)
Mobile phase: MeCN:MeOH:50 mM pH 7.0 sodium acetate buffer 40:8:50
Flow rate: 1
Injection volume: 100
Detector: F ex 350 em 480

CHROMATOGRAM
Retention time: 4
Internal standard: α-methylnorepinephrine (3)
Limit of detection: 2 pg/mL

OTHER SUBSTANCES
Extracted: dihydroxybenzylamine, dopamine, isoproterenol, norepinephrine

KEY WORDS
plasma; derivatization; comparison with electrochemical detection

REFERENCE
van der Hoorn,F.A.J.; Boomsma,F.; Man in 't Veld,A.J.; Schalekamp,M.A.D.H. Determination of catecholamines in human plasma by high-performance liquid chromatography: comparison between a new method with fluorescence detection and an established method with electrochemical detection, *J.Chromatogr.*, **1989**, *487*, 17–28.

SAMPLE
Matrix: blood

Sample preparation: Pack a 65 × 15 SPE column with 50 mg WA-4 alumina (Sigma). Add 500 μL plasma to the SPE column, add 1 mL buffer, rotate for 15 min, wash three times with water (aspirating to dryness each time), centrifuge to dryness, add 200 μL 100 mM pH 1.2 perchloric acid, mix, let stand for 15 min, centrifuge the SPE column at 1000 *g* for 3 min, inject an aliquot of the effluent. (Buffer was 45 g Tris and 5 g EDTA in 200 mL water, pH adjusted to 8.6 with concentrated HCl.)

HPLC VARIABLES
Guard column: 50 × 4.6 5 μm reversed-phase
Column: 250 × 4.6 5 μm ODS Spherisorb
Mobile phase: Buffer contained 1.4% monochloroacetic acid, 0.47% NaOH, and 0.075% EDTA, finally pH adjusted to 3.0 with NaOH or monochloroacetic acid and 6 mg% sodium octylsulfate added.
Column temperature: 35
Flow rate: 1
Injection volume: 100
Detector: E, Bioanalytical Systems LC-4B, TL-5 transducer with a glassy carbon electrode, +650 mV, 1 nA, Ag/AgCl reference electrode

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OTHER SUBSTANCES
Extracted: norepinephrine, dopamine

KEY WORDS
plasma; rabbit; human; SPE

REFERENCE
Ganhao,M.F.; Hattingh,J.; Hurwitz,M.L.; Pitts,N.I. Evaluation of a simple plasma catecholamine extraction procedure prior to high-performance liquid chromatography and electrochemical detection, *J.Chromatogr.*, **1991**, *564*, 55–66.

SAMPLE
Matrix: blood
Sample preparation: Plasma. 1 mL Plasma + 125 μL 2 ng/mL α-methylnorepinephrine + 1 mL buffer + 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol, shake for 2 min, centrifuge at 1000 g for 5 min, freeze in dry ice/acetone. Remove the organic phase and add it to 2 mL 1-octanol (saturated with 80 mM acetic acid) and 200 μL 80 mM acetic acid, shake, centrifuge at 1000 g for 5 min. Freeze the aqueous layer and remove the organic layer. Add 1 mL 10 mM HCl, 1 mL buffer, and 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol to the aqueous phase. Shake, centrifuge, freeze, remove the organic layer and add it to 2 mL 2 M pH 8.6 ammonia-ammonium chloride buffer containing 13.4 mM EDTA (but no complex). Freeze, remove the organic layer and add it to 2 mL 1-octanol and 150 μL 80 mM acetic acid, shake, centrifuge at 1000 g for 5 min. Freeze, remove the organic layer and add the aqueous layer to 200 μL MeCN, 50 μL 1.75 M pH 6.95 bicine buffer containing 1% EDTA, 100 μL 100 mM 1,2-diphenylethylenediamine in 100 mM HCl, and 20 μL 20 mM potassium ferricyanide in water. Heat at 37° in the dark for 1 h, inject a 75 μL aliquot (keep it in the dark in the autosampler). Urine. 100 μL Urine + 1 mL 10 mM HCl + 125 μL 40 ng/mL α-methylnorepinephrine + 1 mL buffer + 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol, shake for 2 min, centrifuge at 1000 g for 5 min, freeze in dry ice/acetone. Remove the organic phase and add it to 2 mL 1-octanol (saturated with 80 mM acetic acid) and 200 μL 80 mM acetic acid, shake, centrifuge at 1000 g for 5 min. Freeze the aqueous layer and remove the organic layer. Add 1 mL 10 mM HCl, 1 mL buffer, and 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol to the aqueous phase. Shake, centrifuge, freeze, remove the organic layer and add it to 2 mL 1-octanol and 150 μL 80 mM acetic acid, shake, centrifuge at 1000 g for 5 min. Freeze, remove the organic layer and add the aqueous layer to 200 μL MeCN, 50 μL 1.75 M pH 6.95 bicine buffer containing 1% EDTA, 100 μL 100 mM 1,2-diphenylethylenediamine in 100 mM HCl, and 20 μL 20 mM potassium ferricyanide in water. Heat at 37° in the dark for 1 h, inject a 50 μL aliquot (keep it in the dark in the autosampler). (Buffer was a 2 M pH 8.6 ammonia-ammonium chloride buffer containing 8.9 mM diphenyl borate-ethanolamine complex and 13.4 mM EDTA.)

HPLC VARIABLES
Column: 100 × 4.6 3 μm PhaseSep C18 ODS2

Mobile phase: Gradient. A was MeCN:MeOH:50 mM pH 7.0 sodium acetate buffer 20:4:76. B was MeCN:MeOH:50 mM pH 7.0 sodium acetate buffer 60:10:30. A:B 40:60 for 3 min, go to 0:100 over 0.5 min, stay at 0:100 for another 4.5 min. (After the last sample flush column with 60 mL MeCN:MeOH:water 70:10:20.)
Flow rate: 1
Injection volume: 50-75
Detector: F ex 350 em 480

CHROMATOGRAM
Retention time: 3.5
Internal standard: α-methylnorepinephrine (2.5)
Limit of detection: 0.3-0.6 pg

OTHER SUBSTANCES
Simultaneous: norepinephrine, dopamine, epinine
Interfering: α-methyldopa

KEY WORDS
plasma

REFERENCE
Boomsma,F.; Alberts,G.; van der Hoorn,F.A.J.; Man in 't Veld,A.J.; Schalekamp,M.A.D.H. Simultaneous determination of free catecholamines and epinine and estimation of total epinine and dopamine in plasma and urine by high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.*, **1992**, *574*, 109–117.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 5 mg alumina + 10 μL 10 μM IS in 10 mM perchloric acid + 100 μL pH 8.7 Tris-HCl buffer, stir for 10 min, centrifuge at 3000 g for 1 min, discard the supernatant. Wash the alumina with two 500 μL portions of water, add 100 μL 100 mM perchloric acid, mix for 1 min, centrifuge at 3000 g for 1 min, inject a 50 μL aliquot of the supernatant. (Heat 20 g alumina (WA-4, Sigma) with 200 mL 2 M HCl at 100° for 1 h with gentle mixing, decant the supernatant, wash with twenty 200 mL portions of water, filter (Toyo Roshi No. 2 paper), dry at 120° overnight.)

HPLC VARIABLES
Column: 150 × 4.6 catecholpak (JASCO)
Mobile phase: MeCN:50 mM pH 3.20 potassium acetate:50 mM pH 3.20 potassium phosphate buffer 3:92.15:4.85 containing 1 mM sodium hexanesulfonate
Column temperature: 40
Flow rate: 0.5
Injection volume: 50
Detector: Chemiluminescence (Kenko filter Y-46) following post-column reaction. The column effluent mixed with reagent 1 pumped at 0.25 mL/min and the mixture flowed through a 15 m × 0.5 mm i.d. knitted PTFE coil at 80°. The effluent from the coil mixed with reagent 2 pumped at 1.4 mL/min and this mixture flowed to the detector. (Reagent 1 was 105 mM ethylenediamine (semiconductor grade) and 175 mM imidazole in MeCN:EtOH 90:10. Reagent 2 was 0.25 mM bis[4-nitro-2-(3,6,9-trioxadecyloxycarbonyl)phenyl] oxalate (Wako), 150 mM hydrogen peroxide, and 110 mM trifluoroacetic acid in dioxane:ethyl acetate 50:50 (Caution! Dioxane is a carcinogen!).)

CHROMATOGRAM
Retention time: 16
Internal standard: 3,4-dihydroxybenzylamine (17)
Limit of detection: 1 fmole

OTHER SUBSTANCES
Extracted: dopamine, norepinephrine

KEY WORDS
human; rat; plasma; SPE; post-column reaction

REFERENCE
Higashidate,S.; Imai,K. Determination of femtomole concentrations of catecholamines by high-performance liquid chromatography with peroxyoxalate chemiluminescence detection, *Analyst*, **1992**, *117*, 1863–1868.

SAMPLE
Matrix: blood
Sample preparation: Filter (Ultrafree-MC with 10000 molecular mass cut-off, Millipore) 100 μL plasma while centrifuging at 15000 g for 15 min. Mix 50 μL ultrafiltrate and 10 μL 140 ng/mL 3-methoxytyramine in Ringer solution, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 150 × 1 5 μm Inertsil-2 ODS
Mobile phase: MeCN:THF:water 6:0.8:93.2 containing 0.48 g/L sodium 1-octanesulfonate, 2 g/L NaH_2PO_4, 8.82 g/L sodium citrate, 10 mg/L EDTA, and 1 mL/L diethylamine, pH adjusted to 3.2 with concentrated orthophosphoric acid.
Flow rate: 0.06
Injection volume: 5
Detector: E, Bioanalytical Systems BAS-4C, glassy carbon working electrodes, upstream +0.75 V, downstream +0.05 V (measuring electrode), Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 3.5
Internal standard: 3-methoxytyramine (11)
Limit of detection: 0.2-0.5 pg

OTHER SUBSTANCES
Extracted: dopamine, norepinephrine, 3,4-dihydroxyphenylacetic acid, serotonin, 5-hydroxyindoleacetic acid, homovanillic acid

KEY WORDS
plasma; microbore; rat; ultrafiltrate

REFERENCE
Cheng,F.-C.; Yang,L.-L.; Kuo,J.-S.; Yang,M.C.M.; Yu,P.-C. Rapid assay of the monoamine content in small volumes of rat plasma, *J.Chromatogr.B*, **1994**, *653*, 9–16.

SAMPLE
Matrix: blood, food, peptides, plants, tissue
Sample preparation: Hydrolyze peptide with 6 M HCl containing 0.2% 3,3'-thiodipropionic acid at 110° for 24 h, evaporate to dryness, reconstitute with 50-200 μL 0.1% HCl containing 0.2% 3,3'-thiodipropionic acid. Homogenize (Ultra-Turrax) 0.1-1 g food, tissue, plant material, lyophilized plasma, or lyophilized tissue in 10 mL 250 nM IS in 100 mM HCl containing 0.2% 3,3'-thiodipropionic acid at 20000 rpm for 2 min, sonicate for ≤30 min, centrifuge at 5000 g for 20 min, discard fat layer, filter (Millipore ultrafiltration insert (MW cutoff 5000) prewashed with 200 μL 100 mM HCl containing 0.2% 3,3'-thiodipropionic acid) 3 mL supernatant while centrifuging at 3500 g for 1 h. Mix 20 μL deproteinized sample (or 10 μL peptide hydrolysate) with 180 μL buffer, vortex, add 200 μL reagent, mix, heat at 70° for 15 min with mixing at 1 min and 12 min, cool in an ice bath for 5 min, centrifuge at 10000 g for 10 s, add 400 μL diluent, mix thoroughly, centrifuge at 15000 g for 5 min, inject a 10 μL aliquot of the supernatant. (Prepare buffer by dissolving 630 mg sodium bicarbonate in 40 mL water, adjusting pH to 8.6 with NaOH, and making up to 50 mL with water. Prepare reagent by sonicating 40 mg dabsyl chloride in 10 mL acetone for 10 min, then filtering into brown vials and storing at -20°. Prepare diluent by mixing 50 mL MeCN, 25 mL EtOH, and 25 mL mobile phase A.)

HPLC VARIABLES
Guard column: present but not specified
Column: 150 × 3.9 4 μm Novapak C18
Mobile phase: Gradient. A was DMF:9 mM NaH_2PO_4 containing 0.16% triethylamine, adjusted to pH 6.55 with phosphoric acid. B was MeCN:water 80:20. A:B 92:8 for 2 min, to 80:20 over 5 min (Waters convex curve 5), to 65:35 over 28 min (Waters concave curve 7), to 50:50 over 10 min, to 0:100 over 21 min, maintain at 0:100 for 11 min, return to initial conditions over 0.5 min, re-equilibrate for 12.5 min.

Column temperature: 50
Flow rate: 1
Injection volume: 10
Detector: UV 436

CHROMATOGRAM
Retention time: 70.61
Internal standard: norleucine (40.90), norvaline (35.06)

OTHER SUBSTANCES
Extracted: amino acids dopamine, histamine, norepinephrine, taurine

KEY WORDS
rinse glass and plasticware with 70% EtOH and water and dry before use; derivatization; cheese; meat; sausage; fish; plasma

REFERENCE
Krause,I.; Bockhardt,A.; Neckermann,H.; Henle,T.; Klostermeyer,H. Simultaneous determination of amino acids and biogenic amines by reversed-phase high-performance liquid chromatography of the dabsyl derivatives, *J.Chromatogr.A*, **1995**, *715*, 67–79.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. Add 1 mL serum to 100 mg activated aluminum oxide suspended in 1 mL pH 8.7 Tris-HCl buffer, stir, let stand for 10 min. Discard the supernatant and wash the solid three times with 5 mL portions of water, wash the solid with 3 mL MeOH, dry under reduced pressure, elute with 3 mL 4 M acetic acid. Evaporate the eluate to dryness under reduced pressure, reconstitute the residue with 90 μL water, inject a 10 μL aliquot. Urine. 5 mL Urine + 5.3 mL 2 M HCl, heat at 100° for 20 min, cool to room temperature, add 1 mL 50 mM disodium EDTA, adjust the pH to 8.5 with dilute ammonia, add 500 mg 200 mesh aluminum oxide (Wako), shake for 10 min, filter, wash the solid with 10 mL water, elute with 5 mL 300 mM acetic acid, inject an aliquot of the eluate.

HPLC VARIABLES
Column: 250 × 3.6 10-25 μm Hitachi 3011 C resin
Mobile phase: 50 mM K_2HPO_4 containing 0.05% phosphoric acid
Column temperature: 45
Flow rate: 0.6
Injection volume: 10
Detector: F ex 383 em 486 following post-column reaction. The column effluent mixed with 1% 2-cyanoacetamide in water pumped at 0.5 mL/min and with buffer pumped at 1 mL/min and the mixture flowed through a 5 m × 0.5 mm ID PTFE coil at 100 ± 1° to the detector. (Buffer was 600 mM boric acid containing 750 mM KOH.)

CHROMATOGRAM
Retention time: 7
Limit of detection: 0.28 pmole

OTHER SUBSTANCES
Extracted: dopamine, norepinephrine

KEY WORDS
post-column reaction; serum; SPE

REFERENCE
Honda,S.; Takahashi,M.; Araki,Y.; Kakehi,K. Postcolumn derivatization of catecholamines with 2-cyanoacetamide for fluorimetric monitoring in high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *274*, 45–52.

SAMPLE
Matrix: blood, urine

Sample preparation: Condition a 150 μL Toyopak IC-SP S (sulfopropyl resin, H^+ form) SPE cartridge (Tosoh) with 10 mL water. Plasma. 700 μL Plasma + 50 μL 700 nM 3,4-dihydroxybenzylamine + 350 μL 2 M perchloric acid, mix, centrifuge at 4° at 1000 g for 15 min. Remove a 700 μL aliquot of the supernatant and add it to 30 μL 2 M potassium carbonate, centrifuge at 4° at 1000 g for 5 min. Add a 500 μL aliquot of the supernatant to the SPE cartridge, wash with 1 mL water, wash with 500 μL EtOH:water 50:50, wash with 5 mL water, elute with 500 μL 2 M sodium perchlorate, filter (0.2 μm), inject a 50 μL aliquot of the filtrate. Urine. Acidify urine collected over 24 h with 10 mL 6 M HCl. 500 μL Urine + 25 μL 10 μM 3,4-dihydroxybenzylamine + 25 μL 40 μM ferulic acid + 500 μL 1 M perchloric acid, mix, centrifuge at 4° at 1000 g for 15 min. Remove a 700 μL aliquot of the supernatant and add it to 30 μL 2 M potassium carbonate, centrifuge at 4° at 1000 g for 5 min, add a 500 μL aliquot of the supernatant to the SPE cartridge, wash with 1.5 mL water, wash with 500 μL EtOH:water 50:50, wash with 5 mL water, elute with 500 μL 2 M sodium perchlorate, filter (0.2 μm), inject a 50 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 150 × 4.6 5 μm TSK-gel ODS-80TM (Tosoh)

Mobile phase: Gradient. A was buffer. B was MeCN:MeOH:buffer 8:12:80, pH 3.1. A:B 100:0 for 4 min, to 60:40 over 8 min, to 0:100 over 2 min, maintain at 0:100 for 16 min, return to initial conditions (step gradient), re-equilibrate for 20 min. Buffer was 60 mM pH 3.1 citric acid containing 32 mM Na_2HPO_4, 1.7 mM sodium hexanesulfonate, and 0.1 mM disodium EDTA (J. Chromatogr. 1989, 467, 237).

Flow rate: 1

Injection volume: 50

Detector: F ex 345 em 480 following post-column reaction. The column effluent passed through a Hitachi 655A electrochemical detector with carbon cloth electrodes; working electrode at +0.68 V versus reference electrode (200 mM equimolar mixture of potassium hexacyanoferrate(II) and potassium hexacyanoferrate(III) containing 200 mM potassium nitrate and 200 mM KOH). The effluent from the electrochemical detector mixed with 20 mM meso-1,2-diphenylethylenediamine in 50 mM HCl pumped at 0.4 mL/min and with 1 M glycine containing 490 mM KOH and 3 mM potassium hexacyanoferrate(III) pumped at 0.4 mL/min. This mixture flowed through a 10 m × 0.47 mm ID coil at 80° to the detector (J. Chromatogr. 1989, 467, 237).

CHROMATOGRAM

Retention time: 10.5

Internal standard: 3,4-dihydroxybenzylamine (12.5)

Limit of detection: 1 nM

OTHER SUBSTANCES

Extracted: dopamine, levodopa, metanephrine, 3-methoxytyramine, norepinephrine

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KEY WORDS

post-column reaction; plasma; SPE

REFERENCE

Nohta,H.; Yamaguchi,E.; Ohkura,Y.; Watanabe,H. Measurement of catecholamines, their precursor and metabolites in human urine and plasma by solid-phase extraction followed by high-performance liquid chromatography with fluorescence derivatization, *J.Chromatogr.*, **1989**, *493*, 15–26.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 1 mL Plasma + 3 mL ice-cold MeOH:500 mM perchloric acid 98:2, centrifuge at 4° at 4000 g for 3 min. Remove 200 μL of the supernatant and add it to 100 μL 80 ng/mL N-methyldopamine, evaporate to dryness under vacuum, reconstitute in 200 μL mobile phase, inject a 5-20 μL aliquot. Urine. 1 mL Urine + 50 mL water, inject a 10 μL aliquot. (To deconjugate adjust pH to 1, flush with nitrogen, heat in a boiling water bath for 1 h, dilute with 50 mL water, inject a 10 μL aliquot.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Supelcosil LC-18

Mobile phase: MeOH:13 mM sodium acetate containing 0.5 mM sodium 1-octanesulfonate and 0.5 mM disodium EDTA 14:86, pH 3.10

Flow rate: 1
Injection volume: 5-20
Detector: E, ESA Model 5100 A Coulochem, Model 5011 A analytical cell, first electrode +0.40 V, second electrode -0.30 V

CHROMATOGRAM
Retention time: 6
Internal standard: N-methyldopamine (11)

OTHER SUBSTANCES
Extracted: methyldopa, norepinephrine, dopamine, dihydroxyphenylacetic acid, 3-O-methylmethyldopa, homovanilic acid

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Lucarelli,C.; Betto,P.; Ricciarello,G.; Grossi,G. High-performance liquid chromatographic determination of L-3-(3,4-dihydroxyphenyl)-2-methylalanine (α-methyldopa) in human urine and plasma, *J.Chromatogr.*, **1991**, *541*, 285–296.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Toyopak IC-SP S sulfopropyl resin, H^+ form, SPE cartridge (Tosoh) with 10 mL water and 2 mL 200 mM pH 5.0 sodium phosphate buffer. Plasma. 700 μL Plasma + 30 μL 700 nM isoproterenol + 50 μL 7 μM 3,4-dihydroxyphenylpropanoic acid + 350 μL 2 M perchloric acid, mix, centrifuge at 4° at 1000 g for 15 min. Remove a 700 μL aliquot of the supernatant and adjust the pH to 1.5-2.0 with about 150 μL 2 M potassium carbonate, centrifuge at 4° at 1000 g for 5 min, add the supernatant to the SPE cartridge, wash with 10 mL water, elute with 300 μL MeOH:2 M sodium perchlorate 7:93, filter (cellulose acetate membrane), inject a 100 μL aliquot of the filtrate. Urine. Collect human urine for 24 h in the presence of 10 mL 6 M HCl. 500 μL Urine + 10 μL 15 μM isoproterenol + 25 μL 800 μM 3,4-dihydroxyphenylpropanoic acid + 500 μL 1 M perchloric acid, mix, centrifuge at 4° at 1000 g for 15 min. Remove a 700 μL aliquot of the supernatant and adjust the pH to 1.5-2.0 with about 130 μL 2 M potassium carbonate, centrifuge at 4° at 1000 g for 5 min, add the supernatant to the SPE cartridge, wash with 1.5 mL water, wash with 500 μL EtOH:water 50:50, wash with 5 mL water, elute with 500 μL 1.5 M KCl in MeOH:100 mM HCl 7:93, filter (cellulose acetate membrane), inject a 100 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm TSK-gel ODS-80TM (Tosoh)
Mobile phase: MeOH:buffer 7:93 (Buffer was 30 mM pH 2.5 citrate buffer containing 0.4 mM sodium octanesulfonate.)
Flow rate: 0.8
Injection volume: 100
Detector: F ex 350 em 480 following post-column reaction. The column effluent mixed with reagent A pumped at 0.3 mL/min and the mixture flowed through a 3 m × 0.5 mm ID stainless steel coil at 90°. The effluent from this coil mixed with reagent B pumped at 0.3 mL/min and the mixture flowed through a 10 m × 0.5 mm ID stainless steel coil at 90° and through a 1 m × 0.5 mm ID stainless steel cooling coil to the detector (Anal. Sci. 1991, 7, 257). (Reagent A was 10 mM sodium periodate containing 3 mM potassium ferricyanide. Reagent B was 30 mM meso-1,2-diphenylethylenediamine in EtOH:water 70:30 containing 130 mM sodium methylate.)

CHROMATOGRAM
Retention time: 20
Internal standard: isoproterenol (60)
Limit of detection: 0.7 nM

OTHER SUBSTANCES
Extracted: dopamine, levodopa, metanephrine, 3-methoxytyramine, norepinephrine, normetanephrine

KEY WORDS
post-column reaction; plasma; SPE

REFERENCE
Jeon,H.-K.; Nohta,H.; Ohkura,Y. High-performance liquid chromatographic determination of catecholamines and their precursor and metabolites in human urine and plasma by postcolumn derivatization involving chemical oxidation followed by fluorescence reaction, *Anal.Biochem.*, **1992**, *200*, 332–338.

SAMPLE
Matrix: blood, urine
Sample preparation: 2 mL Plasma or 1 mL urine + dihydroxybenzylamine + 20 mg Sigma WA4 alumina + 200 µL 1 M pH 8.6 Tris-EDTA buffer, mix for 10 min, discard plasma. Wash the alumina three times with 3 mL water and dry it. Add 125 µL 500 mM phosphoric acid, after 1 min inject a 100 µL aliquot. (Ann. Clin. Biochem. 1985, 22, 194-203)

HPLC VARIABLES
Column: 250 × 4.5 5 µm Ultratechsphere
Mobile phase: Per liter 75 mmol citric acid, 58.5 mmol NaH_2PO_4, 0.2 mmol disodium EDTA, and 4.4 mmol heptanesulfonic acid, pH adjusted to 3.4, made up to a final volume of 2 L, add 200 mL MeOH
Flow rate: 1
Injection volume: 100
Detector: E, ESA Coulochem conditioning cell +0.35 V, first electrode +0.05 V, second electrode -0.35 V

CHROMATOGRAM
Retention time: 8.18
Internal standard: dihydroxybenzylamine (10.53)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Simultaneous: levodopa, metanephrine, norepinephrine, 3-methoxytyrosine, normetanephrine, dihydroxyphenylacetic acid, dopamine

KEY WORDS
plasma

REFERENCE
Dutton,J.; Copeland,L.G.; Playfer,J.R.; Roberts,N.B. Measuring L-dopa in plasma and urine to monitor therapy of elderly patients with Parkinson disease treated with L-dopa and a dopa decarboxylase inhibitor, *Clin.Chem.*, **1993**, *39*, 629–634.

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

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

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

CHROMATOGRAM
Retention time: 2.87

KEY WORDS
whole blood

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

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 5 mg amino acids in 10 mL MeCN:water:triethylamine 50:50:0.55. Remove a 50 μL aliquot and add it to 50 μL 0.66% 2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl isothiocyanate (Fluka) in MeCN, shake mechanically for 30 min, add 10 μL 0.26% ethanolamine in MeCN, shake for 10 min, make up to 1 mL with MeCN, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 25 × 4 (sic) 5 μm LiChrospher 100 RP-18
Mobile phase: MeOH:water 70:30
Flow rate: 1
Injection volume: 10
Detector: UV 231

CHROMATOGRAM
Retention time: k' 22.61, k' 25.67 (enantiomers)

OTHER SUBSTANCES
Simultaneous: phenylephrine

KEY WORDS
derivatization; chiral

REFERENCE
Lobell,M.; Schneider,M.P. 2,3,4,6-Tetra-O-benzoyl-β-D-glucopyranosyl isothiocyanate: an efficient reagent for the determination of enantiomeric purities of amino acids, β-adrenergic blockers and alkyloxiranes by high-performance liquid chromatography using standard reversed-phase columns, *J.Chromatogr.*, **1993**, *633*, 287–294.

SAMPLE
Matrix: cell cultures
Sample preparation: Centrifuge at 20000 g for 5 min, dilute the supernatant 10-50-fold, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 125 × 3 3 μm Nucleosil 100C18
Mobile phase: MeOH:buffer 5:95, pH adjusted to 3.0 with 5 M NaOH (Buffer was 50 mM citric acid containing 30 mM phosphoric acid, 0.75 mM octylsulfate, and 0.5 mM EDTA.)
Column temperature: 40
Flow rate: 0.7
Injection volume: 30
Detector: E, Waters 460, glassy carbon electrode

CHROMATOGRAM
Limit of detection: 10 nM

OTHER SUBSTANCES
Extracted: norepinephrine

REFERENCE
Ghindilis,A.L.; Michael,N.; Makower,A. A new sensitive and simple method for detection of catecholamines from adrenal chromaffin cells, *Pharmazie*, **1995**, *50*, 599–600.

SAMPLE
Matrix: enzyme incubations
Sample preparation: Prepare a SPE column by adding 500 μL of a 20% suspension of 19-40 μm Toyopak SP (strong cation-exchange sulfopropyl resin, Na^+ (Toyo Soda)) in water to a 35 × 6 column, wash with two 2 mL portions of 2 M NaOH, wash with 5 mL water, wash with 2 mL 2 M HCl, wash with 10 mL water. 350 μL Enzyme incubation at pH 8.5 + 50 μL 3 M trichloroacetic acid + 50 μL 2 μM isoproterenol, centrifuge at 4° at 1000 g for 10 min, add a 300 μL aliquot of the supernatant to the SPE column, wash with 10 mL water, wash with 3 mL 200 mM pH 5.5 phosphate buffer, wash with 10 mL water, elute with 2 mL EtOH:1 M NaCl 70:30. Add 100 μL 100 mM pH 6.7 1,2-diphenylethylenediamine in 100 mM HCl and 100 μL 15 mM potassium ferricyanide to the eluate, heat at 37° for 40 min, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 TSK-gel ODS-120T (Toyo Soda)
Mobile phase: MeCN:MeOH:50 mM pH 7.0 Tris-HCl buffer 52:3:45 (Wash with MeCN:MeOH: water 52:3:45 for 25 min at the end of each day.)
Flow rate: 1
Injection volume: 100
Detector: F ex 360 em 480

CHROMATOGRAM
Retention time: 8
Internal standard: isoproterenol (13)

KEY WORDS
derivatization; SPE

REFERENCE
Lee,M.; Nohta,H.; Ohkura,Y.; Yoo,B. Determination of phenylethanolamine N-methyltransferase by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1985**, *348*, 407–415.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Whatman PXS ODS-3 C18
Mobile phase: MeCN:MeOH:water:85% phosphoric acid: sodium octanesulfonate 10:20:70:0.5: 0.108 (v/v/v/v/w)
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6.5

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. Dissolve powdered tablets in 10 mM HCl, filter if necessary, inject an aliquot. Injections, solutions. Dilute with 10 mM HCl, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Partisil-5 ODS-3
Mobile phase: MeOH:buffer 30:70 (Buffer was 10 mM sodium 1-octanesulfonate in 0.2% acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 220

CHROMATOGRAM

Retention time: 11.5
Limit of detection: 33 ng

OTHER SUBSTANCES

Simultaneous: norepinephrine, levonordefrin, isoproterenol, phenylephrine, metaraminol, impurities

KEY WORDS

tablets; injections; ophthalmic solutions; inhalation solutions

REFERENCE

Smela,M.J.,Jr.; Stromberg,R. Liquid chromatographic determination of six sympathomimetic drugs in dosage forms, *J.Assoc.Off.Anal.Chem.*, **1991**, *74*, 289–291.

SAMPLE

Matrix: formulations
Sample preparation: Tablets. Grind tablets, weigh out a portion, dissolve in 50 mL mobile phase, sonicate, filter (No. 4 sintered glass plate), dilute, inject an aliquot. Capsules. Dissolve 10 capsules (without opening) in 100 mL mobile phase, sonicate, inject an aliquot. Injections, ampules, sprays. Dilute, inject an aliquot.

HPLC VARIABLES

Column: 120 × 4.6 Spherisorb C18 ODS-2
Mobile phase: Isopropanol:buffer 5:95 (Buffer was 100 mM sodium dodecyl sulfate containing 25 mM Na_2HPO_4, pH adjusted to 3.0 with HCl.)
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: k' 3.2
Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Simultaneous: carbidopa, dopamine, hydrochlorothiazide, isoproterenol, levodopa, methyldopa, norepinephrine, phenylephrine

KEY WORDS

tablets; capsules; injections; ampules; sprays

REFERENCE

Villanueva Camañas,R.M.; Sanchis Mallols,J.M.; Torres Lapasió,J.R.; Ramis-Ramos,G. Analysis of pharmaceutical preparations containing catecholamines by micellar liquid chromatography with spectrophotometric detection, *Analyst*, **1995**, *120*, 1767–1772.

SAMPLE

Matrix: solutions
Sample preparation: Dilute with 5% dextrose, inject a 20 μL aliquot.

HPLC VARIABLES

Column: Waters microparticulate C18
Mobile phase: MeOH:350 mM acetic acid and 5 mM sodium heptanesulfonate 20:80
Flow rate: 1.6-2.0
Injection volume: 20
Detector: F ex 285 em 315

CHROMATOGRAM

Retention time: 4.13

OTHER SUBSTANCES

Interfering: dopamine

REFERENCE

Williams,D.A.; Fung,E.Y.Y.; Newton,D.W. Ion-pair high-performance liquid chromatography of terbutaline and catecholamines with aminophylline in intravenous solutions, *J.Pharm.Sci.*, **1982**, *71*, 956–958.

SAMPLE

Matrix: solutions
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: 2.15 (tails)

OTHER SUBSTANCES

Simultaneous: monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodeine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine, pemoline, benzphetamine, diethylpropion, mazindol, tranylcypromine, caffeine, fenethyline, phendimetrazine, methylphenidate, phenelzine, chlorphentermine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine, norpipanone, levallorphan
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: pipradol, phenylpropanolamine, fencamfamin, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine

REFERENCE

Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 250 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 5-C18
Mobile phase: 50 mM Potassium perchlorate containing 250 μL/L 3% copper acetate in water and 10 g/L sodium acetate, pH adjusted to 4.45 with acetic acid
Flow rate: 1
Injection volume: 250
Detector: F ex 400 em 500 following post-column reaction. The column effluent flowed through the reactor then through a 3.5 m × 0.8 mm ID coil of PTFE tubing at 30°. The effluent from the coil mixed with reducing solution pumped at 1 (?) mL/min and this mixture flowed through a 2 m × 0.8 mm ID PTFE coil at 30° to the detector. (Prepare the reactor as follows. Dissolve 75.3 g manganese nitrate in 500 mL water, add 50 g 18-35 mesh silica gel (Macherey-Nagel), stir vigorously, slowly add 31.6 g potassium permanganate in 500 mL water, stir for 30 min, filter (500 μm sieve), wash until no permanganate color is left, dry in a desiccator, pack in a 50 × 2.1 stainless steel tube. The reducing solution was 266 g NaOH, 13.4 g anhydrous sodium sulfite, and 9 mL 2-mercaptoethanol in 1 L water. Note that some Nucleosil 5-C18 column packings do not give separation at pH 4.45. In this case it is necessary to use 50 mM perchloric acid as mobile phase and mix the column effluent with pH 4.4 sodium acetate buffer before it enters the reactor.)

CHROMATOGRAM
Retention time: 10
Limit of detection: 0.4 ng/mL

OTHER SUBSTANCES
Extracted: norepinephrine

KEY WORDS
post-column reaction

REFERENCE
Rüter,J.; Kurz,U.P.; Neidhart,B. Solid phase reactors as an analytical tool in the determination of urinary noradrenaline and adrenaline, *J.Liq.Chromatogr.*, **1985**, *8*, 2475–2496.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Partisil ODS-3
Mobile phase: MeOH:buffer 30:70 (Buffer was 10 mM octanesulfonic acid in 0.2% acetic acid.)
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 11.5

OTHER SUBSTANCES
Simultaneous: isoproterenol, levonordefrin, metaraminol, phenylephrine

REFERENCE
Phenomenex Catalog, **1994**, p. 1.077.

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, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Chirex 3022 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 55:35:10 (EtOH/trifluoroacetic acid was premixed 20:1.)

Flow rate: 0.7-1
Injection volume: 20
Detector: UV 282

KEY WORDS
chiral; α = 1.10 for enantiomers

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

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (glass potters) with 5 volumes of 100 mM perchloric acid containing 1.9 mM sodium bisulfite, centrifuge at 10000 g at 4° for 30 min. Filter (0.22 μm) the supernatant, add dihydroxybenzylamine, inject a 5-20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 Hypersil H5 ODS
Mobile phase: EtOH:buffer 2:98 (Buffer was 13.8 g NaH_2PO_4, 60 mg disodium EDTA, and 20 mg 1-octanesulfonic acid in 1 L water, pH adjusted to 3.70 with phosphoric acid.)
Flow rate: 1
Injection volume: 5-20
Detector: E, Unicam PU 4022, 70 mV

CHROMATOGRAM
Retention time: 9
Internal standard: dihydroxybenzylamine

OTHER SUBSTANCES
Extracted: dopamine, norepinephrine

KEY WORDS
adrenal; fetal

REFERENCE
Garciá,J.C.; Blanco,L.; McPherson,M.; Leiva,A.; Maciás,R. High-performance liquid chromatographic determination of norepinephrine, epinephrine and dopamine in human foetal adrenal gland, *J.Chromatogr.B*, **1994**, *656*, 77–80.

SAMPLE
Matrix: urine
Sample preparation: Acidify urine with 1% (v/v) 6 M HCl. 6-10 mL Swine urine or 1-2.5 mL rat urine, centrifuge at 4000 g for 30 min, add 200 ng 3,4-dihydroxybenzylamine hydrobromide and 15 mL 1g/L EDTA, adjust to pH 6.45-6.55 with HCl or NaOH. Add the mixture to a cation-exchange resin SPE cartridge (Bio-Rad), wash twice with 10 mL water and with 5 ml water, elute with 8 mL 10 g/L boric acid. Dilute boric acid eluate with an equal volume of mobile phase, inject a 60 μL aliquot. (Procedure for determining methoxycatecholamines is also described.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Kromasil C8
Mobile phase: MeOH:buffer 15:85 (Mobile phase was 300 mL MeOH, 1.5 mL 200 mg/mL 1-octanesulfonic acid, 100 mL 1 M sodium acetate, and about 1 L water. The pH was adjusted to pH 3.8 with citric acid and made up to 2 L with water.)
Flow rate: 0.6
Injection volume: 60
Detector: E, Bioanalytical Systems, glassy carbon electrode + 650 mV, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 8.23

Internal standard: 3,4-dihydroxybenzylamine hydrobromide (10.31)
Limit of detection: 40 pg

OTHER SUBSTANCES
Extracted: dopamine, norepinephrine

KEY WORDS
pig; rat; SPE; pharmacokinetics

REFERENCE
Hay,M.; Mormède,P. Determination of catecholamines and methoxycatecholamines excretion patterns in pig and rat urine by ion-exchange liquid chromatography with electrochemical detection, *J.Chromatogr.B*, **1997**, *703*, 15–23.

SAMPLE
Matrix: urine
Sample preparation: Acidify urine by adding 1% 6 M HCl. 5 mL Acidified urine + 1 mL 7.5% disodium EDTA, adjust pH to 8.5 with 1 M NaOH, add 250 mg alumina (previously treated with 2 M HCl), shake for 5 min, decant the supernatant, wash the alumina three times with 5 mL portions of water. Place the alumina in a 4 mm ID glass column, elute with 250 mM acetic acid in water, collect 2.5 mL eluate, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 1000 × 2.1 Zipax SCX (DuPont)
Mobile phase: MeCN:50 mM NaH_2PO_4 5:95
Column temperature: 40
Flow rate: 0.8
Injection volume: 100
Detector: F ex 400 em 490 following post-column reaction. The column effluent mixed with the reagent pumped at 0.4 mL/min and the mixture flowed through a 10 m × 0.5 mm PTFE coil at 75 ± 0.1° to the detector. (Reagent was 500 mM borate buffer adjusted to pH 9.7 with NaOH.)

CHROMATOGRAM
Retention time: 9
Limit of quantitation: 0.25 ng

OTHER SUBSTANCES
Extracted: norepinephrine

KEY WORDS
post-column reaction; SPE

REFERENCE
Nimura,N.; Ishida,K.; Kinoshita,T. Novel post-column derivatization method for the fluorimetric determination of norepinephrine and epinephrine, *J.Chromatogr.*, **1980**, *221*, 249–255.

SAMPLE
Matrix: urine
Sample preparation: Add disodium EDTA and sodium metabisulfite to urine. 100 μL Urine + 2 mL water, vortex, add 1 mL reagent 1, add 5 mL reagent 2, shake vigorously for 2 min, centrifuge at 2000 g for 2 min, freeze in dry ice/acetone. Remove the organic layer and add it to 200 μL 80 mM acetic acid and 2 mL n-octanol saturated with acetic acid, shake vigorously for 2 min, centrifuge at 2000 g for 2 min, freeze in dry ice/acetone until the aqueous layer is just solid, remove the organic layer. Thaw out the aqueous layer and add it to 1 mL reagent 1 and 5 mL reagent 2, shake vigorously for 2 min, centrifuge at 2000 g for 2 min, freeze in dry ice/acetone. Remove the organic layer and add it to 200 μL 80 mM acetic acid and 2 mL n-octanol saturated with acetic acid, shake vigorously for 2 min, centrifuge at 2000 g for 2 min, freeze in dry ice/acetone until the aqueous layer is just solid, remove the organic layer. Thaw out the aqueous layer and add 100 μL Bicine buffer, 250 μL MeCN, and 100 μL 100 mM 1,2-diphenylethylenediamine in 100 mM HCl, vortex, add 20 μL 20 mM potassium ferricyanide, vortex, heat at 37° for 40 min, cool to room temperature, inject a 100 μL aliquot. (Prepare

reagent 1 by dissolving 214 g ammonium chloride and 10 g disodium EDTA in 2 L water, adjust pH to 8.3-8.5 with concentrated ammonium hydroxide, add 4.0 g diphenylborate-ethanolamine complex, stir for several hours until a clear solution is obtained. Prepare reagent 2 by dissolving 2.5 g tetraoctylammonium bromide and 10 mL n-octanol (saturated with acetic acid) in 1 L n-heptane. Prepare Bicine buffer by dissolving 14.3 g Bicine (N,N-bis(2-hydroxyethyl)glycine) and 359 mg anhydrous sodium acetate in 45 mL water, stir overnight until dissolved, adjust pH to 7.30 with concentrated NaOH, make up to 50 mL with water. Note that concentration of 1,2-diphenylethylenediamine is not given in paper. Other authors have used 100 mM (J.Chromatogr. 1989, 487, 17; 1992, 574, 109; 1992, 583, 236).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. A was MeCN:MeOH:50 mM pH 7.0 sodium acetate buffer 40:10:50. B was MeCN:MeOH:50 mM pH 7.0 sodium acetate buffer 50:10:40. A:B 75:25 for 1 min, to 10:90 over 7 min, return to initial conditions over 1 min.
Flow rate: 1
Injection volume: 100
Detector: F ex 365 em 418 (cutoff filter)

CHROMATOGRAM

Retention time: 5
Limit of detection: <0.4 nM

OTHER SUBSTANCES

Extracted: dopamine, norepinephrine
Simultaneous: isoproterenol

KEY WORDS

derivatization; protect from light

REFERENCE

Moleman,P.; van Dijk,J. Determination of urinary norepinephrine and epinephrine by liquid chromatography with fluorescence detection and pre-column derivatization, *Clin.Chem.*, **1990**, *36*, 732–736.

SAMPLE

Matrix: urine
Sample preparation: Add 10-15 mL 6 M HCl to a 24 h volume of urine. 2 mL 3 M Tris buffer containing 30 mM EDTA + 500 μL 10 μM dihydrobenzylamide in 100 mM perchloric acid + 2 mL 3 M tris buffer containing 30 mM EDTA + 100 μL 5 M NaOH + 4 mL acidified urine, mix, add to a 1 mL SPE column containing 200 mg alumina (70-230 mesh-ASTM (Touzart et Matignon) at 3 mL/min, wash with 9 mL water at 12 mL/min, force through 0.5 mL air, elute with 1 mL 150 mM perchloric acid at 0.75 mL/min, mix eluate, inject a 100 μL aliquot. (The pH of the Tris buffer is such that the pH of the mixture applied to the SPE column is 7.75-8.0.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Nucleosil 100/C18
Mobile phase: MeOH:buffer 36:64 (Buffer was 75 mM NaH_2PO_4, 0.15 mM EDTA, and 6 mM sodium heptanesulfonate, pH 3.96.)
Flow rate: 1.25
Injection volume: 100
Detector: F ex 280 em 310

CHROMATOGRAM

Retention time: 7.6
Internal standard: dihydrobenzylamide hydrobromide (9.6)
Limit of detection: 10 nM

OTHER SUBSTANCES

Extracted: dopamine, norepinephrine
Simultaneous: levodopa, methyldopa

KEY WORDS
SPE

REFERENCE
Said,R.; Robinet,D.; Barbier,C.; Sartre,J.; Huguet,C. Fully automated high-performance liquid chromatographic assay for the analysis of free catecholamines in urine, *J.Chromatogr.*, **1990**, *530*, 11–18.

SAMPLE
Matrix: urine
Sample preparation: 100 μL Urine + 125 μL 218.6 nM α-methylnorepinephrine in 10 mM HCl + 1 mL 10 mM HCl + 1 mL reagent + 5 mL 4.6 mM tetraoctylammonium bromide in n-heptane: 1-octanol 99:1, shake for 2 min, centrifuge at 20° at 1000 g for 5 min, freeze in dry ice/acetone. Remove the organic phase and add it to 2 mL 1-octanol saturated with 80 mM acetic acid and 200 μL 80 mM acetic acid, shake, centrifuge at 20° at 1000 g for 5 min, freeze in dry ice/acetone. Discard the organic layer and add 1 mL 10 mM HCl to the aqueous layer, add 2 mL 1-octanol saturated with 80 mM acetic acid, add 150 μL 80 mM acetic acid, shake, centrifuge at 20° at 1000 g for 5 min, freeze in dry ice/acetone. Discard the organic layer and add 200 μL MeCN and 50 μL buffer to the aqueous layer, add 100 μL 100 mM 1,2-diphenylethylenediamine in 100 mM HCl, add 20 μL 20 mM potassium ferricyanide in water, heat at 37° in the dark for 1 h, inject a 50 μL aliquot. (Reagent was 8.9 mM diphenylborate-ethanolamine complex in 2 M pH 8.6 ammonia/ammonium chloride buffer containing 13.4 mM EDTA. Buffer was 1.75 M pH 7.05 bicine in water containing 1% EDTA. Recrystallize 1,2-diphenylethylenediamine from toluene:light petroleum (bp 60-80°) 10:90, dry overnight at 60°.)

HPLC VARIABLES
Column: 100 × 4.6 3 μm Cp MicroSpher C18 (Chrompack)
Mobile phase: MeCN:MeOH:50 mM pH 7.0 sodium acetate 40:8:50 (At the end of the day flush column with 60 mL MeCN:MeOH:water 70:10:20.)
Flow rate: 1
Injection volume: 50
Detector: F ex 350 em 480

CHROMATOGRAM
Retention time: 4
Internal standard: α-methylnorepinephrine (Janssen, Beerse, Belgium) (3)
Limit of quantitation: 1.6 nM

OTHER SUBSTANCES
Extracted: dopamine, norepinephrine

KEY WORDS
derivatization; protect from light

REFERENCE
van der Hoorn,F.A.J.; Boomsma,F.; Man in 't Veld,A.J.; Schalekamp,M.A.D.H. Improved measurement of urinary catecholamines by liquid-liquid extraction, derivatization and high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.*, **1991**, *563*, 348–355.

SAMPLE
Matrix: urine
Sample preparation: Condition a 100 mg Bakerbond C-18 SPE cartridge with 2 mL MeOH and 2 mL buffer I. Heat urine at 50°, centrifuge, remove a 500 μL aliquot and add it to 1 mL buffer II (?), add 20 μL 860 ng/mL dihydroxybenzylamine, shake for 5 min, add a 1 mL aliquot to the SPE cartridge, wash with 2 mL buffer I, wash with 1 mL MeOH:buffer I 50:50, wash with 500 μL water, elute with 2 mL 1 M acetic acid, inject a 20 μL aliquot. (Buffer I was 200 mM ammonium chloride containing 0.05% EDTA and 0.4% tetrabutylammonium iodide, pH adjusted to 8.0 ± 0.1. Buffer II was 2 M ammonium chloride containing 0.5% EDTA and 1.2% tetrabutylammonium iodide, pH adjusted to 8.0 ± 0.1.)

HPLC VARIABLES
Column: 150 × 3 5 μm Separon SGX C-18 (Tessek)

Mobile phase: MeOH:buffer 5:95-7:93 (Buffer was 50 mM pH 3.0 ± 0.1 phosphate buffer containing 50 mM EDTA, 1 mM sodium octanesulfonate, and 1 mM NaCl.)
Injection volume: 20
Detector: E, AMOR 400 mV (SunChrom)

CHROMATOGRAM
Internal standard: dihydroxybenzylamine
Limit of detection: 3 ng/mL

OTHER SUBSTANCES
Extracted: dopamine, norepinephrine

KEY WORDS
SPE

REFERENCE
Brandsteterova,E.; Krajnak,K.; Skacani,I. HPLC analysis of urinary catecholamines using affinity SPE procedure, *Pharmazie*, **1995**, *50*, 825–826.

SAMPLE
Matrix: urine
Sample preparation: Adjust urine to pH 3.0 with 6 M HCl, centrifuge at 1600 g for 10 min. 900 μL Supernatant + 100 μL 2.5 μM N-methyldopamine, mix, inject a 100 μL aliquot on to column A and elute to waste with mobile phase A, after 5 min elute the contents of column A on to column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 35 × 4 TSK-precolumn-CA 2 (Tosoh); B 150 × 4.6 mixed mode (C18/cation exchange) (Alltech)
Mobile phase: A 15 mM pH 6.0 citric acid/trisodium citrate buffer; B 200 mM pH 6.0 citric acid/trisodium citrate buffer
Column temperature: 35
Flow rate: 0.7
Injection volume: 100
Detector: E, Mitsubishi 8 channel, 150 mV

CHROMATOGRAM
Retention time: 11.5
Internal standard: N-methyldopamine (30 mV) (20)
Limit of detection: 0.5 nM

OTHER SUBSTANCES
Extracted: dopamine (30 mV), metanephrine (380 mV), 3-methoxytyramine (340 mV), norepinephrine (150 mV), normetanephrine (380 mV), serotonin (150 mV)

KEY WORDS
column-switching

REFERENCE
Mashige,F.; Matsushima,Y.; Miyata,C.; Yamada,R.; Kanazawa,H.; Sakuma,I.; Takai,N.; Shinozuka,N.; Ohkubo,A.; Nakahara,K. Simultaneous determination of catecholamines, their basic metabolites and serotonin in urine by high-performance liquid chromatography using a mixed-mode column and an eight-channel electrochemical detector, *Biomed.Chromatogr.*, **1995**, *9*, 221–225.

SAMPLE
Matrix: urine
Sample preparation: Condition a 10 × 2 20 mg 15-25 μm PLRP-S polymer-based SPE cartridge (Spark Holland) with 1 mL MeOH, with 0.5 mL water, and with 1.5 mL 200 mM pH 8.5 ammonia/ammonium chloride buffer containing 0.05% EDTA. Collect 24 h urine with 10 mL 6 M HCl, final pH 1-3. Dilute 4-fold with buffer. Inject a 200 μL aliquot onto the SPE cartridge, wash with 1 mL 200 mM pH 8.5 ammonia/ammonium chloride buffer containing 0.05% EDTA,

wash with 250 μL MeOH:200 mM pH 8.5 ammonia/ammonium chloride buffer 20:80, wash with water at 1 mL/min for 2.25 min. Elute the contents of the SPE cartridge onto column A with the mobile phase for 30 s then remove the SPE cartridge from the circuit, elute column A with mobile phase onto column B, after 1.25 min elute column A to waste with mobile phase and elute column B with mobile phase, monitor the effluent from column B. (Buffer was 2 M pH 8.5 ammonia/ammonium chloride containing 0.5% EDTA, 0.1% diphenylborate, and 18 ng/mL dihydroxybenzylamine.)

HPLC VARIABLES
Column: A 30 × 4.6 C18 (Brownlee); B 250 × 4.6 5 μm Ultrasphere IP C18
Mobile phase: MeCN:MeOH:buffer 15:8:100, apparent pH adjusted to 3.2 with 1.5 M orthophosphoric acid (Buffer was 50 mM KH_2PO_4 containing 1 mM sodium heptane sulphate and 0.07 mM EDTA.)
Flow rate: 0.8
Injection volume: 200
Detector: E, ESA Model 5100A, Model 5021 conditioning cell, Model 5011 analytical cell, oxidizing electrode +350 mV, screen electrode +100 mV, quantifying electrode -300 mV

CHROMATOGRAM
Retention time: 7
Internal standard: dihydroxybenzylamine (8)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Extracted: norepinephrine, dopamine

KEY WORDS
SPE; column-switching

REFERENCE
Pastoris,A.; Cerutti,L.; Sacco,R.; De Vecchi,L.; Sbaffi,A. Automated analysis of urinary catecholamines by high-performance liquid chromatography and on-line sample pretreatment, *J.Chromatogr.B*, **1995**, *664*, 287–293.

SAMPLE
Matrix: urine
Sample preparation: Acidify urine to pH 2.0-3.5 with 5 M HCl, centrifuge at 7000 g for 10 min, inject a 10-500 μL aliquot on to column A and elute to waste with mobile phase A, after 10 min backflush the contents of column A on to column B with mobile phase B, elute with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A nitrophenylboronic acid modified copolymer (U.S. Patent 4 767 529 (Chem.Abs. 1988, 108, 71698t)); B 53 × 4.6 1.5 μm MICRA NPS RP-18 (MICRA Scientific, Northbrook)
Mobile phase: A 20 mM $(NH_4)_2HPO_4$ containing 10 mM EDTA, adjusted to pH 8.7 with 25% ammonia solution; B 10 mM NaH_2PO_4 containing 0.1 mM dodecanesulfonic acid, adjusted to pH 2.5 with orthophosphoric acid
Flow rate: A 0.5; B from 0.2 to 0.5 over 2 min, maintain at 0.5
Injection volume: 10-500
Detector: F ex 275 em 330

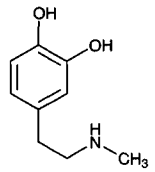
CHROMATOGRAM
Retention time: 6
Limit of detection: 0.76 pmole
Limit of quantitation: 1.75 pmole

OTHER SUBSTANCES
Extracted: dopamine, norepinephrine

KEY WORDS
column-switching

REFERENCE
Rudolphi,A.; Boos,K.-S.; Seidel,D. Coupled-column HPLC analysis of free urinary catecholamines using restricted access affinity precolumn and micro-particulate nonporous silica analytical column, *Chromatographia*, **1995**, *41*, 645–650.

Epinine



Molecular formula: $C_9H_{13}NO_2$
Molecular weight: 167.21
CAS Registry No.: 501-15-5

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

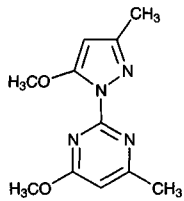
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, 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, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine,

recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

Epirizole



Molecular formula: $C_{11}H_{14}N_4O_2$
Molecular weight: 234.26
CAS Registry No.: 18694-40-1
Merck Index: 3659

SAMPLE
Matrix: solutions

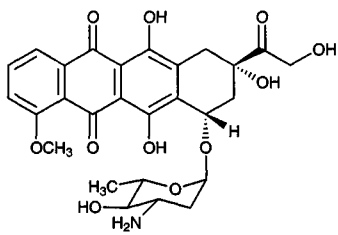
HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 45:55 containing 300 mM KCl
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 251

OTHER SUBSTANCES
Also analyzed: albendazole, prochlorperazine

REFERENCE

Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

Epirubicin



Molecular formula: $C_{27}H_{29}NO_{11}$
Molecular weight: 543.53
CAS Registry No.: 56420-45-2, 56390-09-1 (HCl)
Merck Index: 3660

SAMPLE
Matrix: blood
Sample preparation: Slowly add 200 μL MeCN:100 mM orthophosphoric acid 80:20 to 200 μL plasma in a centrifuge tube. Vortex the white precipitate for 30 s and then centrifuge at 1500

g until a compact pellet and clear supernatant is obtained, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 10 mm long C18
Column: 250 × 4.6 5μm Spherisorb ODS1
Mobile phase: MeCN:buffer 35:65. (Buffer was 60 mM Na_2HPO_4 containing 0.05% triethylamine, adjusted to pH 4.2 with citric acid.)
Flow rate: 1
Injection volume: 20
Detector: F ex 480 em 560

CHROMATOGRAM
Retention time: 9.03
Limit of quantitation: 8.6 pmol/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Barker,I.K.; Crawford,S.M.; Fell,A.F. Determination of plasma concentrations of epirubicin and its metabolites by high-performance liquid chromatography during a 96-h infusion in cancer chemotherapy, *J.Chromatogr.B*, **1996**, *681*, 323–329.

SAMPLE
Matrix: blood
Sample preparation: Condition an Oasis HLB SPE cartridge (Waters) with 1 mL mobile phase: water 1:3. 200 μL Plasma + 500 mL mobile phase:water 1:3 + 100 μL 120 ng/mL IS in water, vortex, add to the SPE cartridge, wash with 1 mL mobile phase:water 1:3, elute with 600 μL MeCN:mobile phase 1:1, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 10 μm LiChrosorb RP18
Mobile phase: MeCN:water 29:71 containing 50 mM Na_2HPO_4 and 0.05% (v/v) triethylamine adjusted to pH 4.6 with citric acid
Flow rate: 1.0
Injection volume: 20
Detector: E, ESA Coulochem II, Model 5014 high-performance analytical cell containing amperometric electrode +400 mV coupled with coulometric electrode -300 mV, palladium reference electrode

CHROMATOGRAM
Retention time: 9.4
Internal standard: doxorubicin (12.0)
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Ricciarello,R.; Pichini,S.; Pacifici,R.; Altieri,I.; Pellegrini,M.; Fattorossi,A.; Zuccaro,R. Simultaneous determination of epirubicin, doxorubicin and their principal metabolites in human plasma by high-performance liquid chromatography and electrochemical detection, *J.Chromatogr.B*, **1998**, *707*, 219–225.

SAMPLE
Matrix: blood
Sample preparation: Plasma + 100 ng daunorubicin hydrochloride + 1 mL pH 8.4 phosphate buffer + 8 mL chloroform:1-heptanol 90:10, shake mechanically for 30 min, centrifuge at 3300 rpm for 10 min. Remove the lower organic layer and evaporate it to 2 mL under a stream of nitrogen. Add the residue to 200 μL 300 mM phosphoric acid, vortex. Remove the aqueous phase and add it to 2 mL n-hexane, vortex, centrifuge, inject a 100-150 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: 250 × 4 7 μm Lichrosorb RP-18
Mobile phase: MeOH:water 70:30 containing 0.5% acetic acid and 2.5 mM sodium heptanesulfonate
Flow rate: 1.2
Injection volume: 100-150
Detector: UV 254

CHROMATOGRAM
Internal standard: daunorubicin
Limit of detection: 4 ng

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Hu,O.Y.-P.; Chang,S.-P.; Jame,J.-M.; Chen,K.-Y. Pharmacokinetic and pharmacodynamic studies with 4'-epidoxorubicin in nasopharyngeal carcinoma patients, *Cancer Chemother.Pharmacol.*, **1989**, *24*, 332–337.

SAMPLE
Matrix: blood
Sample preparation: Condition a C2 SPE cartridge with 1 mL MeOH, 500 μL water and 500 μL buffer. 1 mL Plasma + daunorubicin + 500 μL water, mix, add to SPE cartridge, wash with 500 μL buffer, elute contents of SPE cartridge with mobile phase onto column for 1 min, remove SPE cartridge, elute column with mobile phase, monitor the effluent. (Buffer was 19 mM NaH_2PO_4 adjusted to pH 4.0 with 100 mM phosphoric acid:MeCN 90:10.)

HPLC VARIABLES
Guard column: 50 × 5 10 μm LiChrosorb RP-18
Column: 100 × 5 5 μm Apex II ODS (Jones Chromatography)
Mobile phase: MeCN:buffer 1:2.25 (Buffer was 19 mM NaH_2PO_4 adjusted to pH 4.0 with 100 mM phosphoric acid.)
Flow rate: 1
Injection volume: 1000
Detector: F ex 480 em 580

CHROMATOGRAM
Retention time: 7.5
Internal standard: daunorubicin (22)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; SPE

REFERENCE
Dobbs,N.A.; Twelves,C.J. Measurement of epidoxorubicin and its metabolites by high-performance liquid chromatography using an advanced automated sample processor, *J.Chromatogr.*, **1991**, *572*, 211–217.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 50 ng doxorubicin + 5 mL chloroform:isopropanol 80:20, extract, centrifuge at 3000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 30° in the dark, reconstitute the residue in 100 μL mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 Nucleosil 100-5 C18
Mobile phase: MeCN:10 mM pH 4 ammonium formate buffer 30:70
Flow rate: 1.5
Injection volume: 25
Detector: F ex 480 em 560

CHROMATOGRAM
Retention time: 5.6
Internal standard: doxorubicin (4.5)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Jakobsen,P.; Steiness,E.; Bastholt,L.; Dalmark,M.; Lorenzen,A.; Petersen,D.; Gjedde,S.B.; Sandberg,E.; Rose,C.; Nielsen,O.S. Multiple-dose pharmacokinetics of epirubicin at four different dose levels: studies in patients with metastatic breast cancer, *Cancer Chemother.Pharmacol.*, **1991**, *28*, 63–68.

SAMPLE
Matrix: blood, cells
Sample preparation: Thaw cell samples, sonicate (Branson B-12) at 50 W for 20 s. 400 μL Cell sample or plasma + 200 μL 200 nM daunorubicin in 100 mM pH 9.3 borate buffer, add 1.8 mL chloroform:MeOH 80:20, extract, inject a 200-500 μL aliquot of the organic phase.

HPLC VARIABLES
Column: 250 × 4 Lichrosorb Si-60
Mobile phase: Chloroform:MeOH:glacial acetic acid:0.3 mM magnesium chloride 72:21:2:3
Flow rate: 1.5
Injection volume: 200-500
Detector: F ex 480 em 560

CHROMATOGRAM
Retention time: 3.9
Internal standard: daunorubicin (3.3)
Limit of detection: 0.5 nM

OTHER SUBSTANCES
Extracted: metabolites, doxorubicin

KEY WORDS
plasma; pharmacokinetics; normal phase

REFERENCE
Tidefelt,U.; Sundman-Engberg,B.; Paul,C. Comparison of the intracellular pharmacokinetics of doxorubicin and 4'-epi-doxorubicin in patients with acute leukemia, *Cancer Chemother.Pharmacol.*, **1989**, *24*, 225–229.

SAMPLE
Matrix: blood, tissue

Sample preparation: Plasma. Condition a 100 mg C8 Bond Elut SPE cartridge with 2 mL MeOH and 2 mL 10 μg/mL desipramine hydrochloride in 50 mM pH 7 sodium phosphate buffer. Add 300 μL plasma to the SPE cartridge, wash with 1 mL water, wash with 1 mL 10 μg/mL desipramine hydrochloride in 50 mM pH 7 sodium phosphate buffer, elute with 500 μL MeOH. Evaporate the eluate under a stream of nitrogen, reconstitute in 300 μL mobile phase, inject a 100 μL aliquot. Tissue. Tissue + 1 volume MeOH + 2 volumes 1 M pH 8.5 Tris buffer, homogenize, let stand on ice for 15 min, add 7 volumes MeCN containing daunorubicin, vortex, let stand at room temperature for 15 min, centrifuge, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Supelcosil LC-8-DB
Mobile phase: MeCN:50 mM pH 1.8 phosphoric acid 27:73
Flow rate: 1.5
Injection volume: 100
Detector: F ex 473 em 593

CHROMATOGRAM

Retention time: 4.5
Internal standard: daunorubicin (for tissue)
Limit of detection: 4 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; rat; liver; pharmacokinetics; SPE

REFERENCE

Hall,K.S.; Endresen,L.; Schjerven,L.; Rugstad,H.E. The influence of partial hepatectomy on the pharmacokinetics of preoperatively injected 4'-epidoxorubicin in rats, *Cancer Chemother.Pharmacol.*, **1990**, *26*, 444–448.

SAMPLE

Matrix: blood, tissue
Sample preparation: Homogenize (UltraTurrax T25) tissue with 5 volumes of MeOH:water 5:95 at 13500 rpm for about 2 min, centrifuge at 3200 g for about 2 min, remove the supernatant, centrifuge at 4000 g for 2 min, dilute 1:10 with MeOH:water 5:95. Inject a 50 μL aliquot of plasma or tissue homogenate onto column A with mobile phase A, elute column A with mobile phase A to waste for 10 min, backflush the contents of column A onto column B with mobile phase B, after 5 min remove column A from the circuit and flush it to waste with mobile phase A, elute column B with mobile phase B and monitor the effluent.

HPLC VARIABLES

Column: A 20 × 4 25 μm C4-alkyl-diol silica (Pinkerton type); B 250 × 4 5 μm LiChrospher 60 RP-Select B
Mobile phase: A MeOH:water 5:95; B MeCN:buffer 30:70 (Buffer was 0.1% triethylamine in water adjusted to pH 2.0 with trichloroacetic acid.)
Flow rate: 1
Injection volume: 50
Detector: F ex 445 em 560

CHROMATOGRAM

Retention time: 9
Limit of detection: 25 pg
Limit of quantitation: 50 pg

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

column-switching; protect from light; tumor; liver; plasma; pharmacokinetics

REFERENCE

Rudolphi,A.; Vielhauer,S.; Boos,K.-S.; Seidel,D.; Bäthge,I.-M.; Berger,H. Coupled-column liquid chromatographic analysis of epirubicin and metabolites in biological material and its application to optimization of liver cancer therapy, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 615–623.

SAMPLE

Matrix: blood, urine

Sample preparation: Condition a C18 Sep-Pak SPE cartridge with 3 mL MeOH, 3 mL MeOH:water 50:50, and 10 mL 50 mM pH 8.9 Na_2HPO_4. 1 mL Plasma or urine + 200 ng daunorubicin + 2 mL 0.9% NaCl, mix, add to the SPE cartridge, wash with 3 mL 50 mM pH 8.9 Na_2HPO_4, elute with four 500 μL aliquots of chloroform:MeOH 2:1. Evaporate the eluates to dryness under vacuum while centrifuging, dissolve the residue in 200 μL MeCN:7 mM pH 2.6 Na_2HPO_4 40:60, vortex gently, centrifuge at 3000 g for 15 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Column: 100 × 8 μBondapak phenyl Radial-Pak

Mobile phase: MeCN:buffer 30:70 (Buffer was 7 mM Na_2HPO_4 adjusted to pH 2.6 with formic acid.)

Flow rate: 3

Detector: F ex 480 em 550

CHROMATOGRAM

Internal standard: daunorubicin

Limit of quantitation: 0.6 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; pharmacokinetics; SPE

REFERENCE

Tjuljandin,S.A.; Doig,R.G.; Sobol,M.M.; Watson,D.M.; Sheridan,W.P.; Morstyn,G.; Mihaly,G.; Green,M.D. Pharmacokinetics and toxicity of two schedules of high dose epirubicin, *Cancer Res.*, **1990**, *50*, 5095–5101.

SAMPLE

Matrix: formulations

Sample preparation: Emulsion. 500 μL Emulsion + 10 mL 400 μg/mL hydroquinone in MeOH + 40 mL 0.1% 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: 11.2

Internal standard: hydroquinone (4.2)

Limit of detection: 1 μg/mL

OTHER SUBSTANCES

Simultaneous: carboplatin, 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.

Epoetin

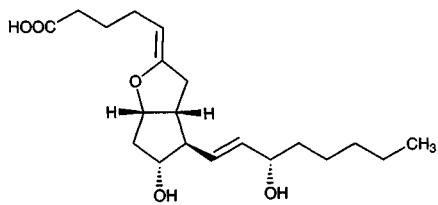
Molecular formula: $C_{809}H_{1301}N_{229}O_{240}S_5$
Molecular weight: 30400 ± 400
CAS Registry No.: 113427-24-0 (alfa), 122312-54-3 (beta)
Merck Index: 3729

SAMPLE
Matrix: dialysate
Sample preparation: Equilibrate 7 mL pre-swollen DEAE-Sephacel gel (Pharmacia) with acetate buffer (40 mM acetic acid + 2.5 mM calcium chloride adjusted to pH 4.5 with 1 M NaOH), pour the gel into a 1 cm diameter column. Add the dialysate (corresponding to 3.7 mg epoetin) to the column at a flow rate of 0.5 mL/min. Elute starting with pH 4.5 acetate buffer and continuing with pH 4.5 acetate buffer containing 15, 30, 60, 150, 350, and 1000 mM NaCl. Collect seven fractions at elution volumes of about 0-35 mL, 35-70 mL, 70-125 mL, 125-160 mL, 160-185 mL, 185-195 mL, and 195-210 mL, adjust the pH of each fraction to 7.5 with 1 M Tris, concentrate by ultra-filtration on PM 10 Amicon membranes, inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 600 × 7.5 TSK G 2000 SW (Pharmacia)
Mobile phase: 20 mM pH 7.4 HEPES-NaOH buffer containing 150 mM NaCl
Flow rate: 0.5
Injection volume: 100
Detector: UV 280

REFERENCE
Gokana,A.; Winchenne,J.J.; Ben-Ghanem,A.; Ahaded,A.; Cartron,J.P.; Lambin,P. Chromatographic separation of recombinant human erythropoietin isoforms, *J.Chromatogr.A*, **1997**, *791*, 109–118.

Epoprostenol



Molecular formula: $C_{20}H_{32}O_5$
Molecular weight: 352.47
CAS Registry No.: 35121-78-9, 61849-14-7 (sodium salt)
Merck Index: 8061
Lednicer No.: 3 10

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in 10 mM NaOH, inject a 20-100 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.1 10 μm PRP-1 styrene-divinylbenzene copolymer (Hamilton)
Mobile phase: MeCN:10 mM NaOH 21:79, measured pH 12.3 (At the end of each day clean the column with 40 mL water and 20 mL MeCN.)
Flow rate: 1
Injection volume: 20-100

Detector: UV 206

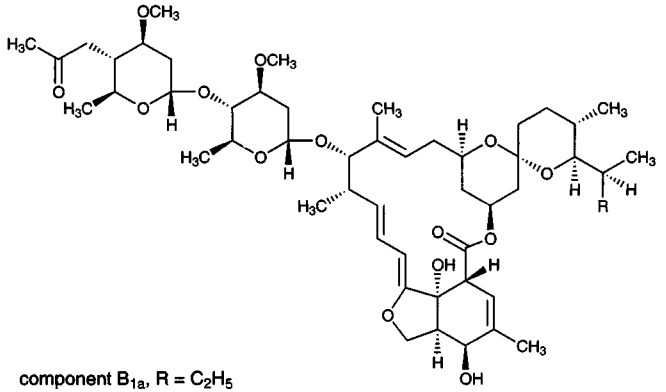
CHROMATOGRAM
Retention time: 4.5
Limit of quantitation: 5 μg/mL

OTHER SUBSTANCES
Simultaneous: 6-ketoprostaglandin F1α, 6-ketoprostaglandin E1, prostaglandin A2, prostaglandin B2, prostaglandin D2, dinoprost, dinoprostone, thromboxane B2

REFERENCE
Skrinska,V.; Thomas,G. High-performance liquid chromatography of prostacyclin, *J.Chromatogr.*, **1983**, *277*, 287–291.

Eprinomectin

Molecular formula: $C_{50}H_{75}NO_{14}$ (component B_{1a}), $C_{49}H_{73}NO_{14}$ (component B_{1b})
Molecular weight: 914.14 (component B_{1a}), 900.12 (component B_{1b})
CAS Registry No.: 123997-26-2, 133305-88-1 (component B_{1a}), 133305-89-2 (component B_{1b})
Merck Index: 3667



component B_{1a}, R = C_2H_5

component B_{1b}, R = CH_3

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL plasma with 2 ng IS, add 3 mL MeCN, vortex for 60 s centrifuge at 3000 rpm for 12 min. Add 1 mL water, vortex, centrifuge. Condition a 3 mL 200 mg C18 SPE cartridge (Waters) with 4 mL MeCN, 4 mL MeCN:chloroform 1:1 containing 0.1% 1-methylimidazole, 4 mL MeCN and 4 mL 100 mM sodium phosphate buffer. Add the supernatant to the SPE cartridge, wash with two 4 mL portions of MeCN:water 1:2, dry under positive nitrogen pressure for 3 min. Elute with 5 mL MeCN:chloroform 50:50 containing 0.1% 1-methylimidazole, evaporate the eluate to dryness under a stream of nitrogen at 45° for 55 min. Reconstitute the dry residue in 100 μL MeCN:1-methylimidazole 70:30 and vortex. Add 150 μL MeCN:trifluoroacetic anhydride 70:30, mix, inject a 100 μL aliquot. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES
Guard column: Zorbax ODS
Column: 250 × 4.6 Zorbax RX-C18
Mobile phase: Gradient. A was MeCN:MeOH:water:triethylamine:orthophosphoric acid 50:44:6:0.2:0.2. B was MeCN:MeOH:water:triethylamine:orthophosphoric acid 50:40:10:0.2:0.2. A:B 0:100 for 12 min, to 100:0 over 2 min, 100:0 for 11 min, re-equilibrate at 0:100 for 3 min.
Column temperature: 30

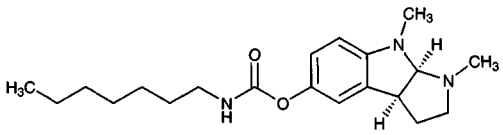
Flow rate: 1
Injection volume: 100
Detector: F ex 365 em 475

CHROMATOGRAM
Retention time: 11.5
Internal standard: L-648 548 (Merck) (24)
Limit of quantitation: 50 pg/mL

KEY WORDS
cow; plasma; derivatization; SPE

REFERENCE
Antonian,L.; DeMontigny,P.; Wislocki,P.G. An automated method for the determination of subnanogram concentrations of eprinomectin in bovine plasma, *J.Pharm.Biomed.Anal.*, **1998**, *16*, 1363–1371.

Eptastigmine



Molecular formula: $C_{21}H_{33}N_3O_2$
Molecular weight: 359.51
CAS Registry No.: 101246-68-8, 121652-76-4 (tartrate)
Merck Index: 3672

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 40 μL 50 ng/mL physostigmine + 1 mL 500 mM sodium bicarbonate + 5 mL n-hexane, 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 at 30°, reconstitute the residue in 100 μL MeCN:MeOH 50:50, inject a 70 μL aliquot.

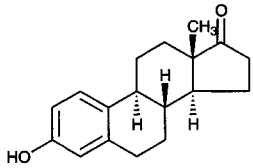
HPLC VARIABLES
Column: 250 × 4.6 5 μm silica (Violet)
Mobile phase: MeCN:MeOH:80 mM ammonium nitrate 50:40:10, pH 8.90
Flow rate: 1
Injection volume: 70
Detector: E, glassy carbon electrode +0.75 V

CHROMATOGRAM
Retention time: 5
Internal standard: physostigmine (6)
Limit of quantitation: 0.2 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Imbimbo,B.P.; Licini,M.; Schettino,M.; Mosca,A.; Onelli,E.; Zecca,L.; Giustina,A. Relationship between pharmacokinetics and pharmacodynamics of eptastigmine in young healthy volunteers, *J.Clin.Pharmacol.*, **1995**, *35*, 285–290.

Equilin



Molecular formula: $C_{18}H_{20}O_2$
Molecular weight: 268.36
CAS Registry No.: 474-86-2
Merck Index: 3676

SAMPLE

Matrix: blood

Sample preparation: Mix 9.7 mL antiserum (*J. Steroid Biochem.* 1980, 13, 1291) with 34 mL 0.4% rivanol solution, vortex on ice for 10 min, centrifuge at 3000 rpm at 4° for 10 min. Add 1.1 g activated charcoal to the supernatant, stir for 15 min on ice, centrifuge at 3000 rpm for 10 min, filter (0.45 μm). Lyophilize, add 3.5 mL pH 7.6 sodium phosphate buffer, determine the content of protein, add 2.2 mL Affi-gel 10 (Bio-Rad, pre-rinsed with 10 mL water and pH 7.6 sodium phosphate buffer). Stir the suspension at 4° overnight, add 220 μL mL 1 M pH 8.0 ethanolamine, stir the adsorbent for 1 h, wash with 200 mL water, 200 mL MeCN:water 90:10, and 100 mL sodium phosphate buffer until the absorbance of the eluate at 280 nm disappears. Pack a 1 mL portion into a 6 mm diameter pipette tip (silanized with trichloromethylsilane), wash with 10 mL water and 10 mL pH 7.3 phosphate buffer. Add 1 mL plasma to the immunoaffinity column, wash with 5 mL water. Elute with 4 mL MeCN:water 90:10, inject a 50 μL aliquot.

HPLC VARIABLES

Column: A 150 × 4.6 5 μm Cosmosil AR (Nacalai Tesque Inc.); B 150 × 1 5 μm Capcell Pak C18

Mobile phase: A MeCN:MeOH:60 mM pH 5.0 acetate buffer 30:9:60; B MeOH:100 mM pH 5.0 ammonium acetate buffer 10:40

Flow rate: 1 (A),0.005 (B)

Injection volume: 50

Detector: E, ESA Coulochem 510 A, + 100 mV for first electrode, + 800 mV for the second electrode (A); MS, Hitachi M-1000H, ESI, drift voltage -50 V, nebulizer 150°, m/z 413 (B)

CHROMATOGRAM

Retention time: 21 (A), 16 (B)

OTHER SUBSTANCES

Extracted: metabolites

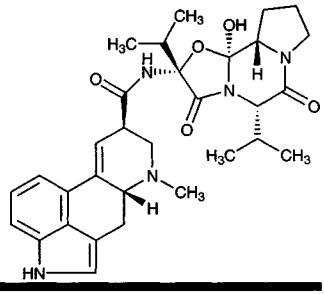
KEY WORDS

plasma; pharmacokinetics

REFERENCE

Ikegawa,S.; Itoh,M.; Murao,N.; Kijima,H.; Suzuki,M.; Fujiyama,T.; Goto,J.; Tohma,M. Immunoaffinity extraction for liquid chromatographic determination of equilin and its metabolites in plasma, *Biomed.Chromatogr.*, **1996**, *10*, 73–77.

Ergocornine



Molecular formula: $C_{31}H_{39}N_5O_5$
Molecular weight: 561.68
CAS Registry No.: 564-36-3
Merck Index: 3685

SAMPLE

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

HPLC VARIABLES

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

CHROMATOGRAM

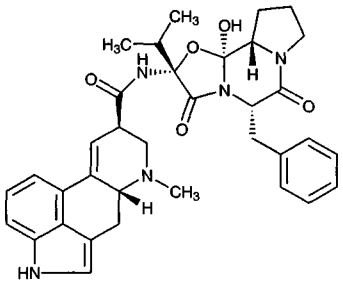
Retention time: 1.2

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, 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, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

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

Ergocristine



Molecular formula: $C_{35}H_{39}N_5O_5$
Molecular weight: 609.73
CAS Registry No.: 511-08-0
Merck Index: 3687

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

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

CHROMATOGRAM
Retention time: 1.1

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pi-

renzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

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

Ergocristinine

Molecular formula: $C_{35}H_{39}N_5O_5$
Molecular weight: 609.73
CAS Registry No.: 511-07-9
Merck Index: 3688

SAMPLE

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

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 1.1

OTHER SUBSTANCES

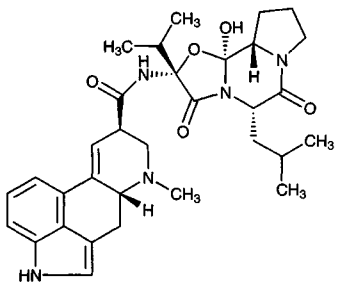
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortrip-

tyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

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

Ergocryptine



Molecular formula: $C_{32}H_{41}N_5O_5$
Molecular weight: 575.71
CAS Registry No.: 511-09-1 (α) 20315-46-2 (β)
Merck Index: 3689

SAMPLE

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

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 1.2

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, 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,

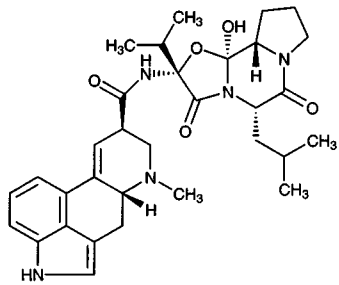
droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

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

Ergoloid mesylates

Molecular formula: $C_{36}H_{45}N_5O_8S$
Molecular weight: 707.85
CAS Registry No.: 8067-24-1
Merck Index: 3692



Dihydroergocomine R = $CH(CH_3)_2$

Dihydroergocristine R = $CH_2C_6H_5$

Dihydro-α-ergocryptine R = $CH_2CH(CH_3)_2$

Dihydro-β-ergocryptine R = $CH(CH_3)CH_2CH_3$

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 50 μL 1 μg/mL dihydroergotamine mesylate in water + 30 μL 5 M NaOH + 7 mL chloroform, shake on a reciprocal shaker for 10 min, centrifuge at 2000 g for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 10-30 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 10 μm RP-8 (Merck)
Mobile phase: MeCN:buffer 60:40 (Buffer was 9 mM NaH_2PO_4 and 9 mM Na_2HPO_4, pH 7.2.)
Flow rate: 1

Injection volume: 10-30
Detector: UV 223 or F ex 295 em 350

CHROMATOGRAM
Retention time: 7.9 (dihydroergocristine)
Internal standard: dihydroergotamine mesylate (5.2)
Limit of detection: 0.5-0.7 ng/mL (F), 5-10 ng/mL (UV)

OTHER SUBSTANCES
Simultaneous: dihydroergocornine, dihydroergocryptine

KEY WORDS
plasma; rat

REFERENCE
Zecca,L.; Bonini,L.; Bareggi,S.R. Determination of dihydroergocristine and dihydroergotamine in plasma by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1983**, *272*, 401–405.

SAMPLE
Matrix: blood, urine, tissue
Sample preparation: Homogenize tissue with 4 volumes of water, centrifuge. 1 mL Plasma, urine, or tissue homogenization supernatant + 50 μL 1 (plasma) or 10 (urine, tissue) μg/mL α-dihydroergocristine + 100 μL 1 M HCl, vortex, add 5 mL hexane, extract, centrifuge. Remove the aqueous phase and add it to 100 μL 2 M NaOH, extract with 7 mL chloroform, centrifuge. Remove 5 mL of the organic layer and evaporate it to dryness, reconstitute the residue in 70 μL mobile phase, inject a 50 μL aliquot.

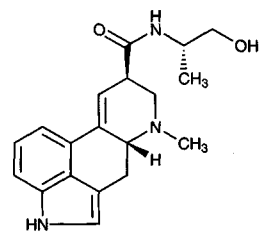
HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 100 RP 18
Mobile phase: MeCN:buffer 43:57 (Buffer was 10 mM pH 7.2 Na_2HPO_4/KH_2PO_4.)
Flow rate: 1
Injection volume: 50
Detector: F ex 295 em 350

CHROMATOGRAM
Retention time: 8.9 (dihydroergocriptine)
Internal standard: α-dihydroergocristine (10.2)
Limit of detection: 2 ng/g (tissue), 10 ng/mL (urine), 0.1 ng/mL (plasma)

KEY WORDS
rat; plasma; kidney; heart; lung; spleen; liver; brain; pharmacokinetics

REFERENCE
Coppi,G.; Silingardi,S. Pharmacokinetics of α-dihydroergocriptine in rats after single intravenous and single and repeated oral administrations, *Biopharm.Drug Dispos.*, **1995**, *16*, 333–342.

Ergonovine



Molecular formula: $C_{19}H_{23}N_3O_2$
Molecular weight: 325.41
CAS Registry No.: 60-79-7, 129-51-1 (maleate)
Merck Index: 3694

SAMPLE
Matrix: blood

Sample preparation: 300 μL Plasma + 300 μL MeCN, centrifuge at 11000 g for 4 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 75 × 2.1 10 μm pellicular reversed phase (Chrompack no. 028653)
Column: 250 × 4.6 5 μm Spherisorb 5-ODS
Mobile phase: MeCN:buffer 35:65 (Buffer was 67 mM KH_2PO_4 containing 0.5 mL/L (?) triethylamine.)
Flow rate: 1.2
Injection volume: 100
Detector: F ex 315 em 430

CHROMATOGRAM

Retention time: 5.26
Limit of quantitation: 75 pg/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

de Groot,A.N.J.A.; Vree,T.B.; Hekster,Y.A.; Baars,A.M.; Van den Biggelaar-Martea,M.; van Dongen,P.W.J. High-performance liquid chromatography of ergometrine and preliminary pharmacokinetics in plasma of men, *J.Chromatogr.*, **1993**, *613*, 158–161.

SAMPLE

Matrix: formulations
Sample preparation: Dilute formulation 1:10. Remove a 1 mL aliquot and add it to 1.5 mL water and 2.5 mL 20 μg/mL ephedrine, inject an 80 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:buffer 35:65 containing 0.05% sodium tetradecyl sulfate (Buffer was 0.83 mM phosphoric acid adjusted to pH 5.0 with triethylamine. Use a 50 × 4.6 5-25 μm LiChroprep Si 60 column before the injector. Wash column with MeCN:83 mM phosphoric acid 40:60 after use.)
Flow rate: 2.5
Injection volume: 80
Detector: UV

CHROMATOGRAM

Retention time: 18
Internal standard: ephedrine (10)

OTHER SUBSTANCES

Simultaneous: oxytocin

KEY WORDS

injections

REFERENCE

Pask-Hughes,R.A.; Corran,P.H.; Calam,D.H. Assay of the combined formulation of ergometrine and oxytocin by high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *214*, 307–315.

SAMPLE

Matrix: formulations
Sample preparation: Injections. 1 mL Injection (200 μg/mL) + 300 mg NaCl + 200 μL 10% ammonia + 5 mL dichloromethane, shake vigorously for 10 min, let stand for a few min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 4 mL water, mix an aliquot with an equal volume of 20 μg/mL 17α-hydroxyprogesterone in MeOH, inject a 20 μL aliquot. Tablets. Weigh out amount of powdered tablets equivalent to about 200 μg compound, add 1 mL water, sonicate for 2 min, add 300 mg

NaCl, add 200 μL 10% ammonia, add 5 mL dichloromethane, shake vigorously for 10 min, let stand for a few min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 4 mL water, mix an aliquot with an equal volume of 20 μg/mL 17α-hydroxyprogesterone in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 5 μm LiChrosorb RP-18
Mobile phase: MeCN:50 mM pH 3.5 acetate buffer 40:60 containing 1.5 mM triethylamine
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7.5
Internal standard: 17α-hydroxyprogesterone (12)

OTHER SUBSTANCES
Simultaneous: benzyl alcohol, methylergonovine
Noninterfering: ascorbic acid

KEY WORDS
injections; tablets

REFERENCE
Tokunaga,H.; Kimura,T.; Kawamura,J. Determination of ergometrine maleate and methylergometrine maleate in pharmaceutical preparations by high-performance liquid chromatography, *Chem.Pharm.Bull.(Tokyo)*, **1983**, *31*, 3988–3993.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablet, add 100 μL 90% formic acid for each 100 μg ergotamine tartrate, swirl to thoroughly wet sample, make up to 100 mL with MeOH, mix, filter (paper), dilute filtrate with MeOH (if necessary) so that the ergotamine tartrate concentration is 3 mg/L, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 C18 (Alltech)
Mobile phase: MeCN:water:triethylamine 70:30:0.05
Flow rate: 1
Injection volume: 20
Detector: F ex 250 em 430

CHROMATOGRAM
Retention time: 2.5

OTHER SUBSTANCES
Simultaneous: ergotamine, ergotaminine
Noninterfering: caffeine

KEY WORDS
tablets

REFERENCE
Cieri,U.R. Determination of ergotamine tartrate in tablets by liquid chromatography with fluorescence detection, *J.Assoc.Off.Anal.Chem.*, **1987**, *70*, 538–540.

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

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

CHROMATOGRAM
Retention time: 1.2

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, 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, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

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

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

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 20:1.5:0.5:78
Flow rate: 1.5

Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 2.69

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 mobile phase, inject a 25-150 μL aliquot.

HPLC VARIABLES
Column: 250 mm long 5 μm Hypersil C18 ODS
Mobile phase: MeCN:10 mM ammonium carbonate 30:70
Flow rate: 2
Injection volume: 25-150
Detector: F ex 328 em 415

CHROMATOGRAM
Retention time: 2.4

OTHER SUBSTANCES
Simultaneous: methylergonovine (methylergometrine), methysergide

REFERENCE
Bredberg,U.; Paalzow,L. Pharmacokinetics of methysergide and its metabolite methylergometrine in the rat, *Drug Metab.Dispos.*, **1990**, *18*, 338–343.

SAMPLE
Matrix: wheat
Sample preparation: Grind wheat to pass 2 mm screen. 25 g Ground wheat + 10 mL 4% ammonia in water + 100 mL ethyl acetate, shake vigorously on a wrist-action shaker for 15 min, filter (paper). Extract 50 mL filtrate twice with 25 mL 1% sulfuric acid. Combine aqueous extracts, add 50 mL 4% ammonia in water, extract twice with 25 mL dichloromethane shaking gently for 30 s each time. Dry extracts over 10-20 g anhydrous sodium sulfate for 10 min in the dark, filter, wash solid with 25 mL dichloromethane. Evaporate filtrate to near dryness at 40° under reduced pressure, rinse into a tube with two 2 mL portions of dichloromethane, evaporate to dryness under a stream of nitrogen, reconstitute in 1 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 10 μm PRP-1 polystyrene-divinylbenzene (Hamilton)
Mobile phase: MeCN:water 45:55 containing 6.6 g/L $(NH_4)_2HPO_4$
Flow rate: 0.3
Injection volume: 20
Detector: F ex not specified em 418 (cut-off filter)

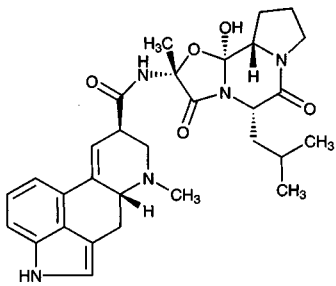
CHROMATOGRAM
Retention time: 7
Limit of detection: < 18 ng/g

OTHER SUBSTANCES
Extracted: ergonovinine, ergotamine, ergocryptine, ergocristine, ergotaminine, ergocryptinine, ergocristinine

REFERENCE
Ware,G.M.; Carman,A.S.; Francis,O.J.; Kuan,S.S. Liquid chromatographic determination of ergot alkaloids in wheat, *J.Assoc.Off.Anal.Chem.*, **1986**, *69*, 697–699.

Ergosine

Molecular formula: $C_{30}H_{37}N_5O_5$
Molecular weight: 547.65
CAS Registry No.: 561-94-4
Merck Index: 3695



SAMPLE

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

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 1.1

OTHER SUBSTANCES

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

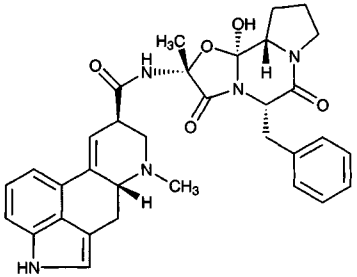
zodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

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

Ergotamine

Molecular formula: $C_{33}H_{35}N_5O_5$
Molecular weight: 581.67
CAS Registry No.: 113-15-5, 379-79-3 (tartrate)
Merck Index: 3703



SAMPLE

Matrix: formulations
Sample preparation: Grind tablet, add 100 μL 90% formic acid for each 100 μg ergotamine tartrate, swirl to thoroughly wet sample, make up to 100 mL with MeOH, mix, filter (paper), dilute filtrate with MeOH (if necessary) so that the ergotamine tartrate concentration is 3 mg/L, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 C18 (Alltech)
Mobile phase: MeCN:water:triethylamine 70:30:0.05
Flow rate: 1
Injection volume: 20
Detector: F ex 250 em 430

CHROMATOGRAM

Retention time: 4

OTHER SUBSTANCES

Simultaneous: ergonovine, ergotaminine
Noninterfering: caffeine

KEY WORDS

tablets

REFERENCE

Cieri,U.R. Determination of ergotamine tartrate in tablets by liquid chromatography with fluorescence detection, *J.Assoc.Off.Anal.Chem.*, **1987**, *70*, 538–540.

SAMPLE

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

HPLC VARIABLES

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

CHROMATOGRAM
Retention time: 1.3

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

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

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

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 60:1.5:0.5:38
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 1.55

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 10 μm Nucleosil C18
Mobile phase: MeCN:10 mM ammonium carbonate 50:50
Flow rate: 1
Detector: UV 240

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: impurities, ergocristine, ergosine, 8-hydroxyergotamine

REFERENCE
Gazdag,M.; Szepesi,G. Selection of high-performance liquid chromatographic methods in pharmaceutical analysis. IV. Selection of most applicable separation system, *J.Chromatogr.*, **1989**, *464*, 279–288.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: MeCN:0.1% pH 3.25 acetic acid containing 1.25 mM heptanesulfonic acid 65:35
Flow rate: 1.3
Injection volume: 20
Detector: UV 254

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
Sample preparation: Pass 20 mL of a solution in water (?) through an Empore C18 SPE disc. Wash with 2.5 mL water, add 1 mL MeCN:25 mM pH 3.0 phosphate buffer 65:35, let soak for 3 min, elute, inject an aliquot of the eluate.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb S5 ODS2
Mobile phase: MeCN:10 mM KH_2PO_4 30:70
Flow rate: 1.3
Detector: UV 254

OTHER SUBSTANCES
Noninterfering: excipients

KEY WORDS
comparison with capillary electrophoresis; SPE

REFERENCE
Lucangioli,S.E.; Rodriguez,V.G.; Fernandez Otero,G.C.; Vizioli,N.M.; Carducci,C.N. Development and validation of capillary electrophoresis methods for pharmaceutical dissolution assays, *J.Capillary Electrophor.*, **1997**, *4*, 27–31.

SAMPLE
Matrix: wheat
Sample preparation: Grind wheat to pass 2 mm screen. 25 g Ground wheat + 10 mL 4% ammonia in water + 100 mL ethyl acetate, shake vigorously on a wrist-action shaker for 15 min, filter (paper). Extract 50 mL filtrate twice with 25 mL 1% sulfuric acid. Combine aqueous extracts, add 50 mL 4% ammonia in water, extract twice with 25 mL dichloromethane shaking gently for 30 s each time. Dry extracts over 10-20 g anhydrous sodium sulfate for 10 min in the dark, filter, wash solid with 25 mL dichloromethane. Evaporate filtrate to near dryness at 40° under reduced pressure, rinse into a tube with two 2 mL portions of dichloromethane, evaporate to dryness under a stream of nitrogen, reconstitute in 1 mL mobile phase, inject a 20 μL aliquot.

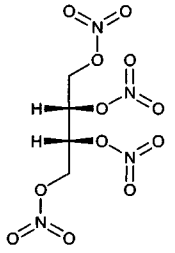
HPLC VARIABLES
Column: 150 × 4.6 10 μm PRP-1 polystyrene-divinylbenzene (Hamilton)
Mobile phase: MeCN:water 45:55 containing 6.6 g/L $(NH_4)_2HPO_4$
Flow rate: 0.3
Injection volume: 20
Detector: F ex not specified em 418 (cut-off filter)

CHROMATOGRAM
Retention time: 15
Limit of detection: < 95 ng/g

OTHER SUBSTANCES
Extracted: ergonovinine, ergonovine, ergocryptine, ergocristine, ergotaminine, ergocryptinine, ergocristinine

REFERENCE
Ware,G.M.; Carman,A.S.; Francis,O.J.; Kuan,S.S. Liquid chromatographic determination of ergot alkaloids in wheat, *J.Assoc.Off.Anal.Chem.*, **1986**, *69*, 697–699.

Erythrityl tetranitrate



Molecular formula: $C_4H_6N_4O_{12}$
Molecular weight: 302.11
CAS Registry No.: 7297-25-8
Merck Index: 3716

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets, weigh out a portion equivalent to 3 mg erythrityl tetranitrate, add to 10 mL 75 μg/mL nitroglycerin in MeOH, sonicate for 2 min, shake mechanically for 30 min, filter, inject an aliquot

HPLC VARIABLES
Guard column: 40 × 4.6 μBondapak C18/Corasil
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water 40:60
Flow rate: 1
Injection volume: 20
Detector: UV 220

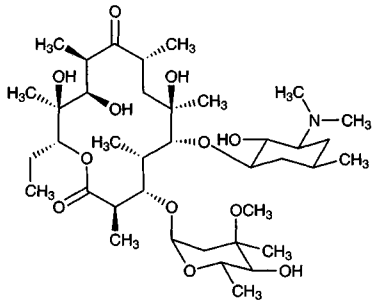
CHROMATOGRAM
Retention time: 31
Internal standard: nitroglycerin (14)

OTHER SUBSTANCES
Simultaneous: pentaerythritol tetranitrate, isosorbide dinitrate

KEY WORDS
tablets

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.

Erythromycin



Molecular formula: $C_{37}H_{67}NO_{13}$
Molecular weight: 733.94
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)
Merck Index: 3720

SAMPLE
Matrix: blood, tissue
Sample preparation: Homogenize (Physcotron) liver with 4 volumes of ice-cold saline. Evaporate 100 μL 3 μg/mL oleandomycin in MeOH to dryness under dry nitrogen. Put 200 μL plasma or liver homogenate into the tube. Add 2 mL MTBE and 5 μL 1 M NaOH and shake mechanically for 5 min. Centrifuge at 1500 g for 10 min, transfer the upper layer transfer into a glass tube and evaporate it to dryness under dry nitrogen. Rinse the inner wall of the tube with 200 μL MeOH and evaporate to dryness. Dissolve the residue in 30 μL MeOH and inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Cosmosil 5-C18 (Nacalai Tesque)
Mobile phase: MeCN:100 mM pH 6.6 sodium acetate buffer 50:50
Flow rate: 0.6
Injection volume: 10
Detector: E, 1.1 V, Ag/AgCl reference electrode IS oleandomycin (8.5)

CHROMATOGRAM
Retention time: 12.8
Limit of detection: 100 μg/mL (plasma); 0.5 μg/g (liver)

KEY WORDS
rat; plasma; liver; pharmacokinetics

REFERENCE
Hanada,E.; Ohtani,H.; Kotaki,H.; Sawada,Y.; Iga,T. Determination of erythromycin concentrations in rat plasma and liver by high-performance liquid chromatography with amperometric detection, *J.Chromatogr.B*, **1997**, *692*, 478–482.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Erythromycin estolate bulk. Dissolve the bulk powder in mobile phase B for a final concentration of 5-6 mg/mL and sonicate for 30 s. Inject an aliquot. Erythromycin estolate capsules. Powder the contents of capsules. Prepare a 5 mg/mL suspension in mobile

phase B, sonicate for 2 min, filter (0.45 μm), discard the first 5 mL of the filtrate. Inject an aliquot. Erythromycin ethylsuccinate powder. Prepare as described for estolate. Erythromycin ethylsuccinate tablets. Powder the tablets, prepare a 15 mg/mL suspension in mobile phase B, sonicate for 2 min, filter (0.45 μm). Discard the first few mL of filtrate. Inject an aliquot. Erythromycin ethylsuccinate powder for oral suspension. Prepare a 500 mg/mL suspension in 100 mL mobile phase B, sonicate for 15 min, make up the supernatant to 100 mL with mobile phase B, filter (0.45 μm). Inject an aliquot. Erythromycin stearate powder. Prepare a 5-6 mg/mL solution in MeOH, inject an aliquot. Erythromycin stearate tablets. Powder the tablets and prepare a 30 mg/mL suspension in MeOH, sonicate for 5 min, filter (0.45 μm), discard the first few mL of the filtrate, inject an aliquot.

HPLC VARIABLES

Guard column: 5 μm Inertsil ODS-2
Column: 150 × 4.6 5 μm Inertsil ODS-2 (A) or 250 × 4.6 5 μm Inertsil ODS-2 (B)
Mobile phase: Gradient. A was MeCN:buffer 10:90. B was MeCN:buffer 75:25. A:B from 90:10 to 0:100 over 10 min, maintain at 0:100. (Prepare mobile phase A as follows. Mix 60 mL 200 mM pH 6.5 ammonium phosphate buffer with 60 mL 200 mM pH 6.5 tetrabutylammonium sulfate buffer and 200 mL water. Add 100 mL MeCN and make up to 1 L with water. Prepare mobile phase B as described for A except use 750 mL MeCN.)
Column temperature: 50
Flow rate: 1.3
Injection volume: 50
Detector: UV 205

CHROMATOGRAM

Retention time: 9.5 (estolate, column A), 14.5 (ethylsuccinate, column A), ca. 26 (stearate, column B), ca. 26 (gluceptate, column B), ca. 26 (lactobionate, column B)

OTHER SUBSTANCES

Simultaneous: degradation products

KEY WORDS

powder; capsules; tablets

REFERENCE

Nasr,M.M.; Stanley,C.M High performance liquid chromatographic assay of erythromycin salts and esters in bulk and pharmaceutical dosage forms, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1147–1160.

SAMPLE

Matrix: tissue
Sample preparation: Condition a 1 mL 100 mg Bond-Elut diol SPE cartridge with 1 mL chloroform (Caution! Chloroform is a carcinogen!). Mix 2 g minced muscle tissue with 800 μL water. Stir, vortex for 1 min at maximum speed, let stand for 15 min. Add 2 mL pH 8 buffer, mix briefly, add 10 mL chloroform. Stir at 100 rpm for 15 min, centrifuge at 4000 g for 10 min, discard the aqueous layer, filter the chloroform layer through glass wool. Add the filtrate to the SPE cartridge, wash with 500 μL chloroform, dry under vacuum, elute with three 200 μL portions of MeOH:100 mM ammonium acetate 50:50, inject a 200 μL aliquot of the eluate. (Buffer was 33.46 g K_2HPO_4 and 1.046 g KH_2PO_4 in 1 L water.)

HPLC VARIABLES

Guard column: 4 × 4 5 μm C18
Column: 125 × 4 5 μm Lichrospher RP18
Mobile phase: Gradient. A was MeCN. B was MeOH. C was 0.1% trifluoroacetic acid in water. A:B:C from 20:20:60 to 25:55:20 in 10 (?) min
Flow rate: 0.5
Injection volume: 200
Detector: MS, HP Model 5989 A, desolvation chamber 60°, source 280° and 300° in negative and positive chemical ionization mode, respectively, with methane as reagent, quadrupole 100°, particle beam nebulizer helium 345 kPa, scan m/z 442.3-619.4 in NCI and 734.4-576.2 in PCI

CHROMATOGRAM

Retention time: 6.4

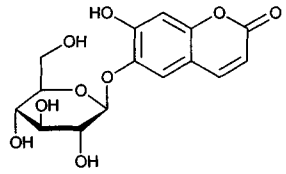
Limit of detection: 50 ng/g

OTHER SUBSTANCES
Extracted: josamycin, spiramycin, tilmicosin, tylosin

KEY WORDS
muscle; cow; SPE

REFERENCE
Delépine,B.; Hurtaud-Pessel,D.; Sanders,P. Multiresidue method for confirmation of macrolide antibiotics in bovine muscle by liquid chromatography/mass spectrometry, *J.AOAC Int.*, **1996**, *79*, 397–404.

Esculin



Molecular formula: $C_{15}H_{16}O_9$
Molecular weight: 340.29
CAS Registry No.: 531-75-9
Merck Index: 3739

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

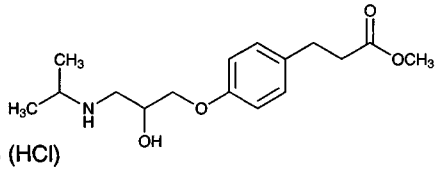
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.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

CHROMATOGRAM
Retention time: 5.277

KEY WORDS
whole blood

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

Esmolol



Molecular formula: $C_{16}H_{25}NO_4$
Molecular weight: 295.38
CAS Registry No.: 81147-92-4, 103598-03-4, 81161-17-3 (HCl)
Merck Index: 3741
Lednicer No.: 4 27

SAMPLE

Matrix: blood
Sample preparation: 1 mL Blood + 6 mL dichloromethane + 100 μL 200 mM NaOH + 5 μL 50 μg/mL IS in water, vortex for 10 s, shake on a mechanical shaker for 10 min, centrifuge at 1000 g for 10 min. Remove the organic layer and add it to 100 μL 2.5 mM sulfuric acid, vortex for 1 min, centrifuge for 5 min, inject a 50 μL portion of the aqueous layer.

HPLC VARIABLES

Guard column: 30 × 3.2 37-53 μm Whatman C18 guard column
Column: 100 × 5 10 μm Radial-Pak CN (Waters)
Mobile phase: MeOH:60 mM KH_2PO_4:triethylamine 25:75:0.1 adjusted to pH 3.15 with 85% phosphoric acid
Flow rate: 1.8
Injection volume: 50
Detector: UV 221

CHROMATOGRAM

Retention time: 2.6
Internal standard: 3-methyloxy-O-demethylencainide (3.9)
Limit of detection: 5 ng/mL
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: amiodarone, atropine, caffeine, cimetidine, digoxin, diazepam, disopyramide (norpace), encainide, flecainide, imipramine, lidocvaine, nimodipine, prazepam, procainamide, propafenone, propranolol, quinidine, theophylline
Interfering: captopril

KEY WORDS

pig; whole blood

REFERENCE

Fan,C.-D.; Zhao,H.; Chow,M.S.S. Simple and rapid high-performance liquid chromatographic assay for esmolol, *J.Chromatogr.*, **1991**, *570*, 217–223.

SAMPLE

Matrix: blood
Sample preparation: 1 mL plasma + 5 mL dichloromethane, shake for 10 min, centrifuge at 4° at 1900 g for 10 min. Remove 4 mL of the organic phase and add it to 600 μL 100 mM pH 2.8 NaH_2PO_4, shake, centrifuge, inject a 100 μL aliquot. (To determine metabolites mix 500 μL aqueous phase (left after initial extraction) with 500 μL 7% perchloric acid, centrifuge for 5 min, inject a 100 μL aliquot of the supernatant.)

HPLC VARIABLES

Guard column: 5 μm Lichrocart 4-4 RP18 (Merck)
Column: 150 × 3.9 5 μm Nova-Pak C18
Mobile phase: MeCN:10 mM pH 2.4 NaH_2PO_4 40:60 containing 0.2 mM sodium dodecylsulfate (For metabolites use MeCN:10 mM pH 2.4 NaH_2PO_4 17.5:82.5 containing 0.2 mM sodium dodecylsulfate.)
Flow rate: 1
Injection volume: 100

Detector: UV 229

CHROMATOGRAM
Retention time: 5.4
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE
Jahn,P.; Eckrich,B.; Schneidrowski,B.; Volz-Zang,C.; Schulte,B.; Mutschler,E.; Palm,D. β_1-Adrenoceptor subtype selective antagonism of esmolol and its major metabolite in vitro and in man. Investigations using tricresylphosphate as red blood cell carboxylesterase inhibitor, *Arzneimittelforschung*, **1995**, *45*, 536–541.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with water to an expected esmolol hydrochloride concentration of 10 μg/mL. Remove a 100 μL aliquot and add it to 100 μL 15 μg/mL IS, inject a 15 μL aliquot.

HPLC VARIABLES
Guard column: μBondapak C18 Guard-Pak
Column: 100 × 8 μBondapak C18 Radial-Pak
Mobile phase: MeOH:10 mM pH 2.69 KH_2PO_4 40:60
Flow rate: 2.8
Injection volume: 15
Detector: UV 229

CHROMATOGRAM
Retention time: 4.76
Internal standard: methyl-4-[4-[2-hydroxy-3-[(2-methylethyl)amino]propoxy]phenyl]butyrate hydrochloride (8.36)

KEY WORDS
stability-indicating; 5% dextrose; injections

REFERENCE
Wiest,D.B.; Garner,S.S.; Childress,L.M. Stability of esmolol hydrochloride in 5% dextrose injection, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 716–718.

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' 4.42

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

REFERENCE
Hamoir,T.; Verlinden,Y.; Massart,D.L. Reversed-phase liquid chromatography of β-adrenergic blocking drugs in the presence of a tailing suppressor, *J.Chromatogr.Sci.*, **1994**, *32*, 14[94]20.

SAMPLE
Matrix: solutions

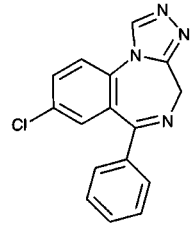
HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 4.43

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, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

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

Estazolam



Molecular formula: $C_{16}H_{11}ClN_4$
Molecular weight: 294.74
CAS Registry No.: 29975-16-4
Merck Index: 3744

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 20 μL 20 μg/mL IS + 200 μL 1 M potassium carbonate + 3 mL chloroform, mix for 2 min, centrifuge at 1200 g for 5 min, aspirate aqueous phase. Evaporate the organic phase under a stream of nitrogen at 40°. Dissolve the residue in 100 μL mobile phase, inject a 20 μL aliquot. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES
Column: 100 × 4.6 2 μm TSK gel Super-ODS (A) or 100 × 4.6 5 μm Hypersil ODS-C18 (B)
Mobile phase: MeCN:5 mM pH 6 NaH_2PO_4 45:55
Flow rate: 0.65
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 10.7 (A), 41.3 (B)
Internal standard: diazepam (29.8 (A), 77.5 (B))
Limit of quantitation: 5 ng/mL (A)

OTHER SUBSTANCES

Extracted: bromazepam, chlordiazepoxide, clonazepam, etizolam, flutazolam, haloxazolam, lorazepam, nitrazepam, oxazolam, triazolam
Simultaneous: alprazolam
Noninterfering: barbital, carbamazepine, cloxazolam, ethosuximide, hexobarbital, mexazolam, oxazepam, pentobarbital, phenobarbital, phenytoin, primidone, trimethadione

KEY WORDS

serum

REFERENCE

Tanaka,E.; Terada,M.; Misawa,.; Wakasugi,C. Simultaneous determination of twelve benzodiazepines in human serum using a new reversed-phase chromatographic column on a 2-μm porous microspherical silica gel, *J.Chromatogr.B*, **1996**, *682*, 173–178.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 2 mL water + 2 mL 100 mM NaOH, mix gently, add 8 mL diethyl ether, shake for 15 min, centrifuge at 2500 rpm for 5 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, vortex for 30 s, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 50 × 4.6 Shim-pack FLC-C8 (Shimadzu)
Mobile phase: MeOH:buffer 53:47 (Buffer was 5 mM Na_2HPO_4 adjusted to pH 6.0 with phosphoric acid.)
Flow rate: 0.6
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 4
Internal standard: estazolam

OTHER SUBSTANCES

Extracted: diazepam, nordiazepam, clorazepate, temazepam, oxazepam
Simultaneous: sulpride, bromazepam, nitrazepam, flunitrazepam
Noninterfering: haloperidol, trihexyphenidyl
Interfering: triazolam

KEY WORDS

serum; estazolam is IS

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: 500 μL Plasma + 20 μL 2.5 μg/mL norprazepam in MeOH + 50 μL buffer + 6 mL diethyl ether:dichloromethane 2:1, agitate, centrifuge. Remove the organic phase and evaporate to dryness under vacuum at 45°, dissolve the residue in 50 μL MeOH, inject a 20 μL aliquot. (Prepare buffer as follows. Solution A was 6.18 g boric acid + 7.46 g KCl in 100 mL water. Solution B was 10.6 g sodium carbonate in 100 mL water. Mix 63 mL solution A and 37 mL solution B and adjust pH to 9.5.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Nova Pak C18
Mobile phase: MeCN:MeOH:buffer 23:13:64 (Buffer was 94 mL 200 mM NaH_2PO_4 + 6 mL 200 mM Na_2HPO_4, adjusted to pH 5.0 with 100 mM HCl.)

Flow rate: 1.3
Injection volume: 20
Detector: UV 242

CHROMATOGRAM
Retention time: 10.2
Internal standard: norprazepam (18.6)
Limit of quantitation: 30 ng/mL

OTHER SUBSTANCES
Simultaneous: alprazolam, bromazepam, chlordiazepoxide, clobazam, diazepam, flumazenil, flunitrazepam, loflazepate, nitrazepam, norflunitrazepam, tofizopam, triazolam
Noninterfering: acepromazine, aceprometazine, amylobarbital, aprobarbital, barbital, brallobarbital, butalbital, caffeine, carbamazepine, chlorpromazine, cyclobarbital, ethosuximide, heptabarbital, hexobarbital, loprazolam, medazepam, midazolam, pentobarbital, phenobarbital, phenytoin, prazepam, secobarbital, theophylline, thiopental, vinylbarbital
Interfering: oxazepam, clonazepam, lorazepam

KEY WORDS
plasma

REFERENCE
Boukhabza,A.; Lugnier,A.A.; Kintz,P.; Mangin,P. Simultaneous HPLC analysis of the hypnotic benzodiazepines nitrazepam, estazolam, flunitrazepam, and triazolam in plasma, *J.Anal.Toxicol.*, **1991**, *15*, 319–322.

SAMPLE
Matrix: blood
Sample preparation: Inject 100-200 μL plasma onto column A with mobile phase A and elute to waste, after 5 min backflush the contents of column A onto column B with mobile phase B, after 5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Wash column A with MeCN:water 60:40 at 1 mL/min for 6 min then re-equilibrate with pH 7.5 buffer for 10 min.

HPLC VARIABLES
Column: A 45 × 4 12 μm TSK-gel G 3 PW (Tosohass); B 75 × 4.6 Ultrasphere ODS C18 3 μm
Mobile phase: A 50 mM pH 7.5 phosphate buffer; B Gradient. A was MeCN. B was 65 mM KH_2PO_4 + 1% diethylamine adjusted to pH 5.4 with phosphoric acid. A:B 22:78 for 5 min, to 25:75 over 10 min, to 60:40 over 15 min.
Flow rate: 1
Injection volume: 100-200
Detector: UV 230

CHROMATOGRAM
Retention time: 21.5

OTHER SUBSTANCES
Extracted: alprazolam, bromazepam, chlordiazepoxide, clobazam, clonazepam, clorazepate, clotiazepam, desmethylclobazam, desmethyldiazepam, diazepam, flunitrazepam, loflazepate, lorazepam, medazepam, nitrazepam, prazepam, temazepam, tetrazepam, tofisopam, triazolam
Noninterfering: carbamazepine, phenytoin, ethosuximide, phenobarbital, primidone, valproic acid
Interfering: oxazepam

KEY WORDS
plasma; column-switching

REFERENCE
Lacroix,C.; Wojciechowski,F.; Danger,P. Monitoring of benzodiazepines (clobazam, diazepam and their main active metabolites) in human plasma by column-switching high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *617*, 285–290.

SAMPLE

Matrix: blood

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

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

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

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 223

CHROMATOGRAM

Retention time: 3.94

Limit of detection: <120 ng/mL

KEY WORDS

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

REFERENCE

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

SAMPLE

Matrix: blood, urine

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

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

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

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

Column temperature: 30

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

Injection volume: 10-30

Detector: UV 221.6

CHROMATOGRAM

Retention time: 16.495

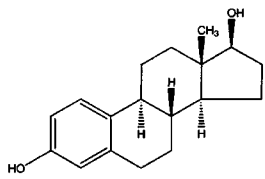
KEY WORDS

whole blood

REFERENCE

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

Estradiol



Molecular formula: $C_{18}H_{24}O_2$

Molecular weight: 272.39

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)

Merck Index: 3746

Lednicer No.: 1 162; 2 136

SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 1 mL 1 mM tetrapentylammonium bromide in 1 M NaOH, mix, add 5 mL 1 mM 1-pyrenesulfonyl chloride (Molecular Probes, Eugene OR) in dichloromethane, vortex for 10 min, centrifuge at 1800 rpm for 10 min. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultramex C8

Mobile phase: MeCN:water 75:25

Flow rate: 1.5

Injection volume: 20

Detector: UV 348, F ex 350 em 385, F ex 325 (Ar laser)

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: equilin, estrone

KEY WORDS
derivatization; serum

REFERENCE
DeSilva,K.H.; Vest,F.B.; Karnes,H.T. Pyrene sulphonyl chloride as a reagent for quantitation of oestrogens in human serum using HPLC with conventional and laser-induced fluorescence detection, *Biomed.Chromatogr.*, **1996**, *10*, 318–324.

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

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

CHROMATOGRAM
Retention time: 18.202

KEY WORDS
whole blood

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

SAMPLE
Matrix: solutions
Sample preparation: Add 25 mL receptor fluid to a 100 mg LiChrolut RP 18 SPE cartridge using vacuum. Elute with 4 mL MeCN, evaporate the eluate, reconstitute the residue in 1 mL MeCN. Inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 µm Lichrospher 100 RP C18
Mobile phase: MeCN:water 60:40
Column temperature: 30
Flow rate: 1.0
Detector: F ex 225 no emission filter

KEY WORDS
SPE

REFERENCE
Rohr,U.D.; Altenburger,R.; Kissel,T. Pharmacokinetics of the transdermal reservoir membrane system delivering β-estradiol: In vitro/in vivo-correlation, *Pharm.Res.*, **1998**, *15*, 877–882.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 200 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:water 60:40
Column temperature: 37
Flow rate: 1.5
Detector: UV 226

CHROMATOGRAM
Retention time: 2.34

OTHER SUBSTANCES
Simultaneous: progesterone

REFERENCE
Kim,D.-D.; Kim,J.L.; Chien,Y.W. Mutual hairless rat skin permeation-enhancing effect of ethanol/water system and oleic acid, *J.Pharm.Sci.*, **1996**, *85*, 1191–1195.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 μBondapack C18
Mobile phase: MeCN:50 mM pH 6 potassium phosphate buffer 47:53
Flow rate: 1.5
Detector: UV 208

CHROMATOGRAM
Retention time: 13-14
Internal standard: estradiol-3-acetate

OTHER SUBSTANCES
Simultaneous: mestranol, 17α-ethinyl estradiol
Noninterfering: ketoconazole, fluconazole, itraconazole, miconazole, α-naphthoflavone, quinidine, sulfaphenazole, troleandomycin

KEY WORDS
estradiol-3-acetate is IS

REFERENCE
Schmider,J.; Greenblatt,D.J.; von Moltke,L.L.; Karsov,D.; Vena,R.; Friedman,H.L.; Shader,R.I. Biotransformation of mestranol to ethinyl estradiol in vitro: The role of cytochrome P-450 2C9 and metabolic inhibitors, *J.Clin.Pharmacol.*, **1997**, *37*, 193–200.

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 17 β-estradiol-HP β CD sublingual tablets, *Pharmazie*, **1996**, *51*, 39–42.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize 2.5 g tissue with 10 mL acetone for 20 s, sonicate for 5 min, centrifuge at 3200 rpm. Decant the supernatant into a silanized tube. Add 8 mL acetone to the pellet and repeat the extraction. Combine the supernatants. Add to a 5 mL pipette tip containing 1.5 g alumina (80-200 mesh, Brockman activity 1) followed by an Econo-Column filled with 1.0 g AGMP-1 resin (Bio-Rad), allow to pass through by gravity. Wash with four 1 mL portions of acetone:water 95:5. Remove the alumina column, wash the ion-exchange column with 1 mL acetone:water 95:5, elute with four 1 mL portions of 10% acetic acid in acetone. Evaporate the combined eluates to dryness with nitrogen at 40°. Add 500 μL water to the residue, extract twice with 2 mL potions of ether. Combine the ether layers and evaporate them to dryness. Reconstitute the residue in mobile phase B. Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelco silica
Mobile phase: Gradient. A was hexane. B was MeOH:hexane:2-propanol 45:40:15. A:B from 100:0 to 60:40 over 15 min.
Flow rate: 2.0
Injection volume: 20
Detector: UV 280

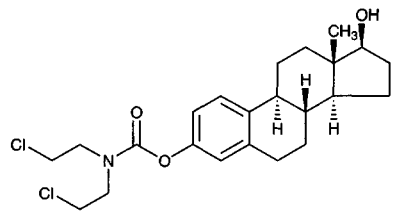
CHROMATOGRAM
Retention time: 10.25
Limit of detection: 10 ng

OTHER SUBSTANCES
Extracted: diethylstilbestrol, zeranol
Simultaneous: estrone, zeralenol, zeralenone, zeralanone

KEY WORDS
chicken; muscle; normal phase; SPE

REFERENCE
Medina,M.B.; Sherman,J.T. High performance liquid chromatographic separation of anabolic oestrogens and ultraviolet detection of 17β-oestradiol, zeranol, diethylstilboestrol or zearalenone in avian muscle tissue extracts, *Food Addit.Contam.*, **1986**, *3*, 263–272.

Estramustine



Molecular formula: $C_{23}H_{31}Cl_2NO_3$
Molecular weight: 440.41
CAS Registry No.: 2998-57-4, 52205-73-9 (phosphate sodium)
Merck Index: 3749
Lednicer No.: 3 83

SAMPLE
Matrix: blood

Sample preparation: 1 mL Plasma + 100 μL EtOH + 2 mL buffer, mix well, add 12 mL hexane, shake slowly for 15 min on a reciprocating shaker, centrifuge at 1200 g at 5° for 10 min. Remove 10 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL hexane:EtOH 92.5:7.5, inject a 20 μL aliquot. (Buffer was 630 mL of a solution containing 61.8 g/L boric acid and 74.6 g/L KCl and 370 mL 106 g/L sodium carbonate solution, shake well, adjust pH to 9.0 with sodium carbonate solution (if necessary), store at 35-37°.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Partisil PXS 5/25 silica gel
Mobile phase: Hexane:EtOH 92.5:7.5
Flow rate: 1.5
Injection volume: 20
Detector: F ex 195 em 250 (cut-off filter)

CHROMATOGRAM
Retention time: 7.2
Limit of detection: 40 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: estrone, estradiol

KEY WORDS
plasma; normal phase; normal phase more sensitive than reverse phase; pharmacokinetics

REFERENCE
Brooks,M.A.; Dixon,R. Determination of estramustine and its 17-keto metabolite in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *182*, 387–394.

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)

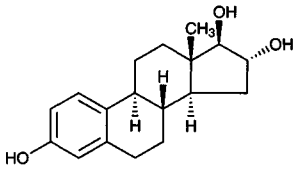
CHROMATOGRAM
Retention time: 7.2
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: estromustine, estrone, estradiol, metabolites

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.

Estriol



Molecular formula: $C_{18}H_{24}O_3$
Molecular weight: 288.39
CAS Registry No.: 50-27-1, 113-22-4 (16,17-bis(sodium hemisuccinate))
Merck Index: 3750

SAMPLE

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

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 13.142

KEY WORDS

whole blood

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

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

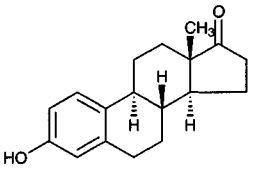
OTHER SUBSTANCES

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

REFERENCE

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

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: 266.34 (equilenin), 272.39 (estradiol), 268.36 (equilin), 270.37 (17α-dihydroequilin), 270.39 (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)

Merck Index: 3216 (17α-dihydroequilin), 3675 (equilenin), 3676 (equilin), 3746 (estradiol), 3751 (estrone)

Lednicer No.: 1 156 (estrone); 1 162 (estradiol); 2 136 (estradiol)

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: 6.2 (estrone)
Limit of detection: 15 ng/mL

OTHER SUBSTANCES

Extracted: estromustine, estradiol, estramustine, metabolites

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

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 (equilenin)
Internal standard: equilenin
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Simultaneous: hydrocortisone, deoxycortisol, triamcinolone, prednisone, dexamethasone, betamethasone

KEY WORDS
plasma; equilenin 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: 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: 28.38 (estrone)
Internal standard: 3,7-dimethoxyflavone (47)

OTHER SUBSTANCES
Extracted: aldosterone, androstenedione, dehydroepiandrosterone, deoxycorticosterone, 11-deoxycortisol, estradiol, 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: solutions

HPLC VARIABLES
Column: 250 × 4.6 3 μm Nucleosil C18
Mobile phase: MeOH:dichloromethane:2-propanol:water 45:5:7.5:42.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 16.80 (17α-dihydroequilenin), 19.30 (17α-dihydroequilin), 22.52 (17α-estradiol), 25.77 (equilenin), 28.62 (equilin), 32.19 (estrone)

REFERENCE
Novakovic,J.; Pacáková,V.; Sevcik,J.; Cserháti,T. Quantitative structure-chromatographic retention relationship study of six underivatized equine estrogens, *J.Chromatogr.B*, **1996**, *681*, 115–123.

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' 1.43 (estrone)

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 10 μm Nucleosil C18
Mobile phase: MeCN:THF:water 12.9:22.4:64.7
Flow rate: 1
Detector: UV 240

CHROMATOGRAM
Retention time: 11.5 (estrone)

OTHER SUBSTANCES
Simultaneous: ethinyl estradiol, mestranol, norethindrone, norethindrone acetate, norgestrel

REFERENCE
Gazdag,M.; Szepesi,G.; Szeleczki,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: 11 (estrone)

OTHER SUBSTANCES
Simultaneous: ethinyl estradiol, mestranol, norethindrone, norethindrone acetate, norgestrel

KEY WORDS
normal phase

REFERENCE
Gazdag,M.; Szepesi,G.; Fábián-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: Inject an aliquot of a solution in MeOH.

HPLC VARIABLES
Column: Radial-PAK μBondapak C18
Mobile phase: MeCN:water 50:50
Flow rate: 2
Injection volume: 100
Detector: UV 254 or 214

CHROMATOGRAM
Retention time: 2.5 (estriol), 5.5 (testosterone), 5.6 (17β-estradiol), 6.9 (estrone), 16.3 (progesterone)

KEY WORDS
testosterone and estradiol interfere

REFERENCE
Erkoc,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: Prepare an aqueous solution, inject a 20 μL aliquot.

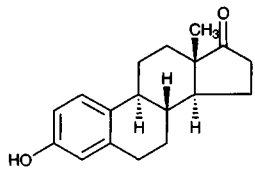
HPLC VARIABLES
Column: 150 × 4.6 3.5 μm Zorbax SB C18
Mobile phase: MeCN:MeOH:buffer 15:45:40 (Buffer was 10 mM KH_2PO_4 and 50 mM tetrabutylammonium chloride, pH adjusted to 3.0 with 1 M HCl.)
Flow rate: 0.9
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 11.2 (estrone), 8.4 (estrone-3-phosphate)

OTHER SUBSTANCES
Simultaneous: estriol, 17β-estradiol, 17β-estradiol-3-phosphate

REFERENCE
Miller,R.B.; Chen,C. A stability-indicating HPLC method for the determination of 17β-estradiol-3-phosphate in an ophthalmic solution, *Chromatographia*, **1995**, *40*, 204–206.

Estrone



Molecular formula: $C_{18}H_{22}O_2$
Molecular weight: 270.37
CAS Registry No.: 53-16-7, 338-67-5 (sodium sulfate), 481-97-0 (hydrogen sulfate)
Merck Index: 3751
Lednicer No.: 1 156

SAMPLE
Matrix: blood

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

HPLC VARIABLES
Column: Radial-PAK μBondapak C18
Mobile phase: MeCN:water 50:50
Flow rate: 2
Injection volume: 100
Detector: UV 254 or 214

CHROMATOGRAM
Retention time: 2.5 (estriol), 5.5 (testosterone), 5.6 (17β-estradiol), 6.9 (estrone), 16.3 (progesterone)

KEY WORDS
testosterone and estradiol interfere

REFERENCE
Erkoc,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: Prepare an aqueous solution, inject a 20 μL aliquot.

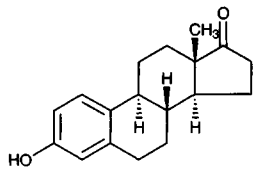
HPLC VARIABLES
Column: 150 × 4.6 3.5 μm Zorbax SB C18
Mobile phase: MeCN:MeOH:buffer 15:45:40 (Buffer was 10 mM KH_2PO_4 and 50 mM tetrabutylammonium chloride, pH adjusted to 3.0 with 1 M HCl.)
Flow rate: 0.9
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 11.2 (estrone), 8.4 (estrone-3-phosphate)

OTHER SUBSTANCES
Simultaneous: estriol, 17β-estradiol, 17β-estradiol-3-phosphate

REFERENCE
Miller,R.B.; Chen,C. A stability-indicating HPLC method for the determination of 17β-estradiol-3-phosphate in an ophthalmic solution, *Chromatographia*, **1995**, *40*, 204–206.

Estrone



Molecular formula: $C_{18}H_{22}O_2$
Molecular weight: 270.37
CAS Registry No.: 53-16-7, 338-67-5 (sodium sulfate), 481-97-0 (hydrogen sulfate)
Merck Index: 3751
Lednicer No.: 1 156

SAMPLE
Matrix: blood

Sample preparation: Hydrolyse serum or plasma with β-glucuronidase and sulfatase, extract with diethyl ether. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 40 μL buffer, add 100 μL 1.5 mg/mL dansyl chloride in acetone, shake vigorously for 30 s, heat at 100° for 5 min, inject a 20 μL aliquot. (Prepare buffer by adjusting the pH of 4 g/L sodium bicarbonate in water to 10.5 with 5 M NaOH.)

HPLC VARIABLES

Column: 250 × 2.6 PAH-10 C18 (Perkin-Elmer)
Mobile phase: Gradient. MeCN:water from 60:40 to 95:5 over 15 min (Perkin-Elmer curve 1), maintain at 95:5 for 10 min. (Flush column with MeCN at 0.1 mL/min overnight.)
Flow rate: 1
Injection volume: 20
Detector: F ex 335 em 540

CHROMATOGRAM

Retention time: 10
Limit of detection: 80 ng

OTHER SUBSTANCES

Extracted: diethylstilbestrol, estriol, hexestrol, zanone, zenone, zeranol

KEY WORDS

derivatization; cow; sheep; plasma; serum; LOD is too high for practical detection of compounds in serum and plasma.

REFERENCE

Rhys Williams,A.T.; Winfield,S.A.; Belloli,R.C. Dns derivatization of anabolic agents with high-performance liquid chromatographic separation and fluorescence detection, *J.Chromatogr.*, **1982**, *240*, 224–229.

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 or F ex 280 em 312

CHROMATOGRAM

Retention time: 41
Limit of detection: 10-100 ng/mL

OTHER SUBSTANCES

Simultaneous: estrone, equilin, estradiol, equilin sulfate, 17-α-dihydroequilin sulfate

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: culture 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 × 3.9 Techopak 10 C18 (HPLC Technology)
Mobile phase: MeOH:0.5% pH 3.0 $(NH_4)H_2PO_4$ 62:39
Flow rate: 0.7
Detector: UV 280, radioactivity

CHROMATOGRAM
Retention time: 17

OTHER SUBSTANCES
Extracted: estradiol

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: Finely powder tablets. Weigh out an amount equivalent to 3 mg piperazine estrone sulfate, add 10 mL mobile phase containing 100 μg/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
Internal standard: biphenyl (k' 11.04)
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Simultaneous: α-estradiol sulfate, β-estradiol sulfate, α-estradiol, β-estradiol, equilin sulfate, estrone, 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: Dissolve in mobile phase

HPLC VARIABLES
Column: 150 × 3.9 4 μm NovaPak C18
Mobile phase: MeOH:25 mM KH_2PO_4 40:60
Flow rate: 0.8
Injection volume: 50
Detector: UV 200

CHROMATOGRAM
Retention time: 19.01

OTHER SUBSTANCES
Simultaneous: 17α-estradiol sulfate, 17β-estradiol sulfate, equilin sulfate, 17α-dihydroequilin sulfate, 17β-dihydroequilin sulfate, equilenin sulfate, 17α-dihydroequilenin sulfate

KEY WORDS
intravenous formulations; tablets

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

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere Octyl
Mobile phase: MeCN:MeOH:buffer 32:18:50 (Buffer was 1.7 mM cetyltrimethylammonium phosphate and 25 mM KH_2PO_4.)
Flow rate: 0.9
Injection volume: 50
Detector: UV 200

CHROMATOGRAM
Retention time: 58.33

KEY WORDS
intravenous formulations; tablets; estrone sulfate; equilin sulfate; 17α-dihydroequilin sulfate; 17β-dihydroequilin sulfate; equilenin sulfate; 17α-dihydroequilenin sulfate; estrone; equilin; 17α-dihydroequilin; 17β-dihydroequilin; equilenin; 17β-dihydroequilenin; 17α-estradiol sulfate; 17β-estradiol sulfate; 17α-estradiol; 17β-estradiol

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: 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 × 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: 50

OTHER SUBSTANCES
Extracted: estradiol, metabolites

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: solutions
Sample preparation: dissolve in ethanol

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) or UV 280

CHROMATOGRAM
Retention time: 12

OTHER SUBSTANCES
Simultaneous: estriol-3-sulfate, 17β-estradiol-3-sulfate, estriol, estrone, 17β-estradiol, estrone-3-sulfate, estriol, estrone

REFERENCE
Simonian,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: solutions
Sample preparation: Prepare a 0.5 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN: water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 22.7

OTHER SUBSTANCES
Simultaneous: acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, diflunisal, doxapram, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, testosterone propionate, tranylcypromine, tripelennamine

Interfering: testosterone

KEY WORDS
details for purification of triethylamine in paper

REFERENCE
Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE
Matrix: solutions

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

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

metin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

SAMPLE

Matrix: solutions

Sample preparation: Mix 10 μL 0.5 mM compound in anhydrous benzene containing 100 mM quinuclidine with 10 μL 25 mM DBD-COCl in anhydrous benzene (Caution! Benzene is a carcinogen!), heat at 60° for 15 min, add 980 μL MeCN:water:acetic acid 50:50:1, inject a 2 μL aliquot. (Purify quinuclidine by sublimation. DBD-COCl was 4-(N-chloroformylmethyl-N-methyl)amino-7-N,N-dimethylaminosulfonyl-2,1,3-benzoxadiazole. Synthesis of DBD-COCl is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene form EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984, 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate: benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !) (Analyst 1989, 114, 413). On a Merck no. 5714 $60F_{254}$ tlc plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products. It is also reported that DBD-F can be purchased from Tokyo Kasei (TCI America, Portland OR). Stir N-methylglycine and 2.3 g sodium carbonate in water at room temperature, add 880 mg DBD-F in 40 mL MeCN dropwise, stir for 1 h, evaporate to remove the MeCN, wash twice with 50 mL portions of ethyl acetate. Acidify the aqueous phase with HCl and extract it twice with 300 mL portions of ethyl acetate. Wash the organic layer twice with 100 mL portions of saturated aqueous NaCl, dry over anhydrous magnesium sulfate, evaporate to dryness under reduced pressure, recrystallize from ethyl acetate to give 4-(N-carboxymethyl-N-methyl)amino-7-dimethylaminosulfonyl-2,1,3- benzoxadiazole (DBD-COOH) as orange-yellow crystals (mp 209-210°). Add 3.5 mL oxalyl chloride and 24 μL DMF to 1 g DBD-COOH in anhydrous benzene, stir at room temperature for 30 min, reflux for 1 h, evaporate to dryness, add 20 mL dry benzene to the residue, filter, evaporate the filtrate to give 4-(N-chloroformylmethyl-N-methyl)amino-7-N,N-dimethylaminosulfonyl-2,1,3-benzoxadiazole (DBD-COCl) as yellow crystals (mp 102°).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Cosmosil 5C18

Mobile phase: Gradient. MeCN:water from 50:50 to 100:0 over 20 min, maintain at 100:0 for 1 h.

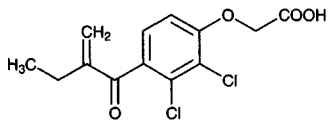
Flow rate: 1
Injection volume: 2
Detector: F ex 440 em 543

CHROMATOGRAM
Retention time: 15
Limit of detection: 40 fmole

KEY WORDS
derivatization

REFERENCE
Imai,K.; Fukushima,T.; Yokosu,H. A novel electrophilic reagent, 4-(*N*-chloroformylmethyl-N-methyl)amino-7-*N*,*N*-dimethylaminosulphonyl-2,1,3-benzoxadiazole (DBD-COCl) for fluorometric detection of alcohols, phenols, amines and thiols, *Biomed.Chromatogr.*, **1994**, *8*, 107–113.

Ethacrynic acid



Molecular formula: $C_{13}H_{12}Cl_2O_4$
Molecular weight: 303.14
CAS Registry No.: 58-54-8, 6500-81-8 (sodium salt)
Merck Index: 3761
Lednicer No.: 1 120

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 20 μL 750 μg/mL 4-(2,4-dichlorophenoxy)butyric acid in MeOH + 20 μL MeOH, vortex briefly, add 3 mL 1 M HCl, vortex, add 5 mL ethyl acetate, rock for 5 min, centrifuge at 400 g for 5 min. Remove the upper ethyl acetate layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 400 μL MeCN:buffer 30:70, inject a 200 μL aliquot. (Buffer was water containing 0.25% triethylamine and 0.25% phosphoric acid.)

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Aquapore C18 (Brownlee)
Column: 100 × 4.6 5 μm Hypersil ODS C18
Mobile phase: MeCN:MeOH:buffer 13:32:55 (Buffer was water containing 0.25% triethylamine and 0.25% phosphoric acid.)
Flow rate: 2
Injection volume: 200
Detector: UV 280

CHROMATOGRAM
Retention time: 8.75
Internal standard: 4-(2,4-dichlorophenoxy)butyric acid (10.1)
Limit of detection: 100 ng/mL
Limit of quantitation: 500 ng/mL

KEY WORDS
plasma

REFERENCE
LaCreta,F.P.; Brennan,J.M.; Tinsley,P.W.; O'Dwyer,P.J. High-performance liquid chromatographic determination of ethacrynic acid in human plasma, *J.Chromatogr.*, **1991**, *571*, 271–276.

SAMPLE
Matrix: blood, urine

Sample preparation: Plasma. 1 mL Plasma + 50 μL 500 ng/mL mefenamic acid or indomethacin + 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°. Redissolve 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: UV 235

CHROMATOGRAM
Retention time: 4.0
Internal standard: mefenamic acid (8) or indomethacin (5)
Limit of detection: 50-250 ng/mL

OTHER SUBSTANCES
Simultaneous: naproxen, flunixin, thiosalicylic acid, phenylbutazone

KEY WORDS
plasma

REFERENCE
Singh,A.K.; Jang,Y.; Mishra,U.; Granley,K. Simultaneous analysis of flunixin, naproxen, ethacrynic acid, indomethacin, phenylbutazone, mefenamic acid and thiosalicyclic 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: Condition a 3 mL 200 mg Bond Elut C18 SPE cartridge with two column volumes of MeOH and one column volume of water. Plasma. Acidify 4 mL plasma with 30 μL 86% phosphoric acid. 1 mL Acidified plasma + 100 μL 1 M HCl + 70 μL 3 μg/mL diclofenac in MeOH, add to the SPE cartridge, wash with 1 column volume water, wash with 4 mL EtOH: water 9:1, elute with 800 μL EtOH. Evaporate the eluate to dryness, reconstitute the residue with 100 μL mobile phase, inject a 70 μL aliquot. Urine. Acidify 10 mL urine with 50 μL 86% phosphoric acid. 500 μL Acidified urine + 100 μL 1 M HCl + 70 μL 7 μg/mL 4-(2,4-dichlorophenoxy)butyric acid, add to the SPE cartridge, wash with 1 column volume water, wash with 4 mL EtOH:water 9:1, elute with 800 μL EtOH. Evaporate the eluate to dryness, reconstitute the residue with 100 μL mobile phase, inject a 70 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 3 μm Spherisorb ODS II
Column: 125 × 4.6 3 μm Spherisorb ODS II
Mobile phase: MeCN:MeOH:THF:0.2% phosphoric acid 32:13:1.5:50 (plasma) or 32:13:2:55 (urine)
Flow rate: 0.9 (plasma), 0.8 (urine)
Injection volume: 70
Detector: UV 275

CHROMATOGRAM
Retention time: 19 (plasma), 30 (urine)
Internal standard: 4-(2,4-dichlorophenoxy)butyric acid (32), diclofenac (37)
Limit of quantitation: 20 ng/mL

KEY WORDS
SPE; plasma; pharmacokinetics

REFERENCE
Voith,B.; Spahn-Langguth,H.; Mutschler,E. New specific and sensitive HPLC-assays for ethacrynic acid and its main metabolite -the cysteine conjugate -in biological material, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1373–1382.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets, weigh out amount equivalent to 50 mg ethacrynic acid, add 100 mL EtOH:water 50:50, stir for 15 min, filter. Dilute a 5 mL aliquot of the filtrate to 20 mL with EtOH:water 50:50. Add a 3 mL aliquot of this solution to 1.5 mL 6 μg/mL 4-acetylbiphenyl in EtOH:water 50:50, make up to 20 mL with mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Hypersil RP-18
Mobile phase: MeCN:50 mM pH 3.0 ammonium phosphate buffer 56:44
Flow rate: 1
Injection volume: 10
Detector: UV 270

CHROMATOGRAM
Internal standard: 4-acetylbiphenyl

KEY WORDS
tablets

REFERENCE
Cavrini,V.; Bonazzi,D.; Di Pietra,A.M.; Gatti,R. Determination of ethacrynic acid in pharmaceutical formulations by difference ultraviolet spectrophotometry after derivatisation with N-acetylcysteine, *Analyst*, **1989**, *114*, 1307–1310.

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, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone,

hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

SAMPLE

Matrix: solutions
Sample preparation: Prepare a solution in MeOH:water 80:20, inject a 6 μL aliquot.

HPLC VARIABLES

Guard column: 5 × 4 10 μm LiChrosorb RP-8
Column: 100 × 4.6 5 μm Spheri RP-18 (Brownlee)
Mobile phase: MeOH:water 80:20 containing 2 g/L lithium perchlorate
Flow rate: 0.5
Injection volume: 6
Detector: E, ESA Model 5100A Coulochem, model 5020 guard cell +950 mV, Model 5010 analytical cell + 400 mV, palladium reference electrode, following post-column photolysis. The effluent from the column flowed through a 10 m × 0.3 mm coil of PTFE tubing and was irradiated at 254 nm with a Sylvania GTE 8 W low-pressure lamp.

CHROMATOGRAM

Limit of detection: 1.33 μg/mL

OTHER SUBSTANCES

Also analyzed: bendroflumethiazide, butizide, chlorthalidone, furosemide, hydrochlorothiazide

KEY WORDS

post-column reaction; post-column photochemical derivatization

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: urine

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

HPLC VARIABLES

Column: 250 × 4.6 5 μm HP Hypersil ODS (A) or HP LiChrosorb RP-18 (B)
Mobile phase: Gradient. MeCN:buffer from 15:85 at 2 min to 80:20 at 20 min (Buffer was 50 mM NaH_2PO_4 containing 16 mM propylamine hydrochloride, adjusted to pH 3 with concentrated phosphoric acid.)
Flow rate: 1
Injection volume: 5
Detector: UV 230, UV 275

CHROMATOGRAM

Retention time: 14.6 (A), 15.3 (B)
Internal standard: β-hydroxyethyltheophylline (3.7 (A), 4.4 (B))
Limit of detection: 5000 ng/mL

OTHER SUBSTANCES

Extracted: furosemide, metolazone, amiloride, acetazolamide, chlorothiazide, hydrochlorothiazide, quinethazone, triamterene, hydroflumethiazide, chlorthalidone, dichlorphenamide, trichloromethiazide, methyclothiazide, benzthiazide, cyclothiazide, polythiazide, bendroflumethiazide, probenecid, spironolactone, canrenone, flumethiazide
Noninterfering: acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil
Interfering: bumetanide

REFERENCE

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

SAMPLE

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

HPLC VARIABLES

Column: 100 × 4 5 μm SGE 100 GL-4 C18P (Scientific Glass Engineering)
Mobile phase: MeCN:MeOH:water:trifluoroacetic acid 15:15:70:0.5
Flow rate: 0.8 or 1
Injection volume: 10-20
Detector: MS, ZAB2-SEQ (VG), PSP source coupled to LC, source 250°, probe 240-260°, scan m/z 200-550 or UV 270

CHROMATOGRAM

Retention time: 4.8
Limit of detection: 50 ng (by MS)

OTHER SUBSTANCES

Extracted: probenecid, bumetanide, spironolactone

REFERENCE

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

SAMPLE

Matrix: urine

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

HPLC VARIABLES

Column: A 20 × 4 5 μm Hypersil octadecylsilica ODS; B 200 × 4.6 5 μm Shiseido SG-120 polymer-based C18

Mobile phase: A water; B Gradient. MeCN:buffer from 7:93 to 15:85 over 3.5 min, to 50:50 over 8.5 min, maintain at 50:50 for 11 min (Buffer was 6.9 g $NaH_2PO_4.H_2O$ in 1 L water, pH adjusted to 3.1 with phosphoric acid.)

Flow rate: 1

Injection volume: 40

Detector: UV 230

CHROMATOGRAM

Retention time: 19.2

Limit of detection: 500 ng/mL

OTHER SUBSTANCES

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

KEY WORDS

column-switching; optimum detection wavelengths vary for each drug

REFERENCE

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

SAMPLE

Matrix: urine

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

HPLC VARIABLES

Column: 75 × 4.6 3 μm Ultrasphere ODS

Mobile phase: Gradient. MeCN:100 mM ammonium acetate adjusted to pH 3 with concentrated phosphoric acid. From 10:90 to 15:85 over 2 min to 55:45 over 3 min to 60:40 over 3 min. Kept at 60:40 for 1 min, decreased to 10:90 over 1 min and equilibrated at 10:90 for 2 min.

Flow rate: 1

Injection volume: 20

Detector: UV 270

CHROMATOGRAM

Retention time: 6.9

Internal standard: 7-propyltheophylline (4.5)

Limit of detection: 200 ng/mL

OTHER SUBSTANCES

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

Interfering: polythiazide, bendroflumethiazide

REFERENCE

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

SAMPLE

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

HPLC VARIABLES

Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 250 × 4 5 μm Hypersil ODS-C18
Mobile phase: A Water; B Gradient. MeCN:buffer 15:85 for 1.5 min then to 80:20 over 8 min. Keep at 80:20 for 2.5 min then re-equilibrate with 15:85. (Buffer was 50 mM NaH_2PO_4 + 1.4 mL propylamine hydrochloride per liter adjusted to pH 3 with concentrated phosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: UV 230

CHROMATOGRAM

Retention time: 10
Limit of detection: 200 ng/mL.

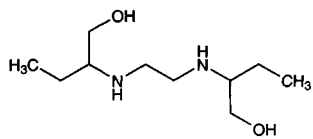
OTHER SUBSTANCES

Simultaneous: acetazolamide, amiloride, bendroflumethiazide, chlorthalidone, cyclothiazide, furosemide, hydrochlorothiazide, probenecid, spironolactone, triamterene
Interfering: bumetanide

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.

Ethambutol



Molecular formula: $C_{10}H_{24}N_2O_2$
Molecular weight: 204.31
CAS Registry No.: 74-55-5, 1070-11-7 (di HCl)
Merck Index: 3764
Lednicer No.: 1 222

SAMPLE

Matrix: blood
Sample preparation: Add 100 μL 8 ng/mL octylamine in MeCN and 100 μL 4 M NaOH to 100 μL plasma, extract with 4 mL chloroform (Caution! Chloroform is a carcinogen!), agitate for 10 min, centrifuge at 3000 rpm for 5 min, discard the aqueous phase. Add 100 μL 400 ng/mL phenylethylisocyanate in MeCN to the organic layer, mix for 1 min, evaporate to dryness under a stream of nitrogen, reconstitute the residue with 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 C18 (Interchim, France)
Column: 150 × 4.6 3 μm Hypersil C18
Mobile phase: MeOH:water 70:30
Flow rate: 1
Injection volume: 20
Detector: UV 200

CHROMATOGRAM
Retention time: 8.9
Internal standard: octylamine (13.7)
Limit of detection: 70 ng/mL
Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES
Noninterfering: amphotericin B, dapsone, didanosine, fluconazole, isoniazid, itraconazole, pyrazinamide, pyrimethamine, rifampicin, rifabutine, sparfloxacin, zidovudine

KEY WORDS
derivatization; plasma

REFERENCE
Chenevier,P.; Massias,L.; Gueylard,D.; Farinotti,R. Determination of ethambutol in plasma by high-performance liquid chromatography after pre-column derivatization, *J.Chromatogr.B*, **1998**, *708*, 310–315.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 2 mL 4 M NaOH + 100 μL 1 mg/mL IS + 8 mL chloroform, agitate for 20 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeCN, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm LiChrosorb Si 60
Mobile phase: MeCN:water 50:50 containing 0.04 mM copper sulfate and 2 M ammonia (The mobile phase was saturated with silica using a 250 × 4.6 column packed with 50 μm high-porosity silica (Alltech).)
Flow rate: 1.5
Injection volume: 50
Detector: UV 270

CHROMATOGRAM
Retention time: 5.7
Internal standard: (S,S)-N,N'-bis(hydroxymethyl-1-ethyl)ethylenediamine (7) (Reflux 5 mmoles 1,2-dibromoethane with 50 mmole (S)-2-aminopropan-1-ol at 110° for 25 min, cool to 30°, add 12 mmoles potassium in 5 mL propanol, cool in an ice bath, filter, concentrate the filtrate under reduced pressure, take up the residue in acetone:propanol 1:1, cool in an ice bath, filter, concentrate the filtrate under reduced pressure, add 1 mL EtOH, filter off the product, dry the IS.)
Limit of detection: 150 ng/mL

OTHER SUBSTANCES
Noninterfering: isoniazid, pyrazinamide, rifampin

KEY WORDS
plasma; derivatization; complexation; copper complexes

REFERENCE
Lacroix,C.; Cerutti,F.; Nouveau,J.; Menager,S.; Lafont,O. Détermination de l'éthambutol plasmatique par chromatographie liquide et détection spectrophotométrique ultraviolette [Determination of ethambutol in plasma by liquid chromatography and ultraviolet spectrophotometric detection], *J.Chromatogr.*, **1987**, *415*, 85–94.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 200 μL Plasma + 300 μL 5 M NaOH + 5 mL diethyl ether, vortex for 1 min, centrifuge at 1200 g for 3 min, remove the organic layer, repeat the extraction. Combine the organic layers and add them to 200 μL 10 mM phosphoric acid, vortex for 1 min, centrifuge at 1200 g for 3 min. Remove the aqueous phase and add it to 300 μL 200 mM pH 7.5 borate buffer, vortex for 10 s, add 40 μL 4 mg/mL 4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole

in MeCN, heat at 80° for 30 min, add 50 μL 1 M phosphoric acid, cool in dry ice/acetone for 1 min, add 2 mL ethyl acetate, vortex for 1 min, centrifuge at 1200 g for 3 min. Discard the upper organic layer and add 50 μL 5 M NaOH to the lower aqueous phase, add 2 mL ethyl acetate:MeOH 90:10, mix for 1 min, centrifuge at 1200 g for 3 min. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 250 μL 10 mM pH 2.5 phosphoric acid, inject a 200 μL aliquot. Urine. Dilute 100 μL urine to 20 mL with water. 200 μL Diluted urine + 300 μL 200 mM pH 7.5 borate buffer, vortex for 10 s, add 40 μL 4 mg/mL 4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole in MeCN, heat at 80° for 30 min, add 50 μL 1 M phosphoric acid, cool in dry ice/acetone for 1 min, add 2 mL ethyl acetate, vortex for 1 min, centrifuge at 1200 g for 3 min. Discard the upper organic layer and add 50 μL 5 M NaOH to the lower aqueous phase, add 2 mL ethyl acetate:MeOH 90:10, mix for 1 min, centrifuge at 1200 g for 3 min. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 1 mL 10 mM pH 2.5 phosphoric acid, inject a 200 μL aliquot.

HPLC VARIABLES

Guard column: 37-53 μm Pellicular ODS (Whatman)
Column: 250 × 4.6 5 μm Spherisorb CN
Mobile phase: MeCN:buffer 30:70 (Buffer was 10 mM phosphoric acid adjusted to pH 2.5 with 10 M KOH.)
Flow rate: 1
Injection volume: 200
Detector: F ex 490 em 540

CHROMATOGRAM

Retention time: 14
Limit of quantitation: 10 ng/mL (plasma), 10 μg/mL (urine)

KEY WORDS

derivatization; plasma; 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole is a less reactive derivatizing reagent

REFERENCE

Breda,M.; Marrari,P.; Pianezzola,E.; Strolin Benedetti,M. Determination of ethambutol in human plasma and urine by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.A*, **1996**, *729*, 301–307.

SAMPLE

Matrix: solutions
Sample preparation: Mix a 2 mL aliquot of a 25 nM (?) solution of ethambutol in benzene (Caution! Benzene is a carcinogen!) with 5 mL 400 mM (R)-(-)-α-methoxyphenylacetyl chloride in benzene and 300 μL pyridine, stir vigorously at 40° for 30 min, pour into 5 mL ether and 5 mL water. Remove the ether layer and wash it with 5 mL 5% HCl, dry over anhydrous magnesium sulfate, filter, evaporate to dryness under reduced pressure, reconstitute with 500 μL chloroform, inject a 5 μL aliquot. (Synthesis of (R)-(-)-α-methoxyphenylacetyl chloride is as follows. Reflux (R)-(-)-α-methoxyphenylacetic acid with a 5 to 10-fold excess of thionyl chloride on a steam bath for 5 min, add benzene, evaporate under reduced pressure, repeat this procedure 3 or 4 times to remove excess thionyl chloride, distil at 70-75°/0.2 mm Hg to give (R)-(-)-α-methoxyphenylacetyl chloride (J. Org. Chem. 1968, 33, 1142).)

HPLC VARIABLES

Column: 250 × 4 5 μm LiChrosorb Si 60
Mobile phase: Chloroform:ethyl acetate 90:10
Flow rate: 0.7
Injection volume: 5
Detector: UV 254

CHROMATOGRAM

Retention time: 9 (-), 9.5 (+), 10.2 (meso)

OTHER SUBSTANCES

Simultaneous: 2-amino-1-butanol

KEY WORDS
derivatization; normal phase; chiral

REFERENCE
Gamberini,G.; Ferioli,V. Determination of optical purity by high performance liquid chromatography of compounds of pharmaceutical interest, *Farmaco.[Prat].*, **1988**, *43*, 357–363.

SAMPLE
Matrix: solutions
Sample preparation: Mix 1 mL of a 16-320 μg/mL solution in pH 4.4 KH_2PO_4 buffer with 100 μL 1.32 mg/mL L-glycine in KH_2PO_4, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Chiral-Si100 D-ValCu (Serva)
Mobile phase: 50 mM pH 4.4 KH_2PO_4 containing 1 mM copper(II) sulfate
Column temperature: 25
Flow rate: 0.3
Injection volume: 20
Detector: UV 260

CHROMATOGRAM
Retention time: 12.7 (+), 14.2 (meso)
Internal standard: L-glycine
Limit of detection: 1 μg/mL

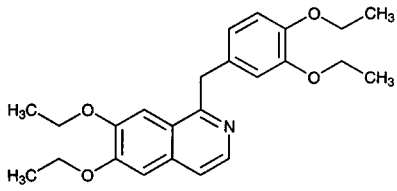
OTHER SUBSTANCES
Simultaneous: impurities, 2-amino-1-butanol

KEY WORDS
derivatization; complexation; chiral; D-enantiomer is not separated from L-enantiomer but they are separated from meso-form

REFERENCE
Ferioli,V.; Gamberini,G.; Rustichelli,C.; Vezzalini,F. Direct determination of non-UV-absorbing compounds by high-performance liquid chromatography, *Farmaco*, **1994**, *49*, 411–413.

Ethaverine

Molecular formula: $C_{24}H_{29}NO_4$
Molecular weight: 395.50
CAS Registry No.: 486-47-5, 985-13-7 (HCl)
Merck Index: 3773



SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 15 μL 2 μg/mL papaverine HCl in MeOH + 200 μL 4 M NaOH + 5 mL diethyl ether, vortex for 5 min, centrifuge at 2000 g for 10 min, remove the organic layer, extract the aqueous layer with 2 mL diethyl ether, centrifuge. Combine the organic layers and add them to 500 μL 1 M HCl, vortex for 1 min, centrifuge at 2000 g for 10 min. Remove the aqueous layer and add it to 500 μL 4 M NaOH, vortex for 1 min, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 25 μL MeOH, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil 10 ODS
Mobile phase: MeOH:0.1% KH_2PO_4 65:35
Flow rate: 2

Detector: UV 238

CHROMATOGRAM
Retention time: 8.5
Internal standard: papaverine (3.5)
Limit of detection: 2 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Brodie,R.R.; Chasseaud,L.F.; Walmsley,L.M.; Soegtrop,H.H.; Darragh,A.; O'Kelly,D.A. Determination of the antispasmodic agent ethaverine in human plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *182*, 379–386.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + 200 μL water + 100 (plasma) or 500 (urine) μL 500 mM pH 8.5 TRIS-HCl buffer + 2 g NaCl + 5 mL butyl chloride:isopropanol 95:5, vortex for 15 s, centrifuge at 3000 g for 10 min, repeat the extraction. Combine the organic layers and evaporate them to dryness in a vortex-evaporator at 45°, reconstitute the residue in 50 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax Sil
Mobile phase: EtOH:water:methanesulfonic acid 500:25:0.5 (plasma) or 500:10:0.25 (urine)
Flow rate: 1.2
Detector: UV 270

CHROMATOGRAM
Retention time: 6.9 (plasma), 7.6 (urine)
Internal standard: ethaverine

OTHER SUBSTANCES
Extracted: encainide
Simultaneous: quinidine
Noninterfering: acetaminophen, alprazolam, aspirin, cephalexin, chloral hydrate, diazepam, digoxin, dipyridamole, docusate, flurazepam, furosemide, hydrochlorothiazide, ibuprofen, isosorbide dinitrate, lidocaine, lorazepam, meclizine, mexiletine, nifedipine, norfloxacin, oxazepam, ranitidine, tocainide, triamterene, triazolam

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KEY WORDS
plasma; ethaverine is IS

REFERENCE
Turgeon,J.; Funck-Brentano,C.; Gray,H.T.; Roden,D.M. Improved high-performance liquid chromatographic assay for encainide and its metabolites in human body fluids, *J.Chromatogr.*, **1989**, *490*, 165–174.

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

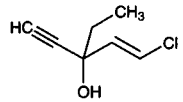
HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelco C18
Mobile phase: MeCN:buffer 70:30 (Buffer contained 2.88% sodium lauryl sulfate and 1.248% NaH_2PO_4 adjusted to pH 3 with orthophosphoric acid.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6.58

OTHER SUBSTANCES
Simultaneous: moxaverine, drotaverine, papaverine, codeine

REFERENCE
Girgis,E.H. Ion-pair reversed-phase liquid chromatographic identification and quantitation of papaverine congeners, *J.Pharm.Sci.*, **1993**, *82*, 503–505.

Ethchlorvynol



Molecular formula: C_7H_9ClO
Molecular weight: 144.60
CAS Registry No.: 113-18-8
Merck Index: 3774

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 200 μL 50 μg/mL hexobarbital in MeCN + 25 μL glacial acetic acid, vortex for 10 s, centrifuge for 1 min, inject a 30-100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: Gradient. MeCN:7.5 g/L NaH_2PO_4 adjusted to pH 3.2 with phosphoric acid 5:95 to 22:78 over 24 min, to 45:55 over 10 min, maintain at 45:55 for 5 min. Re-equilibrate with 5:95 for 5 min.
Column temperature: 50
Flow rate: 3
Injection volume: 30-100
Detector: UV 210

CHROMATOGRAM
Retention time: 16.5
Internal standard: hexobarbital (20.6)
Limit of detection: 200-2000 ng/mL

OTHER SUBSTANCES
Extracted: acetaminophen, amobarbital, butabarbital, butalbital, chlordiazepoxide, diazepam, flurazepam, glutethimide, methaqualone, methyprylon, nitrazepam, pentobarbital, phenobarbital, phenytoin, primidone, salicylic acid, secobarbital, theophylline
Simultaneous: amitriptyline, caffeine, clomipramine, codeine, desipramine, ethotoin, imipramine, lidocaine, methsuximide, nirvanol, nortriptyline, oxazepam, procainamide, phenylpropanolamine, propranolol, quinidine
Interfering: mesantoin

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
Sample preparation: Inject a 6-10 μL aliquot.

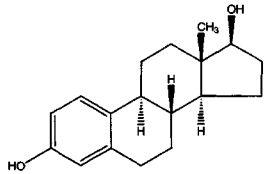
HPLC VARIABLES
Guard column: 20 × 4.6 Supelguard LC-1 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil LC-1 (Supelco)
Mobile phase: MeOH:MeCN:buffer 17.5:17.5:65 (Buffer was 2.72 g KH_2PO_4 in 1.9 L water, pH adjusted to 6.3 with about 2 mL 1 M NaOH, made up to 2 L.)
Flow rate: 2
Injection volume: 6-10
Detector: UV 204

CHROMATOGRAM
Retention time: 5.63
Internal standard: 5-ethyl-5-p-tolybarbituric acid (tolybarb) (4.80)

OTHER SUBSTANCES
Simultaneous: acetaminophen, acetanilide, barbital, butabarbital, butalbital, caffeine, carbamazepine, chloramphenicol, cimetidine, cyheptamide, diazoxide, diflunisal, disopyramide, ethchlorvynol, ethosuximide, glutethimide, heptabarbital, hexobarbital, ibuprofen, indomethacin, ketoprofen, mefenamic acid, mephenytoin, methaqualone, methyl salicylate, methyprylon, naproxen, nirvanol, oxphenylbutazone, phenacetin, phenobarbital, phensuximide, phenylbutazone, phenytoin, primidone, salicylamide, secobarbital, sulindac, theophylline, thiopental, tolmetin
Noninterfering: N-acetylcysteine, N-acetylprocainamide, amikacin, ampicillin, aspirin, chlorpropamide, codeine, diphylline, gentamicin, gentisic acid, meprobamate, morphine, netilmicin, quinidine, salicylic acid, sulfamethoxazole, tetracycline, tobramycin, trimethoprim, valproic acid, vancomycin
Interfering: methsuximide, procainamide, mephobarbital, pentobarbital

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.

Ethinyl estradiol



Molecular formula: $C_{20}H_{24}O_2$
Molecular weight: 296.41
CAS Registry No.: 57-63-6
Merck Index: 3780
Lednicer No.: 1 162

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 500 μL 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 μL mobile phase, inject aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Novapack C18
Mobile phase: MeCN:MeOH:buffer 35:15:50 (Buffer was 50 mM KH_2PO_4 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: estriol, estradiol, 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 ethynyloestradiol in rabbit plasma, *J.Chromatogr.*, **1993**, *619*, 143–147.

SAMPLE
Matrix: blood
Sample preparation: 100 µL Plasma + 10 µL IS in water, extract twice by shaking for 1 min with 1.2 mL dichloromethane, evaporate organic layer below 40° under reduced pressure, dissolve residue in 100 µL MeCN. Add 10 µL reagent 1, add 10 µL reagent 2, heat at 50° for 15 min, cool to room temperature, add 100 µL water, add 200 µL MeOH:water 1:1, add to Sep-Pak C18 cartridge, wash vial with 2 mL MeOH:water 1:1 and add washings to cartridge, wash cartridge with 40 mL MeOH:water 1:1, elute with 5 mL MeOH. Concentrate eluent to 500 µL by evaporation at 40° under reduced pressure, inject 20 µL aliquot. (Reagent 1 was 30 mg 2-(4-carboxyphenyl)-5,6-dimethylbenzimidazole in 3 mL pyridine, add 700 mg 4-piperidinopyridine, dilute to 10 mL with MeCN. Reagent 2 was 700 mg 1-isopropyl-3-(3-dimethylaminopropyl)carbodiimide perchlorate in 10 mL MeCN.)

HPLC VARIABLES
Guard column: 50 × 4 5 µm Wakosil 5C18
Column: 300 × 4 5 µm 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: estriol, estradiol, equilin, equilenin, estrone, estetrol, 4-hydroxyestradiol, 2-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 µL 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 µL mobile phase, inject a 50 µL aliquot.

HPLC VARIABLES
Column: LiChrocart 100 RP-18
Mobile phase: MeOH:isopropanol:dichloromethane:water 40:9:4:47
Flow rate: 1
Injection volume: 50

Detector: UV 220

CHROMATOGRAM
Internal standard: ethinyl estradiol (17α-ethynylestradiol)

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: 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: norgestimate, degradation products

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.

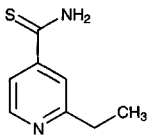
SAMPLE
Matrix: solutions
Sample preparation: Extract 15 mL water with dichloromethane, evaporate organic layer, take up residue in 3 mL mobile phase, inject 50 μL aliquot.

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.

Ethionamide



Molecular formula: $C_8H_{10}N_2S$
Molecular weight: 166.25
CAS Registry No.: 536-33-4
Merck Index: 3783
Lednicer No.: 1 255

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL octadecyl SPE cartridge (J.T. Baker) with one volume of MeOH and one volume of water. 200 μL Serum + 4 μL 200 μg/mL prothionamide in MeCN: 20 mM Na_2HPO_4 50:50, vortex for 5 s, add to the SPE cartridge, wash with 400 μL water, wash with 50 μL MeOH:water 90:10, dry under vacuum for 15 min, elute with 1 mL MeOH. Evaporate the eluate under nitrogen at 40°, reconstitute in 200 μL MeCN:20 mM Na_2HPO_4 25:75, vortex for 5 s, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 4.6 5 μm Hypersil ODS
Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:20 mM Na_2HPO_4 25:75
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 4.28
Internal standard: prothionamide (7.24)
Limit of detection: 200 ng/mL

OTHER SUBSTANCES

Noninterfering: acetaminophen, p-aminosalicylic acid, aspirin, amikacin, capreomycin, ethambutol, isoniazid, kanamycin, pyrazinamide, streptomycin, rifampin
Interfering: phenobarbital

KEY WORDS

serum; SPE

REFERENCE

Peloquin,C.A.; James,G.T.; McCarthy,E. Improved high-performance liquid chromatographic assay for the determination of ethionamide in serum, *J.Chromatogr.*, **1991**, *563*, 472–475.

SAMPLE

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

HPLC VARIABLES

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

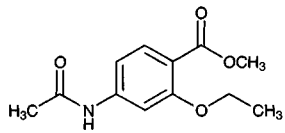
CHROMATOGRAM
Retention time: 8.93
Internal standard: heptanophenone (19.2)
Limit of quantitation: 300 ng/mL

OTHER SUBSTANCES
Extracted: acetazolamide, ampicillin, bromazepam, caffeine, carbamazepine, chloramphenicol, chlorothiazide, diazepam, droperidol, 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.

Ethopabate



Molecular formula: $C_{12}H_{15}NO_4$
Molecular weight: 237.26
CAS Registry No.: 59-06-3
Merck Index: 3791

SAMPLE
Matrix: meat
Sample preparation: Homogenize (Ultra-Turrax TP 18/10) 3 g tissue with 1 mL water and 4 mL acetone for 6 s, centrifuge at 5000 rpm for 3 min. Transfer 4 mL supernatant, add 5 mL dichloromethane, mix for 5 s, centrifuge at 3000 rpm for 3 min. Evaporate the organic layer to dryness under a stream of nitrogen at 60°. Dissolve residue in 500 µL MeOH:buffer 70:30, place in freezer at -20° for 5 min. Filter (Costar Spin-X with 0.2 µm nylon membrane) while centrifuging at 5600 g for 3 min. Inject a 20 µL aliquot of the filtrate. (Prepare buffer by dissolving 4.45 g sodium heptanesulfonate and 1.8 g $Na_2HPO_4.2H2O$ in 750 mL water, adjust pH to 6.3 with 5 M phosphoric acid, adjust pH to 6.0 with 1 M phosphoric acid, make up to 1 L with water.)

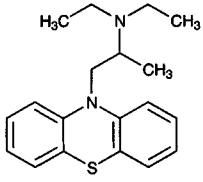
HPLC VARIABLES
Guard column: 20 × 4.6 5 µm Supelcosil LC-ABZ+Plus
Column: 250 × 4.6 5 µm Supelcosil LC-ABZ+Plus
Mobile phase: MeCN:water 35:65
Flow rate: 0.8
Injection volume: 20
Detector: F ex 300 em 350

CHROMATOGRAM
Retention time: ca. 8.3
Limit of quantitation: 1 ng/g

KEY WORDS
chicken meat

REFERENCE
Hormazabal,V.; Yndestad,M. Rapid assay for the determination of residues of amprolium and ethopabate in chicken meat by HPLC, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2517–2525.

Ethopropazine



Molecular formula: $C_{19}H_{24}N_2S$
Molecular weight: 312.48
CAS Registry No.: 522-00-9, 1094-08-2 (HCl)
Merck Index: 3793
Lednicer No.: 1 373

SAMPLE

Matrix: blood
Sample preparation: Add IS and 300 μL MeCN to 100 μL plasma, vortex, centrifuge for 2 min. Remove the supernatant and add it to 300 μL pH 5.9 sodium phosphate buffer and 3 mL hexane and vortex for 45 s. Centrifuge at 2500g for 3 min. Evaporate the supernatant under a stream of nitrogen and reconstitute with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 100 × 4.6 ODS
Mobile phase: MeCN:MeOH:25 mM potassium phosphate 25:25:50 containing 0.75 mL/L 2 M sulfuric acid and 0.25 mL/L triethylamine
Flow rate: 1.0
Detector: UV 250

CHROMATOGRAM

Retention time: 7.7
Internal standard: orphenadrine (5.7)
Limit of quantitation: 12.5 mg/mL

KEY WORDS

plasma; rat; pharmacokinetics

REFERENCE

Padovani,P.K.; Timby,D.M.; Wright,M.R.; Kapil,R.P. Quantitative analysis of DMP 851 in rat and dog plasma by liquid-liquid extraction and reverse-phase high performance liquid chromatography with ultraviolet detection (Abstract 3318), *Pharm.Res.*, **1997**, *14*, S568.

SAMPLE

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

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 3.1

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextro-

propoxyphene, 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, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

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

SAMPLE

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

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 2.70

OTHER SUBSTANCES

Simultaneous: triflupromazine, carphenazine, methotrimeprazine, promazine, perphenazine, chlorprothixene, thiothixene, reserpine, acetophenazine, promethazine, propiomazine, deserpidine
Interfering: methotrimeprazine

KEY WORDS

SFC; pressure 200 bar

REFERENCE

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

SAMPLE
Matrix: solutions

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

CHROMATOGRAM
Retention time: 16.60 (A), 8.25 (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, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
also details of plasma extraction

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

SAMPLE
Matrix: solutions
Sample preparation: Make up a 500 ng/mL solution in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm KK-CARNU (α-R-naphthyl)ethylurea (YMC)
Mobile phase: Hexane:1,2-dichloroethane:EtOH:trifluoroacetic acid 400:150:100:1
Flow rate: 1
Injection volume: 100

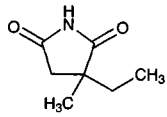
Detector: F ex 254 em 280 (filter)

CHROMATOGRAM
Retention time: 10.94, 12.37 (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.

Ethosuximide



Molecular formula: $C_7H_{11}NO_2$
Molecular weight: 141.17
CAS Registry No.: 77-67-8
Merck Index: 3794
Lednicer No.: 1 228

SAMPLE
Matrix: blood
Sample preparation: Mix 500 μL plasma with 500 μL MeCN and 2 μg IS for 30 s, centrifuge at 2700 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeCN:MeOH:10 mM pH 7.4 phosphate buffer 15:35:50
Column temperature: 25
Flow rate: 1
Detector: UV 219

CHROMATOGRAM
Internal standard: 2-hydroxy-2-ethyl-2-phenylacetamide (17)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: carbamazepine, clonazepam, D,L-2-hydroxy-2-ethyl-2-phenylpropionamide (HEPP), phenobarbital, phenytoin, primidone

KEY WORDS
rat; plasma

REFERENCE
Martínez de Muñoz,D.; Arenas,R.; Chávez González,O. Liquid chromatographic assay in plasma of one of the members of a new series of anticonvulsants: D,L-3-hydroxy-3-ethyl-3-phenylpropionamide, *J.Chromatogr.B*, **1996**, *678*, 377–383.

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

HPLC VARIABLES
Column: 100 × 4.6 5-10 μm Silicalite (by sieving Silicalite, 3M Co.(?))
Mobile phase: MeNC:20 mM pH 6.9 phosphate buffer 10:90

Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 2.45

OTHER SUBSTANCES
Extracted: methamphetamine, sulfamethoxazole, primidone

KEY WORDS
serum

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

SAMPLE
Matrix: blood
Sample preparation: Add 200 μL 2 μg/mL thymol in MeCN to 200 μL serum, vortex for 10 s, centrifuge at 7000 g for 5 min, inject 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Resolve C18-5 (Waters)
Mobile phase: MeCN:isopropanol:50 mM pH 3.0 phosphate buffer 25:15:60
Column temperature: 30
Flow rate: 0.7
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 2.3
Internal standard: thymol (18.5)

OTHER SUBSTANCES
Extracted: primidone, phenobarbital, phenytoin, carbamazepine, valproic acid

KEY WORDS
human; plasma

REFERENCE
Kondo,K.; Nakamura,M.; Nishioka,R.; Kawai,S. Direct method of determination of valproic acid in serum by high performance liquid chromatography, *Anal.Sci.*, **1985**, *1*, 385–387.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 100 μL heptabarbital in MeOH + 500 μL 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 μL MeOH, inject a 3 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 2.1 Whatman Co:Pell ODS
Column: 125 × 4.5 5 μm 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: 3.4
Internal standard: heptabarbital (9.8)
Limit of quantitation: 16 μM

OTHER SUBSTANCES
Extracted: primidone, phenobarbital, pheneturide, carbamazepine, phenytoin
Simultaneous: phenylethylmalonamide, sulthiame, sulfamethoxazole, ethotoin, butabarbital, pentobarbital, methsuximide, cyclobarbital, ethylphenacemide, amobarbital, glutethimide, secobarbital
Interfering: barbital

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 μL Serum or plasma + 200 μL 20 μg/mL IS in MeOH:water 10:90 + 75 μL 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 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 2.1 Permaphase ETH (DuPont)
Column: 250 × 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: 2.3
Internal standard: alphenal (5-allyl-5-phenylbarbituric acid) (4.4)
Limit of quantitation: 1300 ng/mL

OTHER SUBSTANCES
Extracted: phenytoin, carbamazepine, primidone, phenobarbital
Simultaneous: amobarbital, chlordiazepoxide, codeine, cortisol, ethotoin, glutethimide, hexobarbital, mephenytoin, mephobarbital, metharbital, methsuximide, nitrazepam, pentobarbital, phenacetin, phensuximide, secobarbital
Noninterfering: acetaminophen, acetazolamide, amphetamine, bilirubin, caffeine, diazepam, dimenhydrinate, meperidine, meprobamate, methamphetamine, methaqualone, methylphenidate, nicotine, propoxyphene, theophylline, valproate
Interfering: barbital

KEY WORDS
plasma; serum

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

Sample preparation: 500 μL Serum + 50 μL 7 μg/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 μL mobile phase, inject a 50-100 μL aliquot. (Buffer was 10 mM sodium acetate:10 mM acetic acid 88.5:11.5, pH 5.5.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Partisil 5 ODS-3
Mobile phase: MeCN:buffer 28:72 (Buffer was 300 μL 1 M KH_2PO_4 and 50 μL 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: 1.8
Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (11.5)

OTHER SUBSTANCES
Extracted: carbamazepine, phenytoin, secobarbital
Simultaneous: mephobarbital, paramethadione, phenobarbital
Noninterfering: chlorazepate, clonazepam, diazepam, thioridazine, valproic acid
Interfering: primidone

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 μL Serum or plasma + 400 μL 10 μg/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 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:MeOH:buffer 17:28:55, final pH 6.8-7.0 (Buffer was 400 μL 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: 6.8
Internal standard: tolybarb (5-ethyl-5-(p-methylphenyl)barbituric acid) (13.8)

OTHER SUBSTANCES
Extracted: carbamazepine, N-desmethylmethsuximide, phenobarbital, phenytoin, primidone
Simultaneous: acetaminophen, butalbital, caffeine, hexobarbital, methsuximide, phenacetin, phenylethylmalonamide, salicylic acid

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KEY WORDS
plasma; serum

REFERENCE
Szabo,G.K.; Browne,T.R. Improved isocratic liquid-chromatographic simultaneous measurement of phenytoin, phenobarbital, primidone, carbamazepine, ethosuximide, and N-desmethylmethsuximide in serum, *Clin.Chem.*, **1982**, *28*, 100–104.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 50 μL 10 μg/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 × 8 5 μm Nova Pak C18 Radial pak
Mobile phase: MeCN:MeOH:acetone:buffer 8:21:10:61 adjusted to pH 7.95 ± 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: 1.70
Internal standard: tolybarb (5-ethyl-5-(p-methylphenyl)barbituric acid) (4.89)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: primidone, phenobarbital, carbamazepine, phenytoin, metabolites
Simultaneous: acetaminophen, N-acetylprocainamide, aspirin, ampicillin, caffeine, cephapirin, 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: 100 μL Serum + 100 μL buffer + 1.5 mL IS in 5% isopropanol in chloroform, vortex for 30 s, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of air at room temperature, reconstitute the residue in 100 μL mobile phase, inject a 6-10 μL aliquot. (Buffer was 13.6 g KH_2PO_4 in 90 mL water, pH adjusted to 6.8 with about 3 mL 10 M NaOH, made up to 100 mL.)

HPLC VARIABLES
Guard column: 20 × 4.6 Supelguard LC-1 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil LC-1 (Supelco)
Mobile phase: MeOH:MeCN:buffer 17.5:17.5:65 (Buffer was 2.72 g KH_2PO_4 in 1.9 L water, pH adjusted to 6.3 with about 2 mL 1 M NaOH, made up to 2 L.)
Flow rate: 2
Injection volume: 6-10
Detector: UV 204

CHROMATOGRAM
Retention time: 2.57

Internal standard: methsuximide (5.30)

OTHER SUBSTANCES

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

Also analyzed: acetanilide, N-acetylcysteine, N-acetylprocainamide, ampicillin, aspirin, butabarbital, butalbital, chlorpropamide, codeine, cyheptamide, diazoxide, diflunisal, diphylline, disopyramide, ethchlorvynol, gentisic acid, glutethimide, heptabarbital, hexobarbital, ibuprofen, indomethacin, ketoprofen, mefenamic acid, mephenytoin, methaqualone, 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

Interfering: barbital, cimetidine

KEY WORDS

serum

REFERENCE

Meatherall,R.; Ford,D. Isocratic liquid chromatographic determination of theophylline, acetaminophen, chloramphenicol, caffeine, anticonvulsants, and barbiturates in serum, *Ther.Drug Monit.*, **1988**, *10*, 101–115.

SAMPLE

Matrix: blood

Sample preparation: Prepare an SPE cartridge by plugging the end of a 1 mL disposable pipette tip with glass wool and adding about 100 mg Chromosorb P/NAW. Add 50 μL plasma then 50 μL 10 μg/mL tolylphenobarbital in 200 mM HCl to the SPE cartridge, let stand for 2 min, elute with 1 mL chloroform:isopropanol 6:1. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject a 15 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Supelcosil-LC-8
Mobile phase: MeCN:water 20:80
Flow rate: 3.3
Injection volume: 15
Detector: UV 208

CHROMATOGRAM

Retention time: 1.37
Internal standard: tolylphenobarbital (7.57)
Limit of detection: 50-100 ng/mL

OTHER SUBSTANCES

Extracted: theophylline, caffeine, primidone, carbamazepinediol, phenacemide, methyprylon, nirvanol, phenobarbital, chloramphenicol, butabarbital, carbamazepine epoxide, mephenytoin, pentobarbital, amobarbital, carbamazepine, glutethimide, phenytoin, secobarbital, methaqualone

Noninterfering: acetaminophen, amikacin, amitriptyline, clonazepam, cyclosporine,desipramine, diazepam, digoxin, disopyramide, gentamicin, imipramine, lidocaine, methotrexate, N-acetylprocainamide, netilmicin, nortriptyline, procainamide, quinidine, salicylic acid, sulfamethoxazole, tobramycin, trimethoprim, valproic acid, p-hydroxyphenobarbital, vancomycin

Interfering: barbital

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: 400 μL Plasma + 100 μL water, mix for 10 s, add 1 mL isopropanol, vortex for 30 s, centrifuge at 1800 g for 5 min. Remove a 1 mL aliquot of the supernatant and add it to 300 μL 10 mM NaOH, evaporate to dryness under a stream of nitrogen at 50°, add 200 μL 2.5 mM 4-(bromomethyl)-7-methoxycoumarin in MeCN, add 300 μL of a solution of 2,2'-dinitrobiphenyl in MeCN, add 100 mg potassium carbonate, shake at 70° for 1.5 h, inject a 15 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeCN:MeOH:water 20:20:60
Flow rate: 1.3
Injection volume: 15
Detector: UV 320

CHROMATOGRAM
Retention time: 10
Internal standard: 2,2'-dinitrobiphenyl (14)
Limit of detection: 7 pmole

OTHER SUBSTANCES
Noninterfering: acetazolamide, carbamazepine, phenobarbital, primidone, valproic acid

KEY WORDS
derivatization; plasma

REFERENCE
Chen,S.-H.; Wu,H.-L.; Wu,J.-K.; Kou,H.-S.; Wu,S.-M. Determination of ethosuximide in plasma by derivatization and high performance liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 1579–1589.

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

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

CHROMATOGRAM
Retention time: 10.485

KEY WORDS
whole blood

REFERENCE

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

SAMPLE

Matrix: microsomal incubations
Sample preparation: Mix 1 mL microsomal incubation with 500 mg/mL ice-cold 200 mg/mL trichloroacetic acid solution and 500 μL 200 μg/mL IS, centrifuge at 9600 g for 10 min. Filter the supernatant (0.2 μm nylon syringe filter), inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 C18 NovaPak
Mobile phase: Gradient. MeCN:water from 3:97 to 35:65 over 6.4 min, return to initial condition over 0.6 min, re-equilibrate for 9 min
Flow rate: 2.0
Injection volume: 20
Detector: UV 195

CHROMATOGRAM

Retention time: 6.7
Internal standard: 3,3-dimethylglutarimide (6.2)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

liver; rat

REFERENCE

Sarver,J.G.; Bachmann,K.A.; Zhu,D.; Klis,W.A. Ethosuximide is primarily metabolized by CYP3A when incubated with isolated rat liver microsomes, *Drug Metab.Dispos.*, **1998**, *26*, 78–82.

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 213, 240

CHROMATOGRAM

Retention time: 2.0
Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES

Also analyzed: aspirin, caffeine, carbamazepine, chlordiazepoxide, chlorprothixene, clonazepam, diazepam, doxylamine, 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

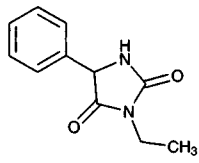
OTHER SUBSTANCES

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, 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, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

Ethotoin



Molecular formula: $C_{11}H_{12}N_2O_3$
Molecular weight: 204.23
CAS Registry No.: 86-35-1
Merck Index: 3795
Lednicer No.: 1 245

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL water + 1 mL phenytoin solution + 5 mL 100 mM trisodium phosphate + 10 mL ether, extract, centrifuge. Discard the ether layer and adjust the pH of the aqueous phase to 6.8 with 400 μL 2 M HCl, extract with 10 mL ether, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 250 μL MeCN, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 4 μBondapak C18
Mobile phase: MeCN:10 mM pH 4.4 sodium phosphate 30:70
Flow rate: 2.2
Injection volume: 10
Detector: UV 195

CHROMATOGRAM

Retention time: 2.5
Internal standard: phenytoin (6.3)

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Meyer,M.C.; Holcombe,B.J.; Burckart,G.J.; Raghow,G.; Yau,M.K. Nonlinear ethotoin kinetics, *Clin.Pharmacol.Ther.*, **1983**, *33*, 329–334.

SAMPLE

Matrix: blood
Sample preparation: 100 μL Serum + 1 mL saturated NaCl solution + 1 mL chloroform, vortex for 10 s, centrifuge. Remove the organic layer and evaporate it to dryness under vacuum at 50°, reconstitute the residue in 50 μL EtOH, inject a 25 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Chiralcel CA-1
Mobile phase: EtOH:water 95:5
Column temperature: 35
Flow rate: 0.5
Injection volume: 25
Detector: UV 254

CHROMATOGRAM

Retention time: 11.9 (d), 14.6 (l)

OTHER SUBSTANCES

Simultaneous: primidone, phenytoin
Interfering: carbamazepine, phenobarbital

KEY WORDS

serum; chiral; pharmacokinetics

REFERENCE
Inotsume,N.; Fujii,J.; Honda,M.; Nakano,M.; Higashi,A.; Matsuda,I. Stereoselective analysis of the enantiomers of ethotoin in human serum using chiral stationary phase liquid chromatography and gas chromatography-mass spectrometry, *J.Chromatogr.*, **1988**, *428*, 402–407.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 3 μL aliquot of a MeOH solution.

HPLC VARIABLES
Guard column: 50 × 2.1 Whatman Co:Pell ODS
Column: 125 × 4.5 5 μm 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: 6.7
Internal standard: heptabarbital (9.8)

OTHER SUBSTANCES
Simultaneous: amobarbital, barbital, butabarbital, carbamazepine, cyclobarbital, ethotoin, ethylphenacemide, glutethimide, methsuximide, pentobarbital, pheneturide, phenobarbital, phenylethylmalonamide, phenytoin, primidone, secobarbital, sulfamethoxazole, sulthiame

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: solutions

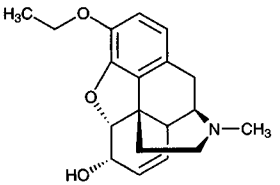
HPLC VARIABLES
Column: 100 × 4.6 3 μm 208HS3410 (Vydac)
Mobile phase: Gradient. MeCN:water from 15:85 to 60:40 over 10 min.
Flow rate: 1.5
Detector: UV 210 (?)

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Simultaneous: barbital, carbamazepine, diazepam, mephenytoin, methsuximide, phenacemide, phenobarbital, phensuximide

REFERENCE
Vydac HPLC Catalog, **1994**, p. 26.

Ethylmorphine



Molecular formula: $C_{19}H_{23}NO_3$
Molecular weight: 313.40
CAS Registry No.: 76-58-4, 6746-59-4 (HCl, dihydrate), 6696-59-9 (methiodide)
Merck Index: 3876
Lednicer No.: 1 287

SAMPLE

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

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 211.1

CHROMATOGRAM

Retention time: 8.735

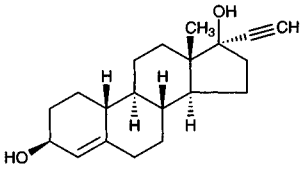
KEY WORDS

whole blood

REFERENCE

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

Ethynodiol



Molecular formula: $C_{20}H_{28}O_2$
Molecular weight: 300.44
CAS Registry No.: 1231-93-2, 297-76-7 (diacetate)
Merck Index: 3905
Lednicer No.: 1 165

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 or 280

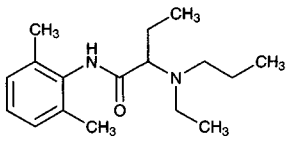
CHROMATOGRAM
Retention time: k' 22.65 (ethynodiol diacetate)
Internal standard: BHT (butylated hydroxytoluene) (k' 16.54)

OTHER SUBSTANCES
Simultaneous: mestranol, ethinyl estradiol, degradation products

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.

Etidocaine



Molecular formula: $C_{17}H_{28}N_2O$
Molecular weight: 276.42
CAS Registry No.: 36637-18-0
Merck Index: 3907
Lednicer No.: 2 95

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 1 M NaOH, vortex for 15 s, add 5 mL diethyl ether, shake on a reciprocating shaker for 10 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject an 80 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.2 10 μm μBondapak C18
Mobile phase: MeCN:50 mM pH 5.80 Na_2HPO_4 25:75
Flow rate: 0.9
Injection volume: 80
Detector: UV 210

CHROMATOGRAM
Retention time: 12.0
Internal standard: etidocaine

OTHER SUBSTANCES
Extracted: 2,6-pipecolylxylidine, lidocaine, bupivacaine, mepivacaine
Noninterfering: metabolites, 2,3-chloroprocaine, theophylline, mexiletine, quinidine, disopyramide, verapamil, phenobarbital, phenytoin, carbamazepine, ethosuximide, digoxin, theobromine, caffeine, furosemide, phenprocoumon, aldactone

KEY WORDS
plasma; etidocaine is IS

REFERENCE
Ha,H.-R.; Funk,B.; Gerber,H.R.; Follath,F. Determination of bupivacaine in plasma by high-performance liquid chromatography, *Anesth.Analg.*, **1984**, *63*, 448–450.

SAMPLE
Matrix: blood
Sample preparation: Condition a diol AASP SPE cartridge (Jones Chromatography) with 1 mL MeOH, 1 mL water, 1 mL mobile phase, 0.5 mL MeOH, 0.5 mL toluene. 200 μL Plasma + 100 μL 100 mM K_2HPO_4, mix, add 1 mL toluene, vortex for 1 min, centrifuge at 12000 g for 1 min. Add 750 μL of the toluene layer to the SPE cartridge, wash with 500 μL MeCN, elute the contents of the SPE onto the column with mobile phase (MeCN used for purge (6 strokes) and afterwash (10 strokes), valve reset time 2 min).

HPLC VARIABLES
Guard column: 20 × 2 30-40 μm Co:Pell ODS
Column: 150 × 4.6 Spherisorb 5 CN
Mobile phase: MeCN:water 40:60 containing 10 mM phosphoric acid
Flow rate: 1
Detector: E, Environmental Science Associates Model 5100A Coulochem, screen mode, electrode 1 +0.7 V, electrode 2 +0.9 V, palladium reference electrode, guard cell +1.2 V (before injection valve)

CHROMATOGRAM
Retention time: 10.8
Internal standard: etidocaine

OTHER SUBSTANCES
Extracted: prilocaine

KEY WORDS
plasma; SPE; etidocaine is IS

REFERENCE
Whelpton,R.; Dudson,P.; Cannell,H.; Webster,K. Determination of prilocaine in human plasma samples using high-performance liquid chromatography with dual-electrode electrochemical detection, *J.Chromatogr.*, **1990**, *526*, 215–222.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 1 M NaOH + 3 mL heptane:ethyl acetate 90:10, shake for 2 min, centrifuge at 1200 g for 10 min. Remove the organic phase and add it to 50 μL 50 mM sulfuric acid, shake for 2 min, centrifuge at 1200 g for 5 min. Remove the aqueous phase and add it to 820 μg sodium acetate, inject a 40 μL aliquot. (The sodium acetate was measured out by adding 50 μL 200 mM sodium acetate in MeOH to the tube and evaporating the MeOH.)

HPLC VARIABLES
Column: 250 × 4 10 μm μBondapak C18
Mobile phase: MeCN:10 mM NaH_2PO_4 20:80, adjusted to pH 2.1
Column temperature: 30
Flow rate: 1
Injection volume: 40
Detector: UV 205

CHROMATOGRAM
Retention time: 10
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: bupivacaine

KEY WORDS
plasma; rabbit

REFERENCE
Le Guévello,P.; Le Corre,P.; Chevanne,P.; Le Verge,R. High-performance liquid chromatographic determination of bupivacaine in plasma samples for biopharmaceutical studies and application to seven other local anaesthetics, *J.Chromatogr.*, **1993**, *622*, 284–290.

SAMPLE
Matrix: blood, urine
Sample preparation: 2 mL Whole blood, plasma, or urine + 1 mL saturated sodium carbonate + 20 μL 100 μg/mL dibucaine, add to a 3 mL Extrelut SPE cartridge, elute with 15 mL dichloromethane. Evaporate eluate to dryness under a stream of nitrogen at 40°, reconstitute in 100 μL 10 mM HCl, add 3 mL diethyl ether, vortex for 20 s, centrifuge at 2800 g for 5 min, inject a 40 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 5 × 6 μBondapak Guard Pak
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:100 mM ammonium acetate 50:50
Flow rate: 1.5
Injection volume: 40
Detector: UV 230

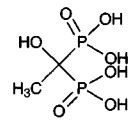
CHROMATOGRAM
Retention time: 14
Internal standard: dibucaine (18)
Limit of detection: 40 ng/mL

OTHER SUBSTANCES
Extracted: prilocaine, lidocaine, bupivacaine,
Also analyzed: procaine, butacaine, tetracaine, p-aminobenzoic acid, articaine, o-toluidine, caffeine, amphetamine, ephedrine, epinephrine, morphine, monoacetylmorphine, diamorphine, ethylmorphine, codeine, acetylcodeine

KEY WORDS
whole blood; plasma; SPE

REFERENCE
Rop,P.P.; Grimaldi,F.; Bresson,M.; Fornaris,M.; Viala,A. Liquid chromatographic analysis of cocaine, benzoylecgonine, local anaesthetic agents and some of their metabolites in biological fluids, *J.Liq.Chromatogr.*, **1993**, *16*, 2797–2811.

Etidronic acid



Molecular formula: $C_2H_8O_7P_2$
Molecular weight: 206.03
CAS Registry No.: 2809-21-4, 7414-83-7 (disodium salt)
Merck Index: 3908

SAMPLE
Matrix: formulations
Sample preparation: Dilute with water to a concentration of 400 μg/mL, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 75 × 4.6 6 μm IC-Pak HR anion-exchange (Waters)
Mobile phase: 7.2 mM nitric acid:7.2 mM potassium nitrate 40:60, pH 2.7

Flow rate: 0.8
Injection volume: 50
Detector: UV 240

CHROMATOGRAM
Retention time: 8.5
Limit of detection: 1000 ng/mL

KEY WORDS
injections; indirect UV detection; rugged

REFERENCE
Tsai,E.W.; Chamberlin,S.D.; Forsyth,R.J.; Bell,C.; Ip,D.P.; Brooks,M.A. Determination of bisphosphonate drugs in pharmaceutical dosage formulations by ion chromatography with indirect UV determination, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 983–991.

SAMPLE
Matrix: formulations
Sample preparation: Dilute injections 100-fold, inject a 20 μL aliquot. Disintegrate a 5 mg tablet in 100 mL water, sonicate for 5 min, centrifuge an aliquot at 3600 g for 4 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 10 μm IC-PAK Anion HC (Waters)
Mobile phase: 1.5 mM Nitric acid containing 0.5 mM copper(II) nitrate (Prepare column by pumping ILC Regenerant A (Waters) and 100 mM nitric acid for 30 min.)
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 245

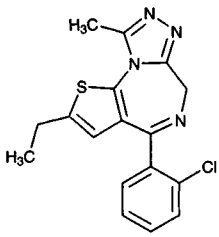
CHROMATOGRAM
Retention time: 5.8

OTHER SUBSTANCES
Simultaneous: alendronate, clodronate, neridronate, olpadronate, pamidronate

KEY WORDS
derivatization; complexation; injections; tablets

REFERENCE
Sparidans,R.W.; Den Hartigh,J.; Vermeij,P. High-performance ion-exchange chromatography with in-line complexation of bisphosphonates and their quality control in pharmaceutical preparations, *J.Pharm. Biomed.Anal.*, **1995**, *13*, 1545–1550.

Etizolam



Molecular formula: $C_{17}H_{15}ClN_4S$
Molecular weight: 342.85
CAS Registry No.: 40054-69-1
Merck Index: 3919

SAMPLE
Matrix: blood

Sample preparation: 500 μL Serum + 20 μL 20 μg/mL IS + 200 μL 1 M potassium carbonate + 3 mL chloroform, mix for 2 min, centrifuge at 1200 g for 5 min, aspirate aqueous phase. Evaporate the organic phase under a stream of nitrogen at 40°. Dissolve the residue in 100 μL mobile phase, inject a 20 μL aliquot. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES
Column: 100 × 4.6 2 μm TSK gel Super-ODS (A) or 100 × 4.6 5 μm Hypersil ODS-C18 (B)
Mobile phase: MeCN:5 mM pH 6 NaH_2PO_4 45:55
Flow rate: 0.65
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 18.4 (A), 67.1 (B)
Internal standard: diazepam (29.8 (A), 77.5 (B))
Limit of quantitation: 5 ng/mL (A)

OTHER SUBSTANCES
Extracted: bromazepam, chlordiazepoxide, clonazepam, estazolam, flutazolam, haloxazolam, lorazepam, nitrazepam, oxazolam, triazolam
Simultaneous: alprazolam
Noninterfering: barbital, carbamazepine, cloxazolam, ethosuximide, hexobarbital, mexazolam, oxazepam, pentobarbital, phenobarbital, phenytoin, primidone, trimethadione

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KEY WORDS
serum

REFERENCE
Tanaka,E.; Terada,M.; Misawa,.; Wakasugi,C. Simultaneous determination of twelve benzodiazepines in human serum using a new reversed-phase chromatographic column on a 2-μm porous microspherical silica gel, *J.Chromatogr.B*, **1996**, *682*, 173–178.

SAMPLE
Matrix: urine
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH, 10 mL dichloromethane:MeOH 9:1, 5 mL MeOH, and 10 mL water. Condition a Sep-Pak Silica SPE cartridge with 20 mL dichloromethane, 20 mL dichloromethane:MeOH 9:1, and 20 mL dichloromethane, then air dry. 10 mL Urine adjusted to pH 5 with acetic acid, add 2.5 mL 500 mM pH 5.0 acetate buffer, add 3000 U β-glucuronidase, incubate at 37° for 24 h, make alkaline with ammonia, centrifuge at 1200 g for 15 min, add the supernatant to the C18 SPE cartridge, wash with 5 mL water, wash with 5 mL MeOH:water 20:80, wash with 2 mL water, elute with 7 mL dichloromethane:MeOH 9:1. Evaporate the eluate to dryness under vacuum, dissolve the residue in 5 mL dichloromethane:MeOH 99:1, add to the silica SPE cartridge, wash with 20 mL dichloromethane, wash with 25 mL dichloromethane:MeOH 99:1, elute with 20 mL dichloromethane:MeOH 9:1. Evaporate the eluate to dryness under vacuum, dissolve the residue in 100 μL mobile phase, inject a 20 μL aliquot. (MeOH for silica SPE cartridge was distilled and dried over 3 Å molecular sieve, then 0.1% water added just before use.)

HPLC VARIABLES
Column: 100 × 8 10 μm Radial-Pak C18
Mobile phase: MeOH:10 mM pH 8.0 phosphate buffer 65:35
Flow rate: 1
Injection volume: 20
Detector: UV 220

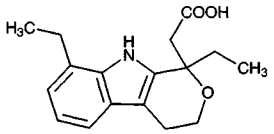
CHROMATOGRAM
Retention time: 17
Internal standard: etizolam

OTHER SUBSTANCES
Simultaneous: triazolam

KEY WORDS
SPE; etizolam is IS

REFERENCE
Inoue,T.; Suzuki,S.-I. High-performance liquid chromatographic determination of triazolam and its metabolites in human urine, *J.Chromatogr.*, **1987**, *422*, 197–204.

Etodolac



Molecular formula: $C_{17}H_{21}NO_3$
Molecular weight: 287.36
CAS Registry No.: 41340-25-4
Merck Index: 3920

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

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 225.2

CHROMATOGRAM
Retention time: 21.503

KEY WORDS
whole blood

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

SAMPLE
Matrix: cell suspensions
Sample preparation: Centrifuge cell suspension at 2000 g for 4 min. Remove a 2 mL aliquot of the supernatant and add it to 200 μL 100 μg/mL IS in DMF, mix, add 200 μL 5 M HCl, extract twice with 3 mL portions of toluene. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL dichloromethane, add 20 μL 10 mg/mL 1-hydroxybenzotriazole in dichloromethane:pyridine 99:1, add 300 μL 10 mg/mL 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in dichloromethane, add 300 μL 10 mg/mL (-)-(S)-α-methylbenzylamine in dichloromethane, let stand for 30 min, evaporate to dryness, reconstitute with 500 μL mobile phase, inject a 10 μL aliquot.

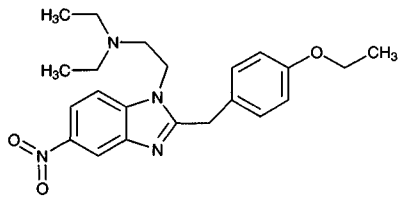
HPLC VARIABLES
Guard column: 10 mm long Techsphere ODS (HPLC Technology, Macclesfield UK)
Column: 250 × 5 5 μm Techsphere ODS (HPLC Technology, Macclesfield UK)
Mobile phase: MeCN:7.5 mM NaH_2PO_4 65:35, containing 5 mM sodium pentanesulfonate, pH adjusted to 2.8 with phosphoric acid
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 5.67, 6.08 (enantiomers)
Internal standard: (R)-ibuprofen (k' 5.21)
Limit of detection: 1 μg/mL

KEY WORDS
derivatization; chiral

REFERENCE
Thomason,M.J.; Hung,Y.-F.; Rhys-Williams,W.; Hanlon,G.W.; Lloyd,A.W. Indirect enantiomeric separation of 2-arylpropionic acids and structurally related compounds by reversed phase HPLC, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1765–1774.

Etonitazene



Molecular formula: $C_{22}H_{28}N_4O_3$
Molecular weight: 396.49
CAS Registry No.: 911-65-9
Merck Index: 3929
Lednicer No.: 1 325

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

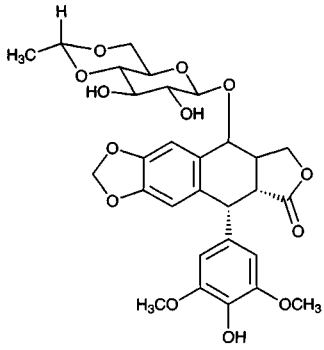
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, 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, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fen-

proporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

Etoposide



Molecular formula: $C_{29}H_{32}O_{13}$
Molecular weight: 588.57
CAS Registry No.: 33419-42-0, 117091-64-2 (phosphate)
Merck Index: 3931

SAMPLE

Matrix: blood
Sample preparation: Mix 500 µL serum, pleural effusion, or urine with 100 µL IS solution and 400 µL pH 9.0 ammonium acetate. Add to an Extrelut-1 SPE cartridge. After 15 min, elute with 3 mL and 4 mL portions of dichloromethane. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue with 100 µL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 µm LiChrospher 100 CN
Mobile phase: MeCN:100 mm pH 3.5 acetate buffer 30:70
Column temperature: 40
Flow rate: 1

Injection volume: 20
Detector: F ex 290 em 365

CHROMATOGRAM
Retention time: 4.5
Internal standard: BRL 43693A (Smith Kleine Beecham) (9.5)

OTHER SUBSTANCES
Extracted: granisetron
Also analyzed: domperidone, metoclopramide, ondansetron
Noninterfering: cisplatin, carboplatin, dexamethasone

KEY WORDS
serum; SPE; pharmacokinetics

REFERENCE
Wada,I.; Satoh,M.; Takeda,T.; Nakabayashi,T.; Honma,T.; Saitoh,H.; Takada,M.; Hirano,K. A rapid assay of granisetron in biological fluids from cancer patients, *Biol.Pharm.Bull.*, **1998**, *21*, 535–537.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL 500 mg Bond-Elut PH SPE cartridge with 6 mL MeOH and 3 mL 20 mM pH 5.5 ammonium acetate. Mix 500 μL serum with 500 μL 20 mM pH 5.5 ammonium acetate, 50 μL 760 mM sodium dodecyl sulfate and 50 μL 800 ng/mL IS in MeOH. Add to the SPE cartridge, wash with 3 mL 20 mM ammonium acetate and 3 mL MeOH:water 10:90. Elute with 2 mL MeOH, evaporate to dryness at 43° under reduced pressure, reconstitute in 150 μL MeOH:water 36:64.

HPLC VARIABLES
Column: 300 × 3.9 Bondclone 10 C18 (Phenomenex, Torrance, CA, USA)
Mobile phase: MeOH:40 mM pH 6.9 KH_2PO_4:0.14 mM 1-heptanesulfonic acid 40:60:0.6
Flow rate: 2
Detector: F ex 230 em 330

CHROMATOGRAM
Retention time: 14
Internal standard: podofilox (28)
Limit of detection: 200 ng/mL
Limit of quantitation: 500 ng/mL

KEY WORDS
serum; SPE; pharmacokinetics

REFERENCE
Manouilov,K.K.; McGuire,T.R.; Gordon,B.G.; Gwilt,P.R. Assay for etoposide in human serum using solid-phase extraction and high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.B*, **1998**, *707*, 342–346.

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL plasma with 200 μL 500 ng/mL 2-acetamidophenol in water. Add 4 mL 500 mM pH 7.2 NaH_2PO_4, add with 5 mL ethyl ether:dichloromethane 2:1, vortex for 1 min. Centrifuge at 4500 rpm for 5 min. Evaporate the organic layer to dryness under a stream of nitrogen at 45°, reconstitute with 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 40 × 4 35-50 μm Corasil C18
Column: 150 × 3.9 4 μm Nova-Pak C8
Mobile phase: MeOH:75 mM pH 3.8 acetate buffer 45:55
Flow rate: 1
Injection volume: 100

Detector: E, Bioanalytical Systems LC-4B, glassy carbon electrode +800 mV, Ag/AgCl reference electrode

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CHROMATOGRAM
Retention time: 5.6
Internal standard: 2-acetomidophenol (3.0)
Limit of detection: 5 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Pérez-Urizar,J.; Picazo,Y.F.; Navarro-González,B.; Flores-Murrieta,F.J.; Castañeda-Hernández,G. A new rapid and economical high performance liquid chromatographic assay with electrochemical detection for the determination of etoposide (VP-16) in human plasma samples, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 939–947.

SAMPLE
Matrix: blood
Sample preparation: Filter 1 mL plasma (Centrifree micropartition device, molecular mass cut-off 30000, Amicon, USA) using a 33° fixed angle centrifuge (Beckman ModelG56R) at 2000 g and 25° for 30 min. To the ultrafiltrate add 50 μL 10 μg/mL teniposide in MeOH and 1 mL chloroform (Caution! Chloroform is a carcinogen!), agitate slowly for 20 min, centrifuge at 1000 g for 5 min. Evaporate the organic phase to dryness under vacuum at 40°, dissolve the dry extract in 50 μL MeOH, inject a 25 μL aliquot. Alternatively, add 10 μg teniposide and 8 mL chloroform (Caution! Chloroform is a carcinogen!) to 1 mL plasma, agitate slowly for 20 min, centrifuge at 1000 g for 5 min, evaporate the organic phase to dryness, dissolve the dry extract in 100 μL MeOH, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm 125 Å μBondapak Phenyl (Waters)
Mobile phase: MeCN:water:glacial acid 35:64:1
Flow rate: 1
Injection volume: 25
Detector: F ex 288 em 328

CHROMATOGRAM
Retention time: 6.5
Internal standard: teniposide (18)
Limit of detection: 10 ng/mL
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Noninterfering: alizapride, doxorubicin, furosemide, idarubicin, ranitidine, vinblastine, vinorelbine

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KEY WORDS
plasma; ultrafiltrate

REFERENCE
Robieux,I.; Aita,P.; Sorio,R.; Toffoli,G.; Boiocchi,M. Determination of unbound etoposide concentration in ultrafiltered plasma by high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.B*, **1996**, *686*, 35–41.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 150 × 3.9 5 μm Symmetry C8
Mobile phase: MeCN:20 mM NaH_2PO_4 27:73
Flow rate: 1

Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6.5

OTHER SUBSTANCES
Simultaneous: idarubicin

KEY WORDS
0.9% NaCl; injections

REFERENCE
Zhang,H.; Ye,L.; Stewart,J.T. HPLC determination of idarubicin-etoposide and idarubicin-ondansetron mixtures in 0.9% sodium chloride injection USP, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 979–988.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm 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

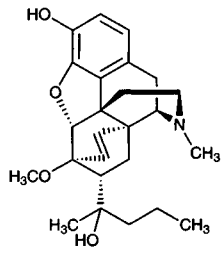
CHROMATOGRAM
Retention time: 6.19

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.

Etorphine



Molecular formula: $C_{25}H_{33}NO_4$
Molecular weight: 411.54
CAS Registry No.: 14521-96-1
Merck Index: 3932
Lednicer No.: 2 321

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

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica

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

CHROMATOGRAM

Retention time: 1.4

OTHER SUBSTANCES

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

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

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

Detector: UV 210

OTHER SUBSTANCES

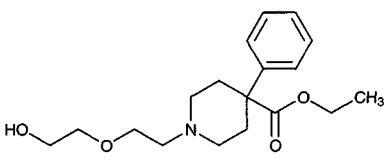
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine,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

REFERENCE

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

Etoxeridine

Molecular formula: $C_{18}H_{27}NO_4$
Molecular weight: 321.42
CAS Registry No.: 469-82-9



SAMPLE

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

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 2.3

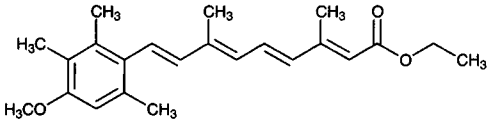
OTHER SUBSTANCES

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

REFERENCE

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

Etretinate



Molecular formula: $C_{23}H_{30}O_3$
Molecular weight: 354.49
CAS Registry No.: 54350-48-0
Merck Index: 3935

SAMPLE

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

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 21.5
Internal standard: retinyl acetate (25.0)
Limit of quantitation: 160 ng/mL

OTHER SUBSTANCES

Extracted: acitretin, cis-acitretin, metabolites

KEY WORDS

rat; liver; plasma; protect from light

REFERENCE

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

SAMPLE

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

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 32

Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Extracted: 13-cis-acitretin, tretinoin, 4-oxo-13-cis-retinoic acid, isotretinoin, acitretin
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 μL Plasma + 10 μL 9.8 μg/mL retinyl palmitate in MeOH + 1.5 mL EtOH + 500 μL 2 M HCl, vortex for 30 s, add 5 mL water, vortex for 30 s, add 7.5 mL n-hexane, rotate for 15 min, centrifuge at 1500 g for 6 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 150 μL mobile phase, inject a 50 μL aliquot.

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

CHROMATOGRAM
Retention time: 6.5
Internal standard: retinyl palmitate (6)
Limit of quantitation: 3 ng/mL

OTHER SUBSTANCES
Extracted: 13-cis acitretin, all-trans-acitretin

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

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

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

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

CHROMATOGRAM
Retention time: 3.2

Internal standard: retinoic acid (5.2)
Limit of quantitation: 3 ng/mL

OTHER SUBSTANCES
Extracted: acitretin, isoacitretin

KEY WORDS
plasma; rat; normal phase; pharmacokinetics

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

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

HPLC VARIABLES
Column: A 14 × 4.6 37-50 μm Bondapak C18 Corasil (column fitted with 3 μm sieves not glass fiber filters); B 30 × 4 5 μm Spherisorb ODS 1 + 125 × 4 5 μm Spherisorb ODS 1
Mobile phase: A MeCN:1% ammonium acetate 10:90; B Gradient. A was MeCN:water:10% ammonium acetate:acetic acid 600:400:4:30. B was MeCN:water:10% ammonium acetate:acetic acid 850:146:4:10. A:B 100:0 to 0:100 over 8 min, stay at 0:100 for 11 min.
Flow rate: A 1.5; B 1
Injection volume: 500
Detector: UV 360

CHROMATOGRAM
Retention time: 21
Internal standard: Ro 12-7554 (ethyl all-trans-9-(2,6-dichloro-4-methoxy-m-tolyl)-3,7-dimethyl-2,4,6,8-nonatetraenoate) (24) and isotretinoin (17)
Limit of detection: 0.5-1 ng/mL
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES
Simultaneous: acitretin, 13-cis-acitretin, metabolites

KEY WORDS
plasma; column-switching

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

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

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

CHROMATOGRAM
Retention time: 25.69

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

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

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

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

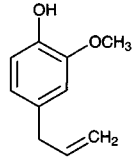
CHROMATOGRAM
Internal standard: retinyl acetate

OTHER SUBSTANCES
Extracted: acitretin

KEY WORDS
do not use PTFE or plastic; rat

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

Eugenol



Molecular formula: $C_{10}H_{12}O_2$
Molecular weight: 164.20
CAS Registry No.: 97-53-0
Merck Index: 3944

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

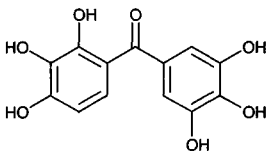
OTHER SUBSTANCES

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, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

Exifone



Molecular formula: $C_{13}H_{10}O_7$
Molecular weight: 278.22
CAS Registry No.: 52479-85-3
Merck Index: 3958

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 1 mL Plasma + 1 mL 100 mM citric acid + 8 mL diethyl ether, shake for 10 min, centrifuge at 4° at 2400 g for 5 min. Remove the organic layer and evaporate

it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot. Urine. 1 mL Urine + 1 mL pH 7 phosphate buffer (Normadose, Prolabo) + 8 mL diethyl ether, shake for 10 min, centrifuge at 4° at 2400 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 250 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: Guard-Pack C18 (Waters)
Column: 100 × 5 4 μm Novapak C18 RCM 8x10
Mobile phase: MeCN:300 mM orthophosphoric acid 15:85, final pH 2.2
Flow rate: 0.9
Injection volume: 20
Detector: E, Environmental Sciences Model 5100 A, ESA 5011 analytical cell, oxidative mode, cell II +0.30 V, cell I and guard cell not used

CHROMATOGRAM
Retention time: 8
Limit of quantitation: 1 nM

KEY WORDS
plasma; silanize all glassware; pharmacokinetics

REFERENCE
Descombe,J.-J.; Doumont,G.; Picard,M. Determination of exifone in human plasma and urine by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1989**, *496*, 345–353.

Factor VIII

CAS Registry No.: 9013-56-3
Merck Index: 3963

SAMPLE
Matrix: solutions
Sample preparation: Add 3 mL Factor VIII solution in water to a column of Sephadex G-25 (Pharmacia), elute with buffer, monitor at UV 280, all protein elutes in void volume, inject a 10 mL aliquot. (Buffer was 50 mM imidazole HCl, 150 mM NaCl, 0.02% sodium azide, pH 7.0.)

HPLC VARIABLES
Guard column: 75 × 25 37 mL TSK 6000PW (Toyo Soda)
Column: 600 × 25 300 mL bead size 17 μm TSK 5000PW (Toyo Soda)
Mobile phase: 50 mM imidazole HCl, 150 mM NaCl, 0.02% sodium azide, pH 7.0
Flow rate: 8.5
Injection volume: 10000
Detector: UV 254 or bioassay

CHROMATOGRAM
Retention time: 12

KEY WORDS
Preparative; SEC

REFERENCE
Herring,S.W.; Shitanishi,K.T.; Moody,K.E.; Enns,R.K. Isolation of human factor VIII:C by preparative high-performance size-exclusion chromatography, *J.Chromatogr.*, **1985**, *326*, 217–224.

SAMPLE
Matrix: solutions

it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot. Urine. 1 mL Urine + 1 mL pH 7 phosphate buffer (Normadose, Prolabo) + 8 mL diethyl ether, shake for 10 min, centrifuge at 4° at 2400 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 250 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: Guard-Pack C18 (Waters)
Column: 100 × 5 4 μm Novapak C18 RCM 8x10
Mobile phase: MeCN:300 mM orthophosphoric acid 15:85, final pH 2.2
Flow rate: 0.9
Injection volume: 20
Detector: E, Environmental Sciences Model 5100 A, ESA 5011 analytical cell, oxidative mode, cell II +0.30 V, cell I and guard cell not used

CHROMATOGRAM
Retention time: 8
Limit of quantitation: 1 nM

KEY WORDS
plasma; silanize all glassware; pharmacokinetics

REFERENCE
Descombe,J.-J.; Doumont,G.; Picard,M. Determination of exifone in human plasma and urine by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1989**, *496*, 345–353.

Factor VIII

CAS Registry No.: 9013-56-3
Merck Index: 3963

SAMPLE
Matrix: solutions
Sample preparation: Add 3 mL Factor VIII solution in water to a column of Sephadex G-25 (Pharmacia), elute with buffer, monitor at UV 280, all protein elutes in void volume, inject a 10 mL aliquot. (Buffer was 50 mM imidazole HCl, 150 mM NaCl, 0.02% sodium azide, pH 7.0.)

HPLC VARIABLES
Guard column: 75 × 25 37 mL TSK 6000PW (Toyo Soda)
Column: 600 × 25 300 mL bead size 17 μm TSK 5000PW (Toyo Soda)
Mobile phase: 50 mM imidazole HCl, 150 mM NaCl, 0.02% sodium azide, pH 7.0
Flow rate: 8.5
Injection volume: 10000
Detector: UV 254 or bioassay

CHROMATOGRAM
Retention time: 12

KEY WORDS
Preparative; SEC

REFERENCE
Herring,S.W.; Shitanishi,K.T.; Moody,K.E.; Enns,R.K. Isolation of human factor VIII:C by preparative high-performance size-exclusion chromatography, *J.Chromatogr.*, **1985**, *326*, 217–224.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 100 × 1 Mono Q gel HR 10/10 (Pharmacia)
Mobile phase: Gradient. 20 mM Tris HCl containing 50 mM calcium chloride, pH 6.8 with a linear gradient up to 600 mM NaCl
Flow rate: 2
Detector: UV 280

CHROMATOGRAM
Retention time: 50

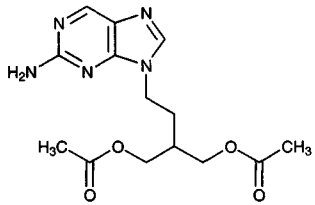
REFERENCE
Andersson,L.O.; Forsman,N.; Huang,K.; Larsen,K.; Lundin,A.; Pavlu,B.; Sandberg,H.; Sewerin,K.; Smart,J. Isolation and characterization of human factor VIII: molecular forms in commercial factor VIII concentrate, cryoprecipitate, and plasma, *Proc.Nat.Acad.Sci.USA*, **1986**, *83*, 2979–2983.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in water, dilute with mobile phase, centrifuge at 10000 g for 10 min, filter (Millex 0.22 μm SLGVO25 BS), inject a 50-500 aliquot.

HPLC VARIABLES
Column: HR10/30 Superose 6 (Pharmacia)
Mobile phase: 20 mM Tris and 20 mM citric acid buffer, pH 7.4 containing 400 mM NaCl (A) or 20 mM pH 7.4 triethanolamine buffer containing 150 mM NaCl (B)
Flow rate: 0.5 (A) or 0.3 (B)
Injection volume: 50-500
Detector: UV 280 or bioassay

REFERENCE
Dawes,J.; Freeman,L.; Dawson,N.J.; Pepper,D.S.; Barrowcliffe,T.W. High molecular weight aggregate content of heated and unheated factor VIII products determined by fast-protein liquid chromatography, *Vox Sang.*, **1990**, *58*, 30–34.

Famciclovir



Molecular formula: $C_{14}H_{19}N_5O_4$
Molecular weight: 321.34
CAS Registry No.: 104227-87-4
Merck Index: 3971

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

HPLC VARIABLES
Guard column: C18 Guard-Pak (Waters)
Column: 100 × 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: 13.5 (as penciclovir, the active metabolite)

Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Extracted: acyclovir

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, urine
Sample preparation: Condition a Bond Elut SCX strong cation exchange SPE cartridge with 1 mL MeOH, 1 mL water, and 1 mL 1 mM pH 7.0 Na_2HPO_4 buffer. 1 mL 500 μL Plasma or 100 μL urine + 100 μL 20-50 μg/mL IS in water + 100-500 μL 16% trichloroacetic acid, add the supernatant to the SPE cartridge, wash with 1 mL MeOH:1 mM pH 7.0 Na_2HPO_4 buffer 20:80, elute with 1 mL MeOH:100 mM pH 11.0 K_2HPO_4 buffer 25:75, inject an aliquot.

HPLC VARIABLES
Column: 3 μm Apex 1 ODS
Mobile phase: Gradient. A was MeOH:10 mM pH 7.0 Na_2HPO_4 buffer 7:93. B was MeOH:10 mM pH 7.0 Na_2HPO_4 buffer 35:65. A:B from 100:0 to 0:100 over 4 min, maintain at 0:100 for 1.5 min, return to 0:100 over 1 min.
Flow rate: 2
Detector: UV 305

CHROMATOGRAM
Retention time: 6
Internal standard: 6-deoxy-9-(4-hydroxy-2-hydroxymethylbut-1-yl)guanine (BRL 44056) (2.8)
Limit of detection: 2000 ng/mL (urine), 500 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: penciclovir, metabolites

KEY WORDS
plasma; human; rat; dog; SPE

REFERENCE
Winton,C.F.; Fowles,S.E.; Pierce,D.M.; Hodge,A.V. Gradient high-performance liquid chromatographic method for the analysis of the pro-drug famciclovir and its metabolites, including the active anti-viral agent penciclovir, in plasma and urine, *Anal.Proc.*, **1990**, *27*, 181–182.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Mix 200 μL microsomal incubation with 200 μL MeOH, centrifuge for 3-5 min in a bench-top microcentrifuge, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: μBondapak C18 Guard-Pack
Column: 250 × 4.6 5 μm Spherisorb ODS 2
Mobile phase: MeCN:0.5 mM pH 4.6 ammonium acetate buffer 9:91
Flow rate: 1.5
Detector: UV 280

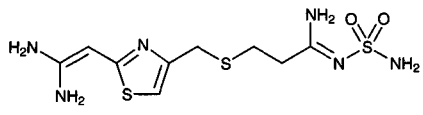
CHROMATOGRAM
Retention time: 20
Limit of quantitation: 10 μM

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
human; guinea pig; rabbit; rat; liver

REFERENCE
Rashidi,M.R.; Smith,J.A.; Clarke,S.E.; Beedham,C. In vitro oxidation of famciclovir and 6-deoxypenciclovir by aldehyde oxidase from human, guinea pig, rabbit, and rat liver, *Drug Metab.Dispos.*, **1997**, *25*, 805–813.

Famotidine



Molecular formula: $C_8H_{15}N_7O_2S_3$
Molecular weight: 337.45
CAS Registry No.: 76824-35-6
Merck Index: 3972
Lednicer No.: 2 37

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

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

CHROMATOGRAM
Retention time: 3.487

KEY WORDS
whole blood

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

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

HPLC VARIABLES
Column: Nova Pak C18
Mobile phase: MeCN:0.1% acetic acid:10 mM pH 7.8 $(NH_4)H_2PO_4$ 10:23:74
Flow rate: 1
Injection volume: 20
Detector: UV 300

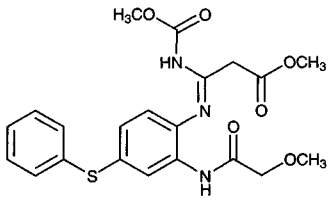
CHROMATOGRAM
Retention time: 13.9

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.

Febantel



Molecular formula: $C_{20}H_{22}N_4O_6S$
Molecular weight: 446.48
CAS Registry No.: 58306-30-2
Merck Index: 3982
Lednicer No.: 4 35

SAMPLE
Matrix: blood
Sample preparation: 4 mL Plasma + 2 mL pH 7.4 phosphate buffer + 20 mL diethyl ether, shake mechanically for 10 min, place in a freezer for 30 min, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 45°, reconstitute the residue in 200 μL DMF, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm RP 8
Mobile phase: Gradient. MeCN:1% phosphoric acid 20:80 to 60:40 over 10 min
Column temperature: 30
Flow rate: 2
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: 10.97
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; sheep

REFERENCE
Delatour,P.; Tiberghien,M.P.; Besse,S. An HPLC procedure for the quantification of five metabolites of febantel in sheep serum, *J.Vet.Pharmacol.Ther.*, **1983**, *6*, 233–235.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 100 μL 10 μg/mL albendazole in MeOH + 200 μL 500 mM ammonium hydroxide (to adjust pH to 11) + 200 mg NaCl + 5 mL distilled diethyl ether, roll for 15 min, remove 4 mL supernatant, repeat extraction, remove 5 mL supernatant. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 60 μL MeOH, sonicate for 2 min, inject a 20 μL aliquot.

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

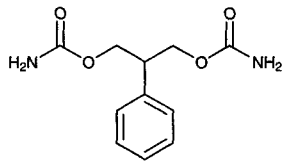
CHROMATOGRAM
Retention time: 6.2
Internal standard: albendazole (1.6)
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Extracted: fenbendazole, oxfendazole, oxfendazole sulfone

KEY WORDS
plasma; sheep

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

Felbamate



Molecular formula: $C_{11}H_{14}N_2O_4$
Molecular weight: 238.24
CAS Registry No.: 25451-15-4
Merck Index: 3988

SAMPLE
Matrix: CSF
Sample preparation: Dilute CSF with an equal volume of a 10 μg/mL solution of IS in MeCN: 30 mM pH 3.0 phosphate buffer 20:80, vortex, centrifuge at about 2000 g at 0° for 10 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 3 μm Spherisorb ODS2
Mobile phase: MeCN:MeOH:30 mM pH 3.0 KH_2PO_4 12:6:82
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 10.5
Internal standard: 2-phenyl-3-carbamoyl-3-oxypropyl allophanate (14.5)
Limit of quantitation: 195 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat

REFERENCE
Romanyshyn,L.A.; Wichmann,J.K.; Kucharczyk,N.; Sofia,R.D. Simultaneous determination of felbamate and four metabolites in rat cerebrospinal fluid by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *622*, 223–228.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma or serum + 100 μL 100 μg/mL IS in MeCN, vortex, let stand for 10 min. Centrifuge at 15000 rpm for 5 min. Mix the supernatant with an equal volume of water and inject an aliquot.

HPLC VARIABLES
Column: 40 × 3.2 3 μm Brownlee VeloSep RP-18
Mobile phase: Gradient. A was MeCN. B was water. A:B from 0:100 to 22:78 over 11 min, to 100:0 over 1.5 min, maintain at 100:0 for 0.5 min, to 0:100 over 1 min, maintain at 0:100 for 6 min.
Flow rate: 0.8 for 11 min, to 1.5 over 2 min, to 0.8 over 6 min, hold at 0.8 for 1 min
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 9.2
Internal standard: 2-methyl-2-phenyl-1,3-propanediol dicarbamate (10.8)

OTHER SUBSTANCES
Extracted: carbamazepine, carbamazepine-10,11-epoxide, clonazepam, ethosuximide, hexobarbital, ibuprofen, phenytoin, valproic acid
Simultaneous: phenobarbital, primidone

KEY WORDS
plasma; serum

REFERENCE
Behnke,C.E.; Reddy,M.N. Determination of felbamate concentration in pediatric samples by high-performance liquid chromatography, *Ther.Drug Monit.*, **1997**, *19*, 301–306.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 500 μL 1 M pH 5.0 sodium acetate buffer + 50 μL 400 μg/mL W-509 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: 3.7
Internal standard: W-509 (4.7)

Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Simultaneous: phenytoin, carbamazepine, 5-(p-hydroxyphenyl)-5-phenylhydantoin, cyheptamide, carbamazepinediol, carbamazepine-10,11-epoxide, metabolites
Also analyzed: ethosuximide, primidone, phenobarbital, ethotoin, lorazepam, phenylethylmalonamide

KEY WORDS

plasma

REFERENCE

Remmel,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: 100 μL Plasma + 200 μL 40 μg/mL IS in MeCN, vortex, centrifuge at 2500 g at 0° for 15 min, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 150 × 4.6 3 μm Spherisorb ODS2
Mobile phase: MeCN:buffer 25:75 (Buffer was 2.2 g K_2HPO_4 in 740 mL water, adjust pH to 6.50 with phosphoric acid, make up to 750 mL with water.)
Column temperature: 40
Flow rate: 0.7
Injection volume: 10
Detector: UV 210

CHROMATOGRAM

Retention time: 5.4
Internal standard: 2-methyl-2-phenyl-1,3-propanediol dicarbamate (7.3)
Limit of quantitation: 150 ng/mL

KEY WORDS

plasma; dog

REFERENCE

Clark,L.A.; Wichmann,J.K.; Kucharczyk,N.; Sofia,R.D. Determination of the anticonvulsant felbamate in beagle dog plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *573*, 113–119.

SAMPLE

Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL 40 μg/mL IS in MeOH:water 4:96 + 1 mL 1 M NaOH saturated with ammonium sulfate, vortex, add 8 mL MTBE:chloroform 75:25, rotate for 45 min, centrifuge at 2000 g at 0° for 15 min. Remove 7-7.5 mL of the organic layer and evaporate it to dryness under vacuum at 50°, reconstitute the residue in 200 μL mobile phase, vortex, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 3 μm Spherisorb ODS2
Mobile phase: MeCN:20 mM pH 6.8 $(NH_4)_2HPO_4$ 15:85
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: UV 210

CHROMATOGRAM

Retention time: 10.9
Internal standard: 2-phenyl-3-carbamoyl-3-oxypropyl allophanate (18.5)

Limit of quantitation: 195 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; rat; dog

REFERENCE
Romanyshyn,L.A.; Wichmann,J.K.; Kucharczyk,N.; Sofia,R.D. Simultaneous determination of felbamate and three metabolites in rat and dog plasmas by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *622*, 229–234.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 200 μL 12 μg/mL acetoacetanilide in MeCN, centrifuge at 14000 g for 15 s, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 125 × 4 5 μm C8 (Merck)
Mobile phase: MeCN:20 mM pH 6.1 phosphate buffer 12.5:87.5
Flow rate: 1.8
Injection volume: 50
Detector: UV 205

CHROMATOGRAM
Retention time: 12.5
Internal standard: acetoacetanilide (8.4)
Limit of detection: 3000 ng/mL

OTHER SUBSTANCES
Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, carbamazepine, digoxin, disopyramide, ethosuximide, lidocaine, phenobarbital, phenytoin, primidone, procainamide, quinidine, salicylic acid, theophylline, valproic acid, vancomycin

KEY WORDS
serum; comparison with capillary electrophoresis

REFERENCE
Shihabi,Z.K.; Oles,K.S. Felbamate measured in serum by two methods: HPLC and capillary electrophoresis, *Clin.Chem.*, **1994**, *40*, 1904–1908.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 50 μL 330 μg/mL IS in MeOH + 3 mL dichloromethane, rotate for 5 min, centrifuge for 5 min. Remove the organic layer and evaporate it to dryness under a stream of air at 40°, reconstitute the residue in 200 μL mobile phase, inject a 35 μL aliquot. Alternatively, condition a 6 mL C18 SPE cartridge (J.T.Baker) with 2 mL MeOH, 2 mL water, 1 mL 1 M KOH, and 1 mL water. 500 μL Serum + 50 μL 330 μg/mL IS in MeOH, add to the SPE cartridge, wash with 1 mL 100 mM pH 7.4 phosphate buffer, wash with 1 mL water, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 35 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax C18
Mobile phase: MeCN:MeOH:THF:buffer 5.25:10.25:12.5:72 (Buffer was 17 g KH_2PO_4 in 250 mL water, pH 7.4.)
Flow rate: 1.5
Injection volume: 35
Detector: UV 254

CHROMATOGRAM
Retention time: 4.98
Internal standard: 2-methyl-2-phenyl-1,3-propanediol dicarbamate (methyl felbamate) (7.0)
Limit of quantitation: 5 μg/mL

OTHER SUBSTANCES
Simultaneous: carbamazepine, carbamazepine epoxide, phenytoin
Noninterfering: amobarbital, butabarbital, butalbital, caffeine, lidocaine, pentobarbital, phenobarbital, primidone, secobarbital, theophylline

KEY WORDS
serum; SPE; valproic acid; comparison with GC

REFERENCE
Gur,P.; Poklis,A.; Saady,J.; Costantino,A. Chromatographic procedures for the determination of felbamate in serum, *J.Anal.Toxicol.*, **1995**, *19*, 499–503.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma or serum + 200 μL IS in MeCN, mix, let stand. centrifuge. Remove the supernatant and wash it with hexane, centrifuge, inject a aliquot.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:phosphate buffer 20:80
Detector: UV 214

CHROMATOGRAM
Retention time: 3.0
Internal standard: W509 (4.5)
Limit of quantitation: 10 μg/mL

OTHER SUBSTANCES
Simultaneous: phenobarbital

KEY WORDS
serum; plasma

REFERENCE
Wong,S.H.Y.; Sasse,E.; Schroeder,J.; Rodgers,J.; Pearson,L.; Neicheril,J.; Radewahn,K.; Morris,G.L. Felbamate monitoring by reversed-phase and automated prestation liquid chromatography (Abstract 201), *Ther.Drug Monit.*, **1995**, *17*, 433–433.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax Tissuemizer) with four volumes of water for 1 min. 100 μL Homogenate + 100 μL 12.5 μg/mL IS in mobile phase + 2 mL water, vortex for 1 min, centrifuge at 600 g for 10 min. 2 mL Supernatant + 8 mL ethyl acetate, vortex for 1 min, centrifuge at 600 g for 10 min. Remove the organic layer and evaporate it to dryness under vacuum at about 50°, reconstitute the residue in 100 μL mobile phase, inject an 80 μL aliquot.

HPLC VARIABLES
Guard column: Resolve C18 (Waters)
Column: 300 × 3.9 5 μm Resolve C18 (Waters)
Mobile phase: MeCN:MeOH:buffer 15:5:80 (Buffer was 2.3 g $K_2HPO_4.3H_2O$ in 950 mL water, adjust pH to 6.8 with phosphoric acid, make up to 1 L with water.)
Column temperature: 50
Flow rate: 1
Injection volume: 80
Detector: UV 210

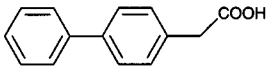
CHROMATOGRAM
Retention time: 11
Internal standard: 2-methyl-2-phenyl-1,3-propanediol dicarbamate (18)
Limit of quantitation: 195 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; brain; heart; pharmacokinetics

REFERENCE
Jacala,A.; Adusumalli,V.E.; Kucharczyk,N.; Sofia,R.D. Determination of the anticonvulsant felbamate and its three metabolites in brain and heart tissue of rats, *J.Chromatogr.*, **1993**, *614*, 285–292.

Felbinac



Molecular formula: $C_{14}H_{12}O_3$
Molecular weight: 212.25
CAS Registry No.: 5728-52-9
Merck Index: 3989
Lednicer No.: 4 32

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL plasma with 20 μL 20 μg/mL IS in MeOH. Vortex for 1 min with dichloromethane:diethyl ether 80:20, centrifuge at 1000 g for 10 min, separate the organic layer. Add 4 mL dichloromethane-diethyl ether 80:20, repeat the same extraction procedure twice, evaporate the organic phase to dryness under a stream of nitrogen, add 100 μL 10 mM NaOH to the residue, shake for 5 min, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 10 μm Vydac AXGU
Column: 250 × 4.6 5 μm Adsorbosphere SAX
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 10:90
Flow rate: 1.2
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 6.8
Internal standard: 2-[4-(2-furoyl)phenyl]propionic acid (3.9)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: fenbufen, pefloxacin

KEY WORDS
plasma

REFERENCE
Carlucci,G.; Palumbo,G.; Mazzeo,P. Simple and rapid analysis of pefloxacin, fenbufen and felbinac in human plasma using high-performance liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1107–1115.

SAMPLE
Matrix: blood

Sample preparation: Mix 1 mL serum with 20 μL 20 μg/mL IS in MeOH and 100 μL 20 mM NaOH. Vortex for 1 min with 4 ml dichloromethane:diethylether 80:20, centrifuge at 2000 g for 10 min, remove 3 mL organic phase. Repeat the same extraction procedure twice. Combine organic layers, evaporate to dryness under a stream of nitrogen. Add 100 μL 20 mM NaOH to the residue, shake for 5 min, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 10 μm Vydac AXGU
Column: 250 × 4.6 5 μm Supelcosil LC-SAX
Mobile phase: MeCN;100 mM pH 7.0 phosphate buffer 10:90
Flow rate: 1.2
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 7.2
Internal standard: 2-[4-(2'-furoyl)phenyl]propionic acid (3.5)
Limit of detection: 30 ng/mL
Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES

Extracted: fenbufen, lomefloxacin

KEY WORDS

plasma

REFERENCE

Carlucci,G.; Mazzeo,P.; Palumbo,G. Simultaneous determination of lomefloxacin, fenbufen and felbinac in human plasma using high performance liquid chromatography, *Chromatographia*, **1996**, *43*, 261–264.

SAMPLE

Matrix: blood
Sample preparation: 50 μL Plasma + 1 mL 100 mM pH 7.0 K_2HPO_4 adjusted to pH 7.0 with 85% orthophosphoric acid + 100 μL 200 μg/mL N-phenylanthranilic 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 μL MeOH:50 mM NaOH 2:1, vortex, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm 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 275

CHROMATOGRAM

Retention time: 9.8
Internal standard: N-phenylanthranilic acid (15.0)
Limit of detection: 450 ng/mL

OTHER SUBSTANCES

Extracted: fenbufen, nalidixic acid, norfloxacin, ofloxacin, 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: 50 μL Plasma + 1 mL 100 mM pH 7.0 K_2HPO_4 adjusted to pH 7.0 with 85% orthophosphoric acid + 100 μL 300 μg/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 μL MeOH:50 mM NaOH 2:1, vortex, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm 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: 10
Internal standard: nalidixic acid (5)
Limit of quantitation: 400 ng/mL

OTHER SUBSTANCES
Extracted: fenbufen, ciprofloxacin

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Naora,K.; Katagiri,Y.; Ichikawa,N.; Hayashibara,M.; Iwamoto,K. Simultaneous high-performance liquid chromatographic determination of ciprofloxacin, fenbufe and felbinac in rat plasma, *J.Chromatogr.*, **1990**, *530*, 186–191.

SAMPLE
Matrix: urine
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL ethyl acetate and dry by aspiration of air. Evaporate an aliquot of 100 μL 20 μg/mL IS in MeOH to dryness at 37°. Add 1 mL urine, vortex, add 250 μL 1 M pH 5.0 acetate buffer, vortex. Add 250 μL of the mixture to the SPE cartridge, dry by aspiration of air, elute with 3 mL ethyl acetate, evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject a 10-30 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:50 mM pH 5.0 phosphate buffer 42:58
Flow rate: 0.9
Injection volume: 10-30
Detector: UV 230

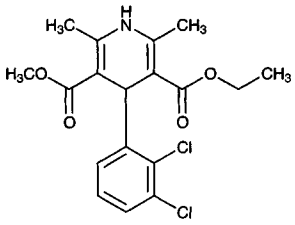
CHROMATOGRAM
Retention time: 8
Internal standard: indomethacin (18.5)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: diclofenac, ibuprofen, fenbufen, flurbiprofen, ketoprofen, loxoprofen, mefenamic acid, naproxen, piroxicam, sulindac

KEY WORDS
SPE

REFERENCE
Hirai,T.; Matsumoto,S.; Kishi,I. Simultaneous analysis of several non-steroidal anti-inflammatory drugs in human urine by high-performance liquid chromatography with normal solid-phase extraction, *J.Chromatogr.B*, **1997**, *692*, 375–388.

Felodipine



Molecular formula: $C_{18}H_{19}Cl_2NO_4$
Molecular weight: 384.26
CAS Registry No.: 72509-76-3
Merck Index: 3991
Lednicer No.: 4 106

SAMPLE
Matrix: bile, urine
Sample preparation: Dilute 20-200 μL bile or urine to 1 mL with water, adjust pH to 2.2 with 1 M phosphate buffer, extract twice with 6 mL ether with gentle inversion for 1 min. Remove the organic phases and evaporate them under a stream of nitrogen, dissolve the residue in 250 μL mobile phase A, inject the whole amount.

HPLC VARIABLES
Column: 60-5 Polygosil C18 (Macherey-Nagel)
Mobile phase: Gradient. A was MeOH:10 mM tetrapropylammonium hydrogen sulfate in 50 mM pH 5.0 phosphate buffer 5:95. B was MeOH:10 mM tetrapropylammonium hydrogen sulfate in 50 mM pH 5.0 phosphate buffer 2:1. A:B 100:0 for 2 min then to 0:100 over 35 min.
Flow rate: 1.2
Injection volume: 250
Detector: UV 254

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
rat

REFERENCE
Sutfin,T.A.; Gabrielsson,M.; Regardh,C.G. Effects of biliary excretion on the disposition of felodipine and metabolites in the rat, *Xenobiotica*, **1987**, *17*, 1203–1214.

SAMPLE
Matrix: blood
Sample preparation: Adjust pH of 200-300 μL whole blood to 12 with 100 μL 1 M NaOH, add 150 μL 10% EtOH, extract with 4 mL diethyl ether for 30 min, freeze in dry ice. Remove the organic phase and evaporate it to dryness under a stream of nitrogen. Reconstitute the residue with 250 μL mobile phase, inject a 200 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.5 5 μm Nucleosil C-18
Mobile phase: MeOH:water 70:30 containing 20 mM perchloric acid and 80 mM sodium chlorate
Flow rate: 1.1
Injection volume: 200
Detector: UV 254

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
whole blood

REFERENCE
Sutfin,T.A.; Gabrielsson,M.; Regardh,C.G. Effects of biliary excretion on the disposition of felodipine and metabolites in the rat, *Xenobiotica*, **1987**, *17*, 1203–1214.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 50 μL 100 ng/mL IS in MeOH, mix, equilibrate for 10 min, add 25 μL 4 M NaOH, mix, add 4 mL n-pentane:dichloromethane 1:1, mix on a Sarstedt CM-9 whirl-mixer for 1 h, centrifuge at 2000 g for 5 min. Remove the organic layer and evaporate it to dryness under vacuum at 30°, dissolve the residue in 40 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2 Perisorb RP-18 (Upchurch)
Column: 250 × 4.6 Chiralcel OJ (Daicel)
Mobile phase: n-Hexane:isopropanol 87.5:12.5
Column temperature: 37
Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 15.9 (S), 21.9 (R)
Internal standard: 3-(2-hydroxy-2-methyl)ethyl-5-methyl-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine dicarboxylate (Bay r 9590)
Limit of detection: 0.1 ng/mL obtainable using off-line GC-ECD detection of HPLC column effluent, 5 ng/mL (UV)

OTHER SUBSTANCES
Simultaneous: metabolites, nitrendipine, nimodipine, nisoldipine, isradipine, niguldipine, nilvadipine, nicardipine

KEY WORDS
plasma; chiral

REFERENCE
Soons,P.A.; Roosemalen,M.C.M.; Breimer,D.D. Enantioselective determination of felodipine and other chiral dihydropyridine calcium entry blockers in human plasma, *J.Chromatogr.*, **1990**, *528*, 343–356.

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

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

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

CHROMATOGRAM
Retention time: 24.423

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149[9]4163.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve tablets in MeCN:1 mM pH 2 KH_2PO_4 50:50, centrifuge, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb C8
Mobile phase: MeCN:buffer 35:65 (Buffer was 1 mM KH_2PO_4 adjusted to pH 2 with phosphoric acid.)
Column temperature: 40
Flow rate: 2.5
Injection volume: 50
Detector: UV 215

CHROMATOGRAM
Retention time: 31

OTHER SUBSTANCES
Simultaneous: enalapril, enalaprilat

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 C_8 column, *J.Chromatogr.A*, **1995**, *707*, 245–254.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Adjust pH to 2.2 with 1 M phosphate buffer, extract with ether for 1 h, centrifuge at 1000 rpm for 15 min, freeze in dry ice/EtOH. Remove the organic phase and evaporate it under a stream of nitrogen, dissolve the residue in 200 μL MeOH, inject a 40 μL aliquot.

HPLC VARIABLES
Guard column: Brownlee Newguard RP-18 pre-column
Column: 150 × 4.5 5 μm Polygosil C18 (Macherey-Nagel)
Mobile phase: Gradient. A was MeOH:10 mM tetrapropylammonium hydrogen sulfate in 50 mM pH 5.0 phosphate buffer 5:95. B was MeOH:10 mM tetrapropylammonium hydrogen sulfate in 50 mM pH 5.0 phosphate buffer 2:1. A:B 65:35 for 2 min then to 0:100 over 35 min, stay at 0:100 for another 27 min.
Flow rate: 0.8
Injection volume: 40
Detector: UV 280

CHROMATOGRAM
Retention time: 43

OTHER SUBSTANCES
Simultaneous: metabolites

REFERENCE
Bäärnhielm,C.; Backman,Å.; Hoffmann,K.J.; Weidolf,L. Biotransformation of felodipine in liver microsomes from rat, dog, and man, *Drug Metab.Dispos.*, **1986**, *14*, 613–618.

SAMPLE
Matrix: microsomal incubations, perfusate
Sample preparation: Extract using a 100 mg 1 mL Bond Elut C2 SPE cartridge, elute with MeOH, evaporate eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 5 Hichrom HiRPB deactivated reverse-phase
Mobile phase: MeOH:25 mM pH 5.0 N,N,N',N'-tetramethylethylenediamine phosphate buffer 75:25
Flow rate: 1
Detector: UV 245

CHROMATOGRAM
Internal standard: felodipine
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: nitrendipine

KEY WORDS
rat; liver; felodipine is IS; SPE

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: perfusate

HPLC VARIABLES
Column: 100 × 8 5 μm Novapak C18 radial compression
Mobile phase: MeCN:10 mM pH 4.5 phosphate buffer 70:30
Flow rate: 2
Detector: UV 237

OTHER SUBSTANCES
Also analyzed: nicardipine, nifedipine, nimodipine, nitrendipine

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: Centrifuge at 2000 g at 37° for 15 min.

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

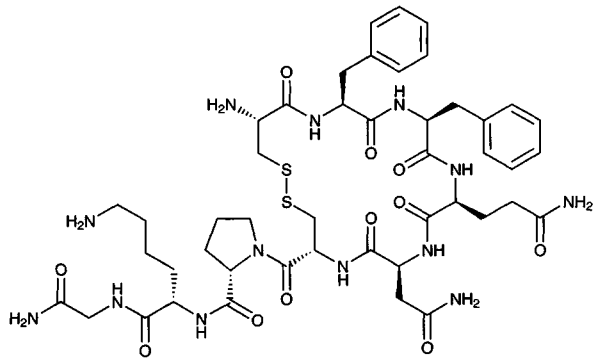
OTHER SUBSTANCES
Also analyzed: nicardipine, nifedipine, nimodipine, nitrendipine

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.

Felypressin

Molecular formula: $C_{46}H_{65}N_{13}O_{11}S_2$
Molecular weight: 1040.24
CAS Registry No.: 56-59-7
Merck Index: 3992



SAMPLE
Matrix: formulations
Sample preparation: Inject a 20 μL aliquot of the undiluted formulation onto column A and elute to waste with mobile phase A, after 14 min elute the contents of column A onto column B with mobile phase B, monitor the effluent from column B, after 9 min re-equilibrate column A with mobile phase A for 7 min.

HPLC VARIABLES
Column: A 25 × 4 4 μm Superspher 60 RP-8; B 50 × 4 4 μm Superspher 60 RP-8e
Mobile phase: A MeCN:50 mM pH 6.0 phosphate buffer 12:88; B MeCN:50 mM pH 6.0 phosphate buffer 20:80
Flow rate: 1
Injection volume: 20
Detector: F ex 390 em 470 following post-column reaction. The column effluent mixed with the reagent pumped at 0.25 mL/min and the mixture flowed through a 5 m × 0.5 mm ID knitted PTFE coil to the detector. (Reagent was 300 μg/mL fluorescamine in MeCN containing 0.1% Brij-35.)

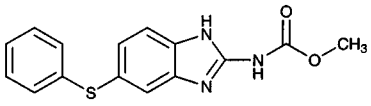
CHROMATOGRAM
Retention time: 18
Limit of detection: 0.6 pmole

OTHER SUBSTANCES
Noninterfering: prilocaine

KEY WORDS
post-column reaction; injections; column-switching; rinse all glass and plastic ware with 1 M acetic acid and then water to minimize adsorption.

REFERENCE
Svensson,M.; Gröningsson,K. Liquid chromatographic determination of felypressin using a column-switching technique and post-column derivatization, *J.Chromatogr.*, **1990**, *521*, 141–147.

Fenbendazole



Molecular formula: $C_{15}H_{13}N_3O_2S$
Molecular weight: 299.35
CAS Registry No.: 43210-67-9
Merck Index: 4000
Lednicer No.: 3 176

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

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

CHROMATOGRAM
Retention time: 10
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: albendazole, oxfendazole, cambendazole, thiabendazole, mebendazole, oxibendazole, parbendazole

KEY WORDS
plasma; sheep

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

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Sep-Pak Vac C18 SPE cartridge with 3 mL MeOH, 1 mL water, and 5 mL 100 mM sodium carbonate. Mix 1.5 mL heparinized plasma with 1.5 mL 100 mM sodium carbonate, add to the SPE cartridge, wash with 5 mL 100 mM sodium carbonate, dry the cartridge in air for 30 s, elute with 750 μL MeCN:0.2% phosphoric acid 50:50, vortex the eluate (protect from light), inject an aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm BST Nucleosil C18
Column: 150 × 3.9 4 μm Novapak C18
Mobile phase: MeCN:buffer 33:67 (Buffer was 50 mM KH_2PO_4 adjusted to pH 3.0 with 20% phosphoric acid.)
Flow rate: 1.5
Injection volume: 50

Detector: UV 220

CHROMATOGRAM
Retention time: 4.5
Limit of detection: 5 ng/mL
Limit of quantitation: 18 ng/mL

OTHER SUBSTANCES
Extracted: praziquantel

KEY WORDS
dog; pharmacokinetics; plasma; SPE

REFERENCE
Morovján,G.; Csokán,P.; Makranaszki,L.; Abdellah-Nagy,E.A.; Tóth,K. Determination of fenbendazole, praziquantel and pyrantel pamoate in dog plasma by high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *797*, 237–244.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 40 μL 10 μg/mL mebendazole in MeOH:DMF 99:1 + 1 mL 50 mM pH 8 borate buffer + 5 mL ethyl acetate, vortex for 20 s, centrifuge at 2500 g for 5 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 50° for about 50 min, reconstitute the residue in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 125 × 4.6 5 μm Hypersil SAS
Mobile phase: MeCN:buffer 30:70 (Buffer was 5 mM phosphoric acid adjusted to pH 5.9 with tetraethylammonium hydroxide.)
Flow rate: 2
Injection volume: 100
Detector: UV 300

CHROMATOGRAM
Retention time: 8.6
Internal standard: mebendazole (3.2)
Limit of detection: 15 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; sheep; pharmacokinetics

REFERENCE
Lehr,K.H.; Damm,P. Simultaneous determination of fenbendazole and its two metabolites and two triclabendazole metabolites in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *382*, 355–360.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 100 μL 10 μg/mL albendazole in MeOH + 200 μL 500 mM ammonium hydroxide (to adjust pH to 11) + 200 mg NaCl + 5 mL distilled diethyl ether, roll for 15 min, remove 4 mL supernatant, repeat extraction, remove 5 mL supernatant. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 60 μL MeOH, sonicate for 2 min, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: present but not specified
Column: 100 × 5 Nucleosil 5C18

Mobile phase: MeCN:1% acetic acid 43:57
Flow rate: 0.9
Injection volume: 20
Detector: UV 292

CHROMATOGRAM
Retention time: 2.6
Internal standard: albendazole (1.6)
Limit of detection: 12.5 ng/mL

OTHER SUBSTANCES
Extracted: febantel, oxfendazole, oxfendazole sulfone

KEY WORDS
plasma; sheep

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

SAMPLE
Matrix: blood, feces, tissue, urine
Sample preparation: Plasma or liver homogenate. 1 mL Plasma or liver homogenate (S9) + 20 μL concentrated ammonium hydroxide, vortex, add to a 1 mL Chem Elut SPE cartridge (Analytichem), rinse tube with 100 μL dichloromethane, add rinse to the SPE cartridge, elute SPE cartridge with two 4 mL portions of dichloromethane. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot. Urine. 1 mL Urine + 20 μL concentrated ammonium hydroxide, vortex, add to a 1 mL Chem Elut SPE cartridge (Analytichem), rinse tube with 100 μL dichloromethane, add rinse to the SPE cartridge, elute SPE cartridge with two 4 mL portions of dichloromethane. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 500 μL 2 M HCl and 1 mL benzene (Caution! Benzene is a carcinogen!), vortex, centrifuge at 2000 rpm for 5 min, wash twice more with benzene. Add the aqueous layer to 500 μL concentrated ammonium hydroxide, extract twice with 1 mL dichloromethane. Combine the extracts and wash them with 1 mL water. Evaporate the dichloromethane layer to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 10 μL aliquot. Feces. 2 g Feces + 8 mL water + 1 mL concentrated ammonia, homogenize by stirring, add to a 10 mL Chem Elut SPE cartridge (Analytichem) with a cotton ball on the top of the column, elute with two 12 mL portions of ethyl acetate. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 2 mL 2 M HCl and 4 mL benzene (Caution! Benzene is a carcinogen!), vortex, centrifuge at 2000 rpm for 5 min, wash three more times with benzene. Add the aqueous layer to 1 mL concentrated ammonium hydroxide, extract twice with 1 mL dichloromethane. Combine the extracts and wash them with 1 mL water. Evaporate the dichloromethane layer to dryness under a stream of nitrogen, reconstitute the residue in 500 μL mobile phase, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 MicroPak MCH 10 octadecylsilane
Mobile phase: MeCN:0.05 N phosphoric acid 78:22
Flow rate: 0.35 for 14 min, increasing to 2 over 2 min, maintain at 2
Injection volume: 5-20
Detector: UV 290

CHROMATOGRAM
Retention time: 17
Limit of detection: 720 ng/g (goat feces), 200 ng/g (duck feces), 10 ng/mL (urine, plasma, tissue)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; goat; duck; chicken; rat; rabbit; liver; sheep; SPE

REFERENCE

Barker,S.A.; Hsieh,L.C.; Short,C.R. Methodology for the analysis of fenbendazole and its metabolites in plasma, urine, feces, and tissue homogenates, *Anal.Biochem.*, **1986**, *155*, 112–118.

SAMPLE

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

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 7.8

OTHER SUBSTANCES

Simultaneous: albendazole, niclosamide, oxyclozanide

KEY WORDS

tablets; powder; liquid formulations

REFERENCE

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

SAMPLE

Matrix: milk
Sample preparation: Condition a 3 mL Bond Elut silica SPE cartridge with 2 mL dichloromethane. 10 g Milk + 5 mL 1 M sodium carbonate, mix, add 150 mL ethyl acetate, add 1 mL 10 mg/mL BHT in ethyl acetate, blend (tissuemizer) at high speed for 5 min, add 10 g anhydrous sodium sulfate, blend for 1 min, let settle for 2-3 min, filter (No. 41 paper), add another 150 mL ethyl acetate to the sodium sulfate, blend for 2 min, filter. Combine the filtrates and evaporate them to dryness under vacuum. Rinse out flask with two 10 mL portions of hexane and two 10 mL portions of 1 M phosphoric acid. Combine rinses, shake vigorously for 2 min, extract the hexane layer with two 10 mL portions of 1 mL phosphoric acid. Combine all the aqueous layers and wash them with 5 mL hexane, adjust the pH to 8-9 by slowly adding about 9 mL 10 M KOH (use an ice bath), add 50 mL ethyl acetate, shake vigorously for 2 min, repeat extraction. Filter the organic layers through 40 g anhydrous sodium sulfate, wash the sodium sulfate with 25 mL ethyl acetate. Combine the organic layers, add 200 μL 10 mg/mL BHT in ethyl acetate, evaporate to dryness under vacuum. Take up the residue in two 3 mL portions of dichloromethane and add them to the SPE cartridge, wash with 5 mL dichloromethane, elute with 5 mL dichloromethane:MeOH 75:25. Evaporate the eluate to dryness under nitrogen, reconstitute in 1 mL mobile phase, vortex, filter (0.2 μm), inject an aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 pellicular C18 (Alltech)
Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:buffer 70:30 (Buffer was 1.15 g $(NH_4)H_2PO_4$ in 950 mL water, adjust pH to 7.0 with dilute ammonia, make up to 1 L with water.)
Flow rate: 1
Injection volume: 50
Detector: UV 298

CHROMATOGRAM

Retention time: 13
Limit of detection: 0.5 ppb

KEY WORDS
cow; SPE

REFERENCE
Tai,S.S.; Cargile,N.; Barnes,C.J. Determination of thiabendazole, 5-hydroxythiabendazole, fenbendazole, and oxfendazole in milk, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 368–373.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 120 × 4.6 7 μm LiChrosorb RP8
Mobile phase: MeOH:33 mM phosphoric acid 50:50
Flow rate: 2
Injection volume: 75
Detector: UV 245 following post-column reaction. The column effluent flowed through a 20 m × 0.5 mm ID knitted PTFE coil irradiated by a 15 w low-pressure mercury lamp (Original Hanau TNN 15/32) to the detector., F ex 300 em 342 following post-column reaction. The column effluent flowed through a 20 m × 0.5 mm ID knitted PTFE coil irradiated by a 15 w low-pressure mercury lamp (Original Hanau TNN 15/32) to the detector.

CHROMATOGRAM
Retention time: 10
Limit of detection: 5 ng

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
post-column reaction; post-column photochemical derivatization

REFERENCE
Uihlein,M.; Schwab,E. A novel reactor for photochemical post-column derivatization in HPLC, *Chromatographia*, **1982**, *15*, 140–146.

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, dox-

apram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

SAMPLE

Matrix: tissue

Sample preparation: Condition a 1 g silica SPE cartridge with 6 mL ethyl acetate-hexane 20:80. 5 g tissue + 500 µL 4 M potassium carbonate, vortex for 10 s, add 10 mL ethyl acetate, vortex for 10 s, shake at 500 rpm for 10 min, centrifuge at 3400 g for 6 min. Decant the supernatant. Repeat the extraction with another 10 mL ethyl acetate. Combined extracts + 80 mL hexane + 2 g anhydrous sodium sulfate, shake, let stand until it becomes transparent. Filter over an S & S 589.1 filter paper circle. Add the filtrate to the SPE cartridge, dry the column in a stream of nitrogen for 10 min. Elute with 3 mL acetic acid:MeOH 3:97, evaporate to dryness under a stream of nitrogen at 37°. Dissolve the residue in 550 µL 50 mM ammonium phosphate, add 280 µL MeCN and 170 µL MeOH, sonicate for 5 min, centrifuge at 15800 g for at least 8 min. Inject a 50 µL aliquot.

HPLC VARIABLES

Guard column: 10 × 2.1 40 µm pellicular reversed phase stainless steel

Column: two 100 × 3.0 5 µm Lichrosorb RP8 glass columns in series

Mobile phase: MeCN:MeOH:50 mM pH 5.0 phosphate buffer 28:17:55 (Prepare buffer as follows. Dissolve 5.75 g ammonium phosphate in 950 mL water, adjust to pH 5.0 with 1 M NaOH. Make up to 1000 mL with water.)

Flow rate: 0.6

Injection volume: 50

Detector: UV 297

CHROMATOGRAM

Retention time: 15

Limit of detection: 500 pg (on column), 3.0 µg/kg (in muscle tissue)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS
muscle; trout; eel; metabolites; SPE

REFERENCE
Iosifidou,E.G.; Haagsma,N. High-performance liquid chromatographic determination of fenbendazole and its metabolites, sulphoxide and sulphone, in fish muscle tissue, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1819–1830.

SAMPLE
Matrix: tissue
Sample preparation: Wash 22 g bulk 40 μm 18% load end-capped C18 material (Analytichem) in a syringe barrel with 100 mL hexane, with 100 mL dichloromethane, and with 100 mL MeOH and dry under vacuum aspiration. Gently blend 2 g C18 material, 0.5 g liver, and 10 μL 40 μg/mL mebendazole in DMF in a glass pestle for 1 min until homogeneous in appearance. Place in a 10 mL syringe barrel plugged with filter paper (Whatman No. 1), cover with filter paper, compress to 4.5 mL, place a 100 μL pipette tip on the barrel to restrict flow, wash with 8 mL hexane, elute with 8 mL MeCN. Pass the eluate through 0.5 g activated alumina (EM Science Type F-20 80-200 mesh) between filter paper in a 10 mL syringe barrel (wash column with 4 mL MeCN just before use). Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH and 400 μL 17 mM phosphoric acid, sonicate for 5-10 min, centrifuge at 17000 g for 5 min, filter the supernatant (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm Micro Pak ODS (Varian)
Mobile phase: MeCN:17 mM phosphoric acid 40:60
Column temperature: 45
Flow rate: 1
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: 14
Internal standard: mebendazole (9)
Limit of detection: 100 ng/g

OTHER SUBSTANCES
Extracted: albendazole, thiafendazole, oxfendazole

KEY WORDS
matrix solid-phase dispersion; liver

REFERENCE
Long,A.R.; Malbrough,M.S.; Hsieh,L.C.; Short,C.R.; Barker,S.A. Matrix solid phase dispersion isolation and liquid chromatographic determination of five benzimidazole anthelmintics in fortified beef liver, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 860–863.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 2.8 mL 500 mg 40 μm 60 Å Bond Elut silica SPE cartridge with 2 mL dichloromethane. 10 g Minced tissue + 5 mL 1 M sodium carbonate + 150 mL ethyl acetate + 1 mL 10 mg/mL BHT in ethyl acetate, blend (Waring) at high speed for 5 min, add 80 g anhydrous sodium sulfate, blend at low speed for 1 min. Decant the organic layer and filter it (No. 41 paper), add 150 mL ethyl acetate to material remaining in blender, blend at low speed for 2-3 min, filter, wash solid with 10 mL EtOH. Combine all the filtrates and evaporate them to dryness under vacuum at 30-35° (beware of bumping). Rinse out flask with two 10 mL portions of hexane and two 10 mL portions of 1 M phosphoric acid, combine rinses, shake vigorously for 2 min, allow to separate for 10 min, extract the hexane layer twice more with 10 mL portions of 1 M phosphoric acid. Combine all the aqueous layers and wash them with 10 mL hexane, adjust the pH of the aqueous layer to 8.5 ± 1.0 by slowly adding about 9 mL 10 M KOH while using an ice bath. Extract twice with 50 mL ethyl acetate (2 min shaking), pass ethyl acetate layers through 40 g anhydrous sodium sulfate, wash the sodium sulfate with 25 mL ethyl acetate. Combine the ethyl acetate layers, add 200 μL 10 mg/mL BHT in ethyl

acetate, evaporate to dryness under vacuum at 30-35° (beware of bumping). Rinse out flask with three 3 mL portions of dichloromethane, add rinses to the SPE cartridge, wash with 5 mL dichloromethane, elute with 5 mL dichloromethane:MeOH 75:25. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase, vortex, filter (0.2 μm), inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 Brownlee RP-18 Spheri-10 MPLC
Column: 250 × 4.6 5 μm C18 (Alltech)
Mobile phase: MeOH:buffer 70:30 (Buffer was 1.15 g $(NH_4)H_2PO_4$ in 950 mL water, adjust pH to 7.0 with dilute ammonia, make up to 1 L with water.)
Flow rate: 1
Injection volume: 50
Detector: UV 298

CHROMATOGRAM
Retention time: 14
Limit of detection: <800 ppb

OTHER SUBSTANCES
Simultaneous: chloramphenicol
Noninterfering: amprolium, chlortetracycline, erythromycin, levamisole, morantel, oxytetracycline, phenothiazine, sulfadimethoxine, sulfamethazine, sulfaquinoxaline

KEY WORDS
cow; liver; SPE

REFERENCE
LeVan,L.W.; Barnes,C.J. Liquid chromatographic method for multiresidue determination of benzimidazoles in beef liver and muscle: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1991**, *74*, 487–493.

SAMPLE
Matrix: tissue
Sample preparation: 3 g Pulverized tissue + 7 mL water, homogenize (Silverson) for 1 min, add 20 mL MeOH, sonicate for 15 min, centrifuge at 2000 g at 5° for 10 min. Remove 10 mL of the supernatant and add it to 8 mL light petroleum (bp 40-60°), shake gently for 30 s, centrifuge. Add the aqueous phase to 14 mL 500 mM NaH_2PO_4 and 8 mL diethyl ether:ethyl acetate 60:40, shake gently for 30 s, centrifuge, repeat the extraction with 5 mL and 3 mL portions of diethyl ether:ethyl acetate 60:40. Combine the upper organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 μL MeCN:water 50:50, sonicate for 5 min, inject a 50 μL aliquot.

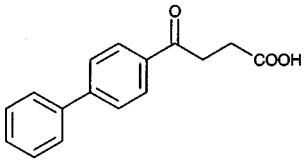
HPLC VARIABLES
Column: 125 × 4 LiChrosorb RP18
Mobile phase: MeCN:THF:water 50:10:40 containing 50 mM ammonium acetate
Flow rate: 1
Injection volume: 50
Detector: MS, Vestec Model 201A, thermospray, positive-ion chemical ionization, electron beam 250 μA, electron multiplier 2000 V, source block 250°, tip heater 250°, lens assembly 150°, vaporizer probe 10° below take-off point, m/z 300

CHROMATOGRAM
Retention time: 2.7
Limit of detection: 50 ng/g

KEY WORDS
sheep; muscle; liver; pharmacokinetics; LC-MS

REFERENCE
Blanchflower,W.J.; Cannavan,A.; Kennedy,D.G. Determination of fenbendazole and oxfendazole in liver and muscle using liquid chromatography-mass spectrometry, *Analyst*, **1994**, *119*, 1325–1328.

Fenbufen



Molecular formula: $C_{16}H_{14}O_3$
Molecular weight: 254.29
CAS Registry No.: 36330-85-5
Merck Index: 4003
Lednicer No.: 2 126

SAMPLE

Matrix: blood
Sample preparation: Vortex 1 mL Plasma with 500 μL 50 mM pH 7.0 phosphate buffer for 1 min, add 2 mL dichloromethane, vortex for 1 min, shake for 5 min. Centrifuge at 100 g for 10 min, separate the organic layer, repeat the extraction procedure twice, evaporate the combined organic layers to dryness under reduced pressure. Reconstitute the residue with 200 μL 20 mM NaOH, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 10 μm Vydac AXGU
Column: 250 × 4.6 5 μm Supelcosil LC-SAX
Mobile phase: MeCN:50 mM pH 7.0 phosphate buffer 10:90
Flow rate: 1.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 3.5
Internal standard: fenbufen
Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Extracted: furprofen, rufloxacin

KEY WORDS

plasma; fenbufen is IS

REFERENCE

Carlucci,G.; Mazzeo,P. Simultaneous determination of furprofen and rufloxacin in human plasma by high-performance liquid chromatography, *J.Chromatogr.Sci.*, **1996**, *34*, 182–184.

SAMPLE

Matrix: blood
Sample preparation: Mix 1 mL plasma with 20 μL 20 μg/mL IS in MeOH. Vortex for 1 min with dichloromethane:diethyl ether 80:20, centrifuge at 1000 g for 10 min, separate the organic layer. Add 4 mL dichloromethane-diethyl ether 80:20, repeat the same extraction procedure twice, evaporate the organic phase to dryness under a stream of nitrogen, add 100 μL 10 mM NaOH to the residue, shake for 5 min, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 10 μm Vydac AXGU
Column: 250 × 4.6 5 μm Adsorbosphere SAX
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 10:90
Flow rate: 1.2
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 5.3
Internal standard: 2-[-(2-furoyl)phenyl]propionic acid (3.9)
Limit of detection: 300 ng/mL

OTHER SUBSTANCES
Extracted: felbinac, pefloxacin

KEY WORDS
plasma

REFERENCE
Carlucci,G.; Palumbo,G.; Mazzeo,P. Simple and rapid analysis of pefloxacin, fenbufen and felbinac in human plasma using high-performance liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1107–1115.

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL serum with 20 μL 20 μg/mL IS in MeOH and 100 μL 20 mM NaOH. Vortex for 1 min with 4 ml dichloromethane:diethylether 80:20, centrifuge at 2000 g for 10 min, remove 3 mL organic phase. Repeat the same extraction procedure twice. Combine organic layers, evaporate to dryness under a stream of nitrogen. Add 100 μL 20 mM NaOH to the residue, shake for 5 min, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 10 μm Vydac AXGU
Column: 250 × 4.6 5 μm Supelcosil LC-SAX
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 10:90
Flow rate: 1.2
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 5.5
Internal standard: 2-[4-(2'-furoyl)phenyl]propionic acid (3.5)
Limit of detection: 20 ng/mL
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Extracted: felbinac, lomefloxacin

KEY WORDS
plasma

REFERENCE
Carlucci,G.; Mazzeo,P.; Palumbo,G. Simultaneous determination of lomefloxacin, fenbufen and felbinac in human plasma using high performance liquid chromatography, *Chromatographia*, **1996**, *43*, 261–264.

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

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 285

CHROMATOGRAM

Retention time: 5.44
Limit of detection: <120 ng/mL

KEY WORDS

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

REFERENCE

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

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL Bond-Elut C18 SPE cartridge with 1 mL MeOH. 1 mL Serum + 6 μL 1 mg/mL ketoprofen in MeOH + 1 mL water + 20 μL saturated ammonium sulfate solution + 60 μL concentrated HCl, vortex for 3 min, add to the SPE cartridge, wash with three 1 mL portions of water, allow to dry for 3 min, elute with five 500 μL portions of MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 5 μm Spheri-5 cyano
Mobile phase: MeCN:MeOH:water:phosphoric acid 21:22:56.5:0.5
Flow rate: 0.5
Injection volume: 100
Detector: F ex 248 em 335 (filter) following post-column reaction. The column effluent flowed through a knitted 7.9 m × 0.3 mm ID PTFE coil irradiated with an SC3-9 UV lamp (UVP, San Gabriel CA) and cooled with a fan to the detector.

CHROMATOGRAM
Retention time: 8.5
Internal standard: ketoprofen (6.5)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
post-column reaction; post-column photochemical derivatization; serum; SPE

REFERENCE
Siluveru,M.; Stewart,J.T. Determination of fenbufen and its metabolites in serum by reversed-phase high-performance liquid chromatography using solid-phase extraction and on-line post-column ultraviolet irradiation and fluorescence detection, *J.Chromatogr.B*, **1996**, *682*, 89–94.

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

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

CHROMATOGRAM
Retention time: 19.292

KEY WORDS
whole blood

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

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 40:60 adjusted to pH 5.5 with NaOH
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 283

OTHER SUBSTANCES
Also analyzed: carbamazepine, indomethacin, ketoprofen, α.-naphthoquinone, naproxen, tolmetin

REFERENCE
Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE
Matrix: urine
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL ethyl acetate and dry by aspiration of air. Evaporate an aliquot of 100 μL 20 μg/mL IS in MeOH to dryness at 37°. Add 1 mL urine, vortex, add 250 μL 1 M pH 5.0 acetate buffer, vortex. Add 250 μL of the mixture to the SPE cartridge, dry by aspiration of air, elute with 3 mL ethyl acetate, evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject a 10-30 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:50 mM pH 5.0 phosphate buffer 42:58
Flow rate: 0.9
Injection volume: 10-30
Detector: UV 230

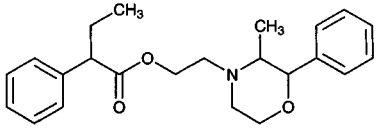
CHROMATOGRAM
Retention time: 9.5
Internal standard: indomethacin (18.5)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: diclofenac, ibuprofen, felbinac, flurbiprofen, ketoprofen, loxoprofen, mefenamic acid, naproxen, piroxicam, sulindac

KEY WORDS
SPE

REFERENCE
Hirai,T.; Matsumoto,S.; Kishi,I. Simultaneous analysis of several non-steroidal anti-inflammatory drugs in human urine by high-performance liquid chromatography with normal solid-phase extraction, *J.Chromatogr.B*, **1997**, *692*, 375–388.

Fenbutrazate



Molecular formula: $C_{23}H_{29}NO_3$
Molecular weight: 367.49
CAS Registry No.: 4378-36-3, 6474-85-7 (HCl)
Merck Index: 4005

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

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

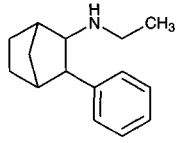
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.2

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

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

Fencamfamine



Molecular formula: $C_{15}H_{21}N$
Molecular weight: 215.34
CAS Registry No.: 1209-98-9, 2240-14-4 (HCl)
Merck Index: 4006
Lednicer No.: 1 74

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

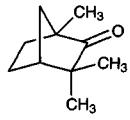
OTHER SUBSTANCES

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, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

Fenchone



Molecular formula: $C_{10}H_{16}O$
Molecular weight: 152.24
CAS Registry No.: 4695-62-9
Merck Index: 4008

SAMPLE
Matrix: solutions

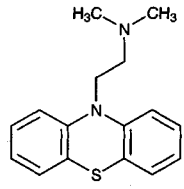
HPLC VARIABLES
Column: 250 × 1 5 μm LiChrosorb RP18
Mobile phase: EtOH:water 35:65 containing 10 mM α-cyclodextrin and 0.5 mM tri-O-methyl-α-cyclodextrin
Column temperature: 25
Flow rate: 0.04
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: k' 10.9

OTHER SUBSTANCES
Extracted: camphor

REFERENCE
Nowakowski,R.; Bielejewska,A.; Duszczyk,K.; Sybilska,D. Chiral discrimination by high-performance liquid chromatography with joint use of two cyclodextrin additives, *J.Chromatogr.A*, **1997**, *782*, 1–11.

Fenethazine



Molecular formula: $C_{16}H_{18}N_2S$
Molecular weight: 270.40
CAS Registry No.: 522-24-7
Merck Index: 4013

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

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

CHROMATOGRAM
Retention time: 4.4

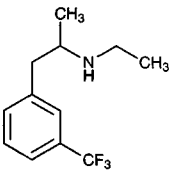
OTHER SUBSTANCES

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

REFERENCE

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

Fenfluramine



Molecular formula: $C_{12}H_{16}F_3N$
Molecular weight: 231.26
CAS Registry No.: 458-24-2, 404-82-0 (HCl)
Merck Index: 4015
Lednicer No.: 1 70

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 1 mL 0.6 M pH 9.8 carbonate buffer + 40 µL 5 µg/mL maprotiline in 10 mM HCl + 5 mL 200 g/L ethyl acetate in n-heptane, mix by rocking for 10 min, centrifuge at 1500 g for 10 min. Remove organic layer and add it to 150 µL 100 mM HCl, mix 10 min, centrifuge at 1500 g for 10 min. Discard organic layer and evaporate aqueous layer at 45° in a vacuum centrifuge for 1 h. Take up residue in 50 µL 1 M pH 10.3 carbonate buffer and 25 µL 10 mg/mL dansyl chloride in MeCN, vortex, allow to react at room temper-

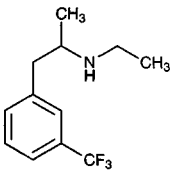
OTHER SUBSTANCES

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

REFERENCE

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

Fenfluramine



Molecular formula: $C_{12}H_{16}F_3N$
Molecular weight: 231.26
CAS Registry No.: 458-24-2, 404-82-0 (HCl)
Merck Index: 4015
Lednicer No.: 1 70

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 1 mL 0.6 M pH 9.8 carbonate buffer + 40 µL 5 µg/mL maprotiline in 10 mM HCl + 5 mL 200 g/L ethyl acetate in n-heptane, mix by rocking for 10 min, centrifuge at 1500 g for 10 min. Remove organic layer and add it to 150 µL 100 mM HCl, mix 10 min, centrifuge at 1500 g for 10 min. Discard organic layer and evaporate aqueous layer at 45° in a vacuum centrifuge for 1 h. Take up residue in 50 µL 1 M pH 10.3 carbonate buffer and 25 µL 10 mg/mL dansyl chloride in MeCN, vortex, allow to react at room temper-

ature for 45 min, evaporate at 45° in a vacuum centrifuge for 20 min, reconstitute in 125 μL MeCN:water 75:25, vortex, centrifuge for 3-5 min, inject a 25-40 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeCN:25 mM KH_2PO_4 75:25 + 500 μL/L orthophosphoric acid + 600 μL/L n-butylamine
Flow rate: 2
Injection volume: 25-40
Detector: F ex 235 em 470 (cut-off)

CHROMATOGRAM

Retention time: 8.21
Internal standard: maprotiline (12.8)

OTHER SUBSTANCES

Simultaneous: fluoxetine, propranolol, clovoxamine, fluvoxamine, amoxapine, desipramine, protriptyline, nortriptyline, sertraline, norfluoxetine
Noninterfering: amitriptyline, imipramine, clomipramine, trimipramine, mianserin, chlordiazepoxide, trazodone, cyclobenzaprine, nomifensine, bupropion, metoprolol, atenolol, pindolol, tranylcypromine, moclobemide, thioridazine, citalopram, clozapine, carbamazepine, doxepin, loxapine

KEY WORDS

plasma

REFERENCE

Suckow,R.F.; Zhang,M.F.; Cooper,T.B. Sensitive and selective liquid-chromatographic assay of fluoxetine and norfluoxetine in plasma with fluorescence detection after precolumn derivatization, *Clin.Chem.*, **1992**, *38*, 1756–1761.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 1.25 μg/mL IS in water + 200 μL water, vortex, add 400 μL 1 M trichloroacetic acid, vortex, centrifuge at 2500 g for 10 min. Remove 900 μL of the supernatant and add it to 250 μL 1 M NaOH, add 2 mL reagent, vortex for 2 s, let stand for 90 min. Remove a 1 mL aliquot of the lower organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL dichloromethane, inject a 35 μL aliquot. (Reagent was 10 μM 3,5-dinitrophenylisocyanate in dichloromethane. Prepare as follows. Stir 6.40 g 3,5-dinitrobenzoyl chloride in 100 mL glacial acetic acid, add 1.80 g sodium azide in small increments, stir for 1 h, add 300 mL cold water. Filter off the 3,5-dinitrobenzyl azide precipitate and wash it with a small portion of water. Dry overnight in a vacuum desiccator. Reflux 25 mg 3,5-dinitrobenzyl azide dissolved in 5 mL toluene for 10 min, cool to room temperature, make up to 50 mL with dichloromethane, dilute an aliquot 1:200 with dichloromethane to give a 10 μM solution of 3,5-dinitrophenylisocyanate. Prepare fresh daily. CAUTION! 3,5-Dinitrobenzyl azide may be explosive and 3,5-dinitrophenylisocyanate may be toxic!)

HPLC VARIABLES

Column: 250 × 4.6 5 μm (R)-naphthylurea Chiral (Supelco)
Mobile phase: Hexane:isopropanol:MeCN 89:9:2
Flow rate: 1.2 for 15 min, 3.5 for 13 min, 1.2 for 7 min
Injection volume: 35
Detector: UV 235

CHROMATOGRAM

Retention time: 10 (d), 11.5 (l)
Internal standard: β-methylphenethylamine (20.7, first peak)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS
plasma; conduct analyses under yellow light; chiral; pharmacokinetics; derivatization

REFERENCE
Zeng,J.-N.; Dou,L.; Duda,M.; Stuting,H.H. New chiral high-performance liquid chromatographic methodology used for the pharmacokinetic evaluation of dexfenfluramine, *J.Chromatogr.B*, **1994**, *654*, 231–248.

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

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 264

CHROMATOGRAM
Retention time: 5.28
Limit of detection: <120 ng/mL

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

lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

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

SAMPLE

Matrix: blood, urine

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

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

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

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

Column temperature: 30

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

Injection volume: 10-30

Detector: UV 207.5

CHROMATOGRAM

Retention time: 13.055

KEY WORDS

whole blood

REFERENCE

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

SAMPLE

Matrix: formulations

Sample preparation: Dissolve the contents of a 150 mg capsule in 1 L water, stir for 1 h, let stand for 30 min, centrifuge an aliquot at 1250 g for 5 min. Dilute 1 mL of the supernatant to 50 mL with water. Remove a 1 mL aliquot and add it to 100 μL 1 μg/mL IS, add 100 μL 100 mM NaOH, add 2 mL reagent, vortex, let stand for 15 min, inject a 30 μL aliquot of upper aqueous layer (sic). (Reagent was 20 μM 3,5-dinitrophenylisocyanate in dichloromethane. Prepare as follows. Stir 6.40 g 3,5-dinitrobenzoyl chloride in 100 mL glacial acetic acid, add 1.80 g sodium azide in small increments, stir for 1 h, add 300 mL cold water. Filter off the 3,5-dinitrobenzyl azide precipitate and wash it with a small portion of water. Dry overnight in a vacuum desiccator. Reflux 25 mg 3,5-dinitrobenzyl azide dissolved in 5 mL toluene for 10 min, cool to room temperature, make up to 50 mL with dichloromethane, dilute an aliquot 1:100 with dichloromethane to give a 20 μM solution of 3,5-dinitrophenylisocyanate. Prepare fresh daily. CAUTION! 3,5-Dinitrobenzyl azide may be explosive and 3,5-dinitrophenylisocyanate may be toxic!)

HPLC VARIABLES

Column: 250 × 4.6 5 μm (R)-naphthylurea Chiral (Supelco)

Mobile phase: Hexane:dichloromethane:MeOH:MeCN 81:17.5:1:0.5 (For l-isomer use 85:13.5:1:0.5 and 200 μM reagent.) (The exact ratios are very important.)

Flow rate: 1.5
Injection volume: 30
Detector: UV 235

CHROMATOGRAM
Retention time: 10.3 (d)
Internal standard: β-methylphenethylamine (17.6)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Simultaneous: impurities

KEY WORDS
conduct analyses under yellow light; chiral; derivatization; capsules

REFERENCE
Dou,L.; Zeng,J.-N.; Gerochi,D.D.; Duda,M.P.; Stuting,H.H. Chiral high-performance liquid chromatography methodology for quality control monitoring of dexfenfluramine, *J.Chromatogr.A*, **1994**, *679*, 367–374.

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: 2.48

OTHER SUBSTANCES
Simultaneous: methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodeine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine, pemoline, benzphetamine, diethylpropion, mazindol, tranylcypromine, caffeine, fenethyline, phendimetrazine, methylphenidate, phenelzine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine, norpipanone, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: chlorphentermine, norpseudoephedrine, methylenedioxyamphetamine, amphetamine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol

REFERENCE
Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

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

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica

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

CHROMATOGRAM

Retention time: 2.1

OTHER SUBSTANCES

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

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 30 × 2.1 Spheri-5 RP-8
Column: 220 × 2.1 Spheri-5 RP-8
Mobile phase: Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95:5 for 2 min, to 0:100 over 15 min (?), maintain at 0:100 for 5 min.
Column temperature: 50
Flow rate: 0.5

Detector: UV 200

CHROMATOGRAM
Retention time: 11.5

OTHER SUBSTANCES
Simultaneous: diethylpropion, phenylpropanolamine, ephedrine, amphetamine, 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, C1-94, **1994**, p. 7.24.

SAMPLE
Matrix: urine
Sample preparation: Condition a Bond Elut C8 SPE cartridge with MeOH, water, and buffer. Dilute human and dog urine with an equal volume of buffer and add to the SPE cartridge, wash with water, elute with 200 μL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute in MeOH, inject an aliquot. Inject mouse and rat urine directly. (Buffer was 100 mM pH 9.0 phosphate buffer.)

HPLC VARIABLES
Mobile phase: Gradient. A was MeOH. B was MeOH:50 mM phosphoric acid 5:95. C was MeOH water 5:95. D was MeOH:water 70:30. A:B:C:D from 30:70:0:0 to 0:0:0:100 over 20 min, to 70:30:0:0 over 5 min, maintain at 70:30:0:0 for 35 min, return to initial conditions over 1 min.
Flow rate: 1
Detector: Radioactivity or UV

CHROMATOGRAM
Retention time: 50

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
mouse; rat; dog; human; pharmacokinetics; SPE

REFERENCE
Marchant,N.C.; Breen,M.A.; Wallace,D.; Bass,S.; Taylor,A.R.; Ings,R.M.J.; Campbell,D.B.; Williams,J. Comparative biodisposition and metabolism of ^{14}C-(±)-fenfluramine in mouse, rat, dog and man, *Xenobiotica*, **1992**, *22*, 1251–1266.

SAMPLE
Matrix: urine
Sample preparation: 1 mL Urine + 200 μL 5 M NaOH + 3 mL diethyl ether, vortex for 2 min, centrifuge at 2200 g for 5 min, freeze in acetone-dry ice. Remove the organic layer and add it to 120 μL 500 mM sulfuric acid, vortex for 2 min, centrifuge at 2200 g for 5 min. Remove the aqueous layer and evaporate traces of ether with a stream of nitrogen, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 20 μm μBondapak C18
Mobile phase: MeCN:50 mM KH_2PO_4 25:75
Flow rate: 1.3
Injection volume: 100
Detector: UV 210

CHROMATOGRAM
Retention time: 14

Limit of detection: 10 ng/mL
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE
Gross,A.S.; Phillips,A.C.; Boutagy,J.; Shenfield,G.M. Determination of dexfenfluramine and nordexfenfluramine in urine by high-performance liquid chromatography using ultraviolet detection, *J.Chromatogr.*, **1993**, *621*, 115–120.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 200 μL 5 μg/mL IS in water + 400 μL water, vortex for 5 s, add 400 μL 1 M NaOH, vortex, centrifuge at 2500 g for 10 min. Remove the supernatant and add it to 5 mL hexane:ethyl acetate 70:30, shake gently by hand for 1 min, let stand for 10 min. Remove 4 mL of the upper organic layer and evaporate it to about 30 μL under a stream of nitrogen, add 1 mL reagent, vortex for 2 s, let stand for 30 min, inject a 35 μL aliquot. (Reagent was 20 μM 3,5-dinitrophenylisocyanate in dichloromethane. Prepare as follows. Stir 6.40 g 3,5-dinitrobenzoyl chloride in 100 mL glacial acetic acid, add 1.80 g sodium azide in small increments, stir for 1 h, add 300 mL cold water. Filter off the 3,5-dinitrobenzyl azide precipitate and wash it with a small portion of water. Dry overnight in a vacuum desiccator. Reflux 25 mg 3,5-dinitrobenzyl azide dissolved in 5 mL toluene for 10 min, cool to room temperature, make up to 50 mL with dichloromethane, dilute an aliquot 1:100 with dichloromethane to give a 20 μM solution of 3,5-dinitrophenylisocyanate. Prepare fresh daily. CAUTION! 3,5-Dinitrobenzyl azide may be explosive and 3,5-dinitrophenylisocyanate may be toxic!)

HPLC VARIABLES
Column: 250 × 4.6 5 μm (R)-naphthylurea Chiral (Supelco)
Mobile phase: Hexane:isopropanol:MeCN 90.5:7.5:2 (d isomer) or 89:9:2 (l isomer)
Column temperature: ambient (l isomer), 35 (d isomer)
Flow rate: 1.2
Injection volume: 35
Detector: UV 235

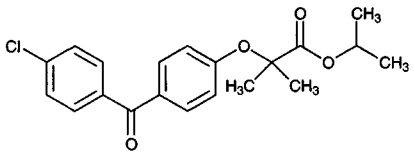
CHROMATOGRAM
Retention time: 9.3 (d isomer), 10.7 (l isomer)
Internal standard: β-methylphenethylamine (19.5, first peak, d-isomer system) (18.8, l-isomer system, first peak)
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
conduct analyses under yellow light; derivatization

REFERENCE
Zeng,J.-N.; Dou,L.; Duda,M.; Stuting,H.H. New chiral high-performance liquid chromatographic methodology used for the pharmacokinetic evaluation of dexfenfluramine, *J.Chromatogr.B*, **1994**, *654*, 231–248.

Fenofibrate



Molecular formula: $C_{20}H_{21}ClO_4$
Molecular weight: 360.84
CAS Registry No.: 49562-28-9
Merck Index: 4019

SAMPLE

Matrix: blood, urine
Sample preparation: Condition a 200 mg Analytichem C8 SPE cartridge with 2 portions of MeOH and 2 portions of water, do not allow to dry. 1 mL Plasma or urine + 5 μg/mL naproxen in MeOH + 500 μL water + 250 μL 1 M HCl, mix, add to the SPE cartridge, wash with 2 portions of water, elute with 500 μL MeCN. Evaporate the eluate to 150 μL under vacuum, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: Bondapak C18/Corasil
Column: 100 × 8 10 μm Radial-Pak C8 (Waters)
Mobile phase: MeCN:buffer 35:65 (Buffer was 2.72 g KH_2PO_4 in 1 L water, pH adjusted to 3 with phosphoric acid.)
Flow rate: 2.5
Injection volume: 100
Detector: UV 254

CHROMATOGRAM

Retention time: 7 (as fenofibric acid)
Internal standard: naproxen (4.5)
Limit of quantitation: 500 ng/mL

KEY WORDS

plasma; SPE

REFERENCE

Ramusino,A.C.; Carozzi,A. Simple and rapid method for determining procetofenic acid, an active metabolite of procetofen, in biological fluids by solid-phase extraction and high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *383*, 419–424.

SAMPLE

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

HPLC VARIABLES

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

CHROMATOGRAM
Retention time: 18.262

KEY WORDS
whole blood

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

SAMPLE
Matrix: feces, urine
Sample preparation: Urine. Add 3-5 mL urine to a C18 Sep-Pak SPE cartridge, wash with 10 mL water, elute with 10 mL MeOH. Evaporate the eluate to dryness under reduced pressure, take up the residue in 200 µL MeOH, inject an aliquot. Feces. Vortex 2 mL homogenized feces with 10 mL MeOH for 90 s, centrifuge, evaporate the supernatant to dryness under reduced pressure, take up the residue in 5 mL water, add to a C18 Sep-Pak SPE cartridge, wash with 10 mL water, elute with 10 mL MeOH. Evaporate the eluate to dryness under reduced pressure, take up the residue in 200 µL MeOH, inject an aliquot.

HPLC VARIABLES
Column: 100 × 8 RadialPAK µBondapak
Mobile phase: MeOH:water 60:40 containing 1% glacial acetic acid
Flow rate: 2.5
Detector: UV 254

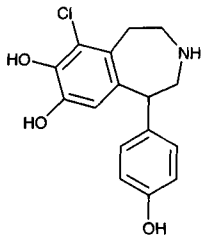
CHROMATOGRAM
Retention time: 68

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
SPE

REFERENCE
Weil,A.; Caldwell,J.; Strolin-Benedetti,M. The metabolism and disposition of 14C-fenofibrate in human volunteers, *Drug Metab.Dispos.*, **1990**, *18*, 115–120.

Fenoldopam



Molecular formula: $C_{16}H_{16}ClNO_3$
Molecular weight: 305.76
CAS Registry No.: 67227-56-9, 67227-57-0 (monomethanesulfonate)
Merck Index: 4020
Lednicer No.: 4 147

SAMPLE
Matrix: blood
Sample preparation: Add 4.75 mL plasma to 250 µL 10% ascorbic acid before storage at -20°. 1 mL Plasma + 50 µL 500 ng/mL IS in 50 mM acetic acid + 5 mL ethyl acetate + 500 µL 0.5 mM Na_2HPO_4, shake on a reciprocal shaker at 60 cycles/min for 10 min, centrifuge at 2000 g for 10 min. Remove 4.5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 µL 200 µg/mL 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate in ethyl acetate (freshly prepared), add 50 µL 1% triethylamine in

ethyl acetate (freshly prepared), let stand at room temperature for 1 h, evaporate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL MeCN:50 mM acetic acid 30:70, inject a 5-50 μL aliquot.

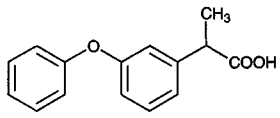
HPLC VARIABLES
Guard column: 30 × 2.1 butylsilica (Pierce)
Column: 220 × 2.1 7 μm Aquapore butylsilica (Pierce)
Mobile phase: MeCN:MeOH:buffer:water 17:15:28:42 (Prepare buffer by dissolving 22 g sodium acetate trihydrate, 21 g citric acid monohydrate, 9.8 g NaOH, and 0.63 g disodium EDTA in 2 L water, pH 5.6. Recycle mobile phase.)
Column temperature: 37
Flow rate: 0.3
Injection volume: 5-50
Detector: E, ESA, guard electrode +0.2 V, working electrode 1 -0.20 V, working electrode 2 +0.20 V

CHROMATOGRAM
Retention time: 3 (S), 6 (R)
Internal standard: 2,3,4,5-tetrahydro-1-phenyl-1H-3-benzazepine-7,8-diol (SK&F 38393-A) (25, 30 (enantiomers))
Limit of detection: 0.25 ng/mL
Limit of quantitation: 0.5 ng/mL

KEY WORDS
derivatization; chiral; plasma

REFERENCE
Boppana,V.K.; Geschwindt,L.; Cyronak,M.J.; Rhodes,G. Determination of the enantiomers of fenoldopam in human plasma by reversed-phase high-performance liquid chromatography after chiral derivatization, *J.Chromatogr.*, **1992**, *592*, 317–322.

Fenoprofen



Molecular formula: $C_{15}H_{14}O_3$
Molecular weight: 242.27
CAS Registry No.: 31879-05-7, 34507-40-5 (calcium salt), 53746-45-5 (calcium salt dihydrate)
Merck Index: 4021
Lednicer No.: 2 67

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 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 ortho-phosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 250

CHROMATOGRAM
Retention time: 6.5

OTHER SUBSTANCES
Simultaneous: ketoprofen, acetaminophen, salicylic acid, naproxen, 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 μL Plasma + 200 μL 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 μL isopropanol:water 2:8, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 100 × 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: 34 (R), 42 (S)
Limit of quantitation: 250 ng/mL

OTHER SUBSTANCES
Simultaneous: ibuprofen

KEY WORDS
plasma; chiral

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: 500 μL Plasma + 50 μL MeOH:water 1:4 + 200 μL 1 M sulfuric acid + 3 mL isooctane:isopropanol 95:5, vortex 30 s, centrifuge at 1800 g for 5 min. Remove organic layer and evaporate it to dryness. Add 300 μL 50 mM triethylamine in MeCN and 50 μL 6 mM ethylchloroformate in MeCN, wait 30 s, add 25 μL 0.1% (S)-naphthylethylamine in MeCN: triethylamine 98:2, after 3 min add 25 μL 2.5% ethanolamine in MeCN, inject 2-30 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm 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: 7.5 (S), 8.8 (R)
Internal standard: fenoprofen

Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: ibuprofen

KEY WORDS
plasma; chiral; UV 232 (see Clin.Chem. 1988; 34; 493); derivatization; fenoprofen is IS

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: 500 μL Plasma + 125 μL 40 mM decanoic acid in MeCN, mix. Dialyze a 100 μL 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 × 2 40 μm Bondesil C18 (Analytichem); B 250 × 3.1 5 μm 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: 500 ng/mL

OTHER SUBSTANCES
Extracted: flurbiprofen (UV 247), ibuprofen (UV 264), 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: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

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

Injection volume: 50
Detector: UV 272

CHROMATOGRAM
Retention time: 7.20
Limit of detection: <120 ng/mL

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

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

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 50 μL water + 200 μL 20% sulfuric acid + 6 mL n-butyl chloride, vortex for 5 min, centrifuge at 950 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 50 mM triethylamine, sonicate for 1 min, vortex for 30 s, add 50 μL 6 mM ethyl chloroformate, let stand for 30 s, add 25 μL 10 mM S-(-)-1-(1-naphthyl)ethylamine, let stand for 3 min, add 25 μL MeCN:ethanolamine 40:1. Evaporate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 250 μL mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Newguard RP-18
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:water (pH 3.0) 66.5:33.5
Column temperature: 27

Flow rate: 1.2
Injection volume: 25
Detector: F ex 280 em 320

CHROMATOGRAM
Retention time: 7.7 (S), 8.5 (R)
Internal standard: fenoprofen

OTHER SUBSTANCES
Extracted: ibuprofen

KEY WORDS
derivatization; chiral; plasma; fenoprofen is IS

REFERENCE
Lau,Y.Y. Determination of ibuprofen enantiomers in human plasma by derivatization and high-performance liquid chromatography with fluorescence detection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2143–2153.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 500 μL Plasma + 50 μL 50 μg/mL ketoprofen in 10 mM NaOH + 100 μL 600 mM sulfuric acid + 3 mL isooctane:isopropanol 95:5, vortex for 30 s, centrifuge at 3000 rpm for 5 min. Remove the organic layer and add it to 2.5 mL water, vortex for 15 s, centrifuge for 3 min. Remove the aqueous layer and add it to 200 μL 600 mM sulfuric acid, add 2.5 mL chloroform, vortex for 15 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 in MeOH containing 1 M triethylamine, after 2 min add 50 μL water, inject a 10-40 μL aliquot. Urine. 500 μL Urine + 250 μL 1 M NaOH, mix, add 300 μL 600 mM sulfuric acid, add 50 μL 50 μg/mL ketoprofen in 10 mM NaOH, add 3 mL isooctane:isopropanol 95:5, vortex for 30 s, centrifuge at 3000 rpm for 5 min. Remove the organic layer and add it to 2.5 mL water, vortex for 15 s, centrifuge for 3 min. Remove the aqueous layer and add it to 200 μL 600 mM sulfuric acid, add 2.5 mL chloroform, vortex for 15 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 in MeOH containing 1 M triethylamine, after 2 min add 50 μL water, inject a 10-40 μL aliquot.

HPLC VARIABLES
Guard column: 20 mm long 37-53 μm reverse phase
Column: 100 × 4.6 5 μm Partisil 5 ODS-3
Mobile phase: MeCN:70 mM KH_2PO_4:triethylamine 65:35:0.02, pH 6.0
Flow rate: 1 (plasma), 1.2 (urine)
Injection volume: 10-40
Detector: UV 275 for 13 min then UV 232

CHROMATOGRAM
Retention time: 16.3 (R, urine), 19.1 (R, plasma or S, urine), 22.0 (S, plasma)
Internal standard: ketoprofen (9, 10 (enantiomers))
Limit of quantitation: 250 ng/mL

KEY WORDS
plasma; derivatization; pharmacokinetics; chiral

REFERENCE
Mehvar,R.; Jamali,F. Stereospecific high-performance liquid chromatographic (HPLC) assay of fenoprofen enantiomers in plasma and urine, *Pharm.Res.*, **1988**, *5*, 53–56.

SAMPLE
Matrix: blood, urine

Sample preparation: Plasma. Adjust pH of 1 mL of plasma immediately to 2-4 with 50 μL 85% phosphoric acid, add 2 mL MeCN, centrifuge, evaporate the MeCN, extract with 3 mL ethyl acetate. Evaporate the organic solvent to dryness, reconstitute in 250 μL mobile phase, 10 μL 0.125 mM ketoprofen, and 20 μL 0.2 mM flunoxaprofen, inject a 100 μL aliquot. Urine. 500 μL Urine + 500 μL mobile phase + 50 μL 0.25 mM ketoprofen, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. MeCN:10 mM pH 2.5 tetrabutylammonium hydrogen sulfate 25:75 for 15 min, 32:68 for 10 min, 38:62 for 12 min (step gradient).
Flow rate: 1
Injection volume: 100
Detector: UV 272

CHROMATOGRAM
Retention time: 45.4
Internal standard: ketoprofen (29.9), flunoxaprofen (42.2)
Limit of detection: 100 ng/mL
Limit of quantitation: 250 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, glucuronides

KEY WORDS
plasma; pharmacokinetics

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: 100 μL Urine or rat plasma or 500 μL human plasma + 50 μL MeOH:10 mM NaOH 10:90 + 200 μL 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 μL 50 mM triethylamine in MeCN, add 50 μL 6 mM ethyl chloroformate in MeCN, vortex for 30 s, add 50 μL 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 μL mobile phase, inject a 10-150 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 37-53 μm reversed-phase
Column: 100 × 4.6 5 μm 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: 11.70, 13.40 (enantiomers)
Internal standard: fenoprofen

OTHER SUBSTANCES
Extracted: ibuprofen

KEY WORDS
derivatization; human; chiral; rat; fenoprofen is IS; plasma

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: blood, urine
Sample preparation: Plasma. 500 μL Plasma + 100 μL 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 μL mobile phase, vortex for 10 s, inject a 100 μL aliquot. Urine. 500 μL Urine + 100 μL 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 μL 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 μL mobile phase, vortex for 10 s, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 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: 5.3, 6.3 (enantiomers)
Internal standard: fenoprofen

OTHER SUBSTANCES
Extracted: ketoprofen

KEY WORDS
plasma; chiral; fenoprofen is IS

REFERENCE
Carr,R.A.; Caillé,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: Plasma. 2 mL Plasma + 250 μL 1 M HCl + 50 μL prenazone solution, vortex briefly, extract twice with 5 mL portions of diethyl ether for 15 min each time. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 36°, reconstitute the residue in 200 μL MeCN:1% aqueous acetic acid 50:50, inject an aliquot. Urine. 500 μL Urine + 250 μL 1 M HCl + 50 μL prenazone solution, mix, extract with 5 mL diethyl ether for 15 min. Remove the organic layer and add it to 1 mL 1% sodium bicarbonate solution (freshly prepared), vortex. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 36°, reconstitute the residue in 200 μL MeCN:1% aqueous acetic acid 50:50, inject an aliquot. (To hydrolyze conjugates mix 500 μL urine, 100 μL 1 M pH 5.2 sodium acetate buffer, and 20 μL enzyme solution, heat at 56° for 2.5 h, cool, proceed as above. The enzyme solution was Suc Helix Pomatia containing 100 000 Fishman U/mL of β-glucuronidase and 1 000 000 Roy U/mL of arylsulfatase (IBF).)

HPLC VARIABLES
Column: 100 × 3 5 μm Nucleosil
Mobile phase: Gradient. MeCN:1% aqueous acetic acid 50:50 for 7 min, to 80:20 over 0.6 min, maintain at 80:20 for 3.4 min, re-equilibrate at initial conditions for 8 min. (For urine isocratic MeCN:1% aqueous acetic acid 50:50.)
Flow rate: 0.5
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Internal standard: prenazone
Limit of quantitation: 200 ng/mL (urine), 50 ng/mL (plasma)

KEY WORDS
horse; plasma; pharmacokinetics

REFERENCE
Delbeke,F.T.; Landuyt,J.; Debackere,M. Disposition of human drug preparations in the horse. IV. Orally administered fenoprofen, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1041–1047.

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

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

CHROMATOGRAM
Retention time: 21.16

KEY WORDS
whole blood

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

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' 2.50 (for first enantiomer)

OTHER SUBSTANCES
Also analyzed: carprofen, cicloprofen, etodolac, flurbiprofen, ibuprofen, ketoprofen, naproxen, pirprofen, tiaprofenic acid

KEY WORDS
derivatization; α = 1.25; 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: cell suspensions
Sample preparation: Centrifuge cell suspension at 2000 g for 4 min. Remove a 2 mL aliquot of the supernatant and add it to 200 µL 100 µg/mL IS in DMF, mix, add 200 µL 5 M HCl, extract twice with 3 mL portions of toluene. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL dichloromethane, add 20 µL 10 mg/mL 1-hydroxybenzotriazole in dichloromethane:pyridine 99:1, add 300 µL 10 mg/mL 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in dichloromethane, add 300 µL 10 mg/mL (-)-(S)-α-methylbenzylamine in dichloromethane, let stand for 30 min, evaporate to dryness, reconstitute with 500 µL mobile phase, inject a 10 µL aliquot.

HPLC VARIABLES
Guard column: 10 mm long Techsphere ODS (HPLC Technology, Macclesfield UK)
Column: 250 × 5 5 µm Techsphere ODS (HPLC Technology, Macclesfield UK)
Mobile phase: MeCN:7.5 mM NaH_2PO_4 55:45, containing 5 mM sodium pentanesulfonate, pH adjusted to 2.8 with phosphoric acid
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 4.95, 5.42 (enantiomers)
Internal standard: (S)-naproxen (100 µg/mL)
Limit of detection: 5 µg/mL

KEY WORDS
derivatization; chiral

REFERENCE
Thomason,M.J.; Hung,Y.-F.; Rhys-Williams,W.; Hanlon,G.W.; Lloyd,A.W. Indirect enantiomeric separation of 2-arylpropionic acids and structurally related compounds by reversed phase HPLC, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1765–1774.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 4 × 4 5 µm LiChroCart LiChrospher 100 RP-18
Column: 125 × 4 5 µm LiChroCart LiChrospher 100 RP-18
Mobile phase: MeCN:pH 4.8 sodium acetate 38:62
Flow rate: 1.5
Injection volume: 50
Detector: UV 223

CHROMATOGRAM
Internal standard: fenoprofen

OTHER SUBSTANCES
Simultaneous: ibuprofen

REFERENCE

Dominkus,M.; Nicolakis,M.; Kotz,R.; Wilkinson,F.E.; Kaiser,R.R.; Chlud,K. Comparison of tissue and plasma levels of ibuprofen after oral and topical administration, *Arzneimittelforschung*, **1997**, *46*, 1138–1143.

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, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

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

SAMPLE
Matrix: solutions
Sample preparation: Mix 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: 14, 21 (enantiomers)

OTHER SUBSTANCES
Also analyzed: benoxaprofen (MeOH:buffer 80:20), carprofen, flurbiprofen, ibuprofen, ketoprofen, pirprofen, 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

HPLC VARIABLES
Column: 125 × 3 Ecocart LiChrospher 100 RP-18
Mobile phase: MeCN:100 mM pH 7.4 KH_2PO_4 30:70
Flow rate: 0.4
Injection volume: 2.5
Detector: F ex 271 em 293

CHROMATOGRAM
Retention time: 4.6

OTHER SUBSTANCES
Simultaneous: lonazolac (F ex 282 em 345)

REFERENCE
Baeyens,W.R.G.; Van Der Weken,G.; Lievens,L.; Van Overbeke,A. LC-study of lonazolac, naproxen and related non-steroidal anti-inflammatory drugs in a classical and a narrow-bore set-up applying UV and fluorescence detection, *Biomed.Chromatogr.*, **1995**, *9*, 263–264.

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: 18.5
Internal standard: ketoprofen (11.6)
Limit of quantitation: 30 ng/mL

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: 8.00 (A), 8.87 (B)

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

KEY WORDS
also details of plasma extraction

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

SAMPLE
Matrix: solutions
Sample preparation: 1 mL 5 mM fenoprofen 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 × 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 230, UV 254

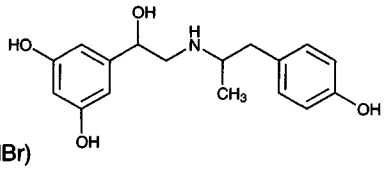
CHROMATOGRAM
Retention time: k' 3.21 (first enantiomer)

OTHER SUBSTANCES
Also analyzed: flurbiprofen, ibuprofen, ketoprofen, tiaprofenic acid

KEY WORDS
derivatization; narrow-bore; chiral; α=1.31; (see Biomed. Chromatogr. 1995; 9; 292)

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.

Fenoterol



Molecular formula: $C_{17}H_{21}NO_4$
Molecular weight: 303.36
CAS Registry No.: 13392-18-2, 1944-12-3 (HBr), 1944-10-1 (HBr)
Merck Index: 4022
Lednicer No.: 2 38

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

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

CHROMATOGRAM
Retention time: 1.6

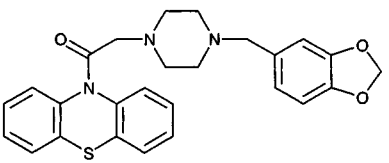
OTHER SUBSTANCES

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

REFERENCE

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

Fenoverine



Molecular formula: $C_{26}H_{25}N_3O_3S$
Molecular weight: 459.57
CAS Registry No.: 37561-27-6
Merck Index: 4023

SAMPLE

Matrix: blood, urine

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

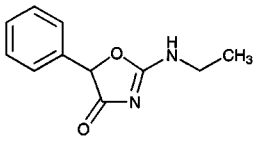
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85: 15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.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

CHROMATOGRAM
Retention time: 15.57

KEY WORDS
whole blood

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

Fenozolone



Molecular formula: $C_{11}H_{12}N_2O_2$
Molecular weight: 204.23
CAS Registry No.: 15302-16-6
Merck Index: 4028

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

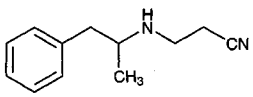
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85: 15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 220.5

CHROMATOGRAM
Retention time: 12.913

KEY WORDS
whole blood

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

Fenproporex



Molecular formula: $C_{12}H_{16}N_2$
Molecular weight: 188.27
CAS Registry No.: 15686-61-0, 18305-29-8 (HCl)
Merck Index: 4036

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

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

CHROMATOGRAM
Retention time: 19.263

KEY WORDS
whole blood

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

SAMPLE
Matrix: solutions

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

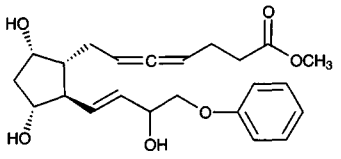
OTHER SUBSTANCES

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

REFERENCE

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

Fenprostalene



Molecular formula: $C_{23}H_{30}O_6$
Molecular weight: 402.49
CAS Registry No.: 69381-94-8
Merck Index: 4037
Lednicer No.: 4 9

SAMPLE

Matrix: solutions

HPLC VARIABLES
Column: 250 × 3.2 10 μm Spherisorb ODS
Mobile phase: MeOH:20 mM acetic acid 55:45
Injection volume: 100
Detector: UV 219 or 270

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Johnson,D.M.; Taylor,W.F.; Thompson,G.F.; Pritchard,R.A. Degradation of fenprostalene in aqueous solution, *J.Pharm.Sci.*, **1983**, *72*, 946–948.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere octyl
Mobile phase: MeCN:water 35:65 to 45:55
Injection volume: 100
Detector: UV 219

CHROMATOGRAM
Retention time: 22

REFERENCE
Johnson,D.M.; Taylor,W.F. Degradation of fenprostalene in polyethylene glycol 400 solution, *J.Pharm.Sci.*, **1984**, *73*, 1414–1417.

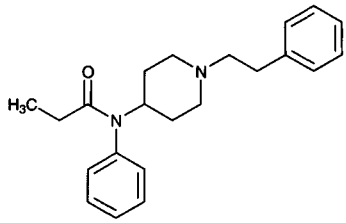
SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Polytron Model PT-10-ST/PT-35) 60 g liver with 240 mL cold water, adjust to pH 2 with 2 M HCl, extract three times with an equal volume of ethyl acetate. Combine the extracts, neutralize with solid sodium bicarbonate, dry over anhydrous sodium sulfate, evaporate to 50 mL under vacuum, evaporate to dryness, take up the residue in 50 mL MeOH:water 90:10, wash with 40 mL hexane, evaporate the MeOH/water phase to dryness, dissolve the residue in MeOH, chromatograph on silica gel 60F-254 tlc plates (EM Science) with ethyl acetate:MeOH 80:20, scrape off the fenprostalene layer, desorb with 100 μL mobile phase, inject a 70 μL aliquot.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeOH:10 mM acetic acid 60:40
Flow rate: 1
Injection volume: 70
Detector: UV 225

KEY WORDS
pig; liver

REFERENCE
Spires,H.R.; Bowen,J.L.; Tomlinson,R.V.; Donahue,D.J. Pharmacokinetic and tissue residue characteristics of fenprostalene, a prostaglandin F2 α analog, in swine, *Am.J.Vet.Res.*, **1990**, *51*, 386–390.

Fentanyl



Molecular formula: $C_{22}H_{28}N_2O$
Molecular weight: 336.48
CAS Registry No.: 437-38-7, 990-73-8 (citrate)
Merck Index: 4043
Lednicer No.: 1 299

SAMPLE

Matrix: blood
Sample preparation: 100 μL Plasma + 50 μL 5 M NaOH + 100 μL MeCN + 600 μL hexane, mix, centrifuge at 2000 rpm for 5 min. Evaporate the organic phase under nitrogen at 30° for about 10 min. Reconstitute the residue in 100 μL MeCN:50 mM pH 3.0 phosphate buffer 50:50, inject a 50 μL aliquot. (Use silanized glassware.)

HPLC VARIABLES

Column: 100 × 8 4 μm cyano column (Waters)
Mobile phase: MeCN:50 mM pH 3.2 phosphate buffer 50:50
Flow rate: 2.5
Injection volume: 50
Detector: UV 210

CHROMATOGRAM

Retention time: 6.2
Limit of detection: 150 pg
Limit of quantitation: 2.5 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Noninterfering: ampicillin, calcium chloride, clafoxan, diazepam, dobutamine, dopamine, furosemide, gentamicin, morphine, midazolam, pavulon, phenytoin, vitamin K

KEY WORDS

plasma

REFERENCE

Bansal,R.; Aranda,J.V. High-performance liquid chromatography microassay for the simultaneous determination of fentanyl and its major metabolites in biological samples, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 353–364.

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL BondElut C18 SPE cartridge once with 1 M HCl, twice with MeOH, and once with water, remove the liquid completely with suction each time. Add 250 μL IS solution and 250 μL serum to the column at 1 mL/min, wash twice with water and once with MeCN draining the column completely after each wash, elute with 250 μL eluting solution, centrifuge for 20 s to remove last of eluate, inject a 5 μL aliquot of the eluate. (Prepare IS solution by adding 40 μL 1 mg/mL N-pentyl-2,6-pipecoloxylidide (1-pentyl-N-(2,6-dimethylphenyl)-2-piperidinecarboxamide, pentyl-PPX) in MeOH to 10 mL 100 mM NaH_2PO_4. Eluting solution was 2.5 mL 35% perchloric acid in 100 mL MeOH.)

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-8 (Applied Biosystems)
Column: 150 × 4.6 5 μm Ultrasphere octyl
Mobile phase: MeCN:10 mM KH_2PO_4 25:80, pH 5.2
Flow rate: 1.5
Injection volume: 5
Detector: UV 205

CHROMATOGRAM
Retention time: 11.4
Internal standard: N-pentyl-2,6-pipecoloxylidide (1-pentyl-N-(2,6-dimethylphenyl)-2-piperidinecarboxamide, pentyl-PPX) (14.5)

OTHER SUBSTANCES
Extracted: bupivacaine, mepivacaine, meperidine
Noninterfering: acetaminophen, codeine, epinephrine, morphine, diazepam

KEY WORDS
serum; SPE

REFERENCE
Gupta,R.N.; Dauphin,A. Column liquid chromatographic determination of bupivacaine in human serum using solid-phase extraction, *J.Chromatogr.B*, **1994**, *658*, 113–119.

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

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

CHROMATOGRAM
Retention time: 2.8
Internal standard: protriptyline (4)

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

KEY WORDS
plasma; SPE

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

SAMPLE
Matrix: blood, urine
Sample preparation: 50 μL Plasma or urine + 50 μL 4 M NaOH + 100 μL MeCN + 500 μL n-hexane, vortex for 30 s, centrifuge at 2000 rpm for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 100 × 8 4 μm Nova pak cyano
Mobile phase: MeCN:5 mM pH 3.2 phosphate buffer 70:30
Flow rate: 2.5
Injection volume: 50
Detector: UV 214

CHROMATOGRAM
Retention time: 6.5
Limit of detection: 2.5 ng/mL

OTHER SUBSTANCES
Extracted: alfentanil, sufentanil

KEY WORDS
plasma

REFERENCE
Bansal,R.; Aranda,J.V. Simultaneous microassay of alfentanil, fentanil, and sufentanil by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 339–348.

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

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 255.8

CHROMATOGRAM
Retention time: 14.202

KEY WORDS
whole blood

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

SAMPLE
Matrix: formulations
Sample preparation: Inject a 20 μL aliquot

HPLC VARIABLES
Column: 100 × 3.9 4 μm Radial Pak phenyl (Waters)
Mobile phase: MeOH:buffer 65:35 (Buffer was 5 mM pH 4.8 phosphate buffer containing 1.4 mM tetrabutylammonium hydroxide.)
Flow rate: 3
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 11.6

OTHER SUBSTANCES
Simultaneous: degradation products, bupivacaine

KEY WORDS
injections; saline; stability-indicating

REFERENCE
Tu,Y.H.; Stiles,M.L.; Allen,L.V.,Jr. Stability of fentanyl citrate and bupivacaine hydrochloride in portable pump reservoirs, *Am.J.Hosp.Pharm.*, **1990**, *47*, 2037–2040.

SAMPLE
Matrix: formulations
Sample preparation: 100 μL Injection solution + 400 μL 2.5 μg/mL haloperidol in water, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Brownlee C18
Mobile phase: MeCN:MeOH:10 mM NaH_2PO_4 24:31:45, pH adjusted to 5.0 with 2 M KOH
Flow rate: 1.7
Injection volume: 100
Detector: UV 210

CHROMATOGRAM
Retention time: 7.88
Internal standard: haloperidol (9.74)

OTHER SUBSTANCES
Extracted: sufentanil

KEY WORDS
injections

REFERENCE
Dewell,W.M.,Jr.; Khandaghabadi,M.; D'Souza,M.J.; Solomon,H.M. High-performance liquid chromatographic determination of fentanyl and sufentanil returned from the operating room, *Am.J.Hosp.Pharm.*, **1993**, *50*, 2374–2375.

SAMPLE
Matrix: formulations

Sample preparation: Dilute 5-fold with mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb phenyl
Mobile phase: MeOH:buffer 65:35 (Buffer was 5 mM pH 4.8 KH_2PO_4 containing 1.4 mM tetrabutylammonium hydroxide.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 229

CHROMATOGRAM
Retention time: 5.1

OTHER SUBSTANCES
Noninterfering: midazolam

KEY WORDS
injections; 5% dextrose; stability-indicating

REFERENCE
Bhatt-Mehta,V.; Johnson,C.E.; Leininger,N.; Agarwal,M. Stability of fentanyl citrate and midazolam hydrochloride during simulated intravenous coadministration, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 511–513.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 500 μL Microsomal incubation + 500 μL DMSO, vortex, centrifuge, filter, inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: Gradient. A was 1 M ammonium acetate. B was MeCN:MeOH:THF:1 M ammonium acetate 30:20:40:10. A:B from 100:0 to 35:65 over 40 min.
Flow rate: 1
Detector: UV 230

CHROMATOGRAM
Retention time: 40

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver; pharmacokinetics

REFERENCE
Tateishi,T.; Wood,A.J.J.; Guengerich,F.P.; Wood,M. Biotransformation of tritiated fentanyl in human liver microsomes. Monitoring metabolism using phenylacetic acid and 2-phenylethanol, *Biochem.Pharmacol.*, **1995**, *50*, 1921–1924.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 3.9 μBondapak C18
Mobile phase: MeCN:water adjusted to pH 3 with phosphoric acid 70:30
Flow rate: 1
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Internal standard: codeine

REFERENCE
Vanbever,R.; Le Boulengé,E.; Préat,V. Transdermal delivery of fentanyl by electroporation. I. Influence of electrical factors, *Pharm.Res.*, **1996**, *13*, 559–565.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 1.46

OTHER SUBSTANCES
Simultaneous: tranylcypromine, caffeine, fenethyline, phendimetrazine, methylphenidate, phenelzine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, nalorphine, phenazocine, norpipanone, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: pemoline, benzphetamine, diethylpropion, mazindol, buprenorphine, dextromoramide, phenoperidine, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene

REFERENCE
Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

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

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

CHROMATOGRAM
Retention time: 1.6

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine,

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

REFERENCE

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

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in mobile phase.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm Applied Biosystems pre-column
Column: 100 × 2 10 μm μPorasil
Mobile phase: MeCN:5 mM pH 3.75 sodium acetate 80:20
Flow rate: 1
Injection volume: 200
Detector: UV 214

CHROMATOGRAM

Retention time: 9.39
Limit of detection: 5.5 ng/mL

OTHER SUBSTANCES

Simultaneous: buprenorphine, nalbuphine, ethylmorphine, morphine, codeine, meperidine, tramadol
Noninterfering: thiopentone, succinylcholine, pancuronium, diazepam, atropine, neostigmine
Interfering: butorphanol

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

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

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

SAMPLE
Matrix: solutions

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 11.40 (A), 6.03 (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, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

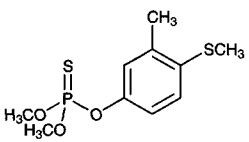
KEY WORDS

also details of plasma extraction

REFERENCE

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

Fenthion



Molecular formula: $C_{10}H_{15}O_3PS_2$
Molecular weight: 278.33
CAS Registry No.: 55-38-9
Merck Index: 4044

SAMPLE

Matrix: blood

Sample preparation: 1.5 mL Serum + 2 mL 200 mM pH 7.0 phosphate buffer, add to an Extrelut No. 3 SPE column, let stand for 10 min, elute with 15 mL n-hexane:diethyl ether 80:20. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue with 150 μL MeOH:water 70:30, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Gradient. MeOH:water from 70:30 to 90:10.
Flow rate: 1
Injection volume: 200
Detector: MS, Hitachi Model M-2000, APCI non-equilibrium interface, vaporizer 250°, nebulizer 400°, ionization needle electrode current 5 μA, drift voltage 230 V, vacuum 0.0001 Pa, ion-source slit 500 μm, collector slit 400 μm, accelerated electrical potential 4 kV, secondary electronic step-up tube potential 1.3 kV, positive-ion mode

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CHROMATOGRAM
Retention time: 10.6
Limit of detection: 20 ng

OTHER SUBSTANCES
Extracted: diazinon, dichlorvos, dimethoate, dimethylvinphos, ediphenphos, IBP, isoxathon, malathion, phenthoate, propaphos, pyridafenthion

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KEY WORDS
serum; SPE; m/z 279

REFERENCE
Kawasaki,S.; Ueda,H.; Itoh,H.; Tadano,J. Screening of organophosphorus pesticides using liquid chromatography-atmospheric pressure chemical ionization mass spectrometry, *J.Chromatogr.*, **1992**, *595*, 193–202.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve in 1 mL 0.5 M NaOH, heat at 75° for 45 min, cool, add 2 drops methyl isobutyl ketone, add 2 drops 0.1% dansyl chloride in acetone, shake well, heat at 65° for 30 min, cool, acidify with 10% HCl, add 300 μL benzene (Caution! Benzene is a carcinogen!), extract, inject a 1-10 μL aliquot.

HPLC VARIABLES
Column: 1000 × 2.4 Zipax coated with 0.5% β,β'-oxydipropionitrile
Mobile phase: Hexane:MeOH 95:5
Flow rate: 0.78
Injection volume: 1-10
Detector: F ex Turner filter no. 811 em Turner filter no. 817

CHROMATOGRAM
Retention time: 3

KEY WORDS
derivatization; normal phase

REFERENCE
Frei,R.W.; Lawrence,J.F. Fluorigenic labelling in high-speed liquid chromatography, *J.Chromatogr.*, **1973**, *83*, 321–330.

SAMPLE
Matrix: solutions
Sample preparation: Condition a 10 × 2 SPE column packed with 40 μm octadecylsilica (Spark Holland) with 10 mL MeCN, 10 mL MeOH, and 10 mL water at 2 mL/min. Add nitric acid to a final concentration of 0.5% to water sample, filter (0.45 μm), add a 150 mL aliquot to the SPE column at 3 mL/min, wash with 3 mL distilled water, elute the contents of the SPE column on to the analytical column with the mobile phase.

HPLC VARIABLES
Column: 250 × 4 4 μm Superspher 60 RP-8 endcapped C8 (Merck)
Mobile phase: Gradient. A was MeCN:MeOH 80:20. B was water. A:B from 10:90 to 40:60 over 10 min, maintain at 40:60 for 5 min, to 90:10 over 33 min, return to initial conditions over 5 min.
Flow rate: 1
Detector: UV 220

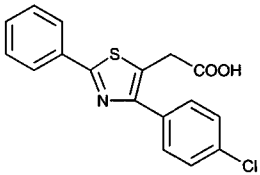
CHROMATOGRAM
Retention time: 39.8
Limit of detection: 65 ng/L

OTHER SUBSTANCES
Simultaneous: azinphos-ethyl, azinphos-methyl, chlorfenvinphos, dichlorvos, fenitrothion, malathion, mevinphos, parathion-ethyl, parathion-methyl
Interfering: diazinon

KEY WORDS
groundwater; wastewater; SPE

REFERENCE
Lacorte,S.; Barceló,D. Improvements in the determination of organophosphorus pesticides in ground- and wastewater samples from interlaboratory studies by automated on-line liquid-solid extraction followed by liquid chromatography-diode array detection, *J.Chromatogr.A*, **1996**, *725*, 85–92.

Fentiazac



Molecular formula: $C_{17}H_{12}ClNO_2S$
Molecular weight: 329.81
CAS Registry No.: 18046-21-4
Merck Index: 4045
Lednicer No.: 4 96

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

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 247

CHROMATOGRAM
Retention time: 13.49
Limit of detection: <120 ng/mL

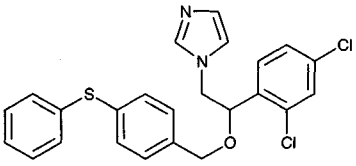
KEY WORDS

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

REFERENCE

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

Fenticonazole



Molecular formula: $C_{24}H_{20}Cl_2N_2OS$
Molecular weight: 455.41
CAS Registry No.: 72479-26-6, 73151-29-8 (nitrate)
Merck Index: 4047
Lednicer No.: 4 93

SAMPLE

Matrix: formulations

Sample preparation: Tablets. Powder tablets, weigh out amount equivalent to about 30 mg, add 100 mL MeOH, sonicate for 5 min, filter. Add a 2 mL aliquot of filtrate to 5 mL of 100 μg/mL ketoconazole in MeOH, make up to 25 mL with MeOH, inject 20 μL aliquot. Cream. Condition a 500 mg Bond-Elut diol cartridge with 6 mL dichloromethane. Weigh out cream equivalent to about 5 mg of drug, add 30 mL dichloromethane, sonicate for 3 min, make up to 100 mL with dichloromethane, filter. Add a 2 mL aliquot to the cartridge, wash with 2 mL dichloromethane:methanol 4:1, wash with 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

CHROMATOGRAM
Retention time: 32
Internal standard: ketoconazole (7)

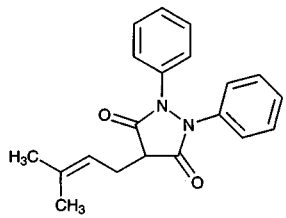
OTHER SUBSTANCES
Simultaneous: clotrimazole, ketoconazole, bifonazole, tioconazole, isoconazole, econazole, miconazole

KEY WORDS
tablets; creams

REFERENCE
Di Pietra,A.M.; Cavrini,V.; Andrisano,V.; Gatti,R. HPLC analysis of imidazole antimycotic drugs in pharmaceutical formulations, *J.Pharm.Biomed.Anal.*, **1992**, *10*, 873–879.

Feprazone

Molecular formula: $C_{20}H_{20}N_2O_2$
Molecular weight: 320.39
CAS Registry No.: 30748-29-9
Merck Index: 4053



SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 2 mL Plasma + 250 μL 1 M HCl + 5 mL diethyl ether, mix, centrifuge at 1100 g for 10 min. Remove the organic phase, repeat the extraction, combine the organic extracts, evaporate under nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, vortex, inject a 20 μL aliquot. Urine. 1 mL Urine + 250 μL 1 M HCl + 5 mL diethyl ether, rotate for 15 min. Remove the organic layer, add 1 mL 1% sodium hydrogen carbonate, vortex for 1 min. Remove the organic layer and evaporate it under nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 3 5 μm Nucleosil
Mobile phase: MeCN:water:acetic acid 40:59.4:0.6
Flow rate: 0.8
Injection volume: 20
Detector: UV 245

CHROMATOGRAM
Retention time: 8.3
Internal standard: feprazone

OTHER SUBSTANCES
Extracted: tiaprofenic acid (UV 310)

Noninterfering: alclofenac, diclofenac, fenoprofen, flunixin, flurbiprofen, ibuprofen, indomethacin, naproxen, oxyphenbutazone, phenylbytazone, piroxicam

KEY WORDS
plasma; feprazone is IS

REFERENCE
Delbeke,F.T.; Baert,K.; De Backer,P. Disposition of human drug preparations in the horse. VI. Tiaprofenic acid, *J.Chromatogr.B*, **1997**, *704*, 207–214.

Fibrinolysin

Molecular weight: about 90000
CAS Registry No.: 9004-09-5, 9001-90-5
Merck Index: 7678

SAMPLE
Matrix: solutions

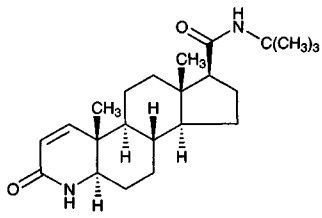
HPLC VARIABLES
Column: 100 × 6 Asahipak GS-520-AHA-ABA (Prepare by suspending 10 g Asahipak-GS gel (Asahi Chemical Industry) in water, sonicate for 5 min, wash with 200 mL water, wash with 200 mL dioxane, suspend in 100 mL dioxane, add 3.24 g 1,1'-carbonyldiimidazole, stir gently for 15 min at room temperature, wash with 200 mL dioxane, suspend in 200 mL 1 M sodium bicarbonate containing 1 M 6-aminohexanoic acid, shake at 4° for 25 h, wash with 200 mL water, wash with 100 mL 1 M NaCl, wash with 200 mL water. Suspend 2 g of the gel in 15 mL 200 mM pH 4.752-(morpholino)ethanesulfonic acid/NaOH buffer, add 288 mg 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide monohydrochloride, stir gently for 30 min, add 28.3 mg p-aminobenzamide monohydrochloride, adjust pH three times to 4.75 with 1 M HCl or 1 M NaOH at 30 min intervals, shake gently at room temperature for 24 h, wash with 150 mL water, wash with 100 mL 50 mM NaOH containing 1 M NaCl, wash with 100 mL 50 mM HCl containing 1 M NaCl, wash with water until washings are neutral. Caution! Dioxane is a carcinogen. It may be possible to use acetone instead.)
Mobile phase: Gradient. Buffer A, after 10 min buffer B, after 25 min buffer B containing 40 mM 6-hexanoic acid, after 40 min buffer B containing 40 mM 6-hexanoic acid and 1 M urea (step gradient). Buffer A was 50 mM pH 6.5 sodium phosphate. Buffer B was 50 mM pH 7.4 sodium phosphate containing 100 mM NaCl.
Flow rate: 1.8
Detector: F ex 285 em 340 (?)

CHROMATOGRAM
Retention time: 50

OTHER SUBSTANCES
Simultaneous: plasminogens

REFERENCE
Ito,N.; Noguchi,K.; Kazama,M.; Shimura,K.; Kasai,K.-I. Separation of human Glu-plasminogen, Lys-plasminogen and plasmin by high-performance affinity chromatography on Asahipak GS gel coupled with *p*-aminobenzamide, *J.Chromatogr.*, **1985**, *348*, 199–204.

Finasteride



Molecular formula: $C_{23}H_{36}N_2O_2$
Molecular weight: 372.55
CAS Registry No.: 98319-26-7
Merck Index: 4125
Lednicer No.: 5 49

SAMPLE

Matrix: blood
Sample preparation: Condition a 6 mL 300 mg Carbograph SPE cartridge (Alltech) with 2 mL water and 4 mL MeOH. Add 100 µL 10 µg/mL IS to 1.0 mL plasma, briefly vortex, add the mixture to the SPE cartridge. Wash with 2 mL water and 4 mL MeOH:water 20:80. Elute with 2 mL MeOH:chloroform 20:80 (Caution! Chloroform is a carcinogen!). Centrifuge eluate at 1000 g for 10 min, filter through a WTP 500 nm filter, evaporate to dryness with a stream of nitrogen under vacuum. Reconstitute the residue in 1.0 mL mobile phase, vortex, inject a 50 µL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 40 µm Pelliguard (Supelco)
Column: 250 × 4.6 5 µm reversed-phase Symmetry (Waters)
Mobile phase: MeCN:40 mM pH 4.0 orthophosphoric acid 45:55
Flow rate: 1.2
Injection volume: 50
Detector: UV 215

CHROMATOGRAM

Retention time: 14.6
Internal standard: 4-androstene-3,17-dione (9.8)
Limit of detection: 5 ng/mL
Limit of quantitation: 10 ng/mL

KEY WORDS

plasma; SPE

REFERENCE

Carlucci,G.; Mazzeo,P. Finasteride in biological fluids: extraction and separation by a graphitized carbon black cartridge and quantification by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *693*, 245–248.

SAMPLE

Matrix: blood
Sample preparation: Non-buffered extraction. 1 mL Plasma + 100 µL 100 ng/mL IS in MeOH. Add 7 mL MTBE, mix and rotate for 15 min, centrifuge, remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°. Reconstitute the residue in 300 µL mobile phase, inject an aliquot. Buffered extraction. 1 mL Plasma + 100 µL 100 ng/mL IS in MeOH. Add 1 mL 200 mM pH 9.8 carbonate buffer and 7 mL MTBE. Extract as described above. Inject an aliquot.

HPLC VARIABLES

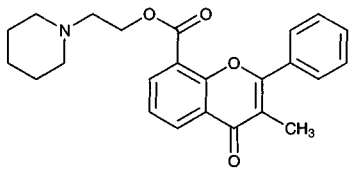
Column: 30 × 4.6 3 µm BDS Hypersil C18 (A), 50 × 2 3 µm BDS Hypersil C18 (B)
Mobile phase: MeCN:water containing 0.1% formic acid 90:10
Column temperature: 60
Flow rate: 1(A), 0.2 (B)
Detector: MS, PE Sciex API IIIplus tandem MS, heated nebulizer 500°, corona discharge needle +4 µA, APCI positive ion, nebulizing gas air 550 kPa, auxiliary gas at 2 mL/min, curtain gas nitrogen at 0.9 mL/min, orifice +50 V, interface heater 60°, collision gas argon, m/z 373 (A), Turbo ion spray, nebulizing gas air 410 kPa, auxiliary gas at 6 mL/min, curtain gas at 1mL/min, orifice +30 V, interface heater 60°, m/z 373 (B)

CHROMATOGRAM
Retention time: 0.5 (A), 1 (B)
Internal standard: finasteride analog (1.1)
Limit of detection: 25 pg/mL (B)

KEY WORDS
plasma

REFERENCE
Matuszewski,B.K.; Constanzer,M.L.; Chavez-Eng,C.M. Matrix effect in quantitative LC/MS/MS analyses of biological fluids: A method for determination of finasteride in human plasma at picogram per milliliter concentrations, *Anal.Chem.*, **1998**, *70*, 882–889.

Flavoxate



Molecular formula: $C_{24}H_{25}NO_4$
Molecular weight: 391.47
CAS Registry No.: 15301-69-6, 3717-88-2 (HCl)
Merck Index: 4135
Lednicer No.: 2 392

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

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

CHROMATOGRAM
Retention time: 2.8

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, 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, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortrip-

tyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 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), 5.94 (B)

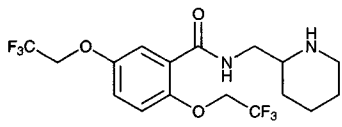
KEY WORDS

also details of plasma extraction; also 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; fluoxetine; fluphenazine; flurazepam; flurbiprofen; fluvoxamine; furosemide; glutethimide; glyburide; guaifenesin; haloperidol; homatropine; hydralazine; hydrochlorothiazide; hydrocodone; hydromorphone; hydroxychloroquine; hydroxyzine; ibuprofen; imipramine; indomethacin; ketoconazole; ketoprofen; ketorolac; labetalol; levorphanol; lidocaine; loratadine; lorazepam; lovastatin; loxapine; mazindol; mefenamic acid; meperidine; mephenytoin; mepivacaine; mesoridazine; metaproterenol; methadone; methdilazine; methocarbamol; methotrexate; methotrimeprazine; methoxamine; methyldopa; methylphenidate; metoclopramide; metolazone; metoprolol; metronidazole; midazolam; moclobemide; morphine; nadolol; nalbuphine; naloxone; naphazoline; naproxen; nifedipine; nizatidine; norepinephrine; nortriptyline; oxazepam; oxycodone; oxymetazoline; paroxetine; pemoline; pentazocine; pentobarbital; pentoxifylline; perphenazine; pheniramine; phenobarbital; phenol; phenolphthalein; phentolamine; phenylbutazone; phenyltoloxamine; phenytoin; pimozide; pindolol; piroxicam; pramoxine; prazepam; prazosin; probenecid; procainamide; procaine; prochlorperazine; procyclidine; promazine; promethazine; propafenone; propantheline; propiomazine; propofol; propranolol; protriptyline; quazepam; quinidine; quinine; racemethorphan; ranitidine; remoxipride; risperidone; salicylic acid; scopolamine; secobarbital; sertraline; sotalol; spironolactone; sulfinpyrazone; sulindac; temazepam; terbutaline; terfena-

dine; tetracaine; theophylline; thiethylperazine; thiopental; thioridazine; thiothixene; timolol; tocainide; tolbutamide; tolmetin; trazodone; triamterene; triazolam; trifluoperazine; trifluopromazine; trimeprazine; trimethoprim; trimipramine; verapamil; warfarin; xylometazoline; yohimbine; zopiclone

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

Flecainide



Molecular formula: $C_{17}H_{20}F_6N_2O_3$
Molecular weight: 414.35
CAS Registry No.: 54143-55-4, 54143-56-5 (acetate)
Merck Index: 4136
Lednicer No.: 3 59

SAMPLE

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

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 3.1
Internal standard: protriptyline (4)

OTHER SUBSTANCES

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

Interfering: diphenhydramine, doxepin, fentanyl, haloperidol, nordoxepin, trazodone

KEY WORDS
plasma; SPE

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

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

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 299

CHROMATOGRAM
Retention time: 5.22
Limit of detection: <120 ng/mL

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

amine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

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

SAMPLE

Matrix: blood, gastric contents, tissue, urine

Sample preparation: Homogenize 15 g liver with water to a final volume of 90 mL, dilute 1 to 10 with water. Homogenize gastric contents, dilute 1 to 100 with water. Dilute urine 1 to 25. 1 mL Whole blood, diluted liver homogenate, diluted gastric contents, or diluted urine + 50 μL 100 μg/mL clomipramine, mix, inject an aliquot.

HPLC VARIABLES

Guard column: 20 mm long Hypersil ODS

Column: 150 mm long Hypersil ODS

Mobile phase: MeCN:10 mM Na_2HPO_4:n-nonylamine 40:60:0.12, pH 3

Flow rate: 1

Detector: UV 295

CHROMATOGRAM

Retention time: 3

Internal standard: clomipramine (5.5)

Limit of quantitation: 1 μg/mL

KEY WORDS

liver; whole blood

REFERENCE

Sadler,D.W.; Quigley,C. Unsuspected self-poisoning with flecainide and alcohol, *J.Forensic Sci.*, **1995**, *40*, 903–905.

SAMPLE

Matrix: blood, urine

Sample preparation: Dilute urine 1:100. 1 mL Plasma or diluted urine + 100 μL 4 μg/mL IS in water + 1 mL water + 1 mL 1 M NaOH + 8 mL distilled diethyl ether, shake for 15 min, centrifuge at 1000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 1 mL freshly prepared 49 mM 1-[(4-nitrophenyl)sulfonyl]-L-prolyl chloride in ethyl acetate, add 100 μL 0.016% triethylamine in ethyl acetate, vortex for 5 s, heat at 80° for 2 h, cool, wash with 3 mL 600 mM HCl, centrifuge for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 40-180 μL aliquot. (Synthesis of 1-[(4-nitrophenyl)sulfonyl]-L-prolyl chloride is as follows. Stir 40-45 mmoles L-(-)-proline in 40 mL THF and 200 mL 10% potassium carbonate, add 37-43 mmoles 4-nitrophenylsulfonyl chloride in 40 mL THF dropwise, heat at 50° for 3 h and maintain at pH 8 or above, cool, acidify to pH 2 and extract into chloroform. Extract with 10% potassium carbonate, acidify the aqueous layer, extract into chloroform. Dry the chloroform extract, evaporate, recrystallize from petroleum ether and benzene (Caution! Benzene is a carcinogen!). Stir 15 mmoles of the 4-nitrophenylsulfonylproline in 100 mL benzene under reflux condenser fitted with a calcium sulfate drying tube, add dropwise a five-fold molar excess of thionyl chloride in 50 mL benzene, heat at 35-40° until formation of the acid chloride is complete (about 48 h, monitor by IR spectroscopy), evaporate, recrystallize product from HPLC-grade heptane (mp 110-110.5°).)

HPLC VARIABLES

Guard column: 50 mm long octadecyl Pellicular ODS (Whatman)

Column: 300 × 3.9 Bondapak C18

Mobile phase: MeCN:water:triethylamine 45:55:0.2

Flow rate: 1

Injection volume: 40-180
Detector: UV 280

CHROMATOGRAM
Retention time: 29.8 (R), 31.9 (S)
Internal standard: (R,S)-N-(2-piperidylmethyl)-2,3-bis-(2,2,2-trifluoroethoxy)benzamide (R, 25.5, S, 27.9)
Limit of quantitation: 50 ng/mL

KEY WORDS
plasma; derivatization; pharmacokinetics; chiral

REFERENCE
Alessi-Severini,S.; Jamali,F.; Pasutto,F.M.; Coutts,R.T.; Gulamhusein,S. High-performance liquid chromatographic determination of the enantiomers of flecainide in human plasma and urine, *J.Pharm.Sci.*, **1990**, *79*, 257–260.

SAMPLE
Matrix: formulations
Sample preparation: Extract ground tablets containing 10-50 mg of the compound with 100 mL MeOH, filter. Add 500 μL 100 μg/mL IS in MeOH to 1 mL filtrate, make up to 10 mL with MeOH. Inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm LiChrosorb
Mobile phase: MeCN:MeOH:buffer 30:60:10 (Buffer was 67 mM KH_2PO_4 adjusted to pH 2.9 with phosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 3.30 (flecainide acetate)
Internal standard: amiodarone (7.46)
Limit of quantitation: 4 μg/mL

KEY WORDS
tablets

REFERENCE
Paw,B.; Przyborowski,L.; Slawik,T. Determination of flecainide acetate in tablets by HPLC and UV-spectrophotometry, *Pharmazie*, **1998**, *53*, 97–98.

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

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

CHROMATOGRAM
Retention time: k' 2.93
Limit of detection: 1 fmole (E), 1 nmole (UV)

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

KEY WORDS
derivatization; complexation

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

SAMPLE
Matrix: solutions
Sample preparation: Mix a 50 μL aliquot of a solution in MeOH:triethylamine 99:1 with 20 μL 0.1% FLOPIC in dry toluene, vortex briefly, let stand at room temperature in the dark for 30 min, add 50 μL 1% ethanolamine in MeOH, let stand at room temperature for 15 min, evaporate to dryness under reduced pressure, reconstitute with 100 μL mobile phase, sonicate for 30 s, inject a 20 μL aliquot. (FLOPIC is (-)-(S)-flunoxaprofen isocyanate; synthesis is as follows. Dissolve 1 g (+)-(S)-flunoxaprofen in 30 mL acetone, cool to 0°, add a solution of 500 μL triethylamine in 2 mL acetone dropwise, add a solution of 370 μL ethyl chloroformate in 2 mL acetone dropwise, stir at 0° for 15 min, add a solution of 250 mg sodium azide in 1 mL water dropwise (Caution! Sodium azide is highly toxic!), stir for 1 h, pour into 60 mL ice water, stir for 10 min, filter, wash the solid with two 50 mL aliquots of ice-water, dry under reduced pressure to obtain flunoxaprofen azide. Dissolve 100 mg flunoxaprofen azide in 3 mL dry toluene, reflux for 10-15 min, cool to room temperature, filter. Evaporate the filtrate to dryness under reduced pressure and dry under reduced pressure to obtain FLOPIC as a crystalline solid (mp 93-94°), store in a desiccator under reduced pressure.)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova Pak C18 (A) or 200 × 4.6 5 μm Nucleosil cyano (B)
Mobile phase: MeOH:water:THF 62:35:3 (A) or n-hexane:isopropanol:diethylamine 95:5:0.05 (B)
Flow rate: 1
Injection volume: 20
Detector: F ex 296 em 356

CHROMATOGRAM
Retention time: 29.0 (R (A)), 31.0 (S (A)), 22.2 (R (A)), 26.8 (S (B))

OTHER SUBSTANCES
Simultaneous: mexiletine (system A only)

KEY WORDS
derivatization; chiral

REFERENCE
Martin,E.; Quinke,K.; Spahn,H.; Mutschler,E. (-)-(S)-Flunoxaprofen and (-)-(S)-naproxen isocyanate: two new fluorescent chiral derivatizing agents for an enantiospecific determination of primary and secondary amines, *Chirality*, **1989**, *1*, 223–234.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 Spherisorb S5SCX
Mobile phase: MeOH:MeCN:water 40:40:20 containing 25 mM perchloric acid
Flow rate: 2
Detector: F ex 215 no emission filter

CHROMATOGRAM
Retention time: 4.5
Internal standard: benzimidazole (7)

OTHER SUBSTANCES
Simultaneous: propranolol

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
Sample preparation: Mix 20 µL of a 1 mM solution in MeOH or water with 50 µL pH 8 borate buffer and 50 µL 18 mM 2-(6-methoxy-2-naphthyl)-1-propyl chloroformate in acetone, vortex, let stand at room temperature for 30 min, add 100 µL 10 mM trans-4-hydroxy-L-proline in water, mix, let stand for 2 min, add 2 mL dichloromethane, vortex for 30 s. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 100 µL mobile phase, inject an aliquot. Prepare 2-(6-methoxy-2-naphthyl)-1-propyl chloroformate as follows. Stir 1.5 mmoles lithium aluminum hydride in THF, slowly add 2 mmoles (S)-naproxen in 20 mL anhydrous THF, reflux for 1 h, evaporate most of the solvent, cautiously add water with stirring, acidify with 6 N HCl, extract three times with diethyl ether. Combine the organic layers and dry them over anhydrous sodium sulfate, evaporate to dryness, chromatograph on silica gel with dichloromethane:MeOH 100:2 (flash chromatography), evaporate eluate to dryness, dry under vacuum over KOH to give 2-(6-methoxy-2-naphthyl)propanol as a white solid (mp 92-3°). Stir 0.5 mmoles 2-(6-methoxy-2-naphthyl)propanol and 0.5 mmoles triethylamine in 10 mL dry toluene at 0°, add 1 mL 20% phosgene in toluene (Caution! Phosgene is highly toxic, perform reaction in a chemical fume hood!) (Fluka), stir for 4 h, filter, evaporate to dryness under reduced pressure, dry under vacuum to give 2-(6-methoxy-2-naphthyl)-1-propyl chloroformate (mp 60°). Store under vacuum over phosphorus pentoxide at room temperature.)

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

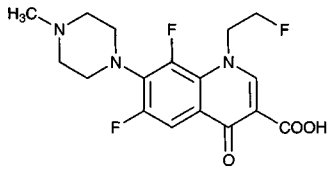
CHROMATOGRAM
Retention time: k' 14.4 (R-(-)), k' 15.2 (S-(+))

OTHER SUBSTANCES
Simultaneous: metoprolol, tocainide
Interfering: propafenone

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.

Fleroxacin



Molecular formula: $C_{17}H_{18}F_3N_3O_3$
Molecular weight: 369.34
CAS Registry No.: 79660-72-3
Merck Index: 4137
Lednicer No.: 5 125

SAMPLE

Matrix: bile, blood, urine
Sample preparation: Dilute urine 1:20. Dilute bile 1:10. 500 μL Serum, diluted urine, or diluted bile + 3.2 mL dichloromethane, vortex, rotate at 20 rpm for 10 min, centrifuge at 1000 g for 10 min. Remove 3 mL of the lower organic phase and add it to 200 μL 100 mM NaOH, rotate at 20 rpm for 30 min, centrifuge at 1000 g for 10 min, inject a 20 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeCN:buffer 10:90, pH adjusted to 2 with 14.6 M phosphoric acid (Buffer was 10 mM NaH_2PO_4 containing 5 mM tetrabutylammonium bromide.)
Flow rate: 2
Injection volume: 20
Detector: F ex 277 em 445

CHROMATOGRAM

Retention time: 3
Limit of detection: 25 ng/mL (bile), 50 ng/mL (urine), 2.5 ng/mL (serum)

OTHER SUBSTANCES

Noninterfering: amikacin, aztreonam, carbamazepine, cephalosporins, ciprofloxacin, clavulanic acid, difloxacin, digitoxin, digoxin, fosfomycin, furosemide, gentamycin, imipenem, lidocaine, netilmicin, norfloxacin, pefloxacin, penicillins, phenobarbital, phenytoin, primidone, procainamide, quinidine, rifampin, salicylic acid, teicoplanin, temafloxacin, theophylline, tobramycin, valproic acid, vancomycin
Interfering: ofloxacin

KEY WORDS

serum; human; rabbit

REFERENCE

Koechlin,C.; Jehl,F.; Linger,L.; Monteil,H. High-performance liquid chromatography for the determination of three new fluoroquinolones, fleroxacin, temafloxacin and A-64730, in biological fluids, *J.Chromatogr.*, **1989**, *491*, 379–387.

SAMPLE

Matrix: blood
Sample preparation: Filter 1 mL plasma using a micropartition system (MPS-1, Amicon, MA) while centrifuging at 2000 g for 20 min at 10°, inject an aliquot of the ultrafiltrate.

HPLC VARIABLES

Column: 250 × 4.6 Spherisorb ODS-2 endcapped
Mobile phase: MeCN:buffer 22:78 containing 5 mM tetrabutylammonium sulfate, adjusted to pH 2.5 with 1 M NaOH (Buffer was 100 mM citric acid containing 200 mM ammonium perchlorate.)
Column temperature: 37
Flow rate: 1
Detector: UV 287

CHROMATOGRAM
Retention time: 4.55
Internal standard: difloxacin (9.5)

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Zlotos,G.; Bücker,A.; Kinzig-Schippers,M.; Sorgel,F.; Holzgrabe,U. Plasma protein binding of gyrase inhibitors, *J.Pharm.Sci.*, **1998**, *87*, 215–220.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 500 μL 7% perchloric acid, vortex for 10 s, centrifuge at >700 g for 10 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeOH:18 mM KH_2PO_4 containing 0.13 mM heptanesulfonic acid:concentrated phosphoric acid 30:70:0.1
Flow rate: 0.8
Injection volume: 20
Detector: F ex 288 em 475 bandpass filter

CHROMATOGRAM
Retention time: 5.5
Limit of detection: 100 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
Sample preparation: Filter (0.2 μm cellulose acetate, Schleicher & Schuell) while centrifuging at 4° at 2500 g for 5 min, maintain filtrate at 4°, inject a 5 μL aliquot of the filtrate onto column A and elute to waste with mobile phase A, collect the effluent containing fleroxacin in a sample loop and backflush the contents of this loop onto column B with mobile phase B, elute column B with mobile phase B and monitor the effluent.

HPLC VARIABLES
Column: A 40 × 6 12 μm TSKgel PWxl methacrylate polymer gel (Toso-Haas); B 250 × 4.6 5 μm Zorbax Rx-C8
Mobile phase: A Isopropanol:30 mM pH 6.2 potassium phosphate buffer 10:90; B MeCN:50 mM pH 2.7 potassium phosphate buffer 18:82
Flow rate: A 0.6; B 1
Injection volume: 5
Detector: UV 287

CHROMATOGRAM
Retention time: 15
Limit of quantitation: 270 ng/mL

OTHER SUBSTANCES
Extracted: Ro 23-9424 (a pro-drug)

KEY WORDS
plasma; column-switching; heart-cut; pharmacokinetics

REFERENCE
Szuna,A.J.; Blain,R.W. Determination of a new antibacterial agent (Ro 23-9424) by multidimensional high-performance liquid chromatography with ultraviolet detection and direct plasma injection, *J.Chromatogr.*, **1993**, *620*, 211–216.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Serum + 20 μL 30 μg/mL pipemidic acid + 250 μL 25% sodium sulfate, vortex, add 3.5 mL chloroform, shake at low speed for 10 min, centrifuge at 1155 g for 10 min. Remove the lower organic layer and add it to 200 μL 1 M NaOH, shake fast for 20 min, centrifuge at 1155 g for 10 min, inject a 150 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 10 μm Nucleosil C18
Mobile phase: MeCN:MeOH:10 mM phosphate buffer containing 5 mM tetrabutylammonium hydrogen sulfate 13:6:81
Flow rate: 1
Injection volume: 150
Detector: F ex 274

CHROMATOGRAM
Internal standard: pipemidic acid
Limit of detection: 50 ng/mL

KEY WORDS
serum; pharmacokinetics

REFERENCE
Bertino,J.S.,Jr.; Nafziger,A.N.; Wong,M.; Stragand,L.; Puleo,C. Effect of a fat- and calcium-rich breakfast on pharmacokinetics of fleroxacin administered in single and multiple doses, *Antimicrob.Agents Chemother.*, **1994**, *38*, 499–503.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Serum + 20 μL (?) 30 μg/mL pipemidic acid + 250 μL 25% sodium sulfate, vortex, add 3.5 mL chloroform, shake at low speed for 10 min, centrifuge at 1155 g for 10 min. Remove the organic layer and add it to 200 μL 1 M NaOH, shake quickly for 20 min, centrifuge at 1155 g for 10 min, inject a 150 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 10 μm Nucleosil C18
Mobile phase: MeCN:MeOH:10 mM phosphate buffer containing 5 mM tetrabutylammonium sulfate 13:6:81
Flow rate: 1
Injection volume: 150
Detector: F ex 274 (?)

CHROMATOGRAM
Internal standard: pipemidic acid
Limit of quantitation: 50 ng/mL

KEY WORDS
pharmacokinetics; serum

REFERENCE
Bertino,J.S.,Jr.; Nafziger,A.N. Pharmacokinetics of oral fleroxacin in male and premenopausal female volunteers, *Antimicrob.Agents Chemother.*, **1996**, *40*, 789–791.

SAMPLE
Matrix: blood, dialysate
Sample preparation: Plasma. 100 μL Plasma + 25 μL MeCN:70% perchloric acid 80:20 containing IS, mix vigorously, centrifuge. Inject a 5 or 50 μL aliquot. Dialysate. Dilute 20 μL dialysate with 980 μL 50 mM sodium dihydrogen phosphate buffer containing IS. Mix thoroughly. Inject a 5 or 10 μL aliquot.

HPLC VARIABLES
Column: Spherisorb ODS II
Mobile phase: MeCN containing 2 mM tetrabutyl ammonium hydrogen sulfate:100 mM citric acid buffer containing 5 mM ammonium perchlorate 87:13, adjusted to pH 2.2
Flow rate: 1.2
Injection volume: 5-50
Detector: F ex 290 em 460

CHROMATOGRAM
Retention time: 6.6
Internal standard: pipemidic acid (3.2)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Uehlinger,G.E.; Schaedeli,F.; Kinzig,M.; Sörgel,F.; Frey,F.J. Pharmacokinetics of fleroxacin after multiple oral dosing in patients receiving regular hemodialysis, *Antimicrob.Agents Chemother.*, **1996**, *40*, 1903–1909.

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 μL aliquot. Serum. Deproteinize serum with MeOH containing ofloxacin, extract with dichloromethane at pH 7.5.

HPLC VARIABLES
Column: 150 × 3.9 Novapack C18
Mobile phase: MeCN:buffer 16:84 (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 290 em 430

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, urine
Sample preparation: 500 μL Plasma + 100 μL 1 μg/mL IS1 in 10 mM HCl + 1 mL pH 7.5 Sörensen buffer + 7 mL dichloromethane:isopropanol 70:30, extract by turning head over head at 70 rotations/min for 10 min, centrifuge at 2000 g for 10 min. Remove 5 mL of the organic

layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in 500 μL mobile phase, vortex, inject an aliquot. Urine. 500 μL Urine + 100 μL 1 mg/mL IS2 in 10 mM HCl + 500 μL 1 M acetic acid + 500 μL 25 mM sodium dodecyl sulfate in water + 7 mL dichloromethane:isopropanol 70:30, extract by turning head over head at 70 rotations/min for 10 min, centrifuge at 2000 g for 10 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in 500 μL mobile phase, vortex, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm TSK-Gel ODS 120T (Toyo Soda)
Mobile phase: MeOH:5 mM tetrabutylammonium hydrogen sulfate 21:79 (plasma) or 24.5:74.5 (urine)
Column temperature: ambient (urine), 27° (plasma)
Flow rate: 0.8
Detector: F ex 290 em 450

CHROMATOGRAM

Retention time: 7.5 (plasma), 9 (urine)
Internal standard: IS1 6-fluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid (12), IS2 pipemidic acid (7)
Limit of quantitation: 10-20 ng/mL

KEY WORDS

plasma; protect from light; pharmacokinetics

REFERENCE

Dell,D.; Partos,C.; Portmann,R. The determination of a new trifluorinated quinolone, fleroxacin, its N-demethyl, and N-oxide metabolites in plasma and urine by high performance liquid chromatography with fluorescence detection, *J.Liq.Chromatogr.*, **1988**, *11*, 1299–1312.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 200 μL Plasma + 1 mL 50 ng/mL IS1 in MeCN:water 95:5, vortex, centrifuge at 14° at 1000 g for 10 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 38°, reconstitute the residue in 200-400 μL mobile phase, inject a 20-30 μL aliquot onto column A and column B in series, after 1.5 min remove column A from the circuit and backflush it to waste, elute column B with mobile phase and monitor the effluent. Urine. 100 μL Urine + 100 μL 100 μg/mL IS2 in water + 5 mL mobile phase, inject a 20 μL aliquot onto column A and column B in series, after 1.5 min remove column A from the circuit and backflush it to waste, elute column B with mobile phase and monitor the effluent.

HPLC VARIABLES

Column: A 10 × 4 Nucleosil 5-C18 (replaced every 60-80 samples); B 250 × 4.6 5 μm TSK ODS-120T (Toyo Soda)
Mobile phase: MeOH:buffer 28:72, pH adjusted to 2.6 with 40% phosphoric acid (Buffer was 50 mM KH_2PO_4 containing 10 mM tetrabutylammonium hydrogen sulfate.)
Column temperature: 28
Flow rate: 1
Injection volume: 20-30
Detector: F ex 290 em 450

CHROMATOGRAM

Retention time: 7.7
Internal standard: IS1 6-fluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid (AM-735) (13.0), IS2 pipemidic acid (6.0)
Limit of detection: 1 ng/mL (plasma)
Limit of quantitation: 1000 ng/mL (urine), 10 ng/mL (plasma)

OTHER SUBSTANCES

Extracted: metabolites
Noninterfering: ciprofloxacin, norfloxacin, pefloxacin

KEY WORDS
plasma; column-switching; protect from light; pharmacokinetics

REFERENCE
Heizmann,P.; Dell,D.; Eggers,H.; Gora,R. Determination of the new fluoroquinolone fleroxacin and its N-demethyl and N-oxide metabolites in plasma and urine by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1990**, *527*, 91–101.

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, 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: milk
Sample preparation: Milk + pipemidic acid + phosphate buffer, extract with dichloromethane: isopropanol 70:30, centrifuge. Remove an aliquot of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in mobile phase, add n-hexane, mix thoroughly, centrifuge, inject an aliquot of the aqueous phase.

HPLC VARIABLES
Column: 250 × 4.6 5 μm ODS-120T (Toyo Soda)
Mobile phase: MeOH:5 mM tetrabutylammonium hydrogen sulfate 28:72
Flow rate: 0.8
Detector: F ex 290 em 450

CHROMATOGRAM
Internal standard: pipemidic acid
Limit of quantitation: 100 ng/mL

KEY WORDS
protect from light; pharmacokinetics

REFERENCE
Dan,M.; Weidekamm,E.; Sagiv,R.; Portmann,R.; Zakut,H. Penetration of fleroxacin into breast milk and pharmacokinetics in lactating women, *Antimicrob.Agents Chemother.*, **1993**, *37*, 293–296.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 4.6 3 μm ODS-Hypersil
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 278

CHROMATOGRAM
Retention time: 4.94

OTHER SUBSTANCES
Simultaneous: photodegradation products, ofloxacin
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: solutions
Sample preparation: Filter (0.45 μm) a solution in MeCN:water 10:90, inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: MeCN:buffer 7:93 (Buffer was 25 mM phosphoric acid adjusted to pH 3.89 with 100 mM tetrabutylammonium hydroxide.)
Flow rate: 1
Injection volume: 10
Detector: UV 280

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: ciprofloxacin, enoxacin, 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: urine
Sample preparation: 250 μL Urine + 20 μL 30 μg/mL pipemidic acid + 250 μL 500 mM pH 7.5 phosphate buffer, vortex, add 3.5 mL chloroform, shake at low speed for 10 min, centrifuge at 1155 g for 10 min. Remove the lower organic layer and add it to 200 μL 1/15 M pH 12.5 sodium phosphate, shake fast for 20 min, centrifuge at 1155 g for 10 min, inject a 150 μL aliquot of the aqueous layer.

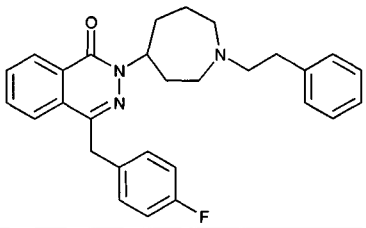
HPLC VARIABLES
Column: 10 μm Nucleosil C18
Mobile phase: MeCN:MeOH:10 mM phosphate buffer containing 5 mM tetrabutylammonium hydrogen sulfate 13:6:81
Flow rate: 1
Injection volume: 150
Detector: F ex 274

CHROMATOGRAM
Internal standard: pipemidic acid
Limit of detection: 50 ng/mL

KEY WORDS
pharmacokinetics

REFERENCE
Bertino,J.S.,Jr.; Nafziger,A.N.; Wong,M.; Stragand,L.; Puleo,C. Effect of a fat- and calcium-rich breakfast on pharmacokinetics of fleroxacin administered in single and multiple doses, *Antimicrob.Agents Chemother.*, **1994**, *38*, 499–503.

Flezelastine



Molecular formula: $C_{29}H_{30}FH_3O$
Molecular weight: 416.58
CAS Registry No.: 135381-77-0

SAMPLE
Matrix: microsomal incubations
Sample preparation: Adjust pH of 750 μL microsomal incubation to 10 with 100 mM NaOH, add 3 mL cyclohexane:ethyl acetate 80:20, shake mechanically for 10 min, centrifuge at 2500 g for 10 min, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute with a solution of azelastine in MeOH, evaporate to dryness under a stream of nitrogen, reconstitute with 200 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher Si-60
Column: 250 × 4 5 μm LiChrospher Si-60
Mobile phase: MeOH containing 0.033% perchloric acid
Flow rate: 0.5 for 17 min then 0.9
Injection volume: 20
Detector: F ex 210 em 360

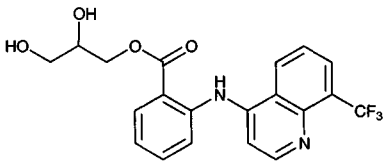
CHROMATOGRAM
Retention time: 15
Internal standard: azelastine (27)
Limit of detection: 125 μg/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; rat; cow; pig; liver

REFERENCE
Paris,S.; Blaschke,G.; Locher,M.; Borbe,H.O.; Engel,J. Investigation of the stereoselective in vitro metabolism of the chiral antiasthmatic/antiallergenic drug flezelastine by high-performance liquid chromatography and capillary zone electrophoresis, *J.Chromatogr.B*, **1997**, *691*, 463–471.

Floctafenine



Molecular formula: $C_{20}H_{17}F_3N_2O_4$
Molecular weight: 406.36
CAS Registry No.: 23779-99-9
Merck Index: 4140
Lednicer No.: 3 184

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 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 353

CHROMATOGRAM

Retention time: 10.73
Limit of detection: <120 ng/mL

KEY WORDS

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

REFERENCE

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

SAMPLE

Matrix: blood, urine

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

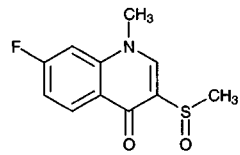
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 209.9

CHROMATOGRAM
Retention time: 17.177

KEY WORDS
whole blood

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

Flosequinan



Molecular formula: $C_{11}H_{10}FNO_2S$
Molecular weight: 239.27
CAS Registry No.: 76568-02-0
Merck Index: 4146
Lednicer No.: 5 127

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 10 µL 100 µg/mL IS in MeCN:MeOH 50:50 + 5 mL chloroform, shake for 10 min, centrifuge at 1800 g for 10 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 MeOH, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 µm Chiralcel OD
Mobile phase: MeOH:EtOH 78:22
Column temperature: 30
Flow rate: 0.7
Detector: UV 320

CHROMATOGRAM
Retention time: 12.1 (R(+)), 7.9 (S(-))
Internal standard: (±)-7-chloro-1-methyl-3-methylsulfinyl-4-quinolone (8.9, 14.6)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; chiral; pharmacokinetics

REFERENCE
Kashiyama,E.; Odomi,M.; Shimizu,T. Stereospecific and simultaneous high-performance liquid chromatographic assay of flosequinan and its metabolites in human plasma, *J.Chromatogr.B*, **1994**, *652*, 179–185.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a 1 mL Baker-10 octadecyl C18 SPE cartridge (J.T. Baker) with MeOH then water. 100 μL Plasma or urine + 25 μL 2 (plasma) or 10 (urine) μg/mL IS in water, add to the SPE cartridge, wash with 2 column volumes of water, elute with 200 μL MeOH, inject a 2 (urine) or 5 (plasma) aliquot of the eluate.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Nova-Pak C18
Mobile phase: MeCN:MeOH:water 7:20:73
Flow rate: 1.2
Injection volume: 2-5
Detector: UV 254

CHROMATOGRAM
Retention time: 3.4
Internal standard: 7-chloro-1-methyl-3-methylsulfinyl-4-quinolone (7.8)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: acetaminophen, amiodarone, diazepam, digoxin, furosemide, lidocaine, nitroglycerin, procainamide, propranolol, quinidine

KEY WORDS
plasma; SPE

REFERENCE
Slegowski,M.B.; Miller,C.; Porter,R.S. Simplified high-performance liquid chromatographic determination of flosequinan and its metabolite in plasma, serum and urine, *J.Chromatogr.*, **1988**, *425*, 227–232.

SAMPLE
Matrix: incubations
Sample preparation: 2 mL Incubation + 10 μg IS + 4 mL chlorobutane:1,2-dichloroethane 80:20, shake for 15 min, centrifuge. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 70°, reconstitute the residue in 800 μL mobile phase, inject a 100 μL aliquot.

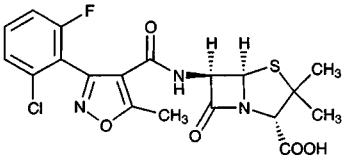
HPLC VARIABLES
Column: 150 × 4.6 Hypersil 3ODS
Mobile phase: MeCN:MeOH:water 7:20:73
Flow rate: 2
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Internal standard: 7-chloro-1-methyl-3-methylsulfinyl-4-quinolone (BTS 49037)

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE
Lee,S.C.; Renwick,A.G. Sulphoxide reduction by rat intestinal flora and by *Escherichia coli in vitro*, *Biochem.Pharmacol.*, **1995**, *49*, 1567–1576.

Floxacillin



Molecular formula: $C_{19}H_{17}ClFN_3O_5S$
Molecular weight: 453.88
CAS Registry No.: 5250-39-5, 1847-24-1 (sodium salt), 34214-51-2 (sodium monohydrate)
Merck Index: 4147

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond Elut C18 SPE cartridge with 2 mL MeCN and 1 mL 10 mM pH 2 Na_2HPO_4. 250 μL Plasma + 100 μL 20 μg/mL dicloxacillin sodium in water, add 400 μL MeCN at -15° while vortexing, add 700 μL 10 mM pH 2 Na_2HPO_4, centrifuge at 8000 g for 10 min. Add the supernatant to the SPE cartridge, wash with 1 mL water, elute with two 500 μL portions of MeCN:water 35:65 containing 10 mM Na_2HPO_4 (pH adjusted to 6 with phosphoric acid), inject a 20 μL aliquot of the eluate.

HPLC VARIABLES
Column: 100 × 2 5 μm ODS Hypersil
Mobile phase: MeCN:water 40:60 containing 10 mM Na_2HPO_4, pH adjusted to 2 with ortho-phosphoric acid
Flow rate: 0.5
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 2.5
Internal standard: dicloxacillin (3.5)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: cloxacillin

KEY WORDS
plasma; pharmacokinetics; SPE

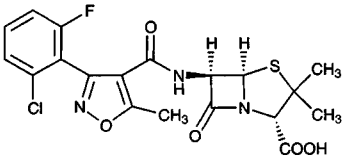
REFERENCE
Hung,C.T.; Lim,J.K.C.; Zoest,A.R.; Lam,F.C. Optimization of high-performance liquid chromatographic analysis for isoxazolyl penicillins using factorial design, *J.Chromatogr.*, **1988**, *425*, 331–341.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL dicloxacillin in water + 25 μL glacial acetic acid + 2 mL ethyl acetate, vortex for 30 s, centrifuge at 2000 g for 5 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 70°, reconstitute the residue in 250 μL mobile phase, inject a 10-20 μL aliquot.

HPLC VARIABLES
Column: 40 × 3.2 RP18 VeloSep (Brownlee)
Mobile phase: MeCN:10 mM pH 7 phosphate buffer 18:82
Flow rate: 1.2
Injection volume: 10-20
Detector: UV 220

REFERENCE
Lee,S.C.; Renwick,A.G. Sulphoxide reduction by rat intestinal flora and by *Escherichia coli in vitro*, *Biochem.Pharmacol.*, **1995**, *49*, 1567–1576.

Floxacillin



Molecular formula: $C_{19}H_{17}ClFN_3O_5S$
Molecular weight: 453.88
CAS Registry No.: 5250-39-5, 1847-24-1 (sodium salt), 34214-51-2 (sodium monohydrate)
Merck Index: 4147

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond Elut C18 SPE cartridge with 2 mL MeCN and 1 mL 10 mM pH 2 Na_2HPO_4. 250 µL Plasma + 100 µL 20 µg/mL dicloxacillin sodium in water, add 400 µL MeCN at -15° while vortexing, add 700 µL 10 mM pH 2 Na_2HPO_4, centrifuge at 8000 g for 10 min. Add the supernatant to the SPE cartridge, wash with 1 mL water, elute with two 500 µL portions of MeCN:water 35:65 containing 10 mM Na_2HPO_4 (pH adjusted to 6 with phosphoric acid), inject a 20 µL aliquot of the eluate.

HPLC VARIABLES
Column: 100 × 2 5 µm ODS Hypersil
Mobile phase: MeCN:water 40:60 containing 10 mM Na_2HPO_4, pH adjusted to 2 with ortho-phosphoric acid
Flow rate: 0.5
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 2.5
Internal standard: dicloxacillin (3.5)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: cloxacillin

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Hung,C.T.; Lim,J.K.C.; Zoest,A.R.; Lam,F.C. Optimization of high-performance liquid chromatographic analysis for isoxazolyl penicillins using factorial design, *J.Chromatogr.*, **1988**, *425*, 331–341.

SAMPLE
Matrix: blood
Sample preparation: 100 µL Plasma + 100 µL dicloxacillin in water + 25 µL glacial acetic acid + 2 mL ethyl acetate, vortex for 30 s, centrifuge at 2000 g for 5 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 70°, reconstitute the residue in 250 µL mobile phase, inject a 10-20 µL aliquot.

HPLC VARIABLES
Column: 40 × 3.2 RP18 VeloSep (Brownlee)
Mobile phase: MeCN:10 mM pH 7 phosphate buffer 18:82
Flow rate: 1.2
Injection volume: 10-20
Detector: UV 220

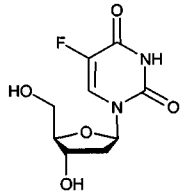
CHROMATOGRAM
Retention time: 2.8
Internal standard: dicloxacillin (4.4)
Limit of detection: 50 ng/mL
Limit of quantitation: 300 ng/mL

OTHER SUBSTANCES
Simultaneous: carbamazepine, phenytoin, phenobarbital
Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, amitriptyline, caffeine, chloramphenicol, cyclosporine, digoxin, ethosuximide, gentamicin, lidocaine, nortriptyline, methotrexate, primidone, procainamide, quinidine, salicylic acid, theophylline, tobramycin, valproic acid, vancomycin, metabolites

KEY WORDS
plasma

REFERENCE
Charles,B.G.; Foo,C.C.; Gath,J. Rapid column liquid chromatographic analysis of flucloxacillin in plasma on a microparticulate pre-column, *J.Chromatogr.B*, **1994**, *660*, 186–190.

Floxuridine



Molecular formula: $C_9H_{11}FN_2O_5$
Molecular weight: 246.20
CAS Registry No.: 50-91-9
Merck Index: 4148

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL water, vortex 10 s, add 200 μL water containing 30% w/v trichloroacetic acid and 30% w/v perchloric acid, vortex 10 s, place in an ice bath for 2 min, centrifuge at 3000 g for 20 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 4.6 5μm Hypersil-ODS
Mobile phase: 20 mM Na_2HPO_4 adjusted to pH 2.0 with orthophosphoric acid
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Internal standard: floxuridine

OTHER SUBSTANCES
Extracted: allopurinol, oxipurinol

KEY WORDS
plasma; floxuridine is IS

REFERENCE
Hung,C.T.; Zoest,A.R.; Perrier,D.G. Analysis of allopurinol and oxipurinol in plasma by reversed phase HPLC, *J.Liq.Chromatogr.*, **1986**, *9*, 2471–2483.

SAMPLE
Matrix: blood

Sample preparation: 100 μL Serum + 5 μL 42 μM 5-chlorouracil in acetone, mix, let stand at room temperature for 3 min, add 500 μL 100 mM pH 3.5 potassium phosphate buffer, add 2 mL ethyl acetate, vortex for 2 min, centrifuge at 1000 g for 5 min. Remove a 1.4 mL aliquot of the organic layer and evaporate it to dryness under reduced pressure. Add 20 mg solid potassium bicarbonate:anhydrous sodium sulfate 1:7 to the residue, add 50 μL 1.3 mM 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone in acetone, add 50 μL 1.5 mM 18-crown-6 in acetone, heat at 50° for 20 min, cool, inject a 10 μL aliquot. (Silanize all glassware. Synthesize 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone as follows. Stir 483 g veratrole in 1.45 L acetic acid at 15° for 1 h, add 683 g concentrated nitric acid (d 1.05) over 1 h (maintain the temperature below 40° by cooling and regulating the rate of addition of the nitric acid). Continue stirring and add 2.127 L fuming nitric acid (d 1.50) over 1 h while maintaining the temperature below 30°, let stand for 2 h, pour into a large volume of cold water, filter, wash the solid with water until the washings are neutral, recrystallize from EtOH to give 4,5-dinitroveratrole (mp 129.5-130.5°) (J. Am. Chem. Soc. 1946, 68, 1536). Reflux 5 g 4,5-dinitroveratrole in 200 mL benzene (Caution! Benzene is a carcinogen!), add 100 g 60 mesh iron powder and 20 mL concentrated HCl in small portions over 1 h, reflux for 4 h, add 10 mL water, reflux for 2 h, cool, make alkaline with 2.5 M NaOH, extract several times with 200 mL portions of benzene. Combine the organic layers and evaporate them to dryness, add 10 mL concentrated HCl, recrystallize from EtOH to give 1,2-diamino-4,5-dimethoxybenzene monohydrochloride as very slightly pink needles (mp 240°) (Anal. Chim. Acta 1982, 134, 39). Heat 2.5 mmoles 1,2-diamino-4,5-dimethoxybenzene hydrochloride and 2.4 mmoles pyruvic acid in 30 mL 500 mM HCl on a boiling water bath for 2 h, cool with ice-water, filter. Wash the precipitate with water and dry it under vacuum, recrystallize from MeOH:water 90:10 to give 6,7-dimethoxy-3-methyl-2(1H)-quinoxalinone as yellow needles (mp 255°) (Chem. Pharm. Bull. 1985, 33, 3493). Treat 1 g 6,7-dimethoxy-3-methyl-2(1H)-quinoxalinone dissolved in 50 mL anhydrous MeOH with a solution of diazomethane in ether, evaporate to dryness under reduced pressure, dissolve the residue in 5 mL ethyl acetate, chromatograph on a 250 × 35 column filled with 130 g 70-230 mesh silica gel 60 (Merck) using n-hexane:ethyl acetate 25:75 to give 6,7-dimethoxy-1,3-dimethyl-2(1H)-quinoxalinone as yellow needles (mp 170-171°). Dissolve 350 mg 6,7-dimethoxy-1,3-dimethyl-2(1H)-quinoxalinone in 3 mL acetic acid, add 350 mg anhydrous sodium acetate, add 2 mL 1.5 M bromine in acetic acid, heat at 100° for 15 min, cool, add 10 mL ether, filter, wash the solid 2 or 3 times with small portions of ether. Combine the filtrate and washings and evaporate them to dryness, dissolve the residue in 5 mL ethyl acetate, chromatograph on a 250 × 35 column filled with 130 g 70-230 mesh silica gel 60 (Merck) using ether, evaporate the main fraction to dryness, recrystallize the residue from n-hexane:ethyl acetate 50:50 to give 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone as yellow needles (mp 161-163°) (J. Chromatogr. 1985, 346, 227). 3-Bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone is also available from Dojindo Molecular Technologies, Inc., 3 Bethesda Metro Center, Suite 700, Bethesda MD 20814; (301) 664-8448; www.dojindo.co.jp.)

HPLC VARIABLES

Column: 100 × 8 10 μm Radial Pak C18 (Waters) (Wash with MeOH at 2 mL/min for 20 min at the end of each day.)
Mobile phase: Gradient. MeOH:water 35:65 for 15 min, 50:50 for 25 min (step gradient), re-equilibrate at initial conditions for 20 min.
Flow rate: 1.5
Injection volume: 10
Detector: F ex 370 em 455

CHROMATOGRAM

Retention time: 28.1
Internal standard: 5-chlorouracil (32.5)
Limit of detection: 25 ng/mL

OTHER SUBSTANCES

Extracted: 5-fluorouracil

KEY WORDS

derivatization; serum; pharmacokinetics

REFERENCE

Yamaguchi,M.; Nakamura,M.; Kuroda,N.; Ohkura,Y. Determination of 5-fluorouracil and 5-fluoro-2'-deoxyuridine in human serum by high-performance liquid chromatography with fluorescence detection, *Anal.Sci.*, **1987**, *3*, 75–79.

SAMPLE
Matrix: blood
Sample preparation: Add 250 μL serum to a 20 × 7 DEAE-Cellulofine AM anion-exchange column (Seikagaku Tokyo), elute with 3.5 mL 1 mM HCl, discard the first 0.5 mL eluate, collect the next 3 mL eluate. Evaporate the eluate to 0.5 mL under reduced pressure, add 15 mL ethyl acetate, shake, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 800 μL anhydrous acetone, add 100 μL 750 μg/mL 4-bromomethyl-6,7-dimethoxycoumarin in acetone, add 100 μL 250 μg/mL 18-crown-6 in acetone, add 1.5 mg anhydrous potassium carbonate, heat at 70° for 15 min (protect from atmospheric moisture with a calcium chloride drying tube), cool, inject an aliquot

HPLC VARIABLES
Column: 200 × 4 5 μm Nucleosil 5 C18
Mobile phase: MeOH:water 60:40
Flow rate: 0.8
Detector: F ex 340 em 420

CHROMATOGRAM
Retention time: 5
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: ftorafur, 5-fluorouracil

KEY WORDS
derivatization; serum; protect from light; SPE

REFERENCE
Yoshida,S.; Adachi,T.; Hirose,S. 4-Bromomethyl-6,7-dimethoxycoumarin as a fluorescence reagent for precolumn derivatization of 5-fluorouracil compounds in high-performance liquid chromatography, *J.Chromatogr.*, **1988**, *430*, 156–162.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL LC-SCX Supelclean strong cation-exchange SPE cartridge (Supelco) with 2 mL MeOH, 1 mL 100 mM copper(II) sulfate solution, and 3 mL 50 mM pH 7 phosphate buffer, do not allow to dry. 300 μL Serum + 5-bromouracil, add to the SPE cartridge, wash with 2 mL 50 mM pH 7 phosphate buffer, wash with 2 mL MeOH, elute with 700 μL 1.7 M ammonia solution, add 70 μL glacial acetic acid to the eluate, mix thoroughly, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelguard LC-18-S (Supelco)
Column: 250 × 4.6 5 μm Supelcosil LC-18-S ODS
Mobile phase: Gradient. A was MeOH:50 mM pH 6.5 phosphate buffer 60:40. B was 50 mM pH 6.5 phosphate buffer.
Flow rate: 1
Injection volume: 20
Detector: UV 269

CHROMATOGRAM
Retention time: 13
Internal standard: 5-bromouracil (12)
Limit of detection: 70 ng/mL

OTHER SUBSTANCES
Extracted: doxifluridine, 5-fluorouracil, 5-fluorouridine monophosphate, metabolites

KEY WORDS
serum; SPE

REFERENCE
Guerrieri,A.; Palmisano,F.; Zambonin,P.G.; De Lena,M.; Lorusso,V. Solid-phase extraction of fluoropyrimidine derivatives on a copper-modified strong cation exchanger: determination of doxifluridine, 5-fluorouracil and its main metabolites in serum by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1993**, *617*, 71–77.

SAMPLE
Matrix: blood, peritoneal fluid
Sample preparation: Plasma. 1 mL Plasma + 10 μL 100 μM bromouridine + 70 μL perchloric acid, mix thoroughly, let stand at 4° for at least 12 h, centrifuge for 5 min. Remove the supernatant and adjust the pH to 7 with 5 M KOH, let stand on ice for 2 h, inject a 20 μL aliquot. Peritoneal fluid. 1 mL Peritoneal fluid + 10 μL 100 μM bromouridine, dilute 1:100 with water, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 12.5 × 4.6 5 μm Zorbax RX
Column: 250 × 4.6 5 μm Zorbax RX
Mobile phase: Gradient. A was 25 mL pH 2.5 ammonium phosphate. B was MeCN:25 mM pH 7.5 ammonium phosphate 7:93. A:B 100:0 for 5 min, to 0:100 over 10 min, maintain at 0:100 for 10 min, return to initial conditions over 1 min, re-equilibrate for 20 min.
Column temperature: 20
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 16-17
Internal standard: bromouridine (18)
Limit of detection: 2.5 nM

OTHER SUBSTANCES
Extracted: 5-fluorouracil

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Smith-Rogers,J.A.; Tong,W.P.; Duafala,M.E.; Markman,M.; Bertino,J.R. High-performance liquid chromatographic method for the simultaneous measurement of floxuridine and fluorouracil in human body fluids, *J.Chromatogr.*, **1991**, *566*, 147–154.

SAMPLE
Matrix: blood, tissue
Sample preparation: Add 100 μL 10% perchloric acid and 20 μL 200 μg/mL IS to 100 μL serum or homogenized tissue. Shake for 2 min and centrifuge at 2000 g for 10 min. Filter (45 μm) supernatant, inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 45 × 4.6 5 μm ODS Hypersil (VDS Optilab)
Column: 250 × 4.6 5 μm ODS Hypersil (VDS Optilab)
Mobile phase: MeOH:99% acetic acid:water 3:0.5:96.95
Column temperature: 30
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 12.75
Internal standard: 5-bromouracil (10.34)
Limit of quantitation: 200 ng/mL (serum); 600 ng/mL (tissue)

OTHER SUBSTANCES
Extracted: metabolites, fluorouracil

KEY WORDS
serum; rat; liver; tumor; kidney; spleen; peritoneum; gastric mucosa; lung; heart; pancreas

REFERENCE
Jung,M.; Berger,G.; Pohlen,U.; Päuser,S.; Reszka,R.; Buhr,H.J. Simultaneous determination of 5-fluorouracil and its active metabolites in serum and tissue by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *702*, 193–202.

SAMPLE
Matrix: blood, tissue
Sample preparation: 0.5 g Tissue or 1 mL plasma + 5 μL 1 M sulfuric acid (lung and heart only) + 2 μL 1 M sulfuric acid (plasma only) + 500 μL 200 mg/mL sodium sulfate (liver and kidney only) + 50 μL 1 M pH 6 sodium acetate (liver only) + 50 μL 1 M pH 5 sodium acetate (kidney only) + 100 μL 2% trichloroacetic acid (lung and heart only) + 15 mL n-propanol:ether (liver 16:84, kidney 20:80, lung 88:12, heart 40:60, plasma 88:12), sonicate for 30 s, shake for 15 min, centrifuge for 15 min. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 1 mL 50 mM ammonium phosphate (liver pH 11, kidney pH 3, lung pH 2.5, heart pH 5, plasma pH 2.5), inject a 20 μL aliquot. (From J. Liq.Chromatogr. 1994, 17, 1621.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb 5 ODS 2
Mobile phase: MeCN:50 mM phosphate buffer 0.5:99.5 (Liver pH 3, kidney pH 6, lung pH 5, heart pH 5, plasma pH 2.5)
Column temperature: 10 (kidney), 35 (lung), 20 (heart), 25 (plasma), 15 (liver)
Flow rate: 1
Injection volume: 20
Detector: UV 200

CHROMATOGRAM
Retention time: 18 (plasma), 28 (lung), 30 (liver), 32 (kidney), 36 (heart)
Internal standard: flucytosine (for plasma) (4), 4-chlorouracil (for tissue) (17 (lung), 18 (liver), 11 (kidney), 19 (heart))
Limit of quantitation: 670 ng/g plasma, 110 ng/g (heart), 90 ng/g (lung), 210 ng/g (kidney), 500 ng/g (liver)

OTHER SUBSTANCES
Extracted: metabolites, 5-fluorouracil

KEY WORDS
plasma; rabbit; liver; kidney; lung; heart; pharmacokinetics

REFERENCE
Del Nozal,M.J.; Bernal,J.L.; Pampliega,A.; Marinero,P.; Pozuelo,M. Determination of the concentrations of 5-fluorouracil and its metabolites in rabbit plasma and tissues by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *656*, 397–405.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 30 mm long 3 μm C18 (Perkin-Elmer)
Mobile phase: MeOH:water 0.5:95.5 (sic)
Flow rate: 1
Injection volume: 10
Detector: UV 268

CHROMATOGRAM
Retention time: 2

KEY WORDS
stability-indicating; saline; injections

REFERENCE
Smith,J.A.; Morris,A.; Duafala,M.E.; Bertino,J.R.; Markman,M.; Kleinberg,M. Stability of floxuridine and leucovorin calcium admixtures for intraperitoneal administration, *Am.J.Hosp.Pharm.*, **1989**, *46*, 985–989.

SAMPLE
Matrix: formulations
Sample preparation: Dilute formulation 1:100 with water, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 5 × 4 35-60 μm Perisorb RP18
Column: 250 × 4 10 μm LiChrosorb RP18
Mobile phase: MeOH:300 mM sodium acetate 2:98
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 10.5

OTHER SUBSTANCES
Simultaneous: fluorouracil

KEY WORDS
injections; water

REFERENCE
Sadjak,A.; Wintersteiger,R. Compatibility of morphine, baclofen, floxuridine and fluorouracil in an implantable medication pump, *Arzneimittelforschung*, **1995**, *45*, 93–98.

SAMPLE
Matrix: solutions
Sample preparation: Prepare 50 mg/mL or 1 mg/mL solutions in 0.9% sodium chloride injection, dilute 1:1000 or 1:100 with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: Bakerbond C18
Mobile phase: MeOH:water 0.5:99.5
Flow rate: 1
Detector: UV 268

CHROMATOGRAM
Retention time: 4.6

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
stability-indicating

REFERENCE
Stiles,M.L.; Allen,L.V.,Jr.; Prince,S.J. Stability of deferoxamine mesylate, floxuridine, fluorouracil, hydromorphone hydrochloride, lorazepam, and midazolam hydrochloride in polypropylene infusion-pump syringes, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 1583–1588.

SAMPLE
Matrix: solutions
Sample preparation: Add 10 mg of 4-bromomethyl-7-methoxycoumarin and 10 mg potassium carbonate to 5 mL of a solution in DMSO, after 5 min at room temperature add 4-nitrobenzoic acid.

HPLC VARIABLES
Column: 200 × 4 5 μm Nucleosil 5 C18
Mobile phase: MeCN:MeOH:water 5:29:66
Flow rate: 0.6
Detector: F ex 346 em 395

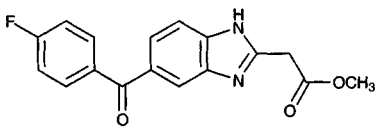
CHROMATOGRAM
Retention time: 12.5
Internal standard: chlorodeoxyuridine (16.5)

OTHER SUBSTANCES
Simultaneous: inosine, thymine, uracil, uridine

KEY WORDS
derivatization; some details of serum extraction in paper

REFERENCE
Yoshida,S.; Hirose,S.; Iwamoto,M. Use of 4-bromomethyl-7-methoxycoumarin for derivatization of pyrimidine compounds in serum analysed by high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.*, **1986**, *383*, 61–68.

Flubendazole



Molecular formula: $C_{16}H_{12}FN_3O_3$
Molecular weight: 313.29
Merck Index: 4154
Lednicer No.: 2 354

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

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85: 15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 211.1

CHROMATOGRAM
Retention time: 16.745

KEY WORDS
whole blood

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

SAMPLE
Matrix: solutions

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

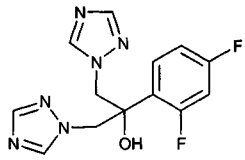
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine,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

thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

Fluconazole



Molecular formula: $C_{13}H_{12}F_2N_6O$
Molecular weight: 306.27
CAS Registry No.: 86386-73-4
Merck Index: 4158

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 700 μg/mL IS + 1.0 mL 1 M KOH, mix, extract twice with 3 mL portions of ethyl acetate. Combine the organic layers and extract with 2 mL 1 M HCl. Add 1.75 mL 1 M KOH to the aqueous layer and extract twice with 3 mL portions of ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of dry nitrogen, reconstitute the residue in 100 μL MeCN:10 mM pH 9.0 ammonium phosphate 50:50, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 3 μm Microsorb C18
Mobile phase: Gradient. MeCN:10 mM pH 9.0 ammonium phosphate 10:90 for 1 min, to 60:40 over 15 min, maintain at 60:40 for 2 min.
Flow rate: 0.5
Injection volume: 20
Detector: UV 260

CHROMATOGRAM

Retention time: 7
Internal standard: α-(2,4-dichlorophenyl)-1H-imidazole-1-ethanol (9)
Limit of quantitation: 12 μg/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Black,D.J.; Kunze,K.L.; Wienkers,L.C.; Gidal,B.E.; Seaton,T.L.; McDonnell,N.D.; Evans,J.S.; Bauwens,J.E.; Trager,W.F. Warfarin-fluconazole II. A metabolically based drug interaction: In vivo studies, *Drug Metab.Dispos.*, **1996**, *24*, 422–428.

SAMPLE

Matrix: blood
Sample preparation: Add 500 μL MeCN containing an excess of sodium carbonate to 500 μL plasma, vortex for 10 s, centrifuge at 2000 g for 10 min, dilute a 350 μL aliquot of the supernatant with 700 μL distilled water, inject an aliquot.

HPLC VARIABLES

Column: 125 × 4 5 μm Ultrabase C8 (SFCC, Neuilly Plaisance, France)
Mobile phase: MeCN:water 28:72
Flow rate: 1

Injection volume: 40
Detector: UV 260

CHROMATOGRAM
Retention time: 3.6
Limit of detection: 150 ng/mL
Limit of quantitation: 400 ng/mL

OTHER SUBSTANCES
Noninterfering: amikacin, amoxicillin, carbamazepine, cilastatin, clavulanic acid, epoxycarbamazepine, flunitrazepam, imipenem, methotrexate, midazolam, ofloxacin, phenobarbital, phenytoin, piperacillin, teicoplanin, theophylline, thiopental, tobramycin, valproic acid, vancomycin

KEY WORDS
plasma

REFERENCE
Cociglio,M.; Brandissou,S.; Alric,R.; Bressolle,F. High-performance liquid chromatographic determination of fluconazole in plasma, *J.Chromatogr.B*, **1996**, *686*, 11–17.

SAMPLE
Matrix: blood
Sample preparation: Condition a 6 mL 500 mg Bakerbond C18 SPE cartridge with 2 column volumes of MeOH and 2 column volumes of buffer. 1 mL Plasma + 100 μL 100 μg/mL IS in buffer + 2 mL buffer, mix, add to the SPE cartridge, wash with 1 mL buffer, wash with 1 mL MeOH:buffer 15:85, dry under vacuum (8-9 inches Hg) for 1 min, elute with 500 μL MeOH without vacuum, elute with 500 μL MeOH under 3-4 inches Hg vacuum. Combine the eluates and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute with 200 μL mobile phase, inject a 15 μL aliquot. (Buffer was 100 mM pH 6.0 sodium phosphate buffer.)

HPLC VARIABLES
Column: 250 × 4 5 μm Nucleosil C18
Mobile phase: MeCN:25 mM pH 7.0 Tris buffer 25:75
Flow rate: 1
Injection volume: 15
Detector: UV 210

CHROMATOGRAM
Retention time: 5
Internal standard: 2-(2,4-difluorophenyl)-1-fluoro-1,3-bis(1H-1,2,4-triazol-1-yl)propan-2-ol (Pfizer UK-54373) (8)
Limit of detection: 100 ng/mL

KEY WORDS
plasma; comparison with capillary electrophoresis; pharmacokinetics; SPE

REFERENCE
von Heeren,F.; Tanner,R.; Theurillat,R.; Thormann,W. Determination of fluconazole in human plasma by micellar electrokinetic capillary chromatography with detection at 190 nm, *J.Chromatogr.A*, **1996**, *745*, 165–172.

SAMPLE
Matrix: blood, dialysate
Sample preparation: Plasma. Extract 100 μL plasma with 5 mL dichloromethane, centrifuge at 1000 g for 15 min. Evaporate the organic to dryness under a stream of nitrogen. Reconstitute the residue with 100 μL mobile phase, inject a 20 μL aliquot. Dialysate. Inject a 10 μL aliquot directly.

HPLC VARIABLES
Column: 200 × 2.1 5 μm HP ODS

Mobile phase: MeCN:buffer 13:87 (Buffer was 20 mm ammonium monobasic phosphate, adjusted to pH 7 with 5 M NaOH.)
Flow rate: 0.5
Injection volume: 10-20
Detector: UV 210

CHROMATOGRAM
Internal standard: UK-51,060 (plasma)

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Yang,H.; Wang,Q.; Elmquist,W.F. Fluconazole distribution to the brain: a crossover study in freely-moving rats using in vivo microdialysis, *Pharm.Res.*, **1996**, *13*, 1570–1575.

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

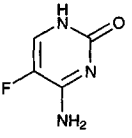
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.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

CHROMATOGRAM
Retention time: 11.398

KEY WORDS
whole blood

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

Flucytosine



Molecular formula: $C_4H_4FN_3O$
Molecular weight: 129.09
CAS Registry No.: 2022-85-7
Merck Index: 4161

SAMPLE
Matrix: blood

Sample preparation: 500 μL Plasma + 50 μL 100 μg/mL 5-chlorouracil in MeOH + 30 μL 1 M pH 4.0 sodium acetate buffer + 300 μL 20% sodium sulfate in water + 8 mL diethyl ether:n-propanol 75:25, vortex for 5 min, centrifuge at 3000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under vacuum using a freeze dryer, reconstitute the residue in 1 mL water, vortex, filter (0.22 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 5 μm YMC A-312 octadecylsilane (Yamamura Chemical)
Mobile phase: MeOH:50 mM pH 3.0 phosphate buffer 1:99
Flow rate: 1.5
Injection volume: 20
Detector: UV 275

CHROMATOGRAM
Retention time: 8
Internal standard: 5-chlorouracil (3)
Limit of quantitation: 200 ng/mL

KEY WORDS
dog; plasma; pharmacokinetics

REFERENCE
Bonny,J.-D.; Kyowa,M. Use of in vitro release tests for the prediction of the in vivo behavior and the development of flucytosine controlled-release capsules, *J.Pharm.Sci.*, **1995**, *84*, 619–623.

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

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

CHROMATOGRAM
Retention time: 3.052

KEY WORDS
whole blood

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

SAMPLE
Matrix: formulations

Sample preparation: Dilute 30 μL of the injection to 100 mL with buffer, add 50 μL 10 mg/mL 5-flucytosine in water, inject a 20 μL aliquot. (Buffer contained 1 g/L $Na_2HPO_4.2H_2O$ and 0.363 g/L KH_2PO_4, pH 7.4.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC 18
Mobile phase: 10 mM KH_2PO_4 adjusted to pH 6.8 with 25% KOH
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 0.9
Internal standard: flucytosine

OTHER SUBSTANCES
Simultaneous: vidarabine phosphate

KEY WORDS
injections; water; flucytosine is IS

REFERENCE
Kwee,M.S.L.; Stolk,L.M.L. Formulation of a stable vidarabine phosphate injection, *Pharm.Weekbl.[Sci].*, **1984**, *6*, 101–104.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Powder tablets, weigh out amount equivalent to 5 mg flucytosine, add 50 mL water, stir for 15 min, filter. 1 mL Filtrate + 1.5 mL 27 μg/mL thymine in buffer, make up to 10 mL with buffer, inject an aliquot. Injections. Dilute with water to a drug concentration of 100 μg/mL. 1 mL Sample + 1.5 mL 27 μg/mL thymine in buffer, make up to 10 mL with buffer, inject an aliquot. (Buffer was 70 mM KH_2PO_4 adjusted to pH 3.0 with HCl.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb CN
Mobile phase: 9 mM Sodium heptanesulfonate adjusted to pH 2.8 with phosphoric acid
Flow rate: 0.8
Injection volume: 20
Detector: UV 266

CHROMATOGRAM
Retention time: 6.3
Internal standard: thymine (4.85)

OTHER SUBSTANCES
Simultaneous: 5-fluorouracil

KEY WORDS
tablets; injections

REFERENCE
Cavrini,V.; Bonazzi,D.; Di Pietra,A.M. Analysis of flucytosine dosage forms by derivative UV spectroscopy and liquid chromatography, *J.Pharm.Biomed.Anal.*, **1991**, *9*, 401–407.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 60 μL to 10 mL with 40 μg/mL p-aminobenzoic acid in MeCN: water 25:75, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeCN:buffer 25:75 (Buffer was 2 mL glacial acetic acid and 700 mg 1-octanesulfonic acid in 750 mL water.)
Flow rate: 1
Injection volume: 5
Detector: UV 285

CHROMATOGRAM
Retention time: 3.2
Internal standard: p-aminobenzoic acid (4.7)

OTHER SUBSTANCES
Noninterfering: degradation products

KEY WORDS
stability-indicating; oral liquids

REFERENCE
Wintermeyer,S.M.; Nahata,M.C. Stability of flucytosine in an extemporaneously compounded oral liquid, *Antimicrob.Agents Chemother.*, **1996**, *40*, 407–409.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 10 μm precolumn (Beckman Instruments Inc.)
Column: 150 × 4.6 5 μm C18 Altex Ultrasphere ODS
Mobile phase: Isocratic. MeOH:buffer 15:85 containing 2.5 mM sodium pentanesulfonate and 2.5 mM sodium heptanesulfonate. Gradient. A was MeOH containing 2.5 mM sodium pentanesulfonate and 2.5 mM sodium heptanesulfonate. B was buffer containing 2.5 mM sodium pentanesulfonate and 2.5 mM sodium heptanesulfonate. A:B 0:100 for 2 min, to 30:70 over 1 min, maintain at 30:70. (Buffer was 50 mM phosphoric acid containing 50 mM KH_2PO_4, pH 2.5.)
Flow rate: 1
Injection volume: 20
Detector: UV 254; UV 285

CHROMATOGRAM
Retention time: 3.76 (isocratic), 9.20 (gradient)
Internal standard: 5-methylcytosine (5.66 (isocratic), 12.62 (gradient))
Limit of quantitation: 12.5 μM

OTHER SUBSTANCES
Simultaneous: barbituric acid, cytosine, fluorouracil, hydroxycytosine, uracil, urea

REFERENCE
Biondi,L.; Nairn,J.G. High performance liquid chromatographic assay for 5-fluorouracil and 5-fluorocytosine, *J.Liq.Chromatogr.*, **1985**, *8*, 1881–1892.

SAMPLE
Matrix: solutions
Sample preparation: Centrifuge and filter cell solutions (0.22 μm), inject an aliquot.

HPLC VARIABLES
Guard column: Guard-PAK C18 (Waters)
Column: 150 × 3.9 5 μm NOVA PAK C18
Mobile phase: MeOH:50 mM pH 6.0 KH_2PO_4 3:97
Flow rate: 1
Detector: UV 280

CHROMATOGRAM
Retention time: 2.5

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: Inject a 20 μL aliquot.

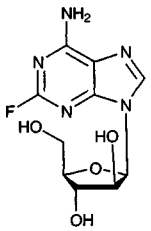
HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb 5 ODS 2
Mobile phase: MeCN:50 mM pH 2.5 phosphate buffer 0.5:99.5
Column temperature: 25
Flow rate: 1
Injection volume: 20
Detector: UV 200

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Simultaneous: 5-fluorouracil, floxuridine

REFERENCE
Del Nozal,M.J.; Bernal,J.L.; Pampliega,A.; Marinero,P.; Pozuelo,M. Determination of the concentrations of 5-fluorouracil and its metabolites in rabbit plasma and tissues by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *656*, 397–405.

Fludarabine



Molecular formula: $C_{10}H_{12}FN_5O_4$
Molecular weight: 285.23
CAS Registry No.: 21679-14-1, 75607-67-9 (phosphate)
Merck Index: 4162
Lednicer No.: 4 167

SAMPLE
Matrix: blood
Sample preparation: Condition a 500 mg Bakerbond SPE cartridge packed with LiChrosorb RP-18 with 2 mL MeOH, 2 mL water and 1 mL MeOH. Mix 500 μL plasma with 100 μL 30 μg/mL mercaptopurine in MeOH, make up to 3 mL with MeOH. Centrifuge at 1100 g for 15 min, add 1.5 mL supernatant to the SPE cartridge, elute at 50 μL/min flow-rate, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 200 × 4 7 μm Lichrosorb RP-18
Mobile phase: MeOH: pH 4.15 phosphate buffer 20:80
Flow rate: 1
Injection volume: 20
Detector: UV 262

CHROMATOGRAM
Retention time: 6.30
Internal standard: mercaptopurine (3.30)
Limit of detection: 50 ng/mL
Limit of quantitation: 100 ng/mL

KEY WORDS
plasma; SPE

REFERENCE
Misztal,G.; Paw,B. Determination of fludarabine phosphate in human plasma using reversed phase high-performance liquid chromatography, *Pharmazie*, **1996**, *51*, 733–734.

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

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil 10 SAX
Mobile phase: Gradient. A was 5 mM pH 2.8 $(NH_4)H_2PO_4$. B was 750 mM pH 3.5 $(NH_4)H_2PO_4$. A:B from 70:30 to 0:100 over 30 min (concave gradient, Waters no. 9). (At the start of each day pump through 20 mL 2 M $(NH_4)H_2PO_4$ then inject 100 μL 100 mM disodium EDTA into the initial mobile phase.)
Flow rate: 3
Injection volume: 50-2000
Detector: UV 262

CHROMATOGRAM
Retention time: 29 (as triphosphate, Fara-ATP)

OTHER SUBSTANCES
Extracted: ara-CTP (cytarabine triphosphate), ATP, CTP, UTP, GTP

KEY WORDS
mononuclear cells

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

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Filter (Amicon CF) while centrifuging at 1000 g for 20 min, filter (0.45 μm) the ultrafiltrate, inject an aliquot. Urine. 250 μL Urine + 500 μL 150 mM $Ba(OH)_2$, vortex, add 500 μL 5% $ZnSO_4.7H_2O$, vortex, centrifuge at 1700 g for 15 min, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeOH:10 mM pH 4.15 $(NH_4)H_2PO_4$ 6:94
Flow rate: 1-1.2
Detector: UV 254

CHROMATOGRAM
Retention time: 55
Limit of detection: 12.5 pmole

OTHER SUBSTANCES
Extracted: fludarabine phosphate

KEY WORDS
plasma; pharmacokinetics; ultrafiltrate

REFERENCE
Hersh,M.R.; Kuhn,J.G.; Phillips,J.L.; Clark,G.; Ludden,T.M.; von Hoff,D.D. Pharmacokinetic study of fludarabine phosphate (NSC 312887), *Cancer Chemother.Pharmacol.*, **1986**, *17*, 277–280.

SAMPLE
Matrix: dialysate

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

CHROMATOGRAM
Retention time: 3

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

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

SAMPLE
Matrix: solutions

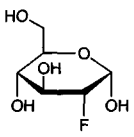
HPLC VARIABLES
Column: 80 × 4.6 3 μm (Perkin-Elmer)
Mobile phase: MeOH:10 mM pH 6.8 potassium phosphate buffer 20:80
Flow rate: 1
Detector: UV 265

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: cladribine, analogs, degradation products

REFERENCE
Reichelova,V.; Liliemark,J.; Albertioni,F. Liquid chromatographic study of acid stability of 2-chloro-2'-*arabino*-fluoro-2'-deoxyadenosine, 2-chloro-2'-deoxyadenosine and related analogues, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 711–714.

Fludeoxyglucose F18



Molecular formula: $C_6H_{11}{}^{18}FO_5$
Molecular weight: 181.15
CAS Registry No.: 105851-17-0
Merck Index: 4163

SAMPLE
Matrix: cell incubations

Sample preparation: Wash cells with PBS, freeze in liquid nitrogen, add 3 mL 400 mM perchloric acid, let stand at 4° for 30 min, centrifuge at 12000 g at 4°, extract with 3 mL 500 mM trioctylamine in 1,1,2-trichlorotrifluoroethane, filter (0.22 μm) the inorganic phase, inject an aliquot.

HPLC VARIABLES
Guard column: LiChrosorb RP 18-5
Column: 100 × 8 Radial-PAK Partisil 10 SAX (Waters)
Mobile phase: Gradient. A was MeOH:15 mM pH 3.8 $(NH_4)H_2PO_4$ 3:97. B was MeOH:750 mM pH 4.8 $(NH_4)H_2PO_4$ 3:97. A:B from 100:0 to 0:100 over 35 min (concave gradient), re-equilibrate for 15 min.
Flow rate: 2
Injection volume: 150
Detector: Radioactivity (Berthold LB 506 C-1) (with Quick Szint Flow 306 pumped at 4 mL/min)

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
C14 labeled; chondrocytes

REFERENCE
Fedders,G.; Kock,R.; Van de Leur,E.; Greiling,H. A radiochemical high-performance liquid chromatographic method for the analysis of 2-fluoro-2-deoxy-D-glucose-derived metabolites in human chondrocytes, *Anal.Biochem.*, **1993**, *211*, 81–86.

SAMPLE
Matrix: reaction mixtures
Sample preparation: Evaporate reaction mixture in the presence of active charcoal and pass through a 100 × 10 column of Merck Kieselgel 60 for dry column chromatography using 70 mL MeCN:water 99.7:0.3, evaporate the eluate, take up the residue in 1 mL water, inject an aliquot.

HPLC VARIABLES
Guard column: 30 × 3.6 Aminex HPX-87C (Bio-Rad)
Column: 300 × 7.8 Aminex HPX-87P (Bio-Rad) (The column contains lead which is washed off. Periodically backflush with 100 mM lead nitrate at 0.1 mL/min overnight.)
Mobile phase: Water
Column temperature: 20
Flow rate: 0.1
Detector: RI (at 18°)

CHROMATOGRAM
Retention time: 68.4

KEY WORDS
F18 labeled

REFERENCE
Oberdorfer,F.; Hull,W.E.; Traving,B.C.; Maier-Borst,W. Synthesis and purification of 2-deoxy-2-[18F]fluoro-D-glucose and 2-deoxy-2-[18F]fluoro-D-mannose: characterization of products by 1H- and 19F-NMR spectroscopy, *Int.J.Rad.Appl.Instrum.[A].*, **1986**, *37*, 695–701.

SAMPLE
Matrix: tissue hydrolyzate
Sample preparation: Centrifuge at 12000 g for 10 min, inject an aliquot.

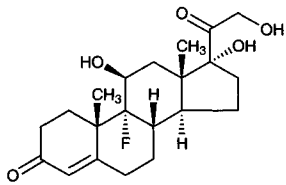
HPLC VARIABLES
Guard column: 20 × 4 APS Hypersil
Column: 250 × 4 BioSil Amino 5S (Bio-Rad)
Mobile phase: MeCN:water 90:10
Flow rate: 1
Detector: Radioactivity (with Quick Szint Flow 302)

CHROMATOGRAM
Retention time: 7

KEY WORDS
C14 labeled

REFERENCE
Fedders,G.; Kock,R.; Van de Leur,E.; Greiling,H. The metabolism of 2-fluoro-2-deoxy-D-glucose in human chondrocytes and its incorporation into keratan sulfate proteoglycans, *Eur.J.Biochem.*, **1994**, *219*, 1063–1071.

Fludrocortisone



Molecular formula: $C_{21}H_{29}FO_5$
Molecular weight: 380.46
CAS Registry No.: 127-31-1, 514-36-3 (acetate)
Merck Index: 4166
Lednicer No.: 1 192

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherex C18 (Phenomenex USA)
Mobile phase: MeOH:THF:water 3:25:72
Flow rate: 1.0
Injection volume: 60
Detector: UV 254

CHROMATOGRAM
Retention time: 15.9

OTHER SUBSTANCES
Simultaneous: 11-deoxycortisol, dexamethasone, hydrocortisone, methylprednisolone, prednisolone

REFERENCE
McWhinney,B.C.; Ward,G.; Hickman,P.E. Improved HPLC method for simultaneous analysis of cortisol, 11-deoxycortisol, prednisolone, methylprednisolone, and dexamethasone in serum and urine, *Clin.Chem.*, **1996**, *42*, 979–981.

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

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil 5-ODS
Mobile phase: THF:water 23:77
Column temperature: 30
Flow rate: 1

Injection volume: 20
Detector: UV 245

CHROMATOGRAM
Retention time: k' 7.87 (fludrocortisone), k' 28.04 (fludrocortisone acetate)
Internal standard: methylprednisolone (k' 11.36)

OTHER SUBSTANCES
Simultaneous: metabolites, betamethasone, corticosterone, cortisone, deflazacort, deoxycorticosterone, dexamethasone, fluorocortisone, fluorocortisone acetate, hydrocortisone, 21-hydroxydeflazacort, 11α-hydroxyprogesterone, methylprednisolone, prednisolone, prednisone, triamcinolone acetonide, triamcinolone

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: 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 μL 500 mM pH 5.0 acetate buffer + 50 μL MeOH + 160 μL 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 μm), 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 μL MeOH, add 20 μL cupric acetate solution, let stand at room temperature for 1 h, add 100 μL reagent, heat at 60° for 40 min, cool, centrifuge briefly at 1000 g, inject a 100 μL aliquot of the supernatant. (Cupric acetate solution was 0.7 g cupric acetate in 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 × 4.6 5 μm 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 for 20 min, re-equilibrate for 20 min.)
Flow rate: 1
Injection volume: 100
Detector: F ex 350 em 390

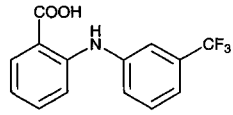
CHROMATOGRAM
Retention time: 35.6
Internal standard: fludrocortisone

OTHER SUBSTANCES
Extracted: hydrocortisone, tetrahydroaldosterone, aldosterone
Noninterfering: cortisone, corticosterone, hydroxycorticosteroids

KEY WORDS
SPE; derivatization; fludrocortisone is IS

REFERENCE
Yoshitake,T.; Ishida,J.; Sonezaki,S.; Yamaguchi,M. High performance liquid chromatographic determination of 3α, 5β-tetrahydroaldosterone and cortisol in human urine with fluorescence detection, *Biomed. Chromatogr.*, **1992**, *6*, 217–221.

Flufenamic acid



Molecular formula: $C_{14}H_{10}F_3NO_2$
Molecular weight: 281.23
CAS Registry No.: 530-78-9
Merck Index: 4167
Lednicer No.: 1 110

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 40 μL 100 μg/mL mefenamic acid in MeOH + 1 mL 1 M hydrochloric acid + 6 mL dichloromethane, shake mechanically for 20 min. Centrifuge at 2000 g for 5 min, evaporate organic phase to dryness under a gentle stream of nitrogen at 40°. Reconstitute the residue with 50 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 5 μm Nucleosil C18
Mobile phase: MeOH:water 77:23
Flow rate: 0.8
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 7.2
Internal standard: mefenamic acid (8.6)
Limit of detection: 100 ng/mL
Limit of quantitation: 500 ng/mL

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Cerretani,D.; Micheli,L.; Fiaschi,L.; Giorgi,G. High-performance liquid chromatography of flufenamic acid in rat plasma, *J.Chromatogr.B*, **1996**, *678*, 365–368.

SAMPLE
Matrix: formulations
Sample preparation: 100-300 mg Gel ointment + 3 mL MeOH, mix vigorously, filter (0.2 μm), inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH
Flow rate: 1
Injection volume: 10
Detector: UV 280

CHROMATOGRAM
Retention time: 4.3

KEY WORDS
ointment

REFERENCE
Yamamura,K.; Yamada,J.-I.; Yotsuyanagi,T. High-performance liquid chromatographic assay of antiinflammatory drugs incorporated in gel ointments. Separation and stability testing, *J.Chromatogr.*, **1985**, *331*, 383–388.

SAMPLE
Matrix: solutions

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

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

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

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

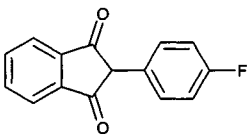
CHROMATOGRAM
Retention time: k' 1.35

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

Fluindione



Molecular formula: $C_{15}H_9FO_2$
Molecular weight: 240.23
CAS Registry No.: 957-56-2
Merck Index: 4168

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL plasma to a siliconized tube containing 50 μL 2 μg/mL IS in MeOH and 100 μL MeCN. Vortex for 30 s, centrifuge at 3000 g for 10 min. Mix a 150 μL aliquot of the supernatant with 150 μL 0.9% NaCl, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeCN:67 mM Na_2HPO_4 buffer adjusted to pH 7.2 with orthophosphoric acid 23:77 (At the end of each chromatographic session rinse the HPLC system with 200 mL MeCN:water 50:50.)
Flow rate: 1.5

Injection volume: 100
Detector: UV 280

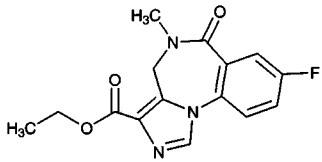
CHROMATOGRAM
Retention time: 4.2
Internal standard: coumarin (8.5)
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Also analyzed: acenocoumarol, brodifacoum, bromadiolone, chlorphacinone, coumatetralyl, difenacoum, diphenadione, ethyl biscoumacetate, phenidione, warfarin

KEY WORDS
plasma

REFERENCE
Aymard,G.; Legrand,M.; Comets,E.; Mentre,F.; Diquet,B. Rapid and simple micromethod for the quantification of fluindione in human plasma using high-performance liquid chromatography, *J.Chromatogr.B*, **1998**, *707*, 169–173.

Flumazenil



Molecular formula: $C_{15}H_{14}FN_3O_3$
Molecular weight: 303.29
CAS Registry No.: 78755-81-4
Merck Index: 4169
Lednicer No.: 4 220

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 25 μL EtOH + 25 μL 3 μg/mL IS1 and 7.6 μg/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 μL mobile phase, inject a 1-15 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 3 μm 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: 5.0
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 climazolam (17.0)
Limit of detection: 0.3 ng/mL

OTHER SUBSTANCES
Extracted: midazolam

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
Sample preparation: 1 mL Serum or plasma + 1 mL saturated Na_2HPO_4 + 100 µL 10 µg/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 × 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: 2.1
Internal standard: N-6-dimethyl-2-(4-methylphenyl)-N-propylimidazo[1,2-α]pyridine-3-acetamide methanesulfonate (Synthelabo France) (6.4)

OTHER SUBSTANCES
Extracted: prothipendyl, zolpidem

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
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: 2.7
Limit of detection: 0.5-2.5 ng/mL

OTHER SUBSTANCES
Simultaneous: atipamezole, atropine, butorphanol, ketamine, medetomidine, midazolam, xylazine

KEY WORDS
plasma; SPE; dog

REFERENCE

Kanazawa,H.; Nagata,Y.; Matsushima,Y.; Takai,N.; Uchiyama,H.; Nishimura,R.; Takeuchi,A. Liquid chromatography-mass spectrometry for the determination of medetomidine and other anaesthetics in plasma, *J.Chromatogr.*, **1993**, *631*, 215–220.

SAMPLE

Matrix: blood

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

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

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

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 244

CHROMATOGRAM

Retention time: 3.26

Limit of detection: <120 ng/mL

KEY WORDS

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

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

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Plasma or urine + 50 μL 4 μg/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 μL MeOH, inject a 30 μL aliquot. (Deconjugate urine as follows. 250 μL Urine + 750 μL pH 5.4 acetate buffer + 500 U β-glucuronidase, heat at 37° for 18 h, add 20 μL 5 M NaOH, centrifuge, proceed as above using 5 mL dichloromethane:diethyl ether.)

HPLC VARIABLES
Guard column: 30 × 4.6 30 μm C8
Column: 100 × 8 4 μm 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: 3.8
Internal standard: flurazepam (18.5)
Limit of detection: 4 ng/mL

OTHER SUBSTANCES
Extracted: midazolam, 4-hydroxymidazolam, 1-hydroxymethylmidazolam
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
Sample preparation: Inject a 25 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb C18
Mobile phase: MeOH:THF:isopropanol:water 30:3.5:1.5:65
Column temperature: 40
Flow rate: 1
Injection volume: 25
Detector: UV 245

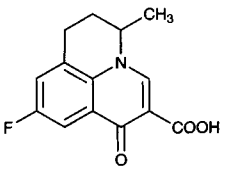
CHROMATOGRAM
Retention time: 6.30

OTHER SUBSTANCES
Simultaneous: methylparaben, propylparaben, phenol, degradation products
Noninterfering: aminophylline, cimetidine, dobutamine, dopamine, famotidine, lidocaine, procainamide, ranitidine

KEY WORDS
injections; 5% dextrose; stability-indicating

REFERENCE
Olsen,K.M.; Gurley,B.J.; Davis,G.A.; Christensen,R.; Monaghan,M.S. Stability of flumazenil with selected drugs in 5% dextrose injection, *Am.J.Hosp.Pharm.*, **1993**, *50*, 1907–1912.

Flumequine



Molecular formula: $C_{14}H_{12}FNO_3$
Molecular weight: 261.25
CAS Registry No.: 42835-25-6
Merck Index: 4172
Lednicer No.: 3 186

SAMPLE
Matrix: blood
Sample preparation: Mix 100 μL plasma with 100 μL pH 6.0 phosphate buffer and 1 mL ethyl acetate, shake, centrifuge at 20000 g for 5 min, collect 800 μL the organic phase, dry under nitrogen at 45°. Add 300 μL pH 7.8 phosphate buffer and 300 μL hexane to the residue, shake, centrifuge at 20000 g for 5 min, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: 4 × 4 C18 (Merck)
Column: 125 × 4 5 μm Lichrospher RP Select B
Mobile phase: MeCN:DMF:water:orthophosphoric acid 18:28:40.5:13.5
Flow rate: 0.8
Injection volume: 100
Detector: UV 324 (A), F ex 320 em 365 (B)

CHROMATOGRAM
Retention time: 6.4
Limit of quantitation: 5 ng/mL (F), 100 ng/mL (UV)

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: ciprofloxacin, danofloxacin, enrofloxacin, marbofloxacin, nalidixic acid, oxolinic acid

KEY WORDS
sheep; plasma

REFERENCE
Delmas,J.M.; Chapel,A.M.; Sanders,P. Determination of flumequine and 7-hydroxyflumequine in plasma of sheep by high-performance liquid chromatography, *J.Chromatogr.B*, **1998**, *712*, 263–268.

SAMPLE
Matrix: tissue
Sample preparation: 2 g Minced tissue + 100 μL 5 μg/mL IS in water + 4 g anhydrous sodium sulfate, mix until homogenized. Add 10 ml ethyl acetate, shake mechanically for 10 min, centrifuge at 1500 g for 10 min. Transfer organic layer into another tube and repeat extraction on the tissue pellet with 10 mL ethyl acetate. Evaporate combined organic phases under a stream of nitrogen at 50°. Dissolve residue in 1 mL MeCN:2.7 mM pH 2.5 oxalic acid 50:50, vortex, sonicate for 5 min, filter through 0.45 μm filter (GHP Acrodisc GF, Gelman Sciences, USA). Inject a 100 μL aliquot. (1 mg/mL IS stock solution was prepared in 20 mM NaOH, it was diluted to working concentration with water immediately before use.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrabase C18 (Shandon, UK)

Mobile phase: Gradient. A was MeCN. B was 2.7 mM pH 2.5 oxalic acid. A:B from 10:90 to 70:30 over 20 min, maintain at 70:30 for 5 min, return to initial conditions over 5 min.
Flow rate: 0.8
Injection volume: 100
Detector: F ex 252 em 356

CHROMATOGRAM
Retention time: 20
Internal standard: ibafloxacin (21.3)
Limit of detection: 15 ng/g
Limit of quantitation: 50 ng/g

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
kidney; pig

REFERENCE
Guyonnet,J.; Pacaud,M.; Richard,M.; Doisi,A.; Spavone,F.; Hellings,P. Routine determination of flumequine in kidney tissue of pig using automated liquid chromatography, *J.Chromatogr.B*, **1996**, *679*, 177–184.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize 1 g tissue with 5 mL acetone, centrifuge, decant and save the supernatant, repeat the extraction. Add 2 mL acetone, 3 mL hexane, and 6 mL 3% NaCl to the combined supernatants, extract, centrifuge, discard the hexane layer. Add the extract to 25 mL chloroform (Caution! Chloroform is a carcinogen!), mix, separate the phases, add 2.5 mL 100 mM pH 9.0 Na_3PO_4 buffer and one drop 1 M NaOH to the chloroform phase, mix, separate the phases, discard the chloroform layer. Wash the aqueous layer with 2.5 mL chloroform, centrifuge the aqueous layer. Dialyze a 740 μL aliquot of the supernatant (in 2 portions) against 3.9 mL 20 mM pH 5.0 sodium phosphate buffer pumped at 0.6 mL/min using a Cuprophan 15000 MW cut-off cellulose acetate membrane. After washing column A with 500 μL MeCN:water 50:50 and 500 μL 20 mM pH 5.0 Na_3PO_4 buffer the dialysate flowed through column A to waste. Wash column A with 500 μL 20 mM pH 5.0 sodium phosphate buffer, elute the contents of column A onto column B with mobile phase, elute with mobile phase, monitor the effluent from column B. (Wash the donor channel with 2 mL 0.01% Triton X-100 in 20 mM pH 5.0 sodium phosphate buffer. Wash the acceptor channel with 3 mL 20 mM pH 5.0 sodium phosphate buffer. Regenerate the membrane with 2 mL 100 mM pH 9.0 sodium phosphate buffer (donor channel) and 3 mL 20 mM pH 5.0 sodium phosphate buffer (acceptor channel).)

HPLC VARIABLES
Column: A 5.8 × 4.6 Hypersil ODS; B 150 × 4.6 PLRP-S (Polymer Labs., UK)
Mobile phase: MeCN:THF:20 mM pH 5.0 Na_3PO_4 buffer 20:15:65
Flow rate: 0.6
Detector: F ex 318 em 364

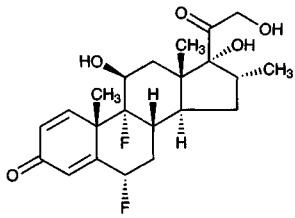
CHROMATOGRAM
Retention time: 12
Limit of detection: 5 ng/g

OTHER SUBSTANCES
Extracted: oxolinic acid

KEY WORDS
column switching; chicken; liver; dialysis

REFERENCE
Eng,G.Y.; Maxwell,R.J.; Cohen,E.; Piotrowski,E.G.; Fiddler,W. Determination of flumequine and oxolinic acid in fortified chicken tissue using on-line dialysis and high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.A*, **1998**, *799*, 349–354.

Flumethasone



Molecular formula: $C_{22}H_{28}F_2O_5$
Molecular weight: 410.46
CAS Registry No.: 2135-17-3, 2002-29-1 (pivalate)
Merck Index: 4173
Lednicer No.: 1 200

SAMPLE

Matrix: blood
Sample preparation: Add 1 mL serum to a Sep Pak C18 SPE cartridge, wash with 4 mL water, elute with 4 mL MeOH, evaporate to dryness under vacuum, reconstitute in 50 μL MeCN:water 30:70, inject whole sample.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:water 30:70
Flow rate: 1
Injection volume: 50
Detector: enzyme immunoassay of fractions

CHROMATOGRAM

Retention time: 18
Limit of detection: 0.3 pg

OTHER SUBSTANCES

Extracted: dexamethasone, betamethasone, triamcinolone
Noninterfering: endogenous steroids

KEY WORDS

serum; SPE; horse

REFERENCE

Friedrich,A.; Schulz,R.; Meyer,H.H. Use of enzyme immunoassay and reverse-phase high-performance liquid chromatography to detect and confirm identity of dexamethasone in equine blood, *Am.J.Vet.Res.*, **1992**, *53*, 2213–2220.

SAMPLE

Matrix: blood
Sample preparation: Condition a Tef Elutor C18 cartridge with two 3 mL portions of MeOH then two 3 mL portions of water. Heat 1 mL plasma at 50° for 10 min, add to cartridge, wash with 2 mL water, 1 mL MeOH:water 10:90, 4 mL acetone:water 20:80, apply suction to cartridge for 10 min to air dry. Elute with 1 mL MeOH, evaporate eluent at 45° under nitrogen, reconstitute with 50 μL mobile phase, inject 25 μL aliquot.

HPLC VARIABLES

Column: 100 × 2 3 μm C18 Hypersil
Mobile phase: MeCN:THF:water 8:10:82, containing 5 mL/L triethylamine, pH adjusted to 6.5 with citric acid
Flow rate: 0.6
Injection volume: 25
Detector: UV 242

CHROMATOGRAM

Retention time: 11.50
Internal standard: flumethasone
Limit of detection: 300 pg/mL

OTHER SUBSTANCES

Simultaneous: dexamethasone, prednisone, hydrocortisone, adrenosterone, prednisolone, estriol, corticosterone, methylprednisolone, cortisone, hydroxyprogesterone, testosterone, deoxycorticosterone, fluorometholone, spironolactone, equilenin, estrone, estradiol, progesterone, diphenhydramine, propranolol, aspirin, theophylline, imipramine, desipramine, indomethacin, amitriptyline, nortriptyline, nordiazepam, diazepam, chlordiazepoxide, tripelennamine, carbamazepine, probenecid, phenobarbital

Noninterfering: caffeine, nicotine, cotinine, chlorothiazide, acetazolamide, phenytoin, pheniramine, cephalothin, primidone, acebutolol, hydrochlorothiazide, quinine, acetophenetidin, furosemide, aldosterone, triamcinolone, ephedrine, allopurinol, phenylephrine

KEY WORDS

plasma; SPE; flumethasone is IS

REFERENCE

Hariharan,M.; Naga,S.; VanNoord,T.; Kindt,E.K. Simultaneous assay of corticosterone and cortisol in plasma by reversed-phase liquid chromatography, *Clin.Chem.*, **1992**, *38*, 346–352.

SAMPLE

Matrix: blood

Sample preparation: Condition a 2 mL 200 mg Tef Elutor C18 SPE cartridge (Versa Prep) with 3 mL MeOH and two 3 mL portions of water. Heat 1 mL plasma at 50° for 10 min, add to SPE cartridge, wash with 2 mL water, wash with 1 mL MeOH:water 10:90, wash with 4 mL acetone:water 20:80, air-dry for 10 min, elute with 1 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 50 μL mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES

Column: 100 × 2 3 μm Hypersil

Mobile phase: MeCN:THF:water 8:10:82 containing 5 mL/L triethylamine, pH adjusted to 6.5 with citric acid

Flow rate: 0.6

Injection volume: 25

Detector: UV 242

CHROMATOGRAM

Retention time: 13

Internal standard: flumethasone

OTHER SUBSTANCES

Extracted: cortisone, corticosterone, hydrocortisone

Simultaneous: acebutolol, acetazolamide, acetophenetidin, adrenosterone, aldosterone, amitriptyline, androsten-3,17-dione, aspirin, carbamazepine, cephalothin, chlordiazepoxide, chlorothiazide, dehydrocorticosterone, deoxycorticosterone, deoxycortisol, desipramine, dexamethasone, diazepam, diphenhydramine, equilenin, estradiol, estriol, estrone, fluorometholone, furosemide, hydrochlorothiazide, hydroxycorticosterone, hydroxyprogesterone, hydroxyprogesterone, imipramine, indomethacin, methylhydroxyprogesterone, methylprednisolone, nandrolone, nordiazepam, nortriptyline, pheniramine, phenobarbital, phenytoin, prednisolone, prednisone, primidone, probenecid, progesterone, propranolol, quinine, spironolactone, testosterone, theophylline, triamcinolone, tripelennamine

Noninterfering: caffeine, nicotine, cotinine, ephedrine, allopurinol, phenylephrine

KEY WORDS

serum; SPE; flumethasone is IS

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: formulations

Sample preparation: Ointment. Weigh out 1-1.5 g ointment, add 10 mL chloroform:hexane 50:50, warm until the ointment dissolves, add to a silica Sep-Pak SPE cartridge, rinse container with 10 mL hexane:chloroform 50:50, add rinse to SPE cartridge, discard all eluates, rinse container with 5 mL MeOH, add rinse to SPE cartridge, elute with 15 mL MeOH, collect all MeOH eluates, make up to 25 mL with MeOH, inject a 25 μL aliquot. Cream. Weigh out 1 g cream and add it to 10 mL MeOH, shake for 5 min, sonicate for 5 min, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 C8 (Brownlee)
Mobile phase: MeOH:THF:water 30:30:40
Flow rate: 1.5
Injection volume: 10-25
Detector: UV 254

CHROMATOGRAM
Retention time: 8.4 (flumethasone pivalate)

KEY WORDS
ointment; cream; SPE

REFERENCE
Lodge,B.A.; Vincent,A. Analysis of flumethasone pivalate formulations by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *301*, 477–480.

SAMPLE
Matrix: liposomal preparations
Sample preparation: Dilute 1000-fold with MeOH/water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: C18
Mobile phase: MeOH:1% acetic acid 70:30
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 5.12

OTHER SUBSTANCES
Interfering: dexamethasone

REFERENCE
Devoisselle,J.-M.; Vion-Dury,J.; Confort-Gouny,S.; Coustaut,D.; Cozzone,P.J. Liposomes containing fluorinated steroids: an analysis based on photon correlation and fluorine-19 nuclear magnetic resonance spectroscopy, *J.Pharm.Sci.*, **1992**, *81*, 249–254.

SAMPLE
Matrix: 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 237

CHROMATOGRAM
Limit of detection: 120 ng/mL

OTHER SUBSTANCES
Also analyzed: betamethasone 17-valerate, dexamethasone

KEY WORDS
for flumethasone 21-acetate; SPE

REFERENCE
Valenta,C.; Janout,H. Corticosteroid analysis by HPLC with increased sensitivity by use of precolumn concentration, *J.Liq.Chromatogr.*, **1994**, *17*, 1141–1146.

SAMPLE
Matrix: synovial fluid
Sample preparation: 100 µL Synovial fluid + 10 µL 10 µg/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 × 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: 6
Internal standard: flumethasone

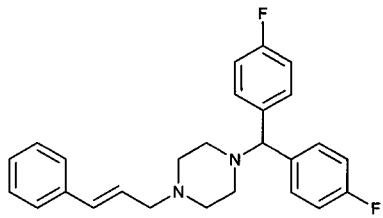
OTHER SUBSTANCES
Extracted: methylprednisolone, methylprednisolone acetate

KEY WORDS
normal phase; cow; flumethasone is IS

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.

Flunarizine

Molecular formula: $C_{26}H_{26}F_2N_2$
Molecular weight: 404.50
CAS Registry No.: 52468-60-7, 30484-77-6 (2.HCl)
Merck Index: 4179
Lednicer No.: 2 31



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

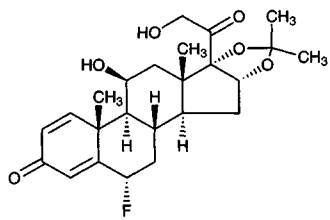
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.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

CHROMATOGRAM
Retention time: 19.317

KEY WORDS
whole blood

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

Flunisolide



Molecular formula: $C_{24}H_{31}FO_6$
Molecular weight: 434.50
CAS Registry No.: 3385-03-3, 77326-96-6 (hemihydrate), 4533-89-5 (acetate)
Merck Index: 4180
Lednicer No.: 2 181

SAMPLE
Matrix: blood
Sample preparation: Add 30 μL IS to 1 mL serum, add 5 mL diethyl ether/dichloromethane (ratio not given), mix for 30 min. Centrifuge and freeze at -80° for 15 min. Evaporate the supernatant under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase. Inject a 50 μL aliquot.

HPLC VARIABLES
Column: Lichrospher RP Select B
Mobile phase: MeCN:20 mM ammonium acetate buffer 80:20
Flow rate: 1
Injection volume: 50
Detector: MS, PE-Sciex API 300, negative ion mode

CHROMATOGRAM
Internal standard: budesonide
Limit of detection: 100 pg/mL

KEY WORDS
serum; pharmacokinetics

REFERENCE
Möllmann,H.; Derendorf,H.; Barth,J.; Meibohm,B.; Wagner,M.; Krieg,M.; Weisser,H.; Knöller,J.; Möllmann,A.; Hochhaus,G. Pharmacokinetic/pharmacodynamic evaluation of systemic effects of flunisolide after inhalation, *J.Clin.Pharmacol.*, **1997**, *37*, 893–903.

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

HPLC VARIABLES
Column: 10 μm Spherisorb ODS
Mobile phase: MeCN:water:acetic acid 35:64:1
Injection volume: 100
Detector: UV 240

CHROMATOGRAM
Internal standard: fluocinonide

KEY WORDS
nasal spray

REFERENCE
Yu,C.D.; Jones,R.E.; Henesian,M. Cascade impactor method for the droplet size characterization of a metered-dose nasal spray, *J.Pharm.Sci.*, **1984**, *73*, 344–348.

SAMPLE
Matrix: solutions

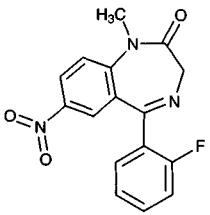
HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:water 55:45
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 10.2

OTHER SUBSTANCES
Simultaneous: metabolites

REFERENCE
Tökés,L.; Cho,D.; Maddox,M.L.; Chaplin,M.D.; Chu,N.I. Isolation and identification of an oxidatively defluorinated metabolite of flunisolide in man, *Drug Metab.Dispos.*, **1981**, *9*, 485–486.

Flunitrazepam



Molecular formula: $C_{16}H_{12}FN_3O_3$
Molecular weight: 313.29
CAS Registry No.: 1622-62-4
Merck Index: 4181
Lednicer No.: 2 406

SAMPLE
Matrix: blood
Sample preparation: Vortex 1 mL plasma with 3 mL toluene:isoamyl alcohol 95:5 at 1000 rpm for 90 s, centrifuge at 2600 g for 10 min. Evaporate a 2.5 mL aliquot of the upper organic layer to dryness under nitrogen at 40°, reconstitute with 100 μL mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 5 μm Nucleosil 120-5 C18

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

HPLC VARIABLES
Column: 10 μm Spherisorb ODS
Mobile phase: MeCN:water:acetic acid 35:64:1
Injection volume: 100
Detector: UV 240

CHROMATOGRAM
Internal standard: fluocinonide

KEY WORDS
nasal spray

REFERENCE
Yu,C.D.; Jones,R.E.; Henesian,M. Cascade impactor method for the droplet size characterization of a metered-dose nasal spray, *J.Pharm.Sci.*, **1984**, *73*, 344–348.

SAMPLE
Matrix: solutions

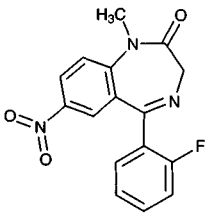
HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:water 55:45
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 10.2

OTHER SUBSTANCES
Simultaneous: metabolites

REFERENCE
Tökés,L.; Cho,D.; Maddox,M.L.; Chaplin,M.D.; Chu,N.I. Isolation and identification of an oxidatively defluorinated metabolite of flunisolide in man, *Drug Metab.Dispos.*, **1981**, *9*, 485–486.

Flunitrazepam



Molecular formula: $C_{16}H_{12}FN_3O_3$
Molecular weight: 313.29
CAS Registry No.: 1622-62-4
Merck Index: 4181
Lednicer No.: 2 406

SAMPLE
Matrix: blood
Sample preparation: Vortex 1 mL plasma with 3 mL toluene:isoamyl alcohol 95:5 at 1000 rpm for 90 s, centrifuge at 2600 g for 10 min. Evaporate a 2.5 mL aliquot of the upper organic layer to dryness under nitrogen at 40°, reconstitute with 100 μL mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 5 μm Nucleosil 120-5 C18

Column: 250 × 4 5 μm Nucleosil 120-5 C 18
Mobile phase: MeOH:buffer 47:53 (Buffer was 100 mM Na_2HPO_4 adjusted to pH 7.8 with orthophosphoric acid.)
Column temperature: 37
Flow rate: 1.2
Injection volume: 25
Detector: UV 302

CHROMATOGRAM
Retention time: 11.4
Internal standard: flunitrazepam

OTHER SUBSTANCES
Extracted: omeprazole

KEY WORDS
plasma; flunitrazepam is IS

REFERENCE
Macek,J.; Ptácek,P.; Klíma,J. Determination of omeprazole in human plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *689*, 239–243.

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

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 221

CHROMATOGRAM
Retention time: 3.92
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acen-

ocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

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

SAMPLE

Matrix: blood, urine

Sample preparation: Condition a 300 mg Bond Elut Certify LRC SPE cartridge with 2 volumes of the elution mixture, MeOH, and pH 6 phosphate buffer. Add 30 μL 10 μg/mL IS in MeOH and 5 mL pH 6 phosphate buffer to 1 mL serum, plasma or urine. Vortex, centrifuge at 10000 rpm for 5 min. Add the supernatant to the SPE cartridge and allow to pass through at 1.0 mL/min. Wash with 5 mL MeOH:water 20:80, 5 mL 1 M orthophosphoric acid, 5 mL MeOH, and 5 mL chloroform. Elute with 2 mL chloroform:isopropanol:ammonia 70:28:2. Add 20 μL ethylene glycol to the effluent, evaporate at 50° under vacuum. Add 30 μL mobile phase to the residue. Inject a 20 μL aliquot. (Caution! Chloroform is a carcinogen! Prepare the SPE elution mixture as follows. Add 400 μL ammonia to 19.6 mL chloroform:isopropanol 70:30.)

HPLC VARIABLES

Column: 250 × 4.0 LiChrospher 60 RP-Select B
Mobile phase: MeCN:20 mM pH 2.0 phosphate buffer 36:64
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 16.19
Internal standard: methylclonazepam (19.97)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

Simultaneous: alprazolam, bromazepam, brotizolam, clobazam, clonazepam, desmethyldiazepam, diazepam, lorazepam, midazolam, methylclonazepam, nitrazepam, oxazepam, temazepam, triazolam

Noninterfering: amphetamine, atropine, barbital, caffeine, cocaine, codeine, dronabinol, fluphenazine, haloperidol, imipramine, morphine, phenobarbital, secobarbital

KEY WORDS

serum; plasma; SPE

REFERENCE

Deinl,I.; Mahr,G.; von Meyer,L. Determination of flunitrazepam and its main metabolites in serum and urine by HPLC after mixed-mode solid-phase extraction, *J.Anal.Toxicol.*, **1998**, *22*, 197–202.

SAMPLE

Matrix: blood, urine

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

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

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

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

Column temperature: 30

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

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 18.558

KEY WORDS

whole blood

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

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

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

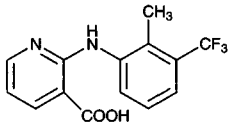
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, 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, fen-

camfamine, fenoprofen, fenproporex, fentanyl, flubendazole, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

Flunixin



Molecular formula: $C_{14}H_{11}F_3N_2O_2$
Molecular weight: 296.25
CAS Registry No.: 38677-85-9, 42461-84-7 (meglumine salt)
Merck Index: 4182
Lednicer No.: 2 281

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma or serum + 4 mL 250 ng/mL naproxen in MeCN, vortex for 30 s, centrifuge at 1000 g for 15 min. Remove 4 mL of the supernatant and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 500 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 50 mm long 30 μm pellicular ODS
Column: 250 mm long 5 μm Spherisorb ODS I
Mobile phase: MeCN:MeOH:1% pH 3.0 acetate buffer 30:20:50
Flow rate: 1.2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 9.0

Internal standard: naproxen (7.7)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: phenylbutazone, oxyphenbutazone

KEY WORDS
plasma; serum; horse; pharmacokinetics; for dogs see Am.J.Vet.Res. 1985; 46; 235

REFERENCE
Hardee,G.E.; Lai,J.-W.; Moore,J.N. Simultaneous determination of flunixin, phenylbutazone, oxyphenbutazone and γ-hydroxyphenylbutazone in equine plasma by high-performance liquid chromatography: With application to pharmacokinetics, *J.Liq.Chromatogr.*, **1982**, *5*, 1991–2003.

SAMPLE
Matrix: blood, milk
Sample preparation: Plasma. 1 mL Plasma + 10 μL 6 μg/mL IS + 1 mL 1 M HCl + 3 mL water, add to a Clin Elut SPE cartridge, elute with two 8 mL portions of dichloromethane. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 200 μL MeOH, vortex for 30 s, inject a 50 μL aliquot. Milk. 25 mL Milk + 2.5 mL 1 M HCl, mix briefly, centrifuge at 10000 rpm in a refrigerated centrifuge for 10 min. Remove the supernatant and wash the pellet with 15 mL 1 M HCl, centrifuge at 10000 rpm in a refrigerated centrifuge for 10 min. Combine the supernatants, add a 5 mL aliquot to 150 μL 10 μg/mL IS and 5 mL hexane, rotate for 5 min, centrifuge at 1000 g for 5 min, add the aqueous phase to a Clin Elut SPE cartridge, elute with two 8 mL portions of dichloromethane. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 200 μL MeOH, vortex for 30 s, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 40 μm CO:PELL ODS
Column: Radial Compression C18 (Waters)
Mobile phase: MeCN:25 mM pH 2.5 potassium phosphate buffer 50:50
Flow rate: 2
Injection volume: 50
Detector: UV 280

CHROMATOGRAM
Retention time: 3.2
Internal standard: Sch 13476 (4.8)
Limit of quantitation: 50 ng/mL

KEY WORDS
plasma; cow; SPE

REFERENCE
Neff-Davis,C.A.; Bosch,K. An HPLC method for the determination of flunixin in bovine plasma and milk, *J.Vet.Pharmacol.Ther.*, **1985**, *8*, 331–334.

SAMPLE
Matrix: blood, urine
Sample preparation: 5 mL Blood or 10 mL urine + 2.5 (blood) or 5 (urine) μg flufenamic acid, acidify with 2 M pH 4.5 acetate buffer, add dichloromethane:EtOH 95:5, agitate for 3 min, centrifuge at 2000 g for 10 min, repeat extraction. Combine the organic phases, wash with saturated sodium bicarbonate solution, evaporate to dryness under a stream of air at 45°, reconstitute in MeOH, inject an aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm Lichrospher 100 RP-18
Column: 125 × 4 5 μm Lichrospher 100 RP-18
Mobile phase: MeOH:buffer 70:30 (Buffer was 200 mM Na_2HPO_4 and 100 mM citric acid, pH 3.2.)

Flow rate: 1
Detector: UV 284

CHROMATOGRAM
Internal standard: flufenamic acid
Limit of quantitation: 100 ng/mL (urine), 250 ng/mL (blood)

KEY WORDS
horse; pharmacokinetics

REFERENCE
Araújo,A.C.; Salvadori,M.C.; Velletri,M.E.; Camargo,M.M.A. Influence of furosemide on the detection of flunixin meglumine in horse urine samples, *J.Anal.Toxicol.*, **1990**, *14*, 146–148.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 50 μL 500 ng/mL mefenamic acid or indomethacin + 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°. Redissolve 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: UV 235

CHROMATOGRAM
Retention time: 2.8
Internal standard: mefenamic acid (8) or indomethacin (5)
Limit of detection: 50-250 ng/mL

OTHER SUBSTANCES
Simultaneous: naproxen, thiosalicylic acid,ethacrynic acid, phenylbutazone

KEY WORDS
plasma

REFERENCE
Singh,A.K.; Jang,Y.; Mishra,U.; Granley,K. Simultaneous analysis of flunixin, naproxen, ethacrynic acid, indomethacin, phenylbutazone, mefenamic acid and thiosalicyclic acid in plasma and urine by high-performance liquid chromatography and gas chromatography-mass spectrometry, *J.Chromatogr.*, **1991**, *568*, 351–361.

SAMPLE
Matrix: milk
Sample preparation: Condition a 3 mL C18 Bakerbond SPE cartridge with 5 mL ethyl acetate and 5 mL water. 5 mL Milk + 200 μL 4000 U/mL β-glucuronidase (type IX-A, Sigma) in 100 mM pH 6.8 phosphate buffer, heat at 37° for 2 h, cool, acidify to pH 3.0-3.5 with about 350-450 μL 1 M HCl, while vortexing gently, add 5.4-5.6 g 60-200 mesh silica gel (Baker No. 3405-1), mix thoroughly for 2 min, place in a 300 × 25 glass column on top off 0.5-1 g untreated silica gel, place 0.5-1 g untreated silica gel on the top of the column, wash with 50 mL water-saturated dichloromethane:hexane 30:70, elute with 50 mL ethyl acetate. Wash the eluate with 25 mL pH 3.5 HCl for 15 s, extract ethyl acetate layer twice with NaOH solution by shaking for 15 s. Combine the aqueous extracts and adjust the pH to 5.0-5.5 with 1 M HCl, add to the SPE cartridge, elute with 5 mL ethyl acetate. Evaporate the eluate to dryness under a stream of nitrogen at 50-55°, reconstitute the residue in 500 μL MeOH:buffer 50:50, sonicate briefly, filter (0.45 μm), inject a 100 μL aliquot of the filtrate. (NaOH solution was 15 mL 100 mM

NaOH and 1 mL 1 M NaOH. Buffer was 2.5 mL 1 M tetrabutylammonium dihydrogen phosphate and 10 mL 100 mM NaOH made up to 500 mL with water, pH 6.)

HPLC VARIABLES
Guard column: 20 × 4 5 μm Hypersil C18 ODS
Column: 200 × 4.6 5 μm Hypersil C18 ODS
Mobile phase: MeOH:buffer 58:42 (Buffer was 2.5 mL 1 M tetrabutylammonium dihydrogen phosphate and 10 mL 100 mM NaOH made up to 500 mL with water, pH 6.)
Column temperature: 45
Flow rate: 1
Injection volume: 100
Detector: UV 285

CHROMATOGRAM
Retention time: 5.7
Limit of quantitation: 1.7 ng/mL

KEY WORDS
cow; SPE

REFERENCE
Rupp,H.S.; Holland,D; Munns,R.K.; Turnipseed,S.B.; Long,A.R. Determination of flunixin in milk by liquid chromatography with confirmation by gas-chromatography/mass spectrometry and selected ion monitoring, *J.AOAC Int.*, **1995**, *78*, 959–967.

SAMPLE
Matrix: urine
Sample preparation: Condition a 3 mL Bond Elut Certify II (C8 plus strong anion exchanger) SPE cartridge with 3 mL MeOH and 3 mL water. Adjust pH of urine to 7 with 1 M NaOH or 1 M HCl, centrifuge at 500 g for 15 min. Add 3 mL of the supernatant to the SPE cartridge at 0.2 mL/min, wash with two 2.5 mL portions of water, wash with two 2 mL portions of MeOH, dry for 20 min under full vacuum, wash with two 2 mL portions of hexane, elute with two 2 mL portions of hexane:acetic acid 90:10, add 2 μg ketoprofen. Evaporate to dryness under a stream of nitrogen, reconstitute in 100 μL hexane:isopropanol 50:50, inject an aliquot.

HPLC VARIABLES
Column: 100 × 2 5 μm Hypersil SI
Mobile phase: Gradient. Hexane:isopropanol containing 5% water from 98:2 to 70:30 over 8 min, to 0:100 over 4 min, maintain at 0:100 for 1 min, re-equilibrate at initial conditions for 5 min.
Column temperature: 45
Flow rate: 0.4
Injection volume: 3
Detector: UV 280 or MS, Hewlett-Packard HP5989A quadrupole, HP 59980B PB interface at 65° with helium pressure of 275 kPa, 10 kV high energy dynode, source 275°, particle beam, EI, CI (methane)

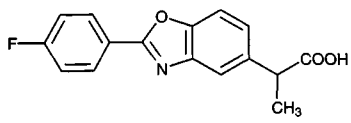
CHROMATOGRAM
Retention time: 4.53
Internal standard: ketoprofen (3.84)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: alclofenac, aminoantipyrine, bumadizone, clonixin, diclofenac, diflunisal, dipyrone, famprofazone, fenbufen, fenclofenac, fenoprofen, floctafenine, flufenamic acid, flurbiprofen, ibufenac, ibuprofen, indomethacin, indoprofen, isopyrin, isoxepac, ketorolac, meclofenamic acid, naproxen, nefopam, niflumic acid, oxaprozin, phenazone, phenazopyridine, phenylbutazone, piroxicam, propyphenazone, salicylamide, sulindac, suprofen, tenoxicam, tiaprofenic acid, tolmetin, zomepirac

KEY WORDS
horse; SPE; normal phase

REFERENCE
Stanley,S.M.; Owens,N.A.; Rodgers,J.P. Detection of flunixin in equine urine using high-performance liquid chromatography with particle beam and atmospheric pressure ionization mass spectrometry after solid-phase extraction, *J.Chromatogr.B*, **1995**, *667*, 95–103.

Flunoxaprofen



Molecular formula: $C_{16}H_{12}FNO_3$
Molecular weight: 285.27
CAS Registry No.: 66934-18-7
Merck Index: 4183

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

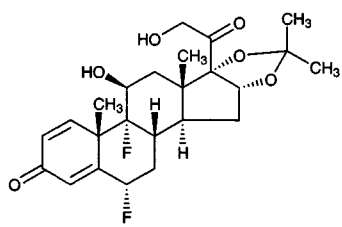
CHROMATOGRAM
Retention time: 21.2 (R), 23.8 (S)
Internal standard: flunoxaprofen

OTHER SUBSTANCES
Extracted: ketoprofen, fenoprofen

KEY WORDS
plasma; chiral; flunoxaprofen is IS; derivatization

REFERENCE
Volland,C.; Sun,H.; Benet,L.Z. Stereoselective analysis of fenoprofen and its metabolites, *J.Chromatogr.*, **1990**, *534*, 127–138.

Fluocinolone acetonide



Molecular formula: $C_{24}H_{30}F_2O_6$
Molecular weight: 452.20
CAS Registry No.: 67-73-2
Merck Index: 4185
Lednicer No.: 1 202; 3 94

SAMPLE
Matrix: blood

Sample preparation: 100 μL Plasma + 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 μL MeCN, add 10 μL reagent 1, add 10 μL reagent 2, heat at 70° for 20 min, cool to room temperature, add 100 μL water, add 200 μL MeOH:water 1:1, 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 μL by evaporation under reduced pressure below 40°, inject 20 μL aliquot. (Reagent 1 was 30 mg 2-(4-carboxyphenyl)-5,6-dimethylbenzimidazole in 3 mL pyridine, add 700 mg 4-piperidinopyridine, dilute to 10 mL with MeCN. Reagent 2 was 700 mg 1-isopropyl-3-(3-dimethylaminopropyl)carbodiimide perchlorate in 10 mL MeCN. Prepare 2-(4-carboxyphenyl)-5,6-dimethylbenzimidazole as follows. Add 13 g 4-carboxybenzaldehyde (terephthalaldehydic acid) in 400 mL EtOH dropwise to 4,5-dimethyl-1,2-phenylenediamine in 400 mL EtOH in an ice bath, after 1 h reflux for 8 h, cool to room temperature, collect the precipitate, recrystallize three times from MeOH:water 50:50 to give 2-(4-carboxyphenyl)-5,6-dimethylbenzimidazole as a white amorphous product (mp >300°) (J.Chromatogr. 1991, 585, 219). 4-Piperidinopyridine is not commercially available but 4-dimethylaminopyridine or 4-pyrrolidinopyridine can be used instead although interferences are greater (J. Chromatogr. 1991, 585, 219). Alternatively 4-piperidinopyridine can be synthesized as follows. Add 200 mmoles piperidine dropwise with stirring to 15 g phosphorus pentoxide and 9.51 g 4-hydroxypyridine, heat at 250° for 7 h, cautiously pour onto 200 g ice, add 400 mL 1 M NaOH, add 200 mL ether. Remove the ether layer and extract the aqueous layer three times with 100 mL portions of ether. Combine the organic layers and dry them over anhydrous potassium carbonate, evaporate, distil the residue, recrystallize from petroleum ether (bp 80-100°) to give 4-piperidinopyridine (bp 167-170°/11 mm Hg; mp 79-80°) (Synthesis 1978, 844). Alternatively, add 1.94 g 4-bromopyridine hydrochloride to 5 mL 50% NaOH, add 5 mL piperidine, add 2.72 g benzyltriethylammonium bromide, heat at 100° for 5 h, remove excess piperidine by distillation, add 25 mL water, extract four times with 25 mL portions of benzene. Combine the organic layers and dry them over anhydrous sodium sulfate, boil the residue with petroleum ether to give 4-piperidinopyridine (mp 80°) (Syn. Commun. 1979, 9, 251). Prepare 1-isopropyl-3-(3-dimethylaminopropyl) carbodiimide perchlorate as follows. Stir 1.41 moles isopropylisocyanate in 750 mL dichloromethane at 5°, add 144 g 3-dimethylaminopropylamine (N,N-dimethyl-1,3-propanediamine) in 250 mL dichloromethane at such a rate that the temperature does not exceed 10°, add 500 mL triethylamine, add 300 g p-toluenesulfonyl chloride in 300 mL dichloromethane at such a rate that the temperature does not exceed 10°, reflux for 3 h, add 400 g anhydrous sodium carbonate, add 3.5 L ice water, stir vigorously for 30 min, remove the organic phase. Extract the aqueous phase three times with 500 mL portions of dichloromethane. Combine the organic layers and dry them over anhydrous sodium sulfate, evaporate under reduced pressure, distil the residue to give 1-isopropyl-3-(3-dimethylaminopropyl)carbodiimide (bp 91-92°/10 mm Hg (Ber. 1941, 74B, 1285)) (cf. Org. Syn. 1973, Coll. Vol. V, 555). Prepare pyridine perchlorate from pyridine and 20% perchloric acid, crystallize from EtOH (Ber. 1926, 59, 446). Add 18 g pyridine perchlorate in portions to 100 mmoles 1-isopropyl-3-(3-dimethylaminopropyl)carbodiimide stirred in 200 mL dichloromethane at 0°, let stand for 30 min, filter, add 200 mL anhydrous diethyl ether to the filtrate. Filter off the precipitate and recrystallize it from dichloromethane/diethyl ether to give 1-isopropyl-3-(3-dimethylaminopropyl)carbodiimide perchlorate (mp 88-90°) (Chem. Pharm. Bull. 1985, 33, 5375).)

HPLC VARIABLES
Guard column: 50 × 4.6 7 μm Zorbax ODS
Column: 250 × 4.6 7 μm Zorbax ODS
Mobile phase: MeOH:water 75:25 containing 5 mM tetramethylammonium hydrogen sulfate
Flow rate: 0.4
Injection volume: 20
Detector: F ex 334 em 418

CHROMATOGRAM
Retention time: 40.7
Internal standard: fluocinolone acetonide
Limit of detection: 0.6 pg/mL

OTHER SUBSTANCES
Extracted: triamcinolone, triamcinolone acetonide
Simultaneous: aldosterone, cortisone, hydrocortisone, corticosterone, fluocinolone acetonide, triamcinolone, triamcinolone acetonide, dexamethasone

KEY WORDS
plasma; derivatization; fluocinolone acetonide is IS

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: 1 mL Serum + 100 μL water containing 5 μg/mL 2,3-diaminonaphthalene and 3.5 μg/mL 18-hydroxy-11-deoxycorticosterone + 1 mL 250 mM NaOH + 7 mL diethyl ether, shake on a rotary shaker for 15 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 30-40°, reconstitute the residue in 70 μL MeOH:100 mM perchloric acid 50:50, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A was 58 mM NaH_2PO_4 containing 6 mM sodium heptanesulfonate, adjusted to pH 3.1 with concentrated phosphoric acid. B was MeCN:MeOH 85:15. A:B from 100:0 to 78:22 over 5 min, to 70:30 over 12 min, maintain at 70:30 for 4 min, to 65:35 over 9 min.
Flow rate: 1
Injection volume: 20
Detector: UV 245, 256, 343

CHROMATOGRAM
Retention time: 24.75 (fluocinolone acetonide)
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, fluendrenolide, fluorometholone, fluprednisolone, hydrocortisone, hydroxychloroquine, 17δ-hydroxyprogesterone, meprednisone, methylprednisolone, methylprednisolone acetate, paramethasone, prednisolone, prednisone, progesterone, triamcinolone
Noninterfering: aspirin, ibuprofen, indomethacin, phenylbutazone, pregnenolone

KEY WORDS
serum

REFERENCE
Volin,P. Simple and specific reversed-phase liquid chromatographic method with diode-array detection for simultaneous determination of serum hydroxychloroquine, chloroquine and some corticosteroids, *J.Chromatogr.B*, **1995**, *666*, 347–353.

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: 8 (fluocinolone acetonide)
Internal standard: norethindrone (12)

OTHER SUBSTANCES
Simultaneous: procinonide, ciprocinonide, fluocinonide

KEY WORDS
creams; column-switching

REFERENCE
Conley,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: 11.25 g Cream + 100 mL cyclohexane + 50 mL MeOH, shake vigorously for 3 min, let stand for 15 min. Remove the lower layer and add it to 140 mL water and 100 mL chloroform, shake for 3 min, allow phases to separate. Remove a 3 mL aliquot of the chloroform layer and add it to 300 μL 0.028% hydrocortisone in 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, 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°).)

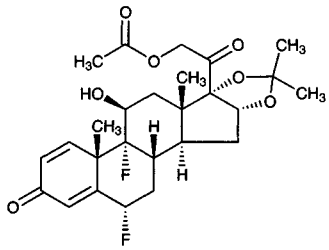
HPLC VARIABLES
Column: 500 × 1 10 μm silica
Mobile phase: Toluene:dioxane 90:10 (Caution! Dioxane is a carcinogen!)
Detector: F ex 230 em 350

CHROMATOGRAM
Internal standard: hydrocortisone

KEY WORDS
derivatization; cream; normal phase; for fluocinolone acetonide

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.

Fluocinonide



Molecular formula: $C_{26}H_{32}F_2O_7$
Molecular weight: 494.53
CAS Registry No.: 356-12-7
Merck Index: 4186

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: 18
Internal standard: norethindrone (12)

OTHER SUBSTANCES

Simultaneous: procinonide, ciprocinonide, fluocinolone acetonide

KEY WORDS

creams; column-switching

REFERENCE

Conley,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: Dissolve in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 10 μm Spherisorb ODS
Mobile phase: MeCN:water:acetic acid 35:64:1
Injection volume: 100
Detector: UV 240

CHROMATOGRAM

Internal standard: fluocinonide

OTHER SUBSTANCES

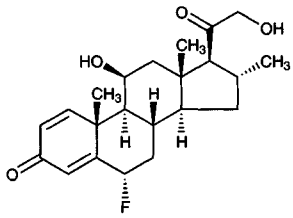
Simultaneous: flunisolide

KEY WORDS

nasal spray; fluocinonide is IS

REFERENCE
Yu,C.D.; Jones,R.E.; Henesian,M. Cascade impactor method for the droplet size characterization of a metered-dose nasal spray, *J.Pharm.Sci.*, **1984**, *73*, 344–348.

Fluocortolone



Molecular formula: $C_{22}H_{29}FO_4$
Molecular weight: 376.47
CAS Registry No.: 152-97-6, 303-40-2 (21-hexanoate)
Merck Index: 4188

SAMPLE
Matrix: blood
Sample preparation: Add 150 µL MeOH to 1 mL plasma. Add 500 µL 100 mM NaOH and 2 mL dichloromethane, shake for 10 min, centrifuge at 2500 g for 10 min, evaporate a 1.9 mL aliquot of the supernatant under a stream of nitrogen at 45°. Reconstitute the residue in 50 µL MeOH, inject 17 µL aliquot.

HPLC VARIABLES
Guard column: 10 × 4 5 µm LiChrospher RP 18
Column: 250 × 4 5 µm Lichrospher RP 18
Mobile phase: MeOH:THF:water 110:2.5:100
Flow rate: 1
Injection volume: 17
Detector: UV 252

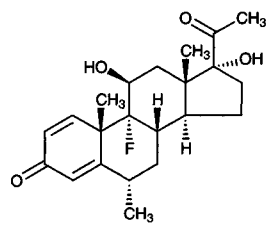
CHROMATOGRAM
Internal standard: fluocortolone

OTHER SUBSTANCES
Extracted: hydrocortisone, triamcinolone

KEY WORDS
plasma

REFERENCE
Doppenschmitt,S.A.; Scheidel,B.; Harrison,F.; Surmann,J.P. Simultaneous determination of triamcinolone acetonide and hydrocortisone in human plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *682*, 79–88.

Fluorometholone



Molecular formula: $C_{22}H_{29}FO_4$
Molecular weight: 376.47
CAS Registry No.: 426-13-1, 3801-06-7 (acetate)
Merck Index: 4213
Lednicer No.: 1 203

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 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 μL mobile phase, add 2 mL hexane, vortex for 1 min, centrifuge at 1000 g for 5 min, discard the hexane layer, inject a 100 μL aliquot of the mobile phase layer.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm Spheri-5 RP-18
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:water:glacial acetic acid 33:62:5
Flow rate: 1
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 14.55
Internal standard: fluorometholone

OTHER SUBSTANCES
Extracted: methylprednisolone, methylprednisolone acetate

KEY WORDS
plasma; fluorometholone is IS

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: 2 mL Plasma + 5 mL hexane, shake horizontally at 60 cycles/min for 10 min, centrifuge at 1000 g for 5 min. Remove the aqueous layer and add it to 8 mL dichloromethane, shake horizontally at 60 cycles/min for 10 min, centrifuge at 1000 g for 5 min, repeat the extraction. Combine the organic layers and add them to 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 μL mobile phase, add 2 mL hexane, vortex for 1 min, centrifuge at 1000 g for 5 min, inject a 100 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm Brownlee guard column
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:water:glacial acetic acid 33:62:5
Flow rate: 1
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 14.6
Internal standard: fluorometholone

OTHER SUBSTANCES
Extracted: methylprednisolone, methylprednisolone acetate

KEY WORDS
plasma; fluorometholone is IS

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 Serum + 100 μL water containing 5 μg/mL 2,3-diaminonaphthalene and 3.5 μg/mL 18-hydroxy-11-deoxycorticosterone + 1 mL 250 mM NaOH + 7 mL diethyl ether, shake on a rotary shaker for 15 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 30-40°, reconstitute the residue in 70 μL MeOH:100 mM perchloric acid 50:50, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18

Mobile phase: Gradient. A was 58 mM NaH_2PO_4 containing 6 mM sodium heptanesulfonate, adjusted to pH 3.1 with concentrated phosphoric acid. B was MeCN:MeOH 85:15. A:B from 100:0 to 78:22 over 5 min, to 70:30 over 12 min, maintain at 70:30 for 4 min, to 65:35 over 9 min.

Flow rate: 1

Injection volume: 20

Detector: UV 245, 256, 343

CHROMATOGRAM

Retention time: 26.15

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, fluendrenolide, fluocinolone acetonide, fluprednisolone, hydrocortisone, hydroxychloroquine, 17δ-hydroxyprogesterone, meprednisone, methylprednisolone, methylprednisolone acetate, paramethasone, prednisolone, prednisone, progesterone, triamcinolone

Noninterfering: aspirin, ibuprofen, indomethacin, phenylbutazone, pregnenolone

KEY WORDS

serum

REFERENCE

Volin,P. Simple and specific reversed-phase liquid chromatographic method with diode-array detection for simultaneous determination of serum hydroxychloroquine, chloroquine and some corticosteroids, *J.Chromatogr.B*, **1995**, *666*, 347–353.

SAMPLE

Matrix: solutions

Sample preparation: Dilute in an appropriate solvent, inject an aliquot.

HPLC VARIABLES

Guard column: RC18 Guardpak (Waters)

Column: 250 × 4.5 μBondapak C18

Mobile phase: MeCN:water 40:60

Flow rate: 1.5

Detector: UV 246

CHROMATOGRAM

Retention time: 6.8

OTHER SUBSTANCES

Simultaneous: 20-α-dihydrofluorometholone, prednisolone

Interfering: prednisolone acetate

REFERENCE

Richman,J.B.; Tang-Liu,D.D.-S. A corneal perfusion device for estimating ocular bioavailability in vitro, *J.Pharm.Sci.*, **1990**, *79*, 153–157.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.5 5 μm 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

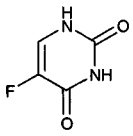
CHROMATOGRAM
Retention time: 3.19
Internal standard: naproxen (3.89)

OTHER SUBSTANCES
Simultaneous: bacitracin, cortisone acetate, diazepam, diclofenac, flurbiprofen, hydrocortisone acetate, imipramine, indomethacin, ketoprofen, ketorolac tromethamine, levobunolol, meclofenamic acid, 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.

Fluorouracil



Molecular formula: $C_4H_3FN_2O_2$
Molecular weight: 130.08
CAS Registry No.: 51-21-8
Merck Index: 4219
Lednicer No.: 3 155

SAMPLE
Matrix: blood
Sample preparation: Mix 150 μL plasma with 150 μL 10% trichloroacetic acid in water, vortex for 30 s, centrifuge at 5000 g for 5 min, inject a 50 μL aliquot of the clear supernatant.

HPLC VARIABLES
Guard column: 9 μm Aminex HPX-87H
Column: 300 × 7.8 9 μm Aminex HPX-87H
Mobile phase: 5 mM sulfuric acid
Column temperature: 60
Flow rate: 0.5
Injection volume: 50
Detector: UV 265

CHROMATOGRAM
Retention time: 24
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Simultaneous: cisplatin, 5-fluoro-2'-deoxyuridine-5'-monophosphate, 5-fluoro-2'-deoxyuridine, 5-fluorouridine, thymidine, uric acid, uridine
Noninterfering: alizapride, cytosine, hypoxanthine, methylprednisolone, methotrexate, metoclopramide, xanthine
Interfering: uracil

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Compagnon,P.; Thiberville,L.; Moore,N.; Thuillez,C.; Lacroix,C. Simple high-performance liquid chromatographic method for the quantitation of 5-fluorouracil in human plasma, *J.Chromatogr.B*, **1996**, *677*, 380–383.

SAMPLE
Matrix: blood
Sample preparation: Mix 500 μL plasma with 50 μL 5 μg/mL 5-chlorouracil, add to an unactivated C18 Chem Elut SPE cartridge (Varian). Elute with four 2 mL portions of MeOH:ethyl acetate 5:95. Flush the SPE cartridge with nitrogen at 1 mL/min. Evaporate collected eluate to dryness under a stream of nitrogen at 40° for 30 min. Reconstitute the residue in 200 μL water, sonicate for 10 min, filter (0.45 μm HV4 membrane, Millipore) Inject a 20 μL aliquot; SPE

HPLC VARIABLES
Column: 150 × 4.6 5 μm Kromasil C18 (Touzart and Matignon, France)
Mobile phase: MeOH:water 3:97
Flow rate: 0.6
Injection volume: 20
Detector: UV 268

CHROMATOGRAM
Retention time: 5.8
Internal standard: 5-chlorouracil (10.7), 5-iodouracil (22.1)
Limit of detection: 10 ng/mL
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites (UV 275)

KEY WORDS
plasma; SPE

REFERENCE
Joulia,J.M.; Pinguet,F.; Grosse,P.Y.; Astre,C.; Bressolle,F. Determination of 5-fluorouracil and its main metabolites in plasma by high-performance liquid chromatography: Application to a pharmacokinetic study, *J.Chromatogr.B*, **1997**, *692*, 427–435.

SAMPLE
Matrix: blood
Sample preparation: Mix 200 μL plasma with 50 μL 500 mM pH 8.0 phosphate buffer, 100 μL 500 ng/mL 5-chlorouracil in water, and 8 mL ethyl acetate. Shake for 30 min and centrifuge at 2200 g for 10 min. Remove the ethyl acetate layer and evaporate it. Reconstitute the residue in 500 μL mobile phase with sonication for 10 min. Inject a 150 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 Develosil 60-3 (Nomura Chemical, Japan)
Mobile phase: n-Hexane:ethyl acetate:formic acid:water 50:0.5:0.5:0.3
Flow rate: 0.9 for 20 min, 0.4 for 27 min, 0.9 for 8 min
Injection volume: 150
Detector: UV 264

CHROMATOGRAM
Internal standard: 5-chlorouracil
Limit of detection: 3 ng/mL

KEY WORDS
plasma; rat

REFERENCE
Fuse,E.; Takai,K.; Okuno,K.; Kobayashi,S. Hepatic extraction ratio of 5-fluorouracil in rats. Dose dependence and effect of uracil and interleukin-2, *Biochem.Pharmacol.*, **1996**, *52*, 561–568.

SAMPLE
Matrix: blood
Sample preparation: Add 50 μL 1 M pH 4.8 sodium acetate buffer to 1 mL plasma (to adjust pH to 6.0), add 250 μL 200 mg/mL saturated sodium sulfate solution, vortex, add 7 mL water-saturated ethyl acetate, vortex vigorously for 90 s, centrifuge for 5 min. Add 1 mL 50 mM pH 11 phosphate buffer to a 6 mL aliquot of the organic layer, vortex, centrifuge. Aspirate the organic layer to waste, flush the residual solvent from the aqueous portion with nitrogen for about 10 min, add 10 μL 1 M sulfuric acid (to adjust the pH to neutral), inject a 125 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm YMC ODS-AQ C18
Mobile phase: Gradient. A was MeOH:10 mM pH 5.5 potassium phosphate buffer 50:50. B was 10 mM pH 5.5 potassium phosphate buffer. A:B 0:100 for 5 min, from 0:100 to 50:50 in 1 min, maintain at 50:50 for 3 min, from 50:50 to 0:100 in 1 min, maintain at 0:100 for 10 min
Column temperature: 40
Flow rate: 1.2
Detector: UV 266

CHROMATOGRAM
Retention time: 6.8-7.3
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Extracted: uracil, 5-chlorouracil

KEY WORDS
plasma

REFERENCE
Coe,R.A.; Earl,R.A.; Johnson,E.T.C.; Lee,J.W. Determination of 5-fluorouracil in human plasma by a simple and sensitive reversed-phase HPLC method, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 1733–1741.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 500 μL physiological saline + 100 μL 500 mM NaH_2PO_4 + 8 mL ethyl acetate, extract, centrifuge. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 1 mL 500 μg/mL 4-bromomethyl-7-methoxycoumarin in acetone:MeCN 1:2 containing 100 μg/mL 18-crown-6 and 1 mg/mL potassium carbonate, reflux in the dark for 45 min, cool, add valeric acid, reflux for 5 min, dilute with acetone, inject an aliquot.

HPLC VARIABLES
Column: 200 × 4 5 μm Nucleosil 5 C18
Mobile phase: MeOH:water 70:30
Flow rate: 0.8
Detector: F ex 346 em 395

CHROMATOGRAM
Retention time: 7
Internal standard: valeric acid (8)

Limit of detection: 100 fmole

OTHER SUBSTANCES
Extracted: tegafur

KEY WORDS
derivatization; serum

REFERENCE
Iwamoto,M.; Yoshida,S.; Hirose,S. Fluorescence determination of 5-fluorouracil and 1-(tetrahydro-2-furanyl)-5-fluorouracil in blood serum by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *310*, 151–157.

SAMPLE
Matrix: blood

Sample preparation: 100 μL Serum + 5 μL 42 μM 5-chlorouracil in acetone, mix, let stand at room temperature for 3 min, add 500 μL 100 mM pH 3.5 potassium phosphate buffer, add 2 mL ethyl acetate, vortex for 2 min, centrifuge at 1000 g for 5 min. Remove a 1.4 mL aliquot of the organic layer and evaporate it to dryness under reduced pressure. Add 20 mg solid potassium bicarbonate:anhydrous sodium sulfate 1:7 to the residue, add 50 μL 1.3 mM 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone in acetone, add 50 μL 1.5 mM 18-crown-6 in acetone, heat at 50° for 20 min, cool, inject a 10 μL aliquot. (Silanize all glassware. Synthesize 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone as follows. Stir 483 g veratrole in 1.45 L acetic acid at 15° for 1 h, add 683 g concentrated nitric acid (d 1.05) over 1 h (maintain the temperature below 40° by cooling and regulating the rate of addition of the nitric acid). Continue stirring and add 2.127 L fuming nitric acid (d 1.50) over 1 h while maintaining the temperature below 30°, let stand for 2 h, pour into a large volume of cold water, filter, wash the solid with water until the washings are neutral, recrystallize from EtOH to give 4,5-dinitroveratrole (mp 129.5-130.5°) (J. Am. Chem. Soc. 1946, 68, 1536). Reflux 5 g 4,5-dinitroveratrole in 200 mL benzene (Caution! Benzene is a carcinogen!), add 100 g 60 mesh iron powder and 20 mL concentrated HCl in small portions over 1 h, reflux for 4 h, add 10 mL water, reflux for 2 h, cool, make alkaline with 2.5 M NaOH, extract several times with 200 mL portions of benzene. Combine the organic layers and evaporate them to dryness, add 10 mL concentrated HCl, recrystallize from EtOH to give 1,2-diamino-4,5-dimethoxybenzene monohydrochloride as very slightly pink needles (mp 240°) (Anal. Chim. Acta 1982, 134, 39). Heat 2.5 mmoles 1,2-diamino-4,5-dimethoxybenzene hydrochloride and 2.4 mmoles pyruvic acid in 30 mL 500 mM HCl on a boiling water bath for 2 h, cool with ice-water, filter. Wash the precipitate with water and dry it under vacuum, recrystallize from MeOH:water 90:10 to give 6,7-dimethoxy-3-methyl-2(1H)-quinoxalinone as yellow needles (mp 255°) (Chem. Pharm. Bull. 1985, 33, 3493). Treat 1 g 6,7-dimethoxy-3-methyl-2(1H)-quinoxalinone dissolved in 50 mL anhydrous MeOH with a solution of diazomethane in ether, evaporate to dryness under reduced pressure, dissolve the residue in 5 mL ethyl acetate, chromatograph on a 250 × 35 column filled with 130 g 70-230 mesh silica gel 60 (Merck) using n-hexane:ethyl acetate 25:75 to give 6,7-dimethoxy-1,3-dimethyl-2(1H)-quinoxalinone as yellow needles (mp 170-171°). Dissolve 350 mg 6,7-dimethoxy-1,3-dimethyl-2(1H)-quinoxalinone in 3 mL acetic acid, add 350 mg anhydrous sodium acetate, add 2 mL 1.5 M bromine in acetic acid, heat at 100° for 15 min, cool, add 10 mL ether, filter, wash the solid 2 or 3 times with small portions of ether. Combine the filtrate and washings and evaporate them to dryness, dissolve the residue in 5 mL ethyl acetate, chromatograph on a 250 × 35 column filled with 130 g 70-230 mesh silica gel 60 (Merck) using ether, evaporate the main fraction to dryness, recrystallize the residue from n-hexane: ethyl acetate 50:50 to give 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone as yellow needles (mp 161-163°) (J. Chromatogr. 1985, 346, 227). 3-Bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone is also available from Dojindo Molecular Technologies, Inc., 3 Bethesda Metro Center, Suite 700, Bethesda MD 20814; (301) 664-8448; www.dojindo.co.jp.)

HPLC VARIABLES
Column: 100 × 8 10 μm Radial Pak C18 (Waters) (Wash with MeOH at 2 mL/min for 20 min at the end of each day.)

Mobile phase: Gradient. MeOH:water 35:65 for 15 min, 50:50 for 25 min (step gradient), re-equilibrate at initial conditions for 20 min.

Flow rate: 1.5

Injection volume: 10

Detector: F ex 370 em 455

CHROMATOGRAM
Retention time: 28.1
Internal standard: 5-chlorouracil (32.5)
Limit of detection: 12.5 ng/mL

OTHER SUBSTANCES
Extracted: floxuridine

KEY WORDS
derivatization; serum; pharmacokinetics

REFERENCE
Yamaguchi,M.; Nakamura,M.; Kuroda,N.; Ohkura,Y. Determination of 5-fluorouracil and 5-fluoro-2'-deoxyuridine in human serum by high-performance liquid chromatography with fluorescence detection, *Anal.Sci.*, **1987**, *3*, 75–79.

SAMPLE
Matrix: blood
Sample preparation: Add 250 μL serum to a 20 × 7 DEAE-Cellulofine AM anion-exchange column (Seikagaku Tokyo), elute with 3.5 mL 1 mM HCl, discard the first 0.5 mL eluate, collect the next 3 mL eluate. Evaporate the eluate to 0.5 mL under reduced pressure, add 15 mL ethyl acetate, shake, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 800 μL anhydrous acetone, add 100 μL 750 μg/mL 4-bromomethyl-6,7-dimethoxycoumarin in acetone, add 100 μL 250 μg/mL 18-crown-6 in acetone, add 1.5 mg anhydrous potassium carbonate, heat at 70° for 15 min (protect from atmospheric moisture with a calcium chloride drying tube), cool, inject an aliquot

HPLC VARIABLES
Column: 200 × 4 5 μm Nucleosil 5 C18
Mobile phase: MeOH:water 60:40
Flow rate: 0.8
Detector: F ex 340 em 420

CHROMATOGRAM
Retention time: 10
Limit of quantitation: 60 ng/mL

OTHER SUBSTANCES
Extracted: floxuridine, ftorafur

KEY WORDS
derivatization; serum; protect from light

REFERENCE
Yoshida,S.; Adachi,T.; Hirose,S. 4-Bromomethyl-6,7-dimethoxycoumarin as a fluorescence reagent for precolumn derivatization of 5-fluorouracil compounds in high-performance liquid chromatography, *J.Chromatogr.*, **1988**, *430*, 156–162.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL LC-SCX Supelclean strong cation-exchange SPE cartridge (Supelco) with 2 mL MeOH, 1 mL 100 mM copper(II) sulfate solution, and 3 mL 50 mM pH 7 phosphate buffer, do not allow to dry. 300 μL Serum + 5-bromouracil, add to the SPE cartridge, wash with 2 mL 50 mM pH 7 phosphate buffer, wash with 2 mL MeOH, elute with 700 μL 1.7 M ammonia solution, add 70 μL glacial acetic acid to the eluate, mix thoroughly, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelguard LC-18-S (Supelco)
Column: 250 × 4.6 5 μm Supelcosil LC-18-S ODS

Mobile phase: Gradient. A was MeOH:50 mM pH 6.5 phosphate buffer 60:40. B was 50 mM pH 6.5 phosphate buffer.
Flow rate: 1
Injection volume: 20
Detector: UV 269

CHROMATOGRAM
Retention time: 6.5
Internal standard: 5-bromouracil (12)
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Extracted: doxifluridine, floxuridine, 5-fluorouridine monophosphate, metabolites

KEY WORDS
serum; SPE

REFERENCE
Guerrieri,A.; Palmisano,F.; Zambonin,P.G.; De Lena,M.; Lorusso,V. Solid-phase extraction of fluoropyrimidine derivatives on a copper-modified strong cation exchanger: determination of doxifluridine, 5-fluorouracil and its main metabolites in serum by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1993**, *617*, 71–77.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 100 μg/mL niacinamide, add 2 mL MeCN dropwise, centrifuge at 3000 g for 30 min. Remove the supernatant and evaporate it to dryness, reconstitute the residue in 1 mL saturated ammonium sulfate, add 5 mL diethyl ether:isopropanol 80:20, shake for 15 min, centrifuge at 3000 g for 15 min, repeat the extraction. Combine the organic phases and evaporate them to dryness, reconstitute the residue in 1 mL mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: 5 μm LiChrosorb RP-18
Column: 250 × 4 5 μm LiChrosorb RP-18
Mobile phase: pH 7.0 phosphate buffer (μ = 0.05)
Flow rate: 1
Injection volume: 100
Detector: UV 265

CHROMATOGRAM
Retention time: 5
Internal standard: niacinamide
Limit of quantitation: 690 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Vandenbosch,C.; van Belle,S.; de Smet,M.; Taton,G.; Bruynseels,V.; Vandenhoven,G.; Massart,D.L. Determination of leucovorin and 5-fluorouracil in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 77–85.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL 20 μg/mL 5-bromouracil + 8 mL ethyl acetate, shake for 20 min, centrifuge at 2500 rpm for 20 min. Remove 7 mL of the organic layer and evaporate it to dryness under nitrogen or at 60°. Dissolve residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 Shimpack CLS-ODS (Shimadzu)

Mobile phase: 0.5 mM phosphoric acid
Column temperature: 40
Flow rate: 1.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Internal standard: 5-bromouracil

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 μL Plasma + 50 μL 15 μg/mL 5-bromouracil + 1 mL 0.365% HCl + 8 mL ethyl acetate, shake for 10 min, centrifuge at 1006 g for 10 min. Remove 7 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 Shimpack CLS-ODS
Mobile phase: Water:phosphoric acid 100:0.1
Column temperature: 40
Flow rate: 1.7
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Internal standard: 5-bromouracil

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Umejima,H.; Kikuchi,A.; Kim,N.-S.; Uchida,T.; Goto,S. Preparation and evaluation of Eudragit gels. VIII. Rectal absorption of 5-fluorouracil from Eudispert hv gels in rats, *J.Pharm.Sci.*, **1995**, *84*, 199–202.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 50 μL 50 μg/mL stavudine + 1 mL ice-cold MeCN, mix vigorously for 30 s, centrifuge at 9000 g for 7 min. Remove the supernatant and add it to excess crystalline magnesium sulfate, mix for 2 min, centrifuge at 9000 g for 10 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 200 μL mobile phase, inject a 15-150 μL aliquot.

HPLC VARIABLES
Guard column: 25 × 2.3 PRP-1 (Hamilton)
Column: 250 × 4.1 10 μm PRP-1 (Hamilton)
Mobile phase: MeCN:5 mM pH 11.1 tetrabutylammonium hydroxide 16:84
Flow rate: 1.5
Injection volume: 15-150
Detector: UV 254

CHROMATOGRAM
Retention time: 4.6
Internal standard: stavudine (5.5)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: 4-deoxy-5-fluorouracil (UV 313), tegafur

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Jarugula,V.R.; Boudinot,F.D. High-performance liquid chromatographic determination of 5-fluorouracil and its prodrugs, tegafur and 4-deoxy-5-fluorouracil, in rat plasma, *J.Chromatogr.B*, **1996**, *677*, 199–203.

SAMPLE
Matrix: blood, peritoneal fluid
Sample preparation: Plasma. 1 mL Plasma + 10 μL 100 μM bromouridine + 70 μL perchloric acid, mix thoroughly, let stand at 4° for at least 12 h, centrifuge for 5 min. Remove the supernatant and adjust the pH to 7 with 5 M KOH, let stand on ice for 2 h, inject a 20 μL aliquot. Peritoneal fluid. 1 mL Peritoneal fluid + 10 μL 100 μM bromouridine, dilute 1:100 with water, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 12.5 × 4.6 5 μm Zorbax RX
Column: 250 × 4.6 5 μm Zorbax RX
Mobile phase: Gradient. A was 25 mL pH 2.5 ammonium phosphate. B was MeCN:25 mM pH 7.5 ammonium phosphate 7:93. A:B 100:0 for 5 min, to 0:100 over 10 min, maintain at 0:100 for 10 min, return to initial conditions over 1 min, re-equilibrate for 20 min.
Column temperature: 20
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 6-7
Internal standard: bromouridine (18)
Limit of detection: 2.5 nM

OTHER SUBSTANCES
Extracted: floxuridine

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Smith-Rogers,J.A.; Tong,W.P.; Duafala,M.E.; Markman,M.; Bertino,J.R. High-performance liquid chromatographic method for the simultaneous measurement of floxuridine and fluorouracil in human body fluids, *J.Chromatogr.*, **1991**, *566*, 147–154.

SAMPLE
Matrix: blood, tissue
Sample preparation: Add 100 μL 10% perchloric acid and 20 μL 200 μg/mL IS to 100 μL serum or homogenized tissue. Shake for 2 min and centrifuge at 2000 g for 10 min. Filter (45 μm) supernatant, inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 45 × 4.6 5 μm ODS Hypersil (VDS Optilab)
Column: 250 × 4.6 5 μm ODS Hypersil (VDS Optilab)
Mobile phase: MeOH:99% acetic acid:water 3:0.5:96.95
Column temperature: 30
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 4.94
Internal standard: 5-bromouracil (10.34)
Limit of quantitation: 100 ng/mL (serum); 300-500 ng/mL (tissue)

OTHER SUBSTANCES
Extracted: metabolites, floxuridine

KEY WORDS
serum; rat; liver; tumor; kidney; spleen; peritoneum; gastric mucosa; lung; heart; pancreas

REFERENCE
Jung,M.; Berger,G.; Pohlen,U.; Päuser,S.; Reszka,R.; Buhr,H.J. Simultaneous determination of 5-fluorouracil and its active metabolites in serum and tissue by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *702*, 193–202.

SAMPLE
Matrix: blood, tissue
Sample preparation: 0.5 g Tissue or 1 mL plasma + 5 μL 1 M sulfuric acid (lung and heart only) + 2 μL 1 M sulfuric acid (plasma only) + 500 μL 200 mg/mL sodium sulfate (liver and kidney only) + 50 μL 1 M pH 6 sodium acetate (liver only) + 50 μL 1 M pH 5 sodium acetate (kidney only) + 100 μL 2% trichloroacetic acid (lung and heart only) + 15 mL n-propanol:ether (liver 16:84, kidney 20:80, lung 88:12, heart 40:60, plasma 88:12), sonicate for 30 s, shake for 15 min, centrifuge for 15 min. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 1 mL 50 mM ammonium phosphate (liver pH 11, kidney pH 3, lung pH 2.5, heart pH 5, plasma pH 2.5), inject a 20 μL aliquot. (From J. Liq.Chromatogr. 1994, 17, 1621.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb 5 ODS 2
Mobile phase: MeCN:50 mM phosphate buffer 0.5:99.5 (Liver pH 3, kidney pH 6, lung pH 5, heart pH 5, plasma pH 2.5)
Column temperature: 10 (kidney), 35 (lung), 20 (heart), 25 (plasma), 15 (liver)
Flow rate: 1
Injection volume: 20
Detector: UV 200

CHROMATOGRAM
Retention time: 5 (plasma), 7 (liver), 7.5 (kidney), 8 (lung), 9 (heart)
Internal standard: flucytosine (for plasma) (4), 4-chlorouracil (for tissue) (18 (liver), 11 (kidney), 17 (lung), 19 (heart))
Limit of quantitation: 300 ng/g plasma, 800 ng/g (heart), 100 ng/g (lung), 240 ng/g (kidney), 570 ng/g (liver)

OTHER SUBSTANCES
Extracted: metabolites, floxuridine

KEY WORDS
plasma; rabbit; liver; kidney; lung; heart; pharmacokinetics

REFERENCE
Del Nozal,M.J.; Bernal,J.L.; Pampliega,A.; Marinero,P.; Pozuelo,M. Determination of the concentrations of 5-fluorouracil and its metabolites in rabbit plasma and tissues by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *656*, 397–405.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject

a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 204

CHROMATOGRAM
Retention time: 3.433

KEY WORDS
whole blood

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

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 1-10 ng 5-fluorouracil in 100 μL DMSO, add 400 μL 2.5 mg/mL 4-bromomethyl-7-methoxycoumarin in DMSO, add 5 mg potassium carbonate, shake at room temperature for 15 min, add 500 μL water, centrifuge at 15600 g for 5 min, inject a 100 μL aliquot of the supernatant onto column A and elute to waste with mobile phase A, after 9.5 min divert the effluent containing the derivatized 5-fluorouracil onto column B, after another 2 min elute column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 150 × 4.6 5 μm CPS-Hypersil cyanopropyl; B 150 × 4.6 5 μm ODS-Hypersil
Mobile phase: A MeOH:water 50:50; B MeOH:water 60:40
Column temperature: 35 (column A only)
Flow rate: 1
Injection volume: 100
Detector: F ex 325 em 395

CHROMATOGRAM
Retention time: 17
Limit of detection: 5 pg

KEY WORDS
derivatization; column-switching; heart-cut

REFERENCE
Kindberg,C.G.; Slavik,M.; Riley,C.M.; Stobaugh,J.F. High-performance liquid chromatography of 5-fluorouracil after derivatization with 4-bromomethyl-7-methoxycoumarin. Characterization of the derivative and the use of column switching for the improvement of resolution and the enhancement of sensitivity, *J.Pharm.Biomed.Anal.*, **1989**, *7*, 459–469.

SAMPLE
Matrix: formulations

Sample preparation: Condition a 500 mg SCX Tech-Elut strong cation exchange SPE cartridge (HPLC Technology) with 6 mL MeOH and five 2 mL portions of buffer. Tablets. Powder tablets, weigh out amount equivalent to 500 mg flucytosine, add 50 mL water, stir for 15 min, filter. 1 mL Filtrate + 1.5 mL 27 μg/mL thymine in buffer, make up to 10 mL with buffer, add 2 mL to the SPE cartridge, wash with 1 mL buffer, collect all the eluates, inject an aliquot. Injections. Dilute with water to a drug concentration of 10 mg/mL. 1 mL Sample + 1.5 mL 27 μg/mL thymine in buffer, make up to 10 mL with buffer, add 2 mL to the SPE cartridge, wash with 1 mL buffer, collect all the eluates, inject an aliquot. (Buffer was 70 mM KH_2PO_4 adjusted to pH 3.0 with HCl.) (To measure flucytosine as well as fluorouracil omit the SPE step.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb CN
Mobile phase: 9 mM Sodium heptanesulfonate adjusted to pH 2.8 with phosphoric acid
Flow rate: 0.8
Injection volume: 20
Detector: UV 266

CHROMATOGRAM
Retention time: 4.0
Internal standard: thymine (4.85)

OTHER SUBSTANCES
Simultaneous: flucytosine (when SPE is omitted)

KEY WORDS
tablets; injections; SPE

REFERENCE
Cavrini,V.; Bonazzi,D.; Di Pietra,A.M. Analysis of flucytosine dosage forms by derivative UV spectroscopy and liquid chromatography, *J.Pharm.Biomed.Anal.*, **1991**, *9*, 401–407.

SAMPLE
Matrix: formulations
Sample preparation: Dilute formulation 1:500 with water, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 5 × 4 35-60 μm Perisorb RP18
Column: 250 × 4 10 μm LiChrosorb RP18
Mobile phase: MeOH:300 mM sodium acetate 2:98
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 4.7

OTHER SUBSTANCES
Simultaneous: floxuridine

KEY WORDS
injections; water

REFERENCE
Sadjak,A.; Wintersteiger,R. Compatibility of morphine, baclofen, floxuridine and fluorouracil in an implantable medication pump, *Arzneimittelforschung*, **1995**, *45*, 93–98.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 1 mL formulation to 10 mL with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 μBondapak phenyl

Mobile phase: MeCN:MeOH:0.01% acetic acid:0.005 N sulfonic acid 20:15:40:25
Flow rate: 1
Detector: UV 230

CHROMATOGRAM
Retention time: 3.5

OTHER SUBSTANCES
Simultaneous: metoclopramide

KEY WORDS
injections; 5% dextrose; stability-indicating

REFERENCE
Wang,D.-P.; Chang,L.-C.; Lee,D.K.T.; Wong,C.-Y. Stability of fluorouracil-metoclopramide hydrochloride admixture, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 98–99.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm C18
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 2.5
Injection volume: 20
Detector: UV 300

CHROMATOGRAM
Retention time: 1.58

OTHER SUBSTANCES
Simultaneous: cytarabine, granisetron

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 10 μm precolumn (Beckman Instruments Inc.)
Column: 150 × 4.6 5 μm C18 Altex Ultrasphere ODS
Mobile phase: Isocratic. MeOH:buffer 15:85 containing 2.5 mM sodium pentanesulfonate and 2.5 mM sodium heptanesulfonate. Gradient. A was MeOH containing 2.5 mM sodium pentanesulfonate and 2.5 mM sodium heptanesulfonate. B was buffer containing 2.5 mM sodium pentanesulfonate and 2.5 mM sodium heptanesulfonate. A:B 0:100 for 2 min, to 30:70 over 1 min, maintain at 30:70. (Buffer was 50 mM phosphoric acid containing 50 mM KH_2PO_4, pH 2.5.)
Flow rate: 1
Injection volume: 20
Detector: UV 254; UV 285

CHROMATOGRAM
Retention time: 2.52 (isocratic), 4.36 (gradient)

Internal standard: 5-methylcytosine (5.66 (isocratic), 12.62 (gradient))
Limit of quantitation: 12.5 μM

OTHER SUBSTANCES
Simultaneous: barbituric acid, cytosine, flucytosine, hydroxycytosine, uracil, urea

REFERENCE
Biondi,L.; Nairn,J.G. High performance liquid chromatographic assay for 5-fluorouracil and 5-fluorocytosine, *J.Liq.Chromatogr.*, **1985**, *8*, 1881–1892.

SAMPLE
Matrix: solutions
Sample preparation: Dilute a 50 mg/mL sample 1:100 with mobile phase and inject an aliquot.

HPLC VARIABLES
Column: Bakerbond C18
Mobile phase: 5 mM pH 7.8 K_2HPO_4
Flow rate: 0.5
Detector: UV 214

CHROMATOGRAM
Retention time: 6.6

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
stability-indicating

REFERENCE
Stiles,M.L.; Allen,L.V.,Jr.; Prince,S.J. Stability of deferoxamine mesylate, floxuridine, fluorouracil, hydromorphone hydrochloride, lorazepam, and midazolam hydrochloride in polypropylene infusion-pump syringes, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 1583–1588.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of an aqueous solution.

HPLC VARIABLES
Column: 100 × 8 Resolve C18
Mobile phase: MeCN:50 mM ammonium dihydrogen phosphate 4:96, pH adjusted to 3.5 with H_3PO_4
Flow rate: 1.7
Detector: UV 268

CHROMATOGRAM
Retention time: 2.8

OTHER SUBSTANCES
Simultaneous: fluorouridine

REFERENCE
Dorta,M.J.; Munguia,O.; Fariña,J.B.; Martin,V.S.; Llabrés,M. Stability indicating high performance liquid chromatography methods for 5-fluorouridine in aqueous solution, *Arzneimittelforschung*, **1997**, *47*, 1388–1392.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX

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

OTHER SUBSTANCES

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

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 mm long 5 μm Microsorb-MV C18
Mobile phase: MeCN:pH 4 sodium acetate buffer 2:98
Flow rate: 1.5

Detector: UV 270

REFERENCE

Phillips,C.A.; Michniak,B.B. Transdermal delivery of drugs with differing lipophilicities using azone analogs as dermal penetration enhancers, *J.Pharm.Sci.*, **1995**, *84*, 1427–1433.

SAMPLE

Matrix: tissue

Sample preparation: Homogenize (Ultra-Turrax) 1 g tissue with 0.05-1 μg IS and 15 mL ice-cold MeCN, rinse homogenizer with 10 mL ice-cold MeCN, centrifuge at 6000 rpm for 15 min, remove the supernatant, wash the pellet with 5 mL MeCN, centrifuge for 5 min. Combine the supernatants, remove a 5 mL aliquot, evaporate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 50 μL n-hexane:ethyl acetate:water 40:80:1, chromatograph on a 125 × 4 5 μm Spherisorb Si column (A) and a 200 × 4.6 5 μm Spherisorb Si column (B) with n-hexane:ethyl acetate:water 40:80:1 at 1 mL/min using column-switching. Initially elute the columns in series, after 3 min (after 5-fluorouracil has eluted from column A to column B) elute only column B, monitor the effluent from column B at 266 nm, collect the appropriate fraction. (Backflush column A to waste for 6 min before the next injection.) Evaporate the eluate to dryness under a stream of nitrogen, reconstitute with 10 μL acetone, add 1 mg freshly-powdered potassium carbonate, add 5 μL 200 μg/mL 18-crown-6 in acetone, add 20 μL 750 μg/mL 4-bromomethyl-7-methoxycoumarin in acetone, heat at 70° for 25 min, inject a 1 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 1.6 3 μm Nukleosil-120 (MZ Analysentechnik)
Column: 125 × 1.6 3 μm Nukleosil-120 (MZ Analysentechnik)
Mobile phase: MeCN:MeOH:water 30:15:50
Flow rate: 0.06
Injection volume: 1
Detector: F ex 305 em 407

CHROMATOGRAM

Retention time: 27
Internal standard: 5-chlorouracil (31)
Limit of detection: 3 ng/g
Limit of quantitation: 30 ng/g

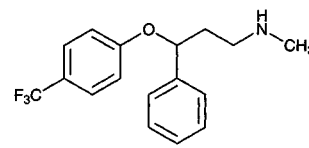
KEY WORDS

derivatization; microbore; human; pig; liver

REFERENCE

Jochheim,C.; Janning,P.; Marggraf,U.; Löffler,T.M.; Hasse,F.; Linscheid,M. A procedure for the determination of 5-fluorouracil in tissue using microbore HPLC and fluorescence detection, *Anal.Biochem.*, **1994**, *217*, 285–291.

Fluoxetine



Molecular formula: $C_{17}H_{18}F_3NO$
Molecular weight: 309.33
CAS Registry No.: 54910-89-3, 59333-67-4 (HCl)
Merck Index: 4222
Lednicer No.: 3 32

SAMPLE

Matrix: blood

Sample preparation: Add 250 μL 2 M sodium carbonate to 500 μL plasma. Add 100 μL 1 μg/mL IS in MeOH, extract with 10 mL n-hexane. Shake for 30 min and centrifuge at 3000 g for

10 min. Cool in a dry ice-acetone bath. Add 200 μL 0.3% phosphoric acid to upper organic layer. Shake for 10 min and centrifuge at 3000 g for 10 min. Separate the organic layer. Inject a 100 μL aliquot of the acidic aqueous layer.

HPLC VARIABLES
Column: 250 × 4.6 5 μm C18 Symmetry (Waters Millipore, USA)
Mobile phase: MeCN:67 mM potassium phosphate buffer adjusted to pH 3.0 with phosphoric acid 35:65 (After each chromatographic session wash the column with 200 mL MeCN:water 50:50.)
Flow rate: 1.2
Injection volume: 100
Detector: UV 226, UV 254, UV 400

CHROMATOGRAM
Retention time: 15.50
Internal standard: clovoxamine (6.5)
Limit of quantitation: 5 ng/mL (UV 226, UV 400); 7 ng/mL (UV 254)

OTHER SUBSTANCES
Extracted: metabolites, amitriptyline, clomipramine, desipramine, imipramine, maprotiline, nortriptyline
Simultaneous: amineptine, carbamazepine, chlordiazepoxide, clorazepate, clozapine, cyamemazine, desmethylmaproptiline, desmethylvenlafaxine, doxepin, flunitrazepam, fluvoxamine, haloperidol, levomepromazine, lorazepam, loxapine, mianserine, sulpiride, trimipramine, venlafaxine, viloxazine, zolpidem, zopiclone
Noninterfering: diazepam, valproic acid
Interfering: chlorpromazine, clonazepam

KEY WORDS
plasma

REFERENCE
Aymard,G.; Livi,P.; Pham,Y.T.; Diquet,B. Sensitive and rapid method for the simultaneous quantification of five antidepressants with their respective metabolites in plasma using high-performance liquid chromatography with diode-array detection, *J.Chromatogr.B*, **1997**, *700*, 183–189.

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL plasma with 400 μL 50 μg/mL IS in MeOH, make up to 2 mL with MeOH, centrifuge at 1100 g for 10 min. Remove a 1 mL aliquot of the supernatant, add 2 mL 1 M NaOH and 2 mL diethyl ether, shake for 10 min, repeat the extraction. Combine the ether extracts and dry them over anhydrous sodium sulfate. Evaporate to dryness under a stream of nitrogen and reconstitute the residue in 1 mL MeOH. Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 200 × 4 10 μm LiChrosorb RP-18
Mobile phase: MeCN:pH 2.7 phosphate buffer 90:10
Flow rate: 1.0
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 2.53
Internal standard: chlorprothixene (5.20)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: amitriptyline, azathioprine, diazepam, doxepin, fludarabine, imipramine, mercaptopurine,
Interfering: methadone, pentazocine, piritramide, tramadol

KEY WORDS
plasma

REFERENCE
Misztal,G.; Hopkala,H. Determination of fluoxetine in human plasma using reversed phase HPLC, *Pharmazie*, **1997**, *52*, 854–856.

SAMPLE
Matrix: blood
Sample preparation: Add 400 μL 330 mM NaOH to 2 mL plasma. Mix for 5 min, extract with 14 mL n-hexane:isoamyl alcohol 98.5:1.5 for 20 min. Centrifuge at 2500 g for 5 min and collect the organic layer. Adjust to pH 2 with 400 μL HCl, shake for 1 min, centrifuge at 1500 g for 10 min. Inject a 100 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 4.6 5 μm LiChrospher RP-18
Column: 250 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: MeCN:5 mM n-octylamine in water 40:60, adjusted to pH 6.4 with orthophosphoric acid (After use, wash the column with water for 15 min, MeCN:water 50:50 for 15 min, and MeCN for 5 min.)
Flow rate: 1
Injection volume: 100
Detector: UV 230

CHROMATOGRAM
Retention time: 24.41
Limit of detection: 4.5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: amisulpride, diazepam, flunitrazepam, fluvoxamine, haloperidol, imipramine, maprotiline, paroxetine, amitriptyline, clomipramine, mianserin

KEY WORDS
plasma

REFERENCE
Gennaro,M.C.; Abrigo,C.; Angelino,S.; Albert,U.; Bogetto,F.; Maina,G.; Prolo,P.; Ravizza,L. Determination of fluoxetine and norfluoxetine in human plasma by ion-interaction RP-HPLC, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 3017–3028.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 20 μL 5 μg/mL IS in MeOH + 100 μL 5 M NaOH, vortex for 30 s, add 2 mL hexane, vortex for 30 s, centrifuge at 3000 g for 3 min. Remove the organic layer and evaporate it under a gentle stream of nitrogen at 20°, reconstitute the residue in 50 μL mobile phase, vortex for 30 s, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: RP C18 (Brownlee)
Column: 150 × 4.6 5 μm Microsorb MV
Mobile phase: MeCN:water 55:45 containing 10 mM triethylamine, adjusted to pH 4.8 with 85% phosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 226

CHROMATOGRAM
Retention time: 4.7
Internal standard: clomipramine (7.4)
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Extracted: norfluoxetine

KEY WORDS
mouse; serum; pharmacokinetics

REFERENCE
Holladay,J.W.; Dewey,M.J; Yoo,S.D. Quantification of fluoxetine and norfluoxetine serum levels by reversed-phase high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1997**, *704*, 259–263.

SAMPLE
Matrix: blood, tissue
Sample preparation: 100 μL Serum or 200 μL brain homogenate + 20 μL 5.0 μg/mL clomipramine in MeOH + 100 μL 5.0 M NaOH + 2 mL hexane, vortex for 30 s, centrifuge at 3000 g for 5 min. Evaporate organic layer under a gentle stream of nitrogen at 20°. Reconstitute residue with 50 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: Microsorb MV C18 (Rainin, Woburn, USA)
Mobile phase: MeCN:water 55:45 containing 10 mM triethylamine, pH adjusted to 4.8 with 85% phosphoric acid
Flow rate: 1.0
Injection volume: 20
Detector: UV 226

CHROMATOGRAM
Internal standard: clomipramine
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; brain; mouse; pharmacokinetics

REFERENCE
Holladay,J.W.; Dewey,M.J.; Yoo,S.D. Pharmacokinetics and antidepressant activity of fluoxetine in transgenic mice with elevated serum α-1-acid glycoprotein levels, *Drug Metab.Dispos.*, **1998**, *26*, 20–24.

SAMPLE
Matrix: blood, tissue
Sample preparation: 100 μL Serum or weighed homogenized brain + 1 mL 600 mM pH 9.8 sodium carbonate-sodium bicarbonate buffer containing 100 ng/mL IS. Add 7 mL ethyl acetate: n-heptane 20:80, mix vigorously for 1.5 min, centrifuge at 3000 g for 10 min. Mix the organic layer with 200 μL 25 mM potassium dihydrogen phosphate adjusted to pH 2.3 with 85% phosphoric acid. Mix for 1 min, centrifuge at 3000 g for 10 min, discard the organic layer, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: 4 × 4 5 μm C8 endcapped (Merck, Germany)
Column: 125 × 4 4 μm C8 endcapped (Merck, Germany)
Mobile phase: MeCN:buffer 42:58 (Buffer was water containing 100 μL/L perchloric acid and 1.5 g/L tetramethylammonium perchlorate.)
Flow rate: 1.2
Injection volume: 50
Detector: UV 227

CHROMATOGRAM
Retention time: 12.7
Internal standard: protriptyline (8.1)

Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: acetopromazine, aceprometazine, amitriptyline, bromazepam, chlorpromazine, ciamemazine, clomipramine, clorazepate, demethyldomipramine, diazepam, flunitrazepam, imipramine, nitrazepam, nordiazepam, nortriptyline

KEY WORDS
serum; brain; mouse; human; pharmacokinetics

REFERENCE
Alvarez,J.-C.; Bothua,D.; Collignon,I.; Advenier,C.; Spreux-Varoquaux,O. Determination of fluoxetine and its metabolite norfluoxetine in serum and brain areas using high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1998**, *707*, 175–180.

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

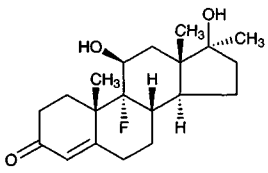
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.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

CHROMATOGRAM
Retention time: 16.185

KEY WORDS
whole blood

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

Fluoxymesterone



Molecular formula: $C_{20}H_{29}FO_3$
Molecular weight: 336.45
CAS Registry No.: 76-43-7
Merck Index: 4223
Lednicer No.: 1 175

Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: acetopromazine, aceprometazine, amitriptyline, bromazepam, chlorpromazine, ciamemazine, clomipramine, clorazepate, demethyldomipramine, diazepam, flunitrazepam, imipramine, nitrazepam, nordiazepam, nortriptyline

KEY WORDS
serum; brain; mouse; human; pharmacokinetics

REFERENCE
Alvarez,J.-C.; Bothua,D.; Collignon,I.; Advenier,C.; Spreux-Varoquaux,O. Determination of fluoxetine and its metabolite norfluoxetine in serum and brain areas using high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1998**, *707*, 175–180.

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

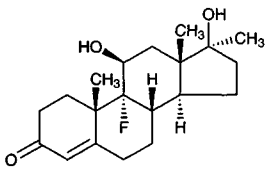
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.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

CHROMATOGRAM
Retention time: 16.185

KEY WORDS
whole blood

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

Fluoxymesterone



Molecular formula: $C_{20}H_{29}FO_3$
Molecular weight: 336.45
CAS Registry No.: 76-43-7
Merck Index: 4223
Lednicer No.: 1 175

SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 10 μL 20 μg/mL 6α-methylprednisolone in MeOH, mix thoroughly, add 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°, reconstitute the residue in 200 μL mobile phase, inject a 50 μL 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 × 4.6 6 μm 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: 14

Internal standard: 6α-methylprednisolone (24)

Limit of detection: 2 ng/mL

KEY WORDS

serum; normal phase; column-switching; pharmacokinetics

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: formulations

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

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax ODS

Mobile phase: MeOH:water 75:25

Flow rate: 1.5

Injection volume: 10

Detector: UV 240

CHROMATOGRAM

Retention time: 4.4

Limit of detection: 5 μg/mL

OTHER SUBSTANCES

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

Interfering: norethindrone, oxandrolone (UV 210), boldenone

KEY WORDS
oils; tablets; suspensions

REFERENCE
Walters,M.J.; Ayers,R.J.; Brown,D.J. Analysis of illegally distributed anabolic steroid products by liquid chromatography with identity confirmation by mass spectrometry or infrared spectrophotometry, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 904–926.

SAMPLE
Matrix: formulations
Sample preparation: Crush tablets, weigh out amount equivalent to 10 mg steroid, dissolve in 10 mL MeOH, sonicate for 15 min, filter. 1 mL Filtrate + 5 mL MeOH + 4 mL water, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: Gradient. MeOH:water from 70:30 to 100:0 over 15 min, maintain at 100:0 for 15 min.
Flow rate: 1
Injection volume: 25
Detector: UV 240

CHROMATOGRAM
Retention time: 7.2

OTHER SUBSTANCES
Simultaneous: boldenone, boldenone acetate, boldenone undecylenate, clostebol acetate, danazol (UV 280), methandriol, methandriol-3-acetate, methandriol dipropionate, methandrostenolone, methyltestosterone, nandrolone, nandrolone decanoate, nandrolone phenylpropionate, nandrolone propionate, stanolone, stanozolol, testosterone, testosterone acetate, testosterone cypionate, testosterone enanthate, testosterone isobutyrate, testosterone propionate, testosterone undecanoate
Noninterfering: oxandrolone, oxymetholone, testosterone decanoate, testosterone isocaproate

KEY WORDS
tablets

REFERENCE
Lurie,I.S.rling,A.R.; Meyers,R.P. The determination of anabolic steroids by MECC, gradient HPLC, and capillary GC, *J.Forensic Sci.*, **1994**, *39*, 74–85.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: μBondapak ODS
Mobile phase: MeCN:water 30:70
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 10
Internal standard: fluoxymesterone

OTHER SUBSTANCES
Simultaneous: triamcinolone acetonide

KEY WORDS
fluoxymesterone is IS

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: 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: 7.7 (phenyl), 12.0 (ODS)

OTHER SUBSTANCES
Simultaneous: triamcinolone acetonide

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
Sample preparation: Dissolve at 100 μg/mL in MeOH.

HPLC VARIABLES
Guard column: 70 × 2.1 Whatman CO:Pell ODS
Column: 300 × 3.9 Bondex C18
Mobile phase: MeOH:water 70:30
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: boldenone, nandrolone, methandrostenolone, testosterone, danazol, methyltestosterone

REFERENCE
Noggle,F.T.,Jr.; Clark,C.R.; DeRuiter,J. Liquid chromatographic and spectral analysis of the 17-hydroxy anabolic steroids, *J.Chromatogr.Sci.*, **1990**, *28*, 162–166.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 0.5 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN:water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 19.6

OTHER SUBSTANCES
Simultaneous: acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, diflunisal, doxapram, estrone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, testosterone, testosterone propionate, tranylcypromine, tripelennamine
Interfering: 2-naphthoxyacetic acid

KEY WORDS
details for purification of triethylamine in paper

REFERENCE
Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE
Matrix: solutions

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

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, 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, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenyl-

butazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

SAMPLE

Matrix: solutions
Sample preparation: Inject a 5 μL aliquot of a 10 μg/mL solution in MeOH.

HPLC VARIABLES

Column: 75 × 4.6 3 μm Ultrasphere ODS
Mobile phase: MeCN:10 mM ammonium acetate buffer 45:55
Flow rate: 0.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM

Retention time: 3.149

OTHER SUBSTANCES

Simultaneous: boldenone, epimethandienone, epitestosterone, 6β-hydroxymethandienone, methandienone, norethindrone, oxymetholone (UV 280), trenbolone

REFERENCE

Barrón,D.; Pascual,J.A.; Segura,J.; Barbosa,J. Prediction of LC retention of steroids using solvatochromic parameters, *Chromatographia*, **1995**, *41*, 573–580.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a solution in MeOH:water 50:50.

HPLC VARIABLES

Column: 250 × 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: 4.5

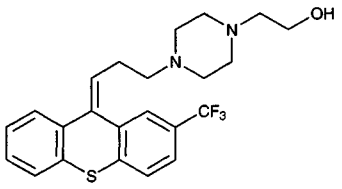
OTHER SUBSTANCES

Simultaneous: medrogestone, mestranol, norethindrone, 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.

Flupentixol



Molecular formula: $C_{23}H_{25}F_3N_2OS$
Molecular weight: 434.53
CAS Registry No.: 2709-56-0, 30909-51-4 (decanoate), 2413-38-9 (2.HCl)
Merck Index: 4224

SAMPLE

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

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 228.7

CHROMATOGRAM

Retention time: 17.358

KEY WORDS

whole blood

REFERENCE

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

SAMPLE

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

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 2.0

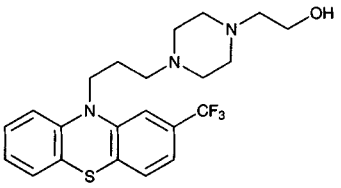
OTHER SUBSTANCES

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

REFERENCE

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

Fluphenazine



Molecular formula: $C_{22}H_{26}F_3N_3OS$
Molecular weight: 437.53
CAS Registry No.: 69-23-8, 146-56-5 (di HCl), 2746-81-8 (enanthate)
Merck Index: 4226
Lednicer No.: 1 383

SAMPLE

Matrix: blood
Sample preparation: 1-5 mL Plasma + 1 mL 1 M NaOH + hexanes, extract for 30 min, centrifuge. Remove a 9 mL aliquot of the organic phase and evaporate it to dryness at 30° under a stream of nitrogen. Dissolve the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 10 μm Micropak CN (Varian)

Mobile phase: MeCN:5 mM ammonium acetate 90:10
Flow rate: 2.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 8.7
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: acetophenazine, amitriptyline, benztropine, butaperazine, carphenazine, chlorpromazine, promethazine, haloperidol, imipramine, mesoridazine, nortriptyline, orphenadrine, piperacetazine, promazine, thioridazine, thiothixene, trifluoperazine, triflupromazine, trihexyphenidyl, trimeprazine, metabolites

KEY WORDS
plasma

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

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 100 μL 1 μg/mL loxapine in isopropanol:diethylamine 99.9:0.1 + 250 μL 25% potassium carbonate containing 0.1% diethylamine + 5 mL hexane: isoamyl alcohol 97:3, vortex for 30 s, centrifuge at 500 g for 3 min. Remove the organic layer and add it to 100 μL 250 mM HCl, vortex for 30 s, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: 50 × 4.6 40 μm C8 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil C8
Mobile phase: MeCN:water:diethylamine:85% phosphoric acid 53.3:45.1:1:0.4, pH adjusted to 7.2 with NaOH or phosphoric acid
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: k' 2.67
Internal standard: loxapine (k' 7.18)

OTHER SUBSTANCES
Extracted: amitriptyline, chlorpromazine, desipramine, desmethldiazepam, desmethylchlordiazepoxide, diazepam, doxepin, haloperidol, imipramine, nortriptyline, thiothixene
Noninterfering: molindone, perphenazine, trifluoperazine
Interfering: chlordiazepoxide, desmethyldoxepin, oxazepam

KEY WORDS
plasma

REFERENCE
Kiel,J.S.; Abramson,R.K.; Morgan,S.L.; Voris,J.C. A rapid high performance liquid chromatographic method for the simultaneous measurement of six tricyclic antidepressants, *J.Liq.Chromatogr.*, **1983**, *6*, 2761–2773.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 40 ng/mL chlorpromazine in water, vortex for a few s, add 500 μL 650 mM sodium carbonate, vortex for a few s, add 7 mL pentane:ethyl acetate 75:25, mix for 15 min, let stand for 5 min. Remove the upper organic layer and evaporate it

to dryness under a stream of nitrogen at 65°, reconstitute the residue in 40 μL MeCN, mix for a few s, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Spherisorb cyano
Mobile phase: MeCN:MeOH:100 mM pH 7 ammonium acetate 90:5:5
Flow rate: 1.5
Injection volume: 30
Detector: E, ESA 5100A, Model 5020 guard cell +1.00 V, model 5011 analytical cell, cell 1 +0.50 V, cell 2 +0.75 V

CHROMATOGRAM
Retention time: 6.00
Internal standard: chlorpromazine (10.87)
Limit of quantitation: 0.025 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Cooper,J.K.; Hawes,E.M.; Hubbard,J.W.; McKay,G.; Midha,K.K. An ultrasensitive method for the measurement of fluphenazine in plasma by high-performance liquid chromatography with coulometric detection, *Ther.Drug Monit.*, **1989**, *11*, 354–360.

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

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 260

CHROMATOGRAM
Retention time: 16.88
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lor-

azepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

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

SAMPLE

Matrix: blood, urine

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

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

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

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

Column temperature: 30

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

Injection volume: 10-30

Detector: UV 259.4

CHROMATOGRAM

Retention time: 17.357

KEY WORDS

whole blood

REFERENCE

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

SAMPLE
Matrix: formulations
Sample preparation: Extract ground tablets containing 1 mg with 10 mL MeOH, shake for 30 min, centrifuge at 2000 rpm for 5 min. Remove a 5 mL aliquot of the supernatant and add it to 10 mL 1.25 mg/mL norephedrine hydrochloride in MeOH, make up to 25 mL with MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax CN
Mobile phase: MeOH:MeCN:25 mM pH 4.5 acetate buffer 30:40:30
Flow rate: 2.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 4.15
Internal standard: norephedrine (2.38)

OTHER SUBSTANCES
Interfering: desipramine, promazine

KEY WORDS
tablets

REFERENCE
Beaulieu,N.; Gagné,C.; Lovering,E.G. Liquid chromatographic determination of identity, content, and content uniformity of desipramine, fluphenazine, and promazine, *J.Assoc.Off.Anal.Chem.*, **1986**, *69*, 178–179.

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

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

CHROMATOGRAM
Retention time: 2.0

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, 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, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine,

mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

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

SAMPLE

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

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 80:1.5:0.5:18
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM

Retention time: k' 0.53 (fluphenazine), k' 3.49 (fluphenazine decanoate), k' 2.49 (fluphenazine enanthate)

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 1 mL 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.

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
Detector: UV 238

CHROMATOGRAM

Retention time: 3.35

OTHER SUBSTANCES

Simultaneous: alprazolam, amitriptyline, chlorpromazine, chlorprothixene, clomipramine, desclomipramine, desmethylimipramine, diazepam, flunitrazepam, imipramine, levomepromazine, maprotiline, nortriptyline, perphenazine, promethazine, thioridazine sulfoxide, thioridazine, thioridazine sulfone, trimipramine, zimeldine
Noninterfering: carbamazepine, clonazepam, lorazepam, nitrazepam, oxazepam, phenytoin
Interfering: haloperidol, protriptyline, zuclopenthixol

KEY WORDS

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: solutions

HPLC VARIABLES

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

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine,

puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 13.60 (A), 7.22 (B)

OTHER SUBSTANCES

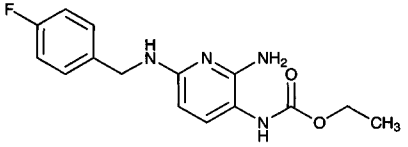
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, 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, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

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.

Flupirtine



Molecular formula: $C_{15}H_{17}FN_4O_2$
Molecular weight: 304.32
CAS Registry No.: 56995-20-1, 75507-68-5 (maleate)
Merck Index: 4227
Lednicer No.: 4 102

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 30 μL 10 μg/mL IS in 10 mM HCl + 40 μL 1 M NaOH + 8 mL diethyl ether, rotate for 15 min, centrifuge. Remove 5 mL of the organic layer and add it to 10 mL n-hexane and 400 μL 1 M HCl, vortex for 1 min, centrifuge at 1000 g, inject a 30-200 μL aliquot of the acidic aqueous phase.

HPLC VARIABLES
Column: 250 × 4.6 5 μm ODS-Hypersil
Mobile phase: Gradient. A was 10 mM pH 3 phosphate buffer. B was MeCN:MeOH 50:50. A:B from 40:60 to 30:70 over 8 min, maintain at 30:70 for 3 min.
Flow rate: 1.4
Injection volume: 30-200
Detector: F ex 323 em 380

CHROMATOGRAM
Retention time: 6.0
Internal standard: [2-amino-6-[[(2,4-dimethylphenyl)methyl]amino]-3-pyridinyl]carbamic acid ethyl ester (9.38)
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Niebch,G.; Borbe,H.O.; Hummel,T.; Kobal,G. Dose-proportional plasma levels of the analgesic flupirtine maleate in man. Application of a new HPLC assay, *Arzneimittelforschung*, **1992**, *42*, 1343–1345.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 500 μL Plasma + 50 μL 2 μg/mL IS in MeOH + 1 mL MeCN, vortex for 5 s, centrifuge at 2000 g for 5 min. Remove the supernatant and evaporate it to dryness under a stream of air at 37°, reconstitute the residue in 150 μL mobile phase, mix for 1 min, centrifuge for 2 min at 2000 g, inject a 20 μL aliquot. Urine. 500 μL Urine + 50 μL 2 μg/mL IS in MeOH + 50 μL 10% ammonium hydroxide, vortex for 5 s, add 5 mL dichloromethane, shake on a reciprocating shaker for 5 min, centrifuge at 2000 g for 5 min. Remove 4 mL of the organic phase and evaporate it to dryness under a stream of air at 37°, reconstitute the residue in 150 μL mobile phase, mix for 1 min, centrifuge for 2 min at 2000 g, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 70 × 2.3 30-38 μm Co:Pell ODS

Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:MeCN:5 mM phosphate buffer 32:32:36, pH adjusted to 6.7
Flow rate: 1.4
Injection volume: 20
Detector: F ex 323 em 370

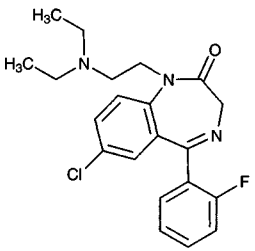
CHROMATOGRAM
Retention time: 3.73
Internal standard: 2-amino-3-carbethoxyamino-6-(2,4-dimethylbenzylamino)pyridine (6.08)
Limit of detection: 30 ng/mL (urine), 10 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: aspirin, carbamazepine, clonazepam, diazepam, methylprednisolone, phenytoin, valproic acid

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Narang,P.K.; Tourville,J.F.; Chatterji,D.C.; Gallelli,J.F. Quantitation of flupirtine and its active acetylated metabolite by reversed-phase high-performance liquid chromatography using fluorometric detection, *J.Chromatogr.*, **1984**, *305*, 135–143.

Flurazepam



Molecular formula: $C_{21}H_{23}ClFN_3O$
Molecular weight: 387.88
CAS Registry No.: 17617-23-1, 1172-18-5 (HCl)
Merck Index: 4233

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 200 μL 50 μg/mL hexobarbital in MeCN + 25 μL glacial acetic acid, vortex for 10 s, centrifuge for 1 min, inject a 30-100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: Gradient. MeCN:7.5 g/L NaH_2PO_4 adjusted to pH 3.2 with phosphoric acid 5:95 to 22:78 over 24 min, to 45:55 over 10 min, maintain at 45:55 for 5 min. Re-equilibrate with 5:95 for 5 min.
Column temperature: 50
Flow rate: 3
Injection volume: 30-100
Detector: UV 210

CHROMATOGRAM
Retention time: 26.7
Internal standard: hexobarbital (20.6)
Limit of detection: 200-2000 ng/mL

OTHER SUBSTANCES
Extracted: acetaminophen, amobarbital, butabarbital, butalbital, chlordiazepoxide, diazepam, ethchlorvynol, 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
Interfering: desmethyldoxepin

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: Basify plasma with 500 mM KOH, extract with diethyl ether:heptane 90:10. Remove the organic layer and evaporate it to dryness, reconstitute the residue in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 mm long 5 μm Spherisorb CN
Mobile phase: MeCN:EtOH:50 mM pH 2.5 phosphate buffer 20:15:65
Detector: UV 200

CHROMATOGRAM
Retention time: 4.86

OTHER SUBSTANCES
Extracted: droperidol

KEY WORDS
flurazepam is IS; plasma

REFERENCE
James,J.; Lowe,D.; Karnes,H.T. Determination of histamine from plasma using derivatization with naphthalene-2,3-dicarboxaldehyde and HPLC with fluorescence detection, *Pharm.Res.*, **1992**, *9*, S21.

SAMPLE
Matrix: blood
Sample preparation: Rock 5 mL whole blood + 10 mL water + 8.5 mL Na_2WO_4 in a 50 mL stoppered tube for 1 min, add 6 mL $NiCl_2$, rock for 5 min, add 15 mL 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. (Na_2WO_4 prepared by mixing 10 g $Na_2WO_4.2H_2O$ in 38 mL of 2 M NaOH and 2.5 g of $NaHCO_3$ and making up to 100 mL. $NiCl_2$ was 17% w/v $NiCl_2$ in water.)

HPLC VARIABLES
Column: 200 × 4.6 5 μm Hypersil C8
Mobile phase: A = MeCN; B = 20 mM n-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: 11
Limit of detection: 0.30 ppm

OTHER SUBSTANCES
Extracted: bromazepam, clonazepam, diazepam, flunitrazepam, medazepam, nitrazepam, oxazepam

Also analyzed: buprenorphine, caffeine, cocaine, codeine, diamorphine, ethylmorphine, lidocaine, methaqualone, morphine, naloxone, noscapine, papaverine, pentazocine, procaine

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: 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: 6.6
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, 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: fluoxetine, 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, urine

Sample preparation: 500 μL Plasma or urine + 50 μL 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 μL MeOH, inject a 30 μL aliquot. (Deconjugate urine as follows. 250 μL Urine + 750 μL pH 5.4 acetate buffer + 500 U β-glucuronidase, heat at 37° for 18 h, add 20 μL 5 M NaOH, centrifuge, proceed as above using 5 mL dichloromethane:diethyl ether.)

HPLC VARIABLES

Guard column: 30 × 4.6 30 μm C8
Column: 100 × 8 4 μm 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: 18.5
Internal standard: flurazepam

OTHER SUBSTANCES

Extracted: flumazenil, midazolam, 4-hydroxymidazolam, 1-hydroxymethylmidazolam
Noninterfering: alfentanil, atropine, bupivacaine, lignocaine, neostigmine

KEY WORDS

plasma; flurazepam is IS

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

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 2.3

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, 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, haloperidol, hy-

droxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 30 × 2.1 Spheri-5 RP-8
Column: 220 × 2.1 Spheri-5 RP-8
Mobile phase: Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95: % 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: 13.3

OTHER SUBSTANCES

Simultaneous: chlordiazepoxide, desalkylflurazepam, diazepam, norchlordiazepoxide, nordiazepam, oxazepam, prazepam

REFERENCE

Rainin Catalog 1991-2, p. 3.26.

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: 13.3

OTHER SUBSTANCES
Simultaneous: norchlordiazepoxide, chlordiazepoxide, nordiazepam, desalkylflurazepam, oxazepam, diazepam, prazepam
Also analyzed: amitriptyline, amphetamine, chlorpromazine, desipramine, desmethyldoxepin, diethylpropion, doxepin, ephedrine, fenfluramine, imipramine, mesoridazine, methamphetamine, nortriptyline, phentermine, phenylpropanolamine, promazine, thioridazine, thiothixene, trifluoperazine

REFERENCE
Rainin Catalog, C1-94, **1994,** p. 7.24.

SAMPLE
Matrix: solutions

HPLC VARIABLES
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: 10.50 (A), 5.53 (B)

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

KEY WORDS
details of plasma extraction

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

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1-10 μg/mL solution in water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil SCX/C18
Mobile phase: MeCN:25 mM pH 3 Na_2HPO_4 50:50
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 4.59

OTHER SUBSTANCES
Also analyzed: amitriptyline, barbital, benzoic acid, butabarbital, clomipramine, clonazepam, desipramine, diazepam, furosemide, imipramine, nitrazepam, phenobarbital, phenol, phenolphthalein, pindolol, propranolol, resorcinol, salicylic acid, secobarbital, terbutaline, xylazine

KEY WORDS
effect of mobile phase pH on capacity factor is discussed

REFERENCE
Walshe,M.; Kelly,M.T.; Smyth,M.R.; Ritchie,H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *708*, 31–40.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a solution in mobile phase.

HPLC VARIABLES
Column: Nova-Pak C18
Mobile phase: MeOH:buffer 85:15 (Buffer was 90.7 mL 66.7 mM Na_2HPO_4 and 9.3 mL 66.7 mM KH_2PO_4 made up to 1 L with water, pH 7.6.)
Flow rate: 5 (sic)
Injection volume: 20
Detector: UV (wavelength not given)

CHROMATOGRAM
Retention time: 10.56
Limit of detection: 100 nM

OTHER SUBSTANCES
Simultaneous: chlordiazepoxide, diazepam, nitrazepam

KEY WORDS
comparison with capillary electrophoresis; capillary GC; and polarography

REFERENCE
McGrath,G.; McClean,S.; O'Kane,E.; Smyth,W.F.; Tagliaro,F. Study of the capillary zone electrophoretic behaviour of selected drugs, and its comparison with other analytical techniques for their formulation assay, *J.Chromatogr.A*, **1996**, *735*, 237–247.

SAMPLE

Matrix: urine

Sample preparation: 2 mL Urine + 3 mL 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 a 9.144 m × 0.5 mm i.d. figure eight Teflon tubing UV irradiation unit maintained at 0-5° with an ice bath

CHROMATOGRAM

Limit of detection: 11 ng/mL

OTHER SUBSTANCES

Also analyzed: midazolam, clonazepam, demoxepam, diazepam, benzophenone

REFERENCE

Selavka,C.M.; Krull,I.S.; Lurie,I.S. Photolytic derivatization for improved LCEC determinations of pharmaceuticals in biological fluids, *J.Chromatogr.Sci.*, **1985**, *23*, 499–508.

SAMPLE

Matrix: urine

Sample preparation: 500 μL Urine + N-ethylnordiazepam + chlorpheniramine + 100 μL buffer, centrifuge at 11000 g for 30 s, inject a 500 μL aliquot onto column A with mobile phase A, after 0.6 min backflush column A with mobile phase A to waste for 1.6 min, elute column A with 250 μL mobile phase B, with 200 μL mobile phase C, and with 1.15 mL mobile phase D. Elute column A to waste until drugs start to emerge then elute onto column B. Elute column B to waste until drugs started to emerge, then elute onto column C. When all the drugs have emerged from column B remove it from the circuit, elute column C with mobile phase D, monitor the effluent from column C. Flush column A with 7 mL mobile phase E, with mobile phase D, and mobile phase A. Flush column B with 5 mL mobile phase E then with mobile phase D. (Buffer was 6 M ammonium acetate adjusted to pH 8.0 with 2 M KOH.)

HPLC VARIABLES

Column: A 10 × 2.1 12-20 μm PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 × 3.2 11 μm Aminex A-28 (Bio-Rad); C 25 × 3.2 5 μm C8 (Phenomenex) + 150 × 4.6 5 μm silica (Macherey-Nagel)

Mobile phase: A 0.1% pH 8.0 potassium borate buffer; B 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid; C MeCN:buffer 40:60 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); D MeCN:buffer 33:67 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); E MeCN: buffer 70:30 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)

Column temperature: ambient (column A), 40 (columns B and C)

Flow rate: A 5; B-E 1

Injection volume: 500

Detector: UV 210, UV 235

CHROMATOGRAM

Retention time: k' 4.2

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

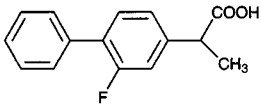
Limit of detection: 300 ng/mL

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

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.

Flurbiprofen



Molecular formula: $C_{15}H_{13}FO_2$
Molecular weight: 244.27
CAS Registry No.: 5104-49-4
Merck Index: 4234
Lednicer No.: 1 86

SAMPLE
Matrix: aqueous humor
Sample preparation: 100 μL Aqueous humor + 500 μL MeCN + 30 μL 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 μL mobile phase by swirl-mixing for 1 min, centrifuge at 3000 g for 20 s, reduce volume to 20-30 μL, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.5 5 μm 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: 6.04
Internal standard: naproxen (3.89)
Limit of detection: 0.4 ng

OTHER SUBSTANCES
Extracted: diclofenac, 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

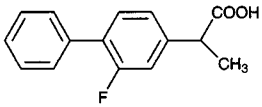
KEY WORDS
human; rabbit

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

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.

Flurbiprofen



Molecular formula: $C_{15}H_{13}FO_2$
Molecular weight: 244.27
CAS Registry No.: 5104-49-4
Merck Index: 4234
Lednicer No.: 1 86

SAMPLE
Matrix: aqueous humor
Sample preparation: 100 μL Aqueous humor + 500 μL MeCN + 30 μL 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 μL mobile phase by swirl-mixing for 1 min, centrifuge at 3000 g for 20 s, reduce volume to 20-30 μL, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.5 5 μm 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: 6.04
Internal standard: naproxen (3.89)
Limit of detection: 0.4 ng

OTHER SUBSTANCES
Extracted: diclofenac, 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: aqueous humor

Sample preparation: 100 μL Aqueous humor + 500 μL MeCN + 30 μL 400 ng/mL (+)-naproxen, mix mechanically for 90 s, centrifuge at 3000 g for 20 min. Remove supernatant and dry it under nitrogen at room temperature. Dissolve residue in 50 μL mobile phase by swirl mixing for 1 min, centrifuge at 3000 g for 20 s. For concentrations of < 20 ng/mL, reduce volume to 20-30 μL under nitrogen.

HPLC VARIABLES

Column: 150 × 4.5 5 μm Ultrasphere octyl

Mobile phase: 505 mL MeCN containing 0.65 mL triethylamine + 495 mL 1.65% glacial acetic acid, apparent pH 4.35

Column temperature: 30

Flow rate: 1

Injection volume: 20

Detector: UV 280

CHROMATOGRAM

Retention time: 6.04

Internal standard: naproxen (3.89)

Limit of detection: 4 ng/mL

OTHER SUBSTANCES

Extracted: diclofenac

Simultaneous: bacitracin, cortisone, diazepam, fluorometholone, hydrocortisone, imipramine, indomethacin, ketoprofen, ketorolac, levobunolol, meclofenamic acid, metipranolol, neomycin, prednisolone, proraracaine, propranolol, salicylic acid, sulfacetamide, suprofen

Noninterfering: acebutolol, acetaminophen, acetazolamide, alprenolol, apraclonidine, atenolol, atropine, betamethasone, betaxolol, bupivacaine, caffeine, cyclopentolate, dexamethasone, diphenhydramine, erythromycin, haloperidol, lidocaine, phenylephrine, polymyxin B, procaine, scopolamine, timolol, tropicamide.

KEY WORDS

rabbit; human

REFERENCE

Riegel,M.; Ellis,P.P. High-performance liquid chromatography assay for antiinflammatory agents diclofenac and flurbiprofen in ocular fluids, *J.Chromatogr.B*, **1994**, *654*, 140–145.

SAMPLE

Matrix: blood

Sample preparation: Add 125 μL MeCN to 50 μL plasma, mix, centrifuge at 9000g for 10 min, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 5 μm C18 (Brownlee)

Column: 250 × 4.6 5 μm RP C18 (Hibar, Merck, Germany)

Mobile phase: MeCN:water:phosphoric acid 60:40:0.05

Flow rate: 1.5

Injection volume: 10

Detector: F ex 250 em 285

CHROMATOGRAM

Retention time: 3.4

Limit of detection: 50 ng/mL

KEY WORDS

rat; plasma; pharmacokinetics

REFERENCE
Park,K.-M.; Gao,Z.-G.; Kim,C.-K. Assay of flurbiprofen in rat plasma using HPLC with fluorescence detection, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 1849–1855.

SAMPLE
Matrix: blood
Sample preparation: Mix 500 μL plasma with 50 μL 200 μg/mL IS in MeOH. Add 500 μL 1 M HCl, extract with 10 mL dichloromethane. Separate the organic layer and evaporate it under a stream of nitrogen at 30°. Reconstitute the residue with 500 μL mobile phase. Inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 10 μm C18 μBondapak
Mobile phase: MeCN:MeOH:1% pH 5.8 glacial acetic acid 19:19:62
Flow rate: 1.8
Injection volume: 50
Detector: UV 260

CHROMATOGRAM
Retention time: 5.65
Internal standard: 2-(2'-chloro-4-biphenyl)propionic acid (7.99)
Limit of detection: 500 ng/mL

KEY WORDS
plasma

REFERENCE
Pargal,A.; Kelkar,M.G.; Nayak,P.J. The effect of food on the bioavailability of ibuprofen and flurbiprofen from sustained release formulations, *Biopharm.Drug Dispos.*, **1996**, *17*, 511–519.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 1 mL water + ibuprofen (15 μg per 100 μL) + 2 mL 10% trichloroacetic acid, mix, add 5 mL hexane, mix for 15 min, centrifuge at 800 g for 5-10 min, repeat extraction. Combine hexane layers and evaporate them under a stream of nitrogen at 37-40°. Reconstitute with 300 μL chloroform, add 200 μL 65 mg/mL 1,1'-carbonyldiimidazole in chloroform, let stand 5-10 min at room temperature, add 10 μL glacial acetic acid, vortex briefly, let stand 5-10 min at room temperature, add 50 μL S-(α)-methylbenzylamine, mix briefly, let stand for 30 min at room temperature, add 3 mL 0.5 M ammonium hydroxide, add 5 mL hexane, mix gently for 15 min. Remove hexane and wash it with 3 mL 1 M HCl, 3 mL 0.5 M ammonium hydroxide, and 3 mL 1 M HCl (with 15 min mixing each time). Evaporate hexane under a stream of nitrogen at 37°, dissolve in 150 μL mobile phase, inject 25 μL aliquot.

HPLC VARIABLES
Guard column: Brownlee RP18
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:water 62:38
Flow rate: 1
Injection volume: 25
Detector: UV 245

CHROMATOGRAM
Retention time: 12 (S), 14 (R)
Internal standard: ibuprofen (17 S, 19 R)
Limit of detection: 10 ng/mL
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
plasma; derivatization; chiral

REFERENCE

Knadler,M.P.; Hall,S.D. High-performance liquid chromatographic analysis of the enantiomers of flurbiprofen and its metabolites in plasma and urine, *J.Chromatogr.*, **1989**, *494*, 173–182.

SAMPLE

Matrix: blood

Sample preparation: 500 μL Plasma + 1.25 μg S-Naproxen + 200 μL 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 μL 30 mM pH 7.5 phosphate buffer, inject 50-100 μL aliquot

HPLC VARIABLES

Column: 100 × 4 5 μm Grom AGP

Mobile phase: 2-Propanol:20 mM pH 6.5 phosphate buffer 5:95 containing 1 mM dimethyloctylamine

Column temperature: 15

Flow rate: 0.8

Injection volume: 50-100

Detector: UV 246

CHROMATOGRAM

Retention time: 7.6 (R), 15.2 (S)

Internal standard: S-naproxen (5.2)

Limit of quantitation: 50 ng/mL

KEY WORDS

plasma; chiral

REFERENCE

Geisslinger,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: 500 μL Plasma + 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

OTHER SUBSTANCES

Extracted: ibuprofen

KEY WORDS

plasma; flurbiprofen is IS

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: 1 mL Plasma + 100 μL 1 M HCl + 100 μL 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 μL 10 mM NaOH, sonicate for 3 min, add 50 μL 100 mM pH 7.0 phosphate buffer containing 0.1% dimethyloctylamine, sonicate for 3 min, inject a 5 μL 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 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: 9.5, 11.6 (enantiomers)
Internal standard: flurbiprofen

OTHER SUBSTANCES
Extracted: ibuprofen

KEY WORDS
plasma; chiral; flurbiprofen is IS

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 α1-acid glycoprotein chiral stationary phase, *J.Liq.Chromatogr.*, **1994**, *17*, 2127–2145.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + + 25 μL 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 μL 5 mg/mL 5-bromoacetyl acenaphthene in MeCN, add 10 μL 3% triethylamine in MeCN, vortex for 30 s, heat at 75° for 5 min, evaporate to dryness under reduced pressure, reconstitute with 25 μL MeCN, inject a 20 μL aliquot. (Prepare 5-bromoacetyl acenaphthene as follows. Add 43 g bromoacetyl chloride 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 × 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 × 4.6 5 μm Hypersil C18
Mobile phase: MeCN:water 90:10
Flow rate: 1
Injection volume: 20
Detector: F ex 250 em 450

CHROMATOGRAM
Internal standard: flurbiprofen

OTHER SUBSTANCES
Extracted: ibuprofen

KEY WORDS
rat; plasma; protect from light; derivatization; flurbiprofen is IS

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 μL Plasma + 125 μL 40 mM decanoic acid in MeCN, mix. Dialyze a 100 μL 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 × 2 40 μm Bondesil C18 (Analytichem); B 250 × 3.1 5 μm 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 247

CHROMATOGRAM
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: fenoprofen (UV 272), ibuprofen (UV 264), 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: 500 μL Plasma + 100 μL 25 μg/mL indomethacin + 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 μL 60 mM ethyl chloroformate in MeCN, vortex for 30 s, add 50 μL 100 mM L-leucinamide in MeOH:triethylamine 100:14, let stand for 2 min, add 50 μL water, inject a 10-50 μL aliquot of the reaction mixture.

HPLC VARIABLES
Column: 250 × 4.6 5 μm 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: 3.5 (R-(-)), 4.4 (S-(+))
Internal standard: indomethacin (5.3)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: ibuprofen (UV 225), ketoprofen

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

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 247

CHROMATOGRAM
Retention time: 8.01
Limit of detection: <120 ng/mL

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

mine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

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

SAMPLE

Matrix: blood, milk

Sample preparation: 200 μL Plasma or milk + 20 μL 30% perchloric acid + 200 μL 2 μg/mL IS in MeOH, vortex for 2 min, add 20 μL 5 M NaOH, centrifuge at 5000 g. Remove a 200 μL aliquot of supernatant and add it to 200 μL mobile phase, mix, centrifuge, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: Waters C18 Guard Pak
Column: 100 × 8 10 μm μBondapak C8 Radial Pak
Mobile phase: MeCN:MeOH:1% acetic acid 30:30:40
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 6
Internal standard: 2-(2'-chloro-4-biphenylyl)propionic acid (7)
Limit of detection: 50 ng/mL

KEY WORDS

plasma

REFERENCE

Johnson,V.A.; Wilson,J.T. Flurbiprofen analysis in plasma and breast milk by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *382*, 367–371.

SAMPLE

Matrix: blood, synovial fluid

Sample preparation: 0.5 mL Plasma or synovial fluid + 200 μL 2 M HCl + 5 mL hexane, tumble 10 min on a rotary mixer, centrifuge at 10 000 g for 5 min. Remove organic layer and evaporate it to dryness under vacuum centrifugation. Reconstitute residue in 150 μL MeOH + 100 μL water, vortex mix, inject aliquot.

HPLC VARIABLES

Guard column: 20 × 2 Perisorb RP18 30-40 μm pellicular
Column: 125 × 4.6 5 μm Spherisorb ODS 1
Mobile phase: MeOH:water 63:37 adjusted to pH 3.3 with phosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 9
Internal standard: flurbiprofen
Limit of detection: <100 ng/mL

OTHER SUBSTANCES

Extracted: diclofenac

KEY WORDS
plasma; flurbiprofen is IS

REFERENCE
Blagbrough,I.S.; Daykin,M.M.; Doherty,M.; Pattrick,M.; Shaw,P.N. High-performance liquid chromatographic determination of naproxen, ibuprofen and diclofenac in plasma and synovial fluid in man, *J.Chromatogr.*, **1992**, *578*, 251–257.

SAMPLE
Matrix: blood, urine
Sample preparation: 100 μL Serum or urine + 50 μL 1 M NaOH, let stand at room temperature for 20 min (to hydrolyse conjugates), add 50 μL 1 M HCl, add 1 mL 1 μg/mL IS in MeCN, add 2 mL 50 mM pH 2.6 potassium phosphate buffer, centrifuge at 2000 rpm for 10 min, inject 100 μL aliquot of supernatant.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm Brownlee RP-8
Column: 300 × 3.9 μBondapak C18
Mobile phase: THF:50 mM pH 2.6 potassium phosphate 45:55
Flow rate: 1.9
Injection volume: 100
Detector: F ex 360 em 320

CHROMATOGRAM
Retention time: 10
Internal standard: (RS)-2-(2-methoxy-4-biphenyl)propionic acid (8)
Limit of quantitation: 100 ng/mL

KEY WORDS
serum

REFERENCE
Adams,W.J.; Bothwell,B.E.; Bothwell,W.M.; VanGiessen,G.J.; Kaiser,D.G. Simultaneous determination of flurbiprofen and its major metabolite in physiological fluids using liquid chromatography with fluorescence detection, *Anal.Chem.*, **1987**, *59*, 1504–1509.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Urine + 100 μL 1 M NaOH (to hydrolyze conjugates), vortex. 500 μL Plasma or 600 μL basified urine + 200 μL 600 mM sulfuric acid, vortex, add 50 μL 100 μg/mL ketoprofen in water + 3 mL isooctane:isopropanol 96:5, mix vigorously for 45 s, centrifuge at 3000 rpm for 5 min. Remove the top layer and add it to 3 mL water, mix vigorously, centrifuge for 5 min, discard the organic layer. Add the aqueous layer to 350 μL 600 mM sulfuric acid, add 3 mL chloroform, mix, centrifuge for 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 100 μL 50 mM triethylamine in MeCN, after 30 s add 50 μL 60 mM ethylchloroformate in MeCN, after 30 s add 50 μL 1 M L-leucinamide, after 2 min add 50 μL water, inject a 10-50 μL aliquot.

HPLC VARIABLES
Guard column: 50 mm long 10 μm Partisil 5 ODS-3
Column: 100 × 4.6 5 μm Partisil 5 ODS-3
Mobile phase: MeCN:67 mM KH_2PO_4:triethylamine 35:65:0.02
Flow rate: 1
Injection volume: 10-50
Detector: UV 250

CHROMATOGRAM
Retention time: 17 (-), 21 (+)
Internal standard: ketoprofen (UV 275) (8 (-), 10 (+))
Limit of quantitation: 250 ng/mL (urine), 100 ng/mL (plasma)

KEY WORDS
plasma; derivatization; pharmacokinetics; chiral

REFERENCE
Berry,B.W.; Jamali,F. Stereospecific high-performance liquid chromatographic (HPLC) assay of flurbiprofen in biological specimens, *Pharm.Res.*, **1988**, *5*, 123–125.

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

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

CHROMATOGRAM
Retention time: 21.337

KEY WORDS
whole blood

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

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. Activate an SPE cartridge filled with 100 mg 40-63 μm silica gel (Merck) with 1 mL MeOH and dry in a hot air oven at 100° for 1 h, equilibrate with 1 mL dichloromethane before use. 500 μL Serum + 100 μL 1 M HCl, mix, add 1 mL 1 M pH 3.8 sodium phosphate buffer, mix, add 3 mL diethyl ether, rock for 20 min, centrifuge at 1000 g for 2 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL 1 mg/mL 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in dichloromethane, add 100 μL 1 mg/mL 1-hydroxybenzotriazole in dichloromethane, add 100 μL 1 mg/mL (R)-(+)-1-(1-naphthyl)ethylamine in dichloromethane, vortex briefly, let stand at room temperature for 2 h, add to the SPE cartridge, elute with two 1 mL portions of dichloromethane:MeCN 90:10. Combine all the eluate and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot. Urine. Activate an SPE cartridge filled with 100 mg 40-63 μm silica gel (Merck) with 1 mL MeOH and dry in a hot air oven at 100° for 1 h, equilibrate with 1 mL dichloromethane before use. 500 μL Urine + 100 μL 1 M HCl, mix, add 1.5 mL 1 M pH 3.8 sodium phosphate buffer, mix, add 5 mL hexane:isopropanol 90:10, rock for 20 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 100 μL 1 mg/mL 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in dichloromethane, add 100 μL 1 mg/mL 1-hydroxybenzotriazole in dichloromethane, add 100 μL 1 mg/mL (R)-(+)-1-(1-naphthyl)ethylamine in dichloromethane,

vortex briefly, let stand at room temperature for 2 h, add to the SPE cartridge, elute with two 1 mL portions of dichloromethane:MeCN 90:10. Combine all the eluate and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 2.1 40-63 μm pellicular C18 (Alltech)
Column: 150 × 3.9 5 μm Resolve C18 (Waters)
Mobile phase: MeCN:10 mM pH 3.5 phosphate buffer 50:50
Flow rate: 1.5
Injection volume: 50
Detector: F ex 290 em 330

CHROMATOGRAM
Retention time: 14.5 (R), 17.8 (S)
Internal standard: flurbiprofen

OTHER SUBSTANCES
Interfering: ibuprofen (a metabolite of ibuprofen interferes with the R enantiomer)

KEY WORDS
flurbiprofen is IS; derivatization; chiral; serum; SPE

REFERENCE
Tan,S.C.; Jackson,S.H.D.; Swift,C.G.; Hutt,A.J. Enantiospecific analysis of ibuprofen by high performance liquid chromatography: Determination of free and total drug enantiomer concentrations in serum and urine, *Chromatographia*, **1997**, *46*, 23–32.

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' 2.27 (for first enantiomer)

OTHER SUBSTANCES
Also analyzed: carprofen, cicloprofen, etodolac, fenoprofen, ibuprofen, ketoprofen, naproxen, pirprofen, tiaprofenic acid

KEY WORDS
derivatization; $\alpha = 1.26$; 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: cell suspensions
Sample preparation: Centrifuge cell suspension at 2000 g for 4 min. Remove a 2 mL aliquot of the supernatant and add it to 200 μL 200 μg/mL IS in DMF, mix, add 200 μL 5 M HCl, extract

twice with 3 mL portions of toluene. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL dichloromethane, add 20 μL 10 mg/mL 1-hydroxybenzotriazole in dichloromethane:pyridine 99:1, add 300 μL 10 mg/mL 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in dichloromethane, add 300 μL 10 mg/mL (-)-(S)-α-methylbenzylamine in dichloromethane, let stand for 30 min, evaporate to dryness, reconstitute with 500 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 10 mm long Techsphere ODS (HPLC Technology, Macclesfield UK)
Column: 250 × 5 5 μm Techsphere ODS (HPLC Technology, Macclesfield UK)
Mobile phase: MeCN:7.5 mM NaH_2PO_4 60:40, containing 5 mM sodium pentanesulfonate, pH adjusted to 2.8 with phosphoric acid
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 4.75, 5.35 (enantiomers)
Internal standard: (S)-naproxen (k' 3.15)
Limit of detection: 500 ng/mL

KEY WORDS
derivatization; chiral

REFERENCE
Thomason,M.J.; Hung,Y.-F.; Rhys-Williams,W.; Hanlon,G.W.; Lloyd,A.W. Indirect enantiomeric separation of 2-arylpropionic acids and structurally related compounds by reversed phase HPLC, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1765–1774.

SAMPLE
Matrix: dialysate
Sample preparation: Inject a 10 μL aliquot of dialysate (pH 7.4 isotonic phosphate buffer) containing 250 ng/mL naproxen.

HPLC VARIABLES
Guard column: 37-50 μm Corasil C18
Column: 100 × 4 5 μm Nucleosil C18
Mobile phase: MeCN:50 mM pH 3.0 phosphate buffer 48:52
Flow rate: 1.1
Injection volume: 10
Detector: F ex 258 em 310

CHROMATOGRAM
Retention time: 4
Internal standard: naproxen (F ex 262 em 356) (2.5)

KEY WORDS
mouse; rat; pharmacokinetics

REFERENCE
Evrard,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: microsomal incubations
Sample preparation: 300 μL Microsomal incubation + 20 μL 6 M HCl, centrifuge at 3000 g for 10 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: μBondapak C18
Column: 250 × 4 5 μm Lichrospher RP-18

Mobile phase: MeCN:water:trifluoroacetic acid 65:145:0.08
Column temperature: 37
Flow rate: 1.5
Injection volume: 100
Detector: UV 273

CHROMATOGRAM
Retention time: 70
Internal standard: 1-naphthol-β-D-glucuronide

OTHER SUBSTANCES
Extracted: metabolites glucuronides

KEY WORDS
rat; human; liver

REFERENCE
Hamdoune,M.; Mounie,J.; Magdalou,J.; Masmoudi,T.; Goudonnet,H.; Escousse,A. Characterization of the in vitro glucuronidation of flurbiprofen enantiomers, *Drug Metab.Dispos.*, **1995**, *23*, 343–348.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb S10-ODS2
Mobile phase: MeCN:water:acetic acid 60:35:0.5
Flow rate: 1
Injection volume: 20
Detector: UV 280

OTHER SUBSTANCES
Simultaneous: mefenamic acid

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

SAMPLE
Matrix: solutions
Sample preparation: Dilute in an appropriate solvent, inject an aliquot.

HPLC VARIABLES
Guard column: RC18 Guardpak (Waters)
Column: 150 × 4.5 5 μm Altex C18
Mobile phase: MeOH:water:acetic acid 67:32.5:0.5
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Retention time: 8

REFERENCE
Richman,J.B.; Tang-Liu,D.D.-S. A corneal perfusion device for estimating ocular bioavailability in vitro, *J.Pharm.Sci.*, **1990**, *79*, 153–157.

SAMPLE
Matrix: solutions
Sample preparation: Mix 100 μL of a 1-200 μM solution of carboxylic acid in dichloromethane with 100 μL 800 μM (-)-APMB in dichloromethane, 100 μL 1.6 mM 2,2'-dipyridyl disulfide in dichloromethane, and 100 μL 1.6 mM triphenylphosphine in dichloromethane, let stand at

room temperature for 20 min. Evaporate to dryness under a stream of nitrogen, reconstitute the residue in 400 μL mobile phase, inject a 10 μL 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 ($CH_3COC_6H_4C(=NH)OC_2H_5.HCl$) by passing dry hydrogen chloride into a mixture of 4-acetylbenzonitrile 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°).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm TSK gel ODS-80TM (Tosoh)
Mobile phase: MeCN:water:acetic acid 60:40:0.1
Flow rate: 1
Injection volume: 10
Detector: F ex 320 em 380

CHROMATOGRAM

Retention time: 16 (S), 18 (R)
Limit of detection: 10 fmole

OTHER SUBSTANCES

Simultaneous: ibuprofen, naproxen

KEY WORDS

derivatization; chiral

REFERENCE

Kondo,J.; Imaoka,T.; Kawasaki,T.; Nakanishi,A.; Kawahara,Y. Fluorescence derivatization reagent for resolution of carboxylic enantiomers by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *645*, 75–81.

SAMPLE

Matrix: solutions
Sample preparation: Mix 100 μL of a solution of the carboxylic acid in MeCN with 100 μL 2 mM (S)-1-(4-dansylaminophenyl)ethylamine in MeCN, add 100 μL 3 mM 2,2'-dipyridyl disulfide (Aldrithiol-2) in MeCN, add 100 μL 3 mM triphenylphosphine in MeCN, vortex, let stand at room temperature for 3 h, inject a 5 μL aliquot. (Synthesis of (S)-1-(4-dansylaminophenyl)ethylamine is as follows. Add 2.2 g di-tert-butyl dicarbonate dropwise to a stirred solution of 2 g (S)-α-methyl-4-nitrobenzylamine hydrochloride ((S)-1-(4-nitrophenyl)ethylamine hydrochloride) and 1.1 g triethylamine in 20 mL MeCN at 0°, stir at room temperature for 1 h, evaporate to dryness under reduced pressure. Dissolve the residue in 50 mL ethyl acetate and wash with 10% aqueous citric acid, wash with water, dry over anhydrous sodium sulfate,

evaporate to dryness under reduced pressure to give (S)-N-tert-butoxycarbonyl-1-(4-nitrophenyl)ethylamine as white crystals (mp 86-89°). Add 200 mg 5% PdC to a solution of 2 g (S)-N-tert-butoxycarbonyl-1-(4-nitrophenyl)ethylamine in 40 mL MeOH, stir, hydrogenate at room temperature for 3 h, filter, evaporate the filtrate to dryness to obtain (S)-N-tert-butoxycarbonyl-1-(4-aminophenyl)ethylamine. Stir 1.4 g (S)-N-tert-butoxycarbonyl-1-(4-aminophenyl) ethylamine in 10 mL MeCN and 50 mL 100 mM pH 9.0 sodium bicarbonate, add a solution of 1.9 g dansyl chloride in 30 mL MeCN dropwise while maintaining the pH of the solution at 9.0 with 1 M NaOH, stir at room temperature for 20 min, stir at 45° for 2 h, cool to room temperature, extract three times with 30 mL portions of ethyl acetate. Combine the organic layers and evaporate them to dryness under reduced pressure, recrystallize the residue from benzene/hexane to obtain (S)-N-tert-butoxycarbonyl-1-(4-dansylaminophenyl)ethylamine as pale-yellow crystals (mp 97-101°) (Caution! Benzene is a carcinogen!). Add 2 mL concentrated HCl to a solution of 1.5 g (S)-N-tert-butoxycarbonyl-1-(4-dansylaminophenyl)ethylamine in 10 mL MeOH, stir at room temperature for 30 min, evaporate to dryness under reduced pressure. Dissolve the residue in 30 mL water and adjust the pH to 8.0 with sodium bicarbonate, extract three times with 30 mL portions of ethyl acetate. Combine the organic layers and evaporate them to dryness under reduced pressure, recrystallize the residue from EtOH to obtain (S)-1-(4-dansylaminophenyl)ethylamine as pale-yellow crystals (mp 157-160°; $[\alpha]_D^{28}$ -11.1° (c = 0.2 in MeCN)). (The (R)-enantiomer can be prepared in an exactly analogous fashion.))

HPLC VARIABLES
Column: 150 × 4.6 5 μm ODS-80TM (Tosoh)
Mobile phase: MeCN:50 mM pH 6.5 sodium acetate buffer 65:35
Flow rate: 1
Injection volume: 5
Detector: F ex 338 em 535

CHROMATOGRAM
Retention time: k' 8.71, k' 10.57 (enantiomers)
Limit of detection: 170 fmole

OTHER SUBSTANCES
Also analyzed: ibuprofen, phenoprofen, 2-phenylpropionic acid, pranoprofen, naproxen

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KEY WORDS
derivatization; chiral

REFERENCE
Iwaki,K.; Bunrin,T.; Kameda,Y.; Yamazaki,M. Resolution and sensitive detection of carboxylic acid enantiomers using fluorescent chiral derivatization reagents by high-performance liquid chromatography, *J.Chromatogr.A*, **1994**, *662*, 87–93.

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: 10, 17 (enantiomers)

OTHER SUBSTANCES
Simultaneous: fenoprofen, ibuprofen, 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

HPLC VARIABLES
Column: Ultrasphere ODS
Mobile phase: MeOH:water:acetic acid 67:32.5:0.5
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Limit of detection: 2 ng

OTHER SUBSTANCES
Simultaneous: flurbiprofen amide

KEY WORDS
rabbit; buffer

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

SAMPLE
Matrix: solutions
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: 27, 35 (enantiomers)

OTHER SUBSTANCES
Also analyzed: benoxaprofen (MeOH:buffer 80:20), carprofen, fenoprofen, ibuprofen, ketoprofen, pirprofen, 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: Mix 1 mL 100 μg/mL compound in dichloromethane with 300 μL 1 mg/mL 1-hydroxybenzotriazole in dichloromethane:pyridine 99:1, 300 μL 1 mg/mL 1-ethyl-3-di-

methylaminopropylcarbodiimide hydrochloride in dichloromethane, and 300 μL 1-1.5 mg/mL benzylamine in dichloromethane, vortex, let stand at room temperature for 1.5 h, wash with 1 mL 250 mM HCl, wash with 1 mL water. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in MeOH, inject an aliquot.

HPLC VARIABLES
Column: 10 μm EXP B101 4-methylbenzoate cellulose on silica gel(Bio-Rad)
Mobile phase: MeOH:50 mM pH 1.5 perchlorate buffer 80:20
Flow rate: 1
Detector: UV 230

CHROMATOGRAM
Retention time: 7, 8

OTHER SUBSTANCES
Simultaneous: ketoprofen

KEY WORDS
derivatization; chiral

REFERENCE
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.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 3 Ecocart LiChrospher 100 RP-18
Mobile phase: Isopropanol:100 mM KH_2PO_4:formic acid 54:100:0.1
Flow rate: 0.6
Detector: UV 254

CHROMATOGRAM
Retention time: 12.0
Limit of quantitation: 200-500 ng/mL

OTHER SUBSTANCES
Simultaneous: acemetacin; diclofenac; 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: 8.01 (A), 8.91 (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, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

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 × 4.6 column containing riboflavin binding proteins (Prepare as follows. Add riboflavin to saturate protein of egg yolk, homogenize with 3 volumes buffer, centrifuge, add the supernatant to a 500 × 30 column of DEAE-cellulose (Whatman) equilibrated with buffer, wash extensively with buffer to remove bound protein, elute riboflavin binding proteins (RFBP) with buffer containing 200 mM NaCl (RFBP has intense yellow color, absorption at 455 nm). Purify RFBP on a Sephadex G-100 column with 50 mM pH 7.5 Tris-HCl buffer as eluent, remove the bound riboflavin by extensive dialysis at pH 3.0. Add 4.5 g N,N-disuccinylimidyl carbonate to 3 g Nucleosil 5NH2 slurried in MeCN, filter, wash with MeCN, wash with 50 mM pH 7.5 phosphate buffer. Suspend 300 mg RFBP in 50 mM phosphate buffer, add the activated silica, mix gently for 2 h using a rotary evaporator, filter, wash with sterile water, wash with isopropanol:water 1:2, pack in a 100 × 4.6 column.) (Buffer was 100 mM pH 5.3 sodium acetate.)
Mobile phase: 50 mM pH 5.5 KH_2PO_4
Flow rate: 0.8
Injection volume: 20
Detector: UV

CHROMATOGRAM

Retention time: k' 6.10

OTHER SUBSTANCES
Simultaneous: isradipine, ketoprofen, nimodipine, suprofen

KEY WORDS
chiral; α = 1.13

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 5 mM flurbiprofen 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 × 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 230, UV 254

CHROMATOGRAM
Retention time: k' 6.46 (first enantiomer)

OTHER SUBSTANCES
Also analyzed: fenoprofen, ibuprofen, ketoprofen, tiaprofenic acid

KEY WORDS
derivatization; narrow-bore; chiral; α=2.18; (see Biomed. Chromatogr. 1995; 9; 292)

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: urine
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL ethyl acetate and dry by aspiration of air. Evaporate an aliquot of 100 µL 20 µg/mL IS in MeOH to dryness at 37°. Add 1 mL urine, vortex, add 250 µL 1 M pH 5.0 acetate buffer, vortex. Add 250 µL of the mixture to the SPE cartridge, dry by aspiration of air, elute with 3 mL ethyl acetate, evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 µL mobile phase, inject a 10-30 µL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 µm Inertsil ODS-2
Mobile phase: MeCN:50 mM pH 5.0 phosphate buffer 42:58
Flow rate: 0.9
Injection volume: 10-30
Detector: UV 230

CHROMATOGRAM
Retention time: 15
Internal standard: indomethacin (18.5)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: diclofenac, ibuprofen, felbinac, fenbufen, ketoprofen, loxoprofen, mefenamic acid, naproxen, piroxicam, sulindac

KEY WORDS
SPE

REFERENCE
Hirai,T.; Matsumoto,S.; Kishi,I. Simultaneous analysis of several non-steroidal anti-inflammatory drugs in human urine by high-performance liquid chromatography with normal solid-phase extraction, *J.Chromatogr.B*, **1997**, *692*, 375–388.

SAMPLE
Matrix: urine
Sample preparation: 1 mL Urine + 1 mL 6 M HCl, heat at 90° for 30 min, cool to room temperature, add ibuprofen (15 μg per 100 μL), add 5 mL dichloroethane, mix 15 min, centrifuge at 800 g for 5-10 min. Evaporate organic layer under a stream of nitrogen at 37-40°. Reconstitute with 300 μL chloroform, add 200 μL 65 mg/mL 1,1'-carbonyldiimidazole in chloroform, let stand 5-10 min at room temperature, add 10 μL glacial acetic acid, vortex briefly, let stand 5-10 min at room temperature, add 50 μL S-(α)-methylbenzylamine, mix briefly, let stand for 30 min at room temperature, add 3 mL 0.25 M ammonium hydroxide, add 5 mL dichloroethane, mix gently for 15 min. Remove organic layer and wash it with 3 mL 0.25 M ammonium hydroxide and twice with 3 mL 1 M HCl (with 15 min mixing each time). Evaporate organic layer under a stream of nitrogen at 37°, dissolve in 150 μL MeCN:water 45:55, inject 20-30 μL aliquot.

HPLC VARIABLES
Guard column: Brownlee RP18
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:50 mM acetic acid 55:45
Flow rate: 1
Injection volume: 20-30
Detector: F ex 200 em 320 (cut-off) (flurbiprofen), UV 232 (ibuprofen)

CHROMATOGRAM
Retention time: 17 (S), 19 (R)
Internal standard: ibuprofen (24 S, 27 R)
Limit of detection: 10 ng/mL
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
derivatization; chiral

REFERENCE
Knadler,M.P.; Hall,S.D. High-performance liquid chromatographic analysis of the enantiomers of flurbiprofen and its metabolites in plasma and urine, *J.Chromatogr.*, **1989**, *494*, 173–182.

SAMPLE
Matrix: urine
Sample preparation: Dilute urine 20-fold with 100 mM pH 2.0 phosphate buffer, extract twice with two volumes of ethyl acetate, centrifuge at 5000 g for 5 min. Combine the organic layers and evaporate them to dryness under a stream of nitrogen below 30°. Reconstitute in 0.2-1 mL mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100 RP-18
Column: 250 × 4 5 μm LiChrospher CH-18

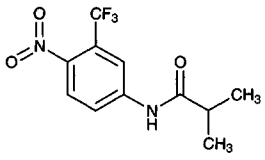
Mobile phase: MeOH:10 mM pH 6.0 phosphate buffer 80:20 containing 2.5 mM cethexonium bromide (Rinse with 100 mL MeOH:EtOH:water 50:25:25 at the end of the day.)
Flow rate: 1
Injection volume: 10
Detector: UV 273

CHROMATOGRAM
Retention time: k' 4.1

OTHER SUBSTANCES
Extracted: glucuronides, pirprofen

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.

Flutamide



Molecular formula: $C_{11}H_{11}F_3N_2O_3$
Molecular weight: 276.22
CAS Registry No.: 13311-84-7
Merck Index: 4242
Lednicer No.: 3 57

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

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

CHROMATOGRAM
Retention time: 22.248

KEY WORDS
whole blood

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

SAMPLE
Matrix: microsomal incubations
Sample preparation: Vigorously mix 500 μL microsomal incubation with 5 mL dichloromethane. Evaporate the dichloromethane layer under a stream of nitrogen at 30°. Reconstitute the residue in 100 μL MeOH. Inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 CP-18 μBondapak
Mobile phase: Gradient. A was MeOH:water 60:40. B was MeOH. A:B from 100:0 to 60:40 over 40 min, flush column with MeOH for 10 min, re-equilibrate at initial conditions
Flow rate: 1
Detector: UV; Radioactivity (Radiometer Flo-1)

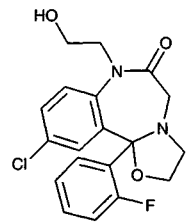
CHROMATOGRAM
Retention time: 18.3

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
radiolabeled

REFERENCE
Shet,M.D.; McPhaul,M.; Fisher,C.W.; Stallings,N.R.; Estabrook,R.W. Metabolism of the antiandrogenic drug (flutamide) by human CYP1A2, *Drug Metab.Dispos.*, **1997**, *25*, 1298–1303.

Flutazolam



Molecular formula: $C_{19}H_{18}ClFN_2O_3$
Molecular weight: 376.81
CAS Registry No.: 27060-91-9
Merck Index: 4243

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 20 μL 20 μg/mL IS + 200 μL 1 M potassium carbonate + 3 mL chloroform, mix for 2 min, centrifuge at 1200 g for 5 min, aspirate aqueous phase. Evaporate the organic phase in a water bath under a stream of nitrogen at 40°. Dissolve residue in 100 μL mobile phase, inject a 20 μL aliquot. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES
Column: 100 × 4.6 2 μm TSK gel Super-ODS (A) or 100 × 4.6 5 μm Hypersil ODS-C18 (B)
Mobile phase: MeCN:5 mM pH 6 NaH_2PO_4 45:55
Flow rate: 0.65
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 32.2 (A), 97.4 (B)
Internal standard: diazepam (29.8 (A), 77.5 (B))
Limit of quantitation: 10 ng/mL (A)

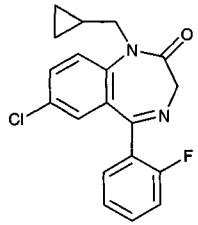
OTHER SUBSTANCES
Extracted: bromazepam, chlordiazepoxide, clonazepam, estazolam, etizolam, haloxazolam, lorazepam, nitrazepam, oxazolam, triazolam
Simultaneous: alprazolam

Noninterfering: barbital, carbamazepine, cloxazolam, ethosuximide, hexobarbital, mexazolam, oxazepam, pentobarbital, phenobarbital, phenytoin, primidone, trimethadione

KEY WORDS
serum

REFERENCE
Tanaka,E.; Terada,M.; Misawa,.; Wakasugi,C. Simultaneous determination of twelve benzodiazepines in human serum using a new reversed-phase chromatographic column on a 2-μm porous microspherical silica gel, *J.Chromatogr.B*, **1996**, *682*, 173–178.

Flutoprazepam



Molecular formula: $C_{15}H_{16}ClFN_2O$
Molecular weight: 342.80
CAS Registry No.: 25967-29-7
Merck Index: 4245

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 25 μL 1 μg/mL o-chlorodiazepam in MeOH, vortex gently, extract twice with 1 mL portions of benzene (Caution! Benzene is a carcinogen!) with shaking. Combine the extracts evaporate them to dryness under a stream of nitrogen at 30-35°, reconstitute the residue in 100 μL mobile phase, inject a 10-90 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:10 mM KH_2PO_4 50:50 adjusted to pH 4.5 with orthophosphoric acid
Flow rate: 1.2
Injection volume: 10-90
Detector: UV 229

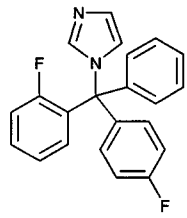
CHROMATOGRAM
Retention time: 16
Internal standard: o-chlorodiazepam
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Conti,I.; Sarati,S.; Caccia,S. Propranolol does not alter flutoprazepam kinetics and metabolism in the rat, *Eur.J.Drug Metab.Pharmacokinet.*, **1991**, *16*, 53–58.

Flutrimazole



Molecular formula: $C_{22}H_{16}F_2N_2$
Molecular weight: 346.38
CAS Registry No.: 119006-77-8
Merck Index: 4247

SAMPLE
Matrix: formulations, tissue
Sample preparation: Formulations. 500 mg Cream + 30 mL EtOH, heat mixture to 50° to melt it, cool, dilute to 50 mL, filter through a 0.45 μm filter. Inject a 25 μL aliquot of the filtrate. Tissue. Add 3 mL MeOH to cleaned and cut skin, sonicate for 15 min, inject an aliquot.

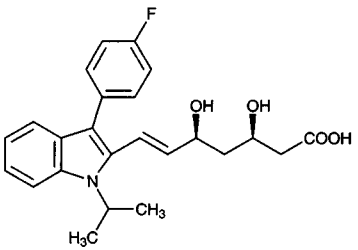
HPLC VARIABLES
Guard column: 10 μm μBondapak C18
Column: 10 μm μBondapak C18
Mobile phase: MeOH:pH 7.0 phosphate buffer 80:20
Flow rate: 1
Injection volume: 25
Detector: UV 225

CHROMATOGRAM
Limit of quantitation: 50 ng/mL

KEY WORDS
cream; skin

REFERENCE
Ramis,J.; Conte,L.; Segado,X.; Forn,J.; Lauroba,J.; Calpena,A.; Escribano,E.; Domenech,J. Influence of formulation on the in vitro transdermal penetration of flutrimazole, *Arzneimittelforschung*, **1997**, *47*, 1139–1144.

Fluvastatin



Molecular formula: $C_{24}H_{26}FNO_4$
Molecular weight: 411.47
CAS Registry No.: 93957-54-1, 93957-55-2 (sodium salt)
Merck Index: 4250
Lednicer No.: 5 105

SAMPLE
Matrix: blood
Sample preparation: Mix 500 μL plasma with 500 μL MeCN and 500 μL pH 6.0 phosphate buffer, extract with 5 mL MTBE by shaking for 30 min, centrifuge at 1200 g for 5 min, evaporate the organic phase under nitrogen. Dissolve the residue in 400 μL MeCN:water 40:60, vortex 3 times for 30 s, inject a 20-300 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Brownlee Si
Column: 250 × 4.6 10 μm Chiralcel OD-R (Daicel Chemical Industries, Japan)
Mobile phase: MeCN:buffer 40:60 (Buffer was pH 2.5 phosphate buffer (I = 0.04).)
Column temperature: 15
Flow rate: 0.5

Injection volume: 100
Detector: F ex 305 em 390

CHROMATOGRAM
Retention time: 28.0 (3S, 5R), 30.0 (3R, 5S)
Limit of quantitation: 5 nM

KEY WORDS
chiral; plasma

REFERENCE
Toreson,H.; Eriksson,B.-M. Determination of fluvastatin enantiomers and the racemate in human blood plasma by liquid chromatography and fluorimetric detection, *J.Chromatogr.A*, **1996**, *729*, 13–18.

SAMPLE
Matrix: blood
Sample preparation: Mix 500 μL plasma with 100 μL 500 nM IS, 500 μL MeCN, and 500 μL pH 6.0 phosphate buffer, extract with 5 mL MTBE by shaking for 30 min, centrifuge at 1200 g for 5 min, evaporate the organic phase under nitrogen. Dissolve the residue in 400 μL mobile phase, vortex 3 times for 30 s, inject an aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Brownlee CN
Column: 150 × 4.6 5 μm Zorbax Rx-C8
Mobile phase: MeOH:pH 6.0 phosphate buffer:100 mM tetrabutylammonium fluoride 60:25:15
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: F ex 305 em 390 following post-column photolysis. The column effluent flowed through a knitted 10 m × 0.3 mm PTFE coil irradiated at 254 nm to the detector.

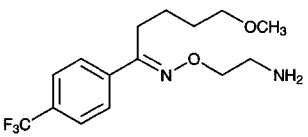
CHROMATOGRAM
Retention time: 15.0
Internal standard: Sandoz compound 63-267 ([R,S,-(E)-] (±)-7-(3-(4-fluorophenyl)-1-(1-methylethyl)-1H-indol-2yl)-(3,5-dihydroxy-6-methyl)-6-heptenoic acid, monosodium salt) (20.5)
Limit of quantitation: 500 pM

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; post-column reaction; post-column photochemical derivatization

REFERENCE
Toreson,H.; Eriksson,B.-M. Determination of fluvastatin enantiomers and the racemate in human blood plasma by liquid chromatography and fluorimetric detection, *J.Chromatogr.A*, **1996**, *729*, 13–18.

Fluvoxamine



Molecular formula: $C_{15}H_{21}F_3N_2O_2$
Molecular weight: 318.34
CAS Registry No.: 54739-18-3
Merck Index: 4251

SAMPLE
Matrix: blood

Sample preparation: 2 mL Plasma + 100 μL 900 ng/mL IS + 4 mL 300 mM trisodium phosphate, extract with 400 μL diisopropyl ether for 20 min, centrifuge. Evaporate upper organic layer to dryness under a stream of nitrogen, dissolve residue in 100 μL MeCN , inject a 50 μL aliquot. (Caution! Diisopropyl ether readily forms explosive peroxides!)

HPLC VARIABLES
Column: 150 × 4.6 3 μm Supelcosil LC-SI
Mobile phase: MeCN:MeOH:concentrated ammonia 87.5:12.0:0.5
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 2.1
Internal standard: 5-(pyrrolidinylpropyliden)-10,11-dihydro-5H-dibenzo[a,d]cyclohepten (4.1)
Limit of detection: 2.2 nM

OTHER SUBSTANCES
Noninterfering: amitriptyline, citalopram, clomipramine, desmethylclomipramine, imipramine, nortriptyline, risperidone

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KEY WORDS
plasma; pharmacokinetics

REFERENCE
Carrillo,J.A.; Dahl,M.-L.; Svensson,J.-O.; Alm,C.; Rodríguez,I.; Bertilsson,L. Disposition of fluvoxamine in humans is determined by the polymorphic CYP2D6 and also by the CYP1A2 activity, *Clin.Pharmacol.Ther.*, **1996**, *60*, 183–190.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 20 μL 10 μg/mL clovoxamine + 120 μL 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 μL 100 mM HCl, shake for 20 min, centrifuge at 3000 g for 10 min, inject a 20 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 120 × 4.6 5 μm 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: 4.3
Internal standard: clovoxamine (3.3)
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Simultaneous: amitriptyline, chlorimipramine, desipramine, doxepin, imipramine, nortriptyline, trimipramine

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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: 2 mL Plasma + 200 μL 1 μg/mL metapramine in MeOH + 2 mL 1 M pH 10.0 phosphate buffer + 6 mL diethyl ether:hexane 50:50, shake for 15 min, centrifuge at 4000 g for 5 min. Remove the organic layer and add it to 2 mL 62.5 mM sulfuric acid, vortex for 5 min, centrifuge at 4000 g for 5 min. Remove the aqueous phase and add it to 1 mL 500 mM NaOH, vortex, add 6 mL hexane:diethyl ether 50:50, shake for 10 min, centrifuge at 4000 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 mM sodium carbonate, add 10 μL 10 mg/mL dansyl chloride in acetone, vortex for 1 min, heat at 45° for 30 min, evaporate under a stream of nitrogen at 50°. Reconstitute the residue in 200 μL MeCN:water 45:55, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.6 5 μm Hypersil ODS

Mobile phase: Gradient. MeCN:water from 45:55 to 65:35 over 10 min, maintain at 65:35 for 20 min

Column temperature: 30

Flow rate: 1.5

Injection volume: 100

Detector: F (Fluorichrom ex 7.54 and 7.60 filters, em 3.71 and 4.76 filters

CHROMATOGRAM

Retention time: 19

Internal standard: metapramine (28)

Limit of detection: 1.5 ng/mL

OTHER SUBSTANCES

Noninterfering: alimemazine, alprazolam, amineptine, amitriptyline, caffeine, clobazam, clomipramine, clorazepate, cyamemazine, diazepam, demethyldiazepam, flunitrazepam, levomepromazine, loprazolam, lorazepam, meprobamate, nitrazepam, oxazepam, triazolam, viloxazine

KEY WORDS

plasma; protect from light; derivatization

REFERENCE

Pommery,J.; Lhermitte,M. High performance liquid chromatographic determination of fluvoxamine in human plasma, *Biomed.Chromatogr.*, **1989**, *3*, 177–179.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 500 μL 2 M sodium bicarbonate + 100 μL 0.1 or 1 μg/mL clovoxamine in MeOH + 10 mL hexane, shake horizontally for 20 min, centrifuge at 2000 g for 10 min. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 5 μm Resolve spherical silica (Waters)

Mobile phase: MeOH:MeCN:THF:water:diethylamine 98.59:1:0.2:0.2:0.01

Flow rate: 1

Injection volume: 50

Detector: UV 254

CHROMATOGRAM

Retention time: 4.7

Internal standard: clovoxamine (5.2)

Limit of detection: 0.5 ng/mL

Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES

Simultaneous: alimemazine, amitriptyline, chlorpromazine, clomipramine, desipramine, fluoxetine, haloperidol, imipramine, levomepromazine, norfluoxetine, nortriptyline, propericiazine, trimipramine, viloxazine

Noninterfering: metabolites, amineptine, buspirone, clobazam, clonazepam, clorazepate, diazepam, flunitrazepam, lorazepam, nitrazepam, oxazepam
Interfering: imipramine

KEY WORDS
plasma; human; rat; normal phase; pharmacokinetics

REFERENCE
Van Der Meersch-Mougeot,V.; Diquet,B. Sensitive one-step extraction procedure for column liquid chromatographic determination of fluvoxamine in human and rat plasma, *J.Chromatogr.*, **1991**, *567*, 441–449.

SAMPLE
Matrix: blood
Sample preparation: Add 10 μL 20 μg/mL oxaprotiline in MeOH to 990 μL plasma or serum. Inject 100 μL plasma or serum onto column A with mobile phase A and elute to waste, after 15 min elute column A onto column B with mobile phase B for 2 min. Remove column A from circuit and re-equilibrate it with mobile phase A for 5 min. Chromatograph on column B with mobile phase B.

HPLC VARIABLES
Column: A 20 × 4.6 10 μm Hypersil MOS C8; B 20 × 4.6 5 μm Hypersil CPS CN + 250 × 4.6 5 μm Nucleosil 100 CN
Mobile phase: A MeOH:water 5:95; B MeOH:MeCN:10 mM pH 6.8 potassium phosphate buffer 188:578:235
Flow rate: 1.5
Injection volume: 100
Detector: UV 214

CHROMATOGRAM
Retention time: 6.2
Internal standard: oxaprotiline (9.5)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Simultaneous: metoclopramide, doxepin, amitriptyline, clomipramine, fluoxetine, imipramine, norfluoxetine, nortriptyline, desipramine, maprotiline
Noninterfering: haloperidol, spiroperidol, pimozide, fluspirilene, trifluperidol, perazine, chlordiazepoxide, clobazam, diazepam, nordiazepam, flurazepam, lorazepam, nitrazepam, oxazepam, carbamazepine
Interfering: clozapine

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: For each 1 mL plasma or serum add 10 μL 14 μg/mL trimipramine in MeOH. Inject serum or plasma directly onto column A with mobile phase A, elute with mobile phase A to waste. After 15 min elute column A onto column B (foreflush) with mobile phase B. After 2 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Re-equilibrate column A with mobile phase A.

HPLC VARIABLES
Column: A 20 × 4.6 10 μm Hypersil MOS C8; B 20 × 4.6 5 μm Hypersil CPS CN + 250 × 4.6 5 μm Nucleosil 100 CN
Mobile phase: A MeOH:water 5:95; B MeCN:MeOH:buffer 578:188:235 (Buffer was 10 mM K_2HPO_4 adjusted to pH 6.8 with 85% phosphoric acid.)

Flow rate: 1.5
Injection volume: 100
Detector: UV 214

CHROMATOGRAM
Retention time: 6.19
Internal standard: trimipramine (6.5)
Limit of detection: 1 ng/mL (with three injections onto column A before switching), 5-10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, amitriptyline, clomipramine, desipramine, doxepin, imipramine, maprotiline, nortriptyline
Noninterfering: chlordiazepoxide, clobazam, clozapine, diazepam, flurazepam, fluspirilene, haloperidol, nitrazepam, oxazepam, perazine, pimozide, spiroperidol, trifluperidol

KEY WORDS
plasma; serum; column-switching

REFERENCE
Härtter,S.; Hiemke,C. Column switching and high-performance liquid chromatography in the analysis of amitriptyline, nortriptyline and hydroxylated metabolites in human plasma or serum, *J.Chromatogr.*, **1992**, *578*, 273–282.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 10 µL 100 µg/mL nortriptyline in water + 100 µL 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 µL 40 mM sodium bicarbonate, add 20 µL 10 mg/mL dansyl chloride in acetone, add 750 µL 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 µL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 15 × 3.2 7 µm NewGuard RP-8 (Brownlee)
Column: 250 × 4.6 5 µm 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: 5.3
Internal standard: nortriptyline (9.7)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: desipramine

KEY WORDS
plasma; derivatization

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 µL 5 µg/mL maprotiline in 10 mM HCl + 5 mL 200 g/L ethyl acetate in n-heptane, mix by rocking for 10 min, centrifuge at 1500 g for 10 min. Remove organic layer and add it to 150 µL 100 mM HCl,

mix 10 min, centrifuge at 1500 g for 10 min. Discard organic layer and evaporate aqueous layer at 45° in a vacuum centrifuge for 1 h. Take up residue in 50 μL 1 M pH 10.3 carbonate buffer and 25 μL 10 mg/mL dansyl chloride in MeCN, vortex, allow to react at room temperature for 45 min, evaporate at 45° in a vacuum centrifuge for 20 min, reconstitute in 125 μL MeCN:water 75:25, vortex, centrifuge for 3-5 min, inject a 25-40 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeCN:25 mM KH_2PO_4 75:25 + 500 μL/L orthophosphoric acid + 600 μL/L n-butylamine
Flow rate: 2
Injection volume: 25-40
Detector: F ex 235 em 470 (cut-off)

CHROMATOGRAM
Retention time: 6.78
Internal standard: maprotiline (12.8)

OTHER SUBSTANCES
Simultaneous: fluoxetine, propranolol, clovoxamine, fenfluramine, amoxapine, desipramine, protriptyline, nortriptyline, sertraline, norfluoxetine
Noninterfering: amitriptyline, imipramine, clomipramine, trimipramine, mianserin, chlordiazepoxide, trazodone, cyclobenzaprine, nomifensine, bupropion, metoprolol, atenolol, pindolol, tranylcypromine, moclobemide, thioridazine, citalopram, clozapine, carbamazepine, doxepin, loxapine

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KEY WORDS
plasma

REFERENCE
Suckow,R.F.; Zhang,M.F.; Cooper,T.B. Sensitive and selective liquid-chromatographic assay of fluoxetine and norfluoxetine in plasma with fluorescence detection after precolumn derivatization, *Clin.Chem.*, **1992**, *38*, 1756–1761.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL BondElut C18 SPE cartridge with 1 mL 1 M HCl, 1 mL MeOH, 1 mL water, and 1 mL 1% potassium carbonate. 700 μL Serum + 50 μL 5 μg/mL trimipramine in 5% potassium bicarbonate + 700 μL MeCN, vortex, centrifuge at 1500 g for 5 min, add supernatant to SPE cartridge (at ca. 1 mL/min). Wash with 2 mL water and 1 mL MeCN, elute with 250 μL MeOH:35% perchloric acid 20:1 by gravity (10 min) then centrifuge for 20 s to remove rest of eluant, inject a 50 μL aliquot of the eluate.

HPLC VARIABLES
Guard column: 15 mm 7 μm Brownlee RP-8
Column: 150 × 4.6 5 μm Ultrasphere Octyl
Mobile phase: MeCN:water 37.5:62.5 containing 0.5 g/L tetramethylammonium perchlorate and 0.5 mL/L 7% perchloric acid
Flow rate: 1.5
Injection volume: 50
Detector: UV 215

CHROMATOGRAM
Retention time: 6.8
Internal standard: trimipramine (9.6)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: amitriptyline, clomipramine, doxepin, fluoxetine, maprotiline, nortriptyline
Interfering: desmethylmaprotiline, desipramine, imipramine, protriptyline

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: 2 mL Serum + 5 mL 300 mM sodium phosphate + 400 μL diisopropyl ether (Caution! Diisopropyl ether readily forms explosive peroxides!) + imipramine, mix for 20 min, centrifuge for 10 min, inject an aliquot of the organic layer.

HPLC VARIABLES
Column: 150 × 4.6 3 μm Apex Silica (Jones Chromatography)
Mobile phase: MeCN:MeOH:25% ammonia 345:65:1.7
Flow rate: 1.3
Detector: UV 254

CHROMATOGRAM
Retention time: 3.5
Internal standard: imipramine
Limit of quantitation: 0.5 nM

KEY WORDS
serum; normal phase; pharmacokinetics

REFERENCE
Spigset,O.; Carleborg,L.; Hedenmalm,K.; Dahlqvist,R. Effect of cigarette smoking on fluvoxamine pharmacokinetics in humans, *Clin.Pharmacol.Ther.*, **1995**, *58*, 399–403.

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

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 253

CHROMATOGRAM
Retention time: 8.71
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; car-

teolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

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

SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + oxprotiline, make alkaline with borate buffer, extract with cyclohexane:dichloromethane 60:40. Remove the organic layer and extract it with acid, inject an aliquot of the acid layer.

HPLC VARIABLES

Column: C18 DB (Supelco)
Mobile phase: MeCN:pH 2.5 phosphate buffer 37:63
Detector: UV 254

CHROMATOGRAM

Internal standard: oxprotiline

OTHER SUBSTANCES

Extracted: bromazepam, clobazam, diazepam, lorazepam, oxazepam

KEY WORDS

serum

REFERENCE

Vandenberghe,H.; MacDonald,J.C. Analysis of fluvoxamine, clobazam and other benzodiazepines on the same HPLC system (Abstract 40), *Ther.Drug Monit.*, **1995**, *17*, 393–393.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the

organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

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

CHROMATOGRAM
Retention time: 15.347

KEY WORDS
whole blood

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

SAMPLE
Matrix: formulations
Sample preparation: Pulverize a tablet, add 100 mL MeOH, shake mechanically for 5 min, centrifuge an aliquot at 3000 rpm for 5 min. Remove a 50 μL aliquot of the supernatant and add it to 50 μL 100 μg/mL propyl paraben in MeOH, make up to 1 mL with MeCN, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 8 10 μm μBondapack C18
Mobile phase: MeCN:50 mM ammonium acetate 60:40 adjusted to pH 5.2 with glacial acetic acid
Flow rate: 0.8
Injection volume: 20
Detector: UV 273

CHROMATOGRAM
Retention time: 9
Internal standard: propyl paraben (7)

KEY WORDS
tablets

REFERENCE
Foda,N.H. Quantitative analysis of fluvoxamine maleate in tablet formulations by HPLC, *J.Liq.Chromatogr.*, **1995**, *18*, 1591–1601.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)

Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 9.97 (A), 5.94 (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, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

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: 8 mL Urine + 2 mL 100 mM pH 9.5 borate buffer, mix, filter (0.2 μm). Remove a 500 μL aliquot of the filtrate and add it to 100 μL 10 mM NaCN in 20 mM pH 9.5 borate buffer, add 500 μL 1 mM naphthalene-2,3-dicarboxaldehyde in MeOH, mix, let stand at room temperature for 20 min, add 100 μL 100 mM glycine in 20 mM pH 9.5 borate buffer, mix, let stand for 10 min, add 500 μL hexane:toluene 50:50, extract. Remove a 400 μL aliquot of the organic layer and add it to 800 μL dichloromethane, mix, inject a 35 μL aliquot.

HPLC VARIABLES

Column: 250 × 3.1 5 μm LiChrosorb Si-60
Mobile phase: Dichloromethane:MeOH 99.8:0.2
Flow rate: 0.5
Injection volume: 35

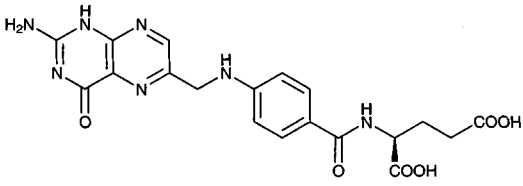
Detector: Chemiluminescence (418 nm cutoff filter) following post-column reaction. The column effluent mixed with 50 mM hydrogen peroxide in MeCN:dichloromethane 50:50 containing 0.5 mM triethylamine pumped at 0.1 mL/min and with 5 mM bis(2,4,6-trichlorophenyl) oxalate in dichloromethane pumped at 0.1 mL/min and the mixture flowed into the detector.

CHROMATOGRAM
Retention time: 7
Limit of detection: 5 nM

KEY WORDS
derivatization; normal phase

REFERENCE
Kwakman,P.J.M.; Koelewijn,H.; Kool,I.; Brinkman,U.A.T.; de Jong,G.J. Naphthalene- and anthracene-2,3-dialdehyde as precolumn labelling reagents for primary amines using reversed- and normal-phase liquid chromatography with peroxyoxalate chemiluminescence detection, *J.Chromatogr.*, **1990**, *511*, 155–166.

Folic acid



Molecular formula: $C_{19}H_{19}N_7O_6$
Molecular weight: 441.40
CAS Registry No.: 59-30-3
Merck Index: 4253

SAMPLE
Matrix: blood, formulations, urine
Sample preparation: Tablets. Powder tablets, dissolve in water, inject a 10 μL aliquot. Injections. Dilute with water, inject a 10 μL aliquot. Plasma, urine. Condition a Lichrolut RP-18 (Merck) SPE cartridge with 3 mL MeOH and 3 mL water. Mix 40 μL plasma or 100 μL urine with twice the volume of MeCN for 2 min, add 100 μL water, centrifuge at 3500 rpm for 15 min, evaporate the supernatant under nitrogen at 45° to remove the organic solvents, add slowly to the SPE cartridge, collect the eluate. Evaporate to dryness under a stream of nitrogen at 45°. Reconstitute the residue with 500 μL MeOH containing 4.2 μg/mL IS. Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Lichrosorb RP-18
Mobile phase: Gradient. A was MeOH. B was 50 mM ammonium acetate. A:B from 5:95 to 15:85 over 6 min, to 30:70 over 7 min, maintain at 30:70 over 7 min
Flow rate: 1
Injection volume: 10
Detector: UV 270

CHROMATOGRAM
Retention time: 9.99
Internal standard: xanthine (4.65)
Limit of detection: 3 ng

OTHER SUBSTANCES
Extracted: ascorbic acid, niacin, niacinamide, riboflavin, vitamin B12

KEY WORDS
plasma; SPE; tablets; injections

REFERENCE
Papadoyannis,I.N.; Tsioni,G.K.; Samanidou,V.F. Simultaneous determination of nine water and fat soluble vitamins after SPE separation and RP-HPLC analysis in pharmaceutical preparations and biological fluids, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 3203–3231.

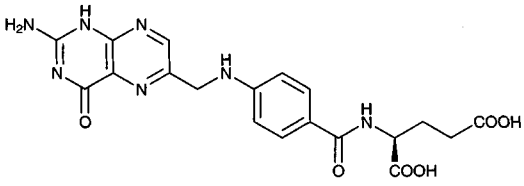
Detector: Chemiluminescence (418 nm cutoff filter) following post-column reaction. The column effluent mixed with 50 mM hydrogen peroxide in MeCN:dichloromethane 50:50 containing 0.5 mM triethylamine pumped at 0.1 mL/min and with 5 mM bis(2,4,6-trichlorophenyl) oxalate in dichloromethane pumped at 0.1 mL/min and the mixture flowed into the detector.

CHROMATOGRAM
Retention time: 7
Limit of detection: 5 nM

KEY WORDS
derivatization; normal phase

REFERENCE
Kwakman,P.J.M.; Koelewijn,H.; Kool,I.; Brinkman,U.A.T.; de Jong,G.J. Naphthalene- and anthracene-2,3-dialdehyde as precolumn labelling reagents for primary amines using reversed- and normal-phase liquid chromatography with peroxyoxalate chemiluminescence detection, *J.Chromatogr.*, **1990**, *511*, 155–166.

Folic acid



Molecular formula: $C_{19}H_{19}N_7O_6$
Molecular weight: 441.40
CAS Registry No.: 59-30-3
Merck Index: 4253

SAMPLE
Matrix: blood, formulations, urine
Sample preparation: Tablets. Powder tablets, dissolve in water, inject a 10 μL aliquot. Injections. Dilute with water, inject a 10 μL aliquot. Plasma, urine. Condition a Lichrolut RP-18 (Merck) SPE cartridge with 3 mL MeOH and 3 mL water. Mix 40 μL plasma or 100 μL urine with twice the volume of MeCN for 2 min, add 100 μL water, centrifuge at 3500 rpm for 15 min, evaporate the supernatant under nitrogen at 45° to remove the organic solvents, add slowly to the SPE cartridge, collect the eluate. Evaporate to dryness under a stream of nitrogen at 45°. Reconstitute the residue with 500 μL MeOH containing 4.2 μg/mL IS. Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Lichrosorb RP-18
Mobile phase: Gradient. A was MeOH. B was 50 mM ammonium acetate. A:B from 5:95 to 15:85 over 6 min, to 30:70 over 7 min, maintain at 30:70 over 7 min
Flow rate: 1
Injection volume: 10
Detector: UV 270

CHROMATOGRAM
Retention time: 9.99
Internal standard: xanthine (4.65)
Limit of detection: 3 ng

OTHER SUBSTANCES
Extracted: ascorbic acid, niacin, niacinamide, riboflavin, vitamin B12

KEY WORDS
plasma; SPE; tablets; injections

REFERENCE
Papadoyannis,I.N.; Tsioni,G.K.; Samanidou,V.F. Simultaneous determination of nine water and fat soluble vitamins after SPE separation and RP-HPLC analysis in pharmaceutical preparations and biological fluids, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 3203–3231.

SAMPLE

Matrix: blood, urine

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

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

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

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

Column temperature: 30

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

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 3.583

KEY WORDS

whole blood

REFERENCE

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

SAMPLE

Matrix: bulk, formulations

Sample preparation: Bulk. Dilute 32 mg bulk drug in 100 mL IS solution, mix well. Remove a 4 mL aliquot and make it up to 100 mL with IS solution, inject an aliquot. Tablets. Powder tablets, weigh out amount equivalent to 300 μg folic acid, add 25 mL IS solution, flush tube with nitrogen, shake vigorously for 5 min, filter (Millipore type HA, 0.45 μm), flush filtrate container with nitrogen, inject an aliquot. Capsules. Weigh out amount of capsule filling equivalent to 300 μg folic acid, add 30 mL hexane, shake vigorously for 5 min, centrifuge at 1070 g for 15 min. Remove the hexane and dry the residue at 60°. Add 25 mL IS solution to the dry residue, flush tube with nitrogen, shake vigorously for 5 min, filter (Millipore type HA, 0.45 μm), flush filtrate container with nitrogen, inject an aliquot. (IS solution was 40 mg methyl paraben, 240 mL MeOH, 650 mL water, 12 mL 40% tetrabutylammonium hydroxide in water, 2.04 g KH_2PO_4, and 30 mL 100 mg/mL pentetic acid in 750 mM ammonium hydroxide made up to 1 L with water.)

HPLC VARIABLES

Column: 300 × 4 10 μm μBondapak C18

Mobile phase: MeOH:buffer 24:76 adjusted to pH 7.0 with phosphoric acid and ammonium hydroxide (Buffer was 7.5 mL 40% tetrabutylammonium hydroxide in water, 2.04 g KH_2PO_4, and 7 mL 1 M phosphoric acid in 760 mL water.)

Column temperature: 35

Flow rate: 1.5

Injection volume: 10

Detector: UV 280

CHROMATOGRAM

Retention time: 12

Internal standard: methyl paraben (17)

OTHER SUBSTANCES
Simultaneous: degradation products, p-aminobenzoic acid
Noninterfering: vitamins A, B6, B12, C, D, E, niacin, thiamine, pantothenic acid

KEY WORDS
protect from light; capsules; tablets

REFERENCE
Tafolla,W.H.; Sarapu,A.C.; Dukes,G.R. Rapid and specific high-pressure liquid chromatographic assay for folic acid in multivitamin-mineral pharmaceutical preparations, *J.Pharm.Sci.*, **1981**, *70*, 1273–1276.

SAMPLE
Matrix: formula, milk
Sample preparation: Mix 8.0 g powdered infant milk with 10 mL water to it. Mix the diluted powder or 10.5 g liquid infant milk with 1 g solid trichloroacetic acid, shake thoroughly with magnetic stirring for 10 min, centrifuge at 1250 g for 10 min, add 3 mL 4% trichloroacetic acid to the solid residue, mix thoroughly for 10 min, centrifuge, discard the solid phase. Combine the two acid extracts and make up to 10 mL with 4% trichloroacetic acid, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Guard column: 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Column: 250 × 4.6 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Mobile phase: MeOH:buffer 15:85 (Buffer was 5 mM octanesulfonic acid and 0.5% triethylamine, pH 3.6.)
Flow rate: 1
Injection volume: 20
Detector: UV 261 for 6 min, UV 287 for 2 min, UV 290 for 5 min, UV 282 for 3 min, UV 268 for 3.5 min, UV 361 for 20.5 min, UV 246 for 20 min

CHROMATOGRAM
Retention time: 13
Limit of quantitation: ≤300 ng/mL

OTHER SUBSTANCES
Extracted: thiamine, riboflavin, pyridoxine, vitamin B12, niacinamide, pyridoxal, pyridoxamine

REFERENCE
Albalá-Hurtado,S.; Veciana-Nogués,M.; Izquierdo-Pulido,M.; Mariné-Font,A. Determination of water-soluble vitamins in infant milk by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *778*, 247–253.

SAMPLE
Matrix: formulations
Sample preparation: Dilute injections with water, inject a 50 μL aliquot. Dissolve tablets or capsule contents in water (warm if necessary), filter (0.5 μm PTFE), inject a 50 μL aliquot of the filtrate. (Dissolve tablets or other formulations containing proteinaceous material in water at 60°, add 5% trichloroacetic acid (to pH 4.4), filter, inject a 50 μL aliquot.)

HPLC VARIABLES
Guard column: pellicular Corasil
Column: 10 μm μBondapak C18
Mobile phase: Gradient. A was prepared by dissolving 1 g sodium dioctylsulfosuccinate in 170 mL MeOH, add 10 mL concentrated formic acid, make up to 800 mL with water, adjust pH to 2.5 with 1 M KOH, make up to 1 L. B was prepared by dissolving 1 g sodium dioctylsulfosuccinate in 450 mL MeOH, add 10 mL concentrated formic acid, make up to 800 mL with water, adjust pH to 4.6, make up to 1 L. A:B 100:0 for 19 min then 0:100 (step gradient) or A:B from 100:0 to 0:100 over 25 min (concave curve 9), maintain at 0:100 for 3 min, return to initial conditions over 2 min.
Flow rate: 1.5
Injection volume: 50
Detector: UV 280

CHROMATOGRAM
Retention time: 7 (step gradient), 8 (curve gradient)

OTHER SUBSTANCES
Simultaneous: niacin (UV 254), niacinamide (UV 254), pyridoxamine, thiamine (UV 254), riboflavin (UV 254), pyridoxine, ascorbic acid

KEY WORDS
injections; capsules; tablets

REFERENCE
Woollard,D.C. New ion-pair reagent for the high-performance liquid chromatographic separation of B-group vitamins in pharmaceuticals, *J.Chromatogr.*, **1984**, *301*, 470–476.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 100 × 4 3 μm Hypersil BDS-C18
Mobile phase: Gradient. MeCN:water adjusted to pH 2.1 from 0.3:99.7 to 25:75 over 11 min
Flow rate: 0.5
Detector: UV 220

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: biotin, caffeine, citric acid, niacinamide, niacin, pantothenic acid, riboflavin, saccharin, thiamine, pyridoxine, vitamin B12, ascorbic acid

KEY WORDS
tablets

REFERENCE
Hewlett Packard Leaflet 12-5091-7351 EUS, **1993**.

SAMPLE
Matrix: formulations
Sample preparation: Dilute liquid multivitamin formulations, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Lichrosorb RP-8
Mobile phase: Gradient. A was 10 mM KH_2PO_4 containing 5 mM sodium hexanesulfonate adjusted to pH 2.8 with phosphoric acid. B was MeOH. A:B from 90:10 to 71.8:28.2 over 4 min, maintain at 71.8:28.2 for 1.5 min, to 50:50 over 6.5 min, maintain at 50:50 for 5 min, return to initial conditions over 5 min
Flow rate: 1
Injection volume: 5
Detector: UV 272

CHROMATOGRAM
Retention time: 10.49
Internal standard: theobromine (8)
Limit of detection: 0.465 ng

OTHER SUBSTANCES
Simultaneous: niacin, niacinamide, thiamine, riboflavin, pyridoxine (UV 290)

KEY WORDS
liquid multivitamins; degas solutions with helium; protect from light

REFERENCE
Blanco,D.; Sánchez,L.A.; Gutiérrez,M.D. Determination of water soluble vitamins by liquid chromatography with ordinary and narrow-bore columns, *J.Liq.Chromatogr.*, **1994**, *17*, 1525–1539.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 30 × 4.6 10 μm octadecylsilica (Brownlee)
Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: Gradient. MeCN:33 mM pH 2.3 sodium phosphate buffer from 7.2:92.8 to 11.3:88.7 over 15 min. (At the end of each day flush system with water then 50-75 mL MeOH. Place a column of 37-53 μm silica (Whatman) between pump and injection valve.)
Flow rate: 1
Injection volume: 100
Detector: F ex 365 em >415 (filter) following post-column reaction. The column effluent mixed with the reagent pumped at 0.23 mL/min and the mixture flowed through a 5 m × 0.8 mm ID coil of PTFE tubing at 60° to the detector. (Reagent was 0.005% calcium hypochlorite (HTH dry chlorine, Olin, Overland KS) in 100 mM K_2HPO_4 containing 200 mM NaCl.)

CHROMATOGRAM
Retention time: 22

KEY WORDS
post-column reaction

REFERENCE
Gregory,J.F.,III; Sartain,D.B.; Day,B.P.F. Fluorometric determination of folacin in biological materials using high performance liquid chromatography, *J.Nutr.*, **1984**, *114*, 341–353.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:50 mM KH_2PO_4 90:10
Flow rate: 1
Detector: UV 210

CHROMATOGRAM
Retention time: 3.3

OTHER SUBSTANCES
Simultaneous: biotin, niacin, pantothenic acid, riboflavin, niacinamide

REFERENCE
MetaChem Catalog, **1995**, p. 21.

SAMPLE
Matrix: tissue

HPLC VARIABLES
Guard column: 20 × 2 pellicular C18
Column: Econosphere C18
Mobile phase: Gradient. A was 16 mM sodium phosphate and 4 mM tetrabutylammonium phosphate, pH 6.0. B was MeCN:16 mM sodium phosphate and 4 mM tetrabutylammonium phosphate, pH 6.0 20:80. A:B 100:0 for 5 min, to 40:60 over 30 min.
Flow rate: 1.5
Injection volume: 50
Detector: UV 290

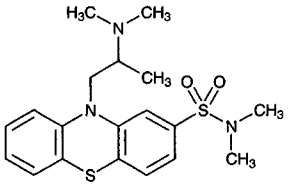
CHROMATOGRAM
Retention time: 21

OTHER SUBSTANCES
Extracted: other folates

KEY WORDS
rat; liver

REFERENCE
Rebello,T. Trace enrichment of biological folates on solid-phase adsorption cartridges and analysis by high-pressure liquid chromatography, *Anal.Biochem.*, **1987**, *166*, 55–64.

Fonazine



Molecular formula: $C_{19}H_{25}N_3O_2S_2$
Molecular weight: 391.56
CAS Registry No.: 7456-24-8, 7455-39-2 (methanesulfonate)
Merck Index: 4260

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

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

CHROMATOGRAM
Retention time: 2.7

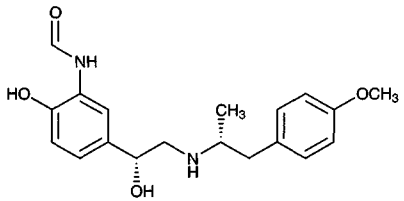
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pe-

cazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

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

Formoterol



Molecular formula: $C_{19}H_{24}N_2O_4$
Molecular weight: 344.41
CAS Registry No.: 73573-87-2, 43229-80-7 (fumarate)
Merck Index: 4272

SAMPLE

Matrix: bulk
Sample preparation: Dissolve in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Chiralcel OJ
Mobile phase: EtOH:heptane 30:70
Injection volume: 20
Detector: UV 365

CHROMATOGRAM

Retention time: k' 1.38 ((RR)-(+)), k' 2.00 ((SS)-(-))

KEY WORDS

chiral

REFERENCE

Francotte,E.R.; Richert,P. Applications of simulated moving-bed chromatography to the separation of the enantiomers of chiral drugs, *J.Chromatogr.A*, **1997**, *769*, 101–107.

SAMPLE

Matrix: bulk
Sample preparation: Dissolve 10 μmole compound (as free base or hydrochloride) in 500 μL MeCN, add 250 μL 5% sodium carbonate (for hydrochlorides only), add 500 μL 100 mM reagent in MeCN, vortex for 1 min, heat at 60° for 2 h, add 100 μmole L-proline, heat at 60° for 30 min. Remove a 100 μL aliquot and dilute it with mobile phase, neutralize with acetic acid, inject a 10 μL aliquot. Prepare the reagent ((R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate) as follows. Add 0.7 mL carbon disulfide to 6 mL (1R,2R)-(-)-1,2-diaminocyclohexane, 12 mL water, and 12 mL EtOH, heat the oil bath to 80°, add 2.8 mL carbon disulfide dropwise (making sure that the product does not start to precipitate), when addition is complete reflux for 1 h, acidify with 500 μL 5 M HCl, reflux for 12 h, cool, filter, wash the solid with a

little cold EtOH to give trans-4,5-tetramethyleneimidazolidine-2-thione as a white fluffy solid (mp 148-150°) (Tetrahedron 1993, 49, 4419). Stir 7.97 g 3,5-dinitrobenzoyl chloride in 30 mL dichloroethane at 50°, add a solution of 6 g trans-4,5-tetramethyleneimidazolidine-2-thione in 120 mL dichloroethane containing a catalytic amount of 4-(dimethylamino)pyridine over 15 min, reflux for 2 h, remove the crystals of (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate by filtration, evaporate the filtrate to dryness and dissolve the residue in 60 mL dichloroethane, reflux for 16 h to obtain more (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate (mp >250°, $[\alpha]_{546}$ = -133° (c = 1) in MeCN).

HPLC VARIABLES

Column: 125 × 4 5 μm Lichrospher 60 RP Select B
Mobile phase: MeCN:20 mM ammonium acetate 55:45
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: k' 2.52, k' 3.69 (enantiomers)

OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, atenolol, carazolol, carvedilol, 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: urine
Sample preparation: Condition a 100 mg silica SPE cartridge with 3 mL MeOH and 3 mL ethyl acetate. Briefly vortex 1 mL urine with 100 μL 2.5 mg/mL IS in MeOH and 100 μL 250 mM pH 8.0 phosphate buffer, add 3 mL ethyl acetate, tumble at 25 rpm for 30 min, centrifuge at 4000 rpm for 10 min. Remove the organic layer and add it to the SPE cartridge, wash with 1 mL ethyl acetate, wash with 10 mL 5% isopropanol in water, centrifuge at 4000 rpm for 10 min. Elute with 3 mL MeOH, evaporate the eluate to dryness at 30° under a stream of nitrogen, reconstitute the residue with 100 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 4 Chiral AGP (Baker, Deventer, Netherlands)
Column: 100 × 4 Chiral AGP (Baker, Deventer, Netherlands)
Mobile phase: Isopropanol:50 mM pH 7.0 phosphate buffer 1.5:100, containing 1 mM KCl and a small quantity of EDTA (sic)
Flow rate: 0.9
Injection volume: 100
Detector: E, Waters EC 460, glassy carbon electrode +0.63 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 8.7 (R,R), 11.3 (S,S)
Internal standard: diastereomeric formoterol (Either R,S or S,R, prepared by preparative HPLC, details in paper) (15.5)
Limit of detection: 60 pmol/L (R,R), 75 pmol/L (S,S)

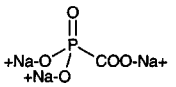
KEY WORDS

SPE; chiral; comparison with GC/MS; pharmacokinetics

REFERENCE

Butter,J.J.; van den Berg,B.T.J.; Portier,E.J.G.; Kaiser,G.; van Boxtel,C.J. Determination by HPLC with electrochemical detection of formoterol RR and SS enantiomers in urine, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 993–1005.

Foscarnet sodium



Molecular formula: CNa_3O_5P
Molecular weight: 191.95
CAS Registry No.: 63585-09-1
Merck Index: 4277

SAMPLE

Matrix: blood, CSF, urine

Sample preparation: Plasma. 100 μL Plasma + 900 μL 10 mM pyrophosphoric acid + 25 mg charcoal, after 30 s filter (Amicon MPS-1 with YMT membrane or Centricon 30 Microcencentrator) while centrifuging at 1000-2000 g for 15 min, dilute the ultrafiltrate 10-fold with 1 mM pyrophosphoric acid, inject a 20 μL aliquot. (For high concentrations of foscarnet charcoal may be omitted.) Urine. 100 μL Urine + 900 μL 10 mM pyrophosphoric acid + 25 mg charcoal, vortex for 30 s, filter (Millipore SJHVL04NS), dilute the filtrate 10-fold with 1 mM pH 5.8 pyrophosphoric acid, inject a 20 μL aliquot. CSF. Dilute CSF 100-fold with 1 mM pH 5.8 pyrophosphoric acid, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 3 μm UltroPac C18 (LKB)

Mobile phase: MeOH:pH 5.8 57 mM phosphate buffer 25:75 containing 1 mM tetrahexylammonium hydrogen sulfate and 0.2 mM pyrophosphoric acid

Flow rate: 1

Injection volume: 20

Detector: E, Environmental Sciences 5100 Coulochem, 5020 guard cell +0.75 V (placed after injector), 5010 analytical cell, cell 1 +0.75 V, cell 2 +0.90 V (monitored)

CHROMATOGRAM

Retention time: 4.66

Limit of quantitation: 30 μM (urine), 500 nM (plasma)

KEY WORDS

plasma; ultrafiltrate

REFERENCE

Pettersson,K.-J.; Nordgren,T.; Westerlund,D. Determination of phosphonoformate (foscarnet) in biological fluids by ion-pair reversed-phase liquid chromatography, *J.Chromatogr.*, **1989**, *488*, 447–455.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. Filter (Amicon Centricon 30) 1 mL plasma while centrifuging at 1500 g for 20 min, heat 200 μL of the ultrafiltrate in a boiling water bath for 20 min, cool, add 25-50 μL 671.8 μM hydrochlorothiazide in MeOH:water 50:50, vortex for 10 s, add 900 μL EtOH, mix for 20 s. Remove a 100 μL aliquot and dilute it to 1 mL with 1 mM pyrophosphoric acid, mix for 20 s, inject a 20 μL aliquot. Urine. 1 mL Urine + 9 mL 10 mM pyrophosphoric acid + 250 mg activated charcoal, vortex for 30 s. Filter (Amicon Centricon-30) 2 mL while centrifuging at 1500 g for 5 min. 200 μL Ultrafiltrate + 200 μL 20 mM NaOH + 400 μL EtOH, heat at 56° for 30 min, cool, add 671.8 μM hydrochlorothiazide in MeOH:water 50:50, mix for 10 s. Remove a 200 μL aliquot and dilute it 10-fold with 1 mM pyrophosphoric acid, mix for 20 s, inject a 20-40 μL aliquot.

HPLC VARIABLES

Column: 4 μm Nova-Pak C18

Mobile phase: MeOH:60 mM pH 5.8 buffer 30:70 containing 1 mM tetrahexylammonium hydrogen sulfate and 0.2 mM pyrophosphoric acid, pH adjusted to 5.8

Flow rate: 0.7

Injection volume: 20-40

Detector: E, ESA Coulochem Model 5100A, guard cell +0.99 V, analytical cell 1 +0.50 V, analytical cell 2 +0.95 V

CHROMATOGRAM
Retention time: 12.0
Internal standard: hydrochlorothiazide (18.9)
Limit of detection: 14 μM
Limit of quantitation: 33 μM

KEY WORDS
plasma; ultrafiltrate; pharmacokinetics

REFERENCE
Hassanzadeh,M.K.; Aweeka,F.T.; Wu,S.; Jacobson,M.A.; Gambertoglio,J.G. Determination of phosphonoformic acid in human plasma and urine by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1990**, *525*, 133–140.

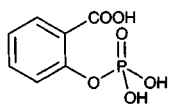
SAMPLE
Matrix: formulations
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeOH:5 mM sulfuric acid 5:95 containing 0.904 g/L tetrahexylammonium hydrogen sulfate
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

KEY WORDS
injections; saline

REFERENCE
Woods,K.; Steinmann,W.; Bruns,L.; Neels,J.T. Stability of foscarnet sodium in 0.9% sodium chloride solution, *Am.J.Hosp.Pharm.*, **1994**, *51*, 88–90.

Fosfosal



Molecular formula: $C_7H_7O_6P$
Molecular weight: 218.10
CAS Registry No.: 6064-83-1
Merck Index: 4281

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep Pak C18 SPE cartridge with 2 mL MeOH. Add 500 μL Plasma to the SPE cartridge, elute with 500 μL water. Collect all the eluate and centrifuge at 2500 rpm for 15 min, inject a 50 μL aliquot of the supernatant.

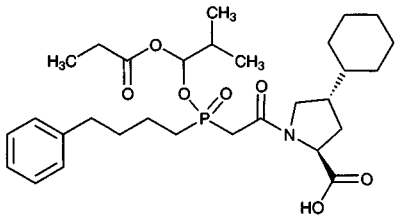
HPLC VARIABLES
Guard column: 10 μm μBondapak C18
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:5 mM tetrabutylammonium phosphate (PIC A) 30:70
Flow rate: 1
Injection volume: 50
Detector: UV 280

CHROMATOGRAM
Retention time: 5.5
Limit of detection: 1000 ng/mL

KEY WORDS
plasma; rat; dog; pharmacokinetics; SPE; also for humans (see Int.J.Clin.Pharmacol.ther.Toxicol. 1988; 26; 421)

REFERENCE
Ramis,J.; Mis,R.; Forn,J. Pharmacokinetics of fosfosal in rats and dogs, *Arzneimittelforschung*, **1989**, *39*, 74–77.

Fosinopril



Molecular formula: $C_{30}H_{46}NO_7P$
Molecular weight: 563.67
CAS Registry No.: 98048-97-6, 97825-24-6, 88889-14-9 (sodium salt)
Merck Index: 4282
Lednicer No.: 5 66

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:0.05% phosphate buffer 65:8:27, pH adjusted to 3.5 with phosphoric acid
Flow rate: 3
Detector: UV

KEY WORDS
perfusate buffer; 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
Sample preparation: Dissolve in mobile phase at a concentration of 100 μg/mL.

HPLC VARIABLES
Column: 150 × 3.9 5 μm RESOLVE (Waters)
Mobile phase: MeCN:water:orthophosphoric acid 4000:15:2
Column temperature: 32
Flow rate: 1
Injection volume: 20
Detector: UV 205

CHROMATOGRAM
Retention time: 5.13

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE
Kirschbaum,J.; Noroski,J.; Cosey,A.; Mayo,D.; Adamovics,J. High-performance liquid chromatography of the drug fosenopril, *J.Chromatogr.*, **1990**, *507*, 165–170.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 3.9 μBondapak phenyl
Mobile phase: MeOH:water:85% phosphoric acid 72:28:0.2
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: solutions

HPLC VARIABLES
Column: 300 × 4 10 μm alkylphenyl (Column Resolution Inc.)
Mobile phase: MeOH:0.2% phosphoric acid 72:28
Flow rate: 2
Injection volume: 50
Detector: UV 215

CHROMATOGRAM
Retention time: 10.5

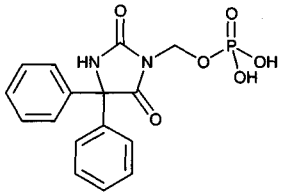
OTHER SUBSTANCES
Simultaneous: fosinoprilat, degradation products

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Lozano,R.; Warren,F.V.,Jr.; Perlman,S.; Joseph,J.M. Quantitative analysis of fosinopril sodium by capillary zone electrophoresis and liquid chromatography, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 139–148.

Fosphenytoin

Molecular formula: $C_{16}H_{15}N_2O_6P$
Molecular weight: 362.28
CAS Registry No.: 93390-81-9



SAMPLE
Matrix: formulations
Sample preparation: Dilute injection with MeCN:water 20:80, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 5 × 6 5 μm Guard-Pak Resolve C18
Column: 150 × 3.9 5 μm Resolve C18
Mobile phase: MeCN:water:85% phosphoric acid 25:75:0.09, containing 5 mM tetrabutylammonium sulfate
Column temperature: 30
Flow rate: 2
Injection volume: 20
Detector: UV 210

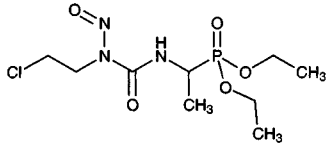
CHROMATOGRAM
Retention time: 9.1
Limit of quantitation: 15 μg/ml

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
injections; stability-indicating

REFERENCE
Fischer,J.H.; Cwik,M.J.; Luer,M.S.; Sibley,C.B.; Deyo,K.L. Stability of fosphenytoin sodium with intravenous solutions in glass bottles, polyvinyl chloride bags, and polypropylene syringes, *Ann.Pharmacother.*, **1997**, *31*, 553–559.

Fotemustine



Molecular formula: $C_9H_{19}ClN_3O_5P$
Molecular weight: 315.69
CAS Registry No.: 92118-27-9
Merck Index: 4285

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg CBA Bond Elut SPE cartridge with 1 mL MeOH and 1 mL water. Centrifuge blood at 5000 g for 2-3 min, freeze plasma in dry ice/hexane within 1 min. Thaw within 3 min by immersion in a 50° water bath. 1 mL Thawed plasma + 500 μL 2.5 μg/mL IS in 100 mM citric acid, vortex for 5 s, centrifuge for 5 min, add a 1 mL aliquot of the supernatant to the SPE cartridge, wash with 1 mL water, elute with 200 μL MeOH into a vial containing 50 μL 100 mM acetic acid, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 125 × 5 5 μm Spherisorb ODS
Mobile phase: MeCN:50 mM ammonium acetate 30:70 adjusted to pH 4.4 with glacial acetic acid
Flow rate: 1
Injection volume: 25
Detector: UV 230

CHROMATOGRAM
Retention time: 9.1
Internal standard: 1-methyl-3-isobutyl-8-vinyl-2,6-dioxopurine (S10338) (7.2)
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Extracted: lomustine, carmustine, metabolites

KEY WORDS
plasma; protect from light; pharmacokinetics; SPE; monkey; human

REFERENCE
Gordon,B.H.; Richards,R.P.; Hiley,M.P.; Gray,A.J.; Ings,R.M.; Campbell,D.B. A new method for the measurement of nitrosoureas in plasma: an h.p.l.c. procedure for the measurement of fotemustine kinetics, *Xenobiotica*, **1989**, *19*, 329–339.

SAMPLE
Matrix: blood

Sample preparation: Add fotemustine in ethanol PBS to plasma. Mix 100 μL plasma with 600 μL diethyl ether, rotate, centrifuge. Remove 400 μL of the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

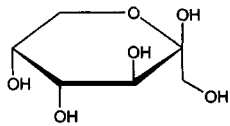
HPLC VARIABLES
Column: 150 mm long 4 μm Novapack
Mobile phase: EtOH:1% acetic acid (pH 3) 25:75
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7.6
Internal standard: carmustine (5.6)
Limit of detection: 1000 ng/mL

KEY WORDS
plasma; rat

REFERENCE
Meulemans,A.; Giroux,B.; Hannoun,P.; Henzel,D.; Bizzari,J.P.; Mohler,J. Permeability of two nitrosoureas, carmustine and fotemustine in rat cortex, *Chemotherapy*, **1989**, *35*, 313–319.

Fructose



Molecular formula: $C_6H_{12}O_6$
Molecular weight: 180.16
CAS Registry No.: 57-48-7
Merck Index: 4295

SAMPLE
Matrix: beverages, plants
Sample preparation: Beverages. Dilute 50-fold, filter (0.22 μm), inject an aliquot of the filtrate. Plants. Heat 1 g barley leaves and 10 mL EtOH:water 80:20 at 100° in a sealed tube for 15-30 min. Evaporate the liquid phase to dryness, reconstitute with water, pass through Analytichem trimethylaminopropyl and cyclohexyl SPE cartridges, inject an aliquot.

HPLC VARIABLES
Column: 300 × 6.5 Sugar-Pak I (Waters)
Mobile phase: water
Column temperature: 70
Flow rate: 0.4
Injection volume: 10
Detector: F ex 360 em 470 following post-column reaction. The effluent from the column passed through a 75 × 3.8 reactor containing Dowex 50 W × 2 sulfonic-acid type styrene divinylbenzene copolymer at 100° and mixed with 30 mM benzamidine in 1 M KOH pumped at 1 mL/min. This mixture flowed through a 530 μL reaction coil (Varian PCR-1) at 100° to the detector.

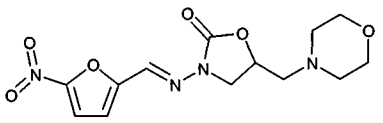
CHROMATOGRAM
Retention time: 22.77
Limit of detection: 60 pmole

OTHER SUBSTANCES
Extracted: dextrose, sucrose

KEY WORDS
barley; SPE; post-column reaction

REFERENCE
Coquet,A.; Haerdi,W.; Degli Agosti,R.; Veuthey,J.-L. Determination of sugars by liquid chromatography with post-column catalytic derivatization and fluorescence detection, *Chromatographia*, **1994**, *38*, 12–16.

Furaltadone



Molecular formula: $C_{13}H_{16}N_4O_6$
Molecular weight: 324.29
CAS Registry No.: 139-91-3
Merck Index: 4315
Lednicer No.: 1 229

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

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 347.4

CHROMATOGRAM
Retention time: 8.927

KEY WORDS
whole blood

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

SAMPLE
Matrix: milk
Sample preparation: Mix 50 mL cow milk with 25 mL 20% trichloroacetic acid, let stand for 15 min. Filter the samples and wash with water. Adjust the pH to 4.5-5 with NaOH, make up to 100 mL with water. Take a 25 mL aliquot and add it to a Sep-Pak Plus C18 SPE cartridge, elute with 2.5 mL mobile phase, pass nitrogen through eluate for at least 2 min (to remove oxygen), inject an aliquot.

HPLC VARIABLES
Guard column: Symmetry C18
Column: 150 × 3.9 4 μm Nova Pak C18

Mobile phase: MeCN:100mM aqueous sodium perchlorate:glacial acetic acid 28:72:0.5
Flow rate: 1
Injection volume: 20
Detector: E, ESA Coulochem II, Model 5011 analytical cell, porous carbon electrode -600 V, Model 5021 conditioning cell

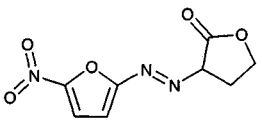
CHROMATOGRAM
Retention time: 2.5
Limit of detection: 4 ppb

OTHER SUBSTANCES
Extracted: furazolidone, nitrofurantoin

KEY WORDS
cow; SPE

REFERENCE
Galeano Diaz,T.; Guiberteau Cabanillas,A.; Acedo Valenzuela,M.I.; Correa,C.A.; Salinas,F. Determination of nitrofurantoin, furazolidone and furaltadone in milk by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.A*, **1997**, *764*, 243–248.

Furazolidone



Molecular formula: $C_8H_7N_3O_5$
Molecular weight: 225.16
CAS Registry No.: 67-45-8
Merck Index: 4320
Lednicer No.: 1 229

SAMPLE
Matrix: blood, eggs
Sample preparation: Dilute 1 mL serum or 0.5 mL egg yolk to 3 mL with water, mix, add to an Extrelut-3 SPE cartridge, elute with 14 mL ethyl acetate. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 500 μL mobile phase, centrifuge at 10000 rpm for 6 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5C18 HG (Wako)
Mobile phase: MeCN:water 40:60
Flow rate: 1
Injection volume: 50
Detector: UV 358

CHROMATOGRAM
Retention time: 3
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Noninterfering: chlortetracycline, oxolinic acid, oxytetracycline, sulfonamides, tylosin

KEY WORDS
pig; serum; chicken; SPE

REFERENCE
Yoshida,K.; Kondo,F. Liquid chromatographic determination of furazolidone in swine serum and avian egg, *J.AOAC Int.*, **1995**, *78*, 1126–1129.

SAMPLE
Matrix: blood, milk
Sample preparation: Extract 1 mL plasma or milk 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

OTHER SUBSTANCES
Simultaneous: nitrofurantoin

KEY WORDS
plasma; furazolidone is IS

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

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 347.4

CHROMATOGRAM
Retention time: 12.24

KEY WORDS
whole blood

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

SAMPLE
Matrix: eggs, tissue
Sample preparation: Add 30 mL MeCN to 10 g homogenized shelled eggs, liver, or muscle, blend at low speed for 2 min, centrifuge at 1000 g for 5 min, add 10 mL 10% NaCl solution and 50 mL dichloromethane to the supernatant, shake for a few min. Filter the lower organic layer through 5 g anhydrous sodium sulfate, evaporate the filtrate to dryness using a rotary vacuum evaporator at 45°, redissolve the residue in 1 mL MeCN:MeOH:20 mM pH 4.6 sodium acetate 10:50:40, inject an aliquot. (Protect from light. Wash the 1 mL of MeCN:MeOH:20 mM pH 4.6 sodium acetate 10:50:40 three times with 1 mL n-hexane before use.)

HPLC VARIABLES
Guard column: 10 × 4.6 μBondapak C18
Column: 150 × 4.6 5 μm Spherisorb ODS2 S5
Mobile phase: MeCN:20 mM pH 4.6 sodium acetate 21:79
Flow rate: 1
Injection volume: 50
Detector: UV 362

CHROMATOGRAM
Retention time: 8.2
Limit of detection: 2.5 ng/g

OTHER SUBSTANCES
Extracted: nitrofurazone, furaltadone

KEY WORDS
chicken; liver; muscle

REFERENCE
Draisci,R.; Giannetti,L.; Lucentini,L.; Palleschi,L.; Brambilla,G.; Serpe,L.; Gallo,P. Determination of nitrofuran residues in avian eggs by liquid chromatography-UV photodiode array detection and confirmation by liquid chromatography-ionspray mass spectrometry, *J.Chromatogr.A*, **1997**, *777*, 201–211.

SAMPLE
Matrix: eggs, tissue
Sample preparation: Add 30 mL MeCN to 10 g homogenized shelled eggs, liver, or muscle, blend at low speed for 2 min, centrifuge at 1000 g for 5 min, add 10 mL 10% NaCl solution and 50 mL dichloromethane to the supernatant, shake for a few min. Filter the lower organic layer through 5 g anhydrous sodium sulfate, evaporate the filtrate to dryness using a rotary vacuum evaporator at 45°, redissolve the residue in 1 mL MeCN:MeOH:20 mM pH 4.6 sodium acetate 10:50:40, inject an aliquot. (Protect from light. Wash the 1 mL of MeCN:MeOH:20 mM pH 4.6 sodium acetate 10:50:40 three times with 1 mL n-hexane before use.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil L C18-DB
Mobile phase: MeCN:water 50:50 containing 1 mM ammonium acetate and 0.025% acetic acid
Flow rate: 0.6
Injection volume: 20
Detector: MS, PESCIEX API I, ionspray interface 5500 V, OR 60 V, m/z 226, split the column effluent so that 0.03 mL/min enters the MS

CHROMATOGRAM
Retention time: 6.3
Limit of detection: 1.6 ng/g

KEY WORDS
chicken; liver; muscle

REFERENCE
Draisci,R.; Giannetti,L.; Lucentini,L.; Palleschi,L.; Brambilla,G.; Serpe,L.; Gallo,P. Determination of nitrofuran residues in avian eggs by liquid chromatography-UV photodiode array detection and confirmation by liquid chromatography-ionspray mass spectrometry, *J.Chromatogr.A*, **1997**, *777*, 201–211.

SAMPLE
Matrix: egs, milk, tissue
Sample preparation: Centrifuge milk at 2000 g for 10 min, freeze at -20° for 15 min, dilute a 10 mL aliquot with 10 mL saline, add 2 mL 1% sodium azide. 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 Cuprophan membrane (10000-15000 dalton cutoff) 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: 11
Limit of detection: 5 ng/mL (milk), 2 ng/g (tissue), 1 ng/g (eggs)

OTHER SUBSTANCES
Extracted: furaltadone, nitrofurantoin, 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 nitrofuran residues in edible products, *J.Chromatogr.*, **1990**, *500*, 453–468.

SAMPLE
Matrix: feed
Sample preparation: Add 15 mL water to 5 g ground feed sample, let stand for 5 min, add 35 mL MeCN:MeOH 50:50, shake on a mechanical shaker for 30 min, filter through a glass fiber filter. Pass the filtrate through a column dry-packed with 4 g neutral alumina (Sigma), discard the first 4 mL of eluate, collect the next 8 mL eluate, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:10 mM pH 6.0 sodium acetate 20:80
Flow rate: 1.5
Injection volume: 20
Detector: UV 365

CHROMATOGRAM
Retention time: 7.2
Limit of detection: 1 μg/g

REFERENCE
McCracken,R.J.; Kennedy,D.G. Determination of furazolidone in animal feeds using liquid chromatography with UV and thermospray mass spectrometric detection, *J.Chromatogr.A*, **1997**, *771*, 349–354.

SAMPLE
Matrix: feed
Sample preparation: Grind feed to pass 20 mesh. 10 g Feed + 5 mL water, swirl, let stand for 5 min, add 50 mL DMF:water 95:5, shake vigorously for 15 s, let stand in the dark at room

temperature overnight, filter (paper). Add 15 mL of the filtrate to 5 g alumina (Alcoa F-20, 80-200 mesh) in a 300 × 10 glass column, discard first several mL of eluate, collect remaining eluate, inject an aliquot

HPLC VARIABLES
Guard column: 100 × 2 μBondapak C18/Corasil
Column: 300 × 4 μBondapak C18
Mobile phase: MeCN:1% acetic acid 20:80
Detector: UV 280, UV 365

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Extracted: carbadox, nitrofurazone

KEY WORDS
protect from light

REFERENCE
Thorpe,V.A. Sample preparation of carbadox, furazolidone, nitrofurazone, and ethopabate in medicated feeds for high pressure liquid chromatography, *J.Assoc.Off.Anal.Chem.*, **1980**, *63*, 981–984.

SAMPLE
Matrix: feed, 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: 1.77
Limit of detection: 4.2 ng

OTHER SUBSTANCES
Simultaneous: nitrofurantoin, furaltadone

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: feeds
Sample preparation: Add 15 mL water to 5 g ground feed sample, let stand for 5 min, add 35 mL MeCN:MeOH 50:50, shake on a mechanical shaker for 30 min, filter through a glass fiber filter. Pass the filtrate through a column dry-packed with 4 g neutral alumina (Sigma), discard the first 4 mL of eluate, collect the next 8 mL eluate, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: RP18 4-4 guard column (Merck)

Column: 125 × 4 5 μm LiChrospher RP18 (end-capped) (Merck)
Mobile phase: MeCN:100 mM ammonium acetate 35:65
Flow rate: 1
Injection volume: 50
Detector: MS, Hewlett-Packard Model HP5989A, positive ion mode, source 200°, thermospray stem 125°, multiplier voltage 2000 V, dynode voltage 8000 V, m/z 243

CHROMATOGRAM
Retention time: 2.75

REFERENCE
McCracken,R.J.; Kennedy,D.G. Determination of furazolidone in animal feeds using liquid chromatography with UV and thermospray mass spectrometric detection, *J.Chromatogr.A*, **1997**, *771*, 349–354.

SAMPLE
Matrix: milk
Sample preparation: Mix 50 mL cow milk with 25 mL 20% trichloroacetic acid, let stand for 15 min. Filter the samples and wash with water. Adjust the pH to 4.5-5 with NaOH, make up to 100 mL with water. Take a 25 mL aliquot and add it to a Sep-Pak Plus C18 SPE cartridge, elute with 2.5 mL mobile phase, pass nitrogen through eluate for at least 2 min (to remove oxygen), inject an aliquot.

HPLC VARIABLES
Guard column: Symmetry C18
Column: 150 × 3.9 4 μm Nova Pak C18
Mobile phase: MeCN:100mM aqueous sodium perchlorate:glacial acetic acid 28:72:0.5
Flow rate: 1
Injection volume: 20
Detector: E, ESA Coulochem II, Model 5011 analytical cell, porous carbon electrode -600 V, Model 5021 conditioning cell

CHROMATOGRAM
Retention time: 2.7
Limit of detection: 5-6 ppb

OTHER SUBSTANCES
Extracted: furaltadone, nitrofurantoin

KEY WORDS
cow; SPE

REFERENCE
Galeano Diaz,T.; Guiberteau Cabanillas,A.; Acedo Valenzuela,M.I.; Correa,C.A.; Salinas,F. Determination of nitrofurantoin, furazolidone and furaltadone in milk by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.A*, **1997**, *764*, 243–248.

SAMPLE
Matrix: shrimp
Sample preparation: Condition a 6 mL Bond Elut C18 SPE cartridge with 5 mL MeOH and 5 mL water. Homogenize (Tissuemizer) 5 g finely-chopped (by hand) shrimp and 20 mL MeCN at medium speed for 45 s, centrifuge at 3000 rpm for 5 min, decant the supernatant. Add 30 mL hexane saturated with MeCN to the supernatant, shake for 30 s, discard the hexane layer. Add 10 mL EtOH to the MeCN layer, evaporate under reduced pressure at 45° to 2-5 mL (until liquid looks milky), add 2 mL EtOH, continue evaporation until there is 2 mL of a thick liquid, add 2 mL EtOH, evaporate to dryness. Add 2 mL water to the residue, sonicate for 5 min, add to the SPE cartridge, wash with 4 mL water, elute at ≤3 mL/min with 5 mL MeCN. Evaporate the eluate to dryness under a stream of nitrogen at ≤45° (remove promptly when dry), reconstitute the residue in 1 mL mobile phase, filter (0.45 μm), inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4 5 μm ODS Hypersil C18

Column: 200 × 4.6 5 μm ODS Hypersil C18
Mobile phase: MeCN:1% aqueous acetic acid 25:75
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: UV 375

CHROMATOGRAM
Retention time: 5
Limit of quantitation: 4 ng/g

OTHER SUBSTANCES
Extracted: nitrofurazone

KEY WORDS
SPE

REFERENCE
Rupp,H.S.; Munns,R.K.; Long,A.R. Simultaneous determination of nitrofurazone and furazolidone in shrimp (*Penaeus vannamei*) muscle tissue by liquid chromatography with UV detection, *J.AOAC Int.*, **1993**, *76*, 1235–1239.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 200 × 4.6 5 μm Hypersil SAS or 150 × 4.6 5 μm Hypersil SAS
Mobile phase: MeCN:buffer 30:70 (Mobile phase was 340 mL 100 mM citric acid, 5 mL 100 mM trisodium citrate, and 5 mL 100 mM Na_2EDTA made up to 500 mL with MeCN.)
Flow rate: 2
Injection volume: 100
Detector: UV 370

CHROMATOGRAM
Retention time: 2.1

OTHER SUBSTANCES
Simultaneous: oxytetracycline, tetracycline, chlortetracycline

REFERENCE
Murray,J.; McGill,A.S.; Hardy,R. Development of a method for the determination of oxytetracycline in trout, *Food Addit.Contam.*, **1987**, *5*, 77–83.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH:water 30:70, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Resolve spherical C18 (Waters)
Mobile phase: MeOH:water 35:65
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 305

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Simultaneous: carbadox, nitrofurazone
Noninterfering: pyrantel

KEY WORDS
protect from light

REFERENCE
Roybal,J.E.; Munns,R.K.; Shimoda,W. Liquid chromatographic determination of carbadox residues in animal feed, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 653–657.

SAMPLE
Matrix: 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: 13.0
Limit of detection: 60 ng/mL

OTHER SUBSTANCES
Simultaneous: degradation products, carbadox, nitrofurazone, nitrofurantoin, furaltadone

REFERENCE
Kaniou,I.; Zachariadis,G.; Kalligas,G.; Tsoukali,H.; Stratis,J. Separation and determination of carbadox, nitrofurazone, nitrofurantoin, furazolidone, and furaltadone in their mixtures by thin layer and high performance liquid chromatography, *J.Liq.Chromatogr.*, **1994**, *17*, 1385–1398.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Waring blender) 10 g muscle, liver, or kidney in 100 mL EtOH for 5 min, let stand for 5 min, filter through 10 g Celite 545 on top of a sintered glass filter, rinse blender with 100 mL EtOH and filter rinse. Add 25 mL 3.6% aqueous metaphosphoric acid to the combined filtrates, evaporate to 25 mL under reduced pressure at 45°. Remove residue, rinse out flask with 5 mL hexane and 3 mL water, combine, centrifuge at 0° at 27000 g for 30 min, discard hexane, rinse surface with 5 mL hexane, discard hexane. Remove aqueous layer, rinse out tube twice with 3 mL portions of water, combine, add 10 mL 1 M KH_2PO_4, make up to 100 mL with water, extract three times for 5 min with 50 mL ethyl acetate. Combine the extracts and dry them over 15 g anhydrous sodium sulfate, filter through glass wool, evaporate to dryness under reduced pressure at 45°. Take up residue in 3 mL ethyl acetate and add to alumina column, rinse flask with 2 mL ethyl acetate and add rinse to column. Elute with 20 mL EtOH:MeOH:ethyl acetate 10:10:80 and combine all the eluate. Evaporate to dryness under reduced pressure at 45°, reconstitute in 500 μL mobile phase, inject a 100 μL aliquot. (Prepare alumina column by slurrying 1 g aluminum oxide (Baker) in 20 mL ethyl acetate and adding to a 200 × 6 glass chromatographic column.)

HPLC VARIABLES
Guard column: Brownlee 10 μm RP-GU MPLC C-8
Column: 250 × 4.6 Brownlee RP-10A C-8
Mobile phase: MeCN:EtOH:10 mM ammonium acetate 25:5:70, pH 6.8
Flow rate: 1
Injection volume: 100
Detector: UV 350

CHROMATOGRAM
Retention time: 8.7
Limit of detection: 2 ng
Limit of quantitation: 10 ng

OTHER SUBSTANCES
Extracted: quinoxaline-2-carboxylic acid, carbadox, nitrofurazone, desoxycarbadox

KEY WORDS
protect from light; pig; muscle; liver; kidney

REFERENCE
MacIntosh,A.I.; Neville,G.A. Liquid chromatographic determination of carbadox, desoxycarbadox, and nitrofurazones in pork tissues, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 958–962.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 500 mg Bond Elut C18 SPE cartridge with 5 mL MeOH and 10 mL water. Homogenize 5 g tissue with 100 mL MeOH:0.2% metaphosphoric acid 40:60 for 2 min, filter through a 1 mm layer of Hyflo Super-Cel. Evaporate the filtrate under reduced pressure at 40° to 10 mL, add the residue to the SPE cartridge, wash with 10 mL water, elute with 10 mL MeOH. Evaporate the eluate to dryness under reduced pressure, take up the residue in 1 mL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 Newguard RP-8
Column: 150 × 4.6 5 μm Inertsil ODS
Mobile phase: MeCN:5 mM oxalic acid 45:55
Flow rate: 0.5
Injection volume: 10
Detector: UV 265

CHROMATOGRAM
Retention time: 7
Limit of detection: 50 ng/g

OTHER SUBSTANCES
Extracted: sulfamonomethoxine, sulfadimethoxine, sulfisozole, nalidixic acid, oxolinic acid, piromidic acid, sodium nifurstyrenate

KEY WORDS
fish; SPE

REFERENCE
Horie,M.; Saito,K.; Hoshino,Y.; Nose,N.; Nakazawa,H.; Yamane,Y. Simultaneous determination of residual synthetic antibacterials in fish by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *538*, 484–491.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax TP 18/2) 3 g ground muscle + 200 μL 1 μg/mL nitrofurazone in water + 6.8 mL MeCN for 6 s, centrifuge at 5000 rpm for 5 min. Remove 6.5 mL of the supernatant and add it to 2 mL 5 M NaCl, shake vigorously for 10 s, centrifuge at 3000 rpm for 2 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 43°, reconstitute the residue in 250 μL MeCN:buffer 20:80, add 1 mL hexane, mix (Whirlimixer), centrifuge for 4 min, discard the hexane layer, filter (Costar Spin-X 0.22 μm cellulose acetate) while centrifuging at 5600 g for 4 min, inject a 20 μL aliquot of the filtrate. (Buffer was 20 mM sodium 1-heptanesulfonate and 10 mM Na_2HPO_4, pH adjusted to 6.0 with phosphoric acid.)

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelcosil LC-ABZ
Column: 250 × 4.6 5 μm Supelcosil LC-ABZ
Mobile phase: MeCN:buffer 25:75 (Buffer was 4.45 g sodium 1-heptanesulfonate and 9.5 g $Na_3PO_4.12H_2O$ in 750 mL water, adjust pH to 2.5 with 5 M phosphoric acid, make up to 1 L with water.)

Flow rate: 1
Injection volume: 20
Detector: UV 365

CHROMATOGRAM
Retention time: 7.5
Internal standard: nitrofurazone (5.5)
Limit of quantitation: 3 ng/g

KEY WORDS
cow; muscle

REFERENCE
Hormazábal,V.; Yndestad,M. Simple and rapid method of analysis for furazolidone in meat tissues by high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 1871–1877.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 500 mg Bond-Elut NH_2 SPE cartridge with 20 mL chloroform: MeOH 70:30 and 10 mL hexane. Homogenize 2 g pulverized tissue with 40 mL MeOH:buffer 30:70 for 1 min, centrifuge at 2000 rpm for 15 min, evaporate to about 15 mL under reduced pressure at 40°, add 25 mL dichloromethane, shake gently for 1 min, centrifuge at 1500 rpm for 10 min. Remove the lower organic layer and evaporate it to near dryness under reduced pressure at 30°. Resuspend the residue in 2 mL dichloromethane and 6 mL hexane, add to the SPE cartridge, wash with 5 mL hexane:dichloromethane 50:50, elute with 5 mL chloroform: MeOH 70:30. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot. (Buffer was 23 g Na_2HPO_4 and 14.2 g citric acid in 1 L water, pH 3.6.)

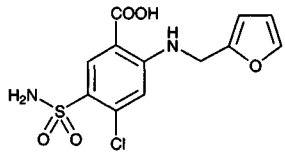
HPLC VARIABLES
Guard column: RP18 4-4 (Merck)
Column: 125 × 4 5 μm Lichrospher RP18 (end-capped)
Mobile phase: MeCN:100 mM ammonium acetate 25:75
Flow rate: 1
Injection volume: 50
Detector: MS, Vestec LC-MS Model 201A, thermospray, positive-ion mode, filament-assisted ionization, electron-beam current 300 μA, block 250°, tip 245°, lens 140°, vaporizer probe 180°, electron multiplier 2000 V, SIM m/z 243 $(M + NH_4)^+$

CHROMATOGRAM
Retention time: 3.6
Limit of detection: 1 ng/g

KEY WORDS
protect from light; pig; liver; kidney; diaphragm; muscle; pharmacokinetics; SPE

REFERENCE
McCracken,R.J.; Blanchflower,W.J.; Rowan,C.; McCoy,M.A.; Kennedy,D.G. Determination of furazolidone in porcine tissue using thermospray liquid chromatography-mass spectrometry and a study of the pharmacokinetics and stability of its residues, *Analyst*, **1995**, *120*, 2347–2351.

Furosemide



Molecular formula: $C_{12}H_{11}ClN_2O_5S$
Molecular weight: 330.75
CAS Registry No.: 54-31-9
Merck Index: 4331
Lednicer No.: 1 134; 2 87

SAMPLE
Matrix: blood
Sample preparation: Filter 900 or 1350 μL serum through a Tosoh Plastic filter (Japan) while centrifuging at 3000 or 5000 g for 15 min. Inject an aliquot.

HPLC VARIABLES
Column: Superspher 100RP-18e
Mobile phase: MeCN:MeOH:water 9:4:491
Flow rate: 1
Detector: UV 285

KEY WORDS
serum; pharmacokinetics; ultrafiltrate

REFERENCE
Takamura,N.; Maruyama,T.; Otagiri,M. Effects of uremic toxins and fatty acids on serum protein binding of furosemide: possible mechanism of the binding defect in uremia, *Clin.Chem.*, **1997**, *43*, 2274–2280.

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL 10 μg/mL nitrazepam in MeOH to 1 mL plasma, vortex, add 500 μL 200 mM pH 9.0 glycine buffer and 5 mL ethyl acetate, extract. Centrifuge at 700 g for 10 min, evaporate 4 mL of the organic phase to dryness under nitrogen at 60°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:buffer 69:31 (Mobile phase was 690 mL MeCN, 310 mL water, and 9 mL glacial acetic acid, adjusted to pH 5.0 with 5 M NaOH.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 5.2
Internal standard: nitrazepam (10.5)
Limit of detection: 10 ng/mL
Limit of quantitation: 30 ng/mL

KEY WORDS
pharmacokinetics; plasma

REFERENCE
Jankowski,A.; Skorek-Jankowska,A.; Lamparczyk,H. Determination and pharmacokinetics of a furosemide-amiloride drug combination, *J.Chromatogr.B*, **1997**, *693*, 383–391.

SAMPLE
Matrix: blood
Sample preparation: Filter 170 μL serum (UFC30HV00 membrane filter, 0.45 μm, Millipore) and inject a 100 μL aliquot of the filtrate onto column A, elute to waste with mobile phase A,

after 4.6 min elute the contents of column A onto column B with mobile phase A:B 82:18, after 7 min elute column B with mobile phase C, monitor the effluent from column B. (After 7 min remove column A from the circuit and wash it with mobile phase A:B 40:60 for 4 min then equilibrate it with mobile phase A for 5 min. After 11 min re-equilibrate column B with mobile phase A for 6 min.)

HPLC VARIABLES
Column: A 10 μm Guard-pak μBondapak C18 (Waters); B 150 × 4.6 5 μm YMC Pack ODS A-type (Yamamura Chemicals, Japan)
Mobile phase: A 20 mM pH 7 phosphate buffer; B MeCN; C MeCN:20 mM pH 7 phosphate buffer 35:65 containing 15 mM tetra-*n*-butylammonium
Column temperature: 40 (column B)
Flow rate: 2
Injection volume: 100
Detector: UV 271

CHROMATOGRAM
Retention time: 14.8
Limit of quantitation: 5 ng/mL

KEY WORDS
column-switching; serum

REFERENCE
Okuda,T.; Yamashita,K.; Motohashi,M. High-performance liquid chromatography using on-line solid-phase extraction: determination of furosemide in human serum, *J.Chromatogr.B*, **1996**, *682*, 343–348.

SAMPLE
Matrix: blood, perilymph, tissue
Sample preparation: Add 5 μL 1100 μg/mL p-nitrophenol to 50 μL serum. Homogenize tissue in MeOH:water 80:20, centrifuge at 4° at 500 g for 5 min, remove 100 μL of the resulting supernatant and add internal standard. Pool perilymph to yield a 3 μL perilymph sample. Acidify all samples with 5 μL 2 M phosphoric acid (except 3 μL for perilymph), extract with 200 μL ethyl acetate, dry under nitrogen, reconstitute in 30 μL mobile phase, inject a 15 μL aliquot. (Perform all extractions in the dark.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm C18 (Beckmann)
Mobile phase: MeOH:10 mM pH 5.5 KH_2PO_4 buffer 37:63
Flow rate: 1.5
Injection volume: 15
Detector: UV 235

CHROMATOGRAM
Retention time: 5.64
Internal standard: p-nitrophenol (8.30)
Limit of detection: <100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, furosemide glucuronide

KEY WORDS
rat; serum; kidney; liver

REFERENCE
Mills,C.D.; Whitworth,C.; Ryback,L.P.; Henley,C.M. Quantification of furosemide from serum and tissues using high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *701*, 65–70.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Mix 500 μL plasma with 30 μL 10 mg/mL warfarin in MeOH, 50 μL 6 M hydrochloric acid and 3 mL diethyl ether, vortex for 30 s, centrifuge at 2000 g for 10

min, evaporate ether layer in a 45° water bath under a stream of nitrogen. Reconstitute the residue in 100 μL MeOH and inject a 20 μL aliquot. Urine. Mix 200 μL urine with 20 μL 6 M hydrochloric acid, 40μL 10 mg/mL warfarin in MeOH and 8 mL diethyl ether, vortex for 30 s, evaporate to dryness in a 45° water bath under a stream of nitrogen. Reconstitute the residue in 100 μL mobile phase and inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 10 μm μBondapak C18 (plasma), 150 × 3.9 5 μm Resolve Spherical C18 (urine)
Mobile phase: MeCN:buffer 38:62 (Buffer was 10 mM potassium dihydrogen phosphate adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 1.5
Injection volume: 20
Detector: F ex 229 em 389

CHROMATOGRAM

Retention time: 3
Internal standard: warfarin (9)
Limit of detection: 2-3 ng/ mL
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES

Simultaneous: quinidine, sulfamethoxazole
Noninterfering: metabolites, carbamazepine, cimetidine, diazepam, disopyramide, fluvoxamine, meclofenamate, metoclopramide, phenobarbital, phenylbutazone, phenytoin, ranitidine, theophylline, trimethoprim

KEY WORDS

pharmacokinetics; plasma

REFERENCE

Abou-Auda,H.S.; Al-Yamani,M.J.; Morad,A.M.; Bawazir,S.A.; Khan,S.Z.; al-Khamis,K.I. High-performance liquid chromatographic determination of furosemide in plasma and urine and its use in bioavailability studies, *J.Chromatogr.B*, **1998**, *710*, 121–128.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. Mix 400 μL plasma with 700 μL MeOH:acetone 60:40, centrifuge at 3000 rpm for 10 min. Add 400 μL of the supernatant to 50 μL 50 ng/mL IS, filter (0.5 μm), inject an aliquot. Urine. Mix 1 mL urine with 700 μL pH 4.8 acetate buffer and 300 μL 100 ng/mL IS, filter (0.5 μm), inject an aliquot.

HPLC VARIABLES

Guard column: 10 × 4 5 μm Shim-pack G-ODS
Column: 150 × 6 5 μm Shim-pack CLC-ODS
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 88:12 for 5 min, 60:40 for 5 min, 50:50 for 10 min, 88:12 for 10 min (step gradient).
Column temperature: 40
Flow rate: 1
Injection volume: 10-50
Detector: F ex 345 em 415

CHROMATOGRAM

Retention time: 13.8
Internal standard: bumetanide (17.4)
Limit of detection: 5 ng/mL (urine), 1 ng/mL (plasma)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Yagi,N.; Kiuchi,T.; Satoh,H.; Terashima,Y.; Kenmotsu,H.; Sekikawa,H.; Takada,M. Bioavailability and diuretic effect of furosemide following administration of tablets and retarded capsules to human subjects, *Biol.Pharm.Bull.*, **1996**, *19*, 616–622.

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

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 234.6

CHROMATOGRAM
Retention time: 15.17

KEY WORDS
whole blood

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

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4.6 5 μm C18
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 1.2
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 20 min, filter, wash solid with MeOH, dilute filtrate with mobile phase, inject a 20 μL 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 μL aliquot.

HPLC VARIABLES
Guard column: μBondapak C18
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water 40:60 containing 5 mM KH_2PO_4/K_2HPO_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: piretanide

KEY WORDS
tablets; pharmacokinetics

REFERENCE
Barroso,M.B.; Jimenez,R.M.; Alonso,R.M.; Ortiz,E. Determination of piretanide and furosemide in pharmaceuticals and human urine by high-performance liquid chromatography with amperometric detection, *J.Chromatogr.B*, **1996**, *675*, 303–312.

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 μL aliquot.

HPLC VARIABLES
Guard column: μBondapak C18
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water 30:70 containing 5 mM KH_2PO_4/K_2HPO_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.Chromatogr.Rel.Technol.*, **1996**, *19*, 231–246.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: THF:glacial acetic acid:water 40:1:59
Flow rate: 1
Detector: UV 276

REFERENCE
Walter,E.; Janich,S.; Roessler,B.J.; Hilfinger,J.M.; Amidon,G.L. HT29-MTX/Caco-2 cocultures as an in vitro model for the intestinal epithelium: In vitro-in vivo correlation with permeability data from rats and humans, *J.Pharm.Sci.*, **1996**, *85*, 1070–1076.

SAMPLE
Matrix: urine
Sample preparation: Inject 5 μL urine onto column A and elute to waste with mobile phase A, after 1 min backflush the contents of column A onto column B with mobile phase B. Monitor the effluent from column B.

HPLC VARIABLES
Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 125 × 4 5 μm LiChrospher 100 RP 18
Mobile phase: A 50 mM pH 3 phosphate buffer; B MeCN:50 mM pH 3 phosphate buffer 60:40 (Prepare buffer as follows. Dissolve 3.45 g NaH_2PO_4 monohydrate in 500 mL water containing 750 μL propylamine hydrochloride, adjust to pH 3 with concentrated phosphoric acid.)
Flow rate: 1
Injection volume: 5
Detector: UV 254, F ex 233 em 389

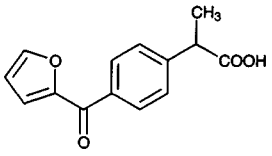
CHROMATOGRAM
Retention time: 7.7
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: amiloride, bumetanide, triamterene

KEY WORDS
column-switching

REFERENCE
Campins-Falcó,P.; Herráez-Hernández,R.; Pastor-Navarro,M.D. Analysis of diuretics in urine by column-switching chromatography and fluorescence detection, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 1867–1885.

Furprofen



Molecular formula: $C_{14}H_{12}O_4$
Molecular weight: 244.25
CAS Registry No.: 66318-17-0

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL plasma with 20 µL 100 µg/mL IS in 20 mM NaOH. Add 500 µL 50 mM pH 7.0 phosphate buffer, vortex for 1 min, add 2 mL dichloromethane, vortex for 1 min, shake for 5 min. Centrifuge at 100 g for 10 min, separate the organic layer, repeat the extraction procedure twice, evaporate the combined organic lowers to dryness under reduced pressure. Reconstitute with 200 µL 20 mM NaOH, inject a 20 µL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 10 µm Vydac AXGU
Column: 250 × 4.6 5 µm Supelcosil LC-SAX
Mobile phase: MeCN:50 mM pH 7.0 phosphate buffer 10:90
Flow rate: 1.5
Injection volume: 20
Detector: UV 280

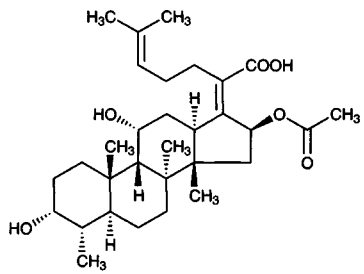
CHROMATOGRAM
Retention time: 4.9
Internal standard: fenbufen (3.5)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: rufloxacin

KEY WORDS
SPE; plasma

REFERENCE
Carlucci,G.; Mazzeo,P. Simultaneous determination of furprofen and rufloxacin in human plasma by high-performance liquid chromatography, *J.Chromatogr.Sci.*, **1996**, *34*, 182–184.

Fusidic acid



Molecular formula: $C_{31}H_{48}O_6$
Molecular weight: 516.72
CAS Registry No.: 6990-06-3, 751-94-0 (sodium salt)
Merck Index: 4340

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

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

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 24.86

KEY WORDS

whole blood

REFERENCE

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

SAMPLE

Matrix: formulations
Sample preparation: Weigh out ointment containing 2.14 mg sodium fusidate, take up in 100 mL MeOH:water 20:80. Remove a 200 μL aliquot and add it to 150 μL 20 mM tetrahexylammonium bromide in 100 mM pH 7.0 phosphate buffer and 100 μL 4.2 mg/mL 2-bromoacetyl-6-methoxynaphthalene in acetone, mix, let stand at room temperature for 5 min, add 150 μL 8.9 μg/mL IS in MeCN, sonicate at room temperature for 1 min, inject a 50 μL aliquot. (Prepare 2-bromoacetyl-6-methoxynaphthalene by stirring equimolar amounts of 2-acetyl-6-methoxynaphthalene (Janssen Chimica, Belgium) and phenyltrimethylammonium tribromide in THF at room temperature for 3 h (Phosphorus and Sulfur 1985, 25, 357), purify by column chromatography on silica gel with chloroform:petroleum ether 50:50 (mp 109-112°) (Chromatographia 1992, 33, 13).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:MeOH:water 51:34:15
Column temperature: 35
Flow rate: 1.6
Injection volume: 50
Detector: F ex 300 em 460

CHROMATOGRAM

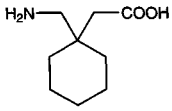
Retention time: 10
Internal standard: nonanoic acid naphthacyl ester (Prepare as follows. Dissolve 2 mmoles nonanoic acid and 1 mmole 2-bromoacetyl-6-methoxynaphthalene in 10 mL anhydrous MeCN, add 500 μL triethylamine, heat to 60° for 30 min, cool, add 30 mL water, extract three times with 10 mL portions of ether. Combine the extracts and wash them with 5% sodium bicarbonate solution and with three 10 mL portions of water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from MeOH/water (mp 66-8°) (Chromatographia 1992, 33, 13).) (5.5)
Limit of detection: 1 pmole

KEY WORDS

derivatization; ointment

REFERENCE
Gatti,R.; Gotti,R.; Bonazzi,D.; Cavrini,V. A comparative evaluation of three detectors in the HPLC analysis of sodium fusidate, *Farmaco*, **1996**, *51*, 115–119.

Gabapentin



Molecular formula: $C_9H_{17}NO_2$
Molecular weight: 171.24
CAS Registry No.: 60142-96-3
Merck Index: 4343

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond Elut C18 SPE cartridge with 1 mL MeOH and 1 mL buffer, do not allow to go dry. Condition an Empore C18 SPE membrane by adding 500 μL MeOH, force through three drops, discard MeOH remaining in reservoir, add 500 μL water, force through three drops, discard water remaining in reservoir. Add 200 μL 3 μg/mL IS in buffer to the SPE cartridge, add 200 μL serum and force through at 1 drop/s, add 200 μL buffer, force all liquid through, elute with 500 μL MeOH. Add 100 μL saturated sodium tetraborate solution and 50 μL 5% 2,4,6-trinitrobenzenesulfonic acid in water to the eluate, mix, heat at 50° for 10 min, add 500 μL 250 mM acetic acid, centrifuge at 12500 g for 2 min, add the supernatant to the SPE membrane, force through using a syringe or by centrifuging at 100-120 g for 5 min, wash with 500 μL MeCN:water 20:80, elute with 75 μL MeCN then 125 μL water, mix the eluates, inject a 50 μL aliquot. (Buffer was saturated sodium tetraborate solution diluted with three volumes of water.)

HPLC VARIABLES
Guard column: 20 × 2 30 μm Permaphase ETH (DuPont)
Column: 250 × 4.6 Ultrasphere C18
Mobile phase: MeCN:water:acetic acid:n-butylamine 52:48:0.1:0.01 (pH should not exceed 4.5) (Connect a 150 × 4.6 37-53 μm silica (Whatman) column between pump and injector.)
Column temperature: 50
Flow rate: 1.2
Injection volume: 50
Detector: UV 340

CHROMATOGRAM
Retention time: 10
Internal standard: 1-(aminomethyl)cycloheptaneacetic acid (13)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, caffeine, carbamazepine epoxide, carbamazepine, chlordiazepoxide, demoxepam, desalkylflurazepam, desmethylchlordiazepoxide, desmethyldiazepam, diazepam, disopyramide, ethosuximide, flurazepam, gentamicin, lidocaine, phenobarbital, phenytoin, primidone, procainamide, quinidine, theophylline, tobramycin, valproic acid, vancomycin

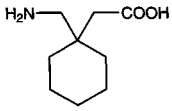
KEY WORDS
SPE; derivatization; pharmacokinetics

REFERENCE
Lensmeyer,G.L.; Kempf,T.; Gidal,B.E.; Wiebe,D.A. Optimized method for determination of gabapentin in serum by, *Ther.Drug Monit.*, **1995**, *17*, 251–258.

SAMPLE
Matrix: blood

REFERENCE
Gatti,R.; Gotti,R.; Bonazzi,D.; Cavrini,V. A comparative evaluation of three detectors in the HPLC analysis of sodium fusidate, *Farmaco*, **1996**, *51*, 115–119.

Gabapentin



Molecular formula: $C_9H_{17}NO_2$
Molecular weight: 171.24
CAS Registry No.: 60142-96-3
Merck Index: 4343

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond Elut C18 SPE cartridge with 1 mL MeOH and 1 mL buffer, do not allow to go dry. Condition an Empore C18 SPE membrane by adding 500 μL MeOH, force through three drops, discard MeOH remaining in reservoir, add 500 μL water, force through three drops, discard water remaining in reservoir. Add 200 μL 3 μg/mL IS in buffer to the SPE cartridge, add 200 μL serum and force through at 1 drop/s, add 200 μL buffer, force all liquid through, elute with 500 μL MeOH. Add 100 μL saturated sodium tetraborate solution and 50 μL 5% 2,4,6-trinitrobenzenesulfonic acid in water to the eluate, mix, heat at 50° for 10 min, add 500 μL 250 mM acetic acid, centrifuge at 12500 g for 2 min, add the supernatant to the SPE membrane, force through using a syringe or by centrifuging at 100-120 g for 5 min, wash with 500 μL MeCN:water 20:80, elute with 75 μL MeCN then 125 μL water, mix the eluates, inject a 50 μL aliquot. (Buffer was saturated sodium tetraborate solution diluted with three volumes of water.)

HPLC VARIABLES
Guard column: 20 × 2 30 μm Permaphase ETH (DuPont)
Column: 250 × 4.6 Ultrasphere C18
Mobile phase: MeCN:water:acetic acid:n-butylamine 52:48:0.1:0.01 (pH should not exceed 4.5) (Connect a 150 × 4.6 37-53 μm silica (Whatman) column between pump and injector.)
Column temperature: 50
Flow rate: 1.2
Injection volume: 50
Detector: UV 340

CHROMATOGRAM
Retention time: 10
Internal standard: 1-(aminomethyl)cycloheptaneacetic acid (13)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, caffeine, carbamazepine epoxide, carbamazepine, chlordiazepoxide, demoxepam, desalkylflurazepam, desmethylchlordiazepoxide, desmethyldiazepam, diazepam, disopyramide, ethosuximide, flurazepam, gentamicin, lidocaine, phenobarbital, phenytoin, primidone, procainamide, quinidine, theophylline, tobramycin, valproic acid, vancomycin

KEY WORDS
SPE; derivatization; pharmacokinetics

REFERENCE
Lensmeyer,G.L.; Kempf,T.; Gidal,B.E.; Wiebe,D.A. Optimized method for determination of gabapentin in serum by, *Ther.Drug Monit.*, **1995**, *17*, 251–258.

SAMPLE
Matrix: blood

Sample preparation: 100 μL Plasma + 100 μL 2 M perchloric acid, vortex for 10 s, centrifuge at 15000 g for 3 min. Remove a 50 μL aliquot and add it to 200 μL MeOH, add 200 μL buffer, add 50 μL reagent, mix, let stand at room temperature for 5 min, inject a 20 μL aliquot. (Prepare buffer weekly by adjusting the pH of 500 mM boric acid to 9.5 with 1 M NaOH. Prepare reagent weekly by dissolving 50 mg o-phthalaldehyde in 4.5 mL MeOH, add 500 μL buffer, add 50 μL 3-mercaptopropionic acid.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere octadecyl
Mobile phase: MeCN:MeOH:buffer 30:30:40 (Prepare the buffer by diluting 7.5 mL glacial acetic acid to 400 mL with water, adding 40 mg EDTA, and adjusting the pH to 3.7 with 3 M NaOH.)
Flow rate: 1.5
Injection volume: 20
Detector: F ex 330 em 440

CHROMATOGRAM
Retention time: 11.5
Internal standard: 1-(aminomethyl)cycloheptaneacetic acid (Parke-Davis) (15)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Noninterfering: alanine, arginine, aspartic acid, carbamazepine, clobazam, clonazepam, cysteine, felbamate, glutamic acid, glycine, histidine, isoleucine, lamotrigine, leucine, lysine, methionine, oxcarbazepine, phenobarbital, phenylalanine, phenytoin, primidone, proline, remacemide, serine, threonine, tiagabine, tyrosine, valine, valproic acid, vigabatrin

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KEY WORDS
derivatization; plasma

REFERENCE
Forrest,G.; Sills,G.J.; Leach,J.P.; Brodie,M.J. Determination of gabapentin in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *681*, 421–425.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 500 μL IS solution + 1 mL MeCN, vortex for 5 min, centrifuge for 15 min. Mix 6 μL buffer, 6 μL reagent, and 6 μL supernatant, let stand for 1 min, inject the whole amount. (Prepare IS solution by dissolving 100 mg gamma-phenyl-gamma-aminobutyric acid and 10 mg 1-(aminomethyl)cycloheptane acetic acid in 500 mL MeCN and 500 mL water. Prepare buffer by dissolving 15.5 mg boric acid in 500 mL water and adjusting to pH 9.5 with concentrated NaOH. Prepare reagent by mixing 100 mg o-phthaldialdehyde, 9 mL MeOH, 1 mL buffer, and 100 μL mercaptoethanol.)

HPLC VARIABLES
Column: 250 × 4 5 μm BANsil C18 (ASMT, Enger, Germany)
Mobile phase: Gradient. A was MeCN:MeOH:0.1% pH 2 phosphoric acid 10:10:80. B was MeCN:MeOH 50:50. A:B 90:10 for 1 min, to 30:70 over 25 min, maintain at 30:70 for 3 min, return to initial conditions over 0.1 min, re-equilibrate for 3.9 min.
Column temperature: 40
Flow rate: 1
Injection volume: 18
Detector: F ex 235 em 435

CHROMATOGRAM
Retention time: 27.0
Internal standard: gamma-phenyl-gamma-aminobutyric acid (Marion Merrel Dow) (23.4), 1-(aminomethyl)cycloheptane acetic acid (Gö-3609, Parke Davis) (28.3)
Limit of detection: 100 ng/mL
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Extracted: vigabatrin

KEY WORDS
derivatization; serum; degas mobile phase continuously with helium

REFERENCE
Juergens,U.H.; May,T.W.; Rambeck,B. Simultaneous HPLC determination of vigabatrin and gabapentin in serum with automated pre-injection derivatization, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1459–1471.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 50 μL IS solution + 200 μL 1.2 M perchloric acid, vortex, centrifuge at 3000 rpm for 5 min. Remove the supernatant and add it to 50 μL 5% 2,4,6-trinitrobenzenesulfonic acid in water, add 50 μL 50% NaOH, vortex, let stand at room temperature for 30 min, add 200 μL 6 M HCl, add 100 μL saturated NaCl, add 6 mL cyclohexane, shake for 10 min, centrifuge at 3000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 55°, reconstitute the residue in 200 μL EtOH:mobile phase 10:90, inject a 75 μL aliquot.

HPLC VARIABLES
Column: 5 μm Spherisorb ODSIII
Mobile phase: MeCN:100 mM pH 4 ammonium acetate 54:46
Column temperature: 40
Flow rate: 1
Injection volume: 75
Detector: UV 350

CHROMATOGRAM
Retention time: 5.2
Internal standard: 1-(aminomethyl)heptaneacetic acid (Parke-Davis) (13.3)
Limit of detection: 25 ng/mL

KEY WORDS
derivatization; plasma; dog; pharmacokinetics

REFERENCE
Stevenson,C.M.; Radulovic,L.L.; Bockbrader,H.N.; Fleisher,D. Contrasting nutrient effects on the plasma levels of an amino acid-like antiepileptic agent from jejeunal administration in dogs, *J.Pharm.Sci.*, **1997**, *86*, 953–957.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 50 μL serum or urine (diluted between 1:50 and 1:200) to 1 mL MeOH containing 3.4 μg γ-phenyl-γ-amino-n-butyric acid, vortex for 15 s, centrifuge at 2000 g for 10 min, mix 6 μL of the supernatant with 3 μL reagent, inject an aliquot. (Reagent was 10 mL 30 mg/mL o-phthaldialdehyde and 200 μL 2-mercaptoethanol made up to 50 mL with 400 mM pH 9.5 borate buffer (Ther. Drug Monit. 1991, 13, 251).)

HPLC VARIABLES
Column: 125 × 3 5 μm Superspher 60 RP-Select B (Merck)
Mobile phase: Gradient. A was MeCN. B was 20 mM KH_2PO_4 buffer. A:B from 22:78 to 37:63 in 12 min, from 37:63 to 55:45 in 6 min, from 55:45 to 80:20 in 1.5 min, maintain at 80:20 for 2 min
Column temperature: 35
Flow rate: 0.7
Detector: F ex 230 em 455

CHROMATOGRAM
Retention time: 17.3
Internal standard: γ-phenyl-γ-amino-n-butyric acid (14.8)
Limit of detection: 500 nM

OTHER SUBSTANCES
Extracted: vigabatrin

KEY WORDS
derivatization; serum; urine

REFERENCE
Wad,N.; Krämer,G. Sensitive high-performance liquid chromatographic method with fluorometric detection for the simultaneous determination of gabapentin and vigabatrin in serum and urine, *J.Chromatogr.B*, **1998**, *705*, 154–158.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 500 μL Plasma + 10 μL IS in water + 5 drops 2 M perchloric acid, vortex vigorously for a few s, centrifuge at 15000 g for 2 min. Remove the supernatant and add it to 500 μL 1 M sodium bicarbonate, add 50 μL 2 M 2,4,6-trinitrobenzenesulfonic acid in water, adjust pH to 8.5 with 100 mM NaOH, let stand for 30 min, add 2 drops of 25% HCl, add 3 mL toluene, shake for 10 min, centrifuge at 5000 g for 2 min. Remove the upper organic layer and evaporate it to dryness under reduced pressure at 40°, reconstitute the residue in 100 μL 200 mM pH 8.5 sodium borate buffer, wash with 1 mL cyclohexane:toluene 90:10 by vortexing for 1 min, inject a 10-50 μL aliquot of the aqueous phase. Urine. 10-100 μL Urine + 10 μL 200 μg/mL IS in water, add 500 μL 1 M sodium bicarbonate, add 50 μL 2 M 2,4,6-trinitrobenzenesulfonic acid in water, adjust pH to 8.5 with 100 mM NaOH, let stand for 30 min, add 2 drops of 25% HCl, add 3 mL toluene, shake for 10 min, centrifuge at 5000 g for 2 min. Remove the upper organic layer and evaporate it to dryness under reduced pressure at 40°, reconstitute the residue in 100 μL 200 mM pH 8.5 sodium borate buffer, wash with 1 mL cyclohexane:toluene 90:10 by vortexing for 1 min, inject a 10-50 μL aliquot of the aqueous phase.

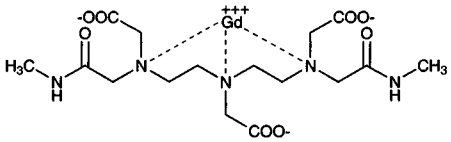
HPLC VARIABLES
Column: 250 × 4 10 μm LiChrosorb RP-18
Mobile phase: MeCN:0.5% acetic acid 58:42
Flow rate: 1
Injection volume: 10-50
Detector: UV

CHROMATOGRAM
Retention time: 10.3
Internal standard: 1-(aminomethyl)cycloheptaneacetic acid (13.2)
Limit of detection: 10 ng/mL
Limit of quantitation: 20 ng/mL

KEY WORDS
plasma; derivatization; pharmacokinetics

REFERENCE
Hengy,H.; Kölle,E.U. Determination of gabapentin in plasma and urine by high-performance liquid chromatography and pre-column labelling for ultraviolet detection, *J.Chromatogr.*, **1985**, *341*, 473–478.

Gadodiamide



Molecular formula: $C_{16}H_{26}GdN_5O_8$
Molecular weight: 573.66
CAS Registry No.: 122795-43-1
Merck Index: 4345

SAMPLE

Matrix: blood, urine
Sample preparation: Serum. Filter (Millipore Ultrafree-MC, type PTGC, 10 000 NMWL Filter unit) serum while centrifuging at 4° at 5000 g for 1 h, inject a 10 μL aliquot of the ultrafiltrate. Urine. Centrifuge urine at 4° at 15000 g for 10 min, dilute a 100 μL aliquot of the supernatant with 400 μL water, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 2.1 5 μm Supelguard LC-19-DB (Supelco)
Column: 250 × 2.1 5 μm Supelcosil LC-19-DB
Mobile phase: 10 mM Triethylammonium acetate containing 2 mM EDTA, pH adjusted to 6.5-7.0 with 1 M acetic acid or 1 M NaOH
Column temperature: 30
Flow rate: 0.3
Injection volume: 10
Detector: UV 658 following post-column reaction with the reagent pumped at 0.3 mL/min. (Reagent was 100 mM nitric acid containing 0.15 mM Arsenazo III and 10 mM urea, filter (0.45 μm), sonicate, discard after 2 days.)

CHROMATOGRAM

Retention time: 4
Limit of detection: 1.1 μM (urine), 0.3 μM (serum)
Limit of quantitation: 10 μM (urine), 2 μM (serum)

OTHER SUBSTANCES

Extracted: gadopentetate dimeglumine

KEY WORDS

serum; ultrafiltrate; post-column reaction

REFERENCE

Hvattum,E.; Normann,P.T.; Jamieson,G.C.; Lai,J.-J.; Skotland,T. Detection and quantitation of gadolinium chelates in human serum and urine by high-performance liquid chromatography and post-column derivatization of gadolinium with Arsenazo III, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 927–932.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 6 Asahipak ODP-50
Mobile phase: MeCN:pH 6.8 Tris-HCl buffer 1.5:98.5 containing 75 μM n-octylamine
Flow rate: 1.2
Detector: UV 215

CHROMATOGRAM

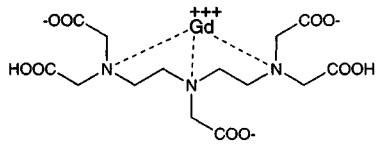
Retention time: 2.4

OTHER SUBSTANCES

Simultaneous: caldiamide, similar chelates of other metals

REFERENCE
Okazaki,O.; Kurata,T.; Yoshioka,N.; Hakusui,H. Pharmacokinetics and stability of caldiamide sodium in rats, *Arzneimittelforschung*, **1996**, *46*, 79–83.

Gadopentetic acid



Molecular formula: $C_{14}H_{20}GdN_3O_{10}$
Molecular weight: 547.58
CAS Registry No.: 80529-93-7, 86050-77-3 (meglumine salt)
Merck Index: 4347

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. Filter (Millipore Ultrafree-MC, type PTGC, 10 000 NMWL Filter unit) serum while centrifuging at 4° at 5000 g for 1 h, inject a 10 μL aliquot of the ultrafiltrate. Urine. Centrifuge urine at 4° at 15000 g for 10 min, dilute a 100 μL aliquot of the supernatant with 400 μL water, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2.1 5 μm Supelguard LC-19-DB (Supelco)
Column: 250 × 2.1 5 μm Supelcosil LC-19-DB
Mobile phase: 10 mM Triethylammonium acetate containing 2 mM EDTA, pH adjusted to 6.5-7.0 with 1 M acetic acid or 1 M NaOH
Column temperature: 30
Flow rate: 0.3
Injection volume: 10
Detector: UV 658 following post-column reaction with the reagent pumped at 0.3 mL/min. (Reagent was 100 mM nitric acid containing 0.15 mM Arsenazo III and 10 mM urea, filter (0.45 μm), sonicate, discard after 2 days.)

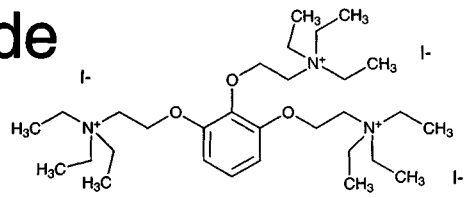
CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Extracted: gadodiamide

KEY WORDS
serum; ultrafiltrate; post-column reaction

REFERENCE
Hvattum,E.; Normann,P.T.; Jamieson,G.C.; Lai,J.-J.; Skotland,T. Detection and quantitation of gadolinium chelates in human serum and urine by high-performance liquid chromatography and post-column derivatization of gadolinium with Arsenazo III, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 927–932.

Gallamine triethiodide



Molecular formula: $C_{30}H_{60}I_3N_3O_3$
Molecular weight: 891.54
CAS Registry No.: 65-29-2
Merck Index: 4361

SAMPLE
Matrix: blood

Sample preparation: 500 μL Plasma + 500 μL MeCN, vortex for 2 min, centrifuge at 15000 g at 4° for 30 min. Remove a 500 μL aliquot of the supernatant and add it to 500 μL mobile phase, mix for 2 min, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak octadecylsilane
Mobile phase: MeCN:buffer 2:98 adjusted to pH 6.0 with 1 M phosphoric acid (Buffer was 5 mM octanesulfonic acid and 5 mM Na_2HPO_4.)
Flow rate: 0.2
Injection volume: 5
Detector: UV 230

CHROMATOGRAM
Retention time: 9.38
Limit of detection: 900 ng/mL

KEY WORDS
plasma

REFERENCE
Shao,M.J.; Fallon,K.D.; Khalil,S.N.; Abouleish,E. Quantitation of gallamine (Flaxedil) in human plasma using high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *345*, 184–186.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 10 μL 100 μg/mL d-tubocurarine chloride in water + 50 μL 10% sodium tungstate:335 mM sulfuric acid 50:50, vortex for 15 s, centrifuge at 12800 g for 2 min, inject a 30 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 50 mm long C18
Column: 250 × 4.6 10 μm μBondapak C18
Mobile phase: MeOH:7.5 mM tetrabutylammonium hydrogen sulfate 10:90
Flow rate: 1.8
Injection volume: 30
Detector: UV 229

CHROMATOGRAM
Retention time: 6.35
Internal standard: d-tubocurarine chloride (10.0)
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Noninterfering: barbiturates, alcuronium, metocurine, neostigmine, edrophonium

KEY WORDS
serum; rat; pharmacokinetics

REFERENCE
Ramzan,I.M. Determination of the neuromuscular blocking drug gallamine in rat serum using high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *417*, 428–433.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 0.5-400 μg/mL solution in mobile phase.

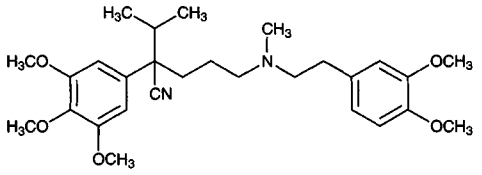
HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil octadecylsilyl
Mobile phase: MeCN:buffer 31:69 containing 100 mM sodium perchlorate (Buffer was 50 mM phosphoric acid adjusted to pH 3 with NaOH.)

Flow rate: 1
Injection volume: 20
Detector: UV 200

CHROMATOGRAM
Retention time: 12
Limit of detection: 35 ng/mL

REFERENCE
Mourier,P.A. Determination of gallamine and its impurities by reversed-phase ion-pair high-performance liquid chromatography and comparison with thin-layer chromatography, *J.Chromatogr.*, **1989**, *462*, 281–292.

Gallopamil



Molecular formula: $C_{28}H_{40}N_2O_5$
Molecular weight: 484.64
CAS Registry No.: 16662-47-8
Merck Index: 4369

SAMPLE
Matrix: blood
Sample preparation: Condition column A with two 995 μL portions of mobile phase at 3 mL/min and then with 995 μL solution B. Wash the donor channel of the dialyser (Gilson ASTED XL fitted with a Cuprophan cellulose acetate membrane with a molecular mass cut-off of 15000) with 2 mL solution A at 3 mL/min and wash the acceptor channel with 2 mL solution B. Dialyze 370 μL plasma against 9 mL solution B is pumped through the acceptor channel at 1 mL/min. Pass the dialysate through column A, backflush the contents of column A onto column B with mobile phase, elute with mobile phase, monitor the effluent from column B. (Solution A was 10 mM pH 3 acetate buffer containing 0.01% Triton X-100 and 50 μg/mL sodium azide. Solution B was 10 mM pH 3 acetate buffer containing 50 μg/mL sodium azide.)

HPLC VARIABLES
Column: A 30 μm Nucleosil CN; B 5 μm LiChrospher 100 RP-18 guard column + 4 μm Superspher 100 RP-18
Mobile phase: MeCN:2-aminoheptane:buffer 25:0.5:75 (Buffer was 10 mM sodium acetate adjusted to pH 3.0 with acetic acid.)
Column temperature: 35
Flow rate: 0.9
Detector: F ex 275 em 310

CHROMATOGRAM
Retention time: 15
Internal standard: gallopamil

OTHER SUBSTANCES
Simultaneous: norverapamil, verapamil

KEY WORDS
dialysate; column-switching; plasma; gallopamil is IS

REFERENCE
Ceccato,A.; Chiap,P.; Hubert,P.; Toussaint,B.; Crommen,J. Automated determination of verapamil and norverapamil in human plasma with on-line coupling of dialysis to high-performance liquid chromatography and fluorimetric detection, *J.Chromatogr.A*, **1996**, *750*, 351–360.

SAMPLE
Matrix: blood

Sample preparation: 1 mL Plasma + 100 μL 60 ng/mL norverapamil in water + 100 μL 2 M KOH, vortex gently for 5 s, add 5 mL hexane:MTBE 80:20, rotate at 45 rpm for 30 min, centrifuge at 1150 g for 5 min, freeze at -80° for 20 min. Remove the organic layer and add it to 100 μL 50 mM pH 3 KH_2PO_4, shake mechanically at high speed for 5 min, centrifuge for 5 min, freeze at -80° for 20 min, discard the organic layer, inject an aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 20 mm long 5 μm Supelcosil LC-18-DB
Column: 250 × 4.6 5 μm Supelcosil LC-18-DB
Mobile phase: MeCN:buffer 38:62 (Buffer was 50 mM KH_2PO_4 containing 5 mM 1-octanesulfonic acid and 1 mM triethylamine, pH adjusted to 3.0 with phosphoric acid.)
Column temperature: 40
Flow rate: 2
Detector: F ex 205 em 0-56 filter (Kopp) (Emission maximum is 310 nm.)

CHROMATOGRAM

Retention time: 10.1
Internal standard: norverapamil (8.0)
Limit of detection: 0.9 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Simultaneous: hydrochlorothiazide, procainamide, propranolol, quinidine, triamterene

KEY WORDS

plasma; pharmacokinetics

REFERENCE

McLean,A.M.; Babcock-Atkinson,E.; Rein,K.; Ruggirello,D.A.; Gonzalez,M.A.; Noonan,P.K. High-performance liquid chromatographic (HPLC) assay using fluorescence detection for the simultaneous determination of gallopamil and norgallopamil in human plasma, *Pharm.Res.*, **1987**, *4*, 327–331.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 0.9% NaCl + 120 μL 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 μL isopropanol, inject a 25 μL aliquot.

HPLC VARIABLES

Guard column: 50 × 4.6 Chiralpak AD (Baker)
Column: 250 × 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: 11 (S-(-)), 14 (R-(+))
Limit of quantitation: 30 ng/mL

OTHER SUBSTANCES

Simultaneous: norverapamil
Also analyzed: verapamil

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: solutions
Sample preparation: Dissolve a sample in water, mix a 1 mL aliquot with 100 μL 13.36 μg/m IS, inject an aliquot.

HPLC VARIABLES

Column: 100 × 4.0 5 μm Chiral-AGP (Baker)
Mobile phase: MeCN:pH 6.8 (I = 0.01) ammonium acetate 11:89 (Buffer was adjusted to pH 6.8 with ammonium hydroxide or acetic acid)
Column temperature: 22
Flow rate: 0.9
Injection volume: 20
Detector: UV 225; MS, Finnigan MAT SSQ 710A, interface particle beam, desolvation chamber 45°, nebulizing gas helium, electron impact mode 70 eV, source 250°, filament current 200 μA, electron multiplier 1500 V, m/z 45-400

CHROMATOGRAM

Retention time: 9.39 ((2R)-(+)), 14.26 ((2S)-(-))
Internal standard: procaine hydrochloride (5.09)
Limit of detection: 154 ng/mL ((2R)-(+)), 163 ng/mL ((2S)-(-))

OTHER SUBSTANCES

Also analyzed: verapamil

KEY WORDS

chiral

REFERENCE

Rustichelli,C.; Ferioli,V.; Gamberini,G. Resolution of the enantiomers of verapamil and gallopamil by chiral liquid chromatography-mass spectrometry, *Chromatographia*, **1997**, *44*, 477–483.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve a sample in water and inject an aliquot.

HPLC VARIABLES

Column: 100 × 4.0 5 μm Chiral-AGP (Baker)
Mobile phase: Gradient. A was MeCN. B was isopropanol. C was pH 6.8 (I = 0.01) ammonium acetate adjusted to pH 6.8 with ammonium hydroxide or acetic acid. A:B:C from 11:1:88 to 7:1:92 over 1.5 min, maintain at 7:1:92 for 35 min, to 11:1:88 over 5 min
Column temperature: 22
Flow rate: 0.9
Injection volume: 20
Detector: UV 225

CHROMATOGRAM

Retention time: 11 ((2R)-(+)), 22 ((2S)-(-))

OTHER SUBSTANCES

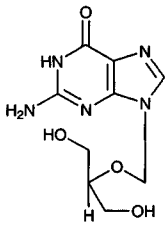
Simultaneous: verapamil

KEY WORDS

chiral

REFERENCE
Rustichelli,C.; Ferioli,V.; Gamberini,G. Resolution of the enantiomers of verapamil and gallopamil by chiral liquid chromatography-mass spectrometry, *Chromatographia*, **1997**, *44*, 477–483.

Ganciclovir



Molecular formula: $C_9H_{13}N_5O_4$
Molecular weight: 255.23
CAS Registry No.: 82410-32-0, 107910-75-8 (sodium salt)
Merck Index: 4374
Lednicer No.: 5 146

SAMPLE
Matrix: blood
Sample preparation: Add 10 μL 200 μg/mL acyclovir in MeOH to 500 μL plasma, add 1 mL MeCN, vortex briefly, centrifuge at 2000 g for 3 min, add 2 mL chloroform (Caution! Chloroform is a carcinogen!) to the supernatant, vortex. Remove the aqueous supernatant layer, remove traces of the organic solvent under a stream of nitrogen at 80° for 3 min, inject an aliquot.

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

CHROMATOGRAM
Retention time: 6
Internal standard: acyclovir (7)
Limit of detection: 3 ng/mL
Limit of quantitation: 10 ng/mL

KEY WORDS
plasma

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

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 2 μg 9-methylxanthine + 50 μL 35% perchloric acid, centrifuge at 4° at 2000 g for 15 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 3 μm Hypersil ODS
Mobile phase: 20 mM pH 3.50 KH_2PO_4
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Internal standard: 9-methylxanthine (4.5)
Limit of detection: 0.5 ng

OTHER SUBSTANCES
Noninterfering: acyclovir, allopurinol, azathioprine, caffeine, guanine, guanosine, hypoxanthine, mercaptopurine, oxypurinol, theophylline, uric acid, xanthine

KEY WORDS
plasma

REFERENCE
Boulieu,R.; Bleyzac,N.; Ferry,S. Modified high-performance liquid chromatographic method for the determination of ganciclovir in plasma from patients with severe renal impairment, *J.Chromatogr.*, **1991**, *571*, 331–333.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 50 μL 35% perchloric acid, centrifuge at 4° at 2000 g for 15 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 3 μm Hypersil ODS
Mobile phase: 20 mM pH 5.25 KH_2PO_4
Flow rate: 1.5
Detector: UV 254

KEY WORDS
plasma

REFERENCE
Boulieu,R.; Bleyzac,N. Stability of ganciclovir in blood samples, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 1205–1207.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 1 mL Plasma + 4 mL MeCN:10 mM pH 3.2 phosphate buffer, mix, centrifuge at 12000 rpm for 15 min. Remove the supernatant, filter, inject an aliquot. Tissue. Homogenize tissue in MeCN/PBS, centrifuge at 12000 rpm for 15 min. Remove the supernatant, filter, inject an aliquot.

HPLC VARIABLES
Guard column: present but not specified
Column: 250 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: MeCN:10 mM KH_2PO_4 20:80 (A) or MeOH:10 mM KH_2PO_4 5:95 containing 1 mM tetramethylammonium perchlorate (B)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 3 (A), 8 (B)
Limit of quantitation: 50 ng/mL

KEY WORDS
plasma; rat; brain; lung; pharmacokinetics

REFERENCE
Brewster,M.E.; Raghavan,K.; Pop,E.; Bodor,N. Enhanced delivery of gangiclovir to the brain through the use of redox targeting, *Antimicrob.Agents Chemother.*, **1994**, *38*, 817–823.

SAMPLE
Matrix: formulations
Sample preparation: Dilute withmobile phase, add hypoxanthine (final hypoxanthine concentration 10 μg/mL), inject a 5 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: 6 mM $(NH_4)H_2PO_4$ adjusted to pH 2.5 with 0.33 M phosphoric acid
Flow rate: 2
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 5.96
Internal standard: hypoxanthine (3.94)

KEY WORDS
injections; 10% dextrose; 25% dextrose; stability-indicating

REFERENCE
Johnson,C.E.; Jacobson,P.A.; Chan,E. Stability of gangiclovir sodium and amino acids in parenteral nutrient solutions, *Am.J.Hosp.Pharm.*, **1994**, *51*, 503–508.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Bioblock stirpack homogenizer) 10 mg tissue in 700 μL ice-cold 600 mM perchloric acid at 6000 rpm for 2.5 min, centrifuge at 4° at 2000 g for 15 min, adjust the pH of the supernatant to 7-8 with NaOH. Remove a 200 μL aliquot and add 10 μL alkaline phosphatase (type VII-NT, 10000 glycine U/mg protein, Sigma), heat at 37° for 30 min, evaporate, reconstitute in 30 μL mobile phase, inject a 20 μL aliquot. (The alkaline phosphatase step may be omitted.)

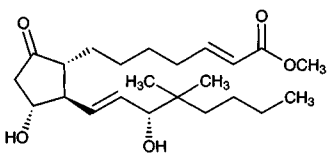
HPLC VARIABLES
Column: 150 × 4.6 3 μm Hypersil ODS
Mobile phase: 20 mM pH 3.5 KH_2PO_4
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Limit of detection: 0.9 pmole/g

KEY WORDS
heart

REFERENCE
Bleyzac,N.; Boulieu,R. High-performance liquid chromatographic determination of ganciclovir nucleotides in human myocardial tissue, *J.Chromatogr.B*, **1994**, *658*, 173–176.

Gemeprost



Molecular formula: $C_{23}H_{38}O_5$
Molecular weight: 394.55
CAS Registry No.: 64318-79-2
Merck Index: 4393
Lednicer No.: 4 11

SAMPLE
Matrix: blood

Sample preparation: Acidify plasma to pH 3.5 with 2 M HCl, pass through a column of Amberlite XAD-2 with a bed volume equal to half the sample volume, wash with water until the eluate is neutral, elute with MeOH (equal to half the original volume), inject an aliquot.

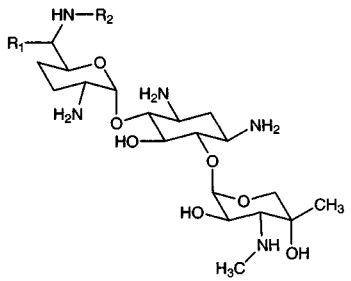
HPLC VARIABLES
Column: 200 × 10 25-40 μm Polygosil 60 C18 glass column
Mobile phase: MeOH:water 83:17
Detector: Radioactivity

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Dimov,V.; Gréen,K.; Bygdeman,M.; Christensen,N.J. Metabolism of 16, 16-dimethyl-*trans*-delta^2-prostaglandin E_1 methyl ester (ONO-802) following intravenous and vaginal administration to pregnant women, *Drug Metab.Dispos.*, **1986**, *14*, 494–502.

Gentamicin

Molecular formula: $C_{19}H_{39}N_5O_7$
Molecular weight: 449.55
CAS Registry No.: 1403-66-3, 1405-41-0 (sulfate)
Merck Index: 4398



Gentamicin C_1 $R_1 = R_2 = CH_3$

Gentamicin C_2 $R_1 = CH_3$, $R_2 = H$

Gentamicin C_{1a} $R_1 = R_2 = H$

SAMPLE
Matrix: blood
Sample preparation: Prepare a column of 150 mg dry silicic acid in a Pasteur pipette plugged with glass wool (10 mm height) and treat with 1 mL water. 500 μL Serum + 1.5 mL water, add to the column, rinse the tube with 1 mL water, add this water to the column, discard the eluate, add 500 μL reagent, let stand for 30 s, elute, discard the eluate, elute with 1.5 mL MeOH. Vortex the eluate, centrifuge, protect from light, inject a 20 μL aliquot. (Elution was performed under positive pressure from a rubber bulb. Reagent was prepared by dissolving 1 g of boric acid in 38 mL water, adjust pH to 10.4 with 450 g/L KOH, add 2 mL 100 mg/mL o-phthalaldehyde in MeOH, add 400 μL 2-mercaptoethanol. Prepare fresh each week.)

HPLC VARIABLES
Guard column: 23 × 3.9 μBondapak C18/Porasil B
Column: 300 × 3.9 10 μm μBondapak
Mobile phase: MeOH:buffer 21:79 (Buffer was 1% triethylamine adjusted to pH 6.2 ± 0.1 with phosphoric acid.)
Flow rate: 2
Injection volume: 25
Detector: F ex 260 em 418

CHROMATOGRAM
Retention time: 4.8 (C1), 6.8 (C1a), 8.6 (C2)
Limit of detection: 80 ng/mL (C1a, C2), 20 ng/mL (C1)

KEY WORDS
serum; derivatization; SPE; pharmacokinetics

REFERENCE
Rumble,R.H.; Roberts,M.S. High-performance liquid chromatographic assay of the major components of gentamicin in serum, *J.Chromatogr.*, **1987**, *419*, 408–413.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 20 μL water + 50 μL buffer, vortex for 15 s, add 200 μL MeCN, vortex for 15 s, centrifuge at 2000 g for 5 min. Filter (0.45 μm, Millex-HV4) the supernatant and add 300 μL of the filtrate to 20 μL 250 mg/mL 1-fluoro-2,4-dinitrobenzene in MeCN. Heat at 80° for 2 h, cool rapidly to room temperature, filter (0.45 μm, Millex-HV4), inject a 50 μL aliquot of the filtrate. (Buffer was prepared by dissolving 3.81 g disodium tetraborate decahydrate in water, adjusting pH to 10 with NaOH, and making up to 100 mL with water.)

HPLC VARIABLES
Guard column: 33 × 4.6 5 μm C18 (Perkin-Elmer)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water:acetic acid 70:30:0.1
Flow rate: 2.2
Injection volume: 50
Detector: UV 365

CHROMATOGRAM
Retention time: 11.0
Internal standard: gentamicin C1a

OTHER SUBSTANCES
Extracted: netilmicin

KEY WORDS
serum; guinea pig; human; derivatization; gentamicin C1a is IS

REFERENCE
Dionisotti,S.; Bamonte,F.; Gamba,M.; Ongini,E. High-performance liquid chromatographic determination of netilmicin in guinea-pig and human serum by fluorodinitrobenzene derivatization with spectrophotometric detection, *J.Chromatogr.*, **1988**, *434*, 169–176.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 20 μL water + 50 μL buffer, vortex for 15 s, add 200 μL MeCN, vortex for 15 s, centrifuge at 2000 g for 5 min. Filter (0.45 μm, Millex-HV4) the supernatant and add 300 μL of the filtrate to 20 μL 250 mg/mL 1-fluoro-2,4-dinitrobenzene in MeCN. Heat at 80° for 2 h, cool rapidly to room temperature, filter (0.45 μm, Millex-HV4), inject a 50 μL aliquot of the filtrate. (Buffer was prepared by dissolving 3.81 g disodium tetraborate decahydrate in water, adjusting pH to 10 with NaOH, and making up to 100 mL with water.)

HPLC VARIABLES
Guard column: 33 × 4.6 5 μm C18 (Perkin-Elmer)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water:acetic acid 70:30:0.1
Flow rate: 2.2
Injection volume: 50
Detector: UV 365

CHROMATOGRAM
Retention time: 11.0
Internal standard: gentamicin C1a

OTHER SUBSTANCES
Extracted: netilmicin

KEY WORDS
serum; guinea pig; human; derivatization; gentamicin C1a is IS

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: blood
Sample preparation: 50 μL Plasma +20 μL water + 50 μL buffer, vortex for 15 s, add 200 μL MeCN, vortex for 20 s, centrifuge at 2000 g for 5 min. Filter (Millex-HV4) the supernatant. Heat 200 μL filtrate and 20 μL 250 mg/mL 1-fluoro-2,4-dinitrobenzene in MeCN at 80° for 1 h, cool, inject a 50 μL aliquot. (Buffer was 3.81 g disodium tetraborate decahydrate in water, adjust pH to 10 with NaOH, make up to 100 mL with water.)

HPLC VARIABLES
Guard column: 25 × 4 10 μm LiChroCART RP 18
Column: 250 × 4 5 μm LiChrosorb RP 18
Mobile phase: MeCN:water 70:30 containing 1 mL/L acetic acid
Flow rate: 2
Injection volume: 50
Detector: UV 365

CHROMATOGRAM
Retention time: 10.0 (gentamicin C1a)
Internal standard: gentamicin C1a

OTHER SUBSTANCES
Extracted: isepamicin
Noninterfering: ampicillin, aspirin, captopril, cefazolin, cefotaxime, ceftazidime, ceftriaxone, cephalosporins, chlorpromazine, diazepam, heparin, propranolol, sulfamethoxazole, sulpiride, trimethoprim, verapamil

KEY WORDS
plasma; guinea pig; human; derivatization; gentamicin C1a is IS

REFERENCE
Dionisotti,S.; Bamonte,F.; Scaglione,F.; Ongini,E. Simple measurement of isepamicin, a new aminoglycoside antibiotic, in guinea pig and human plasma, using high-performance liquid chromatography with ultraviolet detection, *Ther.Drug Monit.*, **1991**, *13*, 73–78.

SAMPLE
Matrix: blood, broth
Sample preparation: Condition a 3 mL 100 mg Isolute CBA-bonded (carboxypropyl) silica SPE cartridge (Jones Chromatography) with 1 mL MeOH and 1 mL 20 mM pH 7.4 phosphate buffer. Add 1 mL plasma or broth to the SPE cartridge, wash with 2 mL 20 mM pH 7.4 phosphate buffer, wash with 4 mL 200 mM pH 9.0 borate buffer, dry with 30 mL air, elute with 1 mL MeCN:200 mM pH 10.5 borate buffer 50:50, force out all the liquid with air. 1 mL Eluate + 200 μL 800 mM boric acid + 200 μL 2.5 mM 9-fluorenylmethyl chloroformate in MeCN, let stand at room temperature for 15 min, add 25 μL 100 mM glycine, let stand for 2 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 3 μm ODS Hypersil
Mobile phase: MeCN:water 87:13
Flow rate: 1
Injection volume: 50
Detector: F ex 260 em 315

CHROMATOGRAM
Retention time: 18 (C_{1a}), 20 (C_2), 22 (C_{2a}), 24 (C_1)

Limit of detection: 10-50 ng/mL

KEY WORDS
derivatization; plasma; SPE

REFERENCE
Stead,D.A.; Richards,R.M.E. Sensitive fluorimetric determination of gentamicin sulfate in biological matrices using solid-phase extraction, pre-column derivatization with 9-fluorenylmethyl chloroformate and reversed-phase high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *675*, 295–302.

SAMPLE
Matrix: blood, dialysate, urine
Sample preparation: Plasma. Condition a 3 mL Baker cyanopropylsilane CN SPE cartridge with 2 mL MeOH, 2 mL water, and 2 mL buffer. 1 mL Plasma + 100 μL 100 μg/mL dibekacin in water, vortex for 15 s, add 1 mL buffer, vortex for 15 s, centrifuge at 3100 g at 4° for 7 min, add to SPE cartridge, wash with 500 μL water, wash with 250 μL mobile phase, elute to dryness. Elute with 250 μL mobile phase, inject an aliquot of the eluate. Urine, dialysate. Dilute 1:100 with water, add 100 μL 100 μg/mL dibekacin per 1 mL of sample, mix well, inject a 100 μL aliquot. (Buffer was 0.94 g sodium hexanesulfonate in 300 mL water, add 500 μL glacial acetic acid, dilute to 500 mL with water.)

HPLC VARIABLES
Guard column: 10 × 4.6 5 μm Hypersil C18
Column: 150 × 4.6 5 μm Hypersil C18
Mobile phase: MeOH:buffer 15:85 (Buffer was 3.48 g sodium hexanesulfonate + 28.4 g sodium sulfate in 2 L water, acidify to pH 3.4 with 2 mL glacial acetic acid.)
Column temperature: 25
Flow rate: 1.1
Injection volume: 100
Detector: F ex 338 em 418 (bandpass filter) following post-column reaction. The column effluent mixed with the reagent pumped at 0.4 mL/min and the mixture flowed through a 3 m × 0.05 mm i.d. knitted PTFE reaction coil at 25° to the detector (Derivatizing reagent was 0.4 g o-phthalaldehyde in 3 mL MeOH added to 390 mL buffer, add 2 mL β-mercaptoethanol, make up to 500 mL with water, store at 4°. Buffer was 1 M pH 10.4 borate from equal volumes of 1 M KOH and boric acid.)

CHROMATOGRAM
Retention time: 11, 17, 17.5, 22
Internal standard: dibekacin

OTHER SUBSTANCES
Simultaneous: kanamycin, isepamicin, tobramycin, netilmicin

KEY WORDS
post-column reaction; SPE; plasma

REFERENCE
Maloney,J.A.; Awni,W.M. High-performance liquid chromatographic determination of isepamicin in plasma, urine and dialysate, *J.Chromatogr.*, **1990**, *526*, 487–496.

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

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

CHROMATOGRAM
Retention time: 14.037

KEY WORDS
whole blood

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

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 2 mg/mL solution in 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 μL aliquot of the lower organic phase.

HPLC VARIABLES
Column: 250 × 4.6 5 μm LiChrosorb SI-100
Mobile phase: Chloroform:THF:water 65:17.5:0.2
Flow rate: 1
Injection volume: 20
Detector: UV 350

CHROMATOGRAM
Retention time: 8, 12, 16

KEY WORDS
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: bulk, formulations
Sample preparation: Bulk. Weigh out amount equivalent to 100 mg gentamicin, dissolve in 100 mL water. Remove a 20 mL aliquot and add it to 10 mL reagent, make up to 50 mL with MeOH, heat at 90° for 15 min, cool for 5 min, inject a 20 μL aliquot. Injections. 500 μL of a 5% injection + 19.5 mL water + 10 mL reagent, heat at 90° for 15 min, cool for 5 min, inject a 20 μL aliquot. (Reagent was 400 mg o-phthalaldehyde in 4 mL MeOH, add 38 mL buffer, add 0.8 mL thioglycolic acid, adjust the pH to 10.4 with 45% KOH. Buffer was 6.18 g boric acid in 200 mL water, adjust pH to 10.4 with 45% KOH, make up to 250 mL with water.)

HPLC VARIABLES
Guard column: 45 × 4.6 Vydac reversed phase
Column: 150 × 4.6 Ultrasphere ODS

Mobile phase: Gradient. A was MeOH:water:acetic acid 700:250:50 containing 5 g/L sodium heptanesulfonate. B was MeOH. A:B 100:0 for 2 min, to 75:25 over 3 min, maintain at 75:25.
Flow rate: 1.5
Injection volume: 20
Detector: UV 330

CHROMATOGRAM
Retention time: 5.07 (C1), 11.06 (C1a), 12.67 (C2a), 13.77 (C2)

KEY WORDS
injections; derivatization

REFERENCE
Albracht,J.H.; de Wit,M.S. Analysis of gentamicin in raw material and in pharmaceutical preparations by high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *389*, 306–311.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Prepare a 100-200 μg/mL solution in water, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4 Carbopac PA-1 anion-exchange (Dionex)
Column: 250 × 4 Carbopac PA-1 anion-exchange (Dionex)
Mobile phase: Gradient. Water:10 mM NaOH from 70:30 to 50:50 over 15 min, re-equilibrate for 5 min.
Flow rate: 1
Injection volume: 20
Detector: E, Dionex PED-1 pulsed amperometric detector, gold working electrode, amperometry mode, E1 0.10 V, t1 300 ms, E2 0.60 V t2 120 ms, E3 -0.80 V t3 300 ms following post-column reaction. The effluent from the column was mixed with 500 mM NaOH pumped at 0.5 mL/min and flowed through a mixing coil (Dionex RDM) to the detector.

CHROMATOGRAM
Retention time: 5.91 (C1a), 6.66 (C2), 8.04 (C2a), 10.05 (C1)
Limit of detection: 20 ng

OTHER SUBSTANCES
Noninterfering: cefazolin, clindamycin, cloxacillin, kanamycin, neomycin, penicillin G, tobramycin

KEY WORDS
post-column reaction; injections

REFERENCE
Kaine,L.A.; Wolnik,K.A. Forensic investigation of gentamicin sulfates by anion-exchange ion chromatography with pulsed electrochemical detection, *J.Chromatogr.A*, **1994**, *674*, 255–261.

SAMPLE
Matrix: formulations
Sample preparation: Mix 2 g cream with 3 mL n-butanol, add 5 mL 2% sulfuric acid, mix thoroughly. Separate lower aqueous layer and re-extract the organic layer with another portion of sulfuric acid. Combine the aqueous layers and make up to 100 mL with water. Filter a portion of the extract through a 0.45 μm Nylon 66 syringe filter. Dilute the filtrate with water, inject a 10 μL. aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Metachem Inertsil C8
Mobile phase: 200 mM Sodium sulfate containing0.3 mM sodium 1-heptanesulfonate and 0.1% acetic acid
Flow rate: 1
Injection volume: 10

Detector: F ex 340 em 440 following post-column reaction. The column effluent mixed with reagent pumped at 1.0 mL/min and this mixture flowed through a 9 m × 0.25 mm I.D. stainless steel coil to the detector. (Reagent was 800 mg o-phthalaldehyde and 1 mL mercaptoethanol in 10 mL MeOH diluted to 1 L with 2.5% boric acid and adjusted to pH 10 with 2.5% KOH.)

CHROMATOGRAM
Retention time: 22.0
Limit of detection: 10 ng

OTHER SUBSTANCES
Also analyzed: paromomycin

KEY WORDS
cream; post-column reaction

REFERENCE
Pick,J.; Olson,L.L.; Ellis,W.Y.; Lim,P. Development and validation of a method to extract and quantitate paromomycin and gentamicin from an Aquaphilic cream formulation, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 131–137.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 150 μL sample to 5 mL, inject an aliquot.

HPLC VARIABLES
Guard column: C18 precolumn filter
Column: 150 × 3.9 4 μm Nova Pak C18
Mobile phase: MeCN:200 mM KH_2PO_4 30:70 adjusted to pH 6.5
Flow rate: 2
Detector: UV 260

CHROMATOGRAM
Retention time: 7.67

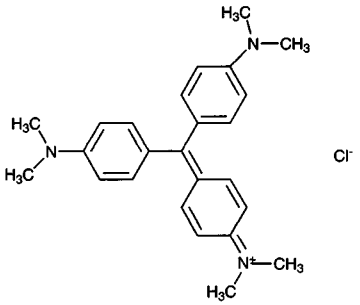
OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
stability-indicating; ophthalmic solutions

REFERENCE
McBride,H.A.; Martinez,D.R.; Trang,J.M.; Lander,R.D.; Helms,H.A. Stability of gentamicin sulfate and tobramycin sulfate in extemporaneously prepared ophthalmic solutions at 8 degrees C, *Am.J.Hosp.Pharm.*, **1991**, *48*, 507–509.

Gentian violet

Molecular formula: $C_{25}H_{30}ClN_3$
Molecular weight: 407.99
CAS Registry No.: 548-62-9
Merck Index: 4401



SAMPLE

Matrix: feed

Sample preparation: Prepare a Sephadex column by slurrying 5 g Sephadex LH-20 with 100 mL MeOH:water 90:10 in a beaker, let stand for 4 h, add to a 300 × 19 column, wash out beaker with MeOH:water 90:10 and add this to the column. Grind feed to pass 1 mm sieve and mix for at least 24 h on a revolving mixer. 10 g Ground feed + 50 mL MeOH:1 M HCl (99:1), shake vigorously on a mechanical shaker for 20 min, centrifuge at 2000 rpm for 15 min, decant, repeat extraction. Combine the extracts, mix well, evaporate an aliquot containing 5 μg gentian violet to dryness under reduced pressure at 48-50°. Reconstitute the residue in 2 mL MeOH:water 90:10, add to the Sephadex column, rinse the flask three times with 2 mL portions and once with a 6 mL portion of MeOH:water 90:10, add the rinses to the column, discard the eluates, elute with 6-6.5 mL MeOH:water 90:10, wash the column tip with 1 mL MeOH:water 90:10, evaporate the eluate to 500 μL under a stream of nitrogen at 48-50°, add 2 mL EtOH, evaporate to dryness under a stream of nitrogen at 48-50°, repeat process, reconstitute the residue in 1 mL MeOH, inject a 3 μL aliquot.

HPLC VARIABLES

Guard column: pellicular C18 (Alltech)
Column: 150 × 3.9 4 μm Nova-Pak RP-C18
Mobile phase: MeOH:buffer 85:15 (Buffer was 10 mM KH_2PO_4 adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 0.75
Injection volume: 3
Detector: UV 588

CHROMATOGRAM

Retention time: 4.4
Limit of detection: 3 ng

KEY WORDS

SPE

REFERENCE

Martinez,E.E.; Shimoda,W. Modified liquid chromatographic method for determination of gentian violet in animal feed, *J.Assoc.Off.Anal.Chem.*, **1989**, *72*, 742–745.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a solution in MeOH, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm CN (Alltech)
Mobile phase: MeOH:100 mM sodium acetate 60:40 containing 50 mg/L disodium EDTA, pH adjusted to 4.5 with aldehyde free glacial acetic acid
Flow rate: 0.8
Injection volume: 5-25
Detector: E, Bioanalytical Systems LC-4B, glassy carbon working electrode +1.000 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 11.2
Limit of quantitation: 0.54 ng

OTHER SUBSTANCES
Simultaneous: metabolites, leucogentian violet
Interfering: methylene blue

REFERENCE
Roybal,J.E.; Munns,R.K.; Hurlbut,J.A.; Shimoda,W. High-performance liquid chromatography of gentian violet, its demethylated metabolites, leucogentian violet and methylene blue with electrochemical detection, *J.Chromatogr.*, **1989**, *467*, 259–266.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 2.1 5 μm Ultracarb C18 (Phenomenex)
Mobile phase: MeCN:100 mM pH 4.5 ammonium acetate 80:20
Flow rate: 0.4
Injection volume: 100
Detector: MS, Hewlett-Packard Model 5989A, source 250°, solvation chamber 45°, particle beam nebulizer helium 40-45°, scan m/z 80-500

OTHER SUBSTANCES
Simultaneous: brilliant green, leucogentian violet, leucomalachite green, malachite green, pentamethyl gentian violet, tetramethyl gentian violet

REFERENCE
Turnipseed,S.B.; Roybal,J.E.; Rupp,H.S.; Hurlbut,J.A.; Long,A.R. Particle beam liquid chromatography-mass spectrometry of triphenylmethane dyes: application to confirmation of malachite green in incurred catfish tissue, *J.Chromatogr.B*, **1995**, *670*, 55–62.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 6 mL neutral alumina SPE cartridge (J.T. Baker) and a 2.8 mL 500 mg Bond Elut PRS SPE cartridge with 5 mL MeCN. Place the alumina SPE cartridge on the top of the PRS SPE cartridge using a Bond Elut adapter. Mix 3 mL 250 mg/mL hydroxylamine hydrochloride in water with 5 mL 50 mM p-toluene sulfonic acid, 20 mL 100 mM ammonium acetate adjusted to pH 4.5 with glacial acetic acid, and 20 g fish tissue. Homogenize at 20000 rpm for 1 min (Ultra-Turrax T25 Tissuemizer, Tekmar, USA). Add 90 mL MeCN to the sample and homogenize for 10 s. Shake vigorously by hand for 1 min. Add 20 g basic alumina (Brockman activity I), shake for 1 min. Centrifuge, decant the supernatant. Add 30 mL MeCN to the residue, extract, combine the supernatants. Add 100 mL water, 50 mL dichloromethane, and 20 mL diethylene glycol to the supernatants, shake vigorously, separate the bottom layer. Again add 50 mL dichloromethane, shake for 1 min, combine the separated dichloromethane layers. Concentrate to 2-3 mL under reduced pressure at 65°. Add 2 mL dichloromethane and 5 mL MeCN then add the mixtures to the SPE cartridges. Rinse the flask twice with 5 mL portions of MeCN and add the rinses to the SPE cartridges. Wash with 5 mL MeCN to waste. Remove the alumina cartridge. Wash the PRS cartridge with 2 mL water and with 1 mL MeCN:100 mM ammonium acetate buffer 50:50 adjusted to pH 4.5 with glacial acetic acid. Elute with 2 mL MeCN:100 mM ammonium acetate buffer 50:50 adjusted to pH 4.5 with glacial acetic acid and collect in a tube containing 500 μL 2.5 mg/mL hydroxylamine hydrochloride in water. Inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2.0 pellicular C18
Column: 150 × 4.6 5 μm SynChropak SCD-100 (SynChrom)
Mobile phase: MeCN:buffer 55:45 (Buffer was 400 mg ammonium acetate and 1 mL triethylamine in 400 mL water, adjusted to pH 3.0 with glacial acetic acid, and made up to 450 mL with water.)
Flow rate: 2.0

Injection volume: 100
Detector: E, ESA Coulochem Model, oxidative electrochemical cell (EC) +900 mV; UV 588; F ex 265 em 360

CHROMATOGRAM
Retention time: 7.9
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, malachite green

KEY WORDS
SPE; catfish; trout

REFERENCE
Rushing,L.G.; Hansen,E.B.,Jr. Confirmation of malachite green, gentian violet and their leuco analogs in catfish and trout tissue by high-performance liquid chromatography utilizing electrochemistry with ultraviolet-visible diode array detection and fluorescence detection, *J.Chromatogr.B*, **1997**, *700*, 223–231.

SAMPLE
Matrix: tissue
Sample preparation: Prepare an alumina column by adding 5 g alumina (Alcoa type F-20, 80-200 mesh, activated chromatographic grade) to a 75 × 16 column. Condition a Bond Elut disposable LRC carboxylic acid SPE cartridge with 10 mL MeCN. 25 g Ground tissue + 100 mL MeCN:buffer 80:20, shake vigorously for 1 min, let stand for 30 min, shake for 1 min, protect from light, let stand overnight, centrifuge at 2000 rpm for 15 min, filter (paper) the supernatant, add 100 mL MeCN:buffer 80:20 to the residue, shake vigorously for 15 s, protect from light, let stand for 1 h, centrifuge, filter (paper). Combine the filtrates, add 25 mL water, add 2 mL diethylene glycol, extract with 100 mL dichloromethane. Evaporate the dichloromethane extract just to dryness, add 5 mL MeCN to the residue, add to the alumina column, rinse flask three times with 5 mL portions of MeCN, add the rinses to the column, elute with 10 mL MeCN, call all the eluates and evaporate them just to dryness. Reconstitute the residue in 25 mL dichloromethane, add 15 mL citrate buffer, shake vigorously for 30 s, remove the organic layer, extract the aqueous layer with two 25 mL portions of dichloromethane. Combine the organic layers and evaporate them just to dryness. Reconstitute with 10 mL MeCN, add a 5 mL aliquot to the SPE cartridge, wash with 5 mL MeCN, discard all eluates, elute with two 2 mL portions of acidic MeOH, evaporate eluate under a stream of nitrogen at 40° to 1 mL, inject a 20 μL aliquot. (Citrate buffer (pH 6-7) was prepared fresh just before use from 1 part 1 M HCl and 2 parts saturated sodium citrate. Acidic MeOH was 95 mL, 5 mL water and 2 mL concentrated HCl, dilute a 1 mL aliquot of this solution to 100 mL with MeOH.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm CN (Alltech)
Mobile phase: MeCN:buffer 50:50 (Buffer was 100 mM sodium acetate adjusted to pH 4.5 with acetic acid containing 50 mg/L EDTA.)
Flow rate: 1
Injection volume: 20
Detector: E, Bioanalytical Systems LC-4B, glassy carbon electrode 1.000 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 16
Limit of detection: <10 PPB

OTHER SUBSTANCES
Extracted: metabolites, leucogentian violet

KEY WORDS
chicken; liver; muscle; SPE; protect from light

REFERENCE

Roybal,J.E.; Munns,R.K.; Hurlbut,J.A.; Shimoda,W. Determination of gentian violet, its demethylated metabolites, and leucogentian violet in chicken tissue by liquid chromatography with electrochemical detection, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 940–command.946.

SAMPLE

Matrix: tissue

Sample preparation: Condition at 6 mL 1 g neutral alumina SPE cartridge (J.T. baker) with 5 mL MeCN. Condition a 2.8 mL 0.5 g Bond Elut PRS SPE cartridge with 5 mL MeCN. 10 g Muscle tissue + 3 mL 250 mg/mL hydroxylamine hydrochloride in water + 5 mL 50 mM p-toluenesulfonic acid + 10 mL 100 mM pH 4.5 ammonium acetate buffer, homogenize (Tekmar Ultra-Turrax T25 tissuemizer) at 20000 rpm for 1 min, add 90 mL MeCN, homogenize for 10 s, shake vigorously by hand for 1 min, add 20 g basic alumina (Brockman activity I, Fisher Scientific), shake vigorously for 1 min, centrifuge, decant, add 30 mL MeCN to the residue, extract, centrifuge, decant. Combine the supernatants and add 100 mL water, add 50 mL dichloromethane, add 2 mL diethylene glycol, shake vigorously by hand for 1 min, let stand for 45 min, remove the lower organic layer, add 50 mL dichloromethane, shake for 1 min, let stand for 5 min, remove the lower organic layers. Combine the organic layers and evaporate them to 2-5 mL under reduced pressure at 65°, add 2 mL dichloromethane, add 5 mL MeCN, add the mixture to the alumina SPE cartridge on top of the PRS SPE cartridge, rinse the flask with two 5 mL portions of MeCN, add the rinses to the SPE cartridges, wash the SPE cartridges with 5 mL MeCN, wash with 1 mL solvent, elute with 1.5 mL solvent, add 500 μL water to the eluate, inject a 100 μL aliquot. (Solvent was MeCN:100 mM ammonium acetate 50:50, adjusted to pH 4.5 with glacial acetic acid.)

HPLC VARIABLES

Guard column: 20 × 2 pellicular CN

Column: 250 × 4.6 5 μm LC-CN (Supelco)

Mobile phase: MeCN:buffer 60:40 (Prepare buffer by dissolving 3.85 g ammonium acetate in 380 mL water, adjust to pH 4.5 with glacial acetic acid, make up to 400 mL with water.)

Flow rate: 1

Injection volume: 100

Detector: UV 588 following post-column oxidation. The column effluent passed through a 20 × 2 column packed with lead(IV) oxide to the detector (Mallinckrodt)

CHROMATOGRAM

Retention time: 12.6

Limit of quantitation: 1 ng/g

OTHER SUBSTANCES

Extracted: leucogentian violet

Simultaneous: methyl violet

KEY WORDS

fish; muscle; catfish; SPE; post-column reaction

REFERENCE

Rushing,L.G.; Webb,S.F.; Thompson,H.C.,Jr. Determination of leucogentian violet and gentian violet in catfish tissue by high-performance liquid chromatography with visible detection, *J.Chromatogr.B*, **1995**, *674*, 125–131.

SAMPLE

Matrix: tissue

Sample preparation: Condition at 6 mL 1 g neutral alumina SPE cartridge (J.T. baker) with 5 mL MeCN. Condition a 2.8 mL 0.5 g Bond Elut PRS SPE cartridge with 5 mL MeCN. 20 g Muscle tissue + 3 mL 250 mg/mL hydroxylamine hydrochloride in water + 5 mL 50 mM p-toluenesulfonic acid in water + 20 mL 100 mM pH 4.5 ammonium acetate buffer, homogenize (Tekmar Ultra-Turrax T25 tissuemizer) at 20000 rpm for 1 min, add 90 mL MeCN, homogenize for 10 s, shake vigorously by hand for 1 min, add 20 g basic alumina (Brockman activity I, Fisher Scientific), shake vigorously for 1 min, centrifuge, decant, add 30 mL MeCN to the residue, extract, centrifuge, decant. Combine the supernatants and add 100 mL water, add 50 mL dichloromethane, add 2 mL diethylene glycol, shake vigorously by hand for 1 min, let stand

for 45 min, remove the lower organic layer, add 50 mL dichloromethane, shake for 1 min, let stand for 5 min, remove the lower organic layer, repeat the extraction with 50 mL dichloromethane. Combine the organic layers and evaporate them to 2-5 mL under reduced pressure at 65°, add 2 mL dichloromethane, add 5 mL MeCN, add the mixture to the alumina SPE cartridge on top of the PRS SPE cartridge, rinse the flask with two 5 mL portions of MeCN, add the rinses to the SPE cartridges, wash the SPE cartridges with 5 mL MeCN. Discard the alumina cartridge, wash the PRS cartridge with 2 mL water, wash with 1 mL solvent, elute with 2 mL solvent, add 500 μL 2.5 mg/mL hydroxylamine hydrochloride in water to the eluate, inject a 100 μL aliquot. (Solvent was MeCN:100 mM ammonium acetate 50:50, adjusted to pH 4.5 with glacial acetic acid.)

HPLC VARIABLES

Guard column: 20 × 2 pellicular C18
Column: 150 × 4.6 5 μm SynChropak SCD-100 (SynChrom)
Mobile phase: MeCN:buffer 55:45 (Prepare buffer by dissolving 400 mg ammonium acetate and 1 mL triethylamine in 400 mL water, adjust to pH 3.6 with glacial acetic acid, make up to 450 mL with water.)
Flow rate: 2
Injection volume: 100
Detector: UV 588 following post-column oxidation. The column effluent passed through a 20 × 2 column packed with lead(IV) oxide (Mallinckrodt) to the detector.

CHROMATOGRAM

Retention time: 8.2
Limit of detection: 1.8 ng/g
Limit of quantitation: 3 ng/g

OTHER SUBSTANCES

Extracted: leucogentian violet, leucomalachite green, malachite green, methyl violet

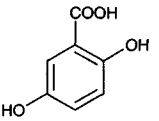
KEY WORDS

fish; muscle; catfish; trout; SPE; post-column reaction

REFERENCE

Rushing,L.G.; Thompson,H.C.,Jr. Simultaneous determination of malachite green, gentian violet and their leuco metabolites in catfish and trout tissue by high-performance liquid chromatography with visible detection, *J.Chromatogr.B*, **1997**, *688*, 325–330.

Gentisic acid



Molecular formula: $C_7H_6O_4$
Molecular weight: 154.12
CAS Registry No.: 490-79-9, 4955-90-2 (sodium salt)
Merck Index: 4404

SAMPLE

Matrix: solutions

HPLC VARIABLES

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

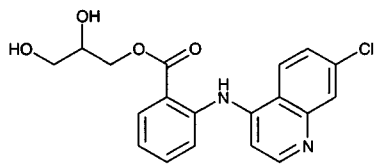
OTHER SUBSTANCES

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

REFERENCE

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

Glafenine



Molecular formula: $C_{19}H_{17}ClN_2O_4$
Molecular weight: 372.81
CAS Registry No.: 3820-67-5
Merck Index: 4443

SAMPLE

Matrix: blood, formulations

Sample preparation: Blood. Add 3 mL MeOH:aqueous ammonia solution 99.5:0.5 to 500 μL serum, shake vigorously, centrifuge at 300 rpm for 15 min, evaporate the supernatant to dry-

ness under a stream of nitrogen, dissolve the residue in 200 μL MeOH, filter (0.2 μm Fluorepore), inject an aliquot. Tablets. Dissolve 200 mg (2 tablets) in 500 mL 100 mM HCl, stir at 100 rpm, filter, dilute 2 mL of the filtrate to 100 mL with 100 mM HCl, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm μBondapak C18
Mobile phase: MeOH:water:H_3PO_4 40:60:0.25, pH adjusted to 3.5 with 100 mM NaOH
Flow rate: 1.5
Injection volume: 10
Detector: UV 360

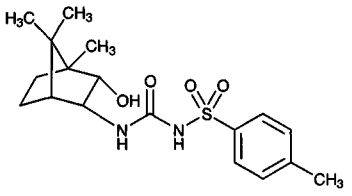
CHROMATOGRAM
Retention time: 7.65
Limit of detection: 5 μg/mL

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
rat; serum; tablets

REFERENCE
Hassan,S.S.M.; Elnemma,E.M.; Abbas,A.B. Determination of glafenine in dosage forms and serum by thin layer densitometry and high performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 215–221.

Glibornuride



Molecular formula: $C_{16}H_{26}N_2O_4S$
Molecular weight: 366.48
CAS Registry No.: 26944-48-9
Merck Index: 4447
Lednicer No.: 2 117

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

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 229

CHROMATOGRAM
Retention time: 5.92
Limit of detection: <120 ng/mL

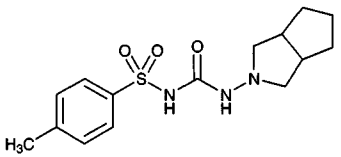
KEY WORDS

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

REFERENCE

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

Gliclazide



Molecular formula: $C_{15}H_{21}N_3O_3S$
Molecular weight: 323.42
CAS Registry No.: 21187-98-4
Merck Index: 4448

SAMPLE

Matrix: blood, urine

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

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

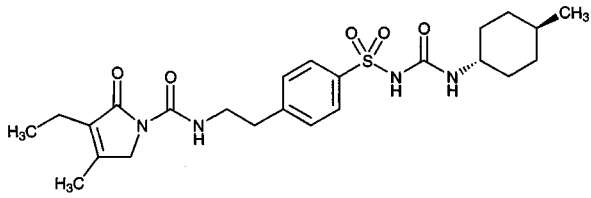
CHROMATOGRAM
Retention time: 20.5

KEY WORDS
whole blood

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

Glimepiride

Molecular formula: $C_{24}H_{34}N_4O_5S$
Molecular weight: 490.62
CAS Registry No.: 93479-97-1
Merck Index: 4449



SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 40 μL 10 μg/mL IS1 in MeOH containing 10 μg/mL IS2 + 1 mL 50 mM pH 1 HCl/KCl buffer + 5 mL diethyl ether, shake for 20 min, centrifuge at 2500 g for 5 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL reagent, heat at 100° for 20 min, evaporate to dryness under a stream of nitrogen at 60°, reconstitute with 200 μL initial mobile phase, inject a 100 μL aliquot. (Prepare reagent by dissolving 30 μL 2,4-dinitrofluorobenzene in 10 mL n-butyl acetate.)

HPLC VARIABLES
Column: 125 × 4.6 5 μm Spherisorb ODS
Mobile phase: Gradient. MeCN:50 mM perchloric acid 40:60 for 6 min, to 58:42 (step gradient), maintain at 58:42 for 8 min, re-equilibrate at initial conditions for 2 min.
Flow rate: 2
Injection volume: 100
Detector: UV 350

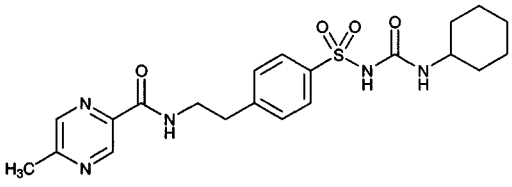
CHROMATOGRAM
Retention time: 11.3
Internal standard: IS1 (1-[4-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)ethyl]phenylsulfonyl]-3-(4-ethylcyclohexyl)urea) (13.4), IS2 (1-[4-[2-(5-chloro-2-methoxyphenyl-1-carboxamido)ethyl]phenylsulfonyl]-3-(4-hydroxycyclohexyl)urea) (3.4)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: glibornuride, glyburide, tolbutamide

KEY WORDS
derivatization; serum; silanize glassware with dichlorodimethylsilane; pharmacokinetics

REFERENCE
Lehr,K.H.; Damm,P. Simultaneous determination of the sulphonylurea glimepiride and its metabolites in human serum and urine by high-performance liquid chromatography after pre-column derivatization, *J.Chromatogr.*, **1990**, *526*, 497–505.

Glipizide



Molecular formula: $C_{21}H_{27}N_5O_4S$
Molecular weight: 445.54
CAS Registry No.: 29094-61-9
Merck Index: 4451
Lednicer No.: 2 117

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

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

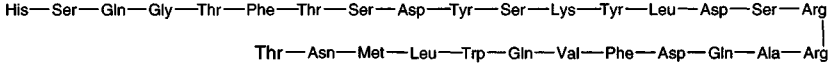
CHROMATOGRAM
Retention time: 17.603

KEY WORDS
whole blood

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

Glucagon

Molecular formula: $C_{153}H_{225}N_{43}O_{49}S$
Molecular weight: 3482.82
CAS Registry No.: 9007-92-5, 16941-32-5
Merck Index: 4455



SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in dilute HCl, inject a 2.5 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm Polygosil C18 (Macherey Nagel) (Condition column as follows. Elute with a linear gradient from MeOH to toluene, pump 10 mL 0.1 g/mL chlorodimethyloctylsilane in toluene through at 0.2 mL/min at 48°, flush (at 1 mL/min) with a linear gradient of MeOH, MeOH:water:trifluoroacetic acid 50:50:0.1, MeOH, and MeOH:chloroform 50:50, flush overnight at 0.2 mL/min with MeOH with a gradient.)
Mobile phase: MeOH:water:trifluoroacetic acid 64:36:0.1
Column temperature: 30
Flow rate: 1.2
Injection volume: 2.5
Detector: UV 205

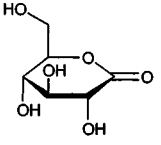
CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Interfering: secretin

KEY WORDS
pig

REFERENCE
Olieman,C.; Sedlick,E.; Voskamp,D. In situ silylation of an octadecylsilyl-silica stationary phase applied to the analysis of peptides, such as secretin and glucagon, *J.Chromatogr.*, **1981**, *207*, 421–424.

Gluconolactone



Molecular formula: $C_6H_{10}O_6$
Molecular weight: 178.14
CAS Registry No.: 90-80-2
Merck Index: 4465

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.1 PRP-X300 (Hamilton)
Mobile phase: 50 mM pH 4.5 NaH_2PO_4
Flow rate: 0.5
Detector: UV 200

CHROMATOGRAM
Retention time: 5.3

OTHER SUBSTANCES
Simultaneous: gluconic acid, dextrose

REFERENCE
Baxter Scientific Products Catalog, 1990-1, p. 123.

SAMPLE
Matrix: urine
Sample preparation: Lyophilize a 5 mL aliquot of urine at -50° for 18 h, reconstitute the residue in 5 mL DMF, centrifuge at 3000 g for 10 min. 1 mL Supernatant + 300 μL phenylisocyanate, heat at 100° for 1 h, cool, add 500 μL MeOH, inject an aliquot.

HPLC VARIABLES
Column: 220 × 4.6 5 μm ODS 224 RP18 (Brownlee)
Mobile phase: MeCN:water 60:40
Flow rate: 2
Injection volume: 10
Detector: UV

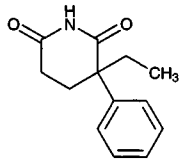
CHROMATOGRAM
Retention time: 9
Limit of detection: 0.4 ng

OTHER SUBSTANCES
Extracted: galactonolactone, galactitol
Simultaneous: dextrose, galactose, allose, myoinositol, sorbitol, mannitol

KEY WORDS
derivatization

REFERENCE
Rakotomanga,S.; Baillet,A.; Pellerin,F.; Baylocq-Ferrier,D. Simultaneous determination of gluconolactone, galactonolactone and galactitol in urine by reversed-phase liquid chromatography: application to galactosemia, *J.Chromatogr.*, **1991**, *570*, 277–284.

Glutethimide



Molecular formula: $C_{13}H_{15}NO_2$
Molecular weight: 217.27
CAS Registry No.: 77-21-4
Merck Index: 4485
Lednicer No.: 1 257

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 200 μL 50 μg/mL hexobarbital in MeCN + 25 μL glacial acetic acid, vortex for 10 s, centrifuge for 1 min, inject a 30-100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: Gradient. MeCN:7.5 g/L NaH_2PO_4 adjusted to pH 3.2 with phosphoric acid 5:95 to 22:78 over 24 min, to 45:55 over 10 min, maintain at 45:55 for 5 min. Re-equilibrate with 5:95 for 5 min.

Column temperature: 50
Flow rate: 3
Injection volume: 30-100
Detector: UV 210

CHROMATOGRAM
Retention time: 24.3
Internal standard: hexobarbital (20.6)
Limit of detection: 200-2000 ng/mL

OTHER SUBSTANCES
Extracted: acetaminophen, amobarbital, butabarbital, butalbital, chlordiazepoxide, diazepam, ethchlorvynol, flurazepam, 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

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KEY WORDS
serum

REFERENCE
Kabra,P.M.; Stafford,B.E.; Marton,L.J. Rapid method for screening toxic drugs in serum with liquid chromatography, *J.Anal.Toxicol.*, **1981**, *5*, 177–182.

SAMPLE
Matrix: blood
Sample preparation: Prepare an SPE cartridge by plugging the end of a 1 mL disposable pipette tip with glass wool and adding about 100 mg Chromosorb P/NAW. Add 50 μL plasma then 50 μL 10 μg/mL tolylphenobarbital in 200 mM HCl to the SPE cartridge, let stand for 2 min, elute with 1 mL chloroform:isopropanol 6:1. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject a 15 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil-LC-8
Mobile phase: MeCN:water 20:80
Flow rate: 3.3
Injection volume: 15
Detector: UV 208

CHROMATOGRAM
Retention time: 11.91
Internal standard: tolylphenobarbital (7.57)
Limit of detection: 50-100 ng/mL

OTHER SUBSTANCES
Extracted: theophylline, caffeine, barbital, ethosuximide, primidone, carbamazepinediol, phenacemide, methyprylon, nirvanol, phenobarbital, chloramphenicol, butabarbital, carbamazepine epoxide, mephenytoin, pentobarbital, amobarbital, carbamazepine, phenytoin, secobarbital, methaqualone
Noninterfering: acetaminophen, amikacin, amitriptyline, clonazepam, cyclosporine, desipramine, diazepam, digoxin, disopyramide, gentamicin, imipramine, lidocaine, methotrexate, N-acetylprocainamide, netilmicin, nortriptyline, procainamide, quinidine, salicylic acid, sulfamethoxazole, tobramycin, trimethoprim, valproic acid, p-hydroxyphenobarbital, vancomycin

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

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

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

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 259

CHROMATOGRAM

Retention time: 3.83

Limit of detection: <120 ng/mL

KEY WORDS

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

REFERENCE

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

SAMPLE
Matrix: microsomal incubations
Sample preparation: Cool 1 mL microsomal incubation to 0°, add 3 mL ethyl acetate, shake in a reciprocal shaker for 10 min, centrifuge at 2500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute with running buffer, inject an aliquot.

HPLC VARIABLES
Guard column: 4 × 4 10 μm RP 8 (Merck)
Column: 250 × 4 5 μm Superspher RP 8
Mobile phase: MeCN:10 mM pH 6.5 tetrabutylammonium hydrogen sulfate 30:70
Flow rate: 0.7
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 33

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
comparison with CE; rat; liver

REFERENCE
Weinz,C.; Blaschke,G.; Schiebel,H.-M. Investigation of the stereoselective in vitro biotransformation of glutethimide by high-performance liquid chromatography and capillary electrophoresis, *J.Chromatogr.B*, **1997**, *690*, 233–242.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 1 5 μm LiChrosorb RP18
Mobile phase: EtOH:water 20:80 containing 20 mM α-cyclodextrin and 0.5 mM tri-O-methyl-α-cyclodextrin
Column temperature: 20
Flow rate: 0.04
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 2.6, k' 2.9 (enantiomers)

OTHER SUBSTANCES
Extracted: mephobarbital

KEY WORDS
chiral

REFERENCE
Nowakowski,R.; Bielejewska,A.; Duszczyk,K.; Sybilska,D. Chiral discrimination by high-performance liquid chromatography with joint use of two cyclodextrin additives, *J.Chromatogr.A*, **1997**, *782*, 1–11.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm 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: 1.87
Internal standard: 8-chlorotheophylline (5.29)

OTHER SUBSTANCES
Simultaneous: acetaminophen, caffeine, cefazolin, cimetidine, ergotamine, heparin, methamphetamine, propranolol, salicylic acid, sulfamethoxazole, theobromine, theophylline, 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

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: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Chiralcel OJ
Mobile phase: Hexane:EtOH 60:40
Column temperature: 23
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 8.73 (R-(+)), 16.20 (S-(-))

OTHER SUBSTANCES
Simultaneous: 4-hydroxyglutethimide

KEY WORDS
chiral

REFERENCE
Aboul-Enein,H.Y.; Islam,M.R. Isocratic high-performance liquid chromatographic resolution of glutethimide enantiomers and their 4-hydroxyglutethimide metabolites using cellulose tribenzoate chiral stationary phase, *J.Chromatogr.Sci.*, **1990**, *28*, 307–310.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 Supelcosil LC-ABZ
Mobile phase: MeCN:25 mM pH 6.9 potassium phosphate buffer 35:65
Flow rate: 1.5
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 4.644

OTHER SUBSTANCES
Also analyzed: 6-acetylmorphine, amiloride, amphetamine, benzocaine, benzoylecgonine, caffeine, cocaine, codeine, doxylamine, fluoxetine, hexobarbital, hypoxanthine, levorphanol, LSD, meperidine, mephobarbital, methadone, methylphenidate, methyprylon, N-norcodeine, oxazepam, oxycodone, phenylpropanolamine, prilocaine, procaine, terfenadine

REFERENCE
Ascah,T.L. Improved separations of alkaloid drugs and other substances of abuse using Supelcosil LC-ABZ column, *Supelco Reporter*, **1993**, *12(3)*, 18–21.

SAMPLE
Matrix: solutions

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

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

faethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)

Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)

Flow rate: 0.6

Injection volume: 25

Detector: UV 229

CHROMATOGRAM

Retention time: 6.60 (A), 6.24 (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, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

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 10 μm Chiralcel OJ
Mobile phase: MeOH
Flow rate: 0.5
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 10 (R-(+)), 16 (S-(-))

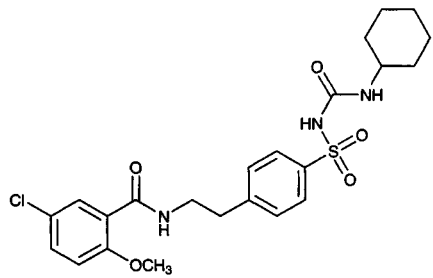
OTHER SUBSTANCES
Simultaneous: metabolites, 5-hydroxyglutethimide

KEY WORDS
chiral

REFERENCE
Weinz,C.; Blaschke,G.; Schiebel,H.-M. Investigation of the stereoselective in vitro biotransformation of glutethimide by high-performance liquid chromatography and capillary electrophoresis, *J.Chromatogr.B*, **1997**, *690*, 233–242.

Glyburide

Molecular formula: $C_{23}H_{28}ClN_3O_5S$
Molecular weight: 494.01
CAS Registry No.: 10238-21-8
Merck Index: 4486
Lednicer No.: 2 139



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

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 229

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 10 μm Chiralcel OJ
Mobile phase: MeOH
Flow rate: 0.5
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 10 (R-(+)), 16 (S-(-))

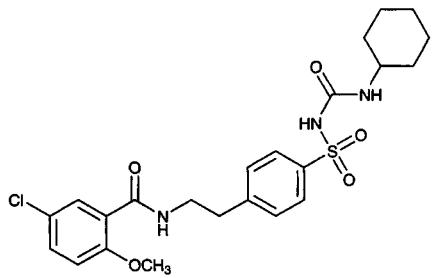
OTHER SUBSTANCES
Simultaneous: metabolites, 5-hydroxyglutethimide

KEY WORDS
chiral

REFERENCE
Weinz,C.; Blaschke,G.; Schiebel,H.-M. Investigation of the stereoselective in vitro biotransformation of glutethimide by high-performance liquid chromatography and capillary electrophoresis, *J.Chromatogr.B*, **1997**, *690*, 233–242.

Glyburide

Molecular formula: $C_{23}H_{28}ClN_3O_5S$
Molecular weight: 494.01
CAS Registry No.: 10238-21-8
Merck Index: 4486
Lednicer No.: 2 139



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

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 229

CHROMATOGRAM
Retention time: 6.25
Limit of detection: <120 ng/mL

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

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

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

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

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

CHROMATOGRAM
Retention time: 21.953

KEY WORDS
whole blood

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

SAMPLE
Matrix: solutions

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

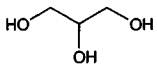
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine,

puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

Glycerin



Molecular formula: $C_3H_8O_3$
Molecular weight: 92.09
CAS Registry No.: 56-81-5
Merck Index: 4493

SAMPLE
Matrix: bulk
Sample preparation: Prepare an aqueous solution, inject an aliquot.

HPLC VARIABLES
Guard column: 10 μm Guard-pak (Waters)
Column: 250 × 4.6 5 μm Ultrasphere ODS C18
Mobile phase: MeCN:water 5:95
Flow rate: 1
Detector: RI

REFERENCE

Cannon,J.M.; Brown,R.D.; Murrill,E.M.; Jameson,C.W. Identification of components in iodinated glycerol, *J.Pharm.Sci.*, **1989**, *78*, 48–51.

SAMPLE
Matrix: bulk
Sample preparation: Prepare an aqueous solution, inject an aliquot.

HPLC VARIABLES
Guard column: 10 μm Guard-pak (Waters)
Column: 250 × 4.6 5 μm Ultrasphere ODS C18
Mobile phase: MeCN:water 5:95
Flow rate: 1
Detector: RI

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: formulations
Sample preparation: Remove the water from 10 μL syrup under reduced pressure for 10 min, reconstitute with 2 mL pyridine. Remove a 25 μL aliquot and add it to 75 μL reagent, shake well, let stand at room temperature for 10 min, evaporate to dryness under reduced pressure

at room temperature, flush the tube with a stream of air or nitrogen, add 2 mL 5% sodium carbonate solution containing 2.5 mg/mL 4-dimethylaminopyridine, shake or sonicate for 5 min, extract with 2 mL chloroform. Wash the extract with 2 mL 5% sodium bicarbonate solution, wash twice with 3 mL portions of 50 mM HCl containing 5% NaCl, inject an aliquot. (Prepare reagent by dissolving 100 mg 4-nitrobenzoyl chloride in pyridine with gentle warming.)

HPLC VARIABLES
Column: 150 × 3 5 μm LiChrosorb SI 60
Mobile phase: n-Hexane:chloroform:MeCN 10:3:1.9 containing 0.1% water
Flow rate: 1.4
Injection volume: 50
Detector: UV 260

CHROMATOGRAM
Retention time: 2.5

OTHER SUBSTANCES
Simultaneous: dextrose, fructose, propylene glycol, saccharose, sorbitol

KEY WORDS
syrup; derivatization; normal phase

REFERENCE
Nachtmann,F.; Budna,K.W. Sensitive determination of derivatized carbohydrates by high-performance liquid chromatography, *J.Chromatogr.*, **1977**, *136*, 279–287.

SAMPLE
Matrix: formulations
Sample preparation: Condition a C18 Sep-Pak SPE cartridge with 2 mL MeOH and 20 mL water. Dilute formulation ten-fold with water, add a 0.5 mL aliquot to the SPE cartridge, elute with three 1 mL portions of water, inject a 10 μL aliquot of the eluate.

HPLC VARIABLES
Column: 8 mm i.d. C18 radial compression (Waters)
Mobile phase: Water
Flow rate: 1
Injection volume: 10
Detector: RI

CHROMATOGRAM
Retention time: 3.4

OTHER SUBSTANCES
Simultaneous: dihydroxyacetone, dioxane, ethylene glycol, formic acid, glyceraldehyde, methylglyoxal, propylene glycol

KEY WORDS
SPE

REFERENCE
Bobin,M.F.; Martini,M.C.; Gudefin,A.; Cotte,J. Dosage de la dihydroxyacétone dans les émulsions [Assay of dihydroxyacetone in emulsions], *Farmaco.[Prat].*, **1983**, *38*, 403–414.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out 1-2 mL, add 20 mL water, mix thoroughly, make up to 100 mL with water, mix thoroughly, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: Micro-Guard ion exclusion cartridge (Bio-Rad)

Column: 300 × 7.8 Aminex HPX-87H (Bio-Rad)
Mobile phase: 6.5 mM sulfuric acid
Column temperature: 65
Flow rate: 0.8
Injection volume: 25
Detector: RI

CHROMATOGRAM
Retention time: 11.62

REFERENCE
Del Grosso,A.V.; May,J.C. Gas chromatographic, liquid chromatographic, and titrimetric procedures for determination of glycerin in allergenic extracts and diagnostic antigens: comparative study, *J.Assoc.Off. Anal.Chem.*, **1987**, *70*, 825–828.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10 μL aliquot of an aqueous solution.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-NH_2
Mobile phase: MeCN:water 75:25
Column temperature: 22
Flow rate: 1
Injection volume: 10
Detector: RI

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: dextrose, fructose, maltose, sucrose

REFERENCE
Johnson,J.M.; Harris,C.H. Selecting the most effective filtration media for HPLC analysis of saccharides, *J.Chromatogr.Sci.*, **1987**, *25*, 267–269.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: ION-300
Mobile phase: 2.5 mM sulfuric acid
Column temperature: 70
Flow rate: 0.4
Detector: RI

CHROMATOGRAM
Retention time: 24

OTHER SUBSTANCES
Simultaneous: acetic acid, citric acid, dextrose, EtOH, fructose, lactic acid, malic acid, MeOH, tartaric acid

REFERENCE
Keystone Scientific Catalog, 1993-4, p. 45.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: Hamilton PRP-X300
Mobile phase: MeCN:0.5 mM sulfuric acid 10:90
Flow rate: 3
Injection volume: 1
Detector: RI

OTHER SUBSTANCES
Simultaneous: i-butanol, n-butanol, s-butanol, t-butanol, EtOH, isopropanol, MeOH, n-propanol

REFERENCE
Keystone Scientific Catalog, 1993-4, p. 23.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: Shodex Sugar SP 0810P and SP 0810
Mobile phase: water
Column temperature: 80
Flow rate: 0.5
Detector: RI

CHROMATOGRAM
Retention time: 27

OTHER SUBSTANCES
Simultaneous: arabinose, dextrose, fructose, galactose, lactose, lactulose, mannitol, pullulan P-10, raffinose, sorbitol, stachyose, sucrose, xylitol

REFERENCE
Majors,R.E. Polymeric liquid chromatography column technology in Japan, *LC.GC*, **1993**, *11*, 778–788.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 100 × 7.8 Sarasep AL-1 (Sarasep, MetaChem)
Mobile phase: 50 mg/L dicalcium EDTA
Column temperature: 85
Flow rate: 0.7
Detector: RI

CHROMATOGRAM
Retention time: 2

OTHER SUBSTANCES
Simultaneous: EtOH, isoamyl alcohol, n-amyl alcohol

REFERENCE
MetaChem Catalog, **1994**, p. 65.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: RSpak DC-613 (Shodex, Phenomenex)
Mobile phase: MeCN:water 80:20
Flow rate: 1

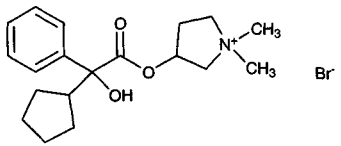
Detector: RI

CHROMATOGRAM
Retention time: 4.5

OTHER SUBSTANCES
Simultaneous: meso-erythrite, arabite, xylite, mannite, sorbite

REFERENCE
Phenomenex Catalog, **1994**, p. 1.109.

Glycopyrrolate



Molecular formula: $C_{19}H_{28}BrNO_3$
Molecular weight: 398.34
CAS Registry No.: 596-51-0
Merck Index: 4511

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

HPLC VARIABLES
Column: 100 × 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: 11.0
Limit of detection: 6.9 μg/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: urine
Sample preparation: Condition a 500 mg 14 mL 40 μm CCX-2 cation-exchange SPE cartridge (Worldwide Monitoring) with two 2.5 mL aliquots of MeOH, two 2.5 mL aliquots of water, and two 2.5 mL aliquots of 100 mM pH 7.00 phosphate buffer, do not allow to dry. 5 mL Urine + 3 mL 100 mM pH 7.00 phosphate buffer + 12.5 ng mepenzolate + 5 mL water, centrifuge at 800 g for 5 min, add to the SPE cartridge, wash with 5 mL MeOH, wash with 5 mL water, dry under vacuum for 5 min, elute with 4 mL MeOH:500 mM pH 3.00 ammonium acetate 95:5 (all flow rates were 1-2 mL/min). Evaporate the eluate under a stream of nitrogen at 60°, reconstitute in 100 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.1 10 μm LiChroma (Chromatographic Specialties)
Mobile phase: MeOH:50 mM pH 3.0 ammonium acetate 80:20
Flow rate: 0.8
Injection volume: 10
Detector: MS, Sciex API III triple quadrupole, ion spray interface, split column effluent 95:5 before entering detector, nebulizing gas air at 550 kPa, collision gas argon, curtain gas nitrogen, positive-ion mode, m/z 318 and 116

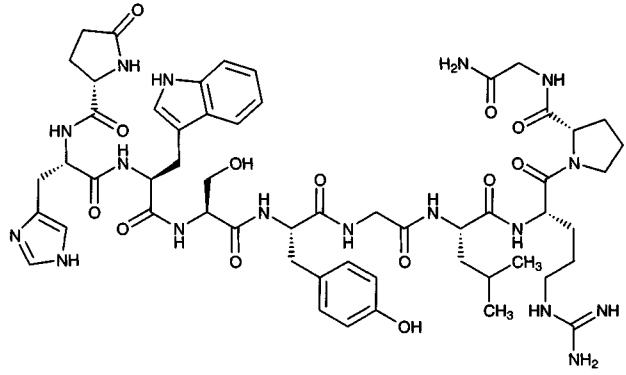
CHROMATOGRAM
Retention time: 2.3
Internal standard: mepenzolate (m/z 340 and 130) (2.1)
Limit of detection: 0.25 ng/mL

KEY WORDS
SPE; horse

REFERENCE
Matassa,L.C.; Woodard,D.; Leavitt,R.K.; Firby,P.; Beaumier,P. Solid-phase extraction techniques for the determination of glycopyrrolate from equine urine by liquid chromatography-tandem mass spectrometry and gas chromatography-mass spectrometry, *J.Chromatogr.*, **1992**, *573*, 43–48.

Gonadorelin

Molecular formula: $C_{55}H_{75}N_{17}O_{13}$
Molecular weight: 1182.31
CAS Registry No.: 33515-09-2, 51952-41-1 (HCl), 52699-48-6 (sulfate)
Merck Index: 5500



SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Analytichem weak cation-exchange (carboxymethylhydrogen form, CBA) SPE cartridge with 1 mL 1% trifluoroacetic acid in MeOH, 1 mL MeOH, and 2 mL water. Add 1 mL plasma to the SPE cartridge, rinse the tube with 1 mL water, add the rinse to the SPE cartridge, wash with 1 mL 1% trifluoroacetic acid in water, wash with 2 mL water, wash with 2 mL MeOH, elute with 2 mL 1% trifluoroacetic acid in MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH:buffer 50:50, inject a 5-75 μL aliquot. (Buffer was 5.7 g monochloroacetic acid, 2.0 g NaOH, and 0.2 g disodium EDTA in 1 L water, pH 3.2.) [Procedure was not necessarily validated for this compound.]; SPE

HPLC VARIABLES
Column: 250 × 2 5 μm Ultrasphere octyl
Mobile phase: Gradient. A was MeOH containing 10 mM sodium octanesulfonate. B was buffer containing 10 mM sodium octanesulfonate. A:B from 45:55 to 70:30 over 30 min, maintain at 70:30 for 1 h. (Buffer was 5.7 g monochloroacetic acid, 2.0 g NaOH, and 0.2 g disodium EDTA in 1 L water, pH 3.2.)
Column temperature: 60
Flow rate: 0.3
Injection volume: 5-75

Detector: F ex 390 em 470 following post-column reaction. The column effluent mixed with 400 mM NaOH pumped at 0.15 mL/min and 0.05% ninhydrin pumped at 0.05 mL/min and the mixture flowed through a 12 m × 0.33 mm i.d. reaction coil at 70° to the detector.

CHROMATOGRAM
Retention time: 22
Limit of detection: 100 fmole

OTHER SUBSTANCES
Simultaneous: adrenocorticotropin, angiotensin I, angiotensin II, angiotensin III, atrial natriuretic peptide, bombesin, bradykinin, somatoliberin, vasopressin

KEY WORDS
plasma; SPE; post-column reaction

REFERENCE
Rhodes,G.R.; Boppana,V.K. High-performance liquid chromatographic analysis of arginine-containing peptides in biological fluids by means of a selective post-column reaction with fluorescence detection, *J.Chromatogr.*, **1988**, *444*, 123–131.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 100 × 5 Nucleosil 5 C8
Mobile phase: MeOH:buffer 23:77 (Buffer was 100 mM phosphoric acid adjusted to pH 3.0 with triethylamine.)
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM
Retention time: 23

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE
Storring,P.L.; Corran,P.H.; Gaines Das,R.E.; Calam,D.H. The International Reference Preparation of Gonadorelin for Bioassay: a comparison with different preparations of synthetic luteinizing hormone releasing hormone using physicochemical methods of analysis, different bioassays and immunoassay, *J.Endocrinol.*, **1982**, *95*, 95–103.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 Zorbax ODS
Mobile phase: MeCN:water:pH 3.0 triethylamine phosphate buffer 14:43:43
Flow rate: 1.5
Detector: UV 210

CHROMATOGRAM
Limit of detection: 100 ng/mL

KEY WORDS
validation

REFERENCE
Bi,M.; Singh,J. Modified HPLC method for quantification of luteinizing hormone-releasing hormone (Abstract 3367), *Pharm.Res.*, **1997**, *14*, S585.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: MeCN:0.1% trifluoroacetic acid 16:84
Flow rate: 1
Injection volume: 20
Detector: UV 214

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Hoitink,M.A.; Beijnen,J.H.; Bult,A.; van der Houwen,O.A.G.J.; Nijholt,J.; Underberg,W.J.M. Degradation kinetics of gonadorelin in aqueous solution, *J.Pharm.Sci.*, **1996**, *85*, 1053–1059.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 4 5 μm Lichrosphere 100 RP-18
Mobile phase: MeCN:0.1% trifluoroacetic acid 16:84
Flow rate: 1
Detector: UV 214

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Hoitink,M.A.; Beijnen,J.H.; Boschma,M.U.S.; Bult,A.; Hop,E.; Nijholt,J.; Versluis,C.; Wiese,G.; Underberg,W.J.M. Identification of the degradation products of gonadorelin and three analogues in aqueous solution, *Anal.Chem.*, **1997**, *69*, 4972–4978.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Gradient. A was 0.1% phosphoric acid. B was MeCN:0.1% phosphoric acid 70:30. A:B from 95:5 to 30:70 over 20 min.
Flow rate: 1
Detector: UV 206 or RIA

CHROMATOGRAM
Retention time: 17

OTHER SUBSTANCES
Simultaneous: protirelin, somatostatin, substance P

REFERENCE
McDermott,J.R.; Smith,A.I.; Biggins,J.A.; Al-Noaemi,M.C.; Edwardson,J.A. Characterization and determination of neuropeptides by high-performance liquid chromatography and radioimmunoassay, *J.Chromatogr.*, **1981**, *222*, 371–379.

SAMPLE
Matrix: solutions

Sample preparation: Cool in ice while mixing 200 μL solution, 100 μL 4 mM benzoin in 2-methoxyethanol, 100 μL mercaptoethanol solution, and 200 μL 2 M KOH, heat on a boiling water bath for 5 min, cool in ice-water for 2 min, add 200 μL 4 M HCl:buffer 50:50, inject a 100 μL aliquot. (Prepare mercaptoethanol solution by dissolving 780 mg β-mercaptoethanol and 2.52 g sodium sulfite in 80 mL water, make up to 100 mL with water. Prepare buffer by dissolving 12.11 g Tris in 80 mL water, adjusting pH to 9.2 with concentrated HCl, and making up to 100 mL with water.)

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: Gradient. MeOH:water:500 mM pH 8.5 Tris-HCl buffer 50:35:15 for 2 min, to 80:5:15 over 24 min, maintain at 80:5:15 for 2 min.
Flow rate: 0.8
Injection volume: 100
Detector: F ex 325 em 425

CHROMATOGRAM

Retention time: 14.5-18.5

OTHER SUBSTANCES

Simultaneous: N-α-acetylarginine, agmatine, angiotensin I, angiotensin II, angiotensin III, arginine, argininosuccinic acid, bradykinin, canavanine, creatine, creatinine, guanidine, guanidinoacetic acid, guanidinobutyric acid, guanidinopropionic acid, guanidinosuccinic acid, homoarginine, methylguanidine, neurotensin, phenylguanidine, taurocyamine, tuftsin

KEY WORDS

derivatization

REFERENCE

Kai,M.; Miyazaki,T.; Yamaguchi,M.; Ohkura,Y. High-performance liquid chromatography of guanidino compounds using benzoin as a pre-column fluorescent derivatization reagent, *J.Chromatogr.*, **1983**, *268*, 417–424.

SAMPLE

Matrix: solutions
Sample preparation: Cool in ice while mixing 100 μL of an aqueous solution, 50 μL 5 mM benzoin in 2-methoxyethanol, 50 μL mercaptoethanol solution, and 100 μL 0.8 M KOH, heat on a boiling water bath for 1.5 min, add 100 μL 1.6 M HCl:1 M pH 8.5 Tris-HCl buffer 50:50, inject a 100 μL aliquot. (Prepare mercaptoethanol solution by dissolving 780 mg β-mercaptoethanol and 2.52 g sodium sulfite in 80 mL water, make up to 100 mL with water.)

HPLC VARIABLES

Column: 15 × 4 (sic) 5 μm LiChrosorb RP-18
Mobile phase: MeCN:50 mM pH 8.5 phosphate buffer 31:69
Flow rate: 0.8
Injection volume: 100
Detector: F ex 325 em 425

CHROMATOGRAM

Retention time: 22

OTHER SUBSTANCES

Simultaneous: angiotensin I, angiotensin II, angiotensin III, leupeptin acid, substance P, tuftsin

KEY WORDS

derivatization

REFERENCE

Kai,M.; Miyazaki,T.; Sakamoto,Y.; Ohkura,Y. Use of benzoin as pre-column fluorescence derivatization reagent for the high-performance liquid chromatography of angiotensins, *J.Chromatogr.*, **1985**, *322*, 473–477.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a solution in saline or Sorensen's buffer.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Econosphere endcapped C18
Mobile phase: MeCN:water 80:20 containing 0.1% trifluoroacetic acid
Detector: UV 278

CHROMATOGRAM
Retention time: 5.2

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE
Miller,L.L.; Kolaskie,C.J.; Smith,G.A.; Rivier,J. Transdermal iontophoresis of gonadotropin releasing hormone (LHRH) and two analogues, *J.Pharm.Sci.*, **1990**, *79*, 490–493.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 TSKgel ODS-120T
Mobile phase: Gradient. A was MeOH:water 20:80 containing 0.05% trifluoroacetic acid. B was MeOH:water 50:50 containing 0.05% trifluoroacetic acid. A:B from 100:0 to 0:100 over 1 h.
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: angiotensin I, angiotensin II, α-endorphin, β-endorphin, calcitonin (human), protirelin (TRH), somatostatin

REFERENCE
Varian Catalog, **1993**, p. 182.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 200 × 3 Spherisorb S5ODS-2
Mobile phase: Gradient. A was 0.05% phosphoric acid containing 0.5% $(NH_4)_2SO_4$. B was MeCN. A:B from 82:18 to 64:36 over 25 min, maintain at 64:36 for 2.5 min, return to initial conditions over 1 min, re-equilibrate for 6.5 min. or Isocratic MeCN:0.05% phosphoric acid containing 0.5% $(NH_4)_2SO_4$ 24:76
Flow rate: 0.5
Detector: UV 210

CHROMATOGRAM
Retention time: 9 (gradient), 2.5 (isocratic)

OTHER SUBSTANCES
Simultaneous: buserelin, deslorelin, goserelin, leuprolide, nafarelin

KEY WORDS
comparison with capillary electrophoresis

REFERENCE

Corran,P.H.; Sutcliffe,N. Identification of gonadorelin (LHRH) derivatives: comparison of reversed-phase high-performance liquid chromatography and micellar electrokinetic chromatography, *J.Chromatogr.*, **1993**, *636*, 87–94.

SAMPLE

Matrix: tissue

Sample preparation: Condition a Bond Elut C18 SPE cartridge with 3 mL water and 3 mL MeCN (in this order ?). Homogenize 400 mg brain tissue with 2 mL 100 mM HCl, add 20 μL 10 μM IS, add 2 mL acetone, mix, centrifuge at 2450 g for 15 min. Remove the supernatant and add it to 220 μL 1 M sodium bicarbonate and 500 μL 100 mM disodium EDTA, centrifuge at 2450 g for 15 min. Remove the supernatant and evaporate it to remove the acetone, dilute the aqueous residue with 2 mL water, add to the SPE cartridge. wash with 1 mL water, wash with 3 mL 100 mM HCl, wash with two 3 mL portions of dichloromethane, wash with 1 mL water, wash with 2 mL 100 mM pH 8.0 phosphate buffer, wash with 2 mL water, elute with 2 mL MeCN:100 mM pH 2.3 phosphate buffer 70:30. Evaporate the eluate under reduced pressure, make up to 400 μL with water, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 200 × 4 5 μm TSKgel ODS-120T (Tosoh)

Mobile phase: Gradient. A was MeCN:300 mM pH 2.3 sodium phosphate buffer:water 1:20:79. B was MeCN:300 mM pH 2.3 sodium phosphate buffer:water 60:20:20. A:B from 90:10 to 55:45 over 33 min, maintain at 55:45 for 7 min, to 0:100 (step gradient), maintain at 0:100.

Flow rate: 1

Injection volume: 100

Detector: F ex 325 em 435 following post-column reaction. The column effluent mixed with 2 mM benzoin in 1.6 M KOH containing 700 mM 2-mercaptoethanol and this mixture flowed through a 15 m × 0.33 mm ID PTFE coil at 76 ± 1°. The effluent from this coil mixed with 500 mM Tris containing 2.1 M HCl pumped at 0.4 mL/min and this mixture flowed to the detector.

CHROMATOGRAM

Retention time: 29.2

Internal standard: [D-Phe^{11}]-neurotensin (40.0)

Limit of detection: 0.5 pmole

OTHER SUBSTANCES

Extracted: bradykinin, dynorphin 1-8, kallidin, leucine enkephalin-Arg, methionine enkephalin-Arg-Gly-Leu, methionine enkephalin-Arg-Phe, α-neoendorphin, β-neoendorphin, neurotensin, substance P, vasopressin

KEY WORDS

post-column reaction; rat; brain; SPE

REFERENCE

Ohno,M.; Kai,M.; Ohkura,Y. High-performance liquid chromatographic determination of substance P-like arginine-containing peptide in rat brain by on-line post-column fluorescence derivatization with benzoin, *J.Chromatogr.*, **1989**, *490*, 301–310.

SAMPLE

Matrix: tissue

Sample preparation: Tissue homogenate + MeCN, vortex for 10 s, centrifuge at 10000 g for 15 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4 10 μm Lichrosorb RP-18

Mobile phase: Gradient. MeCN:0.6% aqueous ethanolamine, pH 3.0 14:86 for 5 min, to 30:70 over 8 min, maintain at 30:70 for 7 min, re-equilibrate at initial conditions for 5 min.

Flow rate: 1

Detector: UV 254

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Extracted: degradation products

KEY WORDS
rabbit; rectal mucosa; vaginal mucosa; nasal mucosa

REFERENCE
Han,K.; Park,J.S.; Chung,Y.B.; Lee,M.J.; Moon,D.C.; Robinson,J.R. Identification of enzymatic degradation products of luteinizing hormone releasing hormone (LHRH)/[D-Ala6] LHRH in rabbit mucosal homogenates, *Pharm.Res.*, **1995**, *12*, 1539–1544.

Gonadotropin

Molecular weight: ca. 39500
CAS Registry No.: 9002-71-3
Merck Index: 2273

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak alkylphenyl
Mobile phase: Gradient. A was 100 mM pH 7.0 ammonium acetate. B was MeCN:water 60:40 containing 15 mM trifluoroacetic acid, pH 2.0. A:B from 100:0 to 0:100 over 90 min.
Flow rate: 1.2
Detector: UV 278

CHROMATOGRAM
Retention time: 30 (α1), 32 (α2), 33 (β1), 34 (β2) (subunits)

REFERENCE
Grego,B.; Hearn,M.T.W. High-performance liquid chromatography of amino acids, peptides and proteins. LXIII. Reversed-phase high-performance liquid chromatographic characterisation of several polypeptide and protein hormones, *J.Chromatogr.*, **1984**, *336*, 25–40.

SAMPLE
Matrix: solutions

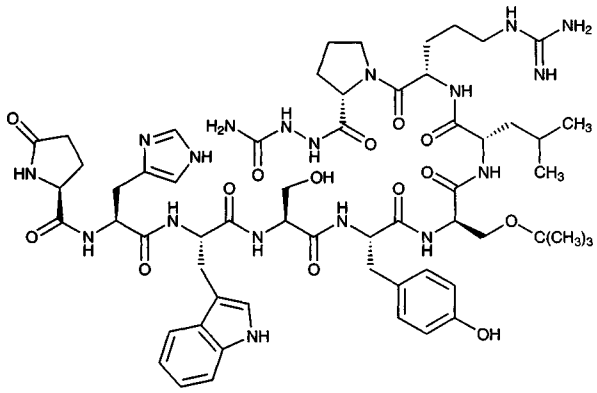
HPLC VARIABLES
Column: 250 × 4.6 Vydac C4 300 A no. 214TP54
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.1% trifluoroacetic acid in MeCN. A:B from 100:0 to 40:60 over 90 min.
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 50 (α-1), 52 (α-2), 50 (β-1), 54 (β-2) (subunits)

REFERENCE
Pollak,S.; Halpine,S.; Chait,B.T.; Birken,S. High resolution high performance liquid chromatography fingerprinting of purified human chorionic gonadotropin demonstrates that oxidation is a cause of hormone heterogeneity, *Endocrinology*, **1990**, *126*, 199–208.

Goserelin

Molecular formula: $C_{59}H_{84}N_{18}O_{14}$
Molecular weight: 1269.43
CAS Registry No.: 65807-02-5
Merck Index: 4547



SAMPLE
Matrix: solutions

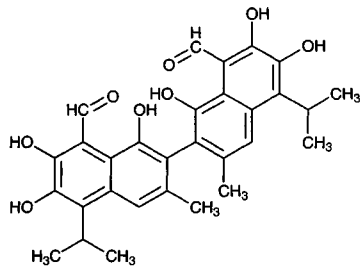
HPLC VARIABLES
Column: 125 × 4 5 μm Lichrosphere 100 RP-18
Mobile phase: MeCN:0.1% trifluoroacetic acid 22:78
Flow rate: 1
Detector: UV 214

OTHER SUBSTANCES
Simultaneous: degradation products
Also analyzed: triptorelin

REFERENCE
Hoitink,M.A.; Beijnen,J.H.; Boschma,M.U.S.; Bult,A.; Hop,E.; Nijholt,J.; Versluis,C.; Wiese,G.; Underberg,W.J.M. Identification of the degradation products of gonadorelin and three analogues in aqueous solution, *Anal.Chem.*, **1997**, *69*, 4972–4978.

Gossypol

Molecular formula: $C_{30}H_{30}O_8$
Molecular weight: 518.56
CAS Registry No.: 303-45-7
Merck Index: 4549



SAMPLE
Matrix: blood
Sample preparation: 400 μL serum + 500 μL saturated disodium EDTA, mix, let stand for 10 min, adjust to pH 8.0 with concentrated NaOH solution using a micro-electrode, add 50 μL 1 g/mL L-phenylalanine methyl ester in MeCN, let stand for 10 min, adjust pH to 7.00 with concentrated sulfuric acid, add 2 mL ether, vortex, centrifuge, repeat extraction. Combine the organic layers and evaporate them to dryness under reduced pressure, reconstitute the residue in 500 μL MeCN, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.5 5 μm Hypersil ODS

Mobile phase: MeCN:THF:buffer 76:2:22 (Buffer was 10 mM KH_2PO_4 adjusted to pH 2.35 with phosphoric acid.)
Flow rate: 2.5
Injection volume: 50
Detector: UV 250

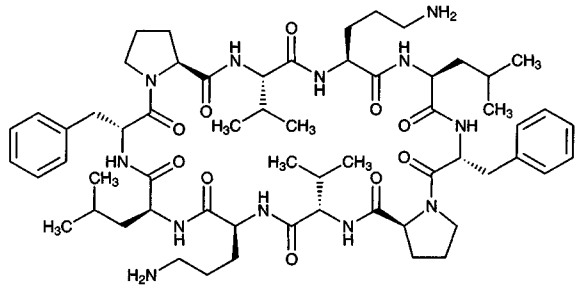
CHROMATOGRAM
Retention time: 11 (-), 17 (+)
Limit of detection: 30 ng/mL

KEY WORDS
serum; derivatization; chiral

REFERENCE
Matlin,S.A.; Belenguer,A.; Vince,P.M.; Stein,R. Analysis of gossypol enantiomers in human serum, *J.Liq.Chromatogr.*, **1990**, *13*, 2261–2268.

Gramicidin

Molecular formula: $C_{60}H_{92}N_{12}O_{10}$
Molecular weight: 1141.47
CAS Registry No.: 1405-97-6, 113-73-5 (gramicidin S)
Merck Index: 4552



SAMPLE
Matrix: saliva
Sample preparation: Mix saliva with an equal volume of EtOH, centrifuge.

HPLC VARIABLES
Column: 200 × 3 5 μm Nucleosil C8
Mobile phase: MeCN:water:phosphoric acid 47.5:52.5:0.01
Column temperature: 70
Flow rate: 2.5
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 13 (valin-gramicidin A)
Limit of detection: 1 μg/mL

KEY WORDS
pharmacokinetics

REFERENCE
Kreuzig,F.; Nahler,G. Salivary levels of gramicidin after use of a tyrothricin lozenge and a tyrothricin gargle/mouth-wash, *Int.J.Clin.Pharmacol.Res.*, **1983**, *3*, 65–70.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 2 μL aliquot.

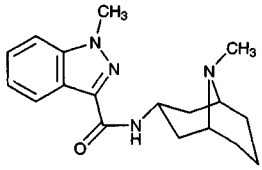
HPLC VARIABLES
Column: 300 × 7.8 Ultrastyragel 1000 Å (Waters)

Mobile phase: THF
Flow rate: 1
Injection volume: 2
Detector: F ex 297 em 330

CHROMATOGRAM
Retention time: 7.9 (dimers), 8.4 (monomer)

REFERENCE
Bañó,M.C.; Braco,L.; Abad,C. New high-performance liquid chromatography-based methodology for monitoring the conformational transitions of self-associating hydrophobic peptides, incorporated into liposomes, *J.Chromatogr.*, **1988**, *458*, 105–116.

Granisetron



Molecular formula: $C_{18}H_{24}N_4O$
Molecular weight: 312.41
CAS Registry No.: 109889-09-0, 107007-99-8 (HCl)
Merck Index: 4557
Lednicer No.: 5 118

SAMPLE
Matrix: blood
Sample preparation: Filter (Amicon Centricon-10 microconcentrator, 10000 daltons molecular weight cutoff) 150 μL plasma while centrifuging at 5° at 5000 g for 100 min, inject a 10 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Spheri-5 silica
Mobile phase: MeCN:25 mM pH 4.2 sodium acetate 40:60
Flow rate: 1
Injection volume: 10
Detector: UV 305

CHROMATOGRAM
Retention time: 3.4
Limit of detection: 19 ng/mL

KEY WORDS
plasma; guinea pig; ultrafiltrate

REFERENCE
Capacio,B.R.; Byers,C.E.; Jackson,T.K.; Matthews,R.L. An HPLC method for the determination of granisetron in guinea pig plasma, *J.Anal.Toxicol.*, **1993**, *17*, 151–155.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond Elut C2 SPE cartridge with two 1 mL portions of MeOH, 1 mL water, and two 1 mL portions of buffer. 1 mL Plasma + 100 μL 40 ng/mL IS in water + 500 μL buffer, add to the SPE cartridge, wash with 1 mL MeCN:water 40:60, remove all wash solvent, elute with 800 μL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 50°. Add 30 μL 10% trimethylsilyldiazomethane in hexane and 30 μL 2% N,N-diisopropylethylamine in MeOH to the residue, heat at 50° for 20 min, cool to room temperature, evaporate to dryness under a stream of nitrogen at 50°, reconstitute in 300 μL MeOH:water 10:90, centrifuge at 1700 g for 5 min, inject a 150 μL aliquot. (Phosphate buffer was 4.33 g Na_2HPO_4 and 3.04 g $NaH_2PO_4.2H_2O$ in 50 mL water.)

HPLC VARIABLES
Guard column: 15 × 3.2 NewGuard RP-18
Column: 250 × 4.6 5 μm Develosil ODS-5 (Nomura Chemical)
Mobile phase: MeOH:buffer 30:70 (Buffer was 15.4 g ammonium acetate and 20 mL tetra-n-butylammonium hydroxide in 1.7 L water, adjust pH to 4.70 with glacial acetic acid, make up to 2 L with water.)
Column temperature: 45
Flow rate: 1
Injection volume: 150
Detector: F ex 310 em 420

CHROMATOGRAM
Retention time: 9.5
Internal standard: 2-methyl-M-(endo-9-methyl-9-azabicyclo[3.3.1]non-3-yl)-2H-indazole-3-carboxamide hydrochloride (6)
Limit of detection: 42 pg/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; SPE; derivatization

REFERENCE
Kudoh,S.; Sato,T.; Okada,H.; Kumakura,H.; Nakamura,H. Simultaneous determination of granisetron and 7-hydroxygranisetron in human plasma by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.B*, **1994**, *660*, 205–210.

SAMPLE
Matrix: blood
Sample preparation: Condition a C2 SPE cartridge (Analytichem) with 1 mL MeOH, 1 mL water, and 1 mL 1 M pH 7.0 phosphate buffer. 1 mL Plasma + 50 μL water + 50 μL 100 ng/mL IS in water + 500 μL 1 M pH 7.0 phosphate buffer, add immediately to SPE cartridge, wash with 1 mL water, wash with 1 mL MeCN:water 40:60, remove wash solvent completely, elute with 1 mL MeOH, elute with 1 mL MeOH:trifluoroacetic acid 99:1. Combine the eluates and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL MeOH:water 10:90, vortex for 30 s, centrifuge at 2000 g for 5 min, transfer to a fresh vial, centrifuge at 2000 g for 5 min, inject a 10-65 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 2.1 C8 (ABI Instruments)
Column: 150 × 2.1 Zorbax Rx C8
Mobile phase: MeCN:buffer 19:81 (Buffer was 0.95 g sodium hexanesulfonate in 405 mL 100 mM pH 4.7 acetate buffer.)
Column temperature: 30
Flow rate: 0.3
Injection volume: 10-65
Detector: E, ESA, E1 0.15 V, E2 0.35 V (measuring electrode), guard cell 0.4 V (before injector) followed by F ex 305 em 360

CHROMATOGRAM
Retention time: 17 (F)
Internal standard: 8-methyl-8-azabicyclo[3.2.1]oct-3-yl 1-methyl-1H-indazole-3-carboxylate (BRL 43704) (21) (F)
Limit of quantitation: 0.1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; SPE; fluorescence detection for granisetron and some metabolites; electrochemical detection for other metabolites

REFERENCE

Boppana,V.K. Simultaneous determination of granisetron and its 7-hydroxy metabolite in human plasma by reversed-phase high-performance liquid chromatography utilizing fluorescence and electrochemical detection, *J.Chromatogr.A*, **1995**, *692*, 195–202.

SAMPLE

Matrix: blood

Sample preparation: 500 μL Plasma + 7 μg IS, vortex for 10 s, add 1.5 mL toluene, add 250 μL buffer, shake for 20 min, centrifuge at 3000 g for 10 min. Remove 1 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 40 μL mobile phase, vortex for 10 s, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Spherisorb CN

Mobile phase: MeCN:buffer 15:85 (Buffer was 100 mM NaH_2PO_4 adjusted to pH 4.5 with orthophosphoric acid.)

Flow rate: 2

Injection volume: 20

Detector: F ex 305 em 365

CHROMATOGRAM

Retention time: 6.64

Internal standard: N-(1-naphthyl)ethylenediamine dihydrochloride (3.60)

Limit of detection: 0.1 ng/mL

Limit of quantitation: 0.3 ng/mL

OTHER SUBSTANCES

Noninterfering: metabolites, anthracyclines, cimetidine, cisplatin, dexamethasone, etoposide, fluorouracil, methotrexate, methylprednisolone, ondansetron

KEY WORDS

plasma

REFERENCE

Pinguet,F.; Bressolle,F.; Martel,P.; Salabert,D.; Astre,C. High-performance liquid chromatographic determination of granisetron in human plasma, *J.Chromatogr.B*, **1996**, *675*, 99–105.

SAMPLE

Matrix: blood, pleural effusion, urine

Sample preparation: Mix 500 μL serum, pleural effusion, or urine with 100 μL IS solution and 400 μL pH 9.0 ammonium acetate. Add to an Extrelut-1 SPE cartridge. After 15 min, elute with 3 mL and 4 mL portions of dichloromethane. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue with 100 μL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm LiChroCART LiChrospher 100 CN

Mobile phase: MeCN:100 mM pH 3.5 acetate buffer 30:70

Column temperature: 40

Flow rate: 1

Injection volume: 20

Detector: F ex 290 em 365

CHROMATOGRAM

Retention time: 11.3

Internal standard: BRL 43693A (Smith Kline Beecham) (9.5)

Limit of detection: 250 pg/mL

Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES

Extracted: etoposide

Simultaneous: domperidone, metoclopramide, ondansetron

Noninterfering: cisplatin, carboplatin, dexamethasone

KEY WORDS
serum; SPE; pharmacokinetics

REFERENCE
Wada,I.; Satoh,M.; Takeda,T.; Nakabayashi,T.; Honma,T.; Saitoh,H.; Takada,M.; Hirano,K. A rapid assay of granisetron in biological fluids from cancer patients, *Biol.Pharm.Bull.*, **1998**, *21*, 535–537.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + 100 μL IS in water + 500 μL 100 mM pH 12 phosphate buffer + 3 mL toluene, shake mechanically for 30 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL mobile phase, inject an 80 μL aliquot.

HPLC VARIABLES
Guard column: 50 mm long unspecified
Column: 250 X 4.5 10 μM Apex CN
Mobile phase: MeOH:buffer 97:3 (Buffer was 50 mM sodium acetate containing 0.25% triethylamine adjusted to pH 6.0.)
Flow rate: 1
Injection volume: 80
Detector: F ex 305 em 360

CHROMATOGRAM
Retention time: 13.5
Internal standard: endo-1-methyl-O-(9-methyl-9-azabicyclo(3,3,1)non-3-yl)-1H-indazole-3-carboxylate (BRL 43704) (19.2)
Limit of detection: 0.1 ng/mL

KEY WORDS
plasma; human; rat; dog

REFERENCE
Clarkson,A.; Coates,P.E.; Zussman,B.D. A specific h.p.l.c. method for the determination of BRL 43694 in plasma and urine, *Br.J.Clin.Pharmacol.*, **1988**, *25*, 136P.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 1-mg granisetron hydrochloride injection with 0.9% NaCl or 5% dextrose to a granisetron concentration of 100 μg/mL, inject a 20 μL aliquot of the solution.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb Cyano
Mobile phase: MeCN:100 mM NaH_2PO_4 15:85
Flow rate: 2
Injection volume: 20
Detector: UV 302

CHROMATOGRAM
Retention time: 9.1

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
injections; stability-indicating

REFERENCE
Quercia,R.A.; Zhang,J.; Fan,C.; Chow,M.S.S. Stability of granisetron hydrochloride in polypropylene syringes, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 2744–2746.

SAMPLE
Matrix: formulations
Sample preparation: Filter (0.2 μm nylon), inject a 50 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Accubond CN (J & W)
Mobile phase: MeCN:buffer 60:40 (Buffer was 20 mM KH_2PO_4 containing 5 mM octanesulfonic acid, adjusted to pH 6.0 with 1 M NaOH.)
Flow rate: 1.5
Injection volume: 50
Detector: UV 305

CHROMATOGRAM
Retention time: 7.5

KEY WORDS
injections; saline; 5% dextrose; stability-indicating

REFERENCE
Chung,K.C.; Chin,M.A.; Gill,M.A. Stability of granisetron hydrochloride in a disposable elastomeric infusion device, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 1541–1543.

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

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb CN
Mobile phase: MeCN:100 mM pH 4.5 NaH_2PO_4 15:85
Injection volume: 20
Detector: UV 300

CHROMATOGRAM
Retention time: 6.8
Internal standard: propylparaben (3.3)

OTHER SUBSTANCES
Simultaneous: dexamethasone, methylprednisolone

KEY WORDS
injections; 5% dextrose; saline; water

REFERENCE
Pinguet,F.; Rouanet,P.; Martel,P.; Fabbro,M.; Salabert,D.; Astre,C. Compatibility and stability of granisetron, dexamethasone, and methylprednisolone in injectable solutions, *J.Pharm.Sci.*, **1995**, *84*, 267–268.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm cyano
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 2
Injection volume: 20

Detector: UV 300

CHROMATOGRAM
Retention time: 5.60

OTHER SUBSTANCES
Simultaneous: dexamethasone (UV 228)

KEY WORDS
stability-indicating; injections; saline; 5% dextrose; apple juice; orange juice; soft drinks

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 3.9 5 μm Symmetry C8 (Waters)
Mobile phase: MeCN:20 mM sodium dihydrogen phosphate 20:80
Flow rate: 1
Injection volume: 20
Detector: UV 307

CHROMATOGRAM
Retention time: 4.5

OTHER SUBSTANCES
Simultaneous: doxorubicin (11.2)

REFERENCE
Zhang,H.; Ye,L.; Stewart,J.T. HPLC determinations of doxorubicin with selected medications in 0.9% sodium chloride injection USP, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 2375–2385.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of an aqueous solution.

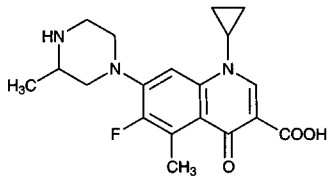
HPLC VARIABLES
Column: 250 × 4 Nucleosil C18
Mobile phase: MeOH:THF:buffer 30:5:65 (Buffer was 100 mM triethylamine adjusted to pH 3.0 with nitric acid.)
Flow rate: 0.8
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6.37

OTHER SUBSTANCES
Simultaneous: ondansetron, 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.

Grepafloxacin



Molecular formula: $C_{19}H_{22}FN_3O_3$
Molecular weight: 359.40
CAS Registry No.: 119914-60-2, 146863-02-7 (±), 161967-81-3 ((±) HCl)
Merck Index: 4567

SAMPLE
Matrix: blood
Sample preparation: Extract 200 µL plasma with dichloromethane:n-butanol 95:5 and evaporate to dryness. Treat the organic layer residue with Mosher's acid chloride (R-(-)- or S-(+)-α-methoxy-α -trifluoromethylphenylacetic acid chloride) and triethanolamine in dichloromethane for 1 hr. Reconstitute, inject an aliquot.

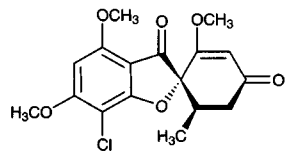
HPLC VARIABLES
Column: 150 × 3.2 5µm ODS
Mobile phase: MeCN:0.2% phosphoric acid 70:30
Column temperature: 35
Detector: F ex 290 em 470

CHROMATOGRAM
Retention time: 12.7 (S-(-)), 13.5 (R-(+))
Internal standard: ciprofloxacin (7.4)
Limit of quantitation: 25 ng/mL

KEY WORDS
derivatization; chiral; plasma

REFERENCE
Tata,P.N.V.; Bramer,S.L. Enantiomeric assay of grepafloxacin in plasma (Abstract 4162), *Pharm.Res.*, **1997**, *14*, S684.

Griseofulvin



Molecular formula: $C_{17}H_{17}ClO_6$
Molecular weight: 352.8
CAS Registry No.: 126-07-8
Merck Index: 4571
Lednicer No.: 1 314

SAMPLE
Matrix: blood
Sample preparation: 100 µL Plasma + 100 µL p-phenylphenol in MeCN, vortex, centrifuge, inject a 20 µL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 mm long 5 µm Novapack
Mobile phase: MeCN:0.1 M acetic acid, pH 3.5 45:55
Flow rate: 1
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: 3.0

Internal standard: p-phenylphenol (4.5)
Limit of detection: 50 ng/mL

KEY WORDS
rat; plasma; pharmacokinetics

REFERENCE
Vudathala,G.K.; Rogers,J.A. Oral bioavailability of griseofulvin from aged griseofulvin: lipid coprecipitates: in vivo studies in rats, *J.Pharm.Sci.*, **1992**, *81*, 1166–1169.

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

HPLC VARIABLES
Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM
Retention time: 13.51
Internal standard: heptanophenone (19)

OTHER SUBSTANCES
Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

KEY WORDS
serum; column-switching

REFERENCE
Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

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

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 292.6

CHROMATOGRAM
Retention time: 18.392

KEY WORDS
whole blood

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

SAMPLE
Matrix: solutions
Sample preparation: Mix an excess of griseofulvin with 8 mL water or with 8 mL 6-7 mM sodium dodecyl sulfate. Shake in a 25° water bath for 48 h, filter through a 0.45 μm filter. Discard the first a few mL, inject a 150 μL aliquot of the rest.

HPLC VARIABLES
Column: 140 × 4.6 5μm cyanopropyl methyl silane bonded silica (Supelco Inc., PA)
Mobile phase: MeOH:water 50:50
Flow rate: 1
Injection volume: 150
Detector: UV 295

REFERENCE
Rao,V.M.; Lin,M.; Larive,C.K.; Southard,M.Z. A mechanistic study of griseofulvin dissolution into surfactant solutions under laminar flow conditions, *J.Pharm.Sci.*, **1997**, *86*, 1132–1137.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Spherisorb 5 ODS
Mobile phase: MeCN:10 mM phosphoric acid 34:66, pH 2.82
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: UV 248

CHROMATOGRAM
Retention time: 12.13
Internal standard: griseofulvin

OTHER SUBSTANCES
Simultaneous: methoxsalen

KEY WORDS
SPE; griseofulvin is IS

REFERENCE
Kucová,D.; Marysková,D.; Davídková,P.; Gasparic,J. High-performance liquid chromatographic determination of methoxsalen in plasma after liquid-solid extraction, *J.Chromatogr.*, **1993**, *614*, 340–344.

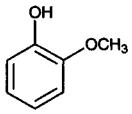
SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm RP 18
Mobile phase: MeOH:water 60:40
Detector: UV 245 and 315

CHROMATOGRAM
Limit of quantitation: 500 ng/mL

REFERENCE
De Smidt,J.H.; Grit,M.; Crommelin,D.J.A. Dissolution kinetics of griseofulvin in mixed micellar solutions, *J.Pharm.Sci.*, **1994**, *83*, 1209–1212.

Guaiacol



Molecular formula: $C_7H_8O_2$
Molecular weight: 124.14
CAS Registry No.: 90-05-1
Merck Index: 4575

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

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

CHROMATOGRAM
Retention time: 13.787

KEY WORDS
whole blood

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

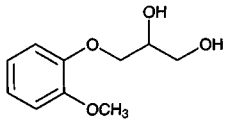
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100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

OTHER SUBSTANCES

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

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

Guaifenesin



Molecular formula: $C_{10}H_{14}O_4$
Molecular weight: 198.22
CAS Registry No.: 93-14-1
Merck Index: 4582
Lednicer No.: 1 118

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 100 μL 2.5 mg/mL O-desmethylnaproxen in MeOH + 400 μL 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 μL mobile phase, inject 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm 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 guaiphenesin glucuronides in plasma and urine of the horse by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *288*, 423–429.

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

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

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

CHROMATOGRAM
Retention time: 11.435

KEY WORDS
whole blood

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

SAMPLE
Matrix: bulk
Sample preparation: Dissolve in mobile phase, inject a 20 μL aliquot.

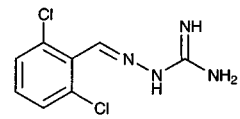
HPLC VARIABLES
Column: 250 × 4.6 10 μm Chiralcel OD
Mobile phase: EtOH:heptane 20:80
Flow rate: 1
Injection volume: 20
Detector: UV 365

CHROMATOGRAM
Retention time: k' 0.57 ((R)-(-)), k'1.72 ((S)-(+))

KEY WORDS
chiral

REFERENCE
Francotte,E.R.; Richert,P. Applications of simulated moving-bed chromatography to the separation of the enantiomers of chiral drugs, *J.Chromatogr.A*, **1997**, *769*, 101–107.

Guanabenz



Molecular formula: $C_8H_8Cl_2N_4$
Molecular weight: 231.08
CAS Registry No.: 5051-62-7, 23256-50-0 (acetate)
Merck Index: 4585
Lednicer No.: 2 123

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 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm 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: 2
Internal standard: carbamazepine (3)

OTHER SUBSTANCES
Simultaneous: mefruside, impurities

KEY WORDS
tablets

REFERENCE
Vio,L.; Mamolo,M.G.; Furlan,G. Quantitative high pressure liquid chromatographic determination of guanabenz and mephruside in pharmaceutical formulations, *Farmaco.[Prat].*, **1988**, *43*, 27–36.

SAMPLE
Matrix: formulations
Sample preparation: Dilute a 500 μL aliquot of syrup with water to give a clonidine concentration of 5 μg/mL, filter (0.22 μm), inject a 15 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax TMS trimethylsilyl
Mobile phase: MeOH:buffer 65:35 (Buffer was 2.2 mM KH_2PO_4 and 16 mM Na_2HPO_4, pH 7.9.)
Flow rate: 1
Injection volume: 15
Detector: UV 254

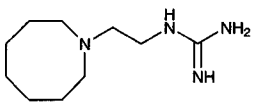
CHROMATOGRAM
Retention time: 7
Internal standard: guanabenz

OTHER SUBSTANCES
Simultaneous: clonidine

KEY WORDS
syrup; guanabenz is IS

REFERENCE
Levinson,M.L.; Johnson,C.E. Stability of an extemporaneously compounded clonidine hydrochloride oral liquid, *Am.J.Hosp.Pharm.*, **1992**, *49*, 122–125.

Guanethidine



Molecular formula: $C_{10}H_{22}N_4$
Molecular weight: 198.31
CAS Registry No.: 55-65-2, 645-43-2 (monosulfate), 60-02-6 (sulfate)
Merck Index: 4589
Lednicer No.: 1 282

SAMPLE

Matrix: blood
Sample preparation: Inject 200 μL serum 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 another 3 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Flush column A with mobile phase C for 10 min, re-equilibrate with mobile phase A for 7 min.

HPLC VARIABLES

Column: A 35 × 4.6 10 μm TSK precolumn BSA-ODS (Tosoh); B 150 × 4.6 5 μm TSKgel ODS-80TM (Tosoh)
Mobile phase: A 50 mM NaH_2PO_4 adjusted to pH 3.0 with 50 mM phosphoric acid; B MeCN:buffer 30:70, containing 7 g/L sodium 1-octanesulfonate (Buffer was 50 mM NaH_2PO_4 adjusted to pH 3.0 with 50 mM phosphoric acid.); C MeCN:water 50:50.
Flow rate: 1
Injection volume: 200
Detector: F ex 39 em 500 following post-column reaction. The effluent from column B mixed with 1 M NaOH pumped at 0.3 mL/min and with 6 g/L ninhydrin in water pumped at 0.3 mL/min and the mixture flowed through a 10 m × 0.5 mm ID PTFE coil at 56° to the detector.

CHROMATOGRAM

Retention time: 16
Limit of detection: 1 ng/mL
Limit of quantitation: 3.1 ng/mL

KEY WORDS

serum; post-column reaction; column-switching; rat; human; pharmacokinetics

REFERENCE

Inamoto,Y.; Inamoto,S.; Hanai,T.; Takahashi,Y.; Kadowaki,K.; Kinoshita,T. Development of automated highly sensitive analytical system for guanethidine sulfate in serum, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2099–2108.

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 μL aliquot and add it to 300 μL 250 μg/mL procaine hydrochloride in water, make up to 10 mL with mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm ASI chromosphere 3869 octadecylsilane (Analytical Sciences, Inc.)
Mobile phase: MeCN:50 mM NaH_2PO_4 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: 3.5

Internal standard: procaine hydrochloride (5.7)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Simultaneous: hydrochlorothiazide

KEY WORDS
tablets; not stability-indicating

REFERENCE
Stewart,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: solutions

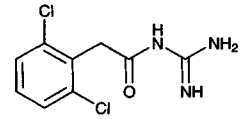
HPLC VARIABLES
Column: μBondapak CN
Mobile phase: MeCN:0.1% pH 7.36 ammonium acetate 50:50
Flow rate: 2
Detector: UV 206

CHROMATOGRAM
Retention time: 2
Limit of detection: 20 ng

KEY WORDS
rabbit; buffer

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

Guanfacine



Molecular formula: $C_9H_9Cl_2N_3O$
Molecular weight: 246.10
CAS Registry No.: 29110-47-2, 29110-48-3 (HCl)
Merck Index: 4590
Lednicer No.: 3 40

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

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

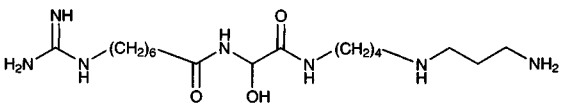
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min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

CHROMATOGRAM
Retention time: 11.387

KEY WORDS
whole blood

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

Gusperimus



Molecular formula: $C_{17}H_{37}N_7O_3$
Molecular weight: 387.53
CAS Registry No.: 104317-84-2, 84937-45-1 ((-)-form tri HCl), 85468-01-5 (tri HCl)
Merck Index: 4610

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 10 μg IS, mix well, make up to 10 mL with water, add to a 50 × 6 column of CM-Sephadex C-25, wash with 10 mL 300 mM NaCl, elute with 10 mL 400 mM NaCl, add the eluate to a Sep-Pak C18 SPE cartridge, wash with 5 mL water, elute with 10 mL MeOH. Evaporate the eluate to dryness under reduced pressure, reconstitute the residue in 200 μL mobile phase, vortex, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: Guard Pak C18 (Waters)
Column: 150 × 4.6 Cosmosil 5C18-P (Nacalai Tesque)
Mobile phase: MeCN:buffer 9:91 (Buffer was 10 mM pH 3 phosphate buffer containing 5 mM sodium pentanesulfonate.)
Flow rate: 1
Injection volume: 50
Detector: UV 205

CHROMATOGRAM
Retention time: 8.6
Internal standard: 1-amino-20-guanidino-11-hydroxy-4,9,12-triazaeicosane-10,13-dione (Heat 33.7 mmole 8-guanidinooctanamide, 30.7 mmole glyoxylspermidine hydrochloride, 33.7 mmole glutaric acid, and 2 g water at 60° for 8 h, dilute with water, chromatograph on CM-Sephadex C-25(Na^+) using gradient elution with water and 1 M NaCl. Evaporate the eluate fraction containing the product to dryness, extract the residue with MeOH, chromatograph the extract on Sephadex LH-20 with MeOH, evaporate the eluate to dryness to get the compound.) (18.6)
Limit of quantitation: 50 ng/mL

KEY WORDS
dog; plasma; SPE; pharmacokinetics

REFERENCE
Nakanuma,R.; Watanabe,K.; Yamashita,K.; Mizuguchi,S.; Hashimoto,Y.; Nakamura,T.; Umezawa,H. High-performance liquid chromatographic determination of deoxyspergualin in dog plasma with ultraviolet detection, *J.Chromatogr.*, **1990**, *527*, 208–213.

SAMPLE
Matrix: blood
Sample preparation: Filter (Amicon MPS-1 with 14 mm YM20 membrane) 1 mL plasma while centrifuging at 800 g for 1 h. Remove a 400 μL aliquot of the ultrafiltrate and add it to 50 μL 500 mM pH 6.8 phosphate buffer, add 50 μL 10 mM NaCN in water, add 100 μL 2 mM naphthalene-2,3-dicarboxaldehyde in MeCN, let stand at room temperature for 15 min, add 50 μL 500 mM pH 3.0 sodium acetate buffer, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm ODS Hypersil
Mobile phase: MeCN:100 mM KH_2PO_4:phosphoric acid 52:48:0.8 containing 18 mM sodium dodecyl sulfate
Column temperature: 40 ± 0.1
Flow rate: 2
Injection volume: 50
Detector: F ex 420 em 490

CHROMATOGRAM
Retention time: 14
Limit of quantitation: 5 ng/mL

KEY WORDS
derivatization; plasma; ultrafiltrate

REFERENCE
Sprancmanis,L.A.; Riley,C.M.; Stobaugh,J.F. Determination of the anticancer drug, 15-deoxyspergualin, in plasma ultrafiltrate by liquid chromatography and precolumn derivatization with naphthalene-2,3-dicarboxaldehyde/cyanide, *J.Pharm.Biomed.Anal.*, **1990**, *8*, 165–175.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 750 μL Plasma + 30 μL 70% perchloric acid, vortex, centrifuge at 15600 g for 5 min, filter (0.22 μm) the supernatant, inject a 10-200 μL aliquot of the filtrate. Urine. Dilute urine 10 to 25-fold with water, centrifuge, filter (0.22 μm) the supernatant, inject a 10-200 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Newguard RP 18
Column: two 100 × 4.6 5 μm RP 18 columns in series (Brownlee)
Mobile phase: Gradient. A was MeCN:100 mM pH 2.55 NaH_2PO_4 containing 8 mM octanesulfonic acid and 0.1 mM EDTA 2:98. B was MeCN:200 mM pH 3.1 NaH_2PO_4 containing 8 mM octanesulfonic acid 30:70. A:B from 55:45 to 25:75 over 20 min.
Flow rate: 1
Injection volume: 10-200
Detector: F ex 340 em 440 following post-column reaction. The column effluent mixed with the reagent pumped at 0.7 mL/min and flowed through a 2 m long reaction coil at 41° to the detector. The reagent was 500 mM pH 8.8 potassium borate buffer containing 0.8 g/L o-phthaldialdehyde, 1% MeOH, and 0.06% 2-mercaptoethanol.

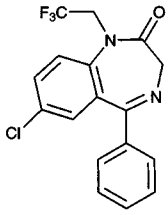
CHROMATOGRAM
Retention time: 22
Limit of quantitation: 100 nM

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
use plasticware; plasma; post-column reaction; pharmacokinetics

REFERENCE
Muindi,J.F.; Lee,S.-J.; Baltzer,L.; Jakubowski,A.; Scher,H.I.; Sprancmanis,L.A.; Riley,C.M.; Vander Velde,D.; Young,C.W. Clinical pharmacology of deoxyspergualin in patients with advanced cancer, *Cancer Res.*, **1991**, *51*, 3096–3101.

Halazepam



Molecular formula: $C_{17}H_{12}ClF_3N_2O$
Molecular weight: 352.74
CAS Registry No.: 23092-17-3
Merck Index: 4619

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond-Elut C8 SPE cartridge with 2 mL MeOH and 2 mL water, do not allow to dry. Add 100 μL 5 ng/mL diazepam in 1 M pH 10.5 glycine buffer then 1 mL plasma to the SPE cartridge, wash with 2 mL water, wash with 50 μL MeOH, elute with three 200 μL aliquots of MeOH. Combine the eluates and evaporate them to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL mobile phase, inject a 50-80 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 3 μm Adsorbosphere C8
Mobile phase: MeOH:20 mM pH 4.0 phosphate buffer 60:40
Flow rate: 1
Injection volume: 50-80
Detector: UV 240

CHROMATOGRAM
Retention time: 9.31
Internal standard: diazepam (7.76)
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES
Extracted: nordiazepam
Simultaneous: alprazolam, chlordiazepoxide, clonazepam, desmethyldiazepam, 3-hydroxyhalazepam, lorazepam, methylclonazepam, oxazepam, prazepam, quazepam, temazepam, triazolam

KEY WORDS
SPE; plasma; pharmacokinetics

REFERENCE
Gupta,S.K.; Ellinwood,E.H. Liquid chromatographic assay and pharmacokinetics of halazepam and its metabolite in humans, *J.Pharm.Sci.*, **1990**, *79*, 822–825.

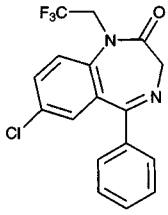
SAMPLE
Matrix: microsomal incubations
Sample preparation: 2.5 mL Microsomal incubation + 2.5 mL acetone, add 30 μL diazepam in MeOH, add 2.5 mL chloroform, centrifuge. Remove the organic layer and evaporate it to dryness under reduced pressure at 40°, reconstitute the residue in 100 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 6.2 7 μm Zorbax silica

KEY WORDS
use plasticware; plasma; post-column reaction; pharmacokinetics

REFERENCE
Muindi,J.F.; Lee,S.-J.; Baltzer,L.; Jakubowski,A.; Scher,H.I.; Sprancmanis,L.A.; Riley,C.M.; Vander Velde,D.; Young,C.W. Clinical pharmacology of deoxyspergualin in patients with advanced cancer, *Cancer Res.*, **1991**, *51*, 3096–3101.

Halazepam



Molecular formula: $C_{17}H_{12}ClF_3N_2O$
Molecular weight: 352.74
CAS Registry No.: 23092-17-3
Merck Index: 4619

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond-Elut C8 SPE cartridge with 2 mL MeOH and 2 mL water, do not allow to dry. Add 100 μL 5 ng/mL diazepam in 1 M pH 10.5 glycine buffer then 1 mL plasma to the SPE cartridge, wash with 2 mL water, wash with 50 μL MeOH, elute with three 200 μL aliquots of MeOH. Combine the eluates and evaporate them to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL mobile phase, inject a 50-80 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 3 μm Adsorbosphere C8
Mobile phase: MeOH:20 mM pH 4.0 phosphate buffer 60:40
Flow rate: 1
Injection volume: 50-80
Detector: UV 240

CHROMATOGRAM
Retention time: 9.31
Internal standard: diazepam (7.76)
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES
Extracted: nordiazepam
Simultaneous: alprazolam, chlordiazepoxide, clonazepam, desmethyldiazepam, 3-hydroxyhalazepam, lorazepam, methylclonazepam, oxazepam, prazepam, quazepam, temazepam, triazolam

KEY WORDS
SPE; plasma; pharmacokinetics

REFERENCE
Gupta,S.K.; Ellinwood,E.H. Liquid chromatographic assay and pharmacokinetics of halazepam and its metabolite in humans, *J.Pharm.Sci.*, **1990**, *79*, 822–825.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 2.5 mL Microsomal incubation + 2.5 mL acetone, add 30 μL diazepam in MeOH, add 2.5 mL chloroform, centrifuge. Remove the organic layer and evaporate it to dryness under reduced pressure at 40°, reconstitute the residue in 100 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 6.2 7 μm Zorbax silica

Mobile phase: Hexane:dichloromethane:isopropanol 77:20:3
Flow rate: 2
Detector: UV 232

CHROMATOGRAM
Retention time: 10
Internal standard: diazepam (12)

OTHER SUBSTANCES
Extracted: metabolites, oxazepam

KEY WORDS
human; liver; normal phase; pharmacokinetics

REFERENCE
Lu,X.-L.; Guengerich,F.P.; Yang,S.K. Stereoselective metabolism of prazepam and halazepam by human liver microsomes, *Drug Metab.Dispos.*, **1991**, *19*, 637–642.

SAMPLE
Matrix: solutions

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

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenyl-

butazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

SAMPLE

Matrix: tissue

Sample preparation: Homogenize brain in 100 mM NaOH (5 mL/g) by sonication. Add 250 μL homogenate (ca. 50 mg tissue equivalent) to 250 μL 100 mM pH 13 NaOH, vortex thoroughly. Add 2 mL toluene, mix (50 inversions), centrifuge at 15000 rpm at 4° for 20 min. Dry the organic phase under a stream of nitrogen. Add 2 mL toluene to the aqueous phase and repeat the extraction. Combine the organic layers and dry under a stream of nitrogen. Reconstitute the residue in 100 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 3 μm Rainin C8 Microsorb
Mobile phase: MeCN:MeOH:25 mM potassium phosphate buffer 18.5:16.5:65
Flow rate: 0.9
Injection volume: 40
Detector: UV 240

CHROMATOGRAM

Retention time: 6.2
Internal standard: halazepam

OTHER SUBSTANCES

Extracted: midazolam

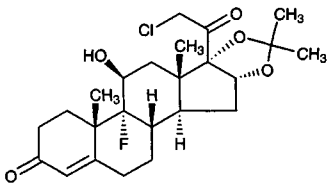
KEY WORDS

brain; rat; halazepam is IS

REFERENCE

Jiang,Q.; Walton,N.Y.; Gunawan,S.; Treiman,D.M. High-performance liquid chromatographic determination of midazolam in rat brain, *J.Chromatogr.B*, **1996**, *683*, 276–280.

Halcinonide



Molecular formula: $C_{24}H_{32}ClFO_5$
Molecular weight: 454.97
CAS Registry No.: 3093-35-4
Merck Index: 4621
Lednicer No.: 2 187

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10-60 μL aliquot.

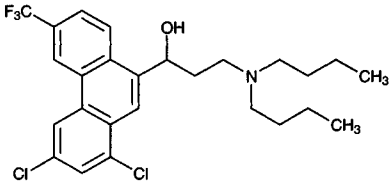
HPLC VARIABLES
Column: 150 × 2 5 μm Spherisorb ODS1
Mobile phase: MeCN:water 50:50
Flow rate: 0.16-0.30
Injection volume: 10-60
Detector: UV 240

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Simultaneous: triamcinolone acetonide

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.

Halofantrine



Molecular formula: $C_{26}H_{30}Cl_2F_3NO$
Molecular weight: 500.43
CAS Registry No.: 69756-53-2, 36167-63-2 (HCl)
Merck Index: 4626
Lednicer No.: 3 76

SAMPLE
Matrix: blood
Sample preparation: Add 1 ml MeCN:EtOH 99:1 to 500 μL erythrocyte pellet, vortex for 1 min, centrifuge at 2000 g for 5 min. Collect the supernatant, evaporate to dryness under nitrogen, reconstitute with 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm AD Chiralpak amylase tris-3,5-dimethyl phenylcarbamate
Mobile phase: Hexane:2-propanol:2-butanol:diethylamine 95:3:2:0.5
Flow rate: 0.3
Injection volume: 20
Detector: F ex 260 em 380

CHROMATOGRAM
Retention time: 16 (+), 24 (-)

Internal standard: (±S)-dichloro-[2-(dibutylamino)methyl]-6-(trifluoromethyl)-9-phenanthrene-methanol (SmithKline Beecham) (21)
Limit of quantitation: 25 ng

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
erythrocytes; chiral; do the extraction procedure in silanized tubes

REFERENCE
Gorichon,E.; Martin,C.; Bangchang,K.N.; Karbwang,J.; Thuiller,A.; Farinotti,R.; Gimenez,F. Chiral chromatographic method to determine the enantiomers of halofantrine and its main chiral desbutyl metabolite in erythrocytes, *J.Chromatogr.B*, **1998**, *712*, 259–262.

SAMPLE
Matrix: blood
Sample preparation: Add 200 µL 2 µg/mL IS to 500 µL plasma. Add 950 µL MeCN, vortex for 1 min, centrifuge at 700 g for 2 min. Add 2-8 mL MTBE, vortex for 2 min, centrifuge at 700 g for 5 min. Remove the upper organic phase and add it to 100 µL 5 mM HCl in MeCN, evaporate under a stream of nitrogen at 35°. Reconstitute the residue with 200 µL MeCN, vortex for 1 min, centrifuge at 700 g for 2 min. Inject a 25 µL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 µm Newguard RP-8 (Perkin Elmer)
Column: 250 × 4.6 5 µm Ultrasphere C8
Mobile phase: MeCN:water 75:25 containing 0.2% sodium dodecyl sulfate (w/v) and 0.2% glacial acetic acid (v/v)
Flow rate: 1.5
Injection volume: 25
Detector: UV 257

CHROMATOGRAM
Internal standard: 2,6-dichloro-6-trifluoromethyl-9-(1-[2-(dibutyl-amino)ethyl])phenanthrene-methanol.HCl (SmithKline Beecham)

KEY WORDS
plasma; dog

REFERENCE
Porter,C.J.H.; Caliph,S.M.; Charman,W.N. Differences in pre- and post-prandial plasma lipid profiles affect the extraction efficiency of a model highly lipophilic drug from beagle dog plasma, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 175–180.

SAMPLE
Matrix: blood
Sample preparation: Add 300 µL MeCN to 100 µL rat plasma with containing IS, vortex. Centrifuge for 2 min and remove the supernatant. Add 200 µL ammonium hydroxide and 2 mL MTBE:hexane 50:50. Vortex for 45 s, centrifuge at 2500 g for 3 min. Evaporate the organic layer to dryness. Add 250 mM (+)-di-O-acetyl L-tartaric acid anhydride in acetic acid:dichloromethane 20:80, heat at 45° for 30 min. Inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 C18
Mobile phase: MeCN:25 mM potassium phosphate/sulfuric acid/triethylamine solution 53.5:46.5 containing 1.8 g/L sodium dodecyl sulfate
Flow rate: 1.2
Detector: UV 254

CHROMATOGRAM
Retention time: 19.3 ((+)-enantiomer), 21.7 ((-)-enantiomer)

Internal standard: imipramine (13.8)
Limit of quantitation: 25 ng/mL

KEY WORDS
plasma; rat; derivatization; chiral

REFERENCE
Padovani,P.K.; Timby,D.M.; Wright,M.R.; Kapil,R.P. Quantitative analysis of DMP 851 in rat and dog plasma by liquid-liquid extraction and reverse-phase high performance liquid chromatography with ultraviolet detection (Abstract 3318), *Pharm.Res.*, **1997**, *14*, S568.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 50 μL 4 μg/mL IS + 1 mL MeCN, vortex for 20 s, centrifuge at 1200 g for 10 min, add to a conditioned 2.8 mL 500 mg Bond Elut C8 SPE cartridge, wash with two 1 mL portions of MeCN, elute with 1 mL MeCN:1 M HCl 90:10. Add the eluate to 1 mL 28% ammonium hydroxide and 6 mL dichloromethane, rotate at 15 rpm for 25 min, centrifuge at 1000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 150 μL mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Ultrasphere C8
Mobile phase: MeCN:water 75:25 containing 0.2% sodium dodecyl sulfate and 0.2% glacial acetic acid
Flow rate: 1.5
Injection volume: 25
Detector: UV 257

CHROMATOGRAM
Retention time: 8.3
Internal standard: 2,4-dichloro-9-(2-dibutylamino-1-hydroxy)ethyl-6-trifluoromethylphenanthrene (BL 22312) (11.5)
Limit of detection: 5 ng/mL
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: chloroquine, dapsone, mefloquine, primaquine, proguanil, pyrimethamine, quinine, sulfadoxine, tetracycline

KEY WORDS
serum; treat glassware with 0.2% aquasil; pharmacokinetics; SPE

REFERENCE
Keeratithakul,D.; Teja-Isavadharm,P.; Shanks,G.D.; Webster,H.K.; Edstein,M.D. An improved high-performance liquid chromatographic method for the simultaneous measurement of halofantrine and desbutylhalofantrine in human serum, *Ther.Drug Monit.*, **1991**, *13*, 64–68.

SAMPLE
Matrix: blood
Sample preparation: Dried blood. Allow 500 μL blood to dry on a strip of filter paper. Store at room temperature, protect from dust and sunlight. Add 15 μL 10 μg/mL IS in water to each strip and allow to dry at 37° for 30 min. Cut strip into small pieces, add 1 mL 10 mM perchloric acid, vortex, let stand at room temperature for 5 min, add 2 mL MeCN, vortex at high speed for 30 s, add 1 mL ammonia, mix thoroughly, add 5 mL hexane, vortex at moderate speed for 1 min, centrifuge at 2000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 120 μL mobile phase, inject a 50 μL aliquot. Whole blood, plasma. 500 μL Whole blood or plasma + 15 μL 10 μg/mL IS in water + 2 mL MeCN, vortex for 30 s, centrifuge at 2000 g for 5 min. Remove the supernatant and add it to 500 μL ammonia, vortex, add 5 mL hexane:diethyl ether 50:50, vortex for 1 min, centrifuge at 2000 g for 5 min. Remove the organic layer and evaporate it to dryness under a

stream of nitrogen at 37°, reconstitute the residue in 120 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 5 μm CN precolumn RP-18 endcapped (Merck)
Column: 250 × 4.6 Hypersil 5 ODS
Mobile phase: MeCN:water:triethylamine 65:35:1 adjusted to pH 4 with orthophosphoric acid
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 8.5
Internal standard: 2,4-dichloro-9-(2-dibutylamino-1-hydroxy)ethyl-6-trifluoromethylphenanthrene (11)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: diazepam
Noninterfering: acetaminophen, chlorcycloguanil, chloroquine, chlorproguanil, cycloguanil, mefloquine, phenobarbital, proguanil, pyrimethamine, quinidine, quinine, sulfadoxine

KEY WORDS
plasma; whole blood; dried blood; pharmacokinetics; diazepam may be used as IS

REFERENCE
Mberu,E.K.; Muhia,D.K.; Watkins,W.M. Measurement of halofantrine and its major metabolite desbutylhalofantrine in plasma and blood by high-performance liquid chromatography: a new methodology, *J.Chromatogr.*, **1992**, *581*, 156–160.

SAMPLE
Matrix: blood
Sample preparation: Condition a 500 mg Bakerbond SPE cartridge with three 3 mL portions of MeCN and three 3 mL portions of solvent. 1 mL Plasma + 3 mL solvent, add to the SPE cartridge, elute with 2 mL solvent. Collect all the eluate and add it to 5 μL 10 μg/mL desipramine hydrochloride in n-hexane:EtOH:2-butanol 93:4.5:2.5, evaporate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 1 mL mobile phase, inject a 200 μL aliquot. (Solvent was MeCN:triethylamine:EtOH 99:1:1 adjusted to pH 4 with 1 M HCl.)

HPLC VARIABLES
Guard column: 50 × 4.6 10 μm Chiralpak AD amylose tris-3,5-dimethylphenylcarbamate (Daicel)
Column: 250 × 4.6 10 μm Chiralpak AD amylose tris-3,5-dimethylphenylcarbamate (Daicel)
Mobile phase: n-Hexane:EtOH:2-butanol:diethylamine 93:4.5:2.5:0.1
Flow rate: 0.3
Injection volume: 200
Detector: F ex 300 em 380

CHROMATOGRAM
Retention time: 15.5 (+), 18 (-)
Internal standard: desipramine (32)
Limit of quantitation: 6 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
SPE; plasma; pharmacokinetics; chiral

REFERENCE

Terefe,H.; Blaschke,G. Direct determination of the enantiomers of the antimalarial drug halofantrine and its active metabolite N-desbutylhalofantrine in human plasma, *J.Chromatogr.B*, **1994**, *657*, 238–242.

SAMPLE

Matrix: blood

Sample preparation: 500 μL Plasma + 30 μL 100 μg/mL IS in MeCN:water 80:20, vortex at high speed and add 2 mL MeCN, centrifuge at 1800 g for 3 min. Remove the supernatant and add it to 500 μL ammonium hydroxide and 5 mL MTBE:hexane 50:50, vortex at high speed for 90 s, centrifuge at 1800 g. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 25°, reconstitute the residue in 300 μL 250 mM (+)-di-O-acetyl-L-tartaric acid anhydride in acetic acid:dichloromethane 20:80 (freshly prepared), heat at 45° for 30 min, add 300 μL MeOH, evaporate to dryness under a stream of nitrogen at 25°, reconstitute with 170 μL mobile phase, inject a 30-100 μL aliquot.

HPLC VARIABLES

Guard column: Guard-Pak ODS (Waters)

Column: 250 × 4.6 Ultrasphere ODS

Mobile phase: MeCN:buffer 53.5:46.5 containing 0.9 g/L sodium dodecyl sulfate (Buffer was 25 mM KH_2PO_4 containing 1.5 mL/L 2 M sulfuric acid and 0.5 mL/L triethylamine, pH 5.0.)

Flow rate: 1.2

Injection volume: 30-100

Detector: UV 254

CHROMATOGRAM

Retention time: 11.5 (+), 13.3 (-)

Internal standard: (±)-2,4-dichloro-α-[2-(dibutylamino)ethyl]-6-(trifluoromethyl)-9-phenanthrenemethanol (SK&F 99123) (17.0, 20.7 (enantiomers))

Limit of quantitation: 12.5 ng/mL

KEY WORDS

plasma; derivatization; pharmacokinetics; chiral

REFERENCE

Brocks,D.R.; Dennis,M.J.; Schaefer,W.H. A liquid chromatographic assay for the stereospecific quantitative analysis of halofantrine in human plasma, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 911–918.

SAMPLE

Matrix: blood

Sample preparation: Condition a 3 mL 200 mg Bond Elut C8 SPE cartridge with 2 mL MeOH and 2 mL buffer. Prepare whole blood by freezing then thawing, dilute with an equal volume of water, centrifuge at 5000 g for 5 min. 1 mL Plasma or whole blood supernatant + 100 μL 10 μg/mL IS in MeCN:water 50:50 + 2 mL MeCN, vortex for 15 s, centrifuge at 1500 g for 10 min, add the supernatant to the SPE cartridge, allow to dry under vacuum for 1 min, wash with two 2 mL portions of buffer, wash with two 2 mL portions of MeOH:water 50:50, elute with four 750 μL portions of ethyl acetate:acetic acid 98:2. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 (plasma) or 100 (whole blood) μL mobile phase, inject a 50 μL aliquot. (Buffer was 1 g/L potassium bicarbonate.)

HPLC VARIABLES

Column: 250 × 4 5 μm Lichrospher 60 RP select B C8

Mobile phase: MeCN:water 35:65 containing 1% triethylamine, adjusted to pH 4 with orthophosphoric acid

Flow rate: 1.1

Injection volume: 50

Detector: F ex 300 em 375

CHROMATOGRAM

Retention time: 6.8

Internal standard: N-tert-butyl-3-hydroxy-(1,3-dichloro-6-trifluoromethyl-9-phenanthryl)propionamide hydrate (S76,395-0, Aldrich) (12.0)

Limit of detection: 10.8 ng/mL (whole blood), 6.1 ng/mL (plasma)

Limit of quantitation: 14.8 ng/mL (whole blood), 12.4 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: chloroquine, mefloquine, proguanil, pyrimethamine, quinine, sulfadoxine

KEY WORDS
whole blood; plasma; SPE

REFERENCE
Gaillard,Y.; Prévosto,J.-M.; Cheminel,V.; Soares,O.; Chaulet,J.-F. New solid-phase extraction for an improved high-performance liquid chromatographic procedure for the quantitation of halofantrine and monodesbutylhalofantrine in blood or plasma, *J.Chromatogr.B*, **1995**, *668*, 315–321.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 1 mL MeCN + 200 μL 2 μg/mL IS in MeCN, vortex for 2 min, centrifuge, add 8 mL MTBE, vortex for 2 min, centrifuge at 700 g for 5 min. Remove 8 mL of the upper organic layer and add it to 100 μL 5 mM HCl in MeCN, evaporate to dryness under a stream of nitrogen at 35°, reconstitute the residue in 200 μL MeCN, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Aquapore (Analytical Biosystems)
Column: 250 × 4.6 5 μm Ultrasphere C8
Mobile phase: MeCN:water 75:25 containing 0.2% sodium dodecyl sulfate and 0.2% glacial acetic acid
Flow rate: 1.5
Injection volume: 25
Detector: UV 257

CHROMATOGRAM
Retention time: 7.8
Internal standard: 2,4-dichloro-6-trifluoromethyl-9-[1-(2-(dibutylamino)ethyl]pehnanthrene-methanol hydrochloride (10.4)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; dog; pharmacokinetics

REFERENCE
Humberstone,A.J.; Currie,G.J.; Porter,C.J.H.; Scanlon,M.J.; Charman,W.N. A simplified liquid chromatography assay for the quantitation of halofantrine and desbutylhalofantrine in plasma and identification of a degradation product of desbutylhalofantrine formed under alkaline conditions, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 265–272.

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

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 258.2

CHROMATOGRAM
Retention time: 22.993

KEY WORDS
whole blood

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

SAMPLE
Matrix: microsomal incubations
Sample preparation: Adjust pH of 1.75 mL microsomal incubation to pH 9 with 100 mM NaOH, add 3.5 mL n-hexane:diethyl ether 70:30, shake mechanically for 15 min, centrifuge at 2500 g for 15 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 1 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 10 μm Chiralcel OD cellulose tris-3,5-dimethylphenylcarbamate
Column: 250 × 4.6 10 μm Chiralcel OD cellulose tris-3,5-dimethylphenylcarbamate
Mobile phase: n-Hexane:isopropanol:diethylamine 90:10:0.1
Flow rate: 0.3
Injection volume: 20
Detector: UV 260

CHROMATOGRAM
Retention time: 27 (+), 31.5 (-)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; liver; chiral

REFERENCE
Terefe,H.; Blaschke,G. Direct determination of the enantiomers of halofantrine and its pharmacologically active metabolite N-desbutylhalofantrine by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *615*, 347–351.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve a sample in MeOH to a concentration of about 1 mg/mL, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Spherisorb SCX
Mobile phase: MeOH:water 80:20 containing 20 mM ammonium formate and 2.3 mL/L trifluoroacetic acid
Flow rate: 1

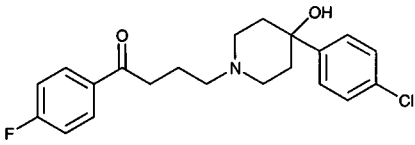
Injection volume: 1-10
Detector: UV 270

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Simultaneous: cimetidine, clomipramine, haloperidol, minoxidil, reserpine, verapamil

REFERENCE
Law,N.; Appleby,J.R.G. Re-evaluation of strong cation-exchange high-performance liquid chromatography for the analysis of basic drugs, *J.Chromatogr.A*, **1996**, *725*, 335–341.

Haloperidol



Molecular formula: $C_{21}H_{23}ClFNO_2$
Molecular weight: 375.87
CAS Registry No.: 52-86-8, 74050-97-8 (decanoate)
Merck Index: 4629
Lednicer No.: 1 306

SAMPLE
Matrix: blood
Sample preparation: Mix 0.5 mL plasma with 25 μL 1 μg/mL IS in MeOH . Vortex for 30 s, add 5 mL ether and 3 mL 100 mM HCl, vortex for 1 min, centrifuge at 3000 rpm for 3 min. Remove the aqueous phase, add 7 mL chloroform (Caution! Chloroform is a carcinogen!) and 500 μL 1 M NaOH. Shake the mixture for 2 min, remove the chloroform phase, evaporate it to dryness under vacuum at 45°. Reconstitute the residue with 500 μL mobile phase, vortex for 1 min, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Resolve C18 (Waters)
Mobile phase: MeOH:buffer 55:45 (Buffer was water containing 200 mM ammonium acetate, adjusted to pH 7.1-7.3 with acetic acid.)
Column temperature: 38
Flow rate: 1.5
Injection volume: 20
Detector: UV 249

CHROMATOGRAM
Retention time: 6.3
Internal standard: diazepam (5.1)
Limit of detection: 5 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
El-Sayed,Y.M.; Khidr,S.H.; Niazy,E.M. High-performance liquid chromatographic assay for the determination of haloperidol in plasma, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 125–134.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 500 μL 600 mM pH 10 sodium carbonate/bicarbonate buffer + 8 mL heptane:isoamyl alcohol 98:2, shake at 250 cycles/min for 5 min, centrifuge at 1500 g for 10 min. Freeze the aqueous layer, evaporate the heptane layer to dryness under a gentle stream of nitrogen at 60°. Dissolve the residue in 75 μL mobile phase, inject a 65 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 LiChroCart
Mobile phase: MeOH:40 mM pH 7.0 ammonium acetate buffer 90:10
Flow rate: 1
Injection volume: 65
Detector: UV 280

CHROMATOGRAM
Retention time: 5.10
Internal standard: haloperidol

OTHER SUBSTANCES
Simultaneous: amitriptyline, citalopram, chlorprothixene, clomipramine, clozapine, desipramine, desmethylcitalopram, desmethylclomipramine, desmethylsertraline, diltiazem, fluoxetine, fluphenazine, 10-hydroxyamitriptyline, 8-hydroxyclomipramine, 8-hydroxydesmethylclomipramine, 10-hydroxynortriptyline, hydroxyzine, imipramine, methotrimeprazine sulfoxide, mianserine, norfluoxetine, nortriptyline, paroxetine, perphenazine, sertraline, zuclopenthixol
Noninterfering: carbamazepine, clonazepam, flunitrazepam, nitrazepam, oxazepam, oxcarbazepine

KEY WORDS
serum; haloperidol is IS

REFERENCE
Olesen,O.V.; Linnet,K. Simplified high-performance liquid chromatographic method for determination of risperidone and 9-hydroxyrisperidone in serum from patients comedicated with other psychotropic drugs, *J.Chromatogr.B*, **1997**, *698*, 209–216.

SAMPLE
Matrix: blood
Sample preparation: After each extraction or wash step, centrifuge the sample at 2000 g for 5 min. 2 mL Plasma + 50 μL 500 ng/mL IS + 500 μL 2 M NaOH + 8 mL n-hexane:isoamyl alcohol 99:1, extract gently for 20 min on rotator in a horizontal position, centrifuge at 2000 g for 5 min. Extract the organic layer with 1 mL 200 mM HCl for 15 min by vigorous shaking, discard the organic layer, wash the aqueous layer with 5 mL n-hexane:isoamyl alcohol 99:1, discard the organic layer. Add 500 μL 2 M NaOH, extract with 7 mL n-hexane:isoamyl alcohol 99:1 for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 50 μL mobile phase, inject a 1 μL aliquot.

HPLC VARIABLES
Column: 150 × 1 5 μm Nucleosil C18
Mobile phase: MeCN:2 mM ammonium formate 45:55 adjusted to pH 3.0 with formic acid
Flow rate: 50 μL/min
Injection volume: 1
Detector: MS, API 100 Sciex, electrospray, nitrogen as the nebulizing and curtain gas, orifice voltages 20, 60, 120, m/z 376.2

CHROMATOGRAM
Retention time: 6.6
Internal standard: chlorohaloperidol (8.7)
Limit of detection: 75 pg/mL
Limit of quantitation: 100 pg/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE

Hoja,H.; Marquet,P.; Verneuil,B.; Lotfi,H.; Dupuy,J.L.; Pénicaut,B.; Lachâtre,G. Determination of haloperidol and its reduced metabolite in human plasma by liquid chromatography-mass spectrometry with electrospray ionization, *J.Chromatogr.B*, **1997**, *688*, 275–280.

SAMPLE

Matrix: blood

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

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 5.1
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

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

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 + 100 μL 1 μg/mL loxapine in isopropanol:diethylamine 99.9:0.1 + 250 μL 25% potassium carbonate containing 0.1% diethylamine + 5 mL hexane: isoamyl alcohol 97:3, vortex for 30 s, centrifuge at 500 g for 3 min. Remove the organic layer and add it to 100 μL 250 mM HCl, vortex for 30 s, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: 50 × 4.6 40 μm C8 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil C8
Mobile phase: MeCN:water:diethylamine:85% phosphoric acid 53.3:45.1:1:0.4, pH adjusted to 7.2 with NaOH or phosphoric acid
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: k' 3.34
Internal standard: loxapine (k' 7.18)

OTHER SUBSTANCES
Extracted: amitriptyline, chlordiazepoxide, chlorpromazine, desipramine, desmethldiazepam, desmethylchlordiazepoxide, desmethyldoxepin, diazepam, doxepin, fluphenazine, imipramine, oxazepam
Noninterfering: molindone, perphenazine, trifluoperazine
Interfering: nortriptyline, 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: Condition a Bond Elut C18 SPE cartridge with 2 mL MeOH, 2 mL water, 2 mL MeOH, and 2 mL water. 1 mL Plasma or serum + 2 mL 100 mM pH 9.0 borate buffer, vortex for 10 s. Add to the SPE cartridge, wash with 5 mL water, elute with 1.6 mL 200 mM HCl in MeOH. Evaporate the eluate to dryness at 80°, reconstitute in 50 μL 600 ng/mL triazolam in mobile phase, centrifuge at 3000 rpm for 5 min, inject a 15 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 X 4 10 μm Hitachi ODS-3056
Mobile phase: MeCN:THF:1% acetate:triethylamine 28.2:1.9:69.5:0.4
Column temperature: 50
Flow rate: 1.6
Injection volume: 15
Detector: UV 245

CHROMATOGRAM
Retention time: 8
Internal standard: triazolam (14)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites, amitriptyline, carpipramine, chlocapramine, clomipramine, chlorpromazine, clorazepam, diazepam, estazolam, flunitrazepam, fluphenazine, haloxazolam, imipramine, levomepromazine, maprotiline, moperone, nitrazepam, perphenazine, promethazine, triazolam, trifluperidol, trimipramine
Interfering: amoxapine

KEY WORDS
plasma; serum; SPE

REFERENCE
Hayakari,M.; Hashimoto,Y.; Kita,T.; Murakami,S. A rapid and simplified extraction of haloperidol from plasma or serum with Bond Elut C18 cartridge for analysis by high performance liquid chromatography, *Forensic Sci.Int.*, **1987**, *35*, 73–81.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 250 μL di-iso-propyl ether:n-butyl alcohol 7:3 containing 400 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: 6
Internal standard: minaprine (5.5)
Limit of detection: 20 ng/mL

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

KEY WORDS
serum

REFERENCE
Mazzi,G. Simple and practical high-performance liquid chromatographic assay of some tricyclic drugs, haloperidol, diltiazem, verapamil, propafenone, and amiodarone, *Chromatographia*, **1987**, *24*, 313–316.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 40 μL 1 μg/mL chlorohaloperidol in MeOH + 2 mL pH 11 Normex buffer, vortex for 1 min, add to a 3 mL Extrelut SPE cartridge, elute with diethyl ether. Evaporate the eluate to dryness under a stream of air at 40°, reconstitute the residue in 100 μL 10 mM HCl, vortex, add 2 mL hexane, shake on a whirlmixer for 20 s, centrifuge at 2800 g for 5 min, inject a 20-40 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:25 mM KH_2PO_4:water 45:50:5
Flow rate: 0.8
Injection volume: 20-40
Detector: UV 220

CHROMATOGRAM
Retention time: 9
Internal standard: chlorohaloperidol (11)
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: alimemazine, amisulpride, amitriptyline, biperiden, caffeine, carbamazepine, clobazam, clomipramine, clorazepate, cyamemazine, desipramine, diazepam, ethybenztropine, ethyl loflazepate, flunitrazepam, imipramine, levomepromazine, loflazepate, lorazepam, nordiazepam, oxazepam, sulpride, sultopride, tiapride, triazolam, trihexiphenidyl, trimipramine, tropazepine, viloxazine
Noninterfering: heptaminol, meprobamate
Interfering: nortriptyline

KEY WORDS
SPE; plasma

REFERENCE
Cahard,C.; Rop,P.P.; Conquy,T.; Viala,A. High-performance liquid chromatographic analysis of haloperidol and hydroxyhaloperidol in plasma after solid-phase extraction, *J.Chromatogr.*, **1990**, *532*, 193–202.

SAMPLE
Matrix: blood

Sample preparation: 1 mL Plasma + 100 μL 200 ng/mL IS in MeOH + 1 mL 50 mM pH 10 borate buffer, vortex briefly, add to an Extrelut 3 SPE cartridge, let stand for 5 min, elute with 15 mL hexane:dichloromethane 50:50. Add the eluate to 3 mL 50 mM sulfuric acid, mix for 10 min, centrifuge at 3000 g for 10 min. Remove the aqueous layer and add it to 6 mL hexane: dichloromethane 50:50, wash for 5 min, centrifuge. Make the aqueous layer basic with 150 μL 28% ammonia, extract twice with 3 mL hexane:dichloromethane 50:50. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 5 μm Spherisorb cyano
Column: 250 × 4.6 5 μm Ultrasphere cyano
Mobile phase: MeCN:buffer 60:40 (Buffer was 50 mM KH_2PO_4 adjusted to pH 6.5 with 28% ammonia.)
Flow rate: 1
Injection volume: 20
Detector: E, 5100 A Coulochem, 5020 guard cell 1.00 V, 5011 analytical cell, detector 1 0.55 V, detector 2 0.80 V, output of detector 2 is monitored

CHROMATOGRAM

Retention time: 21.1 (haloperidol), 14.9 (reduced haloperidol)
Internal standard: methylrisperidone (R68808) (14.3)

OTHER SUBSTANCES

Extracted: chlorpromazine, clomipramine, cyamemazine, desipramine, droperidol, flunitrazepam, imipramine, pipamperone, risperidone, trihexyphenidyl
Noninterfering: alprazolam, bromazepam, carbamazepine, chlorazepate, diazepam, diphenylhydantoin, estazolam, ethylbenzatropine, oxazepam, phenobarbital, triazolam, valproic acid

KEY WORDS

plasma; SPE

REFERENCE

Le Moing,J.P.; Edouard,S.; Levron,J.C. Determination of risperidone and 9-hydroxyrisperidone in human plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1993**, *614*, 333–339.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 40 ng/mL chlorohaloperidol in MeCN + 500 μL saturated sodium carbonate, mix well, add 7 mL pentane:dichloromethane 90:10, shake in a Vibrax shaker for 10 min, centrifuge at 18° at 1725 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 55°, reconstitute the residue in 150 μL MeCN, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere cyano
Mobile phase: MeCN:MeOH:40 mM ammonium acetate 86:6:8
Column temperature: 40
Flow rate: 0.8
Injection volume: 150
Detector: E, ESA Coulochem Model 5100A, Model 5011 porous graphite analytical cell, electrode 1 0.6 V (screening), electrode 2 0.95 V (detection), Model 5020 guard cell 1 V (between pump and injector)

CHROMATOGRAM

Retention time: 12
Internal standard: chlorohaloperidol (11)
Limit of quantitation: 0.1 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

Noninterfering: acetaminophen, benztropine, clonazepam, clozapine, fluphenazine, ibuprofen, lorazepam, pseudoephedrine, trihexiphenidyl

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Aravagiri,M.; Marder,S.R.; Van Putten,T.; Marshall,B.D. Simultaneous determination of plasma haloperidol and its metabolite reduced haloperidol by liquid chromatography with electrochemical detection. Plasma levels in schizophrenic patients treated with oral or intramuscular depot haloperidol, *J.Chromatogr.B*, **1994**, *656*, 373–381.

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

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

CHROMATOGRAM
Retention time: 3.4 (haloperidol), 2.5 (reduced haloperidol)
Internal standard: protriptyline (4)

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

KEY WORDS
plasma; SPE

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

SAMPLE

Matrix: blood

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

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

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

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 221

CHROMATOGRAM

Retention time: 6.08

Limit of detection: <120 ng/mL

KEY WORDS

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

REFERENCE

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

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL Bond Elut Certify SPE cartridge with 2 mL MeOH and 2 mL 100 mM pH 6.0 phosphate buffer, do not allow to dry. 1 mL Blood + 6 mL 100 mM pH 6.0 phosphate buffer, vortex, sonicate, centrifuge, add the supernatant to the SPE cartridge, wash with water, wash with 1 mM pH 3.3 acetic acid, dry by suction, wash with 2 mL acetone: chloroform 50:50, elute with 3 mL ethyl acetate:ammonia 98:2. Evaporate the eluate under a stream of nitrogen at 40°, reconstitute the residue in 50 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pack C18
Mobile phase: Gradient. MeOH:50 mM ammonium acetate 65:35 for 1 min, to 75:25 over 4 min, maintain at 75:25 for 20 min (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 376-165, collision offset -27 V, repeller 100 V, vaporizer 130°, source 200°, filament on 200 μA, argon 2.5 mTorr, multiplier 1500 V, dynode 15 kV, scan time 1.20 s, MSMSC factor 10

CHROMATOGRAM
Retention time: 5.00
Limit of detection: 50 pg

OTHER SUBSTANCES
Extracted: benperidol, dextromoramide, droperidol, methadone, penfluridol, pimozide, pipamperidone, propoxyphene (dextropropoxyphene)

KEY WORDS
SPE; LC/MS

REFERENCE
Verweij,A.M.; Hordijk,M.L.; Lipman,P.J. Quantitative liquid chromatographic thermospray-tandem mass spectrometric analysis of some analgesics and tranquilizers of the methadone, butyrophenone, or diphenylbutylpiperidine groups in whole blood, *J.Anal.Toxicol.*, **1995**, *19*, 65–68.

SAMPLE
Matrix: blood, gastric contents, tissue, urine
Sample preparation: 1 mL Blood, urine, or gastric contents or 1 g tissue homogenate + 500 μL buffer + 8 mL n-hexane:ethyl acetate 70:30, mix on a rotary mixer for 10 min, centrifuge at 3000 g for 8 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL 12.5 mM NaOH in MeOH:water 50:50, inject a 50 μL aliquot. (Buffer was 13.8 g potassium carbonate in 100 mL water, pH adjusted to 9.5 with concentrated HCl.)

HPLC VARIABLES
Guard column: 4 × 4 30 μm LiChrocart Aluspher RP-select B (Merck)
Column: 125 × 4 5 μm Aluspher RP-select B (Merck)
Mobile phase: Gradient. A was 12.5 mM NaOH in MeOH. B was 12.5 mM NaOH in water. A:B 10:90 for 5 min, to 90:10 over 15 min, maintain at 90:10 for 5 min, return to initial conditions over 1 min, re-equilibrate for 5 min.
Flow rate: 1
Injection volume: 50
Detector: UV 230, 254

CHROMATOGRAM
Retention time: 17

OTHER SUBSTANCES

Extracted: alprenolol, amitriptyline, bromazepam, carbamazepine, chlordiazepoxide, chlorpromazine, clonazepam, desipramine, diazepam, flunitrazepam, nitrendipine, nordiazepam, nortriptyline, pindolol, zolpidem

Also analyzed: acebutolol, acetaminophen, alprazolam, amphetamine, atenolol, betaxolol, brotizolam, caffeine, camazepam, captopril, chloroquine, clobazam, clomipramine, clothiapine, clotiazepam, cloxazolam, cocaine, codeine, diclofenac, dihydralazine, dihydrocodeine, dihydroergotamine, diphenhydramine, domperidone, doxepin, droperidol, ergotamine, ethyl loflazepate, fenethylline, fluoxetine, flupentixol, flurazepam, furosemide, gliclazide, hydrochlorothiazide, hydroxyzine, ibuprofen, imipramine, ketazolam, loprazolam, lorazepam, lormetazepam, maprotiline, medazepam, mepyramine, methadone, methaqualone, methyldopa, methylphenidate, metoclopramide, metoprolol, mexiletine, mianserin, midazolam, minoxidil, morphine, nadolol, nitrazepam, oxprenolol, papaverine, pentazocine, phenprocoumon, phenylbutazone, pipamperone, piritramide, practolol, prazepam, prazosin, promazine, promethazine, propoxyphene, propranolol, prothipendyl, quinine, sotalol, sulpride, thioridazine, trazodone, triazolam, trimipramine, tripelennamine, tyramine, verapamil, yohimbine

REFERENCE

Lambert,W.E.; Meyer,E.; De Leenheer,A.P. Systematic toxicological analysis of basic drugs by gradient elution of an alumina-based HPLC packing material under alkaline conditions, *J.Anal.Toxicol.*, **1995**, *19*, 73–78.

SAMPLE

Matrix: blood, tissue

Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM

Retention time: 4.80
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES

Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, imipramine, loxapine, maprotiline, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine

Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

Interfering: meperidine, norpropoxyphene, northiaden

KEY WORDS

serum; whole blood; liver

REFERENCE
McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

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

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

CHROMATOGRAM
Retention time: 14.415

KEY WORDS
whole blood

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

SAMPLE
Matrix: formulations
Sample preparation: Weigh out capsule contents equivalent to 400 μg haloperidol, dissolve in 30 mL MeOH, add 1 mL 100 μg/mL isothipendyl hydrochloride in MeOH, filter (paper), wash filter with MeOH, make up filtrate to 50 mL with MeOH. Dilute a 1 mL aliquot to 10 mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Econosphere C18
Mobile phase: MeOH:water:triethylamine 80:20:0.15, pH adjusted to 7.8 with phosphoric acid
Flow rate: 1.8
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 4
Internal standard: isothipendyl (7)
Limit of quantitation: 800 ng/mL

OTHER SUBSTANCES
Simultaneous: propantheline bromide

KEY WORDS
capsules

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: 100 μL Injection solution + 400 μL 2.5 μg/mL haloperidol in water, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Brownlee C18
Mobile phase: MeCN:MeOH:10 mM NaH_2PO_4 24:31:45, pH adjusted to 5.0 with 2 M KOH
Flow rate: 1.7
Injection volume: 100
Detector: UV 210

CHROMATOGRAM
Retention time: 9.74
Internal standard: haloperidol

OTHER SUBSTANCES
Simultaneous: fentanyl, sufentanil

KEY WORDS
injections; haloperidol is IS

REFERENCE
Dewell,W.M.,Jr.; Khandaghabadi,M.; D'Souza,M.J.; Solomon,H.M. High-performance liquid chromatographic determination of fentanyl and sufentanil returned from the operating room, *Am.J.Hosp.Pharm.*, **1993**, *50*, 2374–2375.

SAMPLE
Matrix: hair
Sample preparation: Wash hair in water, rinse 3 times with MeOH, dry, weigh. 5-25 mg Washed hair + 1 mL 1 M NaOH, heat at 70° for 30 min, adjust pH to 9.5-10. 1 mL Extract + 1 μg protriptyline + 1 mL water + 1 mL 200 mM sodium carbonate buffer, mix, extract with hexane: butanol 95:5 for 20 min. Remove the organic layer and add it to 100 μL 0.2% orthophosphoric acid, mix for 20 min, inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Newguard RP-18
Column: 100 × 4.6 Spheri-5 RP-C18
Mobile phase: MeCN:buffer 40:60 (Buffer was 1.2 L 100 mM pH 7.0 NaH_2PO_4 + 30 mL diethylamine.)
Flow rate: 2
Injection volume: 30
Detector: UV 214

CHROMATOGRAM
Retention time: 2.5
Internal standard: protriptyline (4)

OTHER SUBSTANCES
Extracted: amitriptyline, clomipramine, desipramine, dothiepin, doxepin, imipramine, mianserin, nortriptyline

KEY WORDS
may be interferences

REFERENCE
Couper,F.J.; McIntyre,I.M.; Drummer,O.H. Extraction of psychotropic drugs from human scalp hair, *J.Forensic Sci.*, **1995**, *40*, 83–86.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve a sample in MeOH to a concentration of about 1 mg/mL, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Spherisorb SCX
Mobile phase: MeOH:water 80:20 containing 20 mM ammonium formate and 2.3 mL/L trifluoroacetic acid
Flow rate: 1
Injection volume: 1-10
Detector: UV 270

CHROMATOGRAM
Retention time: 5.5

OTHER SUBSTANCES
Simultaneous: cimetidine, clomipramine, halofantrine, minoxidil, reserpine, verapamil

REFERENCE
Law,N.; Appleby,J.R.G. Re-evaluation of strong cation-exchange high-performance liquid chromatography for the analysis of basic drugs, *J.Chromatogr.A*, **1996**, *725*, 335–341.

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

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

CHROMATOGRAM
Retention time: 2.0

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, 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, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine,

ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 30 × 2 5 μm Hypersil CPS
Column: 250 × 4.6 5 μm Hypersil CPS-5
Mobile phase: MeCN:10 mM pH 5.4 ammonium acetate 67:33
Flow rate: 1
Injection volume: 100
Detector: UV 220, UV 245

CHROMATOGRAM

Retention time: 13 (haloperidol), 11.5 (reduced haloperidol)
Internal standard: pirenzepine (8)
Limit of detection: 150 pmole

OTHER SUBSTANCES

Simultaneous: metabolites

KEY WORDS

comparison with capillary electrophoresis

REFERENCE

Tomlinson,A.J.; Benson,L.M.; Landers,J.P.; Scanlan,G.F.; Fang,J.; Gorrod,J.W.; Naylor,S. Investigation of the metabolism of the neuroleptic drug haloperidol by capillary electrophoresis, *J.Chromatogr.A*, **1993**, *652*, 417–426.

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 215, 241

CHROMATOGRAM
Retention time: 3.0
Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES
Also analyzed: aspirin, caffeine, carbamazepine, chlordiazepoxide, chlorprothixene, clonazepam, diazepam, doxylamine, ethosuximide, furosemide, 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, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primi-

done, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 11.10 (A), 6.17 (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, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

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

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 8.91

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

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

HPLC VARIABLES
Guard column: 10 × 2.1 37-50 μm Bondapak C18
Column: 300 × 3.9 Bondapak C18
Mobile phase: MeCN:water 55:45 containing 2.46 g/L anhydrous sodium acetate, pH adjusted to 6.5 with acetic acid
Flow rate: 1.2
Injection volume: 50

Detector: UV 240

CHROMATOGRAM
Retention time: 8
Limit of detection: 2 ng/g

OTHER SUBSTANCES
Extracted: azaperol, carazolol, acepromazine, xylazine, azaperone, propiomazine, chlorpromazine

KEY WORDS
SPE; pig; kidney

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

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

HPLC VARIABLES
Guard column: Hypersil 5 μm SAS C1
Column: 250 mm long 5 μm Hypersil SAS C1
Mobile phase: MeCN:water 50:50 containing 0.77 g/L ammonium acetate
Flow rate: 2
Detector: E, ESA Model 5100A Coulochem, first electrode +0.4 V, second electrode (which was monitored) +0.7 V, Model 5020 guard cell after pump but before injector at +0.75 V

CHROMATOGRAM
Retention time: 12.5
Limit of detection: 2 ng/g

OTHER SUBSTANCES
Extracted: azaperol, acepromazine, carazolol, azaperone, xylazine, propriomazine, chlorpromazine

KEY WORDS
SPE; pig; kidney; liver

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

Halothane

Molecular formula: $C_2HBrClF_3$
Molecular weight: 197.38
CAS Registry No.: 151-67-7
Merck Index: 4634

SAMPLE
Matrix: solutions
Sample preparation: Mix 50 μL phosphate buffer containing isoflurane and 50 μL 0.05 mM toluene in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: RCSS Guard-Pak μBondapak C18 precolumn cartridge
Column: 100 × 8 4 μm Nova-Pak C18 Radial Compression Module
Mobile phase: MeOH:water 50:50
Flow rate: 3.5
Injection volume: 20
Detector: UV 203

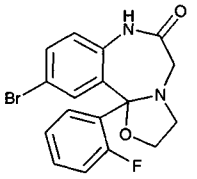
CHROMATOGRAM
Retention time: 6
Internal standard: toluene (12)
Limit of detection: 0.001 mM

OTHER SUBSTANCES
Simultaneous: enflurane, isoflurane

KEY WORDS
buffer

REFERENCE
Janicki,P.K.; Erskine,W.A.R.; James,M.F.M. High-performance liquid chromatographic method for the direct determination of the volatile anaesthetics halothane, isoflurane and enflurane in water and in physiological buffer solutions, *J.Chromatogr.*, **1990**, *518*, 250–253.

Haloxazolam



Molecular formula: $C_{17}H_{14}BrFN_2O_2$
Molecular weight: 377.21
CAS Registry No.: 59128-97-1
Merck Index: 4635

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 20 μL 20 μg/mL IS + 200 μL 1 M potassium carbonate + 3 mL chloroform, mix for 2 min, centrifuge at 1200 g for 5 min, aspirate aqueous phase. Evaporate the organic phase under a stream of nitrogen at 40°. Dissolve the residue in 100 μL mobile phase, inject a 20 μL aliquot. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES
Column: 100 × 4.6 2 μm TSK gel Super-ODS (A) or 100 × 4.6 5 μm Hypersil ODS-C18 (B)
Mobile phase: MeCN:5 mM pH 6 NaH_2PO_4 45:55
Flow rate: 0.65
Injection volume: 20

Detector: UV 254

CHROMATOGRAM
Retention time: 41.5 (A), 100.6 (B)
Internal standard: diazepam (29.8 (A), 77.5 (B))
Limit of quantitation: 50 ng/mL (A)

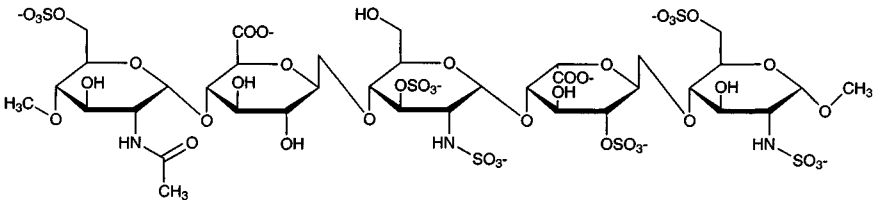
OTHER SUBSTANCES
Extracted: bromazepam, chlordiazepoxide, clonazepam, estazolam, etizolam, flutazolam, lorazepam, nitrazepam, oxazolam, triazolam
Simultaneous: alprazolam
Noninterfering: barbital, carbamazepine, cloxazolam, ethosuximide, hexobarbital, mexazolam, oxazepam, pentobarbital, phenobarbital, phenytoin, primidone, trimethadione

KEY WORDS
serum

REFERENCE
Tanaka,E.; Terada,M.; Misawa,.; Wakasugi,C. Simultaneous determination of twelve benzodiazepines in human serum using a new reversed-phase chromatographic column on a 2-μm porous microspherical silica gel, *J.Chromatogr.B*, **1996**, *682*, 173–178.

Heparin

Molecular weight: 6000-30000
CAS Registry No.: 9005-49-6, 9041-08-1 (Na salt)
Merck Index: 4685



SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 500 μL 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 μL mobile phase, inject aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Novapack C18
Mobile phase: MeCN:MeOH:buffer 35:15:50 (Buffer was 50 mM KH_2PO_4 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: 0.99
Limit of detection: 50 pg/mL

OTHER SUBSTANCES
Simultaneous: ethinylestradiol, estrone, estriol, estradiol
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 ethynyloestradiol in rabbit plasma, *J.Chromatogr.*, **1993**, *619*, 143–147.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm 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: 7.34
Internal standard: 8-chlorotheophylline (5.29)

OTHER SUBSTANCES
Simultaneous: acetaminophen, caffeine, cefazolin, cimetidine, ergotamine, glutethimide, methamphetamine, propranolol, salicylic acid, sulfamethoxazole, theobromine, theophylline, 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

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: solutions

HPLC VARIABLES
Column: 300 × 7.5 Ultropac TSK G 2000 SW (LKB)
Mobile phase: 100 mM NaCl
Flow rate: 0.5
Injection volume: 20
Detector: UV 206

CHROMATOGRAM
Retention time: 15, 21, 29

KEY WORDS
SEC

REFERENCE
de Vries,J.X. Analysis of heparins by size-exclusion and reversed-phase high-performance liquid chromatography with photodiode-array detection, *J.Chromatogr.*, **1989**, *465*, 297–304.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 mg/mL solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: TSK G3000SW and TSK G2000SW in series (Tosoh)
Mobile phase: 500 mM sodium sulfate
Flow rate: 0.5
Injection volume: 20
Detector: UV 234 or RI

KEY WORDS
SEC

REFERENCE
Ahsan,A.; Jeske,W.; Hoppensteadt,D.; Lormeau,J.C.; Wolf,H.; Fareed,J. Molecular profiling and weight determination of heparins and depolymerized heparins, *J.Pharm.Sci.*, **1995**, *84*, 724–727.

SAMPLE
Matrix: urine
Sample preparation: Mix 9 mL urine with 600 μL 5% hexadecylpyridinium chloride, keep at 0° for 4 h. Centrifuge at 2300 g for 15 min, wash the precipitate twice with 1.5 mL 0.1% hexadecylpyridinium chloride. Dissolve the precipitate in 1 mL 2.5 M NaCl. Centrifuge at 2300 g for 15 min. Add 11 mL EtOH:water 85:15 to he supernatant, keep overnight at 0°. Centrifuge at 2300 g for 15 min at 4°. Dry the precipitate under reduced pressure, re-dissolve in 50 μL 200 mM pH 8.0 Tris-HCl buffer. Add 10 μL of an aqueous solution containing 0.1 U chondroitinase ABC, incubate at 37°. for 3 h. Add 250 μL EtOH, keep overnight at 4 dg. Centrifuge at 4° at 2300 g for 15 min. Wash the precipitate with three 1 mL portions of EtOH:water 75:25., dry under reduced pressure. Redissolve in 50 μL 100 mM acetate buffer containing 10 mM pH 7.0 calcium acetate. Mix 10 μL sample with 10 μL 100 mM acetate buffer containing 10 mM pH 7.0 calcium acetate and 30 μL aqueous heparin lyase solution, incubate at 37° for 12 h. Inject a 2 μL aliquot. (The heparin lyase solution contained 4 mU heparin lyase I, 0.4 mU heparin lyase II, and 0.4 mU heparin lyase III.)

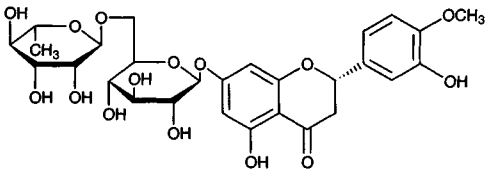
HPLC VARIABLES
Column: 150 × 2.0 5 μm TSK gel Amide-80 column (TOSOH, Japan)
Mobile phase: MeCN:water:200 mM pH 7.0 sodium phosphate buffer:3.0 M ammonium chloride 32:10:1:1
Column temperature: 60
Flow rate: 0.4
Injection volume: 2
Detector: F ex 346 em 410 following post-column reaction. The column effluent mixed with 1% 2-cyanoacetamide solution containing 500 mM NaOH pumped at 0.25 mL/min and this mixture flowed through a 10 m × 0.5 mm reaction coil at 110° and a 2 m × 0.25 mm cooling coil to the detector.

CHROMATOGRAM
Retention time: 7.1 (δ UA-GlcNAc), 8.5 (δ UA-GlcNAc6S), 10.5 (δ UA2S-GlcNac6S), 11.0 (δ UA-GlcNS), 8.9 (δ UA2S-GlcNS), 16.1 (δ UA-GlcNS6S), 21.2 (δ UA2s-GlcNS6S)
Limit of detection: 2 pmol

KEY WORDS
post-column reaction

REFERENCE
Toyoda,H.; Nagashima,T.; Hirata,R.; Toida,T.; Imanari,T. Sensitive high-performance liquid chromatographic method with fluorometric detection for the determination of heparin and heparan sulfate in biological samples: application to human urinary heparan sulfate, *J.Chromatogr.B*, **1997**, *704*, 19–24.

Hesperidin



Molecular formula: $C_{28}H_{34}O_{15}$
Molecular weight: 610.57
CAS Registry No.: 520-26-3
Merck Index: 4705

SAMPLE

Matrix: blood, urine
Sample preparation: Split each plasma and urine sample into two fractions. Mix 3 mL 1 M pH 4.5 sodium acetate buffer with one 3 mL plasma portion or one 10 mL urine portion, incubate at 37° for 20 h with 10,000 U β-glucuronidase (Helix Pomatia, Sigma). Condition a Sep-Pak C18 SPE cartridge with 6 mL EtOH:water 95:5 and 10 mL water. Add the sample to the SPE cartridge. Wash with 4 mL MeOH:water 10:90, elute the flavanones with 3 mL MeOH, reduce the plasma eluent volume to 500 μL under a stream of nitrogen. Inject 25 or 50 μL aliquots.

HPLC VARIABLES

Column: 250 × 4.6 Zorbax ODS
Mobile phase: MeOH:water:glacial acetic acid 47:50.5:2.5
Flow rate: 1
Injection volume: 25-50
Detector: UV 280

CHROMATOGRAM

Retention time: 6.5
Limit of quantitation: 1 μg/mL

OTHER SUBSTANCES

Extracted: hesperitin, naringenin, naringin, narirutin

KEY WORDS

plasma; SPE

REFERENCE

Ameer,B.; Weintraub,R.A.; Johnson,J.V.; Yost,R.A.; Rouseff,R.L. Flavanone absorption after naringin, hesperidin, and citrus administration, *Clin.Pharmacol.Ther.*, **1996**, *60*, 34–40.

SAMPLE

Matrix: blood, urine
Sample preparation: Condition a 2.4 mL 500 mg 40 μm Bond Elut C2 SPE cartridge with 2 mL MeOH and 2 mL water. Adjust pH of 1-2 mL urine or plasma to 5.5 with buffer, add to the SPE cartridge, wash with two 2 mL portions of water, elute with 1 mL MeOH. Add the eluate to 100 μL 4 M pH 5.5 ammonium acetate, centrifuge at 1000 g for 5 min, inject an aliquot of the supernatant. (Buffer was 10 mM ammonium acetate adjusted to pH 5.5 with acetic acid.)

HPLC VARIABLES

Column: 300 × 3.8 10 μm μBondapak C18
Mobile phase: Isopropanol:buffer 20:80 (Buffer was 10 mM ammonium acetate adjusted to pH 5.5 with acetic acid.)
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 303

CHROMATOGRAM

Retention time: 4.69
Internal standard: hesperidin

OTHER SUBSTANCES
Extracted: flavone acetic acid

KEY WORDS
SPE; hesperidin is IS; plasma

REFERENCE
Cummings,J.; Kerr,D.J.; Kaye,S.B.; Smyth,J.F. Optimisation of a reversed-phase high-performance liquid chromatographic method for the determination of flavone acetic acid and its major human metabolites in plasma and urine, *J.Chromatogr.*, **1988**, *431*, 77–85.

SAMPLE
Matrix: fruit
Sample preparation: Grind 1.2 g dried fruit, extract with 50 mL EtOH:water 80:20 at 90° for 2 h. Filter the solution and evaporate it to dryness at vacuum. Dissolve 120 mg residue in 50 mL MeOH, filter (0.45 μm nylon Acrodisk), inject a 3 μL aliquot.

HPLC VARIABLES
Column: 150 × 2.1 5μm Waters Symmetry C18 (Waters USA)
Mobile phase: Gradient. A was water containing 0.6% acetic acid. B was MeOH. A:B 80:20 to 60:40 over 12 min; maintain at 60:40 for 7 min, to 0:100 over 11 min, maintain at 0:100 for 3 min, to 80:20 over 2 min.
Column temperature: 45
Flow rate: 0.2
Injection volume: 3
Detector: UV 290; MS, HP 5989 B electrospray, quadrupole temperature 150°., EM 2173 V, positive mode, drying gas nitrogen, 360°., nebulizing gas nitrogen 0.55 MPa, m/z 611

CHROMATOGRAM
Retention time: 17.6

OTHER SUBSTANCES
Extracted: hesperitin, isonaringin, naringenin, naringin, neohesperidin, nobiletin, tangeritin

KEY WORDS
sour orange; orange

REFERENCE
He,X.-g.; Lian,L.-z.; Lin,L.-z.; Bernart,M.W. High-performance liquid chromatography--electrospray mass spectrometry in phytochemical analysis of sour orange (Citrus aurantium L.), *J.Chromatogr.A*, **1997**, *791*, 127–134.

SAMPLE
Matrix: juice
Sample preparation: Centrifuge 10 mL juice at 2500 g for 10 min, filter 1.2 μm, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 Spheri-5 C18
Column: 125 × 3.6 3 μm C18 (Supelco)
Mobile phase: MeCN:water:glacial acetic acid 20:79.5:0.5
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 13

OTHER SUBSTANCES
Extracted: narirutin, naringin, neohesperidin

KEY WORDS
orange; grapefruit

REFERENCE
Rouseff,R.L. Liquid chromatographic determination of naringin and neohesperidin as a detector of grapefruit juice in orange juice, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 798–802.

SAMPLE
Matrix: juice
Sample preparation: Condition a 360 mg Sep-Pak C18 SPE cartridge with 2 mL MeOH and two 4 mL portions of water. Centrifuge 15 mL orange juice at 8000 rpm for 15 min, add 2 mL of the supernatant to the SPE cartridge, wash with 2 mL water, wash with 1.5 mL MeOH: water 25:75, elute with 1.5 mL THF, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 mm long LC-NH2 (Supelco)
Column: 250 × 4.6 Bondesil C18 (Analytichem)
Mobile phase: Gradient. MeCN:THF:2% acetic acid 5:12:83 for 22 min, to 0:35:65 over 6 min, maintain at 0:35:65 for 12 min, return to initial conditions over 2 min, re-equilibrate for 10 min.
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 13

OTHER SUBSTANCES
Extracted: caffeic acid, coumaric acid, ferulic acid, narirurin, sinapic acid

KEY WORDS
orange; SPE

REFERENCE
Rouseff,R.L.; Dettweiler,G.R.; Swaine,R.M.; Naim,M.; Zehavi,U. Solid-phase extraction and HPLC determination of 4-vinyl guaiacol and its precursor, ferulic acid, in orange juice, *J.Chromatogr.Sci.*, **1992**, *30*, 383–387.

SAMPLE
Matrix: juice
Sample preparation: Grapefruit. 1 g Grapefruit juice concentrate + 1 mL 1.8 mg/mL rhoifolin in MeOH, vortex for 1 min, centrifuge at 25000 g for 15 min, remove the supernatant. Add 1 mL MeOH to the solid and stir with a spatula, vortex for 1 min, centrifuge, repeat extraction. Combine the supernatants and add 2 mL water, filter (1 μm glass fiber), filter (0.2 μm, Anotop), inject a 20 μL aliquot of the filtrate. Orange. 1 g Orange juice concentrate + 0.4 mL 1.8 mg/mL rhoifolin in MeOH + 3 mL MeOH, vortex for 30 s, heat at 55° for 15 min, mix for 30 s, centrifuge at 25000 g for 15 min, remove the supernatant. Add 2 mL MeOH and 1 mL water to the solid, vortex for 30 s, heat at 55° for 15 min, vortex for 30 s, centrifuge. Combine the supernatants and add 4 mL water, vortex, filter (1 μm glass fiber), filter (0.2 μm, Anotop), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Alltima C18 (Alltech)
Mobile phase: MeCN:water:isopropanol:formic acid 11.5:79:9.5:0.1
Flow rate: 0.6
Injection volume: 20
Detector: UV 283

CHROMATOGRAM
Retention time: 21
Internal standard: rhoifolin (UV 335) (30)

OTHER SUBSTANCES
Simultaneous: naringin, narirutin

KEY WORDS
concentrate; orange; grapefruit

REFERENCE
Bronner,W.E.; Beecher,G.R. Extraction and measurement of prominent flavonoids in orange and grapefruit juice concentrates, *J.Chromatogr.A*, **1995**, *705*, 247–256.

SAMPLE
Matrix: plants
Sample preparation: Grind wood, bark, or leaves and extract with MeOH:water 80:20 at room temperature for 24 h, filter, remove the MeOH under reduced pressure, extract the aqueous layer with diethyl ether. Dry the organic layer and evaporate it to dryness, reconstitute with MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: Hypersil ODS
Column: 200 × Hypersil ODS
Mobile phase: Gradient. A was MeOH containing 0.1% phosphoric acid. B was water containing 0.1% phosphoric acid. A:B from 20:80 to 100:0 over 40 min, maintain at 100:0 for 5 min.
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 14

OTHER SUBSTANCES
Extracted: flavanoids, phenolic acids

KEY WORDS
wood; bark; leaves

REFERENCE
Conde,E.; Cadahía,E.; Garcia-Vallejo,M.C. HPLC analysis of flavonoids and phenolic acids and aldehydes in Eucalyptus spp, *Chromatographia*, **1995**, *41*, 657–660.

SAMPLE
Matrix: plants
Sample preparation: Extract leaves eight times with 5 mL MeOH at 60°, combine the extracts, filter, evaporate to dryness, reconstitute in 1 mL MeOH, filter (0.22 μm), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm C18 (Vydac)
Mobile phase: Gradient. MeCN:3% acetic acid from 1:99 to 10:90 over 15 min, to 15:85 over 15 min, to 25:75 over 20 min, to 35:65 over 20 min, to 50:50 over 15 min, maintain at 50:50 for 20 min.
Column temperature: 28
Flow rate: 1
Detector: UV 255 or 280

CHROMATOGRAM
Retention time: 39.5
Internal standard: isorhamnetin 3-rutinoside (38)

OTHER SUBSTANCES
Simultaneous: caffeic acid, chlorogenic acid, daticoside, dihydrorobinetin, robinin, rutin

REFERENCE
Ficarra,R.; Ficarra,P.; Tommasini,S.; Calabrò,M.L.; Ragusa,S.; Barbera,R.; Rapisarda,A. Leaf extracts of some Cordia species: Analgesic and anti-inflammatory activities as well as their chromatographic analysis, *Farmaco*, **1995**, *50*, 245–256.

SAMPLE
Matrix: plants
Sample preparation: 500 mg Plant material + 7 mL MeOH:water 70:30, stir at room temperature for 30 min, centrifuge at 1500 g for 5 min, repeat extraction 3 times. Combine extracts, filter (No. 1 paper), add 2.5 mL 1.172 mg/mL p-tert-octylphenol in MeOH, make up to 25 mL with MeOH:water 70:30, inject a 5 μL aliquot.

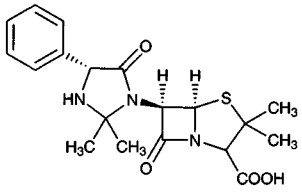
HPLC VARIABLES
Guard column: μBondapak C18
Column: 250 × 4.6 5 μm Cosmosil 5C18 (Nacalai Tesque)
Mobile phase: Gradient. A was MeCN:buffer 10:90. B was MeCN:MeOH:1% acetic acid 45:45:10. A:B from 90:10 to 75:25 over 14 min, maintain at 75:25 for 8 min, to 70:30 over 8 min, to 20:80 over 5 min, to 0:100 over 10 min, maintain at 0:100 over 10 min, return to initial conditions over 5 min. (Buffer contained 20 mM sodium acetate and 419.7 mM acetic acid.)
Flow rate: 0.8
Injection volume: 5
Detector: UV 280

CHROMATOGRAM
Retention time: 28.2
Internal standard: p-tert-octylphenol (54.2)

OTHER SUBSTANCES
Extracted: emodin, gallic acid, honokiol, magnolol, naringin, sennoside A, sennoside B

REFERENCE
Sheu,S.-J.; Lu,C.-F. Determination of eight constituents of Hsiao-cheng-chi-tang by high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *704*, 518–523.

Hetacillin



Molecular formula: $C_{19}H_{23}N_3O_4S$
Molecular weight: 389.48
CAS Registry No.: 3511-16-8, 5321-32-4 (K salt)
Merck Index: 4706
Lednicer No.: 1 414

SAMPLE
Matrix: milk
Sample preparation: 500 μL Milk + 500 μL MeCN:MeOH:water 40:20:40, vortex for 10-15 s, filter (Centricon-10, molecular mass cut-off filter 10000 daltons) with centrifuging at 2677 g for 30 min, inject a 10-100 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 220 × 2.1 5 μm Spheri-5 phenyl microbore (UV detection) or 220 × 4.6 5 μm Spheri-5 phenyl microbore (MS detection)
Mobile phase: MeCN:85% phosphoric acid: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 or MS, Finnigan MAT 4800 quadrupole, thermospray, source 320°, vaporizer 120°, pulsed positive ion negative ion

CHROMATOGRAM
Retention time: 8.3 (UV), 14.5 (MS) [as ampicillin]
Limit of detection: 200 ng/mL (MS), 75 ng/mL (100)

OTHER SUBSTANCES
Also analyzed: cloxacillin, amoxicillin

KEY WORDS
ultrafiltrate; LC-MS; hetacillin is rapidly converted to ampicillin

REFERENCE
Voyksner,R.D.; Tyczkowska,K.L.; Aronson,A.L. Development of analytical methods for some penicillins in bovine milk by ion-paired chromatography and confirmation by thermospray mass spectrometry, *J.Chromatogr.*, **1991**, *567*, 389–404.

SAMPLE
Matrix: solutions
Sample preparation: React the antibiotic, triethylamine, and 1-(2,5-dihydroxyphenyl)-2-bromoethanone in a 1:2:4 molar ratio in DMF at 45° for 2 h (use 18-crown-6 to make the potassium salt soluble), inject a 10 μL aliquot. (Preparation of 1-(2,5-dihydroxyphenyl)-2-bromoethanone is as follows. Stir 27.6 g 1,4-dimethoxybenzene and 28 mL bromoacetyl bromide at 0°, add 53.4 g aluminum bromide over 10 min (an exothermic reactions ensues), let stand at room temperature for 12 h, add 100 mL 48% HBr, add 100 g ice, stir for 1 h, extract twice with 200 mL portions of diethyl ether. Combine the extracts and wash them 3 times with 200 mL portions of water, dry over 40 g anhydrous magnesium sulfate, evaporate to dryness, recrystallize the product 3 times from EtOH to yield 1-(2,5-dihydroxyphenyl)-2-bromoethanone monobromoacetate (mp 105-107°). Dissolve 11 g 1-(2,5-dihydroxyphenyl)-2-bromoethanone monobromoacetate in 200 mL warm dry MeOH saturated with HBr, stir for 18 h, add 200 mL water, cool to -10°. Collect the yellow solid and dry it under vacuum at 50° for 48 h, recrystallize from toluene: heptane 50:50 then toluene to obtain 1-(2,5-dihydroxyphenyl)-2-bromoethanone as yellow needles (mp 117-119°).)

HPLC VARIABLES
Column: 250 × 4 7 μm RP-18 LiChrocart (Merck)
Mobile phase: MeOH:100 mM pH 6.5 sodium acetate 58:42
Flow rate: 1
Injection volume: 10
Detector: E, Bioanalytical Systems Model LC4B, glassy carbon electrode 0.8 V, Ag/AgCl reference electrode

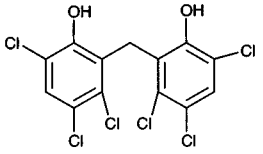
CHROMATOGRAM
Retention time: 7.8

OTHER SUBSTANCES
Simultaneous: carbenicillin, cephapirin, cloxacillin, dicloxacillin, methicillin, nafcillin, oxacillin, penicillin G

KEY WORDS
derivatization

REFERENCE
Munns,R.K.; Roybal,J.E.; Shimoda,W.; Hurlbut,J.A. 1-(4-Hydroxyphenyl)-, 1-(2,4-dihydroxyphenyl)- and 1-(2,5-dihydroxyphenyl)-2-bromoethanones: new labels for determination of carboxylic acids by high-performance liquid chromatography with electrochemical and ultraviolet detection, *J.Chromatogr.*, **1988**, *442*, 209–218.

Hexachlorophene



Molecular formula: $C_{13}H_6Cl_6O_2$
Molecular weight: 406.91
CAS Registry No.: 70-30-4
Merck Index: 4716

SAMPLE
Matrix: solutions
Sample preparation: Dissolve 4-20 μg in 1 mL 5% NaOH, add 30 μL p-anisoyl chloride, vortex for 1 min, let stand at room temperature for 20 min, add 9 mL water, vortex for 2 min, extract three times with 10 mL portions of hexane. Combine the extracts and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL butyl chloride, inject a 10 μL aliquot.

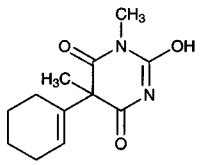
HPLC VARIABLES
Column: 610 × 2.3 36-40 μm Sil-X silica (Nester-Faust)
Mobile phase: Hexane:n-butyl chloride 55:45
Flow rate: 0.7
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 10.5
Limit of detection: 30 ppb

KEY WORDS
derivatization; normal phase

REFERENCE
Porcaro,P.J.; Shubiak,P. Detection of nanogram quantities of hexachlorophene by ultraviolet liquid chromatography, *Anal.Chem.*, **1972**, *44*, 1865–1867.

Hexobarbital



Molecular formula: $C_{12}H_{16}N_2O_3$
Molecular weight: 236.27
CAS Registry No.: 56-29-1, 50-09-9 (sodium salt)
Merck Index: 4742
Lednicer No.: 1 273

SAMPLE
Matrix: solutions

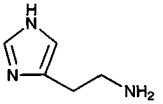
HPLC VARIABLES
Column: 250 × 4.6 5 μm YMC GEL, ODS-AM coated with poly-(R)-1-(α-naphthyl)ethyl methacrylamide (Prepare (R)-1-(α-naphthyl)ethyl methacrylamide by reacting methacryl chloride with (R)-1-(α-naphthyl)ethylamine. Prepare poly-(R)-1-(α-naphthyl)ethyl methacrylamide by polymerizing this compound in anhydrous benzene/THF with 2,2'-azobis(isobutyronitrile) (Caution! Benzene is a carcinogen!). Average molecular weight = 2500. Coat 4 g 5 μm YMC GEL, ODS-AM with 0.8 g of this polymer using dichloromethane as a solvent.)
Mobile phase: MeCN:water 30:70
Flow rate: 0.7

CHROMATOGRAM
Retention time: k' 6.86 (α = 1.08)

KEY WORDS
chiral

REFERENCE
Oi,N.; Hashimoto,S.; Ishizuka,N.; Ohtake,J. Enantiomer separation with poly-(R)-1 (α-naphthyl)-ethyl-methacrylamide coated on ODS silica gel by reversed phase HPLC, *Biomed.Chromatogr.*, **1997**, *11*, 296–297.

Histamine



Molecular formula: $C_5H_9N_3$
Molecular weight: 111.15
CAS Registry No.: 51-45-6, 51-74-1 (phosphate)
Merck Index: 4756

SAMPLE
Matrix: bacterial culture
Sample preparation: Prepare a column by filling a 1 mL pipette tip with 200 mg Extrelut, condition with 250 µL buffer. Add 10 µL bacterial culture and 10 µL IS solution to the column, elute with 1.3 mL ethyl methyl ketone:isopropanol 90:10 (prepared just prior to use) or with 1.3 mL ethyl methyl ketone. Add 200 µL reagent to the eluate, vortex for 15 s, let stand for 30 min, dilute 1 volume of the supernatant with 1 volume of MeOH and 2 volumes of water, inject an aliquot. (Prepare buffer by dissolving 50 mmoles ascorbic acid and 50 mmoles Na_2HPO_4 in 80 mL 1 M NaOH with stirring, adjust pH to 12.5 with 10 mM NaOH, make up to 100 mL with water, store in completely filled vials. Prepare the IS solution by adding 12 µL pentylamine to 100 mL 100 mM HCl. Purify ethyl methyl ketone by passing 20 mL through 4 g acidic aluminum oxide (Merck). Prepare reagent each day by dissolving 10 mg o-phthaldialdehyde and 50 µL ethanethiol in 1 mL MeOH and adding 9 mL 400 mM pH 9.5 sodium borate buffer.)

HPLC VARIABLES
Column: 125 × 2 Superspher 100 RP-18 (Merck)
Mobile phase: MeOH:buffer 85:15 (Buffer was 7% triethylamine adjusted to pH 7.5 with acetic acid.)
Flow rate: 0.2
Injection volume: 1
Detector: F ex 340 em 450

CHROMATOGRAM
Retention time: 2.56
Internal standard: pentylamine (7.45)

OTHER SUBSTANCES
Extracted: cadaverine, isobutylamine, phenethylamine, putrescine, tryptamine, tyramine

KEY WORDS
SPE; derivatization

REFERENCE
Bilic,N. Rapid identification of biogenic amine-producing bacterial cultures using isocratic high-performance liquid chromatography, *J.Chromatogr.A*, **1996**, *719*, 321–326.

SAMPLE
Matrix: beverages

Sample preparation: Condition a 500 mg SAX SPE cartridge (Varian) with two 5 mL portions of MeOH and two 5 mL portions of water. Condition a 1000 mg C18 SPE cartridge with two 5 mL portions of MeOH two 5 mL portions of 200 mM pH 4.5 sodium decanesulfonate. Adjust pH of 15 mL red wine to 8, pass through the SAX SPE cartridge, adjust the pH of the eluate to 4.5, add 100 μL 200 mM sodium decanesulfonate, add this solution to the C18 SPE cartridge, elute with 3 mL MeOH. Mix 10 μL reagent and 2 μL eluate for 1 min, inject the whole amount. (Prepare reagent by dissolving 45 mg o-phthalaldehyde and 200 μL mercaptoethanol in 1 mL MeOH and making up to 10 mL with Buffer. Prepare buffer by dissolving 3.81 g sodium tetraborate in 100 mL water and adjusting pH to 10.5 with 10 M NaOH.)

HPLC VARIABLES

Guard column: ODS Basic (Teknokroma, Barcelona)
Column: 250 × 4.6 5 μm ODS Basic (Teknokroma, Barcelona)
Mobile phase: Gradient. A was THF:50 mM pH 7 (?) sodium acetate 1:99. B was MeOH. A:B from 45:55 to 20:80 over 25 min, maintain at 20:80 for 3 min, return to initial conditions over 2 min.
Column temperature: 60
Flow rate: 1
Injection volume: 12
Detector: F ex 330 em 445

CHROMATOGRAM

Retention time: 4.5
Limit of quantitation: 430 ng/mL

OTHER SUBSTANCES

Extracted: amylamine, butylamine, cadaverine, ethanolamine, ethylamine, hexylamine, isopropylamine, methylamine, 3-methylbutylamine, phenethylamine, propylamine, putrescine, tryptamine, tyramine

KEY WORDS

wine; derivatization; SPE

REFERENCE

Busto,O.; Guasch,J.; Borrull,F. Improvement of a solid-phase extraction method for determining biogenic amines in wines, *J.Chromatogr.A*, **1995**, *718*, 309–317.

SAMPLE

Matrix: blood
Sample preparation: Chill 20 mL whole blood in ice water, add 1 mL reagent, invert several time, centrifuge at 4° at 4000 g for 10 min. Remove the plasma and make it 0.4 M in perchloric acid by adding concentrated perchloric acid, mix, let stand at 4° for 15 min, centrifuge at 4° at 20000 g for 20 min. Remove a 2 mL aliquot and adjust the pH to 7.0 ± 0.2 with 0.5 M KOH, add 400 μL reagent, add 2 g NaCL, add 2 mL ethyl acetate, shake for 1 min, centrifuge at 3400 g, repeat the extraction. Combine the organic layers, add 2 mL 35 mM pH 10.0 ± 0.1 Na_2HPO_4 buffer, shake for 1 min, centrifuge at 3400 g, repeat the wash, evaporate the ethyl acetate layer to 100 μL with a stream of nitrogen, inject a 10-50 μL aliquot. (Prepare reagent as follows. Dissolve 500 mg boric acid in 19 mL water, adjust the pH to 10.40 ± 0.02 with 450 g/L KOH, add 17.5 mg o-phthalaldehyde dissolved in 200 μL MeOH, add 40 μL fresh 2-mercaptoethanol, store under nitrogen at 5°, stable for 7 days (J.Chromatogr. 1979, 162, 293).)

HPLC VARIABLES

Guard column: Co:Pell ODS
Column: 300 × 4 10 μm μBondapak phenyl
Mobile phase: Gradient. MeCN:25 mM pH 5.10 NaH_2PO_4 25:75 for 15 min, then MeOH:25 mM pH 5.10 NaH_2PO_4 45:55 for 35 min (step gradient).
Column temperature: 26
Flow rate: 1.5
Injection volume: 10-50
Detector: F ex 340 em 480

CHROMATOGRAM

Retention time: 6

OTHER SUBSTANCES
Extracted: dopamine, norepinephrine, octopamine, serotonin, tyramine

KEY WORDS
pig; whole blood; derivatization

REFERENCE
Davis,T.P.; Gehrke,C.W.,Jr.; Williams,C.H.; Gehrke,C.W.; Gerhardt,K.O. Pre-column derivatization and high-performance liquid chromatography of biogenic amines in blood of normal and malignant hyperthermic pigs, *J.Chromatogr.*, **1982**, *228*, 113–122.

SAMPLE
Matrix: blood
Sample preparation: Mix plasma with pH 9.7 buffer containing 8.6 mM cyanide and 17.2 mM naphthalene-2,3-dicarboxaldehyde, let stand for 20 min, add 200 mM taurine, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultramex C8
Mobile phase: MeCN:pH 6.8 phosphate buffer 40:60
Detector: F

CHROMATOGRAM
Limit of quantitation: 75 pg

KEY WORDS
derivatization; plasma

REFERENCE
James,J.; Lowe,D.; Karnes,H.T. Determination of histamine from plasma using derivatization with naphthalene-2,3-dicarboxaldehyde and HPLC with fluorescence detection, *Pharm.Res.*, **1992**, *9*, S21.

SAMPLE
Matrix: blood
Sample preparation: Condition a CBA (carboxylic acid) SPE cartridge (Analytichem) with 1 mL MeOH and 2 mL 10 mM pH 7 phosphate buffer. 500 μL Plasma + 100 μL 1 mg/mL betazole + 2 mL ice-cold 10 mM pH 7 phosphate buffer, add to the SPE cartridge at 0.2-0.3 mL/min, dry for 30 s, wash with 1 mL hexane, elute with 1 mL MeOH:100 mM HCl 60:40. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL 200 mM pH 9 sodium borate buffer, add 50 μL 20 μg/mL fluorescamine in MeCN, vortex, store at 4°, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: Pelliguard LC-8 (Supelco)
Column: 250 × 4.6 5 μm Ultramex C8
Mobile phase: MeCN:500 mM pH 7 imidazole buffer 20:80
Flow rate: 1
Injection volume: 50
Detector: F ex 366 em 440

CHROMATOGRAM
Retention time: 15.2
Internal standard: betazole (26.9)
Limit of quantitation: 1 ng/mL

KEY WORDS
plasma; SPE; derivatization

REFERENCE
Lowe,D.R.; March,C.; James,J.E.; Karnes,H.T. A high-performance liquid chromatographic method for histamine in plasma using solid phase extraction and fluorescamine derivatization, *J.Liq.Chromatogr.*, **1994**, *17*, 3563–3570.

SAMPLE
Matrix: blood
Sample preparation: Condition a CBA SPE cartridge with 1 mL MeOH and 2 mL 10 mM pH 7 phosphate buffer. 500 μL Plasma + 2 mL chilled 10 mM pH 7 phosphate buffer, mix, add to the SPE cartridge, dry for 30 min, wash with 1 mL hexane, elute with 1 mL MeOH:100 mM HCl 40:60. Evaporate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL 200 mM pH 9.7 borate buffer. Remove a 10 μL aliquot and add it to 40 μL 20 μg/mL fluorescamine in 200 mM pH 9.7 sodium borate buffer and 50 μL 200 mM pH 9.7 sodium borate buffer, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultramex C-8
Mobile phase: MeCN:500 mM pH 7 imidazole nitrate buffer 20:80
Flow rate: 1
Injection volume: 50
Detector: F ex 366 em 440 (cut-off filter)

CHROMATOGRAM
Limit of detection: 13 pg
Limit of quantitation: 166 pg

KEY WORDS
derivatization; plasma; SPE; details of chemiluminescence detection are also given in paper

REFERENCE
Walters,D.L.; James,J.E.; Vest,F.B.; Karnes,H.T. A comparison of fluorescence versus chemiluminescence detection for analysis of the fluorescamine derivative of histamine by HPLC, *Biomed.Chromatogr.*, **1994**, *8*, 207–211.

SAMPLE
Matrix: blood, food, peptides, plants, tissue
Sample preparation: Hydrolyze peptide with 6 M HCl containing 0.2% 3,3'-thiodipropionic acid at 110° for 24 h, evaporate to dryness, reconstitute with 50-200 μL 0.1% HCl containing 0.2% 3,3'-thiodipropionic acid. Homogenize (Ultra-Turrax) 0.1-1 g food, tissue, plant material, lyophilized plasma, or lyophilized tissue in 10 mL 250 nM IS in 100 mM HCl containing 0.2% 3,3'-thiodipropionic acid at 20000 rpm for 2 min, sonicate for ≤30 min, centrifuge at 5000 g for 20 min, discard fat layer, filter (Millipore ultrafiltration insert (MW cutoff 5000) prewashed with 200 μL 100 mM HCl containing 0.2% 3,3'-thiodipropionic acid) 3 mL supernatant while centrifuging at 3500 g for 1 h. Mix 20 μL deproteinized sample (or 10 μL peptide hydrolysate) with 180 μL buffer, vortex, add 200 μL reagent, mix, heat at 70° for 15 min with mixing at 1 min and 12 min, cool in an ice bath for 5 min, centrifuge at 10000 g for 10 s, add 400 μL diluent, mix thoroughly, centrifuge at 15000 g for 5 min, inject a 10 μL aliquot of the supernatant. (Prepare buffer by dissolving 630 mg sodium bicarbonate in 40 mL water, adjusting pH to 8.6 with NaOH, and making up to 50 mL with water. Prepare reagent by sonicating 40 mg dabsyl chloride in 10 mL acetone for 10 min, then filtering into brown vials and storing at -20°. Prepare diluent by mixing 50 mL MeCN, 25 mL EtOH, and 25 mL mobile phase A.)

HPLC VARIABLES
Guard column: present but not specified
Column: 150 × 3.9 4 μm Novapak C18
Mobile phase: Gradient. A was DMF:9 mM NaH_2PO_4 containing 0.16% triethylamine, adjusted to pH 6.55 with phosphoric acid. B was MeCN:water 80:20. A:B 92:8 for 2 min, to 80:20 over 5 min (Waters convex curve 5), to 65:35 over 28 min (Waters concave curve 7), to 50:50 over 10 min, to 0:100 over 21 min, maintain at 0:100 for 11 min, return to initial conditions over 0.5 min, re-equilibrate for 12.5 min.
Column temperature: 50
Flow rate: 1
Injection volume: 10
Detector: UV 436

CHROMATOGRAM
Retention time: 63.87

Internal standard: norleucine (40.90), norvaline (35.06)

OTHER SUBSTANCES
Extracted: amino acids dopamine, epinephrine, norepinephrine, taurine

KEY WORDS
rinse glass and plasticware with 70% EtOH and water and dry before use; derivatization; cheese; meat; sausage; fish; plasma

REFERENCE
Krause,I.; Bockhardt,A.; Neckermann,H.; Henle,T.; Klostermeyer,H. Simultaneous determination of amino acids and biogenic amines by reversed-phase high-performance liquid chromatography of the dabsyl derivatives, *J.Chromatogr.A*, **1995**, *715*, 67–79.

SAMPLE
Matrix: bulk
Sample preparation: Evaporate 100 μL of a solution of histamine in MeOH to dryness under nitrogen, add 100 μL 0.3 mM N-(4-aminobutyl)-N-ethylisoluminol isothiocyanate in MeCN:water:triethylamine 88:10:2 to the residue, vortex for 10 s, heat at 80° for 1 h. Add 600 μL mobile phase to the reaction mixture, mix, inject a 100 μL aliquot. (Synthesis of N-(4-aminobutyl)-N-ethylisoluminol isothiocyanate is as follows. Dissolve 110 mg N-(4-aminobutyl)-N-ethylisoluminol in 500 mL 100 mM sodium carbonate, add 100 μL thiophosgene, stir at room temperature for 2.5 h, adjust pH to 1 with 100 mL 1 M HCl, extract twice with 250 mL portions of ethyl acetate, combine the extracts and evaporate them to dryness under reduced pressure at 30-35°. Dissolve the residue in 4 mL DMF, add 100 mL ice-cold water, store at 4° overnight, collect the precipitate by filtration (0.45 μm), dry under vacuum for 16 h to obtain N-(4-aminobutyl)-N-ethylisoluminol isothiocyanate. Store at 4° in the dark.)

HPLC VARIABLES
Guard column: 10 × 2.0 10 μm PLRP-S (Polymer Labs., UK)
Column: 250 × 4.0 5 μm Asahipak ODP-50 (Hewlett-Packard)
Mobile phase: Gradient. A was MeCN:10 mM pH 10.5 sodium carbonate buffer 30:70 containing 5 mM tetraheptylammonium bromide. B was MeCN:10 mM pH 10.5 sodium carbonate buffer 70:30. A:B 100:0 for 22 min, to 0:100 (step gradient), maintain at 0:100 for 6 min, re-equilibrate at initial conditions for 30 min
Flow rate: 0.8
Injection volume: 100
Detector: E, ESA Coulochem guard cell, Model 5020, porous graphite electrode, Pd/PdO reference electrode

CHROMATOGRAM
Retention time: 20
Limit of detection: 1.5 pmol

KEY WORDS
derivatization

REFERENCE
Steijger,O.M.; Kamminga,D.A.; Brummelhuis,A.; Lingeman,H. Liquid chromatography with luminol-based electrochemiluminescence detection. Determination of histamine, *J.Chromatogr.A*, **1998**, *799*, 57–66.

SAMPLE
Matrix: bulk
Sample preparation: Mix 0.5-5 nmole histamine with 50 nmole luminarin 2 in 500 μL acetone, add 100 μL 100 mM dimethylaminopyridine in acetone, evaporate to dryness, let stand in the dark for 1.5 h,reconstitute with 200 μL acetone, inject an aliquot. (Dry acetone over 0.4 nm molecular sieve. Luminarin 2, N-[6-[(2,5-dioxo-1-pyrrolidinyl)oxy]-6-oxohexyl]-2,3,6,7-tetrahydro-11-oxo-1H,5H,11H-[1]benzopyrano[6,7,8-ij]quinolizine-9-acetamide, may be obtained from Eurobio, Les Ulis, France. Synthesis is as follows. Reflux (with protection from moisture and with stirring) 2.12 g 8-hydroxyjulolidine, 2.22 g diethyl 1,3-acetonedicarboxylate (oxo-3-glutaric acid ethyl ester, Fluka), 1.71 g anhydrous zinc chloride, and 6 mL EtOH for 24 h, cool, add to 200 mL water, extract with 200 mL ethyl acetate, extract with 100 mL ethyl acetate. Combine

the organic layers and wash them with water, dry over magnesium sulfate, evaporate to dryness, recrystallize from 5 parts ethyl acetate to give ethyl 2,3,6,7-tetrahydro-11-oxo-1H,5H,11H-[1]benzopyrano[6,7,8-ij]quinolizine-9-acetate. Heat 2 g of this compound with 42 mL 1.2% NaOH in water and 40 mL MeOH at 45° for 1 h, cool, wash with 50 mL chloroform, wash with 40 mL chloroform. Degas the aqueous phase and acidify it with 16 mL 3 M HCl, stir for 15 min, adjust pH to 6.5 with 13 mL 2.5 M NaOH, filter. Wash the precipitate with water and dry it to obtain 2,3,6,7-tetrahydro-11-oxo-1H,5H,11H-[1]benzopyrano[6,7,8-ij]quinolizine-9-acetic acid. React 30 g potassium carbonate with 30 g methyl 6-aminohexanoate hydrochloride (Fluka) for 30 h, filter. Stir 11.26 g of 2,3,6,7-tetrahydro-11-oxo-1H,5H,11H-[1]benzopyrano[6,7,8-ij]quinolizine-9-acetic acid, 10.62 g disuccinimidyl oxalate (dihydroxysuccinimide carbonate), 3.81 g anhydrous triethylamine, and 560 mL dry MeCN protected from moisture at room temperature for 1 h, stir at 35-40° for 1 h, add the methyl 6-aminohexanoate filtrate, stir for 8 h, add 20 g ethanolamine, stir for 30 min, filter, wash with water, remove the solvent, chromatograph on a silica gel column with dichloromethane, dichloromethane:THF 85:15, dichloromethane:THF 75:25, recrystallize from ethyl acetate to give methyl N-(2,3,6,7-tetrahydro-11-oxo-1H,5H,11H-[1]benzopyrano[6,7,8-ij]quinolizine-9-yl)acetyl-6-aminohexanoate (yield 36%). Heat 1.25 g of this compound with 42 mL 1.2% NaOH in water and 40 mL MeOH at 45° for 1 h, cool, wash with 50 mL chloroform, wash with 40 mL chloroform. Degas the aqueous phase and acidify it with 16 mL 3 M HCl, stir for 15 min, adjust pH to 6.5 with 13 mL 2.5 M NaOH, filter. Wash the precipitate with water and dry it to obtain N-(2,3,6,7-tetrahydro-11-oxo-1H,5H,11H-[1]benzopyrano[6,7,8-ij]quinolizine-9-yl)acetyl-6-aminohexanoic acid. React 750 mg of this acid with 912 mg triethylamine and 512 mg disuccinimidyl oxalate (dihydroxysuccinimide carbonate) in 27 mL MeCN for 6 h, filter, evaporate, chromatograph on a silica gel column with dichloromethane, dichloromethane:THF 85:15, and dichloromethane:THF 75:25 to obtain Luminarin 2 (yield 19%) (World Pat. 89 12,052; Chem. Abstr. 1990, 113, 23889n).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:5 mM ammonium acetate 26:74
Column temperature: 40 (chemiluminescence only)
Flow rate: 2 (F) or 1.5 (chemiluminescence)
Injection volume: 20
Detector: F ex 390 em 490, Chemiluminescence. 1.1 mM Bis(2,4,6-trichlorophenyl) oxalate in methyl acetate pumped at 0.3 mL/min and 400 mM hydrogen peroxide in THF pumped at 0.3 mL/min mixed in a 292 μL capillary tube and this mixture mixed with the column effluent. The resulting mixture flowed through a 60 μL PTFE capillary at 40° to a Kratos FS970 detector fitted with a 470 nm long-pass filter.

CHROMATOGRAM

Retention time: 15.4
Limit of detection: 100 fmole (F), 50 fmole (chemiluminescence)

KEY WORDS

derivatization; comparison with o-phthalaldehyde derivatization

REFERENCE

Tod,M.; Legendre,J.-Y.; Chalom,J.; Kouwatli,H.; Poulou,M.; Farinotti,R.; Mahuzier,G. Primary and secondary amine derivatization with luminarins 1 and 2: separation by liquid chromatography with peroxyoxalate chemiluminescence detection, *J.Chromatogr.*, **1992**, *594*, 386–391.

SAMPLE

Matrix: cell suspensions
Sample preparation: 500 μL Cell suspension in buffer A containing 0.1% bovine serum albumin + 1.5 mL ice cold buffer A, centrifuge at 4° at 250 g for 10 min, remove the supernatant, add 2 mL buffer A to the pellet, boil this mixture for 3 min, cool on ice, centrifuge at 4° at 500 g for 10 min, remove the supernatant. Dry a 50-200 μL aliquot of each supernatant in a vacuum centrifuge and reconstitute the residue with 100 μL buffer B, add 20 μL reagent, inject an aliquot. (Buffer A contained 150 mM NaCl, 4 mM KCl, 1 mM calcium chloride, 1.2 mM magnesium sulfate, 2.46 mM Na_2HPO_4, 0.615 mM KH_2PO_4, 5.6 mM glucose, and 10 mM HEPES, pH 7.4. Buffer B was THF:100 mM pH 9.5 sodium tetraborate buffer 30:70. The reagent was prepared by mixing equal volumes of 3.8 mM o-phthaldialdehyde in MeOH (freshly prepared) and 2.5 mL/L mercaptoethanol in MeOH.)

HPLC VARIABLES
Column: 100 × 3.2 3 μm Phase-II ODS (Bioanalytical Systems)
Mobile phase: MeCN:MeOH:buffer 14:16:70 containing 1 mM disodium EDTA (Buffer was 100 mM sodium phosphate buffer containing 0.4% triethylamine, pH 6.4.)
Flow rate: 0.6
Detector: E, Bioanalytical Systems Model LC4B, LC17A thin-layer electrochemical cell, glassy carbon working electrode +0.5 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 10
Limit of detection: <0.1 pmole

KEY WORDS
rat mast cells; derivatization

REFERENCE
Jensen,T.B.; Marley,P.D. Development of an assay for histamine using automated high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.B*, **1995**, *670*, 199–207.

SAMPLE
Matrix: cheese, tissue
Sample preparation: Add 1,7-diaminopentane as IS. Homogenize (Polytron) 10 g cheese with two 20 mL portions of 100 mM HCl. Homogenize (Polytron) 10 g tissue with three 15 mL portions of 5% trichloroacetic acid. Saturate the extracts with NaCl, adjust the pH to 11.5. Remove a 5 mL aliquot and add it to 5 mL butanol (cheese) or butanol:chloroform 50:50 (tissue) vortex for 5 min, repeat extraction twice more. Combine the organic extracts and remove a 1 mL aliquot, add 2 drops 1 M HCl, evaporate to dryness under reduced pressure, reconstitute the residue in 1 mL 100 mM HCl, add 0.5 μL saturated sodium bicarbonate, add 1 mL 5 mg/mL dansyl chloride, heat at 40° for 1 h, evaporate to dryness under reduced pressure, reconstitute with MeCN, inject an aliquot.

HPLC VARIABLES
Column: 150 × 1.6 3 μm Spherisorb 3S TG
Mobile phase: Gradient. MeCN:water 65:35 for 1 min, to 80:20 over 4 min, to 90:10 over 1 min.
Flow rate: 0.8
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 3.7
Internal standard: 1,7-diaminopentane (4.2)

OTHER SUBSTANCES
Extracted: cadaverine, 2-phenylethylamine, putrescine, spermidine, spermine, tryptamine, tyramine

KEY WORDS
derivatization; fish; salmon; tuna; salami

REFERENCE
Moret,S.; Conte,L.S. High-performance liquid chromatographic evaluation of biogenic amines in foods. An analysis of different methods of sample preparation in relation to food characteristics, *J.Chromatogr.A*, **1996**, *729*, 363–369.

SAMPLE
Matrix: fish, food, wine
Sample preparation: Filter (0.45 μm) wine, fruit juice or vegetable juice, dilute 1:5 or 1:20. 50 μL Diluted wine, diluted juice, fish extract, or cheese extract (neutralize strongly acidic samples if necessary) + 200 μL 200 mM boric acid adjusted to pH 8.5 with 30% KOH + 200 μL 3 mM 9-fluorenylmethyl chloroformate in acetone, mix for 3 min at room temperature, add 50 μL reagent, mix for 3 min. Remove an 80 μL aliquot and add it to 320 μL initial mobile phase,

inject a 20 μL aliquot. (Reagent was 3 mL heptylamine in 15 mL MeCN, adjusted to pH 7-8 with 175 mL 100 mM HCl. Extract fish as follows. Homogenize 20 g fish and 10 mL 100 mM HCl, add 40 mL 100 mM HCl, centrifuge, decant, extract residue with two 40 mL and one 20 mL portions of 100 mM HCl, filter, make up the extracts to 100 (?) mL with 100 mM HCl. Extract cheese as follows. Homogenize 25 g cheese and 18.75 mL 100 mM HCl, suspend with 40 mL 100 mM HCl, centrifuge, extract the residue twice with 20 mL portions of 100 mM HCl. Combine and filter the extracts, make up to 100 mL with 100 mM HCl.)

HPLC VARIABLES

Column: 250-4 Supersphere 60 RP-8 (Merck)
Mobile phase: Gradient. A was MeCN:100 mM pH 4.4 sodium acetate buffer 50:50. B was MeCN. A:B 100:0 for 7 min, to 90:10 over 5 min, to 70:30 over 15 min, maintain at 70:30 for 6 min, to 10:90 over 7 min, to 0:100 over 3 min, maintain at 0:100 for 9 min, return to 100:0 over 1 min, re-equilibrate for 7 min.
Column temperature: 40
Flow rate: 1.2 (0.05 when not in use)
Injection volume: 20
Detector: F ex 265 em 315

CHROMATOGRAM

Retention time: 27.8
Limit of detection: <20 ng/mL

OTHER SUBSTANCES

Extracted: cadaverine, phenylethylamine, putrescine, spermidine, spermine, tyramine

KEY WORDS

wine; fruit juice; vegetable juice; fish; cheese; derivatization

REFERENCE

Kirschbaum,J.; Luckas,B.; Beinert,W.-D. Pre-column derivatization of biogenic amines and amino acids with 9-fluorenylmethyl chloroformate and heptylamine, *J.Chromatogr.A*, **1994**, *661*, 193–199.

SAMPLE

Matrix: food
Sample preparation: Add 10 mL light petroleum to 5 g natural canned tuna or tuna canned in oil, extract, centrifuge at about 13 g for 15 min, discard the organic layer, extract the remaining solid with two or more 10 mL portions of light petroleum. Homogenize the defatted sample with 10 mL 600 mM perchloric acid, filter, add 10 mL 100 mM NaOH, extract with five 25 mL portions of butanol. Combine the organic layers and extract with 20 mL light petroleum and five 10 mL portions of 100 mM HCl, dilute sample to 50 mL with water. Add 1 mL 1 M NaOH to 2 mL aliquot of the diluted sample, let stand for 5 min, add 1 mL derivatizing solution, let stand 5-10 min until the solution reaches a brown color, add 500 mg NaCl, extract with two 3 mL portions of ethyl acetate, centrifuge, evaporate the organic layer under nitrogen to 100 μL, make up to 2 mL with mobile phase, inject a 20 μL aliquot within 30-40 min. (Prepare the derivatizing solution as follows: Mix 1 g sodium tetraborate heptahydrate, 50 mg o-phthalaldehyde, 50 μL β-mercaptoethanol, and 1 mL MeOH, dilute to 50 mL with 1 M NaOH (pH 10).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb ODS2 RP-18
Mobile phase: MeCN:buffer 30:70 (Buffer was 20 mM KH_2PO_4 adjusted to pH 4.0 with 85% H_3PO_4.)
Column temperature: 34-35
Flow rate: 0.8
Injection volume: 20
Detector: F ex 315 em 415

CHROMATOGRAM

Retention time: 7.5
Limit of detection: 5 μg/g

OTHER SUBSTANCES

Extracted: histidine

KEY WORDS
derivatization; tuna; fish

REFERENCE
Frattini,V.; Lionetti,C. Histamine and histidine determination in tuna fish samples using high-performance liquid chromatography. Derivatization with o-phthalaldehyde and fluorescence detection or UV detection of "free" species, *J.Chromatogr.A*, **1998**, *809*, 241–4]245.

SAMPLE
Matrix: food
Sample preparation: Cheese, chocolate. Homogenize (stomacher) 5 g ground cheese or chocolate with 45 mL 70 mM trisodium citrate at 45° for 5 min. Remove a 3 mL aliquot and add it to 3 mL 600 mM trichloroacetic acid, mix, centrifuge at 4° at 10000 g for 10 min, suspend the pellet in 3 mL 300 mM trichloroacetic acid, centrifuge. Combine the supernatants, filter (0.45 μm), make up the filtrate to 10 mL with water, inject an aliquot. Wine. 3 mL Wine + 3 mL 600 mM trichloroacetic acid, centrifuge, filter the supernatant, inject an aliquot of the filtrate. Fish, sauerkraut. Blend 200 g fish or sauerkraut with 200 mL water for 3 min. Remove a 3 mL aliquot and add it to 3 mL 600 mM trichloroacetic acid, centrifuge, filter the supernatant, inject an aliquot of the filtrate.

HPLC VARIABLES
Guard column: 30 × 3 Corasil C18
Column: 100 × 8 10 μm Nucleosil C18 radial-compression
Mobile phase: DMSO:buffer 47:53 (Prepare mobile phase by dissolving 16 g ninhydrin and 1.2 g hydrindantin in 322 mL DMSO with sonication for 10 min, add 350 mL 1.8 M pH 5.00 sodium acetate buffer, add 2 g sodium dodecyl sulfate dissolved in a mixture of 618 mL DMSO and 710 mL water, flush constantly with nitrogen. Flush column with DMSO:water 50:50 at the end of each day.)
Column temperature: 29
Flow rate: 1
Detector: UV 546 following post-column reaction. The column effluent flowed through a 10 m × 0.25 mm PTFE tube coiled in a figure 8 at 145° to the detector.

CHROMATOGRAM
Retention time: 16
Limit of detection: 800 ng/g (sauerkraut), 300 ng/g (wine)

OTHER SUBSTANCES
Extracted: cadaverine, phenylethylamine, putrescine, tryptamine, tyramine

KEY WORDS
post-column reaction; cheese; chocolate; wine; fish; sauerkraut; tuna

REFERENCE
Joosten,H.M.L.J.; Olieman,C. Determination of biogenic amines in cheese and some other food products by high-performance liquid chromatography in combination with thermo-sensitized reaction detection, *J.Chromatogr.*, **1986**, *356*, 311–319.

SAMPLE
Matrix: food
Sample preparation: Dilute vinegar, wine, or juice 10-fold with water, centrifuge at 2000 g for 15 min. Remove a 20 μL aliquot and mix it with 150 μL buffer and 400 μL 5 mM 2-naphthoxycarbonyl chloride in MeCN, let stand for 3 min, add 50 μL 20 mM glycine in water, let stand for 3 min, add 500 μL MeCN:500 mM pH 4.4 sodium acetate buffer 75:25, mix, inject a 20 μL aliquot. (Prepare buffer by adjusting the pH of 33.43 g boric acid in 950 mL water to 9.0 with 20% KOH, make up to 1 L with water. Synthesis of 2-naphthoxycarbonyl chloride is as follows. Dissolve 30 mmoles 2-naphthol and 30 moles quinoline in 18 g toluene and 5 g dichloromethane, cool to 0°, add 47 mL 1.93 M phosgene in toluene, warm on a steam bath for 10 min, filter, evaporate to remove the solvent, distil the residue (bp 150-152°/9 mm Hg, take up the distillate in ether, crystallize by adding ligroin to obtain 2-naphthoxycarbonyl chloride (mp 66°) (J. Am. Chem. Soc.1951, 73, 2080).)

HPLC VARIABLES
Guard column: present but not specified
Column: 250 × 4.6 4 μm Superspher 60 RP-18e
Mobile phase: Gradient. A was MeCN:100 mM pH 4.4 sodium acetate 40:60. B was MeCN. A:B 77:23 for 7 min, to 65:35 over 31 min, to 30:70 over 3 min, maintain at 30:70 for 5 min, to 0:100 over 1 min, maintain at 0:100 for 6 min, return to initial conditions over 1 min, re-equilibrate for 6 min.
Column temperature: 45
Flow rate: 1.1 for 46 min, to 1.5 over 1 min, maintain at 1.5 for 13 min
Injection volume: 20
Detector: F ex 274 em 335

CHROMATOGRAM
Retention time: 17.15
Limit of detection: 747 ng/g

OTHER SUBSTANCES
Simultaneous: cadaverine, histamine, 2-phenylethylamine, putrescine, spermidine, spermine, tyramine

KEY WORDS
derivatization; vinegar; wine; juice

REFERENCE
Kirschbaum,J.; Busch,I.; Brückner,H. Determination of biogenic amines in food by automated pre-column derivatization with 2-naphthyloxycarbonyl chloride (NOC-Cl), *Chromatographia*, **1997**, *45*, 263–268.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in 10 mM HCl, inject a 1-2 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 7 μm 300 Å Nucleosil C8
Mobile phase: MeOH:buffer 15:85 (Buffer was 50 mM pH 3.1 NaH_2PO_4 containing 0.5 mM disodium EDTA and 5 mM sodium 1-pentanesulfonate or sodium 1-octanesulfonate.)
Flow rate: 1
Injection volume: 1-2
Detector: UV 210

CHROMATOGRAM
Retention time: 13

OTHER SUBSTANCES
Simultaneous: metabolites, methylhistamine, histidine, methylimidazole acetic acid

REFERENCE
Hermann,K.; Frank,G.; Ring,J. High-performance liquid chromatography for the separation of histamine, its precursor, and metabolites: Application to biological samples, *J.Liq.Chromatogr.*, **1995**, *18*, 189–204.

SAMPLE
Matrix: tears
Sample preparation: 10 μL Tears + 40 μL 200 mM pH 9.1 sodium borate buffer + 50 μL 200 μg/mL fluorescamine in MeCN, mix vigorously, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20-40 μm LiChroprep RP-8
Column: 10 μm Nucleosil C8 or 10 μm RP-8 (Merck)
Mobile phase: MeCN:4 mM pH 3.5 KH_2PO_4 65:35
Flow rate: 0.5
Injection volume: 20
Detector: F ex 390 em 480

CHROMATOGRAM
Retention time: 4
Limit of quantitation: 1 ng/mL

KEY WORDS
derivatization

REFERENCE
Bettero,A.; Angi,M.R.; Moro,F.; Benassi,C.A. Histamine assay in tears by fluorescamine derivatization and high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *310*, 390–395.

SAMPLE
Matrix: tears
Sample preparation: 10 μL Tears + 40 μL 100 mM pH 9.1 borate buffer, mix, add 50 μL 200 μg/mL fluorescamine in MeCN with vigorous stirring, inject a 20 μL aliquot on to column A, divert the fraction containing the derivatized histamine on to column B then remove column A from the circuit (details are sketchy).

HPLC VARIABLES
Column: A 20-40 μm RP-8 (Merck); B 10 μm RP-8 (Merck)
Mobile phase: MeCN:4 mM pH 3.5 phosphate buffer 65:35
Flow rate: 0.5
Injection volume: 20
Detector: F ex 390 em 480

CHROMATOGRAM
Retention time: 4
Limit of detection: 0.1 ng/mL

KEY WORDS
derivatization; column-switching; heart cut

REFERENCE
Bettero,A.; Galiano,F.; Benassi,C.A.; Angi,M.R. A rapid HPLC technique for determining levels of histamine in tears from normal and inflamed human eyes, *Food Chem.Toxicol.*, **1985**, *23*, 303–304.

SAMPLE
Matrix: tissue
Sample preparation: Prepare SPE (A) column by washing 100-200 mesh Dowex 50 W with excess 2 M HCl, with water, with 2 M NaOH, and with water. Equilibrate the resin with 200 mM pH 6.5 sodium phosphate buffer and pack in a 16 × 5 glass column. Prepare SPE (B) column by washing cellulose-phosphate fibrous cation-exchanger (Sigma) in a 13 × 17 glass column with excess 100 mM NaOH, with water, with 100 mM HCl, and with water until the pH reaches 5-6. Homogenize (glass homogenizer) rat brain hypothalamus with 500 μL ice-cold 3% perchloric acid, rinse homogenizer 3 times with 500 μL portions of 3% perchloric acid. Combine the homogenate and rinses, add IS, centrifuge at 4° at 10000 g for 30 min, add the supernatant to SPE column (A), wash with 5 mL water, wash with 4 mL 2 M HCl, elute with 2.5 mL 3.5 M HCl. Evaporate the eluate to dryness, reconstitute the residue in 800 μL water, add 150 μL buffer, add 100 μL 20 mM sulfosuccinimidyl-3-(4-hydroxyphenyl)propionate (sulfo B-H, Pierce) in water, vortex for 30 s, adjust pH to 5.5-6.0 with 100 mM HCl, add to SPE column (B), wash with 5 mL water, wash with 6 mL 1 mM HCl, elute with 2.5 mL 100 mM HCl. Discard the first 500 μL eluate, evaporate the next 200 μL to dryness, reconstitute with 1 volume water, add 9 volumes water to a total volume of 0.11-1 mL, inject a 100 μL aliquot. (Prepare buffer by mixing 100 mM sodium carbonate and 100 mM sodium bicarbonate in a 10:1 ratio.)

HPLC VARIABLES
Guard column: μBondapak C18/Corasil
Column: 250 × 4.6 Ultrasphere ODS
Mobile phase: MeCN:140 mM sodium acetate 73:17 containing 3.89 M (sic) 1-octanesulfonic acid and 56 mg/L (?) EDTA, adjusted to pH 3.48 with glacial acetic acid

Flow rate: 1
Injection volume: 100
Detector: E, ESA Coulochem 5100A, Model 5011 analytical cell, cell 1 0.47 V, cell 2 (monitored) 0.56 V, oxidative screen mode

CHROMATOGRAM
Retention time: 8.4
Internal standard: N^{α}-methylhistamine (11.6)
Limit of detection: 0.1 pmole

OTHER SUBSTANCES
Extracted: N^{tau}-methylhistamine
Simultaneous: histidine, spermidine

KEY WORDS
derivatization; rat; brain; SPE

REFERENCE
Mine,K.; Jacobson,K.A.; Kirk,K.L.; Kitajima,Y.; Linnoila,M. Simultaneous determination of histamine and N tau-methylhistamine with high-performance liquid chromatography using electrochemical detection, *Anal.Biochem.*, **1986**, *152*, 127–135.

SAMPLE
Matrix: tissue
Sample preparation: Sonicate 10 g tuna with 50 mL 1200 ppm triethylamine in MeOH for 10 min, filter, filter (0.45 μm) again, add 25 μL filtrate to 10 mg 3,5-dinitrobenzoyl derivatized silica, heat at 60° for 10 min, elute with 1 mL MeCN, inject a 20 μL aliquot of the eluate. (Prepare 3,5-dinitrobenzoyl derivatized silica as follows. Heat 4.7 g 4-hydroxy-3-nitrobenzoic acid, 7.5 mL thionyl chloride, 750 μL pyridine, and 60 mL benzene (Caution! Benzene is a carcinogen!) at 55° for 4 h, cool to room temperature, filter, evaporate under reduced pressure to obtain 4-hydroxy-3-nitrobenzoyl chloride. Store 10 μm LiChrosorb Si-100 silica in a desiccator over saturated aqueous LiCl solution for 2 weeks. 5 g Silica + 8.4 g 62% bis(2-hydroxyethyl)-3-aminopropyltriethoxysilane (Petrarch Systems, Bristol PA) in EtOH, add 40 mL EtOH:pyridine 99.5:0.5 (?), reflux with stirring under nitrogen for 6 h, cool, filter, wash silica with three 25 mL portions of MeOH and four 25 mL portions of dichloromethane, dry under nitrogen at 40° overnight. Heat 5 g 4-hydroxy-3-nitrobenzoyl chloride, 300 μL pyridine, 5 g silica, and 60 mL benzene (previously dried over anhydrous sodium sulfate) at 70-75° for 2 h, filter, wash silica with three 75 mL portions of DMF and three 75 mL portions of dichloromethane, dry under vacuum at 40° for 12 h. Stir 400 mg silica, 600 mg 3,5-dinitrobenzoyl chloride, 25 mL benzene, and 300 μL pyridine at room temperature for 24 h, filter, wash with three 25 mL portions of dichloromethane to give 3,5-dinitrobenzoyl derivatized silica.)

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrosphere C18
Mobile phase: MeCN:water 50:50 containing 0.05% ammonium hydroxide
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 2
Limit of detection: 152 ppb

KEY WORDS
derivatization; tuna; fish

REFERENCE
Zhou.F.-X.,; Wahlberg,J.; Krull,I.S. Silica based 3,5-dinitrobenzoyl (DNB) reagent for off-line derivatization of amine nucleophiles in HPLC, *J.Liq.Chromatogr.*, **1991**, *14*, 1325–1350.

SAMPLE
Matrix: tissue

Sample preparation: Incubate chick embryo retinas in 4 mL medium at 37° for 4 h, centrifuge at 500 g for 1 min. For each 20 mg solid add 1 mL 20 μM 1,7-diaminoheptane in 200 mM perchloric acid, suspend, sonicate (Soniprep 150) with 10 μm amplitude in 10 s bursts, centrifuge at 20000 g for 15 min, neutralize with KOH, centrifuge. Remove a 1 mL aliquot and add it to 1 mL 2 M NaOH, add 5 μL benzoyl chloride, vortex briefly, let stand for 20 min, add 2 mL saturated NaCl, extract with 2 mL diethyl ether, centrifuge. Remove the upper organic phase and wash it with 2 mL 100 μM NaOH, dry the organic layer over a few mg anhydrous sodium sulfate, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 2 mL mobile phase, inject a 20 μL aliquot. (Medium was pH 7.4 serum- and glutamine-free Eagle's minimum essential medium containing 25 mM 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid (HEPES), 100 U/mL penicillin, and 100 μg/mL streptomycin.)

HPLC VARIABLES

Column: 150 × 4.6 3 μm Spherisorb ODS2
Mobile phase: MeOH:water 62:38
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 10.35
Internal standard: 1,7-diaminoheptane (5.4)
Limit of quantitation: 1.25 μM

OTHER SUBSTANCES

Extracted: N-acetylcadaverine, N-acetylputrescine, N^1-acetylspermidine, N^1-acetylspermine, cadaverine, putrescine, spermidine, spermine

KEY WORDS

derivatization; retina; chicken

REFERENCE

Taibi,G.; Schiavo,M.R. Simple high-performance liquid chromatographic assay for polyamines and their monoacetyl derivatives, *J.Chromatogr.*, **1993**, *614*, 153–158.

SAMPLE

Matrix: tissue
Sample preparation: Make a slurry of 40 μm Bakerbond carboxylic acid material in MeOH and prepare SPE columns by adding an aliquot containing 150 mg material to a Pasteur pipette plugged with glass fiber prefilter material (Sartorius). Wash column with 2 mL water, 2 mL 1 M HCl, 2 mL water, and 4 mL 200 mM pH 6.4 sodium phosphate buffer. Homogenize (Ultra-Turrax) rat heart and 500 ng 1-methylhistamine in ice-cold 50 mM pH 8.5 Tris-HCl buffer, sonicate (MSE sonifier, 12 W, 12 μm peak-peak) for 2 min with repeated intervals of 5 s, add perchloric acid to a final concentration of 300 mM, heat at 100° for 5 min, neutralize with KOH, cool to 0°, centrifuge at 4° at 2000 g for 5 min, adjust pH of supernatant to 6.4 with phosphate buffer, add 1-methylhistamine, add to the SPE column, wash with 4 mL 50 mM pH 6.4 disodium EDTA, wash with 4 mL water, elute with 1 mL 1 M HCl. Evaporate the eluate to dryness under a stream of nitrogen at 40° over about 15 min, reconstitute with 100 μL water, add 400 μL 50 mM pH 9.1 sodium borate, with continuous vigorous stirring add 500 μL 200 μg/mL fluorescamine in MeCN (freshly prepared), stir for 1 min, evaporate to dryness under a stream of nitrogen at 40°, reconstitute with 1 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 200 × 3 5 μm Inertsil ODS-2 (see J. Chromatogr. B 1994, 657, 261)
Mobile phase: MeCN:MeOH:water:phosphoric acid 15:10:75:0.2 adjusted to pH 6.87 with ammonium hydroxide (see J. Chromatogr. B 1994, 657, 261)
Column temperature: 20
Flow rate: 0.4
Injection volume: 20
Detector: F ex 360 em 440

CHROMATOGRAM
Retention time: 15
Internal standard: 1-methylhistamine (25)
Limit of detection: 20 pg

OTHER SUBSTANCES
Extracted: 3-methylhistamine

KEY WORDS
SPE; derivatization; rat; heart

REFERENCE
van Haaster,C.M.C.J.; Engels,W.; Lemmens,P.J.M.R.; Hornstra,G.; van der Vusse,G.J. Rapid and highly sensitive high-performance liquid chromatographic method for the determination of histamine and 3-methylhistamine in biological samples using fluorescamine as the derivatizing agent, *J.Chromatogr.*, **1993**, *617*, 233–240.

SAMPLE
Matrix: tissue
Sample preparation: Blend 50 g tissue and 75 mL 5% trichloroacetic acid in a Waring Blendor at high speed for 2 min, centrifuge for 2 min, extract the solid twice more with 75 mL portions of 5% trichloroacetic acid, filter the supernatants through a glass wool plug, wash the funnel with 5% trichloroacetic acid, make up the filtrate to 250 mL with 5% trichloroacetic acid. Remove a 10 mL aliquot and add it to 4 g NaCl, 1 mL 50% NaOH, and 5 mL chloroform:n-butanol 50:50, shake vigorously for 2 min, centrifuge for 5 min, remove the upper organic layer, repeat the extraction twice more with 5 mL portions of chloroform:n-butanol 50:50. Combine the organic layers and add them to 15 mL n-heptane, extract three times with 1 mL portions of 200 mM HCl, inject a 20 μL aliquot (J.Assoc.Off.Anal.Chem. 1978, 61, 139).

HPLC VARIABLES
Column: PRP-X200 Cationic (Hamilton)
Mobile phase: 400 mM pH 4.5 KH_2PO_4
Flow rate: 0.5
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 2
Limit of detection: 10 ng

KEY WORDS
fish

REFERENCE
Kalligas,G.; Kaniou,I.; Zachariadis,G.; Tsoukali,H.; Epivatianos,P. Thin layer and high pressure liquid chromatographic determination of histamine in fish tissues, *J.Liq.Chromatogr.*, **1994**, *17*, 2457–2468.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Polytron) 200 mg tissue with 5 volumes of 1 M perchloric acid, centrifuge at 10000 g for 20 min. Neutralize the supernatant with 10 M KOH, centrifuge at 10000 g for 20 min, remove a 20 μL aliquot of the supernatant and add it to 10 μL reagent, mix well, add 30 μL 10% sodium carbonate in EtOH:water 5:95, mix well, inject a 10 μL aliquot. (Prepare reagent prior to use by mixing equal volumes of 20 mM sulfanilic acid in 1 M HCl and 200 mM sodium nitrite solution.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm TSK-ODS (Toso)
Mobile phase: Gradient. A was EtOH:150 mM pH 6.0 sodium acetate buffer 5:95. B was MeCN:water 60:40. A:B from 100:0 to 55:45 over 30 min.
Column temperature: 40

Flow rate: 1
Injection volume: 10
Detector: UV 420

CHROMATOGRAM
Retention time: 14.58
Limit of detection: <7 nmole/g

OTHER SUBSTANCES
Extracted: carnosine, histidine, tyrosine
Noninterfering: anserine, 1-methylhistidine

KEY WORDS
derivatization; fish; muscle; mackerel

REFERENCE
Sato,M.; Nakano,T.; Takeuchi,M.; Kumagai,T.; Kanno,N.; Nagahisa,E.; Sato,Y. Specific determination of histamine in fish by high-performance liquid chromatography after diazo coupling, *Biosci.Biotechnol.Biochem.*, **1995**, *59*, 1208–1210.

SAMPLE
Matrix: tissue
Sample preparation: 10 g Homogenized fish + 15 mL 600 mM perchloric acid, stir magnetically for 10 min, centrifuge at 3000 rpm for 10 min, remove the supernatant, add 10 mL 600 mM perchloric acid to the residue, stir magnetically for 10 min, centrifuge at 3000 rpm for 10 min. Combine the supernatants, make up to 25 mL with 600 mM perchloric acid, filter (0.45 μm), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova Pak C18
Mobile phase: Gradient. A was 100 mM sodium acetate containing 10 mM sodium octanesulfonate, adjusted to pH 5.20 with acetic acid. B was MeCN:buffer 66:34 (Buffer was 200 mM sodium acetate containing 10 mM sodium octanesulfonate, adjusted to pH 4.50 with acetic acid.) A:B from 80:20 to 20:80 over 50 min, maintain at 20:80 for 2 min, return to initial conditions over 2 min, re-equilibrate for 10 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 340 em 445 following post-column reaction. The column effluent mixed with the reagent pumped at 0.5 mL/min, the mixture flowed through a 200 cm × 0.25 mm i.d. coil of stainless steel tubing to the detector. (Prepare reagent by dissolving 15.5 g boric acid and 13.1 g KOH in 500 mL water, adjust pH to 10.5-11 with 30% KOH (if necessary), add 1.5 mL 30% Brij-35, add 1.5 mL mercaptoethanol, add 2.5 mL 40 μg/mL o-phthalaldehyde in MeOH, mix. Protect from light, prepare fresh daily.)

CHROMATOGRAM
Retention time: 35
Limit of detection: 250 ng/g

OTHER SUBSTANCES
Extracted: agmatine, cadaverine, creatinine, β-phenylethylamine, putrescine, serotonin, spermidine, spermine, tryptamine, tyramine

KEY WORDS
post-column reaction; fish

REFERENCE
Veciana-Nogues,M.T.; Hernandez-Jover,T.; Marine-Font,A.; Vidal-Carou,M.D.C. Liquid chromatographic method for determination of biogenic amines in fish and fish products, *J.AOAC Int.*, **1995**, *78*, 1045–1050.

SAMPLE
Matrix: tissue

Sample preparation: Homogenize (Ultraturrax) 5 g fish, 10 mL 200 mM perchloric acid, and 100 μL 800 μg/mL 1,3-diaminopropane dihydrochloride in water at -20° and 20000 rpm and centrifuge at 2° at 2500 g for 20 min. Remove a 100 μL aliquot of the supernatant and add it to 200 μL saturated sodium bicarbonate solution, add 400 μL 7.5 mg/mL dansyl chloride in acetone, agitate, heat at 60° in the dark for ?, add 100 μL 100 mg/mL L-proline in water, agitate, let stand in the dark at room temperature for 30 min, add 500 μL toluene, agitate. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 300 μL MeCN, filter, inject an aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 5 μm Brownlee C18
Column: 250 × 4.6 5 μm Kromasil C18
Mobile phase: Gradient. MeCN:water from 60:40 to 75:25 over 6 min, maintain at 75:25 for 2 min, to 95:5 over 5 min, maintain at 95:5 for 7 min, re-equilibrate at initial conditions for 10 min.
Column temperature: 25
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: 14.7
Internal standard: 1,3-diaminopropane (12.5)

OTHER SUBSTANCES

Extracted: cadaverine, putrescine, spermidine, spermine

KEY WORDS

derivatization; fish

REFERENCE

Malle,P.; Vallé,M.; Bouquelet,S. Assay of biogenic amines involved in fish decomposition, *J.AOAC Int.*, **1996**, *79*, 43–49.

SAMPLE

Matrix: urine, blood
Sample preparation: Prepare a 500 μL SPE cartridge of Amberlite CG-50 resin and wash it with 2 mL water. Add urine or blood to the SPE cartridge, wash with 2 mL water, wash with two 2 mL portions of 500 mM pH 6.5 sodium acetate, wash with two 2 mL portions of water, elute with 400 μL 2 M perchloric acid, elute with two 1 (urine) or 0.2 (blood) mL portions of mobile phase, inject an aliquot of the eluate.

HPLC VARIABLES

Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:100 mM KH_2PO_4 25:75 containing 56 μg/mL sodium dodecyl sulfate and 3.2 mL/L 10 M NaOH
Flow rate: 1.2
Detector: E, ESA model 5100A, detector 1 +0.85 V, detector 2 +1.12 V, guard cell +1.15 V

CHROMATOGRAM

Retention time: 11
Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: N-methylhistamine

KEY WORDS

guinea pig; whole blood; SPE

REFERENCE

Houdi,A.A.; Crooks,P.A.; Van Loon,G.R.; Schubert,C.A. A simple and sensitive determination of histamine and N^{tau}-methylhistamine in biological fluids by high-performance liquid chromatography with electrochemical detection, *J.Pharm.Sci.*, **1987**, *76*, 398–401.

SAMPLE
Matrix: wine
Sample preparation: 20 μL Wine + 50 μL buffer, mix, add 100 μL 8 mg/mL 9-fluorenylmethyl chloroformate in MeCN, mix, let stand for 3 min, add 50 μL 500 mM ammonia, mix, let stand for 3 min, add 300 μL MeCN:water:acetic acid 80:12:8, inject a 20 μL aliquot. (Prepare buffer by adjusting the pH of 200 mM boric acid to 8.5 with 5 M NaOH.)

HPLC VARIABLES
Column: 200 × 2.1 5 μm ODS Hypersil
Mobile phase: Gradient. A was MeCN:2-octanol 99:1. B was MeCN:water:phosphoric acid:dimethylcyclohexylamine 15:83.12:0.88:1, pH 2.7. A:B 15:85 for 18 min, 38:62 over 0.1 min, to 40:60 over 6.9 min, maintain at 40:60 for 5 min, to 42:58 over 37 min, to 85:15 over 36 min, maintain at 85:15 for 7 min.
Flow rate: 0.3
Injection volume: 2
Detector: F ex 263 em 313

CHROMATOGRAM
Retention time: 100

OTHER SUBSTANCES
Extracted: agmatine, arginine, cadaverine, histidine, ornithine, phenylalanine, phenylethylamine, putrescine, spermidine, spermine, tyramine, tyrosine

KEY WORDS
derivatization

REFERENCE
Bauza,T.; Blaise,A.; Daumas,F.; Cabanis,J.C. Determination of biogenic amines and their precursor amino acids in wines of the Vallée du Rhône by high-performance liquid chromatography with precolumn derivatization and fluorimetric detection, *J.Chromatogr.A*, **1995**, *707*, 373–379.

SAMPLE
Matrix: wine
Sample preparation: Wash 5 g Amberlite CG-50 with water, add 5 mL 10 M NaOH, let stand for 30 min, rinse with water 3 times, add 25 mL 5 M HCl to a pH of 2, wash, mix with 5 mL 10 M NaOH, wash with 1 volume pH 7 buffer. Filter (0.45 μm) wine, add 10 mL to the resin in a 40 × 10 column, wash with water, elute with 10 mL 1 M HCl. Evaporate the eluate to 1 mL under reduced pressure, add heptylamine, derivatize with reagent, inject an aliquot. (Reagent was 45 mg phthalaldehyde in 1 mL MeOH, add 200 μL 2-mercaptoethanol, make up to 10 mL with buffer, prepare daily. Buffer was 3.81 g sodium tetraborate in 100 mL water, adjust to pH 10.5 with 10 M NaOH.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: Gradient. A was THF:water 1:99 containing 0.03% triethanolamine. B was MeOH. A:B from 40:60 to 20:80 over 25 min, re-equilibrate for 3 min. (Every 10 analyses flush with MeCN:80.8 mM acetic acid 30:70 for 1 h.)
Column temperature: 60
Flow rate: 1
Detector: F ex 330 em 445

CHROMATOGRAM
Retention time: 4.5
Internal standard: heptylamine (21.5)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: biogenic amines

KEY WORDS
derivatization; SPE

REFERENCE
Busto,O.; Mestres,M.; Guasch,J.; Borrull,F. Determination of biogenic amines in wine after clean-up by solid-phase extraction, *Chromatographia*, **1995**, *40*, 404–410.

SAMPLE
Matrix: wine
Sample preparation: Mix 10 µL wine with 6 µL pH 8.8 borate buffer (Waters), add 0.5 µL 10 mM 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate in MeCN (Waters), mix, let stand for 5 min, inject a 10 µL aliquot.

HPLC VARIABLES
Guard column: Spherisorb ODS-2
Column: 250 × 4.6 5 µm Spherisorb ODS-2
Mobile phase: Gradient. A was THF:50 mM sodium acetate. B was MeOH. A:B 75:25 for 5 min, to 20:80 over 20 min, to 0:100 (step gradient), maintain at 0:100 for 3 min, return to initial conditions, re-equilibrate for 2 min.
Column temperature: 65
Flow rate: 1
Injection volume: 10
Detector: F ex 250 em 395

CHROMATOGRAM
Retention time: 9
Internal standard: heptylamine
Limit of detection: 100-500 ng/mL

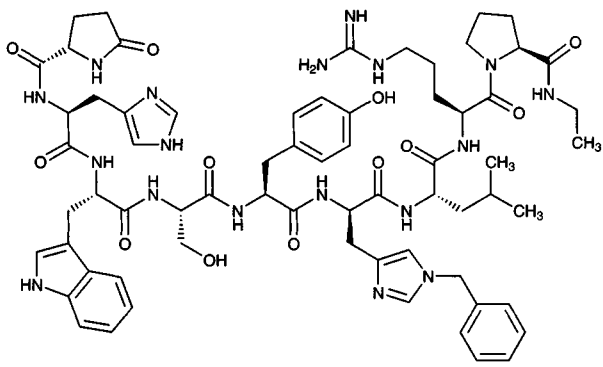
OTHER SUBSTANCES
Extracted: ammonia, amylamine, butylamine, cadaverine, ethanolamine, ethylamine, hexylamine, isopropylamine, methylamine, 3-methylbutylamine, phenethylamine, propylamine, putrescine, pyrrolidine, tyramine

KEY WORDS
derivatization

REFERENCE
Busto,O.; Guasch,J.; Borrull,F. Determination of biogenic amines in wine after precolumn derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, *J.Chromatogr.A*, **1996**, *737*, 205–213.

Histrelin

Molecular formula: $C_{66}H_{86}N_{18}O_{12}$
Molecular weight: 1323.52
CAS Registry No.: 76712-82-8
Merck Index: 4760



SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 µm SP-150 C8 (DuPont)

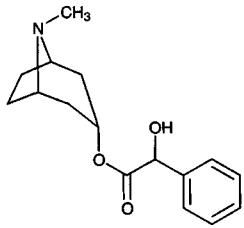
Mobile phase: Gradient. A was 654 mL triethylamine, 444 mL 85% phosphoric acid, and 29.1 g sodium butanesulfonate in 8 L water, pH 2.4. B was MeCN containing enough ethyl acetate to make the absorbance at 210 nm the same as that of mobile phase A. A:B from 85:15 to 78:22 over 25 min (Perkin-Elmer concave curve 4), maintain at 78:22 for 10 min
Flow rate: 1.6
Injection volume: 50
Detector: UV 210, UV 280

CHROMATOGRAM
Retention time: 26

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Oyler,A.R.; Naldi,R.E.; Lloyd,J.R.; Graden,D.A.; Shaw,C.J.; Cotter,M.L. Characterization of the solution degradation products of histrelin, a gonadotropin releasing hormone (LH/RH) agonist, *J.Pharm.Sci.*, **1991**, *80*, 271–275.

Homatropine



Molecular formula: $C_{16}H_{21}NO_3$
Molecular weight: 275.35
CAS Registry No.: 87-00-3, 51-56-9 (HBr)
Merck Index: 4766

SAMPLE
Matrix: plants
Sample preparation: Dissolve alkaloids in 1 mL MeOH, inject aliquot.

HPLC VARIABLES
Column: 150 × 4.1 5 μm Hamilton PRP-1
Mobile phase: MeCN:100 mM pH 10.4 ammonium acetate
Flow rate: 1
Injection volume: 20
Detector: MS thermospray, VG Trio-2, ion source 150°, vaporizer tip 170°, repeller electrode 150 V, m/z 276

CHROMATOGRAM
Internal standard: homatropine
Limit of detection: 2.5 ng/mL

OTHER SUBSTANCES
Simultaneous: hyoscyamine, scopolamine

KEY WORDS
total run time 6 min; homatropine is IS

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
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: 6

OTHER SUBSTANCES
Simultaneous: scopolamine, methscopolamine, tropic acid, atropine methyl, atropine

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 or water to 0.1%.

HPLC VARIABLES
Column: two 250 mm β-cyclodextrin bonded phase columns in series (Advanced Separation Technologies)
Mobile phase: MeOH:1% pH 4.1 aqueous triethylammonium acetate 4:96
Flow rate: 0.5
Injection volume: 1
Detector: UV

CHROMATOGRAM
Retention time: k' 1.98 (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: 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' 0.84 (of first (+) enantiomer)

KEY WORDS
chiral; α 3.13

REFERENCE
Okamoto,Y.; Aburatani,R.; Hatano,K.; Hatada,K. Optical resolution of racemic drugs by chiral HPLC on cellulose and amylose tris(phenylcarbamate) derivatives, *J.Liq.Chromatogr.*, **1988**, *11*, 2147–2163.

SAMPLE
Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 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: 6.77 (A), 3.63 (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, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

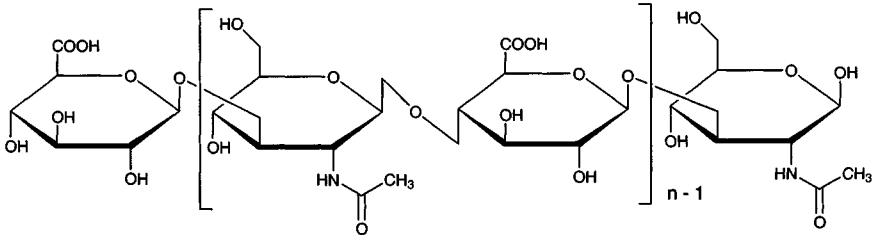
details of plasma extraction

REFERENCE

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

Hyaluronic acid

CAS Registry No.: 9004-61-9
Merck Index: 4793



SAMPLE

Matrix: bulk

Sample preparation: Mix 100 μL of a 1-500 μg/mL solution of hyaluronic acid in water with 40 μL 100 mM pH 5.2 citrate/phosphate buffer, add 10 500 U/mL hyaluronate 4-glycanohydrolase (sheep testis, Type V, Sigma) in water, heat at 37° for 5 h, heat in a boiling water bath for 3 min, evaporate to dryness. Reconstitute with 20 μL 300 mM NaOH, add 20 μL 500 mM 1-(4-methoxy)phenyl-3-methyl-5-pyrazolone in MeOH, heat at 70° for 20 min, add 20 μL 300 mM HCl, add 200 μL water, add 200 μL ethyl acetate saturated with water, shake vigorously, discard the organic phase, repeat the ethyl acetate wash twice more. Evaporate the aqueous phase to dryness and reconstitute the residue in 200 μL MeCN:water 15:85, inject a 20 μL aliquot. (Synthesis of 1-(4-methoxy)phenyl-3-methyl-5-pyrazolone is as follows. Reflux 5.6 g 4-methoxyphenylhydrazine hydrochloride, 5.45 g sodium acetate trihydrate, and 4.16 g ethyl acetoacetate in 40 mL EtOH for 2 h, cool, evaporate to dryness, dissolve the residue in 10 mL EtOH, filter, evaporate the filtrate to dryness, dissolve the residue in a small volume of benzene:ethyl acetate 80:20 (Caution! Benzene is a carcinogen!), chromatograph on a column of 150 g silica gel 60 (Merck) equilibrated with benzene:ethyl acetate 80:20, collect 5 mL fractions (monitor by TLC using Merck silica gel 60 F_{254} eluted with benzene:ethyl acetate 80:20, UV detection, R_f 0.41). Combine the appropriate fractions and evaporate them to dryness, recrystallize the residue from MeOH to give 1-(4-methoxy)phenyl-3-methyl-5-pyrazolone (Anal. Biochem. 1991, 199, 256).)

HPLC VARIABLES

Column: 150 × 6 Cosmosil 5C18-AR
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 15:85
Flow rate: 0.8
Injection volume: 20
Detector: UV 249

CHROMATOGRAM

Retention time: 9 (hexasaccharide), 10 (tetrasaccharide), 16 (disaccharide)

KEY WORDS

derivatization

REFERENCE

Kakehi,K.; Ueda,M.; Suzuki,S.; Honda,S. Determination of hyaluronic acid by high-performance liquid chromatography of the oligosaccharides derived therefrom as 1-(4-methoxy)phenyl-3-methyl-5-pyrazolone derivatives, *J.Chromatogr.*, **1993**, *630*, 141–146.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 1.5 mg/mL solution of sodium hyaluronate in 0.9% saline, filter 0.22 μm, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: TSK G6000 PW (Toyo Soda)

Column: 300 × 7.5 TSK G6000 PW (Toyo Soda)
Mobile phase: 3 mM pH 7.0 NaH_2PO_4 containing 150 mM NaCl and 0.02% sodium azide
Flow rate: 1
Injection volume: 10
Detector: RI

CHROMATOGRAM
Retention time: 8.22

REFERENCE
Beaty,N.B.; Tew,W.P.; Mello,R.J. Relative molecular weight and concentration determination of sodium hyaluronate solutions by gel-exclusion high-performance liquid chromatography, *Anal.Biochem.*, **1985**, *147*, 387–395.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 8 Shodex OHpak KB-803 (Showa Denko)
Mobile phase: 200 mM NaCl
Flow rate: 0.5
Detector: UV 210

CHROMATOGRAM
Retention time: 12

KEY WORDS
GPC

REFERENCE
Nakamura,T.; Majima,M.; Kubo,K.; Takagaki,K.; Tamura,S.; Endo,M. Hyaluronidase assay using fluorogenic hyaluronate as a substrate, *Anal.Biochem.*, **1990**, *191*, 21–24.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10-20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 TSKgel NH2-60
Mobile phase: MeCN:buffer 54:46 (Buffer was 40 mM Tris-HCl borate buffer adjusted to pH 7.5 with HCl containing 5 mM sodium sulfate.)
Flow rate: 0.5
Injection volume: 10-20
Detector: F ex 346 em 410 following post-column reaction. The effluent from the column was mixed with 300 mM NaOH (pumped at 0.25 mL/min) and 1% 2-cyanoacetamide (pumped at 0.25 mL/min). The mixture passed through a 10 m × 0.5 mm i.d. PTFE coil at 105° and a 2 m × 0.25 mm i.d. PTFE coil at 25° to the detector.

CHROMATOGRAM
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: chondroitin sulfate, dermatan sulfate

KEY WORDS
post-column reaction

REFERENCE
Akiyama,H.; Saito,M.; Qiu,G.; Toida,T.; Imanari,T. Analytical studies on hyaluronic acid synthesis by normal human epidermal keratinocytes cultured in a serum-free medium, *Biol.Pharm.Bull.*, **1994**, *17*, 361–364.

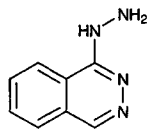
SAMPLE
Matrix: synovial fluid
Sample preparation: Centrifuge synovial fluid, dilute 40 μL to 500 μL with initial mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: LC 18 (Supelco)
Column: 50 × 4.6 Supelcosil LC 318
Mobile phase: Gradient. A was 20 mM NaH_2PO_4 containing 150 mM NaCl, pH 6.5. B was MeCN. A:B from 100:0 to 40:60 over 50 min.
Flow rate: 1
Detector: UV 229

CHROMATOGRAM
Retention time: 0.6

REFERENCE
Brun,P.; De Galateo,A.; Camporese,A.; Cortivo,R.; Abatangelo,G. Analysis of hyaluronic acid in synovial fluid by reversed-phase liquid chromatography, *J.Chromatogr.*, **1990**, *526*, 530–534.

Hydralazine



Molecular formula: $C_8H_8N_4$
Molecular weight: 160.18
CAS Registry No.: 86-54-4, 304-20-1 (HCl)
Merck Index: 4800

SAMPLE
Matrix: blood
Sample preparation: Add 8-12 mL blood to 125 IU lithium heparin in ice-cold tubes, centrifuge at 8000 g for 30 s, add 1 mL plasma to 75 μL 50% sodium nitrite in a tube kept on ice, add 150 μL 16.7 μM 4-methylhydralazine in 10 mM HCl, add 2 mL 20 mM HCl (perform the preceding procedure as rapidly as possible), vortex briefly, allow to stand at 20 ± 1° for 10.0 min, add 1 mL 1 M NaOH/0.6 M sodium tetraborate buffer (pH 10), add chloroform, shake at 110 rpm for 5 min, centrifuge at 1100 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 500 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 10 μm μBondapak phenyl
Mobile phase: MeCN:1.5 mM aqueous phosphoric acid 15:85
Column temperature: 50
Flow rate: 2
Injection volume: 50
Detector: F ex 250 em 360 (cut-off filter)

CHROMATOGRAM
Retention time: 6.7
Internal standard: 4-methylhydralazine (10)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites, propranolol, quinidine

KEY WORDS
plasma; derivatization; pharmacokinetics

REFERENCE

Reece,P.A.; Cozamanis,I.; Zacest,R. Selective high-performance liquid chromatographic assays for hydralazine and its metabolites in plasma of man, *J.Chromatogr.*, **1980**, *181*, 427–440.

SAMPLE

Matrix: blood

Sample preparation: 3 mL Whole blood + 20 μL p-anisaldehyde + 8 μL 5 μg/mL 4-methylhydralazine in 10 mM HCl, vortex for 15 s, let stand at room temperature for 10 min, add 10 mL hexane, shake horizontally at 180 strokes/min for 10 min, centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH, inject the whole amount.

HPLC VARIABLES

Column: 300 × 3.9 μBondapak CN
Mobile phase: MeCN:150 mM pH 3.0 sodium acetate buffer 70:30
Flow rate: 2
Injection volume: 100
Detector: UV 365

CHROMATOGRAM

Retention time: 3.5
Internal standard: 4-methylhydralazine (6)
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES

Noninterfering: propranolol, furosemide, hydrochlorothiazide, digoxin, nitroglycerin

KEY WORDS

whole blood; derivatization

REFERENCE

Ludden,T.M.; Ludden,L.K.; Wade,K.E.; Allerheiligen,S.R. Determination of hydralazine in human whole blood, *J.Pharm.Sci.*, **1983**, *72*, 693–695.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 1 mL 20 mM disodium EDTA + 1 mL 500 mM HCl + 1 mL 10 mM 2-hydroxy-1-naphthaldehyde, vortex for 15 s, keep at 25° for 90 min, add 50 μL 1 μg/mL methyl red, add 7 mL dichloromethane, vortex for 5 min, centrifuge at 4500 rpm for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μL MeCN, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 3 μm Spherisorb ODS-2
Mobile phase: MeCN:buffer 80:20, pH 3 (Buffer was 0.75% phosphoric acid and 0.5% triethylamine in water.)
Flow rate: 0.7
Injection volume: 20
Detector: UV 406

CHROMATOGRAM

Retention time: 6.4
Internal standard: methyl red (6)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Extracted: dihydralazine

KEY WORDS

plasma; derivatization; pharmacokinetics

REFERENCE
Mañes,J.; Mari,J.; Garcia,R.; Font,G. Liquid chromatographic determination of hydralazine in human plasma with 2-hydroxy-1-naphthaldehyde pre-column derivatization, *J.Pharm.Biomed.Anal.*, **1990**, *8*, 795–798.

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 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 + 1.5 mL 0.2% hydrochlorothiazide, make up to 50 mL with water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak 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: 5
Internal standard: hydrochlorothiazide (8)

OTHER SUBSTANCES
Simultaneous: phenylpropanolamine

KEY WORDS
injections; tablets

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: Powder tablets, sonicate for 10 min with enough 5 mM HCl to give a 30 μg/mL solution, filter, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb CN
Mobile phase: MeOH:buffer 20:80 (Buffer was 7 mM sodium heptanesulfonate:50 mM triethylamine adjusted to pH 3.1 with dilute phosphoric acid 80:20.)
Flow rate: 1
Injection volume: 20
Detector: UV 260

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: dihydralazine, phthalazine

KEY WORDS
tablets

REFERENCE
Di Pietra,A.M.; Roveri,P.; Gotti,R.; Cavrini,V. Spectrophotometric and chromatographic (HPLC) analysis of hydralazine, dihydralazine and hydrazine after derivatization with 2-nitrocinnamaldehyde, *Farmaco*, **1993**, *48*, 1555–1567.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets, add MeCN:5 mM sodium octanesulfonate 15:85, sonicate, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:5 mM sodium octanesulfonate:phosphoric acid 15:85:0.045
Flow rate: 2
Detector: UV 220

CHROMATOGRAM
Retention time: 10.86

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
tablets

REFERENCE
Lessen,T.; Zhao,D.C. Interactions between drug substances and excipients. 1. Fluorescence and HPLC studies of triazolophthalazine derivatives from hydralazine hydrochloride and starch, *J.Pharm.Sci.*, **1996**, *85*, 326–329.

SAMPLE
Matrix: perfusate
Sample preparation: 30 μL Perfusate (artificial CSF) + 10 μL 200 mM perchloric acid. Mix a 25 μL aliquot with 12.5 μL reagent, let stand for 2 min, inject an aliquot. (Prepare a stock solution by dissolving 27 mg o-phthalaldehyde in 1 mL MeOH, add 5 μL β-mercaptoethanol, add 9 mL 100 mM pH 9.3 sodium tetraborate containing 10 μM EDTA. This solution is good for 5 days in a sealed amber bottle at room temperature. Prepare the working reagent by diluting 1 mL of the stock solution with 3 mL 100 mM pH 9.3 sodium tetraborate containing 10 μM EDTA, allow to stand for 24 h before use.)

HPLC VARIABLES
Column: two columns 150 × 4.6 5 μm M.S. Gel C18 (ESA)
Mobile phase: MeOH:buffer 8:92 adjusted to pH 3.0 with phosphoric acid (Buffer was 54 mM NaH_2PO_4 containing 1.24 mM sodium heptanesulfonate.)
Column temperature: 33
Flow rate: 1.2
Detector: E, ESA Coulochem Electrode Array System Model 5500, detector temp 33°, oxidation potential 0 V

CHROMATOGRAM
Retention time: 12.50
Limit of quantitation: 0.5 ng/mL

OTHER SUBSTANCES
Extracted: apomorphine, dopamine, isoproterenol, methoxamine, morphine, norepinephrine, phenylephrine

KEY WORDS
rat; derivatization

REFERENCE
Acworth,I.N.; Yu,J.; Ryan,E.; Gariepy,K.C.; Gamache,P.; Hull,K.; Maher,T. Simultaneous measurement of monoamine, amino acid, and drug levels, using high performance liquid chromatography and coulometric array technology: application to in vivo microdialysis perfusate analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 685–705.

SAMPLE
Matrix: solutions
Sample preparation: Make up a solution in 40 mM sodium formate and 62 mM formic acid buffer (pH 3.5), inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 10 μm CN (Waters)
Column: 150 × 4.6 5 μm Ultrasphere CN
Mobile phase: MeOH:buffer 15:85 (Buffer was 40 mM sodium formate and 62 mM formic acid, pH 3.5.)
Flow rate: 1
Injection volume: 20
Detector: UV 258

CHROMATOGRAM
Retention time: 3.7
Internal standard: phenylephrine (2.7)

OTHER SUBSTANCES
Simultaneous: phthalazine, degradation products

REFERENCE
Halasi,S.; Nairn,J.G. Quantitative determination of hydralazine hydrochloride and phthalazine in aqueous solutions by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1989**, *12*, 2397–2403.

SAMPLE
Matrix: solutions
Sample preparation: Add a 1 mL aliquot of a solution in MeOH:water 70:30 to 1 mL pH 4.5 acetate buffer, add 50 μL acetic acid, add 1 mL 2.2 mM 2-nitrocinnamaldehyde in EtOH, heat at 70° for 50 min, cool to room temperature, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.5 5 μm Hypersil C18
Mobile phase: MeCN:buffer 65:35 (Prepare buffer by adjusting the pH of 50 mM triethylamine to 3.3 with dilute phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 390

CHROMATOGRAM
Retention time: 5.1

OTHER SUBSTANCES
Simultaneous: hydrazine (UV 350)

KEY WORDS
derivatization

REFERENCE
Di Pietra,A.M.; Roveri,P.; Gotti,R.; Cavrini,V. Spectrophotometric and chromatographic (HPLC) analysis of hydralazine, dihydralazine and hydrazine after derivatization with 2-nitrocinnamaldehyde, *Farmaco*, **1993**, *48*, 1555–1567.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 12 μm Dynamax C18 (Rainin)
Mobile phase: MeCN:50 mM acetic acid 20:80
Flow rate: 2

Detector: UV 241

CHROMATOGRAM
Retention time: 1.6

OTHER SUBSTANCES
Simultaneous: metabolites, 3-methyl-s-triazolo[3,4-α]phthalazine

REFERENCE
Hickman,D.; Palamanda,J.R.; Unadkat,J.D.; Sim,E. Enzyme kinetic properties of human recombinant arylamine N-acetyltransferase 2 allotypic variants expressed in *Escherichia coli*, *Biochem.Pharmacol.*, **1995**, *50*, 697–703.

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

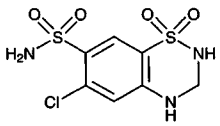
CHROMATOGRAM
Retention time: 6.46 (A), 3.49 (B)

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

KEY WORDS
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.

Hydrochlorothiazide



Molecular formula: $C_7H_8ClN_3O_4S_2$
Molecular weight: 297.74
CAS Registry No.: 58-93-5
Merck Index: 4822
Lednicer No.: 1 358

SAMPLE
Matrix: blood
Sample preparation: 500 µL Serum +100 µL 1.25 µg/mL IS + 5 mL MTBE, vortex for 2 min. Centrifuge at 2700 g for 5 min and evaporate the organic phase to dryness under a stream of nitrogen. Dissolve the residue in 200 µL water, add 3 mL toluene, vortex for 2 min, centrifuge at 2700 g for 10 min, discard the toluene layer . Add 3 mL toluene, vortex, centrifuge, discard the toluene layer. Evaporate the aqueous layer to dryness under a stream of nitrogen. Reconstitute the residue in 200 µL mobile phase. Inject a 100 µL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 µm RP-C18
Column: 250 × 4 5 µm LiChrospher 100 RP-C18
Mobile phase: MeCN:THF:200mM pH 7.5 phosphate buffer 5:10:85
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: UV 273

CHROMATOGRAM
Internal standard: hydroflumethiazide
Limit of detection: 3.3 ng/mL
Limit of quantitation: 11.2 ng/mL

KEY WORDS
serum; pharmacokinetics

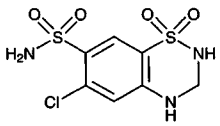
REFERENCE
Vervaet,C.; Remon,J.P. Bioavailability of hydrochlorothiazide from pellets, made by extrusion/spheronisation, containing polyethylene glycol 400 as a dissolution enhancer, *Pharm.Res.*, **1997**, *14*, 1644–1646.

SAMPLE
Matrix: blood
Sample preparation: 200 µL Serum + 20 µL 1 M pH 7.0 phosphate buffer, extract with 5 mL MTBE, vortex for 20 s, centrifuge at 2500 g for 10 min. Remove the organic layer and add it to 10 µL 20 µg/mL IS in MeOH, evaporate to dryness at 80° in a vacuum centrifuge, reconstitute the residue with 180 µL mobile phase, inject a 30 µL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 µm endcapped LichroCART RP18
Mobile phase: MeCN:7.5 mM pH 7.3 phosphate buffer 10:90
Column temperature: 40
Flow rate: 0.8
Injection volume: 30
Detector: E, ESA Coulochem II, coulometric cell 5011, first electrode +450 mV, second electrode +630 mV; UV 254

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

Hydrochlorothiazide



Molecular formula: $C_7H_8ClN_3O_4S_2$
Molecular weight: 297.74
CAS Registry No.: 58-93-5
Merck Index: 4822
Lednicer No.: 1 358

SAMPLE
Matrix: blood
Sample preparation: 500 µL Serum +100 µL 1.25 µg/mL IS + 5 mL MTBE, vortex for 2 min. Centrifuge at 2700 g for 5 min and evaporate the organic phase to dryness under a stream of nitrogen. Dissolve the residue in 200 µL water, add 3 mL toluene, vortex for 2 min, centrifuge at 2700 g for 10 min, discard the toluene layer . Add 3 mL toluene, vortex, centrifuge, discard the toluene layer. Evaporate the aqueous layer to dryness under a stream of nitrogen. Reconstitute the residue in 200 µL mobile phase. Inject a 100 µL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 µm RP-C18
Column: 250 × 4 5 µm LiChrospher 100 RP-C18
Mobile phase: MeCN:THF:200mM pH 7.5 phosphate buffer 5:10:85
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: UV 273

CHROMATOGRAM
Internal standard: hydroflumethiazide
Limit of detection: 3.3 ng/mL
Limit of quantitation: 11.2 ng/mL

KEY WORDS
serum; pharmacokinetics

REFERENCE
Vervaet,C.; Remon,J.P. Bioavailability of hydrochlorothiazide from pellets, made by extrusion/spheronisation, containing polyethylene glycol 400 as a dissolution enhancer, *Pharm.Res.*, **1997**, *14*, 1644–1646.

SAMPLE
Matrix: blood
Sample preparation: 200 µL Serum + 20 µL 1 M pH 7.0 phosphate buffer, extract with 5 mL MTBE, vortex for 20 s, centrifuge at 2500 g for 10 min. Remove the organic layer and add it to 10 µL 20 µg/mL IS in MeOH, evaporate to dryness at 80° in a vacuum centrifuge, reconstitute the residue with 180 µL mobile phase, inject a 30 µL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 µm endcapped LichroCART RP18
Mobile phase: MeCN:7.5 mM pH 7.3 phosphate buffer 10:90
Column temperature: 40
Flow rate: 0.8
Injection volume: 30
Detector: E, ESA Coulochem II, coulometric cell 5011, first electrode +450 mV, second electrode +630 mV; UV 254

CHROMATOGRAM
Retention time: 5.8
Internal standard: p-aminobenzoic acid (1.2)
Limit of quantitation: 5 ng/mL (E)

KEY WORDS
serum

REFERENCE
Richter,K.; Oertel,R.; Kirch,W. New sensitive method for the determination of hydrochlorothiazide in human serum by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.A*, **1996**, *729*, 293–296.

SAMPLE
Matrix: blood, formulations
Sample preparation: Serum. Condition a Bakerbond C18 SPE cartridge with 3 mL MeOH and 3 mL water. Mix 100 μL serum with 200 μl MeCN, vortex for 2 min, add 100 μL 4.08 μg/mL IS in MeOH, mix, centrifuge at 4000 rpm for 15 min. After the removal of the organic solvent add the supernatant to the SPE cartridge, dry under vacuum, wash with 3 mL water, elute with 3 mL MeOH. Evaporate to dryness under a stream of nitrogen at 45°, dilute to 100 μL with MeOH. Inject a 20 μL aliquot. Tablets. Powder tablets. Prepare an 1-3 μg/mL solution of hydrochlorothiazide in MeOH containing 4.08 μg/mL IS. Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:1% acetic acid 20:80
Flow rate: 1.3
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 3.146
Internal standard: hydroflumethiazide (5.28)
Limit of detection: 50 ng/mL
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Noninterfering: captopril

KEY WORDS
tablets; plasma; SPE

REFERENCE
Papadoyannis,I.N.; Samanidou,V.F.; Georga,K.A.; Georgarakis,E. High performance liquid chromatographic determination of hydrochlorothiazide (HCT) in pharmaceutical preparations and human serum after solid phase extraction, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1671–1683.

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

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 226.3

CHROMATOGRAM
Retention time: 9.397

KEY WORDS
whole blood

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

SAMPLE
Matrix: solutions
Sample preparation: Dilute 25 mL 9.6 mg/mL chlorothiazide in MeOH with 1 mL 380 mg/L IS in 0.1% phosphoric acid, inject an aliquot.

HPLC VARIABLES
Column: A 250 × 2 J sphere ODS-M80; B 150 × 4.6 5 μm Beckman Ultrasphere C18
Mobile phase: A Gradient. MeCN:0.1% formic acid from 0:100 to 30:70 over 20 min. B Gradient. MeCN:0.1% phosphoric acid 0:100 to 30:70 over 12 min.
Flow rate: A 0.2; B 1
Detector: A MS, Finnigan Model TSQ-7000 triple-quadrupole, nebulizer nitrogen 260°; B UV 270

CHROMATOGRAM
Retention time: 22
Internal standard: ethylparaben

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
photolysis

REFERENCE
Revelle,L.K.; Musser,S.M.; Rowe,B.J.; Feldman,I.C. Identification of chlorothiazide and hydrochlorothiazide UV-A photolytic decomposition products, *J.Pharm.Sci.*, **1997**, *86*, 631–634.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil ODS1
Mobile phase: MeOH:50 mM pH 3.0 phosphoric acid 10:90
Column temperature: 30
Flow rate: 1.5
Detector: radioactivity detection

OTHER SUBSTANCES
Also analyzed: atenolol, cimetidine, ranitidine

KEY WORDS
14C labeled

REFERENCE
Collett,A.; Sims,E.; Walker,D.; He,Y.-L.; Ayrton,J.; Rowland,M.; Warhurst,G. Comparison of HT29-18-C_1 and Caco-2 cell lines as models for studying intestinal paracellular drug absorption, *Pharm.Res.*, **1996**, *13*, 216–221.

SAMPLE
Matrix: urine
Sample preparation: Dilute 10 mL urine to 15 mL with water, add to Extrelut-20 cartridge, elute with 60 mL ethyl acetate:isopropanol 85:15. Evaporate under vacuum at 50°, filter, dry under nitrogen, reconstitute the residue in 100 µL acetone. Add 100 µL 1 mg/mL 3-bromomethylpropylphenazone in acetone, mix with 1 mg anhydrous potassium carbonate, make up to 200 µL with acetone. Let stand at 105 ± 5° for 60 min. Cool the reaction mixture, dry under a gentle stream of nitrogen. Reconstitute the residue with 500 µL MeCN, shake for 2 min. inject a 10 µL aliquot. (3-Bromomethylpropylphenazone is produced by the reaction of propylphenazone with bromine and recrystallized from chloroform:diethyl ether 1:2. (Caution! Chloroform is a carcinogen!))

HPLC VARIABLES
Column: 250 × 4.6 6 µm Zorbax C8
Mobile phase: MeCN:MeOH:50 mM sodium acetate 34:8:28, adjusted to pH 6.5 with acetic acid
Column temperature: 35
Flow rate: 1
Injection volume: 10
Detector: UV 254

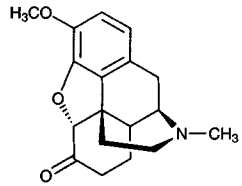
CHROMATOGRAM
Retention time: 27.7 (derivatized), 3.3 (underivatized)

OTHER SUBSTANCES
Extracted: captopril

KEY WORDS
derivatization; SPE

REFERENCE
Khedr,A.; El-Sherief,H. 3-Bromomethyl-propyphenazone as a new derivatization reagent for high performance liquid chromatography of captopril and hydrochlorothiazide with UV-detection, *Biomed.Chromatogr.*, **1998**, *12*, 57–60.

Hydrocodone



Molecular formula: $C_{18}H_{21}NO_3$
Molecular weight: 299.37
CAS Registry No.: 125-29-1, 34195-34-1 (bitartrate hydrate), 143-71-5 (bitartrate)
Merck Index: 4826
Lednicer No.: 1 288

SAMPLE
Matrix: formulations
Sample preparation: 500 µL or 1.0 mL Sample + 1.0 mL water + 500 µL 1 M NaOH + 15 mL dichloromethane, shake at 100 cpm for 20 min. Centrifuge at 2500 rpm for 5 min, evaporate organic layer to dryness under a gentle stream of nitrogen at 35 to 40°. Dissolve residue in 5 mL MeOH, inject a 20 to 80 µL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 µm Alltech C8

Mobile phase: MeCN:pH 4.5 buffer 18:82 (Buffer was 10 mM KH_2PO_4 and 50 mM potassium nitrate.)
Flow rate: 1.4
Injection volume: 20-80
Detector: UV 280

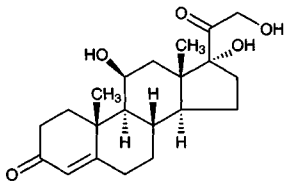
CHROMATOGRAM
Retention time: 14

OTHER SUBSTANCES
Noninterfering: chorpheniramine

KEY WORDS
suspensions

REFERENCE
Hadzija,B.W.; Shrewsbury,R.P. Determination of hydrocodone in Tussionex extended-release suspension by high-performance liquid chromatography (HPLC), *J.Forensic Sci.*, **1996**, *41*, 878–880.

Hydrocortisone



Molecular formula: $C_{21}H_{30}O_5$
Molecular weight: 362.47
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), 2203-97-6 (succinate), 5752489-7 (valerate)
Merck Index: 4828
Lednicer No.: 1 190

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge. Mix 1 mL plasma with 134.0 ng hydrocortisone-d_5 and 74.56 ng cortisone-d_5. Add the sample to the SPE cartridge, wash with 8 mL water, elute with 4 mL ethyl acetate, evaporate the eluate to dryness at 70° under a stream of nitrogen, dissolve the residue in 30 μL mobile phase, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.0 4 μm LiChroCART Superspher 100
Mobile phase: A MeOH:THF:50 mM ammonium formate 17:53:180; B MeCN:50 mM ammonium formate 35:65
Flow rate: 0.6 (A); 1.3 (B)
Injection volume: 20
Detector: MS, Shimadzu LCMS-QP1000EX Model 750 B, thermospray, vaporizer control 155°, vaporizer tip 195°, vapor 274°, ion source block 295°, tip heater 305°, m/z 363

CHROMATOGRAM
Retention time: 13 (A)
Internal standard: hydrocortisone-d_5, cortisone-d_5
Limit of detection: 0.25 ng

OTHER SUBSTANCES
Extracted: cortisone, prednisolone, prednisone

KEY WORDS
plasma; SPE

REFERENCE

Shibasaki,H.; Furuta,T.; Kasuya,Y. Quantification of corticosteroids in human plasma by liquid chromatography-thermospray mass spectrometry using stable isotope dilution, *J.Chromatogr.B*, **1997**, *692*, 7–14.

SAMPLE

Matrix: blood

Sample preparation: Add 100 µL MeOH and 50 µL 1 µg/mL fluocortolone in MeOH to 1 mL plasma. Add 500 µL 100 mM NaOH and 2 mL dichloromethane, shake for 10 min, centrifuge at 2500 g for 10 min, evaporate a 1.9 mL aliquot of the supernatant under a stream of nitrogen at 45°. Reconstitute the residue in 50 µL MeOH, inject 17 µL aliquot.

HPLC VARIABLES

Guard column: 10 × 4 5 µm LiChrospher RP 18
Column: 250 × 4 5 µm Lichrospher RP 18
Mobile phase: MeOH:THF:water 110:2.5:100
Flow rate: 1
Injection volume: 17
Detector: UV 252

CHROMATOGRAM

Internal standard: fluocortolone
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES

Extracted: triamcinolone

KEY WORDS

plasma

REFERENCE

Doppenschmitt,S.A.; Scheidel,B.; Harrison,F.; Surmann,J.P. Simultaneous determination of triamcinolone acetonide and hydrocortisone in human plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *682*, 79–88.

SAMPLE

Matrix: blood, urine

Sample preparation: Condition a 3 mL 500 mg Sep-Pak Vac C18 SPE cartridge with 3 mL MeOH and 3 mL water. 1 mL Serum or urine + 500 µL 200 mM pH 3.85 acetate buffer (serum only) + 400 µL 2.5 µM IS in mobile phase, mix, centrifuge. Add the supernatant to the SPE cartridge, wash with 3 mL acetone:water 20:80, 3 mL water, and 3 mL hexane. Elute with 3 mL diethyl ether into tubes containing 1 mL 200 mM NaOH, vortex, centrifuge. Dry the organic layer under a stream of nitrogen. Reconstitute the residue in 250 µL mobile phase, mix for 5 min. Inject a 60 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Spherex C18 (Phenomenex USA)
Mobile phase: MeOH:THF:water 3:25:72
Flow rate: 1.0
Injection volume: 60
Detector: UV 254

CHROMATOGRAM

Retention time: 12.85
Internal standard: fludrocortisone (15.9)
Limit of detection: 5 nM

OTHER SUBSTANCES

Extracted: 11-deoxycortisol, dexamethasone, methylprednisolone, prednisolone

KEY WORDS

serum; SPE

REFERENCE
McWhinney,B.C.; Ward,G.; Hickman,P.E. Improved HPLC method for simultaneous analysis of cortisol, 11-deoxycortisol, prednisolone, methylprednisolone, and dexamethasone in serum and urine, *Clin.Chem.*, **1996**, *42*, 979–981.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Sep-Pak Plus C18 SPE cartridge with 7 mL MeOH and 14 mL water. Add 40 ng 6β-hydroxycortisone to 400 μL plasma or urine, add the mixture to the SPE cartridge, wash with 6 mL water, 3 mL MeOH:water 12:88, and 3 mL petroleum ether, elute with 5 mL ethyl acetate. Dry the eluate under reduced pressure at 40°, add 200 μL MeCN: triethylamine 90:10 and MeCN:0.1% quinuclidine 20:80 to the residue, vortex. Add 200 μL 0.02% 9-anthroyl nitrile and a few molecular sieves (4A), let stand for 30 min, evaporate under reduced pressure at 40°, dissolve the residue in 200 μL acetone, dilute with 2 mL n-hexane. Add the mixture to a Sep-Pak Plus Silica SPE cartridge, wash with 14 mL 1,2-dichloroethane, elute with 5 mL ethyl acetate. Evaporate the eluate under reduced pressure at 40°, reconstitute the residue in 200 μL mobile phase, inject a 30-60 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Cosmosil 5SL (Nacalai Tesque, Japan)
Mobile phase: Dioxane:ethyl acetate:chloroform:n-hexane:pyridine 58.1:11.6:11.6:16.3:2.4 (Caution! Dioxane and chloroform are carcinogens!)
Flow rate: 1 for 45 min, to 1.2 over 5 min
Injection volume: 30-60
Detector: F ex 360 em 460

CHROMATOGRAM
Retention time: 24
Internal standard: 6β-hydroxycortisone (86)
Limit of detection: 100 pg/mL

OTHER SUBSTANCES
Extracted: cortisone, 6β-hydroxycortisol, 6β-hydroxyprednisolone, prednisolone, prednisone

KEY WORDS
derivatization; plasma; urine; SPE; normal phase

REFERENCE
Shibata,N.; Hayakawa,T.; Takada,K.; Hoshino,N.; Minouchi,T.; Yamaji,A. Simultaneous determination of glucocorticoids in plasma or urine by high-performance liquid chromatography with precolumn fluorimetric derivatization by 9-anthroyl nitrile, *J.Chromatogr.B*, **1998**, *706*, 191–199.

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

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

Injection volume: 10-30
Detector: UV 242.9

CHROMATOGRAM
Retention time: 17.735

KEY WORDS
whole blood

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

SAMPLE
Matrix: solutions
Sample preparation: Prepare solutions in MeCN, dilute to an appropriate concentration with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 120 × 4.6 5 μm octadecyl Bakerbond
Mobile phase: MeCN:water 30:70 containing 16 mM β-cyclodextrin
Column temperature: 5
Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 0.7

OTHER SUBSTANCES
Simultaneous: testosterone, prednisone, cortisone, 17α-methyltestosterone, 17α-hydroxyprogesterone

REFERENCE
Zarzycki,P.K.; Wierzbowska,M.; Lamparczyk,H. The influence of temperature on the high performance liquid chromatographic separation of steroids using mobile phases modified with β-cyclodextrin, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 1305–1311.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 4.1 10 μm Versapack C18 (Alltech)
Mobile phase: MeCN:water 40:60
Flow rate: 1
Injection volume: 50
Detector: UV 254

OTHER SUBSTANCES
Simultaneous: hydrocortisone, 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: urine
Sample preparation: Condition a 10 mL 200 mg MCF Isolute SPE cartridge with two 3 mL portions of EtOH and two 3 mL portions of water. Centrifuge urine at 4000 g for 30 in, filter

through a 0.22 μm filter unit. Dilute 0.75-3mL urine to 4 mL with water. Add 30 ng IS. Add to the SPE cartridge. Wash with three 3 mL portions of water, 3 mL MeOH:10 mM NaOH 30:70, twice with 3 mL water and with 3 mL MeOH:10 mM HCl 30:70. Elute with 3 mL EtOH. Evaporate eluate under vacuum and reconstitute the residue with 150 μL mobile phase. Inject a 100 μL aliquot.

HPLC VARIABLES
Column: 100 × 3.2 5 μm Nucleosil 120-C18
Mobile phase: MeCN:water 24:76
Flow rate: 0.5
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 10.22
Internal standard: dexamethasone (22.01)
Limit of detection: 1.7 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, cortisone

KEY WORDS
SPE; human; pig

REFERENCE
Hay,M.; Mormède,P. Improved determination of urinary cortisol and cortisone, or corticosterone and 11-dehydrocorticosterone by high-performance liquid chromatography with ultraviolet absorbance detection, *J.Chromatogr.B*, **1997**, *702*, 33–39.

SAMPLE
Matrix: urine
Sample preparation: Activate 3-mL 500 mg Bakerbond C18 cartridge with 2 mL MeOH and 2 mL water. Filter sample. Add 25 μL 8 μM IS in MeOH to 2 mL urine, add to the SPE cartridge, wash with two 2 mL portions of 25 mM borate buffer and with 200 mL/L acetone in water. Add 1 mL hexane and air-dry under reduced pressure for 4 min. Elute with two 1 mL portions of ethyl acetate. Dry the eluate under a stream of nitrogen and dissolve in 75 μL 400 mL/L MeOH, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm LiChrospher 100 C18
Mobile phase: MeCN:MeOH:water 3:43:54
Column temperature: 40
Flow rate: 1
Injection volume: 25
Detector: UV 242

CHROMATOGRAM
Retention time: 21.3-21.7
Internal standard: 6 α-methylprednisolone (39.3-40.1)
Limit of detection: 15 nM

OTHER SUBSTANCES
Simultaneous: metabolites, alprazolam, amlodipine, aspirin, carbamazepine, citalopram, corticosterone, cortisone, dexamethasone, digoxin, enalapril, ferrous sulfate, fluoxetine, furosemide, gabapentin, 5-hydroxyindoleacetic acid, lamotrigine, metyrapone, naproxen, oxazepam, oxcarbazepine, oxybutynin, phenobarbital, phenytoin, prednisone, spironolactone, valproic acid, vigabatrin, zopiclone
Noninterfering: octreotide
Interfering: prednisolone

KEY WORDS
SPE; comparison with RIA

REFERENCE
Turpeinen,U.; Markkanen,H.; Välimäki,M.; Stenman,U.-H. Determination of urinary free cortisol by HPLC, *Clin.Chem.*, **1997**, *43*, 1386–1391.

SAMPLE
Matrix: urine
Sample preparation: 10 mL Urine + 40 µL 25 µg/mL corticosterone, vortex briefly, add 1 mL 100 mM NaOH, vortex briefly, add 3 mL dichloromethane, rotate at 20 rpm for 45 min, centrifuge at 1000 g for 15 min, discard the aqueous layer, centrifuge at 1000 g for 10 min, discard the aqueous layer, add 150 mg NaCl, break up emulsion, centrifuge for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 150 µL MeOH, inject an aliquot.

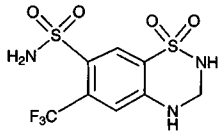
HPLC VARIABLES
Column: 150 × 3.9 4 µm Nova-Pak C18
Mobile phase: Gradient. MeOH:water from 30:70 to 44:56 over 6 min, maintain at 44:56 for 14 min, return to initial conditions over 3 min, re-equilibrate for 5 min.
Flow rate: 1
Detector: UV 246

CHROMATOGRAM
Retention time: 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 11β-hydroxysteroid dehydrogenase, *Clin.Pharmacol.Ther.*, **1996**, *59*, 62–71.

Hydroflumethiazide



Molecular formula: $C_8H_8F_3N_3O_4S_2$
Molecular weight: 331.30
CAS Registry No.: 135-09-1
Merck Index: 4830
Lednicer No.: 1 358

SAMPLE
Matrix: blood
Sample preparation: 500 µL Serum + 5 mL MTBE, vortex for 2 min. Centrifuge at 2700 g for 5 min and evaporate the organic phase to dryness under a stream of nitrogen. Dissolve the residue in 200 µL water, add 3 mL toluene, vortex for 2 min, centrifuge at 2700 g for 10 min, discard the toluene layer. Add 3 mL toluene, vortex, centrifuge, discard the toluene layer. Evaporate the aqueous layer to dryness under a stream of nitrogen. Reconstitute the residue in 200 µL mobile phase. Inject a 100 µL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 µm RP-C18
Column: 250 × 4 5 µm LiChrospher RP-C18
Mobile phase: MeCN:THF:200 mM pH 7.5 phosphate buffer 5:10:85
Flow rate: 1
Injection volume: 100
Detector: UV 273

CHROMATOGRAM
Internal standard: hydroflumethiazide

OTHER SUBSTANCES
Extracted: hydrochlorothiazide

KEY WORDS
hydroflumethiazide is IS; serum

REFERENCE
Vervaet,C.; Remon,J.P. Bioavailability of hydrochlorothiazide from pellets, made by extrusion/spheronisation, containing polyethylene glycol 400 as a dissolution enhancer, *Pharm.Res.*, **1997**, *14*, 1644–1646.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 25 μL 10 mg/mL hydroflumethiazide in water + 1 mL 1 M pH 10 sodium carbonate-bicarbonate buffer + 5 mL ethyl acetate, vortex 1 min, centrifuge at 1250 g for 5 min. Remove the ethyl acetate layer and evaporate at 45° under nitrogen. Dissolve in 100 μL mobile phase, inject 50 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.6 5 μm Spherisorb ODSII
Mobile phase: MeCN:MeOH:buffer 10:9:100 (Buffer was 15.54 g tetraethylammonium hydroxide and 2.9 g 89% orthophosphoric acid in 500 mL water, pH was 2.8.)
Flow rate: 1.2
Injection volume: 50
Detector: F ex 368 em 415 or UV 271

CHROMATOGRAM
Retention time: 7.94
Internal standard: hydroflumethiazide

OTHER SUBSTANCES
Simultaneous: amiloride (detection by F), hydrochlorothiazide (detection by UV)

KEY WORDS
plasma; hydroflumethiazide is IS

REFERENCE
Van der Meer,M.J.; Brown,L.W. Simultaneous determination of amiloride and hydrochlorothiazide in plasma by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *423*, 351–357.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1mL 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 MeOH, sonicate twice at 37° for 1 min, cool at 2-8° for 2 h to obtain a clear solution, inject a 20 μL aliquot. (Buffer was 0.38 g ammonium acetate in 500 mL water and acidified to pH 5.0 with glacial acetic acid.)

HPLC VARIABLES
Guard column: 40 × 4 35-50 μm C18 Corasil
Column: 125 × 4 5 μm 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 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: 12.0
Internal standard: hydroflumethiazide

OTHER SUBSTANCES
Extracted: hydrochlorothiazide
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, pentoxyfylline, phenacetin, phenazone, propyphenazone, phenprocoumon, ranitidine, salicylic acid, sotalol, sulfamethoxazole, trimethoprim, terbutaline, theophylline, tilidine, timolol, triamterene, uric acid, verapamil, ascorbic acid, warfarin, xanthine, purine and pyrimidine bases, nucleosides, nucleotides
Interfering: amiloride

KEY WORDS
plasma; hydroflumethiazide is 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, middle ear fluid
Sample preparation: 75 µL Plasma or middle ear effusion + 50 µL 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 Na_2HPO_4 to the supernatant, inject a 6 µL aliquot.

HPLC VARIABLES
Guard column: 20 × 3.2 Brownlee C8 precolumn
Column: 150 × 4.6 5 µm Zorbax C8
Mobile phase: MeOH:MeCN:10 mM NaH_2PO_4 10:2:88
Column temperature: 40
Flow rate: 1.4
Injection volume: 6
Detector: UV 230

CHROMATOGRAM
Retention time: 6.4
Internal standard: hydroflumethiazide
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Extracted: amoxicillin

KEY WORDS
plasma; chinchilla; hydroflumethiazide is IS

REFERENCE
Erdmann,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: bulk
Sample preparation: Dissolve in solvent, inject an aliquot. (Solvent was 750 mg KCl in 10 mL 1 M HCl, add 400 mL water, add 400 mL MeOH, make up to 1 L with water.)

HPLC VARIABLES
Guard column: 5 × 4 7 µm Nucleosil-100 phenyl

Column: 300 × 4 7 μm Nucleosil-100 phenyl
Mobile phase: MeOH:water 40:60
Column temperature: 35
Flow rate: 1.5
Injection volume: 50
Detector: UV 270

CHROMATOGRAM
Retention time: 3.1

OTHER SUBSTANCES
Simultaneous: bendroflumethiazide, degradation products

REFERENCE
Frontini,R.; Mielck,J.B. Determination and quantitation of bendroflumethiazide and its degradation products using HPLC, *J.Liq.Chromatogr.*, **1992**, *15*, 2519–2528.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 4 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:1% acetic acid 20:80
Flow rate: 1.3
Injection volume: 20 μL
Detector: UV 270

CHROMATOGRAM
Retention time: 5.280

OTHER SUBSTANCES
Simultaneous: hydrochlorothiazide
Noninterfering: captopril

REFERENCE
Papadoyannis,I.N.; Samanidou,V.F.; Georga,K.A.; Georgarakis,E. High performance liquid chromatographic determination of hydrochlorothiazide (HCT) in pharmaceutical preparations and human serum after solid phase extraction, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1671–1683.

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

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 20:1.5:0.5:78
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 2.13

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

SAMPLE
Matrix: urine

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

HPLC VARIABLES

Column: 250 × 4.6 5 μm HP Hypersil ODS (A) or HP LiChrosorb RP-18 (B)
Mobile phase: Gradient. MeCN:buffer from 15:85 at 2 min to 80:20 at 20 min (Buffer was 50 mM NaH_2PO_4 containing 16 mM propylamine hydrochloride, adjusted to pH 3 with concentrated phosphoric acid.)
Flow rate: 1
Injection volume: 5
Detector: UV 230, UV 275

CHROMATOGRAM

Retention time: 8.3 (A), 9.4 (B)
Internal standard: β-hydroxyethyltheophylline (3.7 (A), 4.4 (B))
Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Extracted: furosemide, metolazone, amiloride, acetazolamide, chlorothiazide, hydrochlorothiazide, quinethazone, triamterene, chlorthalidone, dichlorphenamide, trichloromethiazide, methyclothiazide, benzthiazide, cyclothiazide, polythiazide, bendroflumethiazide, ethacrynic acid, bumetanide, probenecid, spironolactone, canrenone, flumethiazide
Noninterfering: acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil

REFERENCE

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

SAMPLE

Matrix: urine
Sample preparation: 1 mL Urine + 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 and acidified to pH 5.0 with glacial acetic acid.)

HPLC VARIABLES

Guard column: 40 × 4 35-50 μm C18 Corasil
Column: 125 × 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: 10.0
Internal standard: hydroflumethiazide

OTHER SUBSTANCES

Extracted: hydrochlorothiazide
Noninterfering: amiloride, 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,

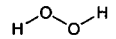
diltiazem, doxepin, doxycycline, enalapril, erythromycin, fenoterol, furosemide, glibenclamide, heparin, hypoxanthine, ibuprofen, indomethacin, isosorbide mononitrate, lisinopril, lovastatin, maprotiline, methyldigoxin, methyldopa, metoclopramide, metoprolol, metronidazole, midazolam, naloxone, nifedipine, nicotine, oxazepam, oxipurinol, penicillin V, pentoxyfylline, phenacetin, phenazone, propyphenazone, phenprocoumon, ranitidine, salicylic acid, sotalol, sulfamethoxazole, trimethoprim, terbutaline, theophylline, tilidine, timolol, triamterene, uric acid, verapamil, ascorbic acid, warfarin, xanthine, purine and pyrimidine bases, nucleosides, nucleotides

Interfering: norfloxacin and ofloxacin

KEY WORDS
hydroflumethiazide is 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.

Hydrogen peroxide



Molecular formula: H_2O_2
Molecular weight: 34.01
CAS Registry No.: 7722-84-1
Merck Index: 4839

SAMPLE
Matrix: beverages
Sample preparation: Mix 3.45 mL 500 mM pH 5.0 potassium phosphate buffer, 250 μL MeOH, and 100 μL antifoaming reagent, pass nitrogen gas through the mixture for a few min, add 1 mL beverage, add 100 μL 1000 U/mL catalase (Boehringer Mannheim) in water (purge with nitrogen before use), add 100 μL 100 mg/mL 4-amino-3-penten-2-one (Fluoral-P, Eastman) in MeCN, bubble nitrogen at 200 mL/min through the mixture, heat at 30° for 10 min, add to a Sep-Pak C18 SPE cartridge, wash with a little water, elute with 5 mL MeCN:water 50:50, inject a 20 μL aliquot of the eluate. (Prepare antifoaming reagent by diluting concentrated silicon polymer (Sigma) with water to give a 0.1% suspension.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeCN:water 50:50
Flow rate: 1
Injection volume: 20
Detector: F ex 410 em 510

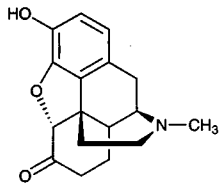
CHROMATOGRAM
Retention time: 3.5
Limit of detection: 50 ppb

OTHER SUBSTANCES
Noninterfering: ascorbic acid

KEY WORDS
derivatization; SPE

REFERENCE
Hamano,T.; Mitsuhashi,Y.; Yamamoto,S. Determination of hydrogen peroxide in beverages by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1987**, *411*, 423–429.

Hydromorphone



Molecular formula: $C_{17}H_{19}NO_3$
Molecular weight: 285.34
CAS Registry No.: 466-99-9, 71-68-1 (HCl)
Merck Index: 4847
Lednicer No.: 1 288

SAMPLE

Matrix: bile, blood, tissue
Sample preparation: 250 μL Bile, 3 mL blood, or 5 mL tissue homogenate + 1 mL 200 μg/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 carefully adding powdered ammonium carbonate until the solution was 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 μL MeOH, vortex for 30 s, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 5 μm RP-18 Spheri-5
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:50 mM pH 7 phosphate buffer 40:60 Place a 70 × 2 30-38 μm 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: 5
Internal standard: nalorphine (14.72)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: codeine, 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.; Sutheimer,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: 1 mL Plasma + 1 mL 1 M sodium bicarbonate + 15 mL diethyl ether, rotate for 20 min, centrifuge at 800 g for 15 min. Remove the organic phase and add it to 200 μL 17 mM phosphoric acid, mix vigorously for 15 s, centrifuge for 5 min. Remove the aqueous phase and evaporate it to dryness under a stream of air at 45°, reconstitute the residue in 200 μL 17 mM phosphoric acid, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 25 × 4.6 5 μm Hi-Chrom reversible octyl (Regis)
Mobile phase: MeCN:30 mM pH 4 KH_2PO_4:0.3% sodium octanesulfonate 15:75:9

Flow rate: 1.5
Injection volume: 100
Detector: E, BAS LC4B, glassy carbon working electrode 0.9 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 8.9
Internal standard: hydromorphone

OTHER SUBSTANCES
Extracted: oxymorphone

KEY WORDS
rat; plasma; hydromorphone is IS

REFERENCE
Lam,G.; Williams,R.M.; Whitney,C.C. Electrochemical determination of oxymorphone in rat plasma by ion-pair reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *413*, 309–314.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Baxter C18 SPE cartridge with 3 mL MeOH and 3 mL water. 1 mL Plasma + 2 mL 500 mM pH 9.3 ammonium sulfate + 30 μL 1 μg/mL naltrexone, add to the SPE cartridge, wash with 3 mL 5 mM pH 9.3 ammonium sulfate, wash with 3 mL water, dry under vacuum, elute with 1 mL MeOH:triethylamine 99.5:0.5. Evaporate the eluate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: C18 (Upchurch)
Column: 100 × 3.2 5 μm Spherisorb C8
Mobile phase: MeOH:50 mM Na_2HPO_4 15:85 containing 3 mM 1-heptanesulfonic acid, pH adjusted to 3.5 with orthophosphoric acid
Flow rate: 0.8
Injection volume: 20
Detector: E, ESA Coulochem, guard cell + 650 mV, analytical cell +250 mV and +600 mV (monitored)

CHROMATOGRAM
Retention time: 8.0
Internal standard: naltrexone (16.4)
Limit of quantitation: 2.5 ng/mL

OTHER SUBSTANCES
Extracted: morphine

KEY WORDS
plasma; SPE

REFERENCE
Bouquillon,A.I.; Freeman,D.; Moulin,D.E. Simultaneous solid-phase extraction and chromatographic analysis of morphine and hydromorphone in plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1992**, *577*, 354–357.

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 μL aliquot

HPLC VARIABLES
Column: μBondapak 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: 5.2

OTHER SUBSTANCES
Simultaneous: hydrocodone, p-aminophenol, acetaminophen, codeine, p-chloroacetanilide

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: formulations
Sample preparation: Dilute 1:5, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4.6 5 μm Spherisorb CN
Mobile phase: MeCN:20 mM KH_2PO_4 50:50, pH adjusted to 5.40 with 1 M NaOH
Flow rate: 1.5
Injection volume: 20
Detector: UV 216

CHROMATOGRAM
Retention time: 7.5

OTHER SUBSTANCES
Simultaneous: morphine, ondansetron

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 βC, *Am.J.Hosp.Pharm.*, **1994**, *51*, 2138–2142.

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

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: MeCN:20 mM KH_2PO_4 adjusted to pH 6.0 with 1 M KOH 50:50
Flow rate: 1
Injection volume: 20
Detector: UV 235

CHROMATOGRAM
Retention time: 10.5
Limit of detection: 253 ng/mL

OTHER SUBSTANCES
Simultaneous: morphine, bupivacaine

KEY WORDS
saline; injections

REFERENCE
Venkateshwaran,T.G.; Stewart,J.T. HPLC determination of morphine-hydromorphone-bupivacaine and morphine-hydromorphone-tetracaine mixtures in 0.9% sodium chloride injection, *J.Liq.Chromatogr.*, **1995**, *18*, 565–578.

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

HPLC VARIABLES
Column: 220 × 4.6 5 μm Brownlee silica (Applied Biosystems)
Mobile phase: MeOH:10 mM KH_2PO_4 adjusted to pH 4.0 with 10% phosphoric acid 25:75
Flow rate: 1
Injection volume: 20
Detector: UV 235

CHROMATOGRAM
Retention time: 8.7
Limit of detection: 338 ng/mL

OTHER SUBSTANCES
Simultaneous: tetracaine, morphine

KEY WORDS
saline; injections

REFERENCE
Venkateshwaran,T.G.; Stewart,J.T. HPLC determination of morphine-hydromorphone-bupivacaine and morphine-hydromorphone-tetracaine mixtures in 0.9% sodium chloride injection, *J.Liq.Chromatogr.*, **1995**, *18*, 565–578.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm cyano
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 1.25
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: 3.72

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: solutions
Sample preparation: Prepare a 10 mg/mL solution in 0.9% sodium chloride, dilute 1:100 with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: Bakerbond C18
Mobile phase: MeOH:buffer 15:85 adjusted to pH 3.5 with o-phosphoric acid (Buffer was 15 mM sodium dihydrogen phosphate containing 3 mM 1-heptanesulfonic acid.)
Flow rate: 0.8
Detector: UV 230

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
stability-indicating

REFERENCE
Stiles,M.L.; Allen,L.V.,Jr.; Prince,S.J. Stability of deferoxamine mesylate, floxuridine, fluorouracil, hydromorphone hydrochloride, lorazepam, and midazolam hydrochloride in polypropylene infusion-pump syringes, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 1583–1588.

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

HPLC VARIABLES
Column: 250 × 4.2 5 μm Ultrasphere C18
Mobile phase: Gradient. A was MeCN containing 1 mg/mL heptanesulfonic acid. B was 50 mM pH 2.2 phosphoric acid containing 1 mg/mL heptanesulfonic acid. A:B 12.5:87.5 for 2.5 min, to 48.5:51.5 over 13.5 min, maintain at 48.5:51.5 for 4 min
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 7.5

OTHER SUBSTANCES
Simultaneous: dexamethasone, diphenhydramine, creatinine, methyl paraben, propyl paraben, degradation products

KEY WORDS
stability-indicating; buffer

REFERENCE
Walker,S.E.; DeAngelis,C.; Iazzetta,J.; Eppel,J.G. Compatibility of dexamethasone sodium phosphate with hydromorphone hydrochloride or diphenhydramine hydrochloride, *Am.J.Hosp.Pharm.*, **1991**, *48*, 2161–2166.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200

mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

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

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

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

Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 5.84 (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, 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, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

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

SAMPLE
Matrix: urine
Sample preparation: 500 μL Urine + N-ethylnordiazepam + chlorpheniramine + 100 μL buffer, centrifuge at 11000 g for 30 s, inject a 500 μL aliquot onto column A with mobile phase A, after 0.6 min backflush column A with mobile phase A to waste for 1.6 min, elute column A with 250 μL mobile phase B, with 200 μL mobile phase C, and with 1.15 mL mobile phase D. Elute column A to waste until drugs start to emerge then elute onto column B. Elute column B to waste until drugs started to emerge, then elute onto column C. When all the drugs have emerged from column B remove it from the circuit, elute column C with mobile phase D, monitor the effluent from column C. Flush column A with 7 mL mobile phase E, with mobile phase D, and mobile phase A. Flush column B with 5 mL mobile phase E then with mobile phase D. (Buffer was 6 M ammonium acetate adjusted to pH 8.0 with 2 M KOH.)

HPLC VARIABLES
Column: A 10 × 2.1 12-20 μm PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 × 3.2 11 μm Aminex A-28 (Bio-Rad); C 25 × 3.2 5 μm C8 (Phenomenex) + 150 × 4.6 5 μm silica (Macherey-Nagel)
Mobile phase: A 0.1% pH 8.0 potassium borate buffer; B 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phos-

phoric acid; C MeCN:buffer 40:60 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); D MeCN:buffer 33:67 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); E MeCN: buffer 70:30 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)
Column temperature: ambient (column A), 40 (columns B and C)
Flow rate: A 5; B-E 1
Injection volume: 500
Detector: UV 210, UV 235

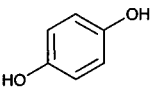
CHROMATOGRAM
Retention time: k' 7.0
Internal standard: N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)
Limit of detection: 300 ng/mL

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

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.

Hydroquinone



Molecular formula: $C_6H_6O_2$
Molecular weight: 110.11
CAS Registry No.: 123-31-9
Merck Index: 4853

SAMPLE
Matrix: air
Sample preparation: Condition a Sep Pak silica SPE cartridge with 10 mL dichloromethane and dry with helium at 5 L/min. Pull air through a 0.80 μm cellulose ester membrane filter and the SPE cartridge at 2 L/min for 1 h, desorb the filter with 5 mL 1% acetic acid with sonication for 10 min, elute the SPE cartridge with 5 mL 1% acetic acid, inject aliquots of the eluates.

HPLC VARIABLES
Guard column: 30 × 4.6 Spheri-5 RP-18
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. A was 1% acetic acid. B was MeCN:acetic acid 99:1. A:B from 0:100 to 90:10 over 10.5 min, to 78:22 to 24.5 min, to 0:100 (step gradient), maintain at 0:100 for 5 min, re-equilibrate for 12 min.
Flow rate: 2
Injection volume: 200
Detector: F ex 304 em 338 for 6.3 min, ex 280 em 325 for 7.7 min, ex 257 em 330 for 5.3 min, ex 342 em 464 for 4.7 min, ex 285 em 310 for 11 min

CHROMATOGRAM
Retention time: 5

Limit of detection: 0.16 μg/cu.m.

OTHER SUBSTANCES
Simultaneous: catechol, cresol, 3-methylcatechol, phenol, scopoletin

KEY WORDS
SPE

REFERENCE
Risner,C.H. The quantification of hydroquinone, catechol, phenol, 3-methylcatechol, scopoletin, m+p-cresol and o-cresol in indoor air samples by high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 4117–4140.

SAMPLE
Matrix: formulations
Sample preparation: Emulsion. 500 μL Emulsion + 10 mL 400 μg/mL hydroquinone in MeOH + 40 mL 0.1% 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: 4.2
Internal standard: hydroquinone
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: carboplatin, epirubicin, iomeprol, mitomycin C

KEY WORDS
emulsions; drug release medium; injections; hydroquinone is IS

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: solutions
Sample preparation: Aqueous food simulants. Pipette 1.0 mL 200 mg/L IS in MeOH into a 25 mL volumetric flask and dilute to the mark with the food simulant obtained from migration experiment, shake. Repeat the procedure to obtain a duplicate sample, filter a portion through a 200 nm membrane filter, inject a 20 μL aliquot. Olive oil simulants. Weigh 25 g olive oil food simulant obtained from migration experiment into a beaker, pour oil into a separating funnel, allow beaker to drain for 30 s. Rinse it with 25 mL hexane and add washes to separating funnel. Add 1.0 mL 200 mg/L IS in MeOH into funnel and mix. Add 10 mL water, shake vigorously by hand for 30 s, allow to stand for 5 min. Collect aqueous phase and reextract oil with a 10 mL water. Combine aqueous extracts, make up to 25 with water, filter the extracts through a small cotton plug to remove any entrained oil. Repeat the procedure to obtain a duplicate sample. Inject a 20 μL aliquot. (Aqueous food simulants were: distilled water, 3% acetic acid in water; EtOH:water 15:85.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil ODS

Mobile phase: MeCN:buffer 15:85 (Prepare mobile phase as follows. Dissolve 7.5 g sodium dihydrogen orthophosphate in 800 mL water, add 150 mL MeCN and adjust to pH 3.6 with glacial acetic acid. Make up to 1000 mL with water.)
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 4.2
Internal standard: 2-methyl-1,3-dihydroxybenzene (7.4)
Limit of detection: 300-500 ng/g

OTHER SUBSTANCES
Extracted: pyrocatechol, resorcinol

KEY WORDS
aqueous food simulants; olive oil simulants

REFERENCE
Philo,M.R.; Jickells,S.M.; Castle,L. Testing for compliance with migration limits: Determination of 1, 2-, 1,3-, and 1,4-dihydroxybenzenes in food-simulating solvents by liquid chromatography, *J.AOAC Int.*, **1996**, *79*, 746–750.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb RP 18
Mobile phase: MeOH:10 mM pH 5.5 potassium phosphate buffer 3.5:96.5
Flow rate: 2-3
Detector: UV 254

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: catechol, phenol, phenyl glucuronide, phenyl glucoside, phenyl galactopyranoside, phenyl sulfate, resorcinol

REFERENCE
Beyer,J.; Frank,G. Hydroxylation and conjugation of phenol by the frog *Rana temporaria*, *Xenobiotica*, **1985**, *15*, 277–280.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: 50 mM pH 3.0 sodium phosphate buffer
Flow rate: 1
Detector: E, ESA, Model 5020 porous graphite analytical cell, T1 0.60 V, T2 0.82 V (monitored), guard cell 0.85 V (before injector)

CHROMATOGRAM
Retention time: 12

OTHER SUBSTANCES
Simultaneous: oxidized glutathione

REFERENCE
O'Gara,C.Y.; Maddipati,K.R.; Marnett,L.J. A sensitive electrochemical method for quantitative hydroperoxide determination, *Chem.Res.Toxicol.*, **1989**, *2*, 295–300.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a solution in 1% acetic acid.

HPLC VARIABLES
Guard column: 30 × 4.6 Spheri-5 RP-18
Column: 250 × 4.6 5 μm Ultrasphere-ODS C18
Mobile phase: Gradient. A was MeCN:acetic acid 99:1. B was 1% acetic acid in water. A:B from 0:100 to 10:90 over 10 min, to 20:80 over 25 min, wash with A for 6 min, re-equilibrate for 14 min.
Flow rate: 2
Injection volume: 20
Detector: F ex 304 em 338

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: catechol (F ex 280 em 325), phenol (ex 274 em 298), resorcinol (F ex 284 em 313)

REFERENCE
Risner,C.H.; Cash,S.L. A high-performance liquid chromatographic determination of major phenolic compounds in tobacco smoke, *J.Chromatogr.Sci.*, **1990**, *28*, 239–244.

SAMPLE
Matrix: urine
Sample preparation: Condition a 500 mg Bond Elut SAX SPE cartridge with 3 mL MeOH and 3 mL water. Dilute 125 μL urine to 4 mL with water, adjust to pH 4.5 with ascorbic acid, add 12.5 μL enzyme solution, heat at 37° for 48 h, add to the SPE cartridge, wash with 3 mL 5 mM pH 7 phosphate buffer. Acidify the eluate to pH <3 with concentrated HCl, add 5 mL ether, vortex, repeat the extraction twice. Combine the organic layers and evaporate them to dryness under reduced pressure at 30°, reconstitute the residue in 1 mL 1% aqueous phosphoric acid, inject a 20 μL aliquot. (The enzyme solutions used to deconjugate glucuronides and sulfate esters were β-glucuronidase/arylsulfatase (Merck, 4114), arylsulfatase (Sigma, S 1629), and β-glucuronidase diluted 1:6 with water (Boehringer, 127051).)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil ODS
Mobile phase: MeOH:5 mM pH 3.4 phosphate buffer 30:70
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 3.2
Limit of detection: 60 μg/mL

OTHER SUBSTANCES
Extracted: catechol, phenol

KEY WORDS
mouse; SPE

REFERENCE
Schad,H.; Schäfer,F.; Weber,L.; Seidel,H.J. Determination of benzene metabolites in urine of mice by solid-phase extraction and high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *593*, 147–151.

SAMPLE
Matrix: urine
Sample preparation: Filter (0.2 μm), inject an aliquot directly. Hydrolyze conjugates by heating with 6 M HCl at 37° for 18 h, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Resolve C18 (Waters)
Mobile phase: MeOH:1.5% trifluoroacetic acid on water 10:90
Flow rate: 0.5
Detector: UV or radioactivity

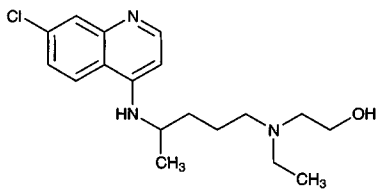
CHROMATOGRAM
Retention time: 1.8

OTHER SUBSTANCES
Extracted: phenol, phenyl glucuronide, phenyl sulfate

KEY WORDS
rat

REFERENCE
Hughes,M.F.; Hall,L.L. Disposition of phenol in rat after oral, dermal, intravenous, and intratracheal administration, *Xenobiotica*, **1995**, *25*, 873–883.

Hydroxychloroquine



Molecular formula: $C_{18}H_{26}ClN_3O$
Molecular weight: 335.88
CAS Registry No.: 118-42-3, 747-36-4 (sulfate)
Merck Index: 4863
Lednicer No.: 1 342

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 500 μL 5 M NaOH + 100 μL 10 μg/mL chloroquine in MeOH + 5 mL hexane:diethyl ether 50:50, vortex for 1 min, centrifuge at 1000 g for 10 min, freeze in dry ice/acetone, remove the organic layer. Thaw out the aqueous layer and repeat the extraction. Combine the organic layers and evaporate them to dryness under vacuum, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 5 μm cyano (Regis)
Column: 75 × 4.6 3 μm Ultremex cyano (Phenomenex)
Mobile phase: 20 mM Dimethyloctylamine phosphate:60 mM ammonium acetate 40:60, pH adjusted to 4.5 (Dimethyloctylamine phosphate was prepared by adding phosphoric acid to N,N-dimethyloctylamine to precipitate the salt.)
Flow rate: 0.6
Injection volume: 50
Detector: UV 320

CHROMATOGRAM
Retention time: 15
Internal standard: chloroquine (23)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

SAMPLE
Matrix: urine
Sample preparation: Filter (0.2 μm), inject an aliquot directly. Hydrolyze conjugates by heating with 6 M HCl at 37° for 18 h, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Resolve C18 (Waters)
Mobile phase: MeOH:1.5% trifluoroacetic acid on water 10:90
Flow rate: 0.5
Detector: UV or radioactivity

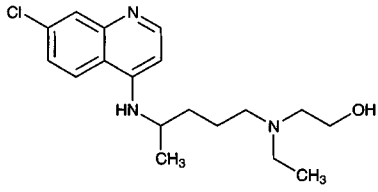
CHROMATOGRAM
Retention time: 1.8

OTHER SUBSTANCES
Extracted: phenol, phenyl glucuronide, phenyl sulfate

KEY WORDS
rat

REFERENCE
Hughes,M.F.; Hall,L.L. Disposition of phenol in rat after oral, dermal, intravenous, and intratracheal administration, *Xenobiotica*, **1995**, *25*, 873–883.

Hydroxychloroquine



Molecular formula: $C_{18}H_{26}ClN_3O$
Molecular weight: 335.88
CAS Registry No.: 118-42-3, 747-36-4 (sulfate)
Merck Index: 4863
Lednicer No.: 1 342

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 500 μL 5 M NaOH + 100 μL 10 μg/mL chloroquine in MeOH + 5 mL hexane:diethyl ether 50:50, vortex for 1 min, centrifuge at 1000 g for 10 min, freeze in dry ice/acetone, remove the organic layer. Thaw out the aqueous layer and repeat the extraction. Combine the organic layers and evaporate them to dryness under vacuum, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 5 μm cyano (Regis)
Column: 75 × 4.6 3 μm Ultremex cyano (Phenomenex)
Mobile phase: 20 mM Dimethyloctylamine phosphate:60 mM ammonium acetate 40:60, pH adjusted to 4.5 (Dimethyloctylamine phosphate was prepared by adding phosphoric acid to N,N-dimethyloctylamine to precipitate the salt.)
Flow rate: 0.6
Injection volume: 50
Detector: UV 320

CHROMATOGRAM
Retention time: 15
Internal standard: chloroquine (23)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; rabbit; pharmacokinetics

REFERENCE
Iredale,J.; Wainer,I.W. Determination of hydroxychloroquine and its major metabolites in plasma using sequential achiral-chiral high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *573*, 253–258.

SAMPLE
Matrix: blood
Sample preparation: Serum. 200 μL Serum + 50 μL 1 μg/mL IS in water + 50 μL 4 M NaOH + 200 μL MTBE, vortex for 30 s, centrifuge at 9950 g for 2 min, inject 100 μL of the organic layer. Whole blood. 100 μL Whole blood + 500 μL water + 50 μL 1 μg/mL IS in water + 50 μL 4 M NaOH + 200 μL MTBE, vortex for 30 s, centrifuge at 9950 g for 2 min, inject 100 μL of the organic layer. Dried blood. Spread 100 μL whole blood on a 70 × 30 mm piece of filter paper, allow to dry, cut paper into 10 × 5 mm strips, add 100 μL 1 μg/mL IS in water, add 1.5 mL 0.5 M NaOH, vortex for 30 s, let stand for 30 min at room temperature, add 300 μL MTBE, vortex for 30 s, centrifuge at 2000 g for 5 min, inject a 100 μL aliquot of the organic layer.

HPLC VARIABLES
Column: 150 × 5 5 μm Spherisorb S5SCX sulfophenylpropyl-modified silica
Mobile phase: MeOH:water 98.5:1.5 containing 9.41 g/L ammonium perchlorate, adjust apparent pH to 8.0 with 220 mL/L 50 mM NaOH in MeOH
Flow rate: 1.5
Injection volume: 100
Detector: F ex 215 em no filter

CHROMATOGRAM
Retention time: 8
Internal standard: 6,8-dichloro-4-(1-methyl-4-diethylaminobutylamino)quinoline (5)
Limit of quantitation: 5 ng/mL (serum), 10 ng/mL (whole blood, dried blood)

OTHER SUBSTANCES
Extracted: chloroquine, quinine, metabolites
Simultaneous: acebutolol, N-acetylprocainamide, atenolol, butriptyline, chlorpromazine, desipramine, flecainide, fluoxetine, imipramine, labetalol, maprotiline, mepacrine, metoprolol, mexiletine, norbutriptyline, normaprotiline, procainamide, propranolol, sotalol
Noninterfering: amitriptyline, amodiaquin, carbamazepine, clomipramine, dapsone, diazepam, dothiepin, doxepin, fluvoxamine, lorazepam, mefloquine, nitrazepam, norclomipramine, nordiazepam, nordothiepin, nordoxepin, nortriptyline, primaquine, proguanil, pyrimethamine

KEY WORDS
serum; whole blood; dried blood

REFERENCE
Croes,K.; McCarthy,P.T.; Flanagan,R.J. Simple and rapid HPLC of quinine, hydroxychloroquine, chloroquine, and desethylchloroquine in serum, whole blood, and filter paper-adsorbed dry blood, *J.Anal.Toxicol.*, **1994**, *18*, 255–260.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Whole blood + 2 mL 20 ng/mL propranolol hydrochloride in water, vortex for 10 s, sonicate for 10 min, centrifuge at 3000 rpm for 20 min. Remove 2 mL of the supernatant and add it to 1 mL 100 mM NaOH and 8 mL ethyl acetate:isopropanol 90:10, vortex for 30 s, centrifuge at 3000 rpm for 15 min. Remove the organic layer and evaporate it to dryness under a stream of air at 35-40°, reconstitute the residue in 250 μL MeOH, vortex for 30 s, inject a 10-100 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 3 7 μm cyano (Applied Biosystems)
Column: 250 × 4.6 5 μm cyanopropyl (Baxter/Burdick & Jackson)

Mobile phase: MeCN:MeOH:buffer 50:30:20 (Buffer was 25 mM K_2HPO_4 adjusted to pH 6.0 with phosphoric acid.)
Column temperature: 50
Flow rate: 2
Injection volume: 10-100
Detector: F ex 230 em 385 (370 nm cut-off filter)

CHROMATOGRAM
Retention time: 9.72
Internal standard: propranolol (4)
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
whole blood

REFERENCE
Wei,Y.; Nygard,G.A.; Khalil,S.K.W. A HPLC method for the separation and quantification of the enantiomers of hydroxychloroquine and its three major metabolites, *J.Liq.Chromatogr.*, **1994**, *17*, 3479–3490.

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

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 222

CHROMATOGRAM
Retention time: 4.65
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acen-

ocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

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

SAMPLE

Matrix: blood

Sample preparation: 1 mL Serum + 100 μL water containing 5 μg/mL 2,3-diaminonaphthalene and 3.5 μg/mL 18-hydroxy-11-deoxycorticosterone + 1 mL 250 mM NaOH + 7 mL diethyl ether, shake on a rotary shaker for 15 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 30-40°, reconstitute the residue in 70 μL MeOH:100 mM perchloric acid 50:50, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18

Mobile phase: Gradient. A was 58 mM NaH_2PO_4 containing 6 mM sodium heptanesulfonate, adjusted to pH 3.1 with concentrated phosphoric acid. B was MeCN:MeOH 85:15. A:B from 100:0 to 78:22 over 5 min, to 70:30 over 12 min, maintain at 70:30 for 4 min, to 65:35 over 9 min.

Flow rate: 1

Injection volume: 20

Detector: UV 245, 256, 343

CHROMATOGRAM

Retention time: 11.93

Internal standard: 2,3-diaminonaphthalene (10.71), 18-hydroxy-11-deoxycorticosterone (15.85)

Limit of detection: 2 ng/mL (343 nm)

OTHER SUBSTANCES

Extracted: betamethasone, chloroquine, corticosterone, cortisone, dexamethasone, fluocinolone acetonide, fluendrenolide, fluorometholone, fluprednisolone, hydrocortisone, 17δ-hydroxyprogesterone, meprednisone, methylprednisolone, methylprednisolone acetate, paramethasone, prednisolone, prednisone, progesterone, triamcinolone

Noninterfering: aspirin, ibuprofen, indomethacin, phenylbutazone, pregnenolone

KEY WORDS

serum

REFERENCE

Volin,P. Simple and specific reversed-phase liquid chromatographic 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, erythrocytes, urine

Sample preparation: Condition a 3 mL Bond Elut C8 SPE cartridge with 2 mL MeOH and 2 mL buffer. Hemolyze erythrocytes in water 1:3. Dilute urine with water 1:99. Add 1 mL plasma, hemolyzed erythrocytes, or diluted urine to the SPE cartridge, wash with 4 mL buffer, wash with 2 mL MeOH:buffer 50:50, elute with 3 mL MeOH:ammonia 99:1. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in the initial mobile phase, vortex, inject a 50 μL aliquot. (Prepare buffer by mixing equal volumes of 100 mM ammonium formate and 100 mM ammonia solution, pH 9.2.)

HPLC VARIABLES

Guard column: 10 × 4 Inertsil
Column: 250 × 4 5 μm Inertsil
Mobile phase: Gradient. A was MeCN. B was MeOH:25% ammonia solution 92.5:7.5. A:B 78:22 for 3 min, then to 65:35 over 2 min (Waters curve no. 3), maintain at 65:35 for 20 min, return to 78:22 over 5 min (Waters curve no. 3).
Flow rate: 0.85
Injection volume: 50
Detector: F ex 325 em 375

CHROMATOGRAM

Retention time: 11.5
Internal standard: hydroxychloroquine

OTHER SUBSTANCES

Extracted: chloroquine, quinine, monodesethylchloroquine, bidesethylchloroquine
Simultaneous: halofantrine, quinidine
Noninterfering: proguanil, cycloguanil, 4-chlorophenylbiguanide, amodiaquine, mefloquine, pyrimethamine, sulfadoxine, cinchonine, cinchonidine

KEY WORDS

SPE; hydroxychloroquine is IS; plasma

REFERENCE

Chaulet,J.-F.; Robet,Y.; Prevosto,J.-M.; Soares,O.; Brazier,J.-L. Simultaneous determination of chloroquine and quinine in human biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *613*, 303–310.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject an aliquot.

HPLC VARIABLES

Guard column: Chiral-AGP
Column: 150 × 4.6 Chiral-AGP (Regis)
Mobile phase: MeCN:EtOH:30 mM pH 7.0 sodium phosphate buffer 1:20:79 containing 5 mM N,N-dimethyloctylamine
Flow rate: 0.9
Injection volume: 50
Detector: UV 320

CHROMATOGRAM

Retention time: 10 (-), 14 (+)
Limit of detection: 5 ng

KEY WORDS

chiral

REFERENCE

Iredale,J.; Wainer,I.W. Determination of hydroxychloroquine and its major metabolites in plasma using sequential achiral-chiral high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *573*, 253–258.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 10-100 μL aliquot.

HPLC VARIABLES
Column: 100 × 4 5 μm Chiral-AGP (ChromTech)
Mobile phase: MeCN:isopropanol:buffer 1:5:94 (Buffer was 50 mM $(NH_4)H_2PO_4$ containing 5 mM dihexylamine, pH adjusted to 7.0 with 3 M NaOH.)
Column temperature: 40
Flow rate: 1
Injection volume: 10-100
Detector: F ex 230 em 385 (370 nm cut-off filter)

CHROMATOGRAM
Retention time: 10 (S(+)), 13 (R(-))

KEY WORDS
chiral

REFERENCE
Wei,Y.; Nygard,G.A.; Khalil,S.K.W. A HPLC method for the separation and quantification of the enantiomers of hydroxychloroquine and its three major metabolites, *J.Liq.Chromatogr.*, **1994**, *17*, 3479–3490.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 5 Spherisorb S5SCX
Mobile phase: MeOH:water 98.5:1.5 containing 80 mM ammonium perchlorate, adjusted to pH 8.0 with 50 mM NaOH in MeOH
Flow rate: 1.5
Detector: F ex 215 no emission filter

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: hydroquinine, quinine, chloroquine

REFERENCE
Croes,K.; McCarthy,P.T.; Flanagan,R.J. HPLC of basic drugs and quaternary ammonium compounds on microparticulate strong cation-exchange materials using methanolic or aqueous methanol eluents containing an ionic modifier, *J.Chromatogr.A*, **1995**, *693*, 289–306.

SAMPLE
Matrix: solutions

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

CHROMATOGRAM
Retention time: 9.60 (A), 3.23 (B)

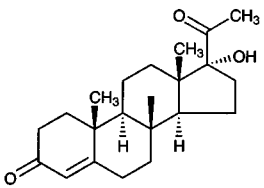
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine,

clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, 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, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
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.

Hydroxyprogesterone



Molecular formula: $C_{21}H_{30}O_3$
Molecular weight: 330.47
CAS Registry No.: 68-96-2
Merck Index: 4886
Lednicer No.: 1 176

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: 2.5 (17-hydroxyprogesterone)
Internal standard: dexamethasone (11.5)
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Extracted: corticosterone, 11-deoxycortisol, hydrocortisone, 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
Sample preparation: Condition a Bond-Elut C18 SPE cartridge with MeOH and water. Add 500 μL plasma to the SPE cartridge, wash with 2 mL water, wash with 2 mL MeOH:water 20:80, elute with two 500 μL aliquots of MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 50 μL MeOH:water 20:80, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 1 5 μm Hypersil ODS
Mobile phase: MeCN:MeOH:water 25:25:50
Flow rate: 0.1
Injection volume: 20
Detector: UV (wavelength not given)

CHROMATOGRAM
Retention time: 8 (17α-hydroxyprogesterone)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Extracted: androstenedione, 20α-hydroxy-4-pregnen-3-one, norethindrone, 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: 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: 36.52
Internal standard: 3,7-dimethoxyflavone (47)

OTHER SUBSTANCES
Extracted: aldosterone, androstenedione, dehydroepiandrosterone, deoxycorticosterone, 11-deoxycortisol, estradiol, estrone, hydrocortisone, 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: 1 mL Serum + 100 μL water containing 5 μg/mL 2,3-diaminonaphthalene and 3.5 μg/mL 18-hydroxy-11-deoxycorticosterone + 1 mL 250 mM NaOH + 7 mL diethyl ether, shake on a rotary shaker for 15 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 30-40°, reconstitute the residue in 70 μL MeOH:100 mM perchloric acid 50:50, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A was 58 mM NaH_2PO_4 containing 6 mM sodium heptanesulfonate, adjusted to pH 3.1 with concentrated phosphoric acid. B was MeCN:MeOH 85:15. A:B from 100:0 to 78:22 over 5 min, to 70:30 over 12 min, maintain at 70:30 for 4 min, to 65:35 over 9 min.
Flow rate: 1
Injection volume: 20
Detector: UV 245, 256, 343

CHROMATOGRAM
Retention time: 29.26 (17δ-hydroxyprogesterone)
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, fluendrenolide, fluocinolone acetonide, fluorometholone, fluprednisolone, hydrocortisone, hydroxychloroquine, 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: formulations
Sample preparation: Make up 1 mL injection to 100 mL with EtOH, remove a 1.2 mL aliquot and add it to 1 mL 1 mg/mL 17-hydroxyprogesterone in EtOH. Dilute this mixture to 50 mL with EtOH, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak CN
Mobile phase: MeOH:20 mM KH_2PO_4 40:60
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7 (hydroxyprogesterone caproate)
Internal standard: 17-hydroxyprogesterone (3)

OTHER SUBSTANCES
Simultaneous: benzyl benzoate
Noninterfering: polyethylene glycol 4000, mystristyl-gamma-picolinium chloride, methylcellulose, thimerosal

KEY WORDS
injections

REFERENCE
Das Gupta,V. Quantitation of hydroxyprogesterone caproate, medroxyprogesterone acetate, and progesterone by reversed-phase high-pressure liquid chromatography, *J.Pharm.Sci.*, **1982**, *71*, 294–297.

SAMPLE
Matrix: formulations
Sample preparation: Injections. 1 mL Injection (200 μg/mL) + 300 mg NaCl + 200 μL 10% ammonia + 5 mL dichloromethane, shake vigorously for 10 min, let stand for a few min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 4 mL water, mix an aliquot with an equal volume of 30 μg/mL 17α-hydroxyprogesterone in MeOH, inject a 20 μL aliquot. Tablets. Weigh out amount of powdered tablets equivalent to about 200 μg compound, add 1 mL water, sonicate for 2 min, add 300 mg NaCl, add 200 μL 10% ammonia, add 5 mL dichloromethane, shake vigorously for 10 min, let stand for a few min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 4 mL water, mix an aliquot with an equal volume of 30 μg/mL 17α-hydroxyprogesterone in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 5 μm LiChrosorb RP-18
Mobile phase: MeCN:50 mM pH 3.5 acetate buffer 40:60 containing 1.5 mM triethylamine
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 12
Internal standard: 17α-hydroxyprogesterone

OTHER SUBSTANCES
Simultaneous: benzyl alcohol, ergonovine, methylergonovine
Noninterfering: ascorbic acid

KEY WORDS
injections; tablets; 17α-hydroxyprogesterone is IS

REFERENCE
Tokunaga,H.; Kimura,T.; Kawamura,J. Determination of ergometrine maleate and methylergometrine maleate in pharmaceutical preparations by high-performance liquid chromatography, *Chem.Pharm.Bull.(Tokyo)*, **1983**, *31*, 3988–3993.

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 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak CN

Mobile phase: MeCN:0.1% phosphoric acid in water 35:65
Flow rate: 3
Injection volume: 25
Detector: UV 225

CHROMATOGRAM
Retention time: 8 (hydroxyprogesterone caproate)
Internal standard: hydroxyprogesterone caproate

OTHER SUBSTANCES
Simultaneous: levothyroxine

KEY WORDS
tablets; hydroxyprogesterone caproate is IS

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: ileostomy effluent
Sample preparation: Dilute ileostomy effluent 1:2 by weight with water. Extract 3 g aliquot three times with 10 mL dichloromethane by shaking for 1 min and centrifuging at 2000 rpm for 2 min. Wash combined extracts successively with 2 mL 0.1 M NaOH and 4 mL water by shaking for 30 s and centrifuging for 1 min then dry the organic layer under air at 40°. Take up the extract in 1 mL MeOH, add 1.1 mL water and apply to C18 Bond Elut SPE cartridge. Wash with 10 mL water, wash with 5 mL MeOH:water 45:55, elute with 2 mL MeOH. Add 50 μL 20 μg/mL progesterone to the eluate, dry at 40°, take up in 100 μL MeOH, inject 10 μL aliquot.

HPLC VARIABLES
Guard column: Bondapak C18/Corasil
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:50 mM pH 3.0 sodium phosphate buffer 55:45
Flow rate: 3
Injection volume: 10
Detector: UV 254 and 238

CHROMATOGRAM
Retention time: 6.0
Internal standard: progesterone (11.6)

OTHER SUBSTANCES
Extracted: beclomethasone alcohol, beclomethasone 17-monopropionate, beclomethasone dipropionate

KEY WORDS
SPE; 17-hydroxyprogesterone is IS

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 5 μm RP-18 C18 (Brownlee)
Mobile phase: MeCN:MeOH:water 30:30:40

Injection volume: 20
Detector: UV 254

KEY WORDS
for 17α-hydroxyprogesterone

REFERENCE
Kane,M.P.; Tsuji,K. Radiolytic degradation scheme for ^{60}Co-irradiated corticosteroids, *J.Pharm.Sci.*, **1983**, *72*, 30–35.

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: 14.6 (17α)

OTHER SUBSTANCES
Simultaneous: cortisone, estriol, cortisol, corticosterone, 11-deoxycortisol, androstenedione, prednisone acetate, 11-deoxycorticosterone, testosterone, dexamethasone acetate, estradiol, estrone, progesterone

REFERENCE
Wei,J.Q.; Wei,J.L.; Zhou,X.T. Optimization of an isocratic reversed phase liquid chromatographic system for the separation of fourteen steroids using factorial design and computer simulation, *Biomed.Chromatogr.*, **1990**, *4*, 3–38.

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' 2.175

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), lynestrenol (UV 210), medroxyprogesterone acetate (UV 240), medroxyprogesterone (UV 240), methandienone (UV 240), methylandrostenediol (UV 210), methylprednisolone acetate (UV 240), methylprednisolone (UV 240), methyltestosterone (UV 240), nandrolone (UV 240), norethisterone (UV 240), prednisolone acetate (UV 240), prednisolone (UV 240), prednisone (UV 240), pregnenolone (UV 210), progesterone (UV 240), testosterone (UV 240)

REFERENCE
Sadlej-Sosnowska,N. Structure retention relationship for steroid hormones. Functional groups as structural descriptors, *J.Liq.Chromatogr.*, **1994**, *17*, 2319–2330.

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

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil 5-ODS
Mobile phase: THF:water 23:77
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 245

CHROMATOGRAM
Retention time: k' 11.86 (11α-hydroxyprogesterone)
Internal standard: methylprednisolone (k' 11.36)

OTHER SUBSTANCES
Simultaneous: metabolites, betamethasone, corticosterone, cortisone, deflazacort, deoxycorticosterone, dexamethasone, fludrocortisone, fludrocortisone acetate, fluorocortisone, fluorocortisone acetate, hydrocortisone, 21-hydroxydeflazacort, methylprednisolone, prednisolone, prednisone, triamcinolone acetonide, triamcinolone

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 × 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: 9.7

OTHER SUBSTANCES
Simultaneous: testosterone, estradiol, norethisterone, hydrocortisone, estriol, 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: 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 form 100:0 to 70:30 over 20 min, to 0:100 over 20 min

Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 25 (17-hydroxyprogesterone)

OTHER SUBSTANCES
Extracted: androstenedione, deoxycortisol, hydrocortisone, testosterone
Simultaneous: estriol, estradiol, pregnenolone, progesterone, testosterone enanthate, testosterone propionate

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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: 3 mL Urine + 1.5 μg betamethasone + 100 mg K_2HPO_4 + 500 mg anhydrous sodium sulfate + 5 mL diethyl ether, shake mechanically for 10 min, centrifuge at 2500 g for 5 min. Remove the organic layer and evaporate it to dryness under vacuum, reconstitute the residue in 200 μL MeOH, filter (0.45 μm), inject a 15 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil ODS
Mobile phase: Gradient. MeCN:water from 4:96 to 30:70 over 10 min, to 45:55 over 5 min, to 50:50 over 3 min
Column temperature: 40
Flow rate: 1
Injection volume: 15
Detector: UV 246

CHROMATOGRAM
Retention time: 14.66
Internal standard: betamethasone (12.83)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: corticosterone, cortisone, deoxycorticosterone, hydrocortisone, prednisolone, prednisone, triamcinolone, triamcinolone acetonide

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REFERENCE
Park,S.-J.; Kim,Y.-J.; Pyo,H.-S.; Park,J. Analysis of corticosteroids in urine by HPLC and thermospray LC/MS, *J.Anal.Toxicol.*, **1990**, *14*, 102–108.

SAMPLE
Matrix: urine
Sample preparation: 3 mL Urine + 0.25 g NaCl, adjust pH to 9.0 with 0.5 g Na_2HPO_4, add 4 mL dichloromethane, vortex 1 min, centrifuge at 3700 g for 3 min. Remove organic phase and dry it over anhydrous sodium sulfate. Evaporate a 3 mL aliquot to dryness under vacuum, reconstitute residue with 200 μL 5 μg/mL IS in MeOH, inject a 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: 19
Internal standard: methylprednisolone (9)

OTHER SUBSTANCES
Simultaneous: triamcinolone, triamcinolone acetonide, prednisolone, corticosterone, hydroxyprogesterone, fluorocortisone acetate, cortisone, hydrocortisone, fluorocortisone, betamethasone, dexamethasone, 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.

Hydroxypropyl methylcellulose

CAS Registry No.: 9004-65-3
Merck Index: 4889

SAMPLE
Matrix: formulations
Sample preparation: Dilute with an equal volume of MeOH:water 40:60, inject an 80 μL aliquot.

HPLC VARIABLES
Column: 300 × 7.8 Ultrahydrogel 250 250 Å cross-linked methacrylate gel (waters)
Mobile phase: MeOH:buffer 20:80 (Buffer was 95 mM boric acid containing 10 mM KCl, 13 μM sodium borate, and 1.5 mM dextrose.) (At the end of each set of analyses rinse instrument and column with 0.5% sodium azide. (Caution! Sodium azide is carcinogenic, highly toxic, and can form explosive heavy metal azides. Do not discharge to the plumbing system!)
Column temperature: 35
Flow rate: 1
Injection volume: 80
Detector: RI

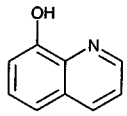
CHROMATOGRAM
Retention time: 10.6

OTHER SUBSTANCES
Simultaneous: PEG 400

KEY WORDS
ophthalmic solutions; SEC

REFERENCE
Delker,G.; Chen,C.; Miller,R.B. Size exclusion chromatographic determination of hydroxypropyl methyl cellulose and polyethylene glycol 400 in an ophthalmic solution, *Chromatographia*, **1995**, *41*, 263–266.

Hydroxyquinoline



Molecular formula: C_9H_7NO
Molecular weight: 145.16
CAS Registry No.: 148-24-3
Merck Index: 4890

SAMPLE

Matrix: solutions

HPLC VARIABLES

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

OTHER SUBSTANCES

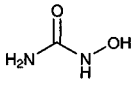
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine,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

metin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

Hydroxyurea



Molecular formula: $CH_4N_2O_2$
Molecular weight: 76.06
CAS Registry No.: 127-07-1
Merck Index: 4896

SAMPLE

Matrix: blood
Sample preparation: Mix 500 μL serum, 20 μL 70% perchloric acid, and 20 μL 15.5 mM methylurea, centrifuge at 12000 g for 5 min. Mix 200 μL supernatant with 700 μL BUN acid reagent (No. 535-3, Sigma) and 600 μL BUN color reagent (No. 535-5, Sigma), place the mixture in a boiling water bath to form colored complexes. After 10 min cool the mixture in ice water, inject a 25-100 μL aliquot of the colored solution.

HPLC VARIABLES

Column: 300 × 3.9 Bondclone 10 C18 (Phenomenex)
Mobile phase: MeCN:water 13:87
Flow rate: 1.7
Injection volume: 25-100
Detector: UV 449

CHROMATOGRAM

Retention time: 6.5
Internal standard: methylurea (12.2)

KEY WORDS

serum; derivatization

REFERENCE

Manouilov,K.K.; McGuire,T.R.; Gwilt,P.R. Colorimetric determination of hydroxyurea in human serum using high-performance liquid chromatography, *J.Chromatogr.B*, **1998**, *708*, 321–324.

SAMPLE

Matrix: blood
Sample preparation: Vortex plasma with two volumes of 5% trichloroacetic acid for 30 s, centrifuge at 7000 g for 5 min, inject a 5 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 100 × 3.2 3 μm Phase-II ODS (Bioanalytical Systems)
Mobile phase: 50 mM sodium acetate containing 5 mM tetrabutylammonium hydroxide, adjusted to pH 6.75 ± 0.02 with 50 mM acetic acid
Flow rate: 0.5
Injection volume: 5
Detector: E, Bioanalytical Systems LC-4B, glassy carbon electrode + 800 mV, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 1.1 (elute for 5 min to remove plasma components)

Limit of detection: 20 μM

OTHER SUBSTANCES
Noninterfering: acetaminophen, caffeine, carbamazepine, ethosuximide, morphine, phenobarbital, phenytoin, salicylic acid, valproic acid

KEY WORDS
plasma

REFERENCE
Havard,J.; Grygiel,J.; Sampson,D. Determination by high-performance liquid chromatography of hydroxyurea in human plasma, *J.Chromatogr.*, **1992**, *584*, 270–274.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 200 μL trichloroacetic acid, vortex for 30 s, centrifuge at 5000 g for 5 min, pass the supernatant through a 1 mL Bond Elut C18 SPE cartridge, inject an aliquot of the eluate.

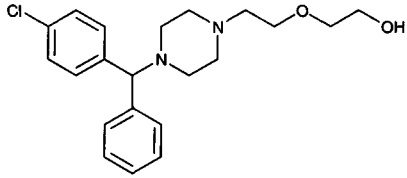
HPLC VARIABLES
Guard column: 20 mm long Supelguard LC 18 (Supelco)
Column: 150 × 4.6 Supelcosil LC 18
Mobile phase: 50 mM Sodium acetate adjusted to pH 6.75 with acetic acid
Flow rate: 0.8
Detector: E, ESA Coulochem II, Model 5020 guard cell 650 mV, Model 5011 analytical cell 600 mV

CHROMATOGRAM
Limit of detection: 600 nM
Limit of quantitation: 1.3 μM

KEY WORDS
pharmacokinetics; serum; SPE

REFERENCE
Villani,P.; Maserati,R.; Regazzi,M.B.; Giacchino,R.; Lori,F. Pharmacokinetics of hydroxyurea in patients infected with human immunodeficiency virus type I, *J.Clin.Pharmacol.*, **1996**, *36*, 117–121.

Hydroxyzine



Molecular formula: $C_{21}H_{27}ClN_2O_2$
Molecular weight: 374.91
CAS Registry No.: 68-88-2, 2192-20-3 (di HCl), 10246-75-0 (pamoate)
Merck Index: 4897
Lednicer No.: 1 59, 4 118

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 100 μL 1 μg/mL triprolidine + 250 μL 10% KOH + 5 mL ether, vortex, centrifuge. Remove ether layer and add it to 100 μL 0.5% phosphoric acid, vortex, centrifuge, remove most of ether layer and discard it, remove traces of ether by nitrogen at room temperature for 2-3 min, inject all of aqueous layer.

HPLC VARIABLES
Column: Waters CN reverse-phase radial compression

Mobile phase: MeCN:buffer 27:73 (Buffer was 75 mM pH 3.0 phosphate buffer containing 20 mM dibutylamine and 50 ng/mL triprolidine.)
Flow rate: 1
Injection volume: 100
Detector: UV 229

CHROMATOGRAM
Retention time: 6.9
Internal standard: triprolidine (3.6)
Limit of detection: 3 ng/mL

KEY WORDS
serum

REFERENCE
Simons,F.E.R.; Simons,K.J.; Frith,E.M. The pharmacokinetics and antihistaminic of the H_1 receptor antagonist hydroxyzine, *J.Allerg.Clin.Immunol.*, **1984**, *73*, 69–75.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 50 μL 3 μg/mL IS + 1 mL 1 M pH 5.0 sodium citrate buffer + 3 mL ethyl acetate, vortex 1 min, centrifuge at 4000 rpm for 15 min, remove organic layer, repeat extraction. Combine organic layers and add 200 μL 1.7% phosphoric acid, vortex 1 min, centrifuge 5 min, remove and discard ethyl acetate layer, remove traces of ethyl acetate from aqueous layer using a stream of nitrogen, inject.

HPLC VARIABLES
Column: radial 4 μm NovoPak C18 radial compression
Mobile phase: MeCN:buffer 46:54 (Buffer was 10 mM pH 2.9 KH_2PO_4 + 20 mM sodium 1-decanesulfonate.)
Flow rate: 1.4
Detector: UV 229

CHROMATOGRAM
Retention time: 3.6
Internal standard: P-265, an ethoxy derivative of cetirizine (6.8)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Simultaneous: cetirizine

KEY WORDS
serum

REFERENCE
Simons,K.J.; Watson,W.T.A.; Chen,X.Y.; Simons,F.E.R. Pharmacokinetic and pharmacodynamic studies of the H_1-receptor antagonist hydroxyzine in the elderly, *Clin.Pharmacol.Ther.*, **1989**, *45*, 9–14.

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

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

Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 230

CHROMATOGRAM

Retention time: 8.62
Limit of detection: <120 ng/mL

KEY WORDS

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

REFERENCE

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

SAMPLE

Matrix: blood, CSF
Sample preparation: Plasma. Centrifuge blood at 7000 rpm, decant 100 μL plasma. Mix 100 μL plasma with 200 μL acetone, centrifuge at 7000 rpm for 5 min. Evaporate the supernatant under a stream of nitrogen, reconstitute the residue with mobile phase, inject an aliquot. CSF. Add 25 μL water to 25 μL CSF, mix with 50 μL acetone, centrifuge at 7000 rpm for 5 min. Evaporate the supernatant under a stream of nitrogen, reconstitute the residue with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: Regis SPS phenyl
Mobile phase: MeCN:pH 3.0 water 21:79 containing 9 mM decanesulfonic acid
Column temperature: 60

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Chou,K.-J.; Donovan,M.D. Distribution of antihistamines into the CSF following intranasal delivery, *Biopharm.Drug Dispos.*, **1997**, *18*, 335–346.

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

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

CHROMATOGRAM
Retention time: 15.267

KEY WORDS
whole blood

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

SAMPLE
Matrix: bulk
Sample preparation: Prepare solutions in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 120 × 4.0 5 μm Lichrosorb RP-18
Mobile phase: MeCN:buffer 30:70 (Buffer was 9.8% triethylamine and 0.35% sodium methanesulfonate with pH adjusted to 2.85 with sulfuric acid.)
Flow rate: 1
Detector: UV 230

CHROMATOGRAM
Retention time: 12.3
Limit of detection: 180 ng/mL
Limit of quantitation: 600 ng/mL

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE

Simpson,D.; Skellern,G.G.; Miller,J.H.MB. Method for the control of known impurities in hydroxyzine hydrochloride, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 1371–1375.

SAMPLE

Matrix: formulations
Sample preparation: 5 mL Formulation + 5 mL IS solution, make up to 50 mL with MeOH, inject 10 μL aliquot. (IS solution was 0.2 mg p-nitroacetophenone and 2.5 mg isobutyrophenone per mL of MeOH.)

HPLC VARIABLES

Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:water:reagent 25:60:15, pH 2.6 (Reagent was MeOH containing 0.06% sulfuric acid, 0.5% sodium sulfate, and 0.02% sodium heptanesulfonate.)
Flow rate: 2
Injection volume: 10
Detector: UV 257

CHROMATOGRAM

Retention time: 9
Internal standard: p-nitroacetophenone (6) and isobutyrophenone (13)

OTHER SUBSTANCES

Simultaneous: benzyl alcohol, benzoic acid, benzaldehyde, p-chlorobenzoic acid, p-chlorobenzaldehyde, p-chlorobenzophenone

KEY WORDS

injections; stability-indicating

REFERENCE

Menon,G.N.; Norris,B.J. Simultaneous determination of hydroxyzine hydrochloride and benzyl alcohol in injection solutions by high-performance liquid chromatography, *J.Pharm.Sci.*, **1981**, *70*, 697–698.

SAMPLE

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

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 2.2

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, er-

gosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

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

SAMPLE

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

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 58:1.5:0.5:40
Flow rate: 1.5
Injection volume: 10
Detector: UV 230

CHROMATOGRAM

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

HPLC VARIABLES

Column: 250 × 4.6 5 μm Lichrosphere cyanopropyl
Mobile phase: Carbon dioxide:MeOH:isopropylamine 90:10:0.05
Column temperature: 50
Flow rate: 3
Injection volume: 5
Detector: UV 220

CHROMATOGRAM

Retention time: 2.42

OTHER SUBSTANCES
Simultaneous: benactyzine, buclizine, perphenazine, thioridazine, amitriptyline, desipramine, imipramine, nortriptyline, protriptyline

KEY WORDS
SFC; pressure 200 bar

REFERENCE
Berger,T.A.; Wilson,W.H. Separation of drugs by packed column supercritical fluid chromatography. 2. Antidepressants, *J.Pharm.Sci.*, **1994**, *83*, 287–290.

SAMPLE
Matrix: solutions

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

CHROMATOGRAM
Retention time: 11.40 (A), 6.27 (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, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

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

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

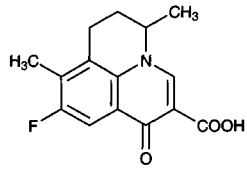
CHROMATOGRAM
Retention time: k' 8.32

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

Ibafloxacin



Molecular formula: $C_{15}H_{14}FNO_3$
Molecular weight: 275.28
CAS Registry No.: 91618-36-9
Merck Index: 4919

SAMPLE
Matrix: tissue
Sample preparation: Mix 2 g minced tissue with 4 g anhydrous sodium sulfate until homogenized. Add 10 ml ethyl acetate, shake mechanically for 10 min, centrifuge at 1500 *g* for 10 min. Transfer organic layer into another tube and repeat extraction on the tissue pellet with 10 mL ethyl acetate. Evaporate combined organic phases under a stream of nitrogen at 50°. Dissolve residue in 1 mL MeCN:2.7 mM pH 2.5 oxalic acid 50:50, vortex, sonicate for 5 min, filter through 0.45 μm filter (GHP Acrodisc GF, Gelman Sciences, USA).. Inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrabase C18 (Shandon, UK)

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

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

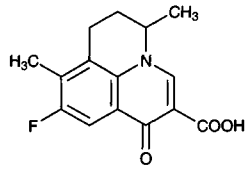
CHROMATOGRAM
Retention time: k' 8.32

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

Ibafloxacin



Molecular formula: $C_{15}H_{14}FNO_3$
Molecular weight: 275.28
CAS Registry No.: 91618-36-9
Merck Index: 4919

SAMPLE
Matrix: tissue
Sample preparation: Mix 2 g minced tissue with 4 g anhydrous sodium sulfate until homogenized. Add 10 ml ethyl acetate, shake mechanically for 10 min, centrifuge at 1500 *g* for 10 min. Transfer organic layer into another tube and repeat extraction on the tissue pellet with 10 mL ethyl acetate. Evaporate combined organic phases under a stream of nitrogen at 50°. Dissolve residue in 1 mL MeCN:2.7 mM pH 2.5 oxalic acid 50:50, vortex, sonicate for 5 min, filter through 0.45 μm filter (GHP Acrodisc GF, Gelman Sciences, USA).. Inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrabase C18 (Shandon, UK)

Mobile phase: Gradient. A was MeCN. B was 2.7 mM pH 2.5 oxalic acid. A:B from 10:90 to 70:30 over 20 min, maintain at 70:30 for 5 min, return to initial conditions over 5 min.
Flow rate: 0.8
Injection volume: 100
Detector: F ex 252 em 356

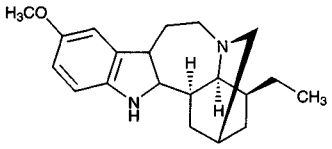
CHROMATOGRAM
Retention time: 21.3
Internal standard: ibafloxacin

OTHER SUBSTANCES
Extracted: flumequine

KEY WORDS
kidney; pig; ibafloxacin is IS

REFERENCE
Guyonnet,J.; Pacaud,M.; Richard,M.; Doisi,A.; Spavone,F.; Hellings,P. Routine determination of flumequine in kidney tissue of pig using automated liquid chromatography, *J.Chromatogr.B*, **1996**, *679*, 177–184.

Ibogaine



Molecular formula: $C_{20}H_{26}N_2O$
Molecular weight: 310.44
CAS Registry No.: 83-74-9
Merck Index: 4920

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

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

CHROMATOGRAM
Retention time: 2.7

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, 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, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine,

ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

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

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

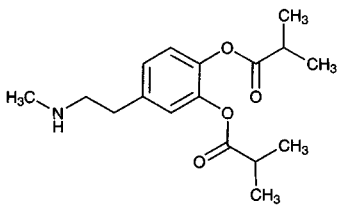
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, 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, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, meth-

azolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

Ibopamine



Molecular formula: $C_{17}H_{25}NO_4$
Molecular weight: 307.39
CAS Registry No.: 66195-31-1
Merck Index: 4921

SAMPLE

Matrix: blood
Sample preparation: 500 µL Plasma + 500 µL pH 7.4 phosphate buffer, mix, add 9 mL dioxane (Caution! Dioxane is a carcinogen!), mix, centrifuge at high speed for 30 s, inject a 20 µL aliquot of the supernatant.

HPLC VARIABLES

Guard column: µBondapak C18 Guard-Pak
Column: 150 × 3.9 Nova-Pak C18
Mobile phase: Dioxane:buffer 35:65 (Caution! Dioxane is a carcinogen!) (Buffer was 100 mM NaH_2PO_4 containing 0.11 mM 9,10-dimethoxyanthracene-2-sulfonate and 0.11 mM 1-heptanesulfonic acid, pH adjusted to 3 with phosphoric acid. Purify 9,10-dimethoxyanthracene-2-sulfonate (Fluka) by Soxhlet extraction with dichloroethane before use.)
Flow rate: 0.5
Injection volume: 20
Detector: F ex 380 em 452 following post-column extraction. The column effluent mixed with dichloroethane pumped at 1 mL/min and the mixture flowed through a 1 m × 0.25 mm i.d. stainless steel coil to a phase separator (Anal. Chim. Acta 1987, 192, 267) then the organic phase flowed through the detector.

CHROMATOGRAM

Retention time: k' 3.3
Limit of detection: 10 ng

OTHER SUBSTANCES

Also analyzed: dibenzoyl dopamine, dibudop, dipivaloyl dopamine

KEY WORDS
plasma; post-column extraction; post-column reaction

REFERENCE
Haas,M.; Moolenaar,F.; Kluppel,A.C.; Dijkstra,D.; Meijer,D.K.; De Zeeuw,D. Determination of dopaminergic prodrugs by high-performance liquid chromatography followed by post-column ion-pair extraction, *J.Chromatogr.B*, **1997**, *693*, 484–488.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 2 mL Plasma + 10 μL 10 μg/mL dihydroxybenzylamine in 200 mM acetic acid + 40 mg alumina (Bioanalytical Systems) + 2 mL tris buffer, vortex, shake on a wrist action shaker for 15 min. Discard the liquid, add 1 mL water to the alumina, vortex for 2 min, discard the liquid, repeat the wash. Add 500 μL 200 mM acetic acid to the alumina, vortex for 15-20 min, inject a 50 μL aliquot of the liquid. Urine. Dilute urine 1:20 with water. Remove a 250 μL aliquot and add it to 10 μL 20 μg/mL α-ethyldopamine in 200 mM acetic acid, add 20 mg alumina (Bioanalytical Systems), add 500 μL tris buffer, vortex, shake on a wrist action shaker for 15 min. Discard the liquid, add 1 mL water to the alumina, vortex for 2 min, discard the liquid, repeat the wash. Add 500 μL 200 mM acetic acid to the alumina, vortex for 15-20 min, inject a 50 μL aliquot of the liquid. (To deconjugate mix 650 μL plasma or urine, 500 μL acetate buffer, 50 μL 40 mg/mL penicillamine in water, and 250 μL sulfatase, heat at 37° for 16 h. Remove a 250 μL aliquot and add 10 μL 10 μg/mL dihydroxybenzylamine in 200 mM acetic acid (plasma) or 20 μg/mL α-ethyldopamine in 200 mM acetic acid (urine), add 20 mg alumina, add 500 μL tris buffer, proceed as above. Tris buffer was 60.5 g tris in 250 mL water, pH adjusted to 8.6 with phosphoric acid. Acetate buffer was 27.2 g sodium acetate trihydrate and 11.6 mL glacial acetic acid in 1 L water, pH 4.7. To prepare sulfatase solution first condition a Sep-Pak C18 SPE cartridge with 15 mL water. Prepare a solution of 15000 U sulfatase H-5 (Sigma) in 2 mL water, add to the SPE cartridge, elute with 3 mL water, collect 5 mL effluent and use this solution.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C18 IP
Mobile phase: MeCN:buffer 1 3:97 (plasma) or MeCN:buffer 2 18:82 (urine) (Buffer 1 was 28.3 g monochloroacetic acid, 9.35 g NaOH, 1.0 g disodium EDTA, and 50 mg sodium octyl sulfate in 2 L water, adjust pH to 3.0 with monochloroacetic acid. Buffer 2 was 22.0 g sodium acetate, 21.0 g citric acid, 9.8 g NaOH, 0.67 g disodium EDTA, and 75 mL glacial acetic acid in 2 L water, adjust pH to 5.0 with 5 M NaOH.)
Flow rate: 1
Injection volume: 50
Detector: E, BAS LC-4B, TL-5 thin-layer glassy-carbon electrode +0.65 V, Ag/AgCl reference electrode

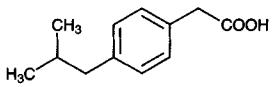
CHROMATOGRAM
Retention time: 23 (plasma), 13 (urine) (of epinine, the active metabolite)
Internal standard: dihydroxybenzylamine (9), α-ethyldopamine (10)
Limit of detection: 0.5 ng/mL

OTHER SUBSTANCES
Extracted: dihydroxyphenylacetic acid

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Gifford,R.; Randolph,W.C.; Heineman,F.C.; Ziemniak,J.A. Analysis of epinine and its metabolites in man after oral administration of its pro-drug ibopamine using high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1986**, *381*, 83–93.

Ibufenac



Molecular formula: $C_{12}H_{16}O_2$
Molecular weight: 192.26
CAS Registry No.: 1553-60-2
Merck Index: 4924

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 33 × 4.6 3 μm C18 (Perkin Elmer, Norwalk, CT)
Mobile phase: MeCN:buffer 40:60 (Prepare mobile phase as follows. Dissolve 4 mL concentrated phosphoric acid in 600 mL water and mix with 400 mL MeCN.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 220

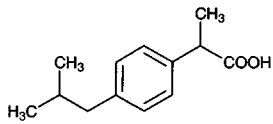
CHROMATOGRAM
Retention time: 2.6

OTHER SUBSTANCES
Simultaneous: dicloxacillin, ibuprofen, ketoprofen
Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, amitriptyline, aspirin, aztreonam, barbituric acid, brompheniramine, cafazolin, caffeine, carbamazepine, carbamazepine epoxide, cephalexin, chlorpheniramine, clonazepam, clotrimazole, desipramine, desmethyldoxepin, digitoxin, digoxin, disopyramide, doxepin, ethosuximide, felbamate, gentamicin, imipenem, imipramine, lidocaine, maprotiline, mephenytoin, mephobarbital, metharbital, methsuximide, methylsuccinimide, nortriptyline, paramethadione, phenacemide, phenobarbital, phensuximide, phenylpropanolamine, phenytoin, primidone, protriptyline, sulfamethoxazole, theophylline, tobramycin, trimethadione, trimethoprim, vancomycin.

KEY WORDS
plasma; rapid HPLC; ibufenac is IS

REFERENCE
Rifai,N.; Lafi,M.; Sakamoto,M.; Law,T. Measurement of plasma ketoprofen by a rapid high-performance liquid chromatography assay, *Ther.Drug Monit.*, **1997**, *19*, 175–178.

Ibuprofen



Molecular formula: $C_{13}H_{18}O_2$
Molecular weight: 206.28
CAS Registry No.: 15687-27-1, 58560-75-1 (± mixture), 61054-06-6 (Al salt), 112017-99-9 (piconol)
Merck Index: 4925
Lednicer No.: 1 86; 2 218, 356

SAMPLE
Matrix: blood
Sample preparation: Mix 500 μL plasma with 50 μL 200 μg/mL IS in MeOH. Add 500 μL 1 M HCl, extract with 10 mL dichloromethane. Separate the organic layer and evaporate it under a stream of nitrogen at 30°. Reconstitute the residue with 500 μL mobile phase. Inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm C18 Novapak
Mobile phase: MeCN:10 mM pH 6.05 dipotassium hydrogen orthophosphate 25:75
Flow rate: 1.4
Injection volume: 50
Detector: UV 225

CHROMATOGRAM
Retention time: 5.2
Internal standard: (4-n-pentylphenyl) acetic acid (7.9)
Limit of detection: 500 ng/mL

KEY WORDS
plasma

REFERENCE
Pargal,A.; Kelkar,M.G.; Nayak,P.J. The effect of food on the bioavailability of ibuprofen and flurbiprofen from sustained release formulations, *Biopharm.Drug Dispos.*, **1996**, *17*, 511–519.

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

HPLC VARIABLES
Column: 100 × 4.6 5-10 μm Silicalite (by sieving Silicalite, 3M Co.(?))
Mobile phase: MeCN:20 mM pH 6.9 phosphate buffer 3:97
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 5.29

KEY WORDS
serum

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

SAMPLE
Matrix: blood
Sample preparation: Centrifuge plasma or serum at 11300 g for 7 min, inject a 200 μL aliquot onto column 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 5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. (Reequilibrate column A with mobile phase A for 4 min.)

HPLC VARIABLES
Column: A 20 × 4.0 BioTrap 500 C18 (ChromTech); B 100 × 4.6 5 μm CT-Sil C18
Mobile phase: A 2-Propanol:30 mM pH 7.0 sodium phosphate buffer containing 10 mM octanoic acid 2:98; B MeCN:30 mM pH 7.0 sodium phosphate buffer 35:65
Flow rate: A 0.8; B 1
Injection volume: 10
Detector: F ex 225 em 535

CHROMATOGRAM
Retention time: 8.0

KEY WORDS
plasma; serum; column-switching

REFERENCE
Hermansson,J.; Grahn,A.; Hermansson,I. Direct injection of large volumes of plasma/serum on a new biocompatible extraction column for the determination of atenolol, propranolol and ibuprofen. Mechanisms for the improvement of chromatographic performance, *J.Chromatogr.A*, **1998**, *797*, 251–263.

SAMPLE
Matrix: blood
Sample preparation: 500 µL Plasma + 50 µL 50 µg/mL fenoprofen in water + 200 µL 20% sulfuric acid + 6 mL n-butyl chloride, vortex for 5 min, centrifuge at 950 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 50 mM triethylamine, sonicate for 1 min, vortex for 30 s, add 50 µL 6 mM ethyl chloroformate, let stand for 30 s, add 25 µL 10 mM S-(-)-1-(1-naphthyl)ethylamine, let stand for 3 min, add 25 µL MeCN:ethanolamine 40:1. Evaporate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 250 µL mobile phase, inject a 25 µL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 µm Newguard RP-18
Column: 150 × 4.6 5 µm Inertsil ODS-2
Mobile phase: MeCN:water (pH 3.0) 66.5:33.5
Column temperature: 27
Flow rate: 1.2
Injection volume: 25
Detector: F ex 280 em 320

CHROMATOGRAM
Retention time: 11.3 (S-(+)), 12.3 (R-(-))
Internal standard: fenoprofen (7.7 (S), 8.5 (R))
Limit of quantitation: 100 ng/mL

KEY WORDS
derivatization; chiral; plasma

REFERENCE
Lau,Y.Y. Determination of ibuprofen enantiomers in human plasma by derivatization and high-performance liquid chromatography with fluorescence detection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2143–2153.

SAMPLE
Matrix: blood, synovial fluid, tissue
Sample preparation: Plasma, synovial fluid. Acidify 1 mL plasma or synovial fluid with 100 µL 1 M HCl, add 100 µL 3 µg/mL IS. Add 10 mL diethyl ether, stir for 10 min, centrifuge at 6000 rpm for 5 min, remove the ether phase, repeat the extraction twice more. Evaporate the organic phase at 40°. Dissolve the residue in 1 mL MeOH, inject a 50 µL aliquot. Tissue. Weigh out about 500 mg frozen tissue, add 5 mL water, 5 mL 100 mM HCL, and 100 µL 3 µg/mL IS. Homogenize the mixture using an Ultraturrax at 13000 rpm, add 30 mL diethyl ether and 2 mL each of Carrez solutions I and II. Vortex, centrifuge at 6000 rpm for 5 min, remove the ether phase. Repeat the same extraction procedure twice with 30 mL portions of diethyl ether. Evaporate the combined ether phases at 40°. Dissolve the residue in 1 mL MeOH, inject a 50 µL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 µm LiChroCart LiChrospher 100 RP-18
Column: 125 × 4 5 µm LiChroCart LiChrospher 100 RP-18
Mobile phase: MeCN:pH 4.8 sodium acetate 38:62
Flow rate: 1.5
Injection volume: 50
Detector: UV 223

CHROMATOGRAM
Internal standard: fenoprofen
Limit of detection: 5 ng/mL

KEY WORDS
plasma; subcutis; musculature; fasciae; pharmacokinetics

REFERENCE
Dominkus,M.; Nicolakis,M.; Kotz,R.; Wilkinson,F.E.; Kaiser,R.R.; Chlud,K. Comparison of tissue and plasma levels of ibuprofen after oral and topical administration, *Arzneimittelforschung*, **1997**, *46*, 1138–1143.

SAMPLE
Matrix: blood, urine
Sample preparation: Filter plasma (0.2 μm membrane) and inject a 100 μL aliquot onto column A, elute with mobile phase A onto column B, after 6 min backflush the contents of column B onto column C with mobile phase B, elute with mobile phase B, monitor the effluent from column C.

HPLC VARIABLES
Column: A 150 × 4.6 5 μm CAPCELL PAK MF (Shiseido, Japan); B 35 × 4.6 5 μm CAPCELL PAK C18 (Shiseido, Japan); C 250 × 4 5μm CAPCELL PAK C18 (Shiseido, Japan)
Mobile phase: A MeCN:50 mM pH 7.0 phosphate buffer 5:95; B MeCN:50 mM pH 7.0 phosphate buffer 27:73
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: UV 223

CHROMATOGRAM
Retention time: 27.2
Limit of detection: 25 ng/mL

KEY WORDS
comparison with capillary electrophoresis; column-switching; plasma; rat

REFERENCE
Kang,S.H.; Chang,S.-Y.; Do,K.-C.; Chi,S.-C.; Chung,D.S. High-performance liquid chromatography with a column-switching system and capillary electrophoresis for the determination of ibuprofen in plasma, *J.Chromatogr.B*, **1998**, *712*, 153–160.

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

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

Detector: UV 200.5

CHROMATOGRAM
Retention time: 23.815

KEY WORDS
whole blood

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

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. Activate an SPE cartridge filled with 100 mg 40-63 μm silica gel (Merck) with 1 mL MeOH and dry in a hot air oven at 100° for 1 h, equilibrate with 1 mL dichloromethane before use. 500 μL Serum + 10 μL 100 μg/mL flurbiprofen in MeCN + 100 μL 1 M HCl, mix, add 1 mL 1 M pH 3.8 sodium phosphate buffer, mix, add 3 mL diethyl ether, rock for 20 min, centrifuge at 1000 g for 2 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL 1 mg/mL 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in dichloromethane, add 100 μL 1 mg/mL 1-hydroxybenzotriazole in dichloromethane, add 100 μL 1 mg/mL (R)-(+)-1-(1-naphthyl)ethylamine in dichloromethane, vortex briefly, let stand at room temperature for 2 h, add to the SPE cartridge, elute with two 1 mL portions of dichloromethane:MeCN 90:10. Combine all the eluate and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot. Urine. Activate an SPE cartridge filled with 100 mg 40-63 μm silica gel (Merck) with 1 mL MeOH and dry in a hot air oven at 100° for 1 h, equilibrate with 1 mL dichloromethane before use. 500 μL Urine + 10 μL 100 μg/mL flurbiprofen in MeCN + 100 μL 1 M HCl, mix, add 1.5 mL 1 M pH 3.8 sodium phosphate buffer, mix, add 5 mL hexane:isopropanol 90:10, rock for 20 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 100 μL 1 mg/mL 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in dichloromethane, add 100 μL 1 mg/mL 1-hydroxybenzotriazole in dichloromethane, add 100 μL 1 mg/mL (R)-(+)-1-(1-naphthyl)ethylamine in dichloromethane, vortex briefly, let stand at room temperature for 2 h, add to the SPE cartridge, elute with two 1 mL portions of dichloromethane:MeCN 90:10. Combine all the eluate and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 2.1 40-63 μm pellicular C18 (Alltech)
Column: 150 × 3.9 5 μm Resolve C18 (Waters)
Mobile phase: MeCN:10 mM pH 3.5 phosphate buffer 50:50
Flow rate: 1.5
Injection volume: 50
Detector: F ex 290 em 330

CHROMATOGRAM
Retention time: 22.2 (R), 25.6 (S)
Internal standard: flurbiprofen (quantitation uses the peak for the (S)-enantiomer) (14.5 (R), 17.8 (S))
Limit of quantitation: 100 ng/mL

KEY WORDS
derivatization; chiral; serum; SPE

REFERENCE
Tan,S.C.; Jackson,S.H.D.; Swift,C.G.; Hutt,A.J. Enantiospecific analysis of ibuprofen by high performance liquid chromatography: Determination of free and total drug enantiomer concentrations in serum and urine, *Chromatographia*, **1997**, *46*, 23–32.

SAMPLE
Matrix: cell suspensions
Sample preparation: Centrifuge cell suspension at 2000 g for 4 min. Remove a 2 mL aliquot of the supernatant and add it to 200 μL 1 μg/mL IS in DMF, mix, add 200 μL 5 M HCl, extract twice with 3 mL portions of toluene. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL dichloromethane, add 20 μL 10 mg/mL 1-hydroxybenzotriazole in dichloromethane:pyridine 99:1, add 300 μL 10 mg/mL 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in dichloromethane, add 300 μL 10 mg/mL (-)-(S)-α-methylbenzylamine in dichloromethane, let stand for 30 min, evaporate to dryness, reconstitute with 500 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 10 mm long Techsphere ODS (HPLC Technology, Macclesfield UK)
Column: 250 × 5 5 μm Techsphere ODS (HPLC Technology, Macclesfield UK)
Mobile phase: MeCN:7.5 mM NaH_2PO_4 60:40, containing 5 mM sodium pentanesulfonate, pH adjusted to 2.8 with phosphoric acid
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 6.63, 7.11 (enantiomers)
Internal standard: (S)-naproxen (k' 3.05)
Limit of detection: 10 μg/mL

KEY WORDS
derivatization; chiral

REFERENCE
Thomason,M.J.; Hung,Y.-F.; Rhys-Williams,W.; Hanlon,G.W.; Lloyd,A.W. Indirect enantiomeric separation of 2-arylpropionic acids and structurally related compounds by reversed phase HPLC, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1765–1774.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water:glacial acetic acid 40:60:0.1
Flow rate: 2.5
Injection volume: 20
Detector: UV 232

CHROMATOGRAM
Retention time: 15.5

OTHER SUBSTANCES
Simultaneous: naproxen

KEY WORDS
plasma

REFERENCE
Niazi,S.K.; Alam,S.M.; Ahmad,S.I. Partial-area method in bioequivalence assessment: Naproxen, *Biopharm.Drug Dispos.*, **1997**, *18*, 103–116.

SAMPLE
Matrix: solutions
Sample preparation: Mix 50 μL of a 0.001-5 mM solution in MeCN with 50 μL 1 mM DNS-APy in MeCN containing 50 mM 2,2'-dipyridyl disulfide and 50 mM triphenylphosphine, let

stand at room temperature for 30 min. Remove a 10 μL aliquot and dilute it to 100 μL with MeCN, inject a 2 μL aliquot. (Synthesis of DNS-APy, 1-(5-dimethylamino-1-naphthalenesulfonyl)-(S)-3-aminopyrrolidine, is as follows. Cool a solution of 16.4 g (R)-(+)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane:acetone 95:5 to obtain (3R)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3R)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65°, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry over anhydrous magnesium sulfate, evaporate to obtain (3S)-3-azido-1-(phenylmethyl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3S)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the minimum amount of EtOH to obtain (3S)-(+)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). (3S)-(+)-Aminopyrrolidine dihydrochloride is also reported to be available from Tokyo Kasei. Stir 800 mg (3S)-(+)-3-aminopyrrolidine dihydrochloride and 2 mL triethylamine in 800 mL MeCN at 0-10°, add a solution of 440 mg dansyl chloride in 80 mL MeCN dropwise, stir in the dark for 30 min, evaporate to dryness under reduced pressure, dissolve the residue in 200 mL 5% HCl, wash twice with 40 mL portions of dichloromethane. Adjust the pH of the organic layer to 13-14 with 5% NaOH, extract twice with 10 mL portions of dichloromethane. Combine the organic layers and wash them with 80 mL water. Dry the organic layer over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, dissolve the residue in dichloromethane:MeOH 90:10, chromatograph on silica gel with dichloromethane:MeOH 90:10. Collect the greenish-yellow fluorescent band and evaporate it under reduced pressure to obtain DNS-APy as a greenish-yellow oil.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm TSK gel ODS-80TM (Tosoh)
Mobile phase: MeCN:water 60:40
Flow rate: 1
Injection volume: 2
Detector: F ex 340 em 530

CHROMATOGRAM

Retention time: 30 ((S)-(+)), 32 ((R)-(-))
Limit of detection: 0.1 pmole

KEY WORDS

derivatization; chiral

REFERENCE

Al-Kindy,S.; Santa,T.; Fukushima,T.; Homma,H.; Imai,K. 1-(5-Dimethylamino-1-naphthalenesulphonyl)-(S)-3-aminopyrrolidine (DNS-Apy) as a fluorescence chiral labelling reagent for carboxylic acid enantiomers, *Biomed.Chromatogr.*, **1997**, *11*, 137–142.

SAMPLE

Matrix: urine
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL ethyl acetate and dry by aspiration of air. Evaporate an aliquot of 100 μL 20 μg/mL IS in MeOH to dryness at 37°. Add 1 mL urine, vortex, add 250 μL 1 M pH 5.0 acetate buffer, vortex. Add 250 μL of the mixture to the SPE cartridge, dry by aspiration of air, elute with 3 mL ethyl acetate, evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject a 10-30 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:50 mM pH 5.0 phosphate buffer 42:58
Flow rate: 0.9
Injection volume: 10-30
Detector: UV 230

CHROMATOGRAM
Retention time: 24
Internal standard: indomethacin (18.5)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: diclofenac, felbinac, fenbufen, flurbiprofen, ketoprofen, loxoprofen, mefenamic acid, naproxen, piroxicam, sulindac

KEY WORDS
SPE

REFERENCE
Hirai,T.; Matsumoto,S.; Kishi,I. Simultaneous analysis of several non-steroidal anti-inflammatory drugs in human urine by high-performance liquid chromatography with normal solid-phase extraction, *J.Chromatogr.B*, **1997**, *692*, 375–388.

SAMPLE
Matrix: urine
Sample preparation: Condition an Isolute HAX SPE cartridge (International Sorbent Technology, UK) with 3 mL MeOH, 3 mL water, and 3 mL 100 mM pH 2.0 acetate buffer. Add 1 mL 100 mM pH 2.0 acetate buffer to 1 mL urine, mix, add to the SPE cartridge. Wash with 2 mL water, 2 mL MeOH:water 40:60, 2 mL hexane containing 10% triethylamine, and 2 mL hexane, elute with 1 mL MeOH containing 10 mM HCl. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute the dry residue with 50 μL MeOH, add 100 μL reagent. Add 10 μL diethyl phosphorocyanidate and let stand for 5 min at room temperature. Add 40 μL water and inject a 10 μL aliquot. (The reagent was 28 mM Nepsilon-dansyl-O-methyl-L-lysine in MeOH. Preparation is as follows. Dissolve 53.2 mg Nepsilon-dansyl-L-lysine in 2 mL MeOH, add 8 mL benzene and 400 μL 10% trimethylsilyldiazomethane in hexane, stir for 30 min at room temperature, evaporate to dryness under a stream of nitrogen at 40°. Reconstitute the dry residue in 5 mL MeOH to a final concentration of 28 mM. (Caution! Benzene is a carcinogen!) The free amino group of the reagent reacts with the free carboxylic acid group of ibuprofen.)

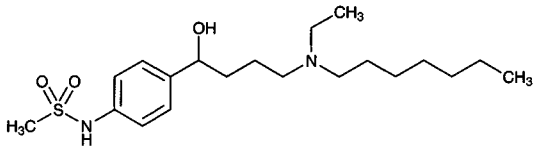
HPLC VARIABLES
Column: 250 × 4.6 5 μm L-column ODS (Chemicals Inspection and Testing Institute, Japan)
Mobile phase: Gradient. MeCN:water from 65:35 to 80:20 over 20 min.
Column temperature: 35
Flow rate: 1
Injection volume: 10
Detector: F ex 340 em 523

CHROMATOGRAM
Retention time: 15 (-), 15.9 (+)
Limit of detection: 4 pmol
Limit of quantitation: 10 ng/mL

KEY WORDS
chiral; SPE; derivatization

REFERENCE
Hayamizu,T.; Kudoh,S.; Nakamura,H. Methylated Nepsilon-dansyl-L-lysine as a fluorogenic reagent for the chiral separation of carboxylic acids, *J.Chromatogr.B*, **1998**, *710*, 211–218.

Ibutilide



Molecular formula: $C_{20}H_{36}N_2O_3S$
Molecular weight: 384.58
CAS Registry No.: 122647-31-8, 122647-32-9 (fumarate)
Merck Index: 4927
Lednicer No.: 5 23

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL 100 mg Bond-Elut C18 SPE cartridge with 1 mL MeCN:acetone:triethylamine 50:50:0.2, with 1 mL 0.1% triethylamine in water, and 1 mL water. 0.1-1 mL Plasma + 1 mL buffer + 100 µL 100-250 ng/mL IS in MeCN:10 mM ammonium acetate 30:70, mix, add to the SPE cartridge, wash with 1 mL water, wash with 2 mL MeCN:MeOH:water 25:25:50, wash with 1 mL water, dry under vacuum for 10 min, wash with 300 µL hexane, dry under vacuum for 5 min, elute with 500 µL MeCN:acetone:triethylamine 50:50:0.2. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 10 µL 0.1% acetic acid in MeCN and 100 µL 0.1% 1-naphthylisocyanate in MeCN, mix, heat at 30° for 10 min, add 600 MeOH:water:trifluoroacetic acid 10:90:0.1, inject a 600 µL aliquot onto column A and elute to waste with mobile phase A, after 8 min backflush the contents of column A onto column B with mobile phase C, after 3.1 min elute column B to waste with mobile phase D, after 6.1 min divert the fraction containing the drugs onto column C, after 2 min elute the contents of column C onto column D with mobile phase E, elute column E with mobile phase E, monitor the effluent from column E. Before the next injection flush column A with mobile phase B for 22.1 min and re-equilibrate with mobile phase A for 2 min. (Buffer was 50 mM pH 7.0 NaH_2PO_4 containing 0.1% triethylamine.)

HPLC VARIABLES

Column: A two 15 × 3.2 7 µm Newguard in series; B 20 × 4 4 µm Sentry Nova-Pak C18 + 75 × 4.6 3 µm Ultremex 3 C8; C 30 × 4.6 Spheri-5 RP-18; D 100 × 4.6 3 µm Pirkle covalent dinitrophenyl-D-phenylglycine (Regis)

Mobile phase: A MeOH:water 40:60; B MeCN:MeOH:water:trifluoroacetic acid:triethylamine 40:40:20:0.1:0.1; C MeCN:MeOH:water:trifluoroacetic acid:triethylamine 25:5:70:0.1:0.1; D MeCN:water:trifluoroacetic acid:triethylamine 52:48:0.1:0.1; E MeOH:trifluoroacetic acid:triethylamine 100:0.3:0.3

Flow rate: 1

Injection volume: 600

Detector: F ex 290 em 345

CHROMATOGRAM

Retention time: 11.7 (+), 12.6 (-)

Internal standard: N-[4-[4-(ethyloctylamino)-1-hydroxybutyl]phenyl]methanesulfonamide (Upjohn U-74747) (IS detected using F ex 224 em 340 (cut-off filter) detector between columns B and C) (4)

Limit of quantitation: 17 pg/mL

KEY WORDS

derivatization; SPE; plasma; column-switching; heart-cut; human; rat; rabbit; dog; chiral

REFERENCE

Hsu,C.-y.L.; Walters,R.R. Assay of the enantiomers of ibutilide and artilide using solid-phase extraction, derivatization, and achiral-chiral column-switching high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *667*, 115–128.

SAMPLE

Matrix: blood

Sample preparation: Basify 1 mL serum with 500 mM pH 10 carbonate buffer, extract with 1-chlorobutane. Extract the organic layer with 37.5 mM sulfuric acid. Basify the sulfuric acid layer (?) and extract it with 1-chlorobutane. Evaporate the organic layer to dryness, add 50 µL

130 ng/mL IS, add 50 μL 0.1% triethylamine in MeCN, evaporate to dryness, add 10 μL 0.1% acetic acid in MeCN, add 100 μL 220 μL/L naphthylisocyanate in MeCN, heat at 30° for 10 min, evaporate to dryness, reconstitute with 100 μL MeCN, use a column-switching technique during the first 6 min.

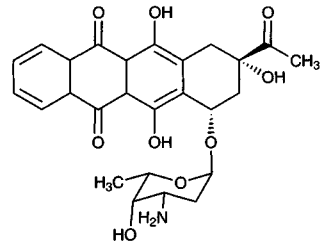
HPLC VARIABLES
Column: 100 × 4.6 3 μm 100 Å D-phenylglycine Pirkle column
Mobile phase: MeOH:triethylamine:trifluoroacetic acid 100:0.038:0.038
Flow rate: 1
Detector: F ex 224 em 340

CHROMATOGRAM
Retention time: 10.5 (+), 11.6 (-)
Internal standard: U-71175
Limit of quantitation: 0.5 ng/mL

KEY WORDS
chiral; derivatization; serum

REFERENCE
Bhandarkar,S.V.; Neau,S.H. Development of a chiral separation assay for suprofen using an α1-acid glycoprotein column (Abstract APQ 1117), *Pharm.Res.*, **1996**, *13*, S32.

Idarubicin



Molecular formula: $C_{26}H_{27}NO_9$
Molecular weight: 497.50
CAS Registry No.: 58957-92-9, 57852-57-0 (HCl)
Merck Index: 4931
Lednicer No.: 5 47

SAMPLE
Matrix: blood
Sample preparation: Condition a 6 mL Bondelut C18 SPE cartridge with 3 mL MeOH and 3 ml MeOH:phosphate buffer 1:2. 1 mL Plasma + 10-100 μL 1 μg/mL daunorubicin hydrochloride in water + 1 mL 10 mM pH 8 phosphate buffer containing 600 nM tetrabutylammonium bromide + 1 mL MeOH, add to the SPE cartridge, wash with 4 mL MeOH:water 25:75, elute with 3 mL 30 mM phosphoric acid in MeOH. Add the eluate to 100 μL 100 mM KH_2PO_4, evaporate to 100-400 μL under vacuum at 25°, inject a 10-100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-CN
Mobile phase: Gradient. A was MeCN:10 mM KH_2PO_4 22:78. B was MeCN:10 mM KH_2PO_4 containing 6 mM phosphoric acid 70:30. A:B from 90:10 to 80:20 over 9 min.
Injection volume: 10-100
Detector: F ex 470 em 580

CHROMATOGRAM
Retention time: 12.1
Internal standard: daunorubicin (10.1)
Limit of detection: 0.2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; SPE

REFERENCE
Camaggi,C.M.; Carisi,P.; Strocchi,E.; Pannuti,F. High-performance liquid chromatographic analysis of idarubicin and fluorescent metabolites in biological fluids, *Cancer Chemother.Pharmacol.*, **1992**, *30*, 303–306.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 150 × 3.9 5 μm Symmetry C8
Mobile phase: MeCN:20 mM NaH_2PO_4 27:73
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 9.6

OTHER SUBSTANCES
Simultaneous: etoposide

KEY WORDS
0.9% NaCl; injections

REFERENCE
Zhang,H.; Ye,L.; Stewart,J.T. HPLC determination of idarubicin-etoposide and idarubicin-ondansetron mixtures in 0.9% sodium chloride injection USP, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 979–988.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: MeCN:20 mM KH_2PO_4 43:57
Flow rate: 1.8
Injection volume: 20
Detector: UV 254

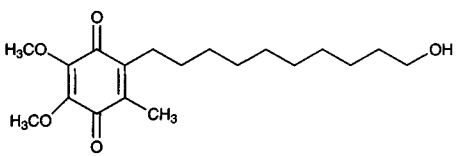
CHROMATOGRAM
Retention time: 7.1

OTHER SUBSTANCES
Simultaneous: ondansetron

KEY WORDS
0.9% NaCl; injections

REFERENCE
Zhang,H.; Ye,L.; Stewart,J.T. HPLC determination of idarubicin-etoposide and idarubicin-ondansetron mixtures in 0.9% sodium chloride injection USP, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 979–988.

Idebenone



Molecular formula: $C_{19}H_{30}O_5$
Molecular weight: 338.44
CAS Registry No.: 58186-27-9
Merck Index: 4932

SAMPLE
Matrix: blood, tissue
Sample preparation: Serum. 100 μL serum + 100 μL 100 ng/mL vitamin K3 in EtOH, shake, add 4 mL cyclohexane:benzene 20:80 (Caution! Benzene is a carcinogen!), vortex for 2 min, centrifuge at 1500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL EtOH, filter (0.45 μm), inject a 20 μL aliquot. Tissue. 50-100 mg Brain tissue + 500 μL 100 mM perchloric acid + 20 μL 100 ng/mL vitamin K3 in EtOH, homogenize in a glass homogenizer at 4°, add 6 mL cyclohexane:benzene 80:20 (Caution! Benzene is a carcinogen!), vortex for 2 min, centrifuge at 1500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL EtOH, filter (0.45 μm), inject a 20 μL aliquot.

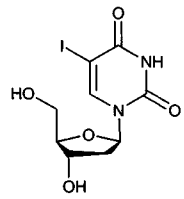
HPLC VARIABLES
Column: 150 × 6 5 μm Shim-pack CLC-ODS (Shimadzu)
Mobile phase: MeOH:water 85:15 containing 50 mM sodium perchlorate
Flow rate: 1
Injection volume: 20
Detector: E, EICOM ECD 100, glassy carbon working electrode +0.7 V, Ag/AgCl reference electrode following post-column catalytic reduction. The column effluent flowed through a 10 × 4.6 column packed with 10 μm 5% platinum on alumina catalyst (Toa Electronics) to the detector. (Purge catalyst column with water at 10 mL/min for 5 min before use.)

CHROMATOGRAM
Retention time: 7.8
Internal standard: vitamin K3 (4.9)
Limit of detection: 5 pg

KEY WORDS
rat; serum; brain; post-column reaction

REFERENCE
Wakabayashi,H.; Nakajima,M.; Yamato,S.; Shimada,K. Determination of idebenone in rat serum and brain by high-performance liquid chromatography using platinum catalyst reduction and electrochemical detection, *J.Chromatogr.*, **1992**, *573*, 154–157.

Idoxuridine



Molecular formula: $C_9H_{11}IN_2O_5$
Molecular weight: 354.10
CAS Registry No.: 54-42-2
Merck Index: 4934

SAMPLE
Matrix: aqueous humor
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: Aquapore RP.300 (Brownlee)
Mobile phase: Water:acetic acid 99:1
Flow rate: 1
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: 12.0

OTHER SUBSTANCES
Extracted: 5-iodo-2'-deoxycytidine

KEY WORDS
rabbit; GPC

REFERENCE
Sincholle,D.; Conduzorgues,J.-P.; Mbatchi,B.; Masse,J.-P. Corneal penetration and metabolism of the antiherpetic 5-iodo-2'-deoxycytidine, *Curr.Eye Res.*, **1985**, *4*, 627–629.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 25 μL 20 μg/mL 5-iodouridine in mobile phase + 1 mL saturated ammonium sulfate + 50 μL pH 6.7 ammonium phosphate + 4 mL ethyl acetate, vortex for 15 min, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to about 500 μL under a stream of air, add 200 μL 500 mM NaOH, vortex for 15 min, centrifuge at 2000 g for 10 min, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: 220 × 4.6 5 μm C18 (Brownlee)
Mobile phase: MeCN:10 mM potassium phosphate buffer 5:95
Flow rate: 1.5
Injection volume: 50
Detector: UV 290

CHROMATOGRAM
Retention time: 10.33
Internal standard: 5-iodouridine (7.74)
Limit of detection: 80 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum

REFERENCE
Belotto,N.; Reiner,V.; Verga,F.; Canobbio,G.; Lietti,F.; Magnani,P.; Lucarelli,C. Determination of 2'-deoxy-5-iodouridine and its metabolite 5-iodouracil by high-performance liquid chromatography with ultraviolet absorbance detection in human serum, *J.Chromatogr.*, **1991**, *572*, 327–332.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 10 μg/mL bromodeoxyuridine in water + 2 mL saturated ammonium sulfate solution + 100 μL pH 6.7 ammonium phosphate buffer + 8 mL ethyl acetate, shake for 15 min, centrifuge at 1200 g for 10 min. Remove the organic layer and evaporate it to 1 mL under a stream of air, add 400 μL 500 mM KOH, shake for 15 min, centrifuge at 1000 g for 10 min, inject a 3-30 μL aliquot of the aqueous phase (J.Chromatogr. 1985, 341, 217).

HPLC VARIABLES
Column: 250 × 4.6 6 μm Zorbax C8
Mobile phase: MeOH:50 mM pH 7.3 ammonium phosphate buffer 12:88
Flow rate: 1
Injection volume: 3-30
Detector: UV 280

CHROMATOGRAM
Internal standard: bromodeoxyuridine
Limit of detection: 300 nM

KEY WORDS
dog; plasma

REFERENCE
Smith,D.E.; Brenner,D.E.; Knutsen,C.A.; Deremer,S.J.; Terrio,P.A.; Johnson,N.J.; Stetson,P.L.; Ensminger,W.D. Mutual kinetic interaction between 5-fluorouracil and bromodeoxyuridine or iododeoxyuridine in dogs, *Drug Metab.Dispos.*, **1993**, *21*, 277–283.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Dilute with water to an idoxuridine concentration of 0.1%. Remove a 15 mL aliquot and add it to 2 mL sulfathiazole solution, make up to 20 mL with water, inject a 10 μL aliquot. (Prepare sulfathiazole solution by dissolving 120 mg sulfathiazole in 10 mL EtOH, make up to 100 mL with water.)

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeOH:water 13:87
Flow rate: 1.7
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 8
Internal standard: sulfathiazole (12.5)

OTHER SUBSTANCES
Simultaneous: impurities

KEY WORDS
eye drops

REFERENCE
Carr,G.P.R. The development of British Pharmacopeia monographs for idoxuridine eye drops using high-pressure liquid chromatography for assay and for controlling related impurities, *J.Chromatogr.*, **1978**, *157*, 171–184.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 2 μL aliquot of a 0.1% solution.

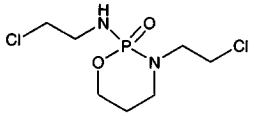
HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:water 10:90
Flow rate: 1
Injection volume: 2
Detector: UV 262

CHROMATOGRAM
Retention time: 18

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Szepesi,G.; Gazdag,M.; Mezei,J. Investigation of the hydrolytic decomposition of 5-iodine-2'-desoxyuridine by high-performance liquid chromatography, *Pharmazie*, **1980**, *35*, 602–604.

Ifosfamide



Molecular formula: $C_7H_{15}Cl_2N_2O_2P$
Molecular weight: 261.09
CAS Registry No.: 3778-73-2
Merck Index: 4937

SAMPLE

Matrix: blood
Sample preparation: Condition a 100 mg CH Bond Elut SPE cartridge with two 1 mL aliquots of MeOH and 1 mL water adjusted to pH 4 with phosphoric acid. 1 mL Serum + 1 mL 25 mM pH 4 phosphate buffer + 200 μL water adjusted to pH 4 with phosphoric acid, add this mixture to the SPE cartridge in two 1 mL aliquots, allow to elute through cartridge for 2-3 min then dry by applying a full vacuum for 5 min. Wash with 1 mL MeCN:water adjusted to pH 4 with phosphoric acid 10:90, elute with 1 mL MeOH, evaporate to dryness under a stream of nitrogen, reconstitute in 250 μL mobile phase, inject a 20 μL aliquot onto column A, after about 6.5 min switch effluent containing ifosfamide from column A onto column B for 42 s, elute column B and monitor the effluent.

HPLC VARIABLES

Column: A 50 × 4.6 5 μm Spherisorb C1; B 100 × 4 Chiral-AGP α_1 acid glycoprotein (Chromtech)
Mobile phase: MeCN:15 mM pH 4 phosphate buffer 1:99
Flow rate: 1
Injection volume: 20
Detector: UV 195

CHROMATOGRAM

Retention time: 11.6 (S), 13.0 (R)
Limit of detection: 2500 ng/mL

KEY WORDS

serum; chiral; SPE; column-switching

REFERENCE

Corlett,S.A.; Chrystyn,H. Enantiomeric separation of R- and S-ifosfamide and their determination in serum from clinical subjects, *J.Chromatogr.B*, **1994**, *654*, 152–158.

SAMPLE

Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with three 1 mL portions of MeOH and three 1 mL portions of 25 μM cyclohexylamine in 500 mM pH 8.0 sodium phosphate buffer containing 1 M NaCl. 500 μL Plasma (stabilized with semicarbazide) + 500 μL 500 mM pH 8.0 sodium phosphate buffer containing 1 M NaCl + 100 μL 100 mg/mL sodium diethyldithiocarbamate, heat at 70° for 30 min, cool to 0°, add to the SPE cartridge, wash three times with 25 μM cyclohexylamine in 500 mM pH 8.0 sodium phosphate buffer containing 1 M NaCl, elute with 1 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL MeCN:water 27.5:72.5, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 125 × 4 5 μm LichroCART RP8
Mobile phase: MeCN:water 32:68 containing 10 mM sodium phosphate buffer and 20 mM cyclohexylamine, pH 7.0
Injection volume: 10
Detector: UV 280

CHROMATOGRAM

Retention time: 4 (as ifosforamide mustard, the active metabolite)
Limit of quantitation: 450 nmoles

KEY WORDS
derivatization; plasma; SPE

REFERENCE
Kaijser,G.P.; Beijnen,J.H.; Rozendom,E.; Bult,A.; Underberg,W.J.M. Analysis of ifosforamide mustard, the active metabolite of ifosfamide, in plasma, *J.Chromatogr.B*, **1996**, *686*, 249–255.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 100 × 4.6 5 μm ODS-Hypersil
Mobile phase: MeCN:water 30:70
Flow rate: 1.5
Detector: UV 210

CHROMATOGRAM
Retention time: 1.5

KEY WORDS
injections; saline; water; stability-indicating

REFERENCE
Muñoz,M.; Girona,V.; Pujol,M.; Durán,S.; Vicente,P.; Solé,L.-A. Stability of ifosfamide in 0.9% sodium chloride solution or water for injection in a portable i.v. pump cassette, *Am.J.Hosp.Pharm.*, **1992**, *49*, 1137–1139.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4.6 5 μm C18
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 1.8
Injection volume: 20
Detector: UV 198

CHROMATOGRAM
Retention time: 2.89

OTHER SUBSTANCES
Simultaneous: granisetron (UV 300)

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE
Matrix: reaction mixtures
Sample preparation: Add solid NaCl to a 500 μL aliquot of the reaction mixture until some solid remains undissolved, add 250 μL MeCN, stir for 5 min, inject a 20 μL aliquot of the upper layer.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Microsorb C8

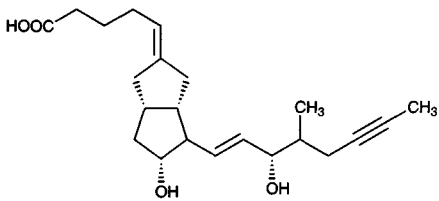
Mobile phase: MeOH:20 mM pH 4.4 KH_2PO_4 25:75
Flow rate: 1
Injection volume: 20
Detector: UV 190

CHROMATOGRAM
Retention time: 13.5
Limit of detection: 10000 ng/mL

OTHER SUBSTANCES
Simultaneous: cyclophosphamide

REFERENCE
Lunn,G.; Sansone,E.B.; Andrews,A.W.; Hellwig,L.C. Degradation and disposal of some antineoplastic drugs, *J.Pharm.Sci.*, **1989**, *78*, 652–659.

Iloprost



Molecular formula: $C_{22}H_{32}O_4$
Molecular weight: 360.49
CAS Registry No.: 73873-87-7
Merck Index: 4940

SAMPLE
Matrix: formulations
Sample preparation: 100 mL 5% Dextrose injection + 25 mL chloroform + 1 mL 10 μg/mL 2-naphthoic acid in MeOH, shake vigorously for 1-2 min. Remove the organic layer and evaporate it to dryness under a stream of air, reconstitute the residue in 1 mL MeOH, inject a 70 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm ODS Hypersil
Mobile phase: MeCN:MeOH:20 mM pH 3.0 KH_2PO_4 40:14.4:45.6
Flow rate: 1.8
Injection volume: 70
Detector: UV 207

CHROMATOGRAM
Retention time: 8.24 (4R), 8.96 (4S)
Internal standard: 2-naphthoic acid (4.5)
Limit of detection: 10 ng

KEY WORDS
injections; 5% dextrose; diastereoisomers

REFERENCE
Scypinski,S.; Lanzano,R.L.; Soltero,R.A. Determination of iloprost in 5% dextrose in water solution by reversed-phase high performance liquid chromatography, *J.Pharm.Sci.*, **1990**, *79*, 934–937.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: Radial Pak C18 (Waters)
Mobile phase: Gradient. MeCN:0.1% acetic acid from 26:74 to 35:65 over 3 min, maintain at 35:65 for 22 min, to 50:50 over 5 min, to 100:0 over 15 min.
Flow rate: 2

Detector: Radioactivity

CHROMATOGRAM
Retention time: 18.5 (R), 20 (S)

KEY WORDS
stereoisomers

REFERENCE
Tsai,A.-l.; Vijjeswarapu,H.; Wu,K.K. Interaction between platelet receptor and iloprost isomers, *Biochim. Biophys.Acta*, **1988**, *942*, 220–226.

SAMPLE
Matrix: urine
Sample preparation: Inject an aliquot directly.

HPLC VARIABLES
Guard column: 50 × 4.6 not otherwise specified
Column: 250 × 4.6 Lichrosorb RP-18
Mobile phase: Gradient. A was MeOH containing 1 mL/L acetic acid. B was water containing 1 mL/L acetic acid. A:B from 30:70 to 48:52 over 60 min, to 55:45 over 80 min, to 80:20 over 60 min.
Flow rate: 2
Detector: Radioactivity

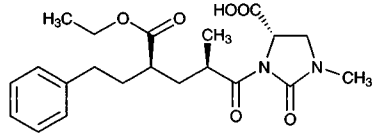
CHROMATOGRAM
Retention time: 170

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; radiolabelled

REFERENCE
Krause,W.; Hümpel,M.; Hoyer,G.-A. Biotransformation of the stable prostacyclin analogue, iloprost, in the rat, *Drug Metab.Dispos.*, **1984**, *12*, 645–651.

Imidapril



Molecular formula: $C_{20}H_{27}N_3O_6$
Molecular weight: 405.45
CAS Registry No.: 89271-37-9
Merck Index: 4947

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a 100 mg Bond Elut C18 SPE cartridge with 3 mL MeOH, with 3 mL water, and with 1 mL 100 mM HCl. Condition a 100 mg Bond Elut SI silica SPE cartridge with 1 mL acetone and 3 mL n-hexane. Plasma. 1 mL Plasma + 200 μL 2 M HCl + 200 μL water + 10 mL acetone, shake for 10 min, centrifuge at 1500 g for 10 min. Remove 10 mL of the supernatant and evaporate it to dryness under reduced pressure at 45°, reconstitute the residue in 1 mL 100 mM HCl, add 10 mL diethyl ether, shake for 5 min, centrifuge at 1500 g for 5 min. Add the aqueous layer to the C18 SPE cartridge, wash with 2 mL 100 mM HCl, wash with 1 mL MeOH:100 mM HCl 20:80, elute with 1 mL MeOH:100 mM HCl 60:40. Evaporate the eluate to dryness, reconstitute in 200 μL MeOH:100 mM HCl 60:40, evaporate to dryness into a vial, reconstitute in 25 μL water and 25 μL acetone, sonicate for 10 min, add

50 μL 0.2% IS1 in acetone, heat at 40° for 1 h, evaporate to dryness under reduced pressure at 45°, dissolve in 50 μL acetone and 1 mL hexane, add to the silica SPE cartridge, wash with 1 mL n-hexane:ethyl acetate 70:30, elute with 1 mL n-hexane:ethyl acetate 50:50, evaporate the eluate to dryness, reconstitute in 300 μL 20 ng/mL 9-anthrylmethyl myristate in MeCN:water 80:20, inject a 200 μL aliquot. Urine. 1 mL Urine + 200 μL water + 200 μL IS2 + 15 mL 500 mM Na_2HPO_4, make up to 25 mL with water, remove a 2 mL aliquot and add it to 10 mL diethyl ether, shake for 5 min, centrifuge at 1500 g for 5 min, discard the organic layer, add 10 mL chloroform, shake for 5 min, centrifuge at 1500 g for 5 min. Add 1 mL of the aqueous layer to the C18 SPE cartridge, wash with 2 mL 100 mM HCl, wash with 1 mL MeOH:100 mM HCl 20:80, elute with 1 mL MeOH:100 mM HCl 60:40. Evaporate the eluate to dryness, reconstitute in 200 μL MeOH:100 mM HCl 60:40, evaporate to dryness into a vial, reconstitute in 25 μL water and 25 μL acetone, sonicate for 10 min, add 50 μL 0.2% 9-anthryldiazomethane in acetone, heat at 40° for 1 h, evaporate to dryness under reduced pressure at 45°, dissolve in 50 μL acetone and 1 mL hexane, add to the silica SPE cartridge, wash with 1 mL n-hexane:ethyl acetate 70:30, elute with 1 mL n-hexane:ethyl acetate 50:50, evaporate the eluate to dryness, reconstitute in 250 μL MeCN:water 80:20, inject a 25 μL aliquot. (Synthesis of 9-anthryldiazomethane is as follows. Stir 8.8 g 9-anthraldehyde and 8.5 g 80% hydrazine hydrate in 150 mL EtOH at room temperature for 3 h, filter off the solid 9-anthraldehyde hydrazone and dry under vacuum (mp 124-6°) (Bull. Chem. Soc. Jpn. 1967, 40, 691). Dissolve 220 mg 9-anthraldehyde hydrazone in 100 mL anhydrous ether, add 800 mg activated manganese dioxide, follow the reaction by reverse-phase HPLC using MeCN at 0.4 mL/min and UV 254. At the end of the reaction filter off the manganese and wash it with 20 mL ether, evaporate the filtrate to obtain 9-anthryldiazomethane (mp 64-6°) (Anal.Biochem. 1980, 107, 116 and 1983, 132 456). Prepare activated manganese dioxide as follows. Stir a solution of 20 g potassium permanganate in 250 mL water at room temperature, add 10 g activated carbon (Nuchar C-190 or C-190N), stir for 16 h, filter (Buchner funnel), wash 4 times with 50 mL portions of water, dry in air, dry in an oven at 105-110° for 8-24 h (J.Org.Chem. 1970, 35, 3971).)

HPLC VARIABLES

Guard column: 4 × 4 5 μm Lichrosorb Si60
Column: 250 × 4 5 μm Hypersil MOS-5 dimethyloctyl
Mobile phase: MeCN:0.02% triethylamine 80:20, pH adjusted to 3.0 with 10% phosphoric acid
Flow rate: 1
Injection volume: 25-200
Detector: F ex 254 em 412

CHROMATOGRAM

Retention time: 23 (plasma), 20 (urine)
Internal standard: 9-anthryldiazomethane (IS1) (17), (S)-3-(N-[(S)-1-ethoxycarbonylundecyl]-L-alanyl)-1-methyl-2-oxoimidazoline-4-carboxylic acid (IS2) (42)
Limit of detection: 10 ng/mL (urine), 0.2 ng/mL (plasma)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; protect from light during and after derivatization; derivatization; SPE

REFERENCE

Tagawa,K.; Hayashi,K.; Mizobe,M.; Noda,K. Highly sensitive determination of imidapril, a new angiotensin I-converting enzyme inhibitor, and its active metabolite in human plasma and urine using high-performance liquid chromatography with fluorescent labelling reagent, *J.Chromatogr.*, **1993**, *617*, 95–103.

SAMPLE

Matrix: bulk
Sample preparation: 5 mg Imidapril hydrochloride + 15 mg L-alanine-β-naphthylamide hydrobromide + 100 μL chloroform + 50 μL pyridine + 2 mL 4.5 g/L N,N'-dicyclohexylcarbodiimide in chloroform, shake vigorously, let stand for 1 h, wash with 2 mL 1 M HCl, wash with two 2 mL portions of water. Remove a 1 mL aliquot and dilute it to 5 mL with chloroform, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Zorbax Sil

Mobile phase: Chloroform:MeOH:EtOH:diethylamine 600:10:2:0.1
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 5 (SRS), 5.5 (SRR), 7.5 (SSS, SSR), 15 (RSS), 17.5 (RRR), 19 (RRS), 21 (RSS)
Limit of detection: 0.05% of larger isomer

KEY WORDS
derivatization; chiral; normal phase

REFERENCE
Nishi,H.; Yamasaki,K.; Kokusenya,Y.; Sato,T. Optical resolution of imidapril hydrochloride by high-performance liquid chromatography and application to the optical purity testing of drugs, *J.Chromatogr.A*, **1994**, *672*, 125–133.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablets, weigh out amount equivalent to 25 mg imidapril, add 40 mL MeOH:water 40:60, shake vigorously for 10 min, make up to 50 mL with MeOH:water 40:60, filter (0.45 μm), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil 5C8
Mobile phase: MeOH:10 mM pH 2.7 phosphate buffer 40:60
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 215

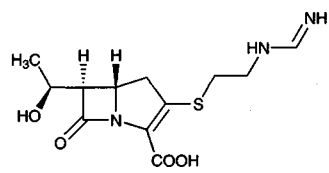
CHROMATOGRAM
Retention time: 4 (SRR, RSS), 6 (SSR, RRS), 7 (SRS, RSR)
Limit of detection: 0.05% for each diastereomer

OTHER SUBSTANCES
Simultaneous: degradation products, imidaprilat

KEY WORDS
tablets

REFERENCE
Nishi,H.; Yamasaki,K.; Kokusenya,Y.; Sato,T. Optical resolution of imidapril hydrochloride by high-performance liquid chromatography and application to the optical purity testing of drugs, *J.Chromatogr.A*, **1994**, *672*, 125–133.

Imipenem



Molecular formula: $C_{12}H_{17}N_3O_4S$
Molecular weight: 317.37
CAS Registry No.: 64221-86-9, 74431-23-5 (monohydrate)
Merck Index: 4954
Lednicer No.: 4 181

SAMPLE
Matrix: blood

Sample preparation: Filter (Ultrafree MC) plasma at 6000 g for 10 min, inject a 10 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova Pak C18
Mobile phase: 200 mM pH 7.2 borate buffer (Prepare the buffer by dissolving 12.4 g boric acid in 1 L water and adjusting pH to 7.2 with 1 M NaOH.)
Flow rate: 1
Injection volume: 10
Detector: UV 300

CHROMATOGRAM
Retention time: 4.44
Limit of detection: 30 ng/mL
Limit of quantitation: 80 ng/mL

KEY WORDS
pharmacokinetics; plasma

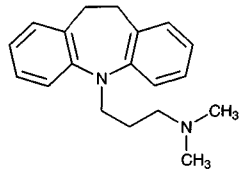
REFERENCE
Garcia-Capdevila,L.; López-Calull,C.; Arroyo,C.; Moral,M.A.; Mangues,M.A.; Bonal,J. Determination of imipenem in plasma by high-performance liquid chromatography for pharmaceutical studies in patients, *J.Chromatogr.B*, **1997**, *692*, 127–132.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10 μL aliquot of a 400 μg/mL solution.

HPLC VARIABLES
Column: 150 × 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

REFERENCE
Connolly,M.; Debenedetti,P.G.; Tung,H.-H. Freeze crystallization of imipenem, *J.Pharm.Sci.*, **1996**, *85*, 174–177.

Imipramine



Molecular formula: $C_{19}H_{24}N_2$
Molecular weight: 280.41
CAS Registry No.: 50-49-7, 113-52-0 (HCl)
Merck Index: 4955
Lednicer No.: 1 401; 2 420; 3 32; 4 146 201 203

SAMPLE
Matrix: blood
Sample preparation: Add 250 μL 2 M sodium carbonate to 500 μL plasma. Add 100 μL 1 μg/mL IS in MeOH, extract with 10 mL n-hexane. Shake for 30 min and centrifuge at 3000 g for 10 min. Cool in a dry ice-acetone bath. Add 200 μL 0.3% phosphoric acid to the upper organic layer. Shake for 10 min and centrifuge at 3000 g for 10 min. Separate the organic layer. Inject a 100 μL aliquot of the acidic aqueous layer.

HPLC VARIABLES
Column: 250 × 4.6 5 μm C18 Symmetry (Waters Millipore, USA)

Sample preparation: Filter (Ultrafree MC) plasma at 6000 g for 10 min, inject a 10 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova Pak C18
Mobile phase: 200 mM pH 7.2 borate buffer (Prepare the buffer by dissolving 12.4 g boric acid in 1 L water and adjusting pH to 7.2 with 1 M NaOH.)
Flow rate: 1
Injection volume: 10
Detector: UV 300

CHROMATOGRAM
Retention time: 4.44
Limit of detection: 30 ng/mL
Limit of quantitation: 80 ng/mL

KEY WORDS
pharmacokinetics; plasma

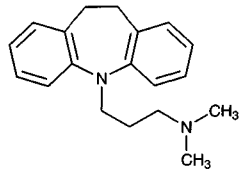
REFERENCE
Garcia-Capdevila,L.; López-Calull,C.; Arroyo,C.; Moral,M.A.; Mangues,M.A.; Bonal,J. Determination of imipenem in plasma by high-performance liquid chromatography for pharmaceutical studies in patients, *J.Chromatogr.B*, **1997**, *692*, 127–132.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10 μL aliquot of a 400 μg/mL solution.

HPLC VARIABLES
Column: 150 × 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

REFERENCE
Connolly,M.; Debenedetti,P.G.; Tung,H.-H. Freeze crystallization of imipenem, *J.Pharm.Sci.*, **1996**, *85*, 174–177.

Imipramine



Molecular formula: $C_{19}H_{24}N_2$
Molecular weight: 280.41
CAS Registry No.: 50-49-7, 113-52-0 (HCl)
Merck Index: 4955
Lednicer No.: 1 401; 2 420; 3 32; 4 146 201 203

SAMPLE
Matrix: blood
Sample preparation: Add 250 μL 2 M sodium carbonate to 500 μL plasma. Add 100 μL 1 μg/mL IS in MeOH, extract with 10 mL n-hexane. Shake for 30 min and centrifuge at 3000 g for 10 min. Cool in a dry ice-acetone bath. Add 200 μL 0.3% phosphoric acid to the upper organic layer. Shake for 10 min and centrifuge at 3000 g for 10 min. Separate the organic layer. Inject a 100 μL aliquot of the acidic aqueous layer.

HPLC VARIABLES
Column: 250 × 4.6 5 μm C18 Symmetry (Waters Millipore, USA)

Mobile phase: MeCN:67 mM potassium phosphate buffer adjusted to pH 3.0 with phosphoric acid 35:65 (After each chromatographic session wash the column with 200 mL MeCN:water 50:50.)
Flow rate: 1.2
Injection volume: 100
Detector: UV 226, UV 254, UV 400

CHROMATOGRAM
Retention time: 9.84
Internal standard: clovoxamine (6.5)
Limit of quantitation: 5 ng/mL (UV 226, UV 400); 7 ng/mL (UV 254)

OTHER SUBSTANCES
Extracted: metabolites, amitriptyline, clomipramine, desipramine, fluoxetine, maprotiline, nortriptyline
Simultaneous: amineptine, chlordiazepoxide, chlorpromazine, clonazepam, clorazepate, clozapine, cyamemazine, desmethylmaproptiline, desmethylvenlafaxine, doxepin, flunitrazepam, haloperidol, levomepromazine, lorazepam, loxapine, mianserine, sulpiride, trimipramine, venlafaxine, viloxazine, zolpidem, zopiclone
Noninterfering: diazepam, valproic acid
Interfering: carbamazepine, fluvoxamine

KEY WORDS
plasma

REFERENCE
Aymard,G.; Livi,P.; Pham,Y.T.; Diquet,B. Sensitive and rapid method for the simultaneous quantification of five antidepressants with their respective metabolites in plasma using high-performance liquid chromatography with diode-array detection, *J.Chromatogr.B*, **1997**, *700*, 183–189.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 1600 ng clomipramine in MeOH + 2 mL 1 M NaOH + 5 mL hexane:isoamyl alcohol 99:1, shake mechanically for 15 min, centrifuge at 1686 g for 5 min. Remove the organic phase and add it to 200 μL 0.05% orthophosphoric acid, shake for 15 min, centrifuge for 5 min, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: μBondapak/Porasil
Column: μBondapak C18
Mobile phase: MeCN:buffer 40:60 (Buffer was 13.68 g KH_2PO_4 in 2 L water, adjusted to pH 4.7 with dilute KOH.)
Column temperature: 50
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 5
Internal standard: clomipramine (7.5)
Limit of detection: 0.6 ng

OTHER SUBSTANCES
Extracted: amitriptyline, desipramine, nortriptyline
Simultaneous: chlordiazepoxide, chlorpromazine, cimetidine, clomipramine, diazepam, doxepin, flurazepam, lorazepam, oxazepam, pentobarbital, perphenazine, phenobarbital, phenytoin, prochlorperazine, propoxyphene, secobarbital, thioridazine, trifluoperazine
Noninterfering: acetaminophen, codeine, meperidine

KEY WORDS
plasma

REFERENCE

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

SAMPLE

Matrix: blood

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

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 10.1
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

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

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 μL 10 μg/mL protriptyline in water + 200 μL 80 g/L $NaHCO_3$ + 5 mL hexane, vortex for 15 s, centrifuge for 5 min. Remove the hexane layer and evaporate it in a stream of nitrogen at 60°. Reconstitute in 100 μL mobile phase, vortex for 15 s, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 300 × 4 10 μm μBondapak CN
Mobile phase: MeCN:MeOH:5 mM phosphate buffer 60:15:25, adjusted to pH 7.0
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 3.62
Internal standard: protriptyline (12.20)
Limit of detection: 6 ng/mL

OTHER SUBSTANCES

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

Noninterfering: caffeine, theophylline, salicylic acid, chlordiazepoxide, methaqualone, diazepam, acetaminophen, trifluoperazine

KEY WORDS
serum

REFERENCE
Koteel,P.; Mullins,R.E.; Gadsden,R.H. Sample preparation and liquid-chromatographic analysis for tricyclic antidepressants in serum, *Clin.Chem.*, **1982**, *28*, 462–466.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond-Elut C18 column with 2 volumes MeOH then 2 volumes water. Add 1 mL serum then 200 μL 700 ng/mL promazine in MeOH:0.1 M HCl 13:87 to each column, wash with 2 volumes water, wash with 2 volumes 0.1 M acetic acid, wash with MeOH/water, add 200 μL 10 mM ammonium acetate in MeOH, wait for 30 s, elute with vacuum, repeat elution process two more times. Combine eluates and evaporate them to dryness at 56-8° under compressed air. Reconstitute with 200 μL mobile phase, vortex 10 s, inject 75-100 μL aliquot. (MeOH/water was 500 mL MeOH:water 65:35 plus 25 μL concentrated HCl.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelco silica
Mobile phase: EtOH:MeCN:t-butylamine 98:2:0.05 (Mix 1 gallon EtOH with 77 mL MeCN and 1.9 mL t-butylamine.)
Flow rate: 2
Injection volume: 75-100
Detector: UV 254

CHROMATOGRAM
Retention time: 4.0
Internal standard: promazine (5.2)
Limit of detection: 2 ng/mL

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

KEY WORDS
serum; normal phase; SPE

REFERENCE
Beierle,F.A.; Hubbard,R.W. Liquid chromatographic separation of antidepressant drugs: I. Tricyclics, *Ther.Drug Monit.*, **1983**, *5*, 279–292.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 100 μL 1 μg/mL loxapine in isopropanol:diethylamine 99.9:0.1 + 250 μL 25% potassium carbonate containing 0.1% diethylamine + 5 mL hexane: isoamyl alcohol 97:3, vortex for 30 s, centrifuge at 500 g for 3 min. Remove the organic layer and add it to 100 μL 250 mM HCl, vortex for 30 s, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: 50 × 4.6 40 μm C8 (Supelco)

Column: 250 × 4.6 5 μm Supelcosil C8
Mobile phase: MeCN:water:diethylamine:85% phosphoric acid 53.3:45.1:1:0.4, pH adjusted to 7.2 with NaOH or phosphoric acid
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: k' 5.00
Internal standard: loxapine (k' 7.18)
Limit of detection: 2.5 ng/mL

OTHER SUBSTANCES
Extracted: amitriptyline, chlordiazepoxide, chlorpromazine, desipramine, desmethldiazepam, desmethylchlordiazepoxide, desmethyldoxepin, doxepin, fluphenazine, haloperidol, nortriptyline, oxazepam, thiothixene
Noninterfering: molindone, perphenazine, trifluoperazine
Interfering: diazepam

KEY WORDS
plasma

REFERENCE
Kiel,J.S.; Abramson,R.K.; Morgan,S.L.; Voris,J.C. A rapid high performance liquid chromatographic method for the simultaneous measurement of six tricyclic antidepressants, *J.Liq.Chromatogr.*, **1983**, *6*, 2761–2773.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Serum or plasma + 100 μL 1 μg/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 μL mobile phase, vortex for 2 min, add 0.5 mL hexane, vortex briefly, centrifuge for 5 min, discard upper hexane layer, inject a 100 μL aliquot of the lower layer.

HPLC VARIABLES
Column: 250 × 4 Bio-Sil ODS-10 (Bio-Rad)
Mobile phase: MeCN:pH 4.5 50 mM phosphate buffer 30:70 (Buffer was 6.9 g KH_2PO_4 in 1 L adjusted to pH 4.5 with orthophosphoric acid.)
Column temperature: 45
Flow rate: 2.5
Injection volume: 100
Detector: UV 202

CHROMATOGRAM
Retention time: 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, nortriptyline, triazolam

KEY WORDS
plasma; serum

REFERENCE
McCormick,S.R.; Nielsen,J.; Jatlow,P. Quantification of alprazolam in serum or plasma by liquid chromatography, *Clin.Chem.*, **1984**, *30*, 1652–1655.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 37 μL 2 μg/mL IS in MeOH + 500 μL pH 10 borate buffer + 1.5 mL hexane:isoamyl alcohol 95:5, shake for 10 min. Evaporate the organic layer to dryness under a stream of nitrogen, reconstitute in 100 μL MeOH, inject a 50 μL aliquot. (The borate buffer was prepared as follows. Prepare a solution of 61.8 g boric acid and 74.6 g KCl in 1 L water. Add 630 mL of this solution to 370 mL 106 g/L sodium carbonate solution. Adjust pH to 10.0 with 6 M NaOH and store at 35-37°.)

HPLC VARIABLES
Column: 250 × 4.6 Zorbax Sil
Mobile phase: MeOH:ammonium hydroxide 998:2
Flow rate: 1.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 5
Internal standard: N-desmethylclomipramine hydrochloride (10)
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Extracted: amitriptyline, nortriptyline, desipramine, 2-hydroxyimipramine, 2-hydroxydesipramine, metabolites
Also analyzed: doxepin, desmethyldoxepin, desmethylclomipramine, clomipramine, maprotiline, protriptyline
Noninterfering: chlordiazepoxide, diazepam, flurazepam, oxazepam, thioridazine

KEY WORDS
plasma

REFERENCE
Sutfin,T.A.; D'Ambrosio,R.; Jusko,W.J. Liquid-chromatographic determination of eight tri- and tetracyclic antidepressants and their major active metabolites, *Clin.Chem.*, **1984**, *30*, 471–474.

SAMPLE
Matrix: blood
Sample preparation: Evaporate 200 μL 1 μg/mL clomipramine in MeOH into a tube, add 2 mL plasma, add 2 mL pH 10 Titrisol buffer (Merck), add 8 mL diethyl ether, shake for 15 min, centrifuge at 2800 g for 5 min. Remove the organic phase and shake it with 100 μL 50 mM phosphoric acid for 15 min, centrifuge at 2800 g for 10 s. Remove the aqueous layer and vortex it with 2 mL diethyl ether for 10 s, centrifuge at 2800 g. Discard the organic layer and inject a 10-50 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:25 mM KH_2PO_4:water 45:50:5
Flow rate: 1
Injection volume: 10-50
Detector: UV 254

CHROMATOGRAM
Retention time: 8.9
Internal standard: clomipramine (13)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Simultaneous: desipramine, trimipramine
Noninterfering: alimemazine, alprazolam, amineptine, amitriptyline, caffeine, carbamazepine, citalopram clobazam, desmethylflunitrazepam, diazepam, dibenzepine, estazolam, ethyl lofla-

zepate, indalpine, loprazolam, lorazepam, meprobamate, nitrazepam, norclobazam, nordiazepam, nortriptyline, oxazepam, triazolam, viloxazine
Interfering: monodesmethyltrimipramine, flunitrazepam, levomepromazine

KEY WORDS
plasma

REFERENCE
Pok Phak,R.; Conquy,T.; Gouezo,F.; Viala,A.; Grimaldi,F. Determination of metapramine, imipramine, trimipramine and their major metabolites in plasma by reversed-phase column liquid chromatography, *J.Chromatogr.*, **1986**, *375*, 339–347.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Analytichem cyanopropyl SPE cartridge with 1 mL water and 1 mL MeOH, do not allow to dry. Add 1 mL serum + 250 μL 50 mM sodium n-heptanesulfonic acid to the SPE cartridge, wash with 1 mL water, 1 mL MeOH:water 50:50, air dry cartridge, elute with 1 mL MeOH:triethylamine 99.2:0.8, evaporate eluate to dryness under a stream of nitrogen at 40°, reconstitute residue with 250 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5μm Spherisorb cyanopropyl
Mobile phase: MeOH:20 mM phosphoric acid containing 0.05% N,N-diethyloctylamine 55:45, pH was 2.4
Flow rate: 1.5
Injection volume: 50
Detector: UV 214

CHROMATOGRAM
Retention time: 9
Internal standard: imipramine

OTHER SUBSTANCES
Simultaneous: doxepin

KEY WORDS
serum; imipramine is IS; SPE

REFERENCE
Emm,T.; Lesko,L.J.; Perkal,M.B. Simultaneous determination of doxepin and nordoxepin in serum using high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *419*, 445–451.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elut C-18 SPE cartridge twice with MeOH and twice with water. 500 μL Serum + 50 μL 1 μg/mL N-propionylprocainamide in 2.5 mM HCl, add to SPE cartridge, wash with 2 volumes water, wash with 2 volumes 0.1 M acetic acid, wash with 1 volume MeOH:2.5 mM HCl 10:90. Add 200 μL 10 mM acetic acid and 5 mM diethylamine in MeOH to column, let stand 1 min, elute under vacuum, repeat, evaporate eluents to dryness under nitrogen at room temperature, reconstitute in 100 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Guard column: Pelliguard LC-CN (Supelco)
Column: 150 × 4.6 5 μm Supelcosil LC-PCN
Mobile phase: MeCN:MeOH:10 mM pH 7.0 phosphate buffer 58:14:28
Flow rate: 1.2
Injection volume: 40
Detector: UV 254

CHROMATOGRAM
Retention time: 10.5
Internal standard: N-propionylprocainamide (6)
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Extracted: amitriptyline, desipramine, doxepin, nortriptyline, protriptyline, trimipramine
Simultaneous: atropine, butalbital, chlorpromazine, maprotiline, methadone, norpropoxyphene, phenylpropanolamine, procainamide, prochlorperazine, promethazine, propranolol, quinidine, trifluoperazine, trimeprazine
Noninterfering: acetaminophen, allopurinol, amikacin, amoxapine, amytal, bretylium, caffeine, carbamazepine, carisoprodol, chloramphenicol, chlordiazepoxide, chlorpropamide, clonazepam, codeine, diazepam, disopyramide, droperidol, ethinamate, ethinamate, ethosuximide, fluphenazine, flurazepam, furosemide, gentamicin, haloperidol, hydrochlorothiazide, hydroxyzine, ibuprofen, kanamycin, lidocaine, loxapine, meperidine, mephobarbital, meprobamate, methaqualone, methotrexate, morphine, nafcillin, naloxone, neomycin, perphenazine, phenacetin, phenobarbital, phenytoin, prazepam, primidone, procaine, propoxyphene, reserpine, salicylamide, salicylic acid, secobarbital, spironolactone, theophylline, thiopental, thioridazine, tobramycin, valproic acid, verapamil
Interfering: hydroxynortriptyline

KEY WORDS
serum; SPE

REFERENCE
Lin,W.-N.; Frade,P.D. Simultaneous quantitation of eight tricyclic antidepressants in serum by high-performance liquid chromatography, *Ther.Drug Monit.*, **1987**, *9*, 448–455.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 250 μL di-iso-propyl ether:n-butyl alcohol 7:3 containing 800 ng/mL minaprine, centrifuge 2 min, shake, centrifuge 5 min, inject 50 μL aliquot of top organic layer.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm Brownlee cyano spheri-5
Column: 250 × 4.6 5 μm Altex ultrasphere cyano
Mobile phase: MeCN:THF:water:2 M ammonium formate (pH 4.0) 700:100:195:5
Column temperature: 20
Flow rate: 1.5
Injection volume: 50
Detector: UV 248

CHROMATOGRAM
Retention time: 7.5
Internal standard: minaprine (5.5)
Limit of detection: 20 ng/mL

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

KEY WORDS
serum

REFERENCE
Mazzi,G. Simple and practical high-performance liquid chromatographic assay of some tricyclic drugs, haloperidol, diltiazem, verapamil, propafenone, and amiodarone, *Chromatographia*, **1987**, *24*, 313–316.

SAMPLE
Matrix: blood

Sample preparation: Inject 200 μL serum onto column A and elute with mobile phase A for 10 min then back-flush column A onto column B with mobile phase B for 4 min. Elute column B with mobile phase B and monitor the effluent. Remove column A from circuit and wash with MeCN:water 60:40 for 6 min then with mobile phase A for 10 min.

HPLC VARIABLES

Column: A 40 × 4 TSKprecolumn PW (Tosoh); B 150 × 4 TSKgel ODS-80TM (Tosoh)
Mobile phase: A 50 mM pH 7.5 potassium phosphate; B MeCN:100 mM pH 2.7 potassium phosphate 32.5:67.5, containing 0.2 g/L sodium 1-heptanesulfonate
Flow rate: 1
Injection volume: 200
Detector: UV 210

CHROMATOGRAM

Retention time: 15
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: amitriptyline, amoxapine, clomipramine, doxepin, desipramine, maprotiline, trimipramine
Interfering: nortriptyline

KEY WORDS

serum; column-switching; use gradient to determine metabolites

REFERENCE

Matsumoto,K.; Kanba,S.; Kubo,H.; Yagi,G.; Iri,H.; Yuki,H. Automated determination of drugs in serum by column-switching high-performance liquid chromatography. IV. Separation of tricyclic and tetracyclic antidepressants and their metabolites, *Clin.Chem.*, **1989**, *35*, 453–456.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Plasma + 100 μL 10 mM HCl + 200 μL 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 μL 10 mM HCl, wash with 2 mL MTBE, wash with 2 mL hexane, inject a 3-20 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 250 × 4.6 5 μm 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: 17.9
Internal standard: imipramine

OTHER SUBSTANCES

Extracted: diltiazem
Simultaneous: alprazolam, amitriptyline, desipramine, loxapine, nortriptyline
Noninterfering: clomipramine

KEY WORDS

plasma; imipramine is IS

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: 1 mL Plasma + 200 μL saturated potassium carbonate, mix, add 5 mL hexane:isopropanol 98:2, shake at 230 rpm for 15 min, centrifuge at 800 g for 10 min. Remove the organic layer and add it to 100 μL 0.5% orthophosphoric acid, shake for 15 min, centrifuge at 800 g at 5° for 10 min, inject a 50 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Spherisorb ODS-1
Mobile phase: MeCN:water:1 M NaH_2PO_4 55:35:10
Flow rate: 1.8
Injection volume: 50
Detector: UV 205

CHROMATOGRAM

Retention time: 6.4
Internal standard: imipramine

OTHER SUBSTANCES

Simultaneous: diphenhydramine

KEY WORDS

plasma; imipramine is IS

REFERENCE

Selinger,K.; Prevost,J.; Hill,H.M. High-performance liquid chromatography method for the determination of diphenhydramine in human plasma, *J.Chromatogr.*, **1990**, *526*, 597–602.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 10 μL 1 mg/mL 8-hydroxychloroimipramine + 500 μL 0.6 M pH 10.4 carbonate buffer + 5 mL ethyl acetate:heptane 20:80, shake for 2.5 min, centrifuge at 3000 g for 10 min. Remove organic layer and add it to 125 μL pH 2.4 25 mM KH_2PO_4, shake for 2.5 min, centrifuge at 3000 g for 10 min. Remove the aqueous layer and put it in a rotary evaporator for 25 min to remove traces of organic solvent. Inject a 50 μL aliquot.

HPLC VARIABLES

Column: 120 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:buffer 30:70 (Buffer was 10 Mm KH_2PO_4 + 5 mM tetramethylammonium chloride adjusted to pH 2.4 with concentrated phosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: E, ESA Coulochem Model 5100A, detector 1 +0.2 V, detector 2, +0.68 V, guard cell 0.70 V, gain 12 × 10, response time 0.4 s; UV 215

CHROMATOGRAM

Retention time: 9.39
Internal standard: 8-hydroxychloroimipramine (6.35)
Limit of detection: 0.5 ng/mL (electrochemical)
Limit of quantitation: 15 ng/mL (UV)

OTHER SUBSTANCES

Simultaneous: 2-hydroxydesipramine, 2-hydroxyimipramine, desipramine, chlorodesipramine, chloroimipramine, mesoridazine
Noninterfering: doxepin, nordoxepin, amitriptyline, fluoxetine, norfluoxetine, triazolam, alprazolam

KEY WORDS
plasma

REFERENCE
Foglia,J.P.; Sorisio,D.; Perel,J.M. Determination of imipramine, desipramine and their hydroxy metabolites by reversed-phase chromatography with ultraviolet and coulometric detection, *J.Chromatogr.*, **1991**, *572*, 247–258.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 3 μL 20 ng/mL clobazam + 1 mL saturated sodium borate (adjusted to pH 11 with 6 M NaOH) + 5 mL n-hexane, mix 2 min, centrifuge at 3000 g for 10 min. Remove organic phase and evaporate to dryness under a stream of helium at 30°. Reconstitute in 20 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 20 mm long Pelliguard LC-8 40 μm (Supelco)
Column: 150 × 4.6 C8 5 μm (Supelco)
Mobile phase: MeCN:buffer 50:50 (Buffer was 1.2 mL butylamine in 1 L 10 mM NaH_2PO_4, pH adjusted to 3 with phosphoric acid.)
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 3.025
Internal standard: clobazam (k' 1.344)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: desipramine, nortriptyline, amitriptyline, clomipramine
Simultaneous: nitrazepam, lorazepam, clonazepam, triazolam, flunitrazepam, alprazolam, diazepam, haloperidol, maprotiline

KEY WORDS
serum

REFERENCE
Segatti,M.P.; Nisi,G.; Grossi,F.; Mangiarotti,M.; Lucarelli,C. Rapid and simple high-performance liquid chromatographic determination of tricyclic antidepressants for routine and emergency serum analysis, *J.Chromatogr.*, **1991**, *536*, 319–325.

SAMPLE
Matrix: blood
Sample preparation: For each 1 mL plasma or serum add 10 μL 14 μg/mL trimipramine in MeOH. Inject serum or plasma directly onto column A with mobile phase A, elute with mobile phase A to waste. After 15 min elute column A onto column B (foreflush) with mobile phase B. After 2 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Re-equilibrate column A with mobile phase A.

HPLC VARIABLES
Column: A 20 × 4.6 10 μm Hypersil MOS C8; B 20 × 4.6 5 μm Hypersil CPS CN + 250 × 4.6 5 μm Nucleosil 100 CN
Mobile phase: A MeOH:water 5:95; B MeCN:MeOH:buffer 578:188:235 (Buffer was 10 mM K_2HPO_4 adjusted to pH 6.8 with 85% phosphoric acid.)
Flow rate: 1.5
Injection volume: 100
Detector: UV 214

CHROMATOGRAM
Retention time: 9.65

Internal standard: trimipramine (6.5)
Limit of detection: 1 ng/mL (with three injections onto column A before switching), 5-10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, amitriptyline, clomipramine, desipramine, doxepin, fluvoxamine, maprotiline, nortriptyline
Noninterfering: chlordiazepoxide, clobazam, clozapine, diazepam, flurazepam, fluspirilene, haloperidol, nitrazepam, oxazepam, perazine, pimozide, spiroperidol, trifluperidol

KEY WORDS
plasma; serum; column-switching

REFERENCE
Härtter,S.; Hiemke,C. Column switching and high-performance liquid chromatography in the analysis of amitriptyline, nortriptyline and hydroxylated metabolites in human plasma or serum, *J.Chromatogr.*, **1992**, *578*, 273–282.

SAMPLE
Matrix: blood
Sample preparation: Add 10 µL 20 µg/mL oxaprotiline in MeOH to 990 µL plasma or serum. Inject 100 µL plasma or serum onto column A with mobile phase A and elute to waste, after 15 min elute column A onto column B with mobile phase B for 2 min. Remove column A from circuit and re-equilibrate it with mobile phase A for 5 min. Chromatograph on column B with mobile phase B.

HPLC VARIABLES
Column: A 20 × 4.6 10 µm Hypersil MOS C8; B 20 × 4.6 5 µm Hypersil CPS CN + 250 × 4.6 5 µm Nucleosil 100 CN
Mobile phase: A MeOH:water 5:95; B MeOH:MeCN:10 mM pH 6.8 potassium phosphate buffer 188:578:235
Flow rate: 1.5
Injection volume: 100
Detector: UV 214

CHROMATOGRAM
Retention time: 9.7
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Simultaneous: clozapine, fluvoxamine, metoclopramide, fluoxetine, norfluoxetine, nortriptyline, desipramine, maprotiline, doxepin, clomipramine, amitriptyline
Noninterfering: haloperidol, spiroperidol, pimozide, fluspirilene, trifluperidol, perazine, chlordiazepoxide, clobazam, diazepam, nordiazepam, flurazepam, lorazepam, nitrazepam, oxazepam, carbamazepine
Interfering: oxaprotiline

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: Condition a 1 mL BondElut C18 SPE cartridge with 1 mL 1 M HCl, 1 mL MeOH, 1 mL water, and 1 mL 1% potassium carbonate. 700 µL Serum + 50 µL 5 µg/mL trimipramine in 5% potassium bicarbonate + 700 µL MeCN, vortex, centrifuge at 1500 g for 5 min, add supernatant to SPE cartridge (at ca. 1 mL/min). Wash with 2 mL water and 1 mL MeCN, elute with 250 µL MeOH:35% perchloric acid 20:1 by gravity (10 min) then centrifuge for 20 s to remove rest of eluant, inject a 50 µL aliquot of the eluate.

HPLC VARIABLES
Guard column: 15 mm 7 μm Brownlee RP-8
Column: 150 × 4.6 5 μm Ultrasphere Octyl
Mobile phase: MeCN:water 37.5:62.5 containing 0.5 g/L tetramethylammonium perchlorate and 0.5 mL/L 7% perchloric acid
Flow rate: 1.5
Injection volume: 50
Detector: UV 215

CHROMATOGRAM
Retention time: 7.1
Internal standard: trimipramine (9.6)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: amitriptyline, clomipramine, desipramine, doxepin, fluoxetine, maprotiline, protriptyline
Interfering: desmethylmaprotiline, fluvoxamine, nortriptyline

KEY WORDS
serum; SPE

REFERENCE
Gupta,R.N. An improved solid phase extraction procedure for the determination of antidepressants in serum by column liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 2751–2765.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 200 ng/mL IS in MeOH + 1 mL 50 mM pH 10 borate buffer, vortex briefly, add to an Extrelut 3 SPE cartridge, let stand for 5 min, elute with 15 mL hexane:dichloromethane 50:50. Add the eluate to 3 mL 50 mM sulfuric acid, mix for 10 min, centrifuge at 3000 g for 10 min. Remove the aqueous layer and add it to 6 mL hexane: dichloromethane 50:50, wash for 5 min, centrifuge. Make the aqueous layer basic with 150 μL 28% ammonia, extract twice with 3 mL hexane:dichloromethane 50:50. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm Spherisorb cyano
Column: 250 × 4.6 5 μm Ultrasphere cyano
Mobile phase: MeCN:buffer 60:40 (Buffer was 50 mM KH_2PO_4 adjusted to pH 6.5 with 28% ammonia.)
Flow rate: 1
Injection volume: 20
Detector: E, 5100 A Coulochem, 5020 guard cell 1.00 V, 5011 analytical cell, detector 1 0.55 V, detector 2 0.80 V, output of detector 2 is monitored

CHROMATOGRAM
Retention time: 19.7
Internal standard: methylrisperidone (R68808) (14.3)

OTHER SUBSTANCES
Extracted: chlorpromazine, clomipramine, cyamemazine, desipramine, droperidol, flunitrazepam, haloperidol, pipamperone, risperidone
Noninterfering: alprazolam, bromazepam, carbamazepine, chlorazepate, diazepam, diphenylhydantoin, estazolam, ethylbenzatropine, oxazepam, phenobarbital, triazolam, valproic acid
Interfering: trihexyphenidyl

KEY WORDS
plasma; SPE

REFERENCE
Le Moing,J.P.; Edouard,S.; Levron,J.C. Determination of risperidone and 9-hydroxyrisperidone in human plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1993**, *614*, 333–339.

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

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

CHROMATOGRAM
Retention time: 4.6
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, lidocaine, methadone, methaqualone, mexiletine, midazolam, norchlorimipramine, nordiazepam, nordoxepin, norfluoxetine, norverapamil, pentazocine, promazine, propafenone, propoxyphene, propranolol, protriptyline, quinidine, temazepam, trazodone, trimipramine
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: maprotiline, nortriptyline, 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: 500 μL Plasma + 100 μL EtOH + 500 μL 500 mM NaOH, mix, add to an Extrelut-1 SPE cartridge, let stand for 10 min, elute with 5 mL n-hexane:isoamyl alcohol 98:2. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 125 × 4.6 5 μm Partisphere silica (Whatman)
Mobile phase: Hexane:EtOH:dichloromethane:diethylamine 77:18:5:0.003
Flow rate: 1.3
Detector: UV 214

CHROMATOGRAM
Retention time: 3
Internal standard: imipramine

OTHER SUBSTANCES
Extracted: clomipramine
Simultaneous: amitriptyline, desipramine, doxepin, fluoxetine

KEY WORDS
plasma; SPE; normal phase; imipramine is IS

REFERENCE
Altieri,I.; Pichini,S.; Pacifici,R.; Zuccaro,P. Improved clean-up procedure for the high-performance liquid chromatographic assay of clomipramine and its demethylated metabolite in human plasma, *J.Chromatogr.B*, **1995**, *669*, 416–417.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 500 mM pH 9 phosphate buffer, vortex briefly, add 7 mL n-heptane:isoamyl alcohol 98.5:1.5, shake on a rotating shaker at 32 rpm for 15 min, centrifuge at 1500 g for 5 min. Remove 6 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 μL mobile phase, vortex for 10 s, centrifuge at 1500 g for 3 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelguard LC-8-DB (Supelco)
Column: 150 × 4.6 5 μm Supelcosil LC-8-DB
Mobile phase: MeCN:MeOH:buffer 20:25:55 (Buffer was 50 mM pH 3.0 KH_2PO_4 containing 0.2% triethylamine.)
Flow rate: 1
Injection volume: 100
Detector: UV 235

CHROMATOGRAM
Retention time: 11.6
Internal standard: imipramine

OTHER SUBSTANCES
Extracted: ticlopidine

KEY WORDS
plasma; imipramine is IS

REFERENCE
Dal Bo,L.; Verga,F.; Marzo,A.; Ambrosoli,L.; Poli,A. Determination of ticlopidine in human plasma by high-performance liquid chromatography and ultraviolet absorbance detection, *J.Chromatogr.B*, **1995**, *665*, 404–409.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of

the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 251

CHROMATOGRAM

Retention time: 8.53
Limit of detection: <120 ng/mL

KEY WORDS

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

REFERENCE

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

SAMPLE

Matrix: blood
Sample preparation: 100 μL Serum + 25 μL 5 μg/mL clomipramine in MeOH, vortex for 30 s, add 100 μL 5 M NaOH, add 2 mL hexane, vortex for 30 s, centrifuge at 3000 g for 3 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 20°, reconstitute the residue in 50 μL mobile phase, vortex for 30 s, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Microsorb MV octadecyl
Mobile phase: MeCN:10 mM triethylamine 60:40, pH adjusted to 3.0 with 85% phosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 260

CHROMATOGRAM
Retention time: 6.0
Internal standard: clomipramine (8.3)
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Extracted: desipramine

KEY WORDS
mouse; serum; pharmacokinetics

REFERENCE
Yoo,S.D.; Holladay,J.W.; Fincher,T.K.; Dewey,M.J. Rapid microsample analysis of imipramine and desipramine by reversed-phase high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1995**, *668*, 338–342.

SAMPLE
Matrix: blood, CSF, tissue
Sample preparation: Homogenize brain tissue in 4 mL 50 mM pH 7.4 Tris-HCl. 500 μL Serum or 200 μL CSF or 1 mL homogenate + 100 μL 2 M NaOH + IS + 5 mL hexane:dichloromethane 60:40, shake for 15 min, centrifuge at 4000 rpm for 10 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm Lichrospher RP select B
Mobile phase: MeCN:0.1% pH 4 diethylamine in water 40:60
Flow rate: 1.5
Injection volume: 30
Detector: UV 254

CHROMATOGRAM
Retention time: 13.85
Internal standard: desmethylclomipramine chlorhydrate (6.85)
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, desipramine

KEY WORDS
rat; serum; brain; pharmacokinetics

REFERENCE
Besret,L.; Debruyne,D.; Rioux,P.; Bonvalot,T.; Moulin,M.; Zarifian,E.; Baron,J.-C. A comprehensive investigation of plasma and brain regional pharmacokinetics of imipramine and its metabolites during and after chronic administration in the rat, *J.Pharm.Sci.*, **1996**, *85*, 291–295.

SAMPLE
Matrix: blood, tissue
Sample preparation: Serum. 1 mL Serum + 800 ng nortriptyline + 4 mL MeOH + 5 mL 2.5% perchloric acid, shake vigorously, centrifuge at 11000 g for 15 min. Add supernatant to 1 mL 4 M KOH, centrifuge. Add supernatant (9 mL) to 10 mL diethyl ether:ethyl acetate 85:15, shake for 15 min. Remove 8 mL of organic phase and evaporate it to dryness under a stream of nitrogen. Dissolve residue in 200 μL mobile phase buffer:MeOH 9:1, inject 100 μL aliquot. Tissue. 2 g Brain tissue + 10 mL 2.5% perchloric acid + 8 mL MeOH + 1.6 μg nortriptyline,

homogenize, centrifuge at 11000 g for 15 min. Add supernatant to 4 mL 4 M KOH, centrifuge. Add supernatant to 20 mL diethyl ether:ethyl acetate 3:1, shake for 15 min. Remove 8 mL of organic phase and evaporate it to dryness under a stream of nitrogen. Dissolve residue in 200 μL mobile phase buffer:MeOH 9:1, inject 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Cosmosil 5C18
Mobile phase: MeOH:THF:buffer 45:17:88 (Buffer was 1% triethylamine adjusted to pH 3.0 with phosphoric acid.)
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 11.2
Internal standard: nortriptyline (14.2)
Limit of detection: 10 ng/g (tissue)

OTHER SUBSTANCES
Simultaneous: desipramine

KEY WORDS
serum; rat

REFERENCE
Sugita,S.; Kobayashi,A.; Suzuki,S.; Yoshida,T.; Nakazawa,K. High-performance liquid chromatographic determination of imipramine and its metabolites in rat brain, *J.Chromatogr.*, **1987**, *421*, 412–417.

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM
Retention time: 17.9
Internal standard: cianopramine (8.93)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, tranylcypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine

Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine
Interfering: thiothixene

KEY WORDS
serum; whole blood; liver

REFERENCE
McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE
Matrix: blood, tissue, urine
Sample preparation: Serum, urine. 500 μL Serum or urine + 100 μL 2 μg/mL diazepam + 200 μL 20% sodium carbonate + 500 μL water + 3 mL n-hexane:isoamyl alcohol 98.5:1.5, mix for 2 min, centrifuge at 1200 g for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, inject a 10 μL aliquot. Tissue. Homogenize 1 g sample with 9 mL 100 mM HCl and 100 μL 20 μg/mL diazepam, centrifuge at 15000 g for 10 min. Add 500 μL 20% sodium carbonate and 4 mL n-hexane:isoamyl alcohol 98.5:1.5 to 1 mL of the supernatant, mix for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, filter by microconcentrator (Microcon-30, Grace). Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 2 μm TSK gel Super-Octyl (A) or 100 × 4.6 5 μm Hypersil MOS-C8 (B), (Yokogawa, Japan)
Mobile phase: MeOH:20 mM pH 7 KH_2PO_4 60:40
Flow rate: 0.6
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 12.8 (A), 23.8 (B)
Internal standard: diazepam (4.4, A)
Limit of quantitation: 50 ng/mL (serum, urine) (A), 500 ng/mL (tissue) (A)

OTHER SUBSTANCES
Extracted: amitriptyline, amoxapine, clomipramine, desipramine, dothiepin, doxepin, maprotiline, melitracen, mianserin, nortriptyline
Noninterfering: barbital, carbamazepine, ethosuximide, hexobarbital, lofepramine, pentobarbital, phenobarbital, phenytoin, primidone, sulpiride, trimethadione, trimipramine

KEY WORDS
serum; brain; liver

REFERENCE
Tanaka,E.; Terada,M.; Nakamura,T.; Misawa,S.; Wakasugi,C. Forensic analysis of eleven cyclic antidepressants in human biological samples using a new reversed-phase chromatographic column of 2 μm porous microspherical silica gel, *J.Chromatogr.B*, **1997**, *692*, 405–412.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + 20 μL 10 μg/mL pericyazine in MeOH, mix, add 0.2 mL 1 M sodium carbonate buffer to adjust pH to 9.6. Add 10 mL distilled diethyl ether, shake on an automatic shaker for 10 min, centrifuge at 1000 g for 10 min in a refrigerated centrifuge. Remove the upper organic layer and add 100μL 100 mM orthophosphoric acid. Shake for 10 min and centrifuge at 1000 g for 10 min. Discard the top layer and inject a 50 μL aliquot of the acid layer.

HPLC VARIABLES
Guard column: 40 × 4.6 10 μm RP-18

Column: 300 × 3.9 Bondclone 10 C18 (Phenomenex)
Mobile phase: MeCN:100 mM K_2HPO_4, adjust pH to 6.0 with orthophosphoric acid
Flow rate: 2
Injection volume: 50
Detector: E, EDT Chromajet, oxidation cell +1.00 V

CHROMATOGRAM
Retention time: 12.3
Internal standard: pericyazine (6.2)
Limit of detection: 3 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, desipramine

KEY WORDS
plasma; pharmacokinetics; silanize glassware

REFERENCE
Chen,A.G.; Wing,Y.K.; Chiu,H.; Lee,S.; Chen,C.N.; Chan,K. Simultaneous determination of imipramine, desipramine and their 2- and 10-hydroxylated metabolites in human plasma and urine by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *693*, 153–158.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + 1 mL 600 mM pH 11.3 K_2CO_3 + 100 μL 5 (plasma) or 20 (urine) μM 2-hydroxydesmethylclomipramine in EtOH + 5 mL heptane:MTBE 1:1 + 5% n-butanol, vortex 1 min, centrifuge at 1400 g for 10 min, freeze at -50° (dry ice/ethanol). Remove organic layer and add it to 1 mL 20 mM HCl, vortex 1 min, centrifuge at 1400 g for 10 min, freeze at -50° (dry ice/ethanol). Discard organic layer. Thaw out aqueous layer and make alkaline (pH 11) by adding 500 μL 600 mM pH 11.3 K_2CO_3. Add 3 mL heptane:MTBE 1:1 + 5% n-butanol, vortex 1 min, centrifuge at 1400 g for 10 min, freeze at -50° (dry ice/ethanol). Remove organic layer and evaporate it to dryness at 50° under a stream of nitrogen. Dissolve residue in 100 μL mobile phase, vortex for 5 s, centrifuge at 1400 g for 1 min, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4 7 μm 120 Å Nucleosil
Column: 250 × 4 5 μm 100 Å Nucleosil RP-phenyl
Mobile phase: MeCN:buffer 30:70 (Buffer was 14.05 g sodium perchlorate and 1.6 mL 60% perchloric acid in 5 L water, pH 2.5.)
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 16.62
Internal standard: 2-hydroxydesmethylclomipramine (10.02)
Limit of detection: 10 nM (urine), 5 nM (plasma)

OTHER SUBSTANCES
Simultaneous: desipramine, metabolites

KEY WORDS
plasma

REFERENCE
Nielsen,K.K.; Brsen,K. High-performance liquid chromatography of imipramine and six metabolites in human plasma and urine, *J.Chromatogr.*, **1993**, *612*, 87–94.

SAMPLE
Matrix: blood, urine

Sample preparation: 100 μL Serum or urine + 25 μL 5 μg/mL clomipramine in MeOH + 100 μL 5 M NaOH + 2 mL hexane, vortex for 30 s, centrifuge at 5000 rpm for 3 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 20°, reconstitute the residue in 50 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 10 × 4.6 5 μm Microsorb MV C18
Mobile phase: MeCN:10 mM triethylamine in water 60:40, pH adjusted to 3.0 with 85% phosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 260

CHROMATOGRAM
Internal standard: clomipramine
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: desipramine

KEY WORDS
mouse; serum; pharmacokinetics

REFERENCE
Yoo,S.D.; Holladay,J.W.; Fincher,T.K.; Baumann,H.; Dewey,M.J. Altered disposition and antidepressant activity of imipramine in transgenic mice with elevated α-1-acid glycoprotein, *J.Pharmacol.Exp.Ther.*, **1996**, *276*, 918–922.

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

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

CHROMATOGRAM
Retention time: 15.113

KEY WORDS
whole blood

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

SAMPLE
Matrix: formulations
Sample preparation: Crush tablet or capsule, to 2 mg amitriptyline add 20 mL MeOH, shake 30 min, centrifuge at 2000 rpm for 5 min, to 5 mL supernatant add 4 mL 1.25 mg/mL norephedrine.HCl in MeOH, dilute to 10 mL with MeOH.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax CN
Mobile phase: MeCN:MeOH:25 mM pH 4.8 sodium acetate-acetic acid buffer 35:45:20
Flow rate: 2.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 3.8
Internal standard: norephedrine (2.7)

OTHER SUBSTANCES
Also analyzed: chlorpromazine, amitriptyline, thioridazine, trifluoperazine

KEY WORDS
tablets; capsules

REFERENCE
Lovering,E.G.; Beaulieu,N.; Lawrence,R.C.; Sears,R.W. Liquid chromatographic method for identity, assay, and content uniformity of five tricyclic drugs, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 168–171.

SAMPLE
Matrix: hair
Sample preparation: Wash hair in water, rinse 3 times with MeOH, dry, weigh. 5-25 mg Washed hair + 1 mL 1 M NaOH, heat at 70° for 30 min, adjust pH to 9.5-10. 1 mL Extract + 1 μg protriptyline + 1 mL water + 1 mL 200 mM sodium carbonate buffer, mix, extract with hexane: butanol 95:5 for 20 min. Remove the organic layer and add it to 100 μL 0.2% orthophosphoric acid, mix for 20 min, inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Newguard RP-18
Column: 100 × 4.6 Spheri-5 RP-C18
Mobile phase: MeCN:buffer 40:60 (Buffer was 1.2 L 100 mM pH 7.0 NaH_2PO_4 + 30 mL diethylamine.)
Flow rate: 2
Injection volume: 30
Detector: UV 214

CHROMATOGRAM
Internal standard: protriptyline (4)

OTHER SUBSTANCES
Extracted: amitriptyline, clomipramine, desipramine, dothiepin, doxepin, haloperidol, mianserin, nortriptyline

KEY WORDS
may be interferences

REFERENCE
Couper,F.J.; McIntyre,I.M.; Drummer,O.H. Extraction of psychotropic drugs from human scalp hair, *J.Forensic Sci.*, **1995**, *40*, 83–86.

SAMPLE
Matrix: microsomal incubations

Sample preparation: Add 200 μL MeCN to 200 μL microsomal incubation, centrifuge at 3000 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 Zorbax Phenyl
Mobile phase: MeCN:buffer 50:50 (Buffer was 20 mM perchloric acid adjusted to pH 2.5 with NaOH.)
Flow rate: 1
Injection volume: 120
Detector: Radioactivity, Inus β-Ram using Inus Tru-Count scintillation fluid at a flow rate of 5 mL/min

CHROMATOGRAM
Retention time: 4.7

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver

REFERENCE
Obach,R.S. Nonspecific binding to microsomes: Impact on scale-up of in vitro intrinsic clearance to hepatic clearance as assessed through examination of warfarin, imipramine, and propranolol, *Drug Metab.Dispos.*, **1997**, *25*, 1359–1369.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Condition a C18 SepPak SPE cartridge with 5 mL MeOH and 5 mL buffer. 1 mL Microsomal incubation + 500 μL buffer, mix, add a 500 μL aliquot to the SPE cartridge, wash with 4 mL MeOH:buffer containing 0.01% ascorbic acid 5:95, elute with 5 mL MeOH, evaporate eluate to dryness, reconstitute in mobile phase, inject an aliquot. (Buffer was 100 mM pH 3.0 potassium acetate buffer containing 5 mM n-heptanesulfonic acid.)

HPLC VARIABLES
Guard column: used but not specified
Column: 100 × 8 4 μm Nova-Pak C18
Mobile phase: Gradient. MeOH:MeCN:buffer 20:35:45 for 20 min, then 30:50:20 for 25 min, then 35:60:5
Flow rate: 0.4
Injection volume: 50-300
Detector: UV 254

CHROMATOGRAM
Retention time: 33

OTHER SUBSTANCES
Extracted: metabolites, desipramine, lofepramine

KEY WORDS
rat; human; liver; SPE

REFERENCE
Strandgården,K.; Gunnarsson,P.O. Metabolism of lofepramine and imipramine in liver microsomes from rat and man, *Xenobiotica*, **1994**, *24*, 703–711.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 1 mL Microsomal incubation + 500 μL 2 M pH 12 sodium carbonate + 5 mL ether, vortex, centrifuge at 1000 g for 10 min. Remove the organic layer and add it to 1

mL 100 mM HCl, vortex, centrifuge. Remove the aqueous layer and add it to 100 μL 2 M pH 12 sodium carbonate and 1 mL ether, extract. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-PCN
Mobile phase: MeCN:MeOH:10 mM pH 7 K_2HPO_4 40:35:25
Flow rate: 1.4
Injection volume: 50
Detector: UV 214

CHROMATOGRAM
Retention time: 7.83

OTHER SUBSTANCES
Extracted: metabolites, desipramine

KEY WORDS
rat; liver; brain

REFERENCE
Sequeira,D.J.; Strobel,H.W. High-performance liquid chromatographic method for the analysis of imipramine metabolism in vitro by liver and brain microsomes, *J.Chromatogr.B*, **1995**, *673*, 251–258.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 50:50 containing150 mM KCl
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 251

REFERENCE
Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

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

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

CHROMATOGRAM
Retention time: 4.5

OTHER SUBSTANCES

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

REFERENCE

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

SAMPLE

Matrix: solutions

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

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 60:1.5:0.5:38
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM

Retention time: k' 2.14

REFERENCE

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

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 500 μg/mL solution in MeOH:water 50:50, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax C8
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L MeCN:water 20:80. A:B from 100:0 to 0:100 over 30 min. (Purify triethylamine as follows. Wash neutral alumina (Merck) 3 times with 2 bed volumes of pentane, 3 times with 2 bed volumes of dichloromethane, and 3 times with 2 bed volumes of MeOH, allow solvent to evaporate in a fume hood overnight, heat alumina at 130° for 2 h. Prepare a 14 cm column of the washed alumina in a 290 × 22 tube, pass through a head volume of MeOH, pass through triethylamine. When triethylamine starts to elute discard the first 20 mL, use the next 20 mL, discard the column.)
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 18.7

OTHER SUBSTANCES
Simultaneous: acetophenone, amphetamine, desipramine, ethylmorphine, mefenamic acid, methamphetamine, morphine, phenylbutazone, salicylic acid

KEY WORDS
also details of isocratic elution

REFERENCE
Hill,D.W. Evaluation of alkyl bonded silica and solvent phase modifiers for the efficient elution of basic drugs on HPLC, *J.Liq.Chromatogr.*, **1990**, *13*, 3147–3175.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Adsorbosphere C18 (PEEK column) (retention times are longer and peaks broader with stainless steel column)
Mobile phase: MeCN:20 mM pH 3.2 KH_2PO_4 23.4:76.6 containing 0.05% nonylamine
Flow rate: 1.2
Detector: UV 214

CHROMATOGRAM
Retention time: 11

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

REFERENCE
Supelco Catalog, **1993**, p. 440.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Econosil C8
Mobile phase: MeCN:buffer 30:70 (Buffer was 20 mM KH_2PO_4 and 14 mM triethylamine adjusted to pH 3.0 with phosphoric acid.)

Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 8.3
Limit of quantitation: < 1000 ng/mL

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

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KEY WORDS
UV spectra given

REFERENCE
Ryan,T.W. Identification and quantification of tricyclic antidepressants by UV-photodiode array detection with multicomponent analysis, *J.Liq.Chromatogr.*, **1993**, *16*, 1545–1560.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 30 × 2.1 Spheri-5 RP-8
Column: 220 × 2.1 Spheri-5 RP-8
Mobile phase: Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95:5 for 2 min, to 0:100 over 15 min (?), maintain at 0:100 for 5 min.
Column temperature: 50
Flow rate: 0.5
Detector: UV 200

CHROMATOGRAM
Retention time: 14

OTHER SUBSTANCES
Simultaneous: desmethyldoxepin, doxepin, desipramine, nortriptyline, amitriptyline
Also analyzed: amphetamine, chlordiazepoxide, chlorpromazine, desalkylflurazepam, diazepam, diethylpropion, ephedrine, fenfluramine, flurazepam, mesoridazine, methamphetamine, norchlordiazepoxide, nordiazepam, oxazepam, phentermine, phenylpropanolamine, prazepam, promazine, thioridazine, thiothixene, trifluoperazine

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REFERENCE
Rainin Catalog, C1-94, **1994**, p. 7.24.

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

HPLC VARIABLES
Column: 250 × 4.6 5 μm Lichrosphere cyanopropyl
Mobile phase: Carbon dioxide:MeOH:isopropylamine 90:10:0.05
Column temperature: 50
Flow rate: 3
Injection volume: 5
Detector: UV 220

CHROMATOGRAM
Retention time: 3.19

OTHER SUBSTANCES
Simultaneous: benactyzine, buclizine, hydroxyzine, perphenazine, thioridazine, amitriptyline, desipramine, nortriptyline, protriptyline

KEY WORDS
SFC; pressure 200 bar

REFERENCE
Berger,T.A.; Wilson,W.H. Separation of drugs by packed column supercritical fluid chromatography. 2. Antidepressants, *J.Pharm.Sci.*, **1994**, *83*, 287–290.

SAMPLE
Matrix: solutions

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

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

thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 14.70 (A), 6.78 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, 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, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

details of plasma extraction

REFERENCE

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

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1-10 μg/mL solution in water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil SCX/C18
Mobile phase: MeCN:25 mM pH 3 Na_2HPO_4 50:50
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 3.37

OTHER SUBSTANCES
Also analyzed: amitriptyline, barbital, benzoic acid, butabarbital, clomipramine, clonazepam, desipramine, diazepam, flurazepam, furosemide, nitrazepam, phenobarbital, phenol, phenolphthalein, pindolol, propranolol, resorcinol, salicylic acid, secobarbital, terbutaline, xylazine

KEY WORDS
effect of mobile phase pH on capacity factor is discussed

REFERENCE
Walshe,M.; Kelly,M.T.; Smyth,M.R.; Ritchie,H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *708*, 31–40.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 6.31

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

SAMPLE
Matrix: urine

Sample preparation: 500 μL Urine + N-ethylnordiazepam + chlorpheniramine + 100 μL buffer, centrifuge at 11000 g for 30 s, inject a 500 μL aliquot onto column A with mobile phase A, after 0.6 min backflush column A with mobile phase A to waste for 1.6 min, elute column A with 250 μL mobile phase B, with 200 μL mobile phase C, and with 1.15 mL mobile phase D. Elute column A to waste until drugs start to emerge then elute onto column B. Elute column B to waste until drugs started to emerge, then elute onto column C. When all the drugs have emerged from column B remove it from the circuit, elute column C with mobile phase D, monitor the effluent from column C. Flush column A with 7 mL mobile phase E, with mobile phase D, and mobile phase A. Flush column B with 5 mL mobile phase E then with mobile phase D. (Buffer was 6 M ammonium acetate adjusted to pH 8.0 with 2 M KOH.)

HPLC VARIABLES

Column: A 10 × 2.1 12-20 μm PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 × 3.2 11 μm Aminex A-28 (Bio-Rad); C 25 × 3.2 5 μm C8 (Phenomenex) + 150 × 4.6 5 μm silica (Macherey-Nagel)
Mobile phase: A 0.1% pH 8.0 potassium borate buffer; B 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid; C MeCN:buffer 40:60 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); D MeCN:buffer 33:67 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); E MeCN:buffer 70:30 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)
Column temperature: ambient (column A), 40 (columns B and C)
Flow rate: A 5; B-E 1
Injection volume: 500
Detector: UV 210, UV 235

CHROMATOGRAM

Retention time: k' 4.2
Internal standard: N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)
Limit of detection: 300 ng/mL

OTHER SUBSTANCES

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

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 μg/mL desmethylclomipramine in water, mix for a few s, add to a C18 SepPak attached to a 5 mL syringe, allow to flow through (10-15 min). Wash with 1 mL 0.1 M NaCl, wash with 1 mL water, wash 3 mL reagent by gravity. Elute with 3 mL MeOH and push air through to remove as much as possible. Evaporate in vacuum at 37°, vortex with 50 μL mobile phase for 1 min, inject 25 μL aliquot. (Reagent was isopropanol:n-heptane:1 M sulfuric acid 40:320:1.)

HPLC VARIABLES

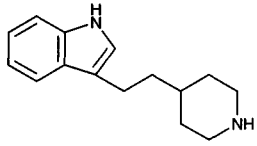
Guard column: 50 × 4.6 30 μm Permaphase ETH
Column: 250 × 4.6 5-6 μm Zorbax cyanopropyl
Mobile phase: MeCN:0.5 M acetic acid:n-butylamine 40:60:0.0022
Flow rate: 2.5
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 22
Internal standard: Desmethylclomipramine
Limit of detection: 16.7 ng/mL

OTHER SUBSTANCES
Simultaneous: amitriptyline, doxepin, nortriptyline, metabolites
Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, caffeine, carbamazepine, chloramphenicol, clonazepam, cyclosporine, diazepam, digoxin, disopyramide, ethosuximide, flurazepam, gentamicin, haloperidol, kanamycin, lidocaine, meprobamate, methapyriline, methaqualone, methotrexate, methyprylon, netilmicin, pentazocine, pentobarbital, phenobarbital, phenytoin, prazepam, primidone, procainamide, propranolol, quinidine, salicylic acid, secobarbital, streptomycin, theophylline, tobramycin, tocainide, valproic acid, vancomycin.

REFERENCE
Evenson,M.A.; Engstrand,D.A. A SepPak HPLC method for tricyclic antidepressant drugs in human vitreous humor, *J.Anal.Toxicol.*, **1989**, *13*, 322–325.

Indalpine



Molecular formula: $C_{15}H_{20}N_2$
Molecular weight: 228.34
CAS Registry No.: 63758-79-2
Merck Index: 4965

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 100 μL 1 μg/mL IS in MeOH + 200 μL 5 M NaOH, vortex for 1 min, add 4 mL dichloromethane, shake for 15 min, centrifuge at 3000 g for 10 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 38°, reconstitute the residue in 50 μL mobile phase, vortex, centrifuge at 3000 g for 5 min, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 3 Bondapak C18/Corasil
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:10 mM K_2HPO_4:acetic acid 50:50:1
Flow rate: 1
Injection volume: 10
Detector: F ex 220 em 370

CHROMATOGRAM
Retention time: 8
Internal standard: 4-[(5-methoxy-3-indolyl)methyl]piperidine (PK 26042) (5)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Jozefczak,C.; Ktorza,N.; Uzan,A. High-performance liquid chromatographic determination of indalpine, a new non-tricyclic antidepressant, in human plasma: identification and simultaneous measurement of its major plasma metabolite, *J.Chromatogr.*, **1982**, *230*, 87–95.

SAMPLE
Matrix: blood, tissue
Sample preparation: 200 μL Plasma + 10 μL 1 μg/mL IS in MeOH + 50 μL 1 M NaOH + 4 mL dichloromethane, shake on an alternating agitator for 20 min, centrifuge at -4° at 2000 g for 15-20 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 38°, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot. Tissue. Mouse brain + 25 μL 1 μg/mL IS in MeOH + 3 mL 1 M perchloric acid, homogenize in a potter apparatus (Ultraturax), centrifuge at 2000 g for 15 min. Remove the supernatant and adjust the pH to 12 with 1 M NaOH, add 4 mL dichloromethane, shake on an alternating agitator for 20 min, centrifuge at -4° at 2000 g for 15-20 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 38°, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Nova-Pack C18
Mobile phase: MeOH:water:5 mM hexanesulfonic acid 52:48:2
Flow rate: 0.8
Injection volume: 50
Detector: E, Metrohm 641 VA, 1000 mV, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 5.8
Internal standard: 5-methoxy-3-(4-piperidylmethyl)indole (3.6) (structure shown is 3-(4-piperidylmethyl)indole)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
mouse; plasma; brain; pharmacokinetics

REFERENCE
Joulin,Y.; Doare,L.; Diquet,B. Micromethod for the determination of indalpine in mouse plasma using high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1986**, *381*, 457–463.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH, inject an aliquot.

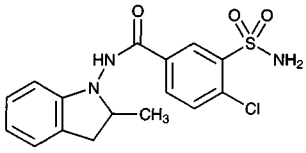
HPLC VARIABLES
Column: 125 × 4.9 5 μm Spherisorb C8
Mobile phase: MeCN:MeOH:buffer 13:35:52 (Buffer was 6.5 g/L (?) KH_2PO_4 adjusted to pH 3 with orthophosphoric acid.)
Column temperature: 45
Flow rate: 1.2
Injection volume: 60
Detector: UV 254

CHROMATOGRAM
Retention time: 2.9

OTHER SUBSTANCES
Simultaneous: amitriptyline, bromazepam, chlorpromazine, clobazam, clomipramine, desipramine, diazepam, flunitrazepam, haloperidol, imipramine, levomepromazine, lorazepam, maprotiline, metapramine, mianserin, oxazepam, prochlorperazine, triazolam
Noninterfering: meprobamate

REFERENCE
Rouquette,C.; Hecquet,D.; Pommereau,X.; Gardere,J.J.; Brachet-Liermain,A. Metapramine overdose: report of two cases and analytical determinations, *J.Anal.Toxicol.*, **1985**, *9*, 275–277.

Indapamide



Molecular formula: $C_{16}H_{16}ClN_3O_3S$
Molecular weight: 365.84
CAS Registry No.: 26807-65-8
Merck Index: 4969
Lednicer No.: 2 349

SAMPLE

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

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 1.0

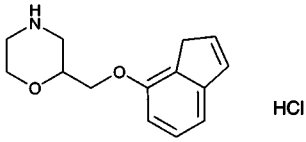
OTHER SUBSTANCES

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

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

Indeloxazine hydrochloride

Molecular formula: $C_{14}H_{18}ClNO_2$
Molecular weight: 267.76
CAS Registry No.: 65043-22-3
Merck Index: 4972
Lednicer No.: 4 59



SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 300 ng/mL viloxazine in water + 500 μL 200 mM ammonium hydroxide + 4 mL ether, extract, centrifuge. Remove the organic layer and evaporate it to dryness at 45°, reconstitute the residue in 100 μL 3 mg/mL sodium bicarbonate and 200 μL 125 μg/mL dansyl chloride in acetone, heat at 45° for 20 min, cool, add 4 mL ether. Wash the ether solution twice with 3 mL water and evaporate it to dryness at 45°. Take up the residue in 100 μL n-heptane, inject a 3-5 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 5 μm LiChrosorb SI-60
Mobile phase: n-Heptane:ethyl acetate 20:3
Flow rate: 1.5
Injection volume: 3-5
Detector: F ex 365 em 505

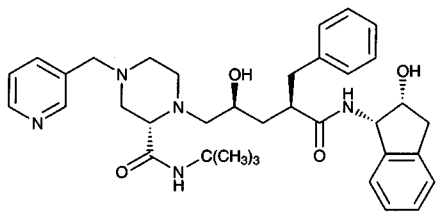
CHROMATOGRAM
Retention time: 3
Internal standard: viloxazine (4)
Limit of detection: 5 ng/mL

KEY WORDS
plasma; normal phase; derivatization

REFERENCE
Kamimura,H.; Sasaki,H.; Yokoi,K.; Kawamura,S. Determination of indeloxazine in plasma by liquid chromatography and gas chromatography-mass spectrometry, *J.Pharm.Sci.*, **1985**, *74*, 559–561.

Indinavir

Molecular formula: $C_{36}H_{47}N_5O_4$
Molecular weight: 613.80
CAS Registry No.: 150378-17-9, 157810-81-6 (sulfate)
Merck Index: 4979

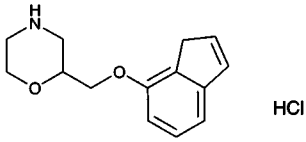


SAMPLE
Matrix: blood
Sample preparation: 300 μL Plasma + 300 μL 50 mM pH 9.0 ammonium dihydrogen phosphate buffer + 30 μL 10.5 μg/mL IS in water, vortex for 10 s, add 3 mL diethyl ether, vortex for 30

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.

Indeloxazine hydrochloride

Molecular formula: $C_{14}H_{18}ClNO_2$
Molecular weight: 267.76
CAS Registry No.: 65043-22-3
Merck Index: 4972
Lednicer No.: 4 59



SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 300 ng/mL viloxazine in water + 500 μL 200 mM ammonium hydroxide + 4 mL ether, extract, centrifuge. Remove the organic layer and evaporate it to dryness at 45°, reconstitute the residue in 100 μL 3 mg/mL sodium bicarbonate and 200 μL 125 μg/mL dansyl chloride in acetone, heat at 45° for 20 min, cool, add 4 mL ether. Wash the ether solution twice with 3 mL water and evaporate it to dryness at 45°. Take up the residue in 100 μL n-heptane, inject a 3-5 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 5 μm LiChrosorb SI-60
Mobile phase: n-Heptane:ethyl acetate 20:3
Flow rate: 1.5
Injection volume: 3-5
Detector: F ex 365 em 505

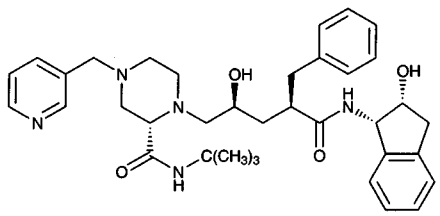
CHROMATOGRAM
Retention time: 3
Internal standard: viloxazine (4)
Limit of detection: 5 ng/mL

KEY WORDS
plasma; normal phase; derivatization

REFERENCE
Kamimura,H.; Sasaki,H.; Yokoi,K.; Kawamura,S. Determination of indeloxazine in plasma by liquid chromatography and gas chromatography-mass spectrometry, *J.Pharm.Sci.*, **1985**, *74*, 559–561.

Indinavir

Molecular formula: $C_{36}H_{47}N_5O_4$
Molecular weight: 613.80
CAS Registry No.: 150378-17-9, 157810-81-6 (sulfate)
Merck Index: 4979



SAMPLE
Matrix: blood
Sample preparation: 300 μL Plasma + 300 μL 50 mM pH 9.0 ammonium dihydrogen phosphate buffer + 30 μL 10.5 μg/mL IS in water, vortex for 10 s, add 3 mL diethyl ether, vortex for 30

s, keep at -20° for 30 min. Remove the ether layer and evaporate it to dryness under nitrogen. Reconstitute the residue with 150 μL 10 mM pH 5.5 ammonium dihydrogen phosphate buffer, centrifuge at 750 g for 5 min, inject a 35 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Delta-pak C4 (Waters)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mM ammonium dihydrogen phosphate and 1 mM 1-heptanesulfonic acid sodium salt, pH adjusted to 4.8 with ammonium hydroxide.)
Flow rate: 0.6
Injection volume: 35
Detector: UV 210

CHROMATOGRAM
Retention time: 13.2 ± 0.5
Internal standard: methylindinavir (methyl at 5 position on pyridine) (14.1 ± 0.7)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: nelfinavir, ritonavir, saquinavir
Noninterfering: didanosine, lamivudine, stavudine, zalcitabine, zidovudine

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Iayewardene,A.L.; Zhu,F.; Aweeka,F.T.; Gambertoglio,J.G. Simple high-performance liquid chromatographic determination of the protease inhibitor indinavir in human plasma, *J.Chromatogr.B*, **1998**, *707*, 203–211.

SAMPLE
Matrix: blood
Sample preparation: Add 50 μL 1 μg/mL IS in MeCN:water 50:50 to 1 mL plasma and vortex. Add 1 mL 100 mM pH 9.5 borate buffer, vortex, add 8 mL isopropanol:chloroform 1:15 (Caution! Chloroform is a carcinogen!), mix on a flat-bed shaker for 15 min, centrifuge at 1500 g for 5 min. Remove the lower organic layer and evaporate it under a stream of nitrogen at 45°. Reconstitute the residue in 1 mL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 50 × 3.9 5 μm Symmetry C8
Mobile phase: MeCN:water 40:60 containing 2 mM ammonium acetate
Flow rate: 1
Injection volume: 50
Detector: MS, PE-SCIEX API IIIplus triple quadrupole, heated nebulizer, corona discharge needle (+4.5 μA), nebulizer 500°, collision gas argon at 260 × 10^{12} atoms/cm^2, nebulizing gas nitrogen at 80 psi and 0.6 mL/min, orifice +75 V, electron multiplier -4.0 kV, dwell time 400 ms with a 30 ms pause time between scans, Q1 at m/z 523, Q2 at m/z 512, Q3 at m/z 273

CHROMATOGRAM
Retention time: 1.5
Internal standard: structure given in paper
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE
Woolf,E.; Haddix,H.M.; Matuszewski,B. Determination of an in vivo metabolite of a human immunodeficiency virus protease inhibitor in human plasma by high-performance liquid chromatography with tandem mass spectrometry, *J.Chromatogr.A*, **1997**, *762*, 311–319.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 50 μL MeCN:water 50:50 + 25 μL 1 μg/mL IS in MeCN, vortex, add 1 mL 100 mM pH 9.5 borate buffer, vortex, add 8 mL MTBE, mix on a Glas Col (Terre Haute, IN) RD 350 rotator for 15 min, centrifuge at 1500 g for 5 min, freeze the aqueous phase in a dry ice-acetone bath. Decant the organic layer and evaporate it under a stream of nitrogen at 42°. Reconstitute the residue in 175 μL mobile phase, inject a 6 μL aliquot.

HPLC VARIABLES
Column: 50 × 2 3 μm BDS Hypersil C8
Mobile phase: MeCN:water 40:60 containing 7 mM pH ammonium acetate, adjusted to pH 4.9 with formic acid
Flow rate: 0.2
Injection volume: 6
Detector: MS, PE-Sciex API IIIplus triple quadrupole, turbo-ion spray, turbo probe at 500°, auxiliary gas nitrogen, nebulizing gas nitrogen at 80 psi, positive ion mode, interface sprayer at +4 kV, sampling orifice at +60 V, m/z 614

CHROMATOGRAM
Retention time: 2.6
Internal standard: indinavir analog (4.2)
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES
Extracted: hexadeuterated indinavir (m/z 620)

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Woolf,E.J.; Matuszewski,B.K. Simultaneous determination of unlabeled and deuterium-labeled indinavir in human plasma by high-performance liquid chromatography ith tandem mass spectrometric detection, *J.Pharm.Sci.*, **1997**, *86*, 193–198.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Add four volumes of MeCN to microsomal incubation, centrifuge at 1500 g for 10 min, evaporate supernatant to dryness under nitrogen at 40°, reconstitute the residue in 100-120 μL mobile phase, inject an 80 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Hypersil 5C18 (Phenomenex)
Mobile phase: Gradient. A was MeCN. B was 0.3% pH 6 triethylamine in water. A:B from 25:75 to 45:55 over 18 min
Flow rate: 1.5
Injection volume: 80
Detector: UV 240

CHROMATOGRAM
Retention time: 16.1

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver

REFERENCE
Koudriakova,T.; Iatsimirskaia,E.; Utkin,I.; Gangl,E.; Vouros,P.; Storozhuk,E.; Orza,D.; Marinina,J.; Gerber,N. Metabolism of the human immunodeficiency virus protease inhibitors indinavir and ritonavir by human intestinal microsomes and expressed cytochrome P4503A4/3A5: Mechanism-based inactivation of cytochrome P4503A by ritonavir, *Drug Metab.Dispos.*, **1998**, *26*, 552–561.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Add four volumes of 1-chlorobutane containing 50 ng/mL 5-methoxypsoralen to microsomal incubation, mix vigorously, centrifuge at 1500 g for 10 min, evaporate 1-chlorobutane extract to dryness under nitrogen, reconstitute the residue in 100 μL mobile phase, inject an 80 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Hypersil 5C18 (Phenomenex)
Mobile phase: Gradient. A was MeCN. B was 0.3% pH 6 triethylamine in water. A:B from 25:75 to 45:55 in 18 min
Flow rate: 1.5
Injection volume: 80
Detector: UV 240

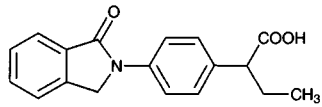
CHROMATOGRAM
Retention time: 16.1
Internal standard: 5-methohypsoralen (14.6)
Limit of detection: 30 nM

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver

REFERENCE
Koudriakova,T.; Iatsimirskaia,E.; Utkin,I.; Gangl,E.; Vouros,P.; Storozhuk,E.; Orza,D.; Marinina,J.; Gerber,N. Metabolism of the human immunodeficiency virus protease inhibitors indinavir and ritonavir by human intestinal microsomes and expressed cytochrome P4503A4/3A5: Mechanism-based inactivation of cytochrome P4503A by ritonavir, *Drug Metab.Dispos.*, **1998**, *26*, 552–561.

Indobufen



Molecular formula: $C_{16}H_{17}NO_3$
Molecular weight: 295.34
CAS Registry No.: 63610-08-2
Merck Index: 4991

SAMPLE
Matrix: blood
Sample preparation: 0.5-1 mL Plasma + 500 μL water + 100 μL 600 mM sulfuric acid + 40 mg NaCl + 4 mL diethyl ether, 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 500 μL MeOH, inject a 10 μL aliquot.

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 275

CHROMATOGRAM
Retention time: 6 (-), 8 (+) (assignment tentative)
Internal standard: indobufen

OTHER SUBSTANCES
Extracted: indoprofen

KEY WORDS
plasma; derivatization; chiral; indobufen is IS

REFERENCE
Björkman,S. Determination of the enantiomrs of indoprofen in blood plasma by high-performance liquid chromatography after rapid derivatization by means of ethyl chloroformate, *J.Chromatogr.*, **1985**, *339*, 339–346.

SAMPLE
Matrix: blood
Sample preparation: 0.5-1 mL Plasma + 100 μL 600 mM sulfuric acid + 40 mg NaCl + 4 mL diethyl ether, extract by rotation, centrifuge at 1200 g. Remove the organic layer and evaporate it to dryness under a stream of air at 30°, add 5 drops of toluene, evaporate to dryness under a stream of air. Reconstitute the residue in 200 μL 50 mM triethylamine in MeCN, add 100 μL 60 mM ethyl chloroformate in MeCN, let stand for 30 s, add 100 μL 1 M leucinamide hydrochloride in MeOH containing 1 M triethylamine, let stand for 2 min, add 500 μL 250 mM HCl, extract with 4 mL ethyl acetate. Remove the organic layer and evaporate it to dryness under a stream of air at 30°, reconstitute in 500 μL MeOH, inject a 10 μL aliquot (J.Chromatogr. 1985, 339, 339).

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:pH 6.4 phosphate buffer 40:60
Flow rate: 1.5
Injection volume: 10
Detector: UV 275

CHROMATOGRAM
Retention time: 6.977 (R), 8.848 (S)

KEY WORDS
chiral; derivatization

REFERENCE
Perrone,G.; Farina,M. High-performance liquid chromatographic method for direct resolution of the indobufen enantiomeric components, *J.Chromatogr.*, **1990**, *520*, 373–378.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Chiralcel OD
Mobile phase: Hexane:isopropanol:formic acid 80:20:0.5
Flow rate: 1.5
Detector: UV 270

CHROMATOGRAM
Retention time: 8.20 (R), 10.91 (S)
Limit of detection: 2 ng

KEY WORDS
chiral; $\alpha = 1.33$

REFERENCE
Perrone,G.; Farina,M. High-performance liquid chromatographic method for direct resolution of the indobufen enantiomeric components, *J.Chromatogr.*, **1990**, *520*, 373–378.

SAMPLE
Matrix: urine
Sample preparation: 500 μL Urine + 50 μL 5000 U/mL β-glucuronidase (Sigma) + 2 mL 100 mM pH 5 acetate buffer, heat at 37° for 16 h, add S-indoprofen, add 1 mL 1 M HCl, extract with diethyl ether. Remove the organic phase and extract it with 500 μL 1 M NaOH. Remove the aqueous phase and add it to 1 mL 1 M HCl, extract with diethyl ether. Remove the organic layer and evaporate it to dryness under a stream of air at 30°, add 5 drops of toluene, evaporate to dryness under a stream of air. Reconstitute the residue in 200 μL 50 mM triethylamine in MeCN, add 100 μL 60 mM ethyl chloroformate in MeCN, let stand for 30 s, add 100 μL 1 M leucinamide hydrochloride in MeOH containing 1 M triethylamine, let stand for 2 min, add 500 μL 250 mM HCl, extract with 4 mL ethyl acetate. Remove the organic layer and evaporate it to dryness under a stream of air at 30°, reconstitute in 500 μL MeOH, inject a 10 μL aliquot (J.Chromatogr. 1985, 339, 339).

HPLC VARIABLES
Column: 125 × 4 4 μm Lichrocart Superspher (Merck)
Mobile phase: MeCN:10 mM pH 6.5 phosphate buffer 35:65
Injection volume: 10
Detector: UV 275

CHROMATOGRAM
Internal standard: indoprofen
Limit of detection: 5 μg/mL

KEY WORDS
chiral; derivatization; pharmacokinetics

REFERENCE
Strolin Benedetti,M.; Frigerio,E.; Tamassia,V.; Noseda,G.; Caldwell,J. The dispositional enantioselectivity of indobufen in man, *Biochem.Pharmacol.*, **1992**, *43*, 2032–2034.

SAMPLE
Matrix: urine
Sample preparation: Inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 LiChrosorb C18
Mobile phase: Gradient. MeCN:water 25:75 containing 0.1% trifluoroacetic acid for 30 min then MeCN:water 40:60 containing 0.1% trifluoroacetic acid for 20 min (step gradient).
Flow rate: 2
Detector: F ex 290 em 440

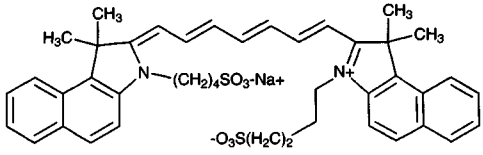
CHROMATOGRAM
Retention time: 36.8

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; mouse

REFERENCE
Grubb,N.; Caldwell,J.; Strolin-Benedetti,M. Excretion balance and urinary metabolism of indobufen in rats and mice, *Biochem.Pharmacol.*, **1993**, *46*, 759–761.

Indocyanine green



Molecular formula: $C_{43}H_{47}N_2NaO_6S_2$
Molecular weight: 774.98
CAS Registry No.: 3599-32-4
Merck Index: 4992

SAMPLE

Matrix: blood
Sample preparation: 0.5 mL Plasma +10 μL 250 μg/mL 1-acetamidopyrene in MeOH + 200 μL 1 M ammonium sulfate + 800 μL cold MeCN, vortex for 30 s, store at -20° for at least 30 min, vortex, centrifuge at 1500 g for 30 min. Remove 400 μL of the upper organic layer and evaporate it under a stream of nitrogen. Reconstitute with 100 μL mobile phase, vortex for 30 s, inject a 75 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:buffer 47:53 (Buffer was 6.805 g potassium monophosphate in 1 L water, adjust pH to 6.00 with 10 M NaOH.)
Flow rate: 1
Injection volume: 75
Detector: UV 214

CHROMATOGRAM

Retention time: 7.2
Internal standard: 1-acetamidopyrene (9.7)
Limit of detection: 39 ng/mL

OTHER SUBSTANCES

Simultaneous: lorazepam, antipyrine
Noninterfering: adenosine, albuterol, alphenal, aspirin, caffeine, carbamazepine, cefazolin, cephalexin, cephalothin, cimetidine, ciprofloxacin, claforan, desipramine, enoxacin, fleroxacin, furosemide, hydralazine, hydrochlorothiazide, minoxidil, norfloxacin, phenytoin, propafenone, sulindac, teicoplanin, theophylline, vancomycin

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: 25 μL Plasma + 25 μL cold (-20°) 60 μg/mL IS in acetone, vortex for 30 s, centrifuge at 13000 g for 1 min, inject a 10-20 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4.6 5 μm octylsilane (Alltech)
Mobile phase: MeCN:50 mM pH 4.0 phosphate buffer 42:58
Flow rate: 1.5
Injection volume: 10-20
Detector: F ex 214 em 370

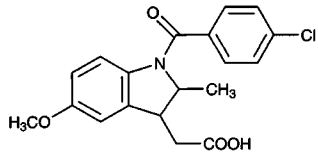
CHROMATOGRAM

Retention time: 7.3
Internal standard: 1,1',3,3,3',3'-hexamethyl-4,4',5,5'-dibenzo-2,2'-indotricarbocyanine perchlorate (Eastman Kodak) (8.7)
Limit of detection: 500 ng/mL

KEY WORDS
rat; plasma; pharmacokinetics

REFERENCE
Pollack,G.M.; Brouwer,K.L.R.; Demby,K.B.; Jones,J.A. Determination of hepatic blood flow in the rat using sequential infusions of indocyanine green or galactose, *Drug Metab.Dispos.*, **1990**, *18*, 197–202.

Indomethacin



Molecular formula: $C_{19}H_{16}ClNO_4$
Molecular weight: 357.79
CAS Registry No.: 53-86-1, 74252-25-8 (sodium salt trihydrate)
Merck Index: 4998
Lednicer No.: 1 318; 2 345; 3 165

SAMPLE
Matrix: aqueous humor
Sample preparation: 100 μL Aqueous humor + 500 μL MeCN + 30 μL 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 μL mobile phase by swirl-mixing for 1 min, centrifuge at 3000 g for 20 s, reduce volume to 20-30 μL, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.5 5 μm 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: 6.64
Internal standard: naproxen (3.89)

OTHER SUBSTANCES
Extracted: diclofenac, flurbiprofen, 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: aqueous humor
Sample preparation: 200 μL Aqueous humor + 200 μL MeCN, vortex for 15 s, centrifuge at 2000 rpm for 10 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 4 × 4 5 μm Lichrospher 100 C18
Column: 125 × 4 5 μm Lichrospher 100 C18
Mobile phase: MeOH:50 mM pH 3 sodium phosphate buffer 65:35
Flow rate: 1
Injection volume: 20
Detector: E, Shimadzu L-ECD-6A, glassy carbon working electrode 1.20 V, Ag/AgCl reference electrode or UV 266

CHROMATOGRAM
Retention time: 7.5
Limit of detection: 50 ng/mL (UV), 20 ng/mL (E)

KEY WORDS
rabbit

REFERENCE
Baudrit,O.; Fabre,H. Evaluation of electrochemical and fluorescence detection in liquid chromatography for the assay of indomethacin in aqueous humor samples, *J.Liq.Chromatogr.*, **1995**, *18*, 3283–3299.

SAMPLE
Matrix: bile, blood, gastric contents, urine
Sample preparation: Plasma. 300 μL Plasma + 1 mL MeCN, vortex for 30 s, centrifuge at 3500 rpm. Remove the supernatant and evaporate it to 100 μL under a stream of nitrogen at 50°, inject a 20 μL aliquot. Urine, bile, gastric fluid. 300 μL Urine, bile, or gastric fluid + 100 μL 5 M NaOH, let stand at room temperature for 15 min, adjust the pH with 100 μL 28.3% phosphoric acid, add 1 (urine, bile) or 1.5 (gastric fluid) mL MeCN, vortex for 30 s, centrifuge at 3500 rpm. Remove the supernatant and evaporate it to 100 μL under a stream of nitrogen at 50°, inject a 20 (urine), 35 (bile), or 40 (gastric fluid) μL aliquot. (Borate buffer was 12.4 g boric acid and 10 mL 1 M NaOH made up to 1 L with water, pH 7.2.)

HPLC VARIABLES
Guard column: Microguard reverse-phase (Bio-Rad)
Column: 100 × 8 Radial-PAK C18 in a radial compression module
Mobile phase: MeCN:buffer 70:30 (Buffer was 6.8 g/L KH_2PO_4 adjusted to pH 3.0 with 85% phosphoric acid.)
Flow rate: 2
Injection volume: 20-40
Detector: UV 340

CHROMATOGRAM
Retention time: 13
Internal standard: indomethacin

OTHER SUBSTANCES
Extracted: sulindac

KEY WORDS
plasma; indomethacin is IS

REFERENCE
Musson,D.G.; Vincek,W.C.; Constanzer,M.L.; Detty,T.E. Analytical methods for the determination of sulindac and metabolites in plasma, urine, bile, and gastric fluid by liquid chromatography using ultraviolet detection, *J.Pharm.Sci.*, **1984**, *73*, 1270–1273.

SAMPLE
Matrix: blood
Sample preparation: Mix 500 μL serum with 100 μL MeOH:50 mM pH 3 sodium phosphate buffer 50:50, vortex for 5 s. Add 1 mL MeCN, vortex for 1 min. Centrifuge the mixture at 14000 rpm for 5 min, decant the clear upper layer. Add 500 μL MeCN to the pellet, mix, centrifuge at 14000 rpm for 5 min. Combine the upper layers and evaporate at 40°. Reconstitute the

residue with 300 μL MeOH:50 mM pH 3.0 sodium phosphate buffer 40:60 containing 0.5% sodium metabisulfate (sic). Vortex for 30 s and inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Techsil C18 (HPLC Technology, Macclesfield)
Mobile phase: MeCN:50 mM phosphate buffer 46:63, adjusted to pH 3.0 with NaOH
Flow rate: 1.2
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 7.17
Internal standard: indomethacin

OTHER SUBSTANCES
Extracted: sulindac

KEY WORDS
indomethacin is IS; serum

REFERENCE
Kanfer,I.; Brown,C.; Koninigs,M.; Swarbrick,J. Absorption of sulindac from a novel (Pro-SorbTM) liquid formulation, *Biopharm.Drug Dispos.*, **1996**, *17*, 209–221.

SAMPLE
Matrix: blood
Sample preparation: Precipitate 100 μL serum with 200 μL 2 μg/mL IS in MeCN, centrifuge at 12 000 g for 5 min. Inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChroCART LiChrospher 60 RP Select B
Column: 125 × 4 5 μm LiChroCART LiChrospher 60 RP Select B
Mobile phase: MeCN:buffer 60:40 (Buffer was 25 mM pH 3.0 triethylammonium phosphate containing 2% MeCN.)
Flow rate: 1
Injection volume: 50
Detector: UV 280

CHROMATOGRAM
Retention time: 2.28
Internal standard: mefenamin (2.94)
Limit of detection: 110 ng/mL

KEY WORDS
serum

REFERENCE
Hannak,D.; Scharbert,F.; Kattermann,R. Stepwise binary gradient high-performance liquid chromatographic system for routine drug monitoring, *J.Chromatogr.A*, **1996**, *728*, 307–310.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 250 μL 1 M sulfuric acid + 5 mL 24 ng/mL p-phenylphenol in dichloromethane, vortex for 10 s, centrifuge at 500 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 100 μL MeOH, inject a 20-30 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax ODS
Mobile phase: Gradient. MeOH:100 mM pH 5 acetate buffer 45:55 for 3 min, to 62:38 over 2 min, maintain at 62:38 for 10 min.

Column temperature: 40
Flow rate: 1.5
Injection volume: 20-30
Detector: UV 254

CHROMATOGRAM
Retention time: 13.83
Internal standard: p-phenylphenol (12.43)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: acetaminophen, caffeine, carbamazepine, ethosuximide, fenoprofen, naproxen, phenobarbital, phenytoin, primidone, quinidine, salicylic acid, sulindac, theophylline, tolmetin, valproic acid
Noninterfering: ibuprofen

KEY WORDS
plasma

REFERENCE
Shimek,J.L.; Rao,N.G.S.; Wahba Khalil,S.K. High performance liquid chromatographic analysis of tolmetin, indomethacin and sulindac in plasma, *J.Liq.Chromatogr.*, **1981**, *4*, 1987–2013.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 100 μL pH 2 dilute sulfuric acid + 1 mL MeCN, vortex for 30 s, centrifuge at 2500 rpm for 5 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax CN
Mobile phase: MeCN:4% aqueous acetic acid 45:55
Flow rate: 1
Injection volume: 10
Detector: UV 340

CHROMATOGRAM
Retention time: 6
Internal standard: indomethacin

OTHER SUBSTANCES
Extracted: sulindac

KEY WORDS
plasma; indomethacin is IS

REFERENCE
Clark,C.R.; McMillian,C.L.; Hoke,J.F.; Campagna,K.D.; Ravis,W.R. Liquid chromatographic determination of sulindac and metabolites in serum, *J.Chromatogr.Sci.*, **1987**, *25*, 247–251.

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 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: 9

OTHER SUBSTANCES
Simultaneous: ketoprofen, acetaminophen, salicylic acid, naproxen, fenoprofen, ibuprofen

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: Wash a Sep-Pak C18 cartridge with 2 mL MeOH, 5 mL water, and 1 mL 0.25 mM pH 3.0 ammonium phosphate buffer. 20-200 μL Plasma + 100 μL MeOH + 20 μL 50 μg/mL indomethacin in MeOH + 100 μL 0.25 mM pH 3.0 ammonium phosphate buffer + 100 μL water, vortex for 2 min, centrifuge at 1800 g for 10 min. Add the supernatant to the cartridge, wash with 5 mL water, elute twice with 5 mL portions of MeOH. Evaporate eluate to dryness under vacuum, dissolve the residue in 1 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Brownlee RP18
Mobile phase: MeOH:buffer 75:25 (Buffer prepared by diluting 0.25 mM ammonium phosphate buffer adjusted to pH 3.0 with orthophosphoric acid.)
Injection volume: 20
Detector: E ESA Coulochem Model 5100 A, + 0.9 V

CHROMATOGRAM
Retention time: 14.6
Internal standard: naproxen (10.0)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Also analyzed: sulindac, piroxicam, diflunisal

KEY WORDS
plasma

REFERENCE
Kazemifard,A.G.; Moore,D.E. Liquid chromatography with amperometric detection for the determination of non-steroidal anti-inflammatory drugs in plasma, *J.Chromatogr.*, **1990**, *533*, 125–132.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 1 M pH 10.0 Tris buffer containing 150 ng/mL indomethacin + 8 mL diethyl ether, vortex 3 min, freeze for 1 h. Remove organic phase and evaporate it to dryness at room temperature. Dissolve residue in 200 μL MeOH, inject 20 μL aliquot.

HPLC VARIABLES
Column: 70 × 4.6 3 μm Ultrasphere XL ODS

Mobile phase: MeOH:20 mM ammonium acetate buffer (pH 5.0) 65:35
Flow rate: 1.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 1.64
Internal standard: indomethacin
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: dipyridamole

KEY WORDS
plasma; indomethacin is IS

REFERENCE
Barberi,M.; Merlin,J.L.; Weber,B. Sensitive determination of free and plasma protein-bound dipyridamole by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *565*, 511–515.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elut phenyl SPE cartridge with 5 mL MeOH and 5 mL water. Adjust pH of 500 μL plasma to 3.4 with 345 mM citrate buffer, add to SPE cartridge, wash with water, dry, elute with 5 mL hexane:diethyl ether 50:50. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL MeOH, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 6 × 4 4 μm Nova-Pack C18
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:20 mM ammonium sulfate 55:45
Flow rate: 1.5
Injection volume: 25
Detector: UV 340

CHROMATOGRAM
Retention time: 7.5
Internal standard: indomethacin

OTHER SUBSTANCES
Extracted: phenylbutazone, oxyphenbutazone, suxibuzone

KEY WORDS
plasma; SPE; indomethacin is IS

REFERENCE
Caturla,M.C.; Cusido,E. Solid-phase extraction for the high-performance liquid chromatographic determination of indomethacin, suxibuzone, phenylbutazone and oxyphenbutazone in plasma, avoiding degradation of compounds, *J.Chromatogr.*, **1992**, *581*, 101–107.

SAMPLE
Matrix: blood
Sample preparation: 20 μL serum + 20 μL MeCN, vortex for a few s, centrifuge at 10000 g for 2 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:25 mM phosphate buffer 35:65 containing 30 mM hydrogen peroxide, adjusted to pH 7.0 with 1 M NaOH

Flow rate: 1
Injection volume: 20
Detector: F ex 358 em 462 following post-column reaction. The column effluent flowed through a 15 m × 0.5 mm ID stainless steel coil at 180° then a 3 m × 0.5 mm ID stainless steel coil at 15° to the detector.

CHROMATOGRAM
Retention time: 10
Limit of detection: 500 ng/mL

KEY WORDS
post-column reaction; serum; pharmacokinetics

REFERENCE
Kubo,H.; Umiguchi,Y.; Kinoshita,T. Fluorometric determination of indomethacin in serum by high performance liquid chromatography using in-line oxidation with hydrogen peroxide, *J.Liq.Chromatogr.*, **1993**, *16*, 465–474.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Plasma + 250 μL 0.75 μg/mL mefenamic acid in MeCN + 50 μL MeCN, vortex, centrifuge at 9000 g for 3 min. Remove 250 μL of the supernatant and evaporate it to dryness under vacuum, dissolve the residue in 50 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 Alltech 5 μm C18 bonded-phase silica
Column: 250 × 4.6 Vydac column packed with Merck 5 μm C18 bonded-phase silica
Mobile phase: MeCN:10 mM phosphoric acid 60:40, pH 2.6
Flow rate: 0.9
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 6.3
Internal standard: mefenamic acid (9.2)
Limit of detection: 60 ng/mL

KEY WORDS
plasma

REFERENCE
Niopas,I.; Mamzoridi,K. Determination of indomethacin and mefenamic acid in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *656*, 447–450.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL 60 μm Separon SGX C18 SPE cartridge with 5 mL MeOH, 5 mL water, and 5 mL buffer. 250 μL Blood + 500 μL 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 supernatant to 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 × 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: 7.8
Internal standard: indomethacin

OTHER SUBSTANCES
Simultaneous: ibuprofen

KEY WORDS
SPE; indomethacin is IS; rabbit; human

REFERENCE
Sochor,J.; Klimes,J.; Zahradnicek,M.; Sedlacek,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 Bond-Elut C2 SPE cartridge with 1 mL MeOH and 1 mL mobile phase. 1 mL Serum + 1 drop saturated ammonium sulfate solution + 1 drop 1 M HCl, vortex for 3 min, add to the SPE cartridge, wash with six 500 μL portions of wash solvent, elute with four 250 μL aliquots of mobile phase, combine the eluates, vortex, inject a 100 μL aliquot. (Wash solvent was MeCN:water adjusted to pH 3.0 with phosphoric acid 20:80.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere C8
Mobile phase: MeCN:68 mM pH 2.5 phosphate buffer 55:45
Flow rate: 0.5
Injection volume: 100
Detector: F ex 232 em 335 (filter) following post-column photolysis. The effluent from the column flowed through a 7.9 m × 0.3 mm i.d. coil of PTFE irradiated by an SC3-9 UV lamp (UVP) (cooled with air) to the detector.

CHROMATOGRAM
Retention time: 12
Internal standard: indomethacin

OTHER SUBSTANCES
Extracted: sulindac

KEY WORDS
serum; post-column reaction; SPE; indomethacin is IS; post-column photochemical derivatization

REFERENCE
Siluveru,M.; Stewart,J.T. Determination of sulindac and its metabolites in human serum by reversed-phase high-performance liquid chromatography using on-line post-column ultraviolet irradiation and fluorescence detection, *J.Chromatogr.B*, **1995**, *673*, 91–96.

SAMPLE
Matrix: blood
Sample preparation: Erythrocytes. 500 μL Erythrocytes + 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. Acidify 500 μL plasma 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 × 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: 7.8
Internal standard: indomethacin

OTHER SUBSTANCES
Extracted: ibuprofen
Simultaneous: diazepam, phenylanthranilic acid

KEY WORDS
plasma; erythrocytes; rabbit; indomethacin is IS

REFERENCE
Sochor,J.; Klimes,J.; Sedlácek,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 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 253

CHROMATOGRAM
Retention time: 8.91
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; bupren-

orphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

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

SAMPLE

Matrix: blood, CSF, gastric contents, urine

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

HPLC VARIABLES

Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)

Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.

Column temperature: 50

Flow rate: 1.5

Detector: UV 220

CHROMATOGRAM

Retention time: 15.27

Internal standard: heptanophenone (19)

OTHER SUBSTANCES

Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

KEY WORDS

serum; column-switching

REFERENCE

Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

SAMPLE

Matrix: blood, tissue

Sample preparation: Plasma. 50 μL Plasma + 150 μL 8 μg/mL mefenamic acid in MeOH, mix, centrifuge at 15000 rpm, filter (0.45 μm), inject an aliquot. Tissue. Homogenize liver in ice-cold 10 mM pH 7.4 phosphate buffer, 1 mL homogenate + 2 mL 8 μg/mL mefenamic acid in MeOH, mix, centrifuge at 15000 rpm, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:MeOH:water:acetic acid 65:10:25:1
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Internal standard: mefenamic acid
Limit of detection: 30 ng/mL

KEY WORDS
plasma; rat; liver

REFERENCE
Ogiso,T.; Iwaki,M.; Kinoshita,T.; Tanino,T.; Paku,T. Pharmacokinetics of indomethacin octyl ester (prodrug) and indomethacin produced from the prodrug, *J.Pharm.Sci.*, **1994**, *83*, 34–37.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 50 μL 500 ng/mL mefenamic acid + 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°. Redissolve 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: UV 235

CHROMATOGRAM
Retention time: 5
Internal standard: mefenamic acid (8)
Limit of detection: 50-250 ng/mL

OTHER SUBSTANCES
Simultaneous: naproxen, flunixin, thiosalicylic acid, ethacrynic acid, phenylbutazone

KEY WORDS
plasma

REFERENCE
Singh,A.K.; Jang,Y.; Mishra,U.; Granley,K. Simultaneous analysis of flunixin, naproxen, ethacrynic acid, indomethacin, phenylbutazone, mefenamic acid and thiosalicyclic 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. 100 μL Plasma + 400 μL 200 mM perchloric acid, centrifuge at 3000 g, inject a 20 μL aliquot of the supernatant. Urine. Dilute 1:1 with water, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 75 × 2.1 pellicular reverse phase (Chrompack no. 28653)
Column: 250 × 4.6 5 μm Cp Spherisorb ODS (Chrompack)
Mobile phase: Gradient; MeCN:5 g/L orthophosphoric acid from 80:20 to 60:40 (sic, probably 20:80 to 40:60) over 30 min then stay there for 5 min, then to initial conditions over 5 min, equilibrate for 2 min before next injection
Flow rate: 1.2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 28.13
Limit of detection: 5.3 ng/mL
Limit of quantitation: 146 ng/mL (urine), 60 ng/mL (plasma)

OTHER SUBSTANCES

Simultaneous: metabolites, glucuronides

KEY WORDS

plasma; pharmacokinetics; also details of preparative procedure

REFERENCE

Vree,T.B.; Van den Biggelaar-Martea,M.; Verwey-van Wissen,C.P.W.G.M. Determination of indomethacin, its metabolites and their glucuronides in human plasma and urine by means of direct gradient high-performance liquid chromatographic analysis. Preliminary pharmacokinetics and effect of probenecid, *J.Chromatogr.*, **1993**, *616*, 271–282.

SAMPLE

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

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 201.7

CHROMATOGRAM

Retention time: 21.748

KEY WORDS

whole blood

REFERENCE

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

SAMPLE
Matrix: bulk
Sample preparation: 500 μL Plasma + 100 μL 5 mg/mL indomethacin in MeOH, filter (Sartorius SM 13243 ultrafiltration unit at 4000 g for 30 min). Add filtrate to a dry Chem Elut column (modified diatomaceous earth), leave 3 to 5 min, elute with 6 mL diethyl ether, evaporate eluant under a stream of nitrogen at room temperature, sonicate residue with 100 μL MeOH for 10 min, vortex, inject 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:100 mM pH 4.0 sodium acetate buffer 55:45
Flow rate: 1
Injection volume: 20
Detector: E, ESA Coulochem 5100A dual electrode with an ESA guard cell, + 0.65 V

CHROMATOGRAM
Retention time: 11.74
Internal standard: indomethacin

OTHER SUBSTANCES
Simultaneous: dipyridamole

KEY WORDS
plasma; indomethacin is IS

REFERENCE
Barberi-Heyob,M.; Merlin,J.L.; Pons,L.; Calco,M.; Weber,B. A sensitive isocratic liquid chromatography assay for the determination of dipyridamole in plasma with electrochemical detection, *J.Liq.Chromatogr.*, **1994**, *17*, 1837–1848.

SAMPLE
Matrix: formulations
Sample preparation: Reconstitute 1 mg indomethacin sodium trihydrate injection with 2 mL water, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.2 5 μm Ultrasphere ODS C18 (Beckman)
Mobile phase: MeCN:50 mM H_3PO_4 60:40
Flow rate: 1
Injection volume: 5
Detector: UV 260

CHROMATOGRAM
Retention time: 6.5-7.0

KEY WORDS
injections

REFERENCE
Walker,S.E.; Gray,S.; Schmidt,B. Stability of reconstituted indomethacin sodium trihydrate in original vials and polypropylene syringes, *Am.J.Health-Syst.Pharm.*, **1998**, *55*, 154–158.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 250 μL Microsomal incubation, 150 μL ice-cold MeCN and 2.5 ng ketoprofen, centrifuge. Extract the mixture with 4 mL ethyl acetate, centrifuge at 3000 rpm for 10 min, remove the organic fraction and evaporate it under a gentle stream of nitrogen at 40°. Dissolve the residue in 30 μL MeOH and dilute to 60 μL with water. Inject a 30 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm RP-18 (Kanto Chemical, Tokyo)

Mobile phase: MeCN:water 40:60 containing 0.6% acetic acid
Column temperature: 35
Flow rate: 1
Injection volume: 30
Detector: UV 260

CHROMATOGRAM
Retention time: 49.0
Internal standard: ketoprofen (18.0)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
liver; pharmacokinetics

REFERENCE
Nakajima,M.; Inoue,T.; Shimada,N.; Tokudome,S.; Yamamoto,T.; Kuroiwa,Y. Cytochrome P450 2C9 catalyzes indomethacin O-demethylation in human liver microsomes, *Drug Metab.Dispos.*, **1998**, *26*, 261–266.

SAMPLE
Matrix: perfusate
Sample preparation: Mix 100 nL perfusate with 600 nL perfusion fluid containing IS, inject an aliquot. (Perfusion fluid contained 104 mM NaCl, 25 mM sodium bicarbonate, 2.3 mM sodium biphosphate, 10 mM sodium acetate, 1.2 mM calcium chloride, 1 mM magnesium sulfate, 5 mM KCl, 5 mM dextrose, and 5 mM alanine.)

HPLC VARIABLES
Column: 300 × 2 10 μm μBondapak C18
Mobile phase: MeOH:water 52:48
Flow rate: 0.13
Injection volume: 0.2
Detector: F ex 295 em 376 following post-column reaction. The column effluent mixed with 4 M NaOH pumped at 0.0013 mL/min and the mixture flowed through a 130 μL PTFE coil at 64° to the detector.

CHROMATOGRAM
Internal standard: phenylbutazone
Limit of detection: 25 ng/mL

KEY WORDS
post-column reaction; microbore

REFERENCE
De Zeeuw,D.; Leinfelder,J.L.; Brater,D.C. Highly sensitive measurement of indomethacin using a high performance liquid chromatographic technique combined with post column in-line hydrolysis, *J.Chromatogr.*, **1986**, *380*, 157–162.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 40:60 adjusted to pH 5.5 with NaOH
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 264

OTHER SUBSTANCES
Also analyzed: carbamazepine, fenbufen, ketoprofen, α.-naphthoquinone, naproxen, tolmetin

REFERENCE

Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE

Matrix: solutions

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: 18

OTHER SUBSTANCES

Simultaneous: tolmetin, aspirin, ibuprofen, fenbufen, naproxen, carprofen, diflunisal

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: Sample + 400 μL 5 mM DBD-PZ + 70 mM diethylphosphorocyanidate in MeCN, react for 6 h, inject a 1 μL aliquot. (Synthesis of 4-(N,N-dimethylaminosulfonyl)-7-N-piperazino-2,1,3-benzoxadiazole (DBD-PZ) is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene form EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ tlc plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei (TCI America, Portland OR). Add 123 mg 4-(N,N-dimethyl-

aminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to 129 mg piperazine in 20 mL MeCN at room temperature, stir for 30 min, evaporate under reduced pressure, dissolve residue in 50 mL 5% HCl, wash three times with 20 mL ethyl acetate, discard ethyl acetate extracts, adjust pH of aqueous solution to 13-14 with 5% NaOH, extract five times with 50 mL ethyl acetate, combine extracts, wash with 20 mL water, dry over anhydrous sodium sulfate, evaporate under vacuum to give 4-(N,N-dimethylaminosulfonyl)-7-N-piperazino-2,1,3-benzoxadiazole as orange crystals (mp 121-2°).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:water 65:35
Column temperature: 40
Flow rate: 1
Injection volume: 1
Detector: F ex 437 em 561

CHROMATOGRAM

Retention time: 8
Limit of detection: 3.9 fmol

OTHER SUBSTANCES

Simultaneous: ibuprofen

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

HPLC VARIABLES

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

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol,

mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 125 × 3 Ecocart LiChrospher 100 RP-18
Mobile phase: Isopropanol:100 mM KH_2PO_4:formic acid 54:100:0.1
Flow rate: 0.6
Detector: UV 254

CHROMATOGRAM

Retention time: 10.7
Limit of quantitation: 200-500 ng/mL

KEY WORDS

solutions acemetacin; diclofenac; flurbiprofen; lonazolac; ketoprofen; naproxen; piroxicam; sulindac; tenoxicam

REFERENCE

Baeyens,W.R.G.; Van Der Weken,G.; Van Overbeke,A.; Zhang,Z.D. Preliminary results on the LC-separation of non-steroidal anti-inflammatory agents in conventional and narrow-bore RP set-ups applying columns with different internal diameters, *Biomed.Chromatogr.*, **1995**, *9*, 261–262.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeCN:water 45:55, pH adjusted to 3.5 with acetic acid
Detector: UV 280

CHROMATOGRAM

Internal standard: clomethacin

OTHER SUBSTANCES

Also analyzed: diclofenac, phenylbutazone

REFERENCE
Guterres,S.S.; Fessi,H.; Barratt,G.; Puisieux,F.; Devissaguet,J.-P. Poly(D,L-lactide) nanocapsules containing non-steroidal anti-inflammatory drugs: Gastrointestinal tolerance following intravenous and oral administration, *Pharm.Res.*, **1995**, *12*, 1545–1547.

SAMPLE
Matrix: solutions

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

CHROMATOGRAM
Retention time: 8.45 (A), 9.22 (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, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

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

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 250 ng/mL solution in pH 2.5 buffer.

HPLC VARIABLES
Column: 150 × 0.32 3 μm Hypersil C18
Mobile phase: MeCN:pH 6.0 acetate/citrate buffer 45:55
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 14
Limit of detection: 0.4 ng/mL
Limit of quantitation: 1.2 ng/mL

KEY WORDS
microcolumn

REFERENCE
Streel,B.; Ceccato,A.; Chiap,P.; Hubert,P.; Crommen,J. Injection-generated solvent and pH gradients for sample enrichment on injection of large volumes in microcolumn liquid chromatography, *Biomed.Chromatogr.*, **1995**, *9*, 254–256.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 10 μm Spherisorb silica
Column: 120 × 2 5 μm Spherisorb ODS
Mobile phase: MeOH:20 mM pH 7.0 phosphate buffer 58:42
Column temperature: 30
Flow rate: 0.378
Injection volume: 10
Detector: UV 254

REFERENCE
Lough,W.J.; Mills,M.J.; Maltas,J. Analyte adsorption in liquid chromatography valve injectors for samples in non-eluting solvents, *J.Chromatogr.A*, **1996**, *726*, 67–75.

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 acetate buffer 50:50
Detector: UV 242

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
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2

Injection volume: 20
Detector: UV 254

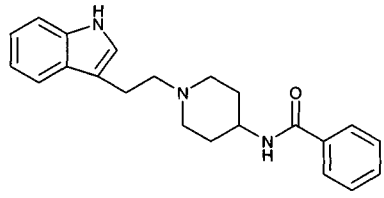
CHROMATOGRAM
Retention time: k' 0.58

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

Indoramin



Molecular formula: $C_{22}H_{25}N_3O$
Molecular weight: 347.46
CAS Registry No.: 26844-12-2
Merck Index: 5000
Lednicer No.: 2 344

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

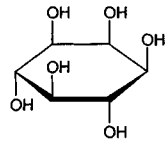
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.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

CHROMATOGRAM
Retention time: 12.533

KEY WORDS
whole blood

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

Inositol



Molecular formula: $C_6H_{12}O_6$
Molecular weight: 180.16
CAS Registry No.: 87-89-8, 573-35-3 (monophosphate)
Merck Index: 5008

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 8.7 Aminex HPX-87C Ca^{++} (Bio-rad)
Mobile phase: Water
Column temperature: 50
Flow rate: 0.6
Detector: E, pulsed amperometric detector (Dionex ?), E1 0.1 V, T1 300 ms, E2 0.6 V, T2 120 ms, E3 -0.8 V, T3 300 ms, following post-column reaction. The column effluent mixed with 100 mM NaOH pumped at 0.2 mL/min and the mixture flowed to the detector.

CHROMATOGRAM
Retention time: 13.64 (myo-inositol), 14.16 (chiro-inositol), 11.16 (scyllo-inositol), 19.58 (neo-inositol)

OTHER SUBSTANCES
Also analyzed: 2-deoxygalactitol, 2-deoxyribositol, dextrose, fucitol, fucose, galactitol, galactose, mannitol, mannose, perseitol, sorbitol

KEY WORDS
post-column reaction

REFERENCE
Wang,W.T.; Safar,J.; Zopf,D. Analysis of inositol by high-performance liquid chromatography, *Anal.Biochem.*, **1990**, *188*, 432–435.

Insulin

Molecular formula: $C_{257}H_{383}N_{65}O_{77}S_6$(human)
Molecular weight: 5807.69 (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)
Merck Index: 5011

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 250 × 4 5 μm Nucleosil RP18
Mobile phase: MeCN:buffer 24:76 (w/w) (Prepare buffer by dissolving 9.8 g 85% phosphoric acid and 28.4 g sodium sulfate in 1 L water.)
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 214

CHROMATOGRAM
Retention time: 10.89

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Kunkel,A.; Günter,S.; Dette,C.; Wätzig,H. Quantitation of insulin by capillary electrophoresis and high-performance liquid chromatography. Method comparison and validation, *J.Chromatogr.A*, **1997**, *781*, 445–455.

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, RNAase

REFERENCE
Ricker,R.D.; Sandoval,L.A.; Permar,B.J.; Boyes,B.E. Improved reversed-phase high performance liquid chromatography columns for biopharmaceutical analysis, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 93–105.

SAMPLE
Matrix: 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.

Interferon

Molecular formula: $C_{860}H_{1353}N_{227}O_{255}S_9$
Molecular weight: 19241.28
CAS Registry No.: 76543-88-9 (αA), 99210-65-8 (α2B), 98059-61-1 (gamma-1B)
Merck Index: 5015
Lednicer No.: 4 1

SAMPLE
Matrix: solutions
Sample preparation: Terminate the enzymatic hydrolysis by adding 10 μL 1% trifluoroacetic acid, 100 μL 8 M guanidine hydrochloride, inject an aliquot of the enzymatic digests.

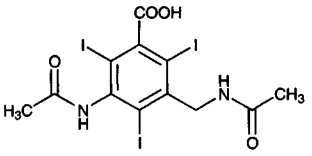
HPLC VARIABLES
Column: 150 × 4.6 5 μm Vydac C18
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid. B was MeCN:water 90:10 containing 0.1% trifluoroacetic acid. A:B from 52:48 to 45:55 in 45 min, to 20:80 over 2.5 min.
Flow rate: 0.6
Detector: UV 214

CHROMATOGRAM
Retention time: 20

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE
Gitlin,G.; Tsarbopoulos,A.; Patel,S.T.; Sydor,W.; Pramanik,B.N.; Jacobs,S.; Westreich,L.; Mittelman,S.; Bausch,J.N. Isolation and characterization of a monomethioninesulfoxide variant of interferon α-2b, *Pharm.Res.*, **1996**, *13*, 762–769.

Iodamide



Molecular formula: $C_{12}H_{11}I_3N_2O_4$
Molecular weight: 627.94
CAS Registry No.: 440-58-4, 18656-21-8 (meglumine salt)
Merck Index: 5031

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 100 µL 500 µg/mL iodopyracet in water + 5 mL MeOH, centrifuge at 1500 g for 20 min. Remove the supernatant and evaporate it to dryness under a stream of air at 65°, reconstitute the residue in 300 µL MeOH:water 90:10, let stand for 10 min, inject a 10 µL aliquot. Urine. 100 µL Urine + 900 µL mobile phase + 100 µL 500 µg/mL iodopyracet in water, inject a 10 µL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 5 µm LiChrosorb RP-8
Mobile phase: MeOH:water 85:15 containing 10 mM tetrabutylammonium hydrogen sulfate and 10 mM Tris
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 8
Internal standard: iodopyracet (11)
Limit of detection: 200 ng/mL

KEY WORDS

plasma

REFERENCE

Hekman,P.; Van Ginneken,C.A. Rapid determination of renal contrast media in biological fluids by means of high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *182*, 492–495.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 0.5-1 mL Plasma + 1 mL 1 M HCl + 5 mL ethyl acetate, shake for 10 min, centrifuge, repeat extraction. Combine the organic layers and evaporate them under a stream of nitrogen at 60°, add 3 mL 100 mM NaOH, shake for 10 min, centrifuge, discard the organic layer. Remove the aqueous layer and add it to 500 µL 1 M HCl, add 5 mL ethyl acetate, shake for 10 min, centrifuge, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 µL mobile phase, inject a 10-20 µL aliquot. Urine. 100 µL Urine + 1 mL 1 M HCl + add 5 mL ethyl acetate, shake for 10 min, centrifuge, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 400 µL mobile phase, inject a 10-20 µL aliquot.

HPLC VARIABLES

Column: 250 × 2.6 10 µm ODS-HC Sil-X-1 (Perkin-Elmer)
Mobile phase: MeCN:water:85% phosphoric acid 4:96:0.03
Flow rate: 1
Injection volume: 10-20
Detector: UV 235

CHROMATOGRAM

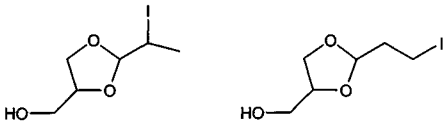
Retention time: 2.5
Internal standard: iodamide

OTHER SUBSTANCES
Extracted: o-iodohippurate, iothalamate

KEY WORDS
plasma; iodamide is IS

REFERENCE
Boschi,S.; Marchesini,B. High-performance liquid chromatographic method for the simultaneous determination of iothalamate and o-iodohippurate, *J.Chromatogr.*, **1981**, *224*, 139–143.

Iodinated glycerol



Molecular formula: $C_6H_{11}IO_3$
Molecular weight: 258.06
CAS Registry No.: 5634-39-9
Merck Index: 5033

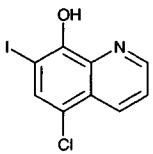
SAMPLE
Matrix: bulk
Sample preparation: Prepare an aqueous solution, inject an aliquot.

HPLC VARIABLES
Guard column: 10 μm Guard-pak (Waters)
Column: 250 × 4.6 5 μm Ultrasphere ODS C18
Mobile phase: MeCN:water 5:95
Flow rate: 1
Detector: RI

KEY WORDS
this procedure determines glycerol, a component of iodinated glycerol

REFERENCE
Cannon,J.M.; Brown,R.D.; Murrill,E.M.; Jameson,C.W. Identification of components in iodinated glycerol, *J.Pharm.Sci.*, **1989**, *78*, 48–51.

Iodochlorhydroxyquin



Molecular formula: C_9H_5ClINO
Molecular weight: 305.50
Merck Index: 5052

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL concentrated perchloric acid, vortex for 30 s, centrifuge at 15° at 5000 rpm for 15 min. Remove 500 μL of the supernatant and add it to 5 mL ether, vortex for 10 s, centrifuge at 15° for 10 min, repeat extraction. Add 10 mL ether to the original precipitate, vortex for 1 min, centrifuge at 15° at 5000 rpm for 15 min. Combine the extracts and dry them over anhydrous sodium sulfate, evaporate to dryness under a stream of nitrogen at 38°, reconstitute the residue in 500 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 40 × 5 RP-18-MPLC (Brownlee)
Column: 250 × 2.6 ODS-HC-SIL-X-I (Perkin-Elmer)

Mobile phase: MeOH:50 mM phosphoric acid 80:20 (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: 5
Limit of quantitation: 1 μg/mL

OTHER SUBSTANCES
Noninterfering: hydrocortisone

KEY WORDS
plasma

REFERENCE
Ezzedeen,F.W.; Masoud,A.N.; Stohs,S.J.; Lerman,S.J. High-performance liquid chromatographic analysis of iodochlorhydroxyquin in plasma, *J.Pharm.Sci.*, **1981**, *70*, 889–891.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Weigh out 30 mg of bulk drug or an amount of cream equivalent to 30 mg iodochlorhydroxyquin, add 70 mL THF, shake vigorously until the cream has dissolved, make up to 100 mL with THF. Remove a 5 mL aliquot and add it to 1 mL pyridine and 1 mL acetic anhydride, heat at 60° for 15 min, cool, add 15 mL 450 μg/mL testosterone acetate in 94:6 butyl chloride:THF, mix thoroughly. Remove a 3 mL aliquot and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 15 mL mobile phase with gentle warming and vigorous shaking, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μPorasil
Mobile phase: Butyl chloride:water-saturated butyl chloride:THF:glacial acetic acid 55:55:3:2
Flow rate: 2-3
Detector: UV 254

CHROMATOGRAM
Retention time: 6
Internal standard: testosterone acetate (8)

OTHER SUBSTANCES
Simultaneous: impurities

KEY WORDS
cream; normal phase; derivatization; acetylation

REFERENCE
Kubiak,E.J.; Munson,J.W. Analysis of iodochlorhydroxyquin in cream formulations and bulk drugs by high-performance liquid chromatography, *J.Pharm.Sci.*, **1982**, *71*, 872–875.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Weigh out ointment, cream, or bulk drug containing 30 mg of iodochlorhydroxyquin, add 40-50 mL THF, dissolve with heating, make up to 100 mL with THF. Remove a 5 mL aliquot and add it to 1 mL 10 mg/mL nickel chloride in MeOH, add 5 mL 0.4 mg/mL diphenylamine in MeOH, make up to 50 mL with MeOH, filter, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak phenyl

Mobile phase: MeCN:MeOH:water 30:20:50 containing 1 mM $NiCl_2$
Flow rate: 1.2
Detector: UV 273

CHROMATOGRAM
Retention time: 7.5
Internal standard: diphenylamine (11)

OTHER SUBSTANCES
Simultaneous: chloroxine, iodoquinol

KEY WORDS
ointment; creams; separated as Ni chelates

REFERENCE
Wojtowicz,E.J. Reverse-phase high-performance liquid chromatographic determination of halogenated 8-hydroxyquinoline compounds in pharmaceuticals and bulk drugs, *J.Pharm.Sci.*, **1984**, *73*, 1430–1433.

SAMPLE
Matrix: formulations
Sample preparation: Ointment. 50 mg Ointment + 10 mL ether, vortex until dissolved. Remove a 200 μL 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 × 5 RP-18-MPLC (Brownlee)
Column: 250 × 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: 7.5
Internal standard: phenyl salicylate (5.25)

OTHER SUBSTANCES
Simultaneous: hydrocortisone

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: feces, tissue, urine
Sample preparation: Urine. 1-5 mL Urine + 1-5 mL diethyl ether, vortex for 10 s, centrifuge at 15° at 3000 g for 10 min. Remove the organic layer and dry it over anhydrous sodium sulfate, evaporate to dryness under a stream of nitrogen at 40°, reconstitute the residue in mobile phase, inject a 20 μL aliquot. (Hydrolyze conjugates by adjusting pH to 5 with 1 M acetate buffer. add β-glucuronidase (Sigma) to a final concentration of 200 U/mL, heat at 37° for 2 h, neutralize with 3 M NaOH, add 1-5 mL diethyl ether, vortex for 10 s, centrifuge at 15° at 3000

g for 10 min. Remove the organic layer and dry it over anhydrous sodium sulfate, evaporate to dryness under a stream of nitrogen at 40°, reconstitute in 500 μL benzene (Caution! Benzene is a carcinogen!), add to an alumina column, wash with 2 mL benzene:pyridine 7:1, wash with 2 mL acetone, wash with 2 mL 100 mM acetic acid in MeOH, wash with 2 mL MeOH. Remove the alumina and add it to 1 mL saturated aqueous sodium fluoride, extract twice with 5 mL diethyl ether. Combine the extracts and evaporate them to dryness, reconstitute the residue in mobile phase, inject a 20 μL aliquot.) Tissue. Homogenize (Potter-Elvehjem) 1 g liver and 2 mL mobile phase, place homogenate in another tube, rinse original tube with 1 mL mobile phase, add rinse to homogenate, add 5 mL diethyl ether, vortex for 1 min, centrifuge at 3000 g for 10 min, repeat extraction twice. Combine the organic layers and evaporate them to dryness, reconstitute in 500 μL benzene (Caution! Benzene is a carcinogen!), add to an alumina column, wash with 2 mL benzene:pyridine 7:1, wash with 2 mL acetone, wash with 2 mL 100 mM acetic acid in MeOH, wash with 2 mL MeOH. Remove the alumina and add it to 1 mL saturated aqueous sodium fluoride, extract twice with 5 mL diethyl ether. Combine the extracts and evaporate them to dryness, reconstitute the residue in mobile phase, inject a 20 μL aliquot. Feces. Homogenize (Potter-Elvehjem) 1 g feces and 5 mL mobile phase, adjust pH to 5 with 1 M acetate buffer, add β-glucuronidase (Sigma) to a final concentration of 200 U/mL, heat at 37° for 2 h, neutralize with 3 M NaOH, add 1-5 mL diethyl ether, vortex for 10 s, centrifuge at 15° at 3000 g for 10 min. Remove the organic layer and dry it over anhydrous sodium sulfate, evaporate to dryness under a stream of nitrogen at 40°, reconstitute in 500 μL benzene (Caution! Benzene is a carcinogen!), add to an alumina column, wash with 2 mL benzene:pyridine 7:1, wash with 2 mL acetone, wash with 2 mL 100 mM acetic acid in MeOH, wash with 2 mL MeOH. Remove the alumina and add it to 1 mL saturated aqueous sodium fluoride, extract twice with 5 mL diethyl ether. Combine the extracts and evaporate them to dryness, reconstitute the residue in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 40 × 5 RP-18-MPLC (Brownlee)
Column: 250 × 2.6 ODS-HC-SIL-X-I (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: 7.5
Limit of detection: 200 ng
Limit of quantitation: 250 ng/g (feces), 500 ng/g (liver), 200 ng/mL (urine)

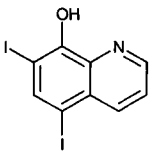
KEY WORDS

liver; dog; SPE

REFERENCE

Ezzedeen,F.W.; Stohs,S.J.; Stublar,M. Analysis of iodochlorhydroxyquin in biological materials by high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *276*, 121–128.

Iodoquinol



Molecular formula: $C_9H_5I_2NO$
Molecular weight: 396.95
CAS Registry No.: 83-73-8
Merck Index: 5063

SAMPLE

Matrix: bulk, formulations
Sample preparation: Cream, bulk. Weigh out cream or bulk drug containing 20 mg of iodoquinol, add 40-50 mL THF, dissolve with heating, make up to 100 mL with THF. Remove a 5

mL aliquot and add it to 1 mL 10 mg/mL nickel chloride in MeOH, add 3 mL 0.4 mg/mL diphenylamine in MeOH, make up to 50 mL with MeOH, filter, inject an aliquot. Tablets. Finely powder tablets, weigh out amount equivalent to 200 mg iodoquinol, add 150 mL THF, haet on a steam bath for a few min, shake mechanically for 30 min, make up to 250 mL with THF. Remove a 25 mL aliquot and make up to 100 mL with THF. Remove a 5 mL aliquot and add it to 1 mL 10 mg/mL nickel chloride in MeOH, add 3 mL 0.4 mg/mL diphenylamine in MeOH, make up to 50 mL with MeOH, filter, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak phenyl
Mobile phase: MeCN:MeOH:water 30:20:50 containing 1 mM $NiCl_2$
Flow rate: 1.2
Detector: UV 273

CHROMATOGRAM
Retention time: 9
Internal standard: diphenylamine (11)

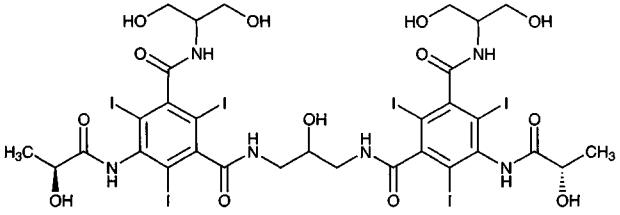
OTHER SUBSTANCES
Simultaneous: chloroxine, iodochlorhydroxyquin

KEY WORDS
tablets; creams; separated as Ni chelates

REFERENCE
Wojtowicz,E.J. Reverse-phase high-performance liquid chromatographic determination of halogenated 8-hydroxyquinoline compounds in pharmaceuticals and bulk drugs, *J.Pharm.Sci.*, **1984**, *73*, 1430–1433.

Iofratol

Molecular formula: $C_{31}H_{36}I_6N_6O_{13}$
Molecular weight: 1462.09
CAS Registry No.: 141660-63-1



SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Add 30 μL 2 mg/mL IS in water to 100 μL plasma. Add 30 μL 35% perchloric acid. Agitate and centrifuge at 3500 g for 10 min. Inject a 10 μL aliquot of the clear supernatant. Urine. Dilute 1 mL urine with 2 mL water, centrifuge at 4500 g for 15 min. Add 100 μL 5 mg/mL IS, 100 μL glacial acetic acid, and an ion-exchange resin mixture (1 g Duolite A-30B + 900 mg Amberlite IR-120). Dilute the suspension to 5 mL with water. Agitate for 30 min and centrifuge at 3500 g for 5 min. Inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 30 × 4 7 μm LiChrosorb RP-8
Column: 250 × 4.6 5 μm LiChrosorb RP-8
Mobile phase: MeCN:5 mM pH 4.5 potassium dihydrogen phosphate 5:95
Column temperature: 45
Flow rate: 1
Injection volume: 10
Detector: UV 242

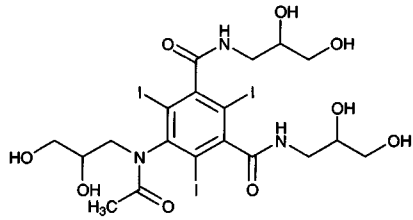
CHROMATOGRAM
Retention time: 12.0
Internal standard: iopamidol (4.1)
Limit of detection: 75 ng/mL (plasma), 430 ng/mL (urine)

KEY WORDS
plasma; rat; human

REFERENCE
Arbughi,T.; Bertani,F.; Celeste,R.; Grotti,A.; Tirone,P. High-performance liquid chromatographic determination of the X-ray imaging contrast agent, iofratol, in plasma and urine, *J.Chromatogr.B*, **1997**, *701*, 103–113.

Iohexol

Molecular formula: $C_{19}H_{26}I_3N_3O_9$
Molecular weight: 821.14
CAS Registry No.: 66108-95-0
Merck Index: 5068



SAMPLE
Matrix: blood
Sample preparation: Mix serum with an equal volume of MeCN, vortex for 15 s, centrifuge at 14000 g for 30 s, dilute the supernatant 100-fold with mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 40 × 3.2 3 μm Velosep RP-18 (Applied Biosystems)
Mobile phase: 8 mM pH 2 Phosphoric acid
Flow rate: 1
Injection volume: 10
Detector: UV 254

KEY WORDS
serum

REFERENCE
Shihabi,Z.K.; Constantinescu,M.S. Iohexol in serum determined by capillary electrophoresis, *Clin.Chem.*, **1992**, *38*, 2117–2120.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 50 μL 250 μg/mL acetaminophen in 100 mM HCl, add to SPE cartridge containing 150 mg 80-100 mesh Chromosorb P/NAW, elute with 1 mL ethyl acetate:MeOH 5:1, add the eluate to 50 μL 100 mM HCl, vortex for 15 s, centrifuge at 10000 g for 3 min, inject a 20 μL aliquot of the lower aqueous phase.

HPLC VARIABLES
Column: 5 μm C8
Mobile phase: MeCN:20 mM pH 3.3 phosphoric acid 2.5:97.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Internal standard: acetaminophen
Limit of detection: <1 μg/mL

OTHER SUBSTANCES
Extracted: aminohippuric acid (PAH)

KEY WORDS
serum; SPE

KEY WORDS
plasma; rat; human

REFERENCE
Arbughi,T.; Bertani,F.; Celeste,R.; Grotti,A.; Tirone,P. High-performance liquid chromatographic determination of the X-ray imaging contrast agent, iofratol, in plasma and urine, *J.Chromatogr.B*, **1997**, *701*, 103–113.

Iohexol

Molecular formula: $C_{19}H_{26}I_3N_3O_9$
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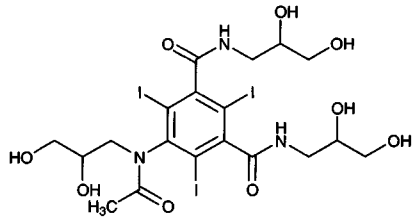


SAMPLE
Matrix: blood
Sample preparation: Mix serum with an equal volume of MeCN, vortex for 15 s, centrifuge at 14000 g for 30 s, dilute the supernatant 100-fold with mobile phase, inject a 10 µL aliquot.

HPLC VARIABLES
Column: 40 × 3.2 3 µm Velosep RP-18 (Applied Biosystems)
Mobile phase: 8 mM pH 2 Phosphoric acid
Flow rate: 1
Injection volume: 10
Detector: UV 254

KEY WORDS
serum

REFERENCE
Shihabi,Z.K.; Constantinescu,M.S. Iohexol in serum determined by capillary electrophoresis, *Clin.Chem.*, **1992**, *38*, 2117–2120.

SAMPLE
Matrix: blood
Sample preparation: 50 µL Serum + 50 µL 250 µg/mL acetaminophen in 100 mM HCl, add to SPE cartridge containing 150 mg 80-100 mesh Chromosorb P/NAW, elute with 1 mL ethyl acetate:MeOH 5:1, add the eluate to 50 µL 100 mM HCl, vortex for 15 s, centrifuge at 10000 g for 3 min, inject a 20 µL aliquot of the lower aqueous phase.

HPLC VARIABLES
Column: 5 µm C8
Mobile phase: MeCN:20 mM pH 3.3 phosphoric acid 2.5:97.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Internal standard: acetaminophen
Limit of detection: <1 µg/mL

OTHER SUBSTANCES
Extracted: aminohippuric acid (PAH)

KEY WORDS
serum; SPE

REFERENCE
Andreeva,M.; Rapondjieva,A.; Deskova,D.; Tishkov,I.; Svinarov,D. Liquid chromatographic determination of iohexol and PAH with Chromosorb P column used for sample preparation (Abstract 175), *Ther.Drug Monit.*, **1995**, *17*, 427.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 50 μL Plasma + 150 μL buffer, centrifuge at 1000 g at 0° for 10 min, inject a 10 μL aliquot. Tissue. Weigh 2 testes, add 5 mL buffer, homogenize (Kinematica type PT 10/35, setting 7.5) for 1 min, vortex, centrifuge at 1000 g at 0° for 10 min. Remove a 50 μL aliquot of the supernatant and add it to 150 μL buffer, centrifuge at 1000 g at 0° for 10 min, inject a 10 μL aliquot. (Buffer was 5 mM metaphosphoric acid and 5 mM disodium EDTA.)

HPLC VARIABLES
Guard column: C18 Bondapak guard column
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:buffer 2.5:97.5–5:95 (Buffer was 100 mM NaH_2PO_4 and 0.2 mM Na_2EDTA adjusted to pH 3.1 with orthophosphoric acid.)
Flow rate: 1
Injection volume: 10
Detector: UV 254

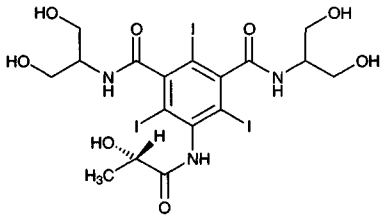
CHROMATOGRAM
Retention time: 7.5 (two isomers seen)
Limit of detection: 670 ng/mL

OTHER SUBSTANCES
Also analyzed: iopamidol, diatrizoate

KEY WORDS
plasma; mouse; testes

REFERENCE
Harapanhalli,R.S.; Yaghmai,V.; Patel,Y.D.; Baker,S.R.; Rao,D.V. Assay of radiographic contrast agents in mice plasma and testes by high-performance liquid chromatography, *Anal.Chem.*, **1993**, *65*, 606–612.

Iopamidol



Molecular formula: $C_{17}H_{22}I_3N_3O_8$
Molecular weight: 777.09
CAS Registry No.: 60166-93-0
Merck Index: 5071

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 50 μL Plasma + 150 μL buffer, centrifuge at 1000 g at 0° for 10 min, inject a 10 μL aliquot. Tissue. Weigh 2 testes, add 5 mL buffer, homogenize (Kinematica type PT 10/35, setting 7.5) for 1 min, vortex, centrifuge at 1000 g at 0° for 10 min. Remove a 50 μL aliquot of the supernatant and add it to 150 μL buffer, centrifuge at 1000 g at 0° for 10 min, inject a 10 μL aliquot. (Buffer was 5 mM metaphosphoric acid and 5 mM disodium EDTA.)

HPLC VARIABLES
Guard column: C18 Bondapak guard column
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:buffer 2.5:97.5–5:95 (Buffer was 100 mM NaH_2PO_4 and 0.2 mM Na_2EDTA adjusted to pH 3.1 with orthophosphoric acid.)

Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 9.5
Limit of detection: 640 ng/mL

OTHER SUBSTANCES
Also analyzed: iohexaol, diatrizoate

KEY WORDS
plasma; mouse; testes

REFERENCE
Harapanhalli,R.S.; Yaghmai,V.; Patel,Y.D.; Baker,S.R.; Rao,D.V. Assay of radiographic contrast agents in mice plasma and testes by high-performance liquid chromatography, *Anal.Chem.*, **1993**, *65*, 606–612.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Add 30 μL water to 100 μL plasma. Add 30 μL 35% perchloric acid. Agitate and centrifuge at 3500 g for 10 min. Inject a 10 μL aliquot of the clear supernatant. Urine. Dilute 1 mL urine with 2 mL water, centrifuge at 4500 g for 15 min. Add 100 μL 5 mg/mL IS, 100 μL glacial acetic acid, and an ion-exchange resin mixture (1 g Duolite A-30B + 900 mg Amberlite IR-120). Dilute the suspension to 5 mL with water. Agitate for 30 min and centrifuge at 3500 g for 5 min. Inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 30 × 4.0 7 μm LiChrosorb RP-8
Column: 250 × 4.6 5 μm LiChrosorb RP-8
Mobile phase: MeCN:5 mM pH 4.5 potassium dihydrogen phosphate 5:95
Column temperature: 45
Flow rate: 1.0
Injection volume: 10
Detector: UV 242

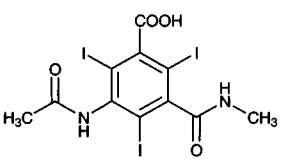
CHROMATOGRAM
Retention time: 4.1

OTHER SUBSTANCES
Extracted: iofratol

KEY WORDS
iopamidol is IS; plasma; rat; human

REFERENCE
Arbughi,T.; Bertani,F.; Celeste,R.; Grotti,A.; Tirone,P. High-performance liquid chromatographic determination of the X-ray imaging contrast agent, iofratol, in plasma and urine, *J.Chromatogr.B*, **1997**, *701*, 103–113.

Iothalamate



Molecular formula: $C_{11}H_9I_3N_2O_4.C_7H_{17}NO_5$ (meglumine), $C_{11}H_8I_3N_2NaO_4$ (sodium salt)
Molecular weight: 809.13 (meglumine), 635.90 (sodium salt)
CAS Registry No.: 13087-53-1, 2276-90-6 (iothalamic acid), 6284-40-8 (meglumine), 17692-74-9 (^{125}I radioactive agent), 15845-98-4 (^{131}I radioactive agent), 1225-20-3 (sodium salt)

SAMPLE
Matrix: blood, urine
Sample preparation: Dilute urine 10-fold with water. Add 200 μL MeCN containing 20 μg/mL p-aminobenzoic acid to 100 μL diluted urine, vortex briefly, centrifuge at 12000 g for 4 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 5 μm Ultrasphere C18
Column: 250 mm 5 μm Primesphere C18 (Torrance, USA)
Mobile phase: MeOH:buffer 18:82 (Buffer was 50 mM NaH_2PO_4 with 0.5 mM tetrabutyl ammonium hydrogen sulfate with an unadjusted pH of 4.11.)
Flow rate: 0.8
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 13
Internal standard: p-aminobenzoic acid (9.8)

OTHER SUBSTANCES
Extracted: p-aminohippuric acid

KEY WORDS
serum

REFERENCE
Agarwal,R. Chromatographic estimation of iothalamate and p-aminohippuric acid to measure glomerular filtration rate and effective renal plasma flow in humans, *J.Chromatogr.B*, **1998**, *705*, 3–9.

SAMPLE
Matrix: formulations
Sample preparation: Mix a dilution of the injectable solution in water with a mixture of 2,4-dinitrobenzenesulfonyl chloride (DNBS-Cl) and sodium carbonate in acetone at ambient temperature for 1 hr. Dilute the reaction mixture with 25% MeOH and inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 4 μm Nova-Pak C18
Mobile phase: MeOH:buffer 25:75 (Buffer was 20 mM tetrapropylammonium hydroxide adjusted to pH 3.4 with orthophosphoric acid.)
Flow rate: 1.3
Injection volume: 20
Detector: UV 262

CHROMATOGRAM
Retention time: 2.75
Limit of quantitation: 1 μg

OTHER SUBSTANCES
Simultaneous: diatrizoate, meglumine

KEY WORDS
injections; only meglumine is derivatized under these conditions

REFERENCE
Lau-Cam,C.A.; Roos,R.W. HPLC method with spectrophotometric detection for the simultaneous assay of meglumine and its counterions iothalamic acid or diatrizoic acid in radiographic solutions for injection (Abstract 3372), *Pharm.Res.*, **1997**, *14*, S586.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma or urine + 0.5-5 μg p-aminobenzoic acid + 200 μL MeCN, vortex for a few s, centrifuge at 800 g for 5 min, inject a 5 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:0.04% pH 2.5 ± 0.05 phosphoric acid 3.5:96.5
Flow rate: 1.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 6
Internal standard: p-aminobenzoic acid (8)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: p-aminohippuric acid

KEY WORDS
plasma; dog; human; pharmacokinetics

REFERENCE
Prueksaritanont,T.; Chen,M.L.; Chiou,W.L. Simple and micro high-performance liquid chromatographic method for simultaneous determination of p-aminohippuric acid and iothalamate in biological fluids, *J.Chromatogr.*, **1984**, *306*, 89–97.

SAMPLE
Matrix: blood
Sample preparation: Add barbital to plasma. 100 μL Plasma + 500 μL MeOH, vortex for 15 s, centrifuge at 2500 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in buffer, inject a 15-20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Octyl C8 (Rainin)
Mobile phase: MeOH:MeCN:buffer 90:10:300 (Buffer was 6.44 g KH_2PO_4, 7.04 g K_2HPO_4, and 14 mL 500 mM dodecyltriethylammonium phosphate (Regis) in 4 L water.)
Flow rate: 1
Injection volume: 15-20
Detector: UV 254

CHROMATOGRAM
Retention time: 18.2
Internal standard: barbital (15.9)
Limit of quantitation: 3000 ng/mL

OTHER SUBSTANCES
Extracted: p-aminohippuric acid

KEY WORDS
plasma

REFERENCE
Jayewardene,A.L.; Seneviratne,A.K.; Gambertoglio,J.G. Paired ion reversed-phase HPLC assay for the simultaneous determination of iothalamic acid and para aminohippuric acid in plasma, *J.Liq.Chromatogr.*, **1994**, *17*, 2395–2412.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 0.5-1 mL Plasma + 18 μg iodamide + 2 μg hippuric acid + 1 mL 1 M HCl + 5 mL ethyl acetate, shake for 10 min, centrifuge, repeat extraction. Combine the organic layers and evaporate them under a stream of nitrogen at 60°, add 3 mL 100 mM NaOH, shake for 10 min, centrifuge, discard the organic layer. Remove the aqueous layer and add it to 500 μL 1 M HCl, add 5 mL ethyl acetate, shake for 10 min, centrifuge, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL mobile phase, inject a 10-20 μL aliquot. Urine. 1 mL Urine + 10 μL 10% iodamide in water, mix. Remove a 100 μL aliquot and add it to 1 mL 1 M HCl, add 5 mL ethyl acetate, shake for 10 min, centrifuge, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 400 μL mobile phase, inject a 10-20 μL aliquot.

HPLC VARIABLES
Column: 250 × 2.6 10 μm ODS-HC Sil-X-1 (Perkin-Elmer)
Mobile phase: MeCN:water:85% phosphoric acid 4:96:0.03
Flow rate: 1
Injection volume: 10-20
Detector: UV 235

CHROMATOGRAM
Retention time: 2.0
Internal standard: iodamide (2.5), hippuric acid (5.6)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: o-iodohippurate

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Boschi,S.; Marchesini,B. High-performance liquid chromatographic method for the simultaneous determination of iothalamate and o-iodohippurate, *J.Chromatogr.*, **1981**, *224*, 139–143.

SAMPLE
Matrix: blood, urine
Sample preparation: 200 μL Plasma + 200 μL 100 mM NaH_2PO_4 + 400 μL MeCN, mix for 5 s, let stand at 4° for 15 min, centrifuge at 10500 g for 1 min. Remove the supernatant and add it to 2 mL dichloromethane, mix for 5 min, centrifuge at 4800 g for 10 min, inject a 5-50 μL aliquot of the upper aqueous phase. Urine. Centrifuge urine at 4800 g for 10 min, dilute 1:10 with 50 mM NaH_2PO_4, inject an aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrosorb RP-18
Mobile phase: MeCN:water 7.5:92.5 containing 5.50 g/L $NaH_2PO_4.H_2O$, 1.80 g/L $Na_2HPO_4.2H_2O$, and 20 mg/L tetrabutylammonium bromide, pH 6.4 (plasma) or MeCN:water 5:95 containing 5.50 g/L $NaH_2PO_4.H_2O$, 1.80 g/L $Na_2HPO_4.2H_2O$, and 22.5 mg/L tetrabutylammonium bromide, pH 6.4 (urine)
Flow rate: 1
Injection volume: 5-50
Detector: UV 254

CHROMATOGRAM
Retention time: 2.3 (plasma), 2.7 (urine)
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Extracted: cefotetan (UV 280)

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Kees,F.; Grobecker,H.; Naber,K.G. High-performance liquid chromatographic analysis of cefotetan epimers in human plasma and urine, *J.Chromatogr.*, **1984**, *305*, 363–371.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 500 μL Plasma + 500 μL 1 M HCl, vortex for 10 s, add 6 mL ethyl acetate, vortex for 20 s, centrifuge at 4° at 1700 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 400 μL 25 mM pH 3 KH_2PO_4, add 500 μL dichloromethane, shake gently horizontally for 5 min, centrifuge at 1700 g for 5 min, inject a 20 μL aliquot of the aqueous phase. Urine. Dilute 1:10 with water. Remove a 1 mL aliquot and add it to 1 mL 1 M HCl, vortex for 10 s, add 6 mL ethyl acetate, vortex for 20 s, centrifuge at 4° at 1700 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 400 μL 25 mM pH 3 KH_2PO_4, add 500 μL dichloromethane, shake gently horizontally for 5 min, centrifuge at 1700 g for 5 min, inject a 20 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: 10 × 3 anion-exchange guard column (Chrompack)
Column: 250 × 4.6 Partisil 10 SAX
Mobile phase: MeCN:25 mM pH 3 phosphate buffer 15:85
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7.05
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: p-aminohippuric acid
Noninterfering: acipimox, allopurinol, aspirin, atenolol, captopril, chlorthalidone, clonidine, digitoxin, digoxin, diltiazem, dipyridamole, enalapril, furosemide, gemfibrozil, hydralazine, hydrochlorothiazide, ibopamine, insulin, inulin, isosorbide dinitrate, α-methyldopa, nicardipine, nifedipine, prazosin, propranolol, salicylic acid, simvastatin, trinitrin, verapamil

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Gaspari,F.; Mainardi,L.; Ruggenenti,P.; Remuzzi,G. High-performance liquid chromatographic determination of iothalamic acid in human plasma and urine, *J.Chromatogr.*, **1991**, *570*, 435–440.

SAMPLE
Matrix: urine
Sample preparation: Dilute urine 1:100 or 1:500. 200 μL Diluted urine + 50 μL barbital solution, vortex for 15 s, inject a 20-30 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C8
Mobile phase: MeCN:MeOH:10 mM pH 7.5 potassium phosphate buffer:0.5 M dodecyl triethylammonium phosphate 6:94:300:0.6 (0.5 M Dodecyl triethylammonium phosphate was Q-12, Ion pair reagent, Regis Chemical Co.)
Flow rate: 1
Injection volume: 20-30

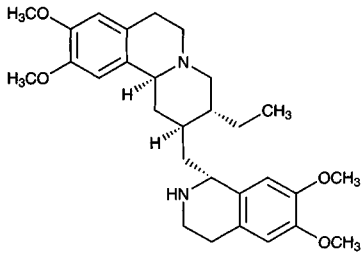
Detector: UV 254

CHROMATOGRAM
Retention time: 16.8
Internal standard: barbital (14.5)
Limit of quantitation: 50000 ng/mL

OTHER SUBSTANCES
Extracted: p-aminohippuric acid

REFERENCE
Seneviratne,A.K.; Jayewardene,A.L.; Gambertoglio,J.G. Paired-ion reversed-phase HPLC assay for the determination of iothalamic acid and para aminohippuric acid in urine, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 1311–1316.

Ipecac



Molecular formula: $C_{28}H_{38}N_2O_4$, $C_{29}H_{40}N_2O_4$
Molecular weight: 466.62, 480.65
CAS Registry No.: 483-17-0, 483-18-1
Merck Index: 5086

SAMPLE
Matrix: blood, emesis, urine
Sample preparation: 2 mL Plasma, whole blood, urine, or emesis (diluted 1:100) + 100 μL 10 ng/mL N-propylprocainamide in water + 2 mL saturated sodium borate + 7 mL n-butyl chloride, vortex for 30 s, centrifuge. Remove the organic phase and add it to 200 μL 10 mM HCl, vortex, centrifuge, inject a 30-50 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:25 mM pH 8.0 Na_2HPO_4 72:28
Flow rate: 1.7
Injection volume: 30-50
Detector: F ex 285 em 316

CHROMATOGRAM
Retention time: 3 (cephaeline), 4.1 (emetine)
Internal standard: N-propylprocainamide (1.7)
Limit of quantitation: 5 ng/mL

KEY WORDS
plasma; whole blood

REFERENCE
Crouch,D.J.; Moran,D.M.; Finkle,B.S.; Peat,M.A. Quantitative analysis of emetine and cephaeline by reversed-phase high performance liquid chromatography with fluorescence detection, *J.Anal.Toxicol.*, **1984**, *8*, 63–65.

SAMPLE
Matrix: blood, vomit
Sample preparation: Mix 250 μL vomit or 500 μL serum with an equal volume of 10% ammonium hydroxide for 5 min, add 4 mL ether, mix. Remove the organic layer and evaporate it

to dryness under vacuum at 25°, reconstitute the residue in 10 (vomit) or 0.2 (serum) mL 0.01% HCl. Mix 1 mL vomit solution with 50 μL 4 mg/mL acrinol or 200 μL serum solution with 50 μL 200 μg/mL acrinol, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm TSK gel ODS-80TM
Mobile phase: MeOH:buffer 54:46 (Buffer was 10 mM sodium 1-heptanesulfonate adjusted to pH 4 with glacial acetic acid.)
Flow rate: 1
Injection volume: 100
Detector: F ex 285 em 316

CHROMATOGRAM
Internal standard: acrinol

KEY WORDS
dog; serum; pharmacokinetics; assay determines cephaeline, a constituent of ipecac

REFERENCE
Teshima,D.; Suzuki,A.; Otsubo,K.; Higuchi,S.; Aoyama,T.; Shimozono,Y.; Saita,M.; Noda,K. Efficacy of emetic and United State Pharmacopoeia ipecac syrup in prevention of drug absorption, *Chem.Pharm.Bull.(Tokyo)*, **1990**, *38*, 2242–2245.

SAMPLE
Matrix: cell cultures
Sample preparation: Dry 1-5 g cell culture at 60°, powder, extract with MeOH in a glass percolator for 72 h. Filter (paper) extract, wash solid with MeOH at 45°, concentrate filtrate under reduced pressure, filter (0.5 μm), dilute filtrate with MeOH, inject a 2.5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm silica gel (Spectra Physics)
Mobile phase: Chloroform:MeOH:diethylamine 90:10:0.2
Flow rate: 0.5
Injection volume: 2.5
Detector: UV 280

CHROMATOGRAM
Retention time: 4.5 (emetine), 5 (cephaeline)

KEY WORDS
normal phase

REFERENCE
Jha,S.; Sahu,N.P.; Mahato,S.B. Production of the alkaloids emetine and cephaeline in callus cultures of *Cephaelis ipecacuanha*, *Planta Med.*, **1988**, *54*, 504–506.

SAMPLE
Matrix: formulations
Sample preparation: Syrup. Dilute syrup with an equal volume of water. 2 mL Diluted syrup + 2 mL 1% dansyl chloride in acetone + 200 μL 1.5 M sodium carbonate, heat at 45 ± 2° in the dark for 20 min, cool, add 3 mL water, add 500 μL benzene (Caution! Benzene is a carcinogen!), shake. Remove the organic layer and evaporate it to dryness, reconstitute the residue in mobile phase, inject a 10 μL aliquot. Capsules. Sonicate the contents of a capsule in 20 mL water for 10 min, centrifuge at 2000 g for 10 min. 2 mL Supernatant + 2 mL 1% dansyl chloride in acetone + 200 μL 1.5 M sodium carbonate, heat at 45 ± 2° in the dark for 20 min, cool, add 3 mL water, add 500 μL benzene (Caution! Benzene is a carcinogen!), shake. Remove the organic layer and evaporate it to dryness, reconstitute the residue in mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 2.8 10 μm silica gel SI 100 (Merck)

Mobile phase: Diisopropyl ether:isopropanol:concentrated ammonia 48:2:0.3 (Caution! Diisopropyl ether readily forms explosive peroxides!)
Injection volume: 10
Detector: UV 254 or F ex 358 em 492 (cephaeline) or ex 356 em 481 (emetine)

CHROMATOGRAM
Retention time: 2.5 (cephaeline), 3.5 (emetine)

OTHER SUBSTANCES
Simultaneous: ephedrine, codeine (not derivatized, detect at UV 254 only)

KEY WORDS
derivatization; syrup; capsules; normal phase

REFERENCE
Frei,R.W.; Santi,W.; Thomas,M. Liquid chromatography of dansyl derivatives of some alkaloids and the application to the analysis of pharmaceuticals, *J.Chromatogr.*, **1976**, *116*, 365–377.

SAMPLE
Matrix: formulations
Sample preparation: Mix 10 g linctus with 10-20 mL 200 μg/mL ethyl 4-hydroxybenzoate in MeCN:mobile phase 10:90, make up to 100 mL with mobile phase, inject a 10-25 μL aliquot. Mix 10 pastilles with 50 mL 200 μg/mL ethyl 4-hydroxybenzoate in MeCN:mobile phase 10:90, make up to 200 mL with mobile phase, inject a 10-25 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 μBondapak C18
Mobile phase: MeOH:water 40:60 containing 1 g/L sodium 1-heptanesulfonate and 1 mL/L orthophosphoric acid
Column temperature: 35
Flow rate: 2
Injection volume: 10-25
Detector: F ex 276 em 304

CHROMATOGRAM
Retention time: 8 (cephaeline), 15 (emetine)
Internal standard: ethyl 4-hydroxybenzoate (5)
Limit of quantitation: 5 μg/g

OTHER SUBSTANCES
Simultaneous: dihydroemetine (UV 214), emetamine (UV 214), O-methylpsychotrine (UV 214), tetrahydroemetine (UV 214)

KEY WORDS
stability-indicating; linctus; pastilles; rugged

REFERENCE
Elvidge,D.A.; Johnson,G.W.; Harrison,J.R. Selective, stability-indicating assay of the major ipecacuanha alkaloids, emetine and cephaeline, in pharmaceutical preparations by high-performance liquid chromatography using spectrofluorimetric detection, *J.Chromatogr.*, **1989**, *463*, 107–118.

SAMPLE
Matrix: formulations
Sample preparation: 2 mL Sample + 1 mL 200 μg/mL emetine hydrochloride in water, make up to 10 mL with mobile phase, filter (0.45 μm), inject a 50-100 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Technosphere RP C-8 (HPLC Technology)
Mobile phase: MeCN:40 mM tetramethylammonium bromide:1 M acetic acid 80:15:5 (apparent pH 4.5)

Flow rate: 1.5
Injection volume: 50-100
Detector: UV 260

CHROMATOGRAM
Retention time: 1.83
Internal standard: emetine

OTHER SUBSTANCES
Simultaneous: benzalkonium (C12, C14, C16), tetrahydrozoline, naphazoline

KEY WORDS
nasal; ophthalmic; emetine is IS

REFERENCE
Santoni,G.; Medica,A.; Gratteri,P.; Furlanetto,S.; Pinzauti,S. High-performance liquid chromatographic determination of benzalkonium and naphazoline or tetrahydrozoline in nasal and ophthalmic solutions, *Farmaco*, **1994**, *49*, 751–754.

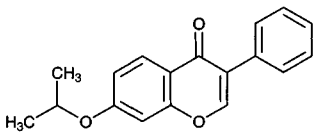
SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 4 C18 Micropak SP
Mobile phase: MeCN:3 mM pH 2.5 phosphoric acid 20:80
Flow rate: 2
Injection volume: 15
Detector: F ex 285 em 315 or UV 205

CHROMATOGRAM
Retention time: 4.1 (cephaeline), 6.2 (emetine)
Limit of detection: 30 ng/mL

REFERENCE
Lachman,M.F.; Romeo,R.; McComb,R.B. Emetine identified in urine by HPLC, with fluorescence and ultraviolet/diode array detection, in a patient with cardiomyopathy, *Clin.Chem.*, **1989**, *35*, 499–502.

Ipriflavone



Molecular formula: $C_{18}H_{16}O_3$
Molecular weight: 280.32
CAS Registry No.: 35212-22-7
Merck Index: 5090

SAMPLE
Matrix: bulk
Sample preparation: Inject a 10 μL aliquot of a solution in MeOH.

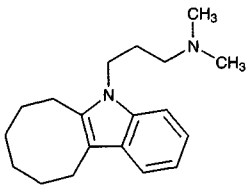
HPLC VARIABLES
Column: 30 × 4.6 3 μm ODS C18 (Perkin-Elmer)
Mobile phase: MeCN:buffer 50:50 (Buffer was 10 mM triethylamine adjusted to pH 2.5 with orthophosphoric acid.)
Flow rate: 1.2
Injection volume: 10
Detector: UV 225

CHROMATOGRAM
Retention time: 3.4

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE
Sustacha,K.; Chacón,M.; Lucero,M.L.; Orjales,A. Determination of ipriflavone and its synthetic impurities by high-performance liquid chromatography using diode-array detection, *J.Chromatogr.A*, **1996**, *719*, 245–250.

Iprindole



Molecular formula: $C_{19}H_{28}N_2$
Molecular weight: 284.44
CAS Registry No.: 5560-72-5, 20432-64-8 (HCl)
Merck Index: 5091
Lednicer No.: 1 318

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

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

CHROMATOGRAM
Retention time: 4.5

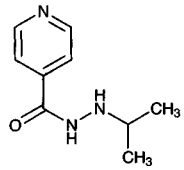
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, 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, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide,

phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

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

Iproniazid



Molecular formula: $C_9H_{13}N_3O$
Molecular weight: 179.22
CAS Registry No.: 54-92-2, 305-33-9 (phosphate)
Merck Index: 5094
Lednicer No.: 1 254

SAMPLE

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

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 264

CHROMATOGRAM

Retention time: 3.00
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam;

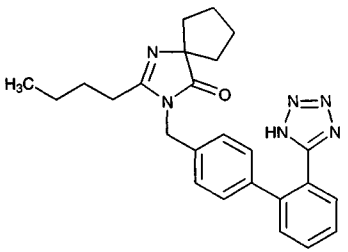
tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

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

Irbesartan

Molecular formula: $C_{25}H_{28}N_6O$
Molecular weight: 428.54
CAS Registry No.: 138402-11-6
Merck Index: 5097



SAMPLE

Matrix: blood, urine

Sample preparation: Condition a 3 mL 100 mg Isolute Cyano SPE cartridge (Jones Chromatography) with 2 mL MeOH and 2 mL 0.85% phosphoric acid. Plasma. Mix 1.0 mL 0.85% phosphoric acid and 100 µL 1 µg/mL IS in 0.85% phosphoric acid with 250 µL plasma. Pass the mixture slowly through the SPE cartridge, wash with 3 mL 0.85% phosphoric acid, wash with 1 mL hexane. Elute with 1 mL of mixture MeOH:0.85% phosphoric acid 50:50. Inject a 20 µL aliquot of the eluate. Urine. Mix 1.0 mL 0.85% phosphoric acid and 100 µL 1 µg/mL IS in 0.85% phosphoric acid with 250 µL urine. Pass the mixture slowly through the SPE cartridge, wash with 4 mL 0.85% phosphoric acid. Elute with 1 mL of mixture MeOH:0.85% phosphoric acid 50:50. Inject a 20 µL aliquot of the eluate.

HPLC VARIABLES

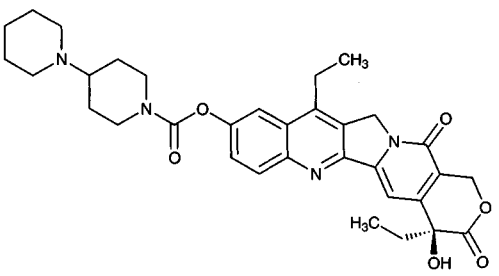
Column: 150 × 4.6 5 µm YMC-ODS-AQ
Mobile phase: MeCN:buffer 50:50 (Prepare buffer by adding 1 mL triethylamine to 1 L water, adjust pH to 3.5 with 85% phosphoric acid.)
Flow rate: 0.8
Injection volume: 20
Detector: F ex 250 em 371

CHROMATOGRAM
Retention time: 6.6
Internal standard: BMS-190462 (9.4)
Limit of quantitation: 1 ng/mL

KEY WORDS
plasma; urine; SPE; pharmacokinetics

REFERENCE
Chang,S.-Y.; Whigan,D.B.; Vachharajani,N.N.; Patel,R. High-performance liquid chromatographic assay for the quantitation of irbesartan (SR 47436/BMS-186295) in human plasma and urine, *J.Chromatogr.B*, **1997**, *702*, 149–155.

Irinotecan



Molecular formula: $C_{33}H_{38}N_4O_6$
Molecular weight: 586.69
CAS Registry No.: 97682-44-5, 100286-90-6 (HCl)
Merck Index: 5104

SAMPLE
Matrix: bile
Sample preparation: Dilute bile, inject an aliquot.

HPLC VARIABLES
Column: 300 × 7.2 TSKgel-ODS 80Tm (Tosoh)
Mobile phase: Gradient. MeOH:100 mM pH 4.0 phosphate buffer from 50:50 to 60:40 over 20 min, maintain at 60:40 for 20 min.
Flow rate: 3
Detector: UV 254

CHROMATOGRAM
Retention time: 16

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; preparative for metabolites

REFERENCE
Atsumi,R.; Suzuki,W.; Hakusui,H. Identification of the metabolites of irinotecan, a new derivative of camptothecin, in rat bile and its biliary excretion, *Xenobiotica*, **1991**, *21*, 1159–1169.

SAMPLE
Matrix: blood
Sample preparation: Mix 10 μL plasma with 100 μM diisopropyl fluorophosphate, 125 ng/mL camptothecin and 375μL MeOH with an automatic mixer (S-100, Taitec, Saitama, Japan) for 30 s, centrifuge at 3000 rpm for 10 min. Evaporate the supernatant on a Speed Vac Plus SC110A (Savant Instruments. Inc., Farmingdale, NY) and dissolve the residue in 200 μL THF: 50 mM pH 2.0 KH_2PO_4 containing 5 mM heptanesulfonate 25:75. Centrifuge at 10000 rpm for 3 min, inject 20 or 50 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.5 5 μm TSK-GEL ODS-80TS (Toyo soda, Tokyo)
Column: 150 × 4.6 5 μm TSK-GEL ODS-80TS (Toyo soda, Tokyo)
Mobile phase: THF:50mM pH 4.0 KH_2PO_4 containing 5mM heptanesulfonate 25:75
Flow rate: 0.8
Injection volume: 20 or 50
Detector: F ex 370 em 430

CHROMATOGRAM
Internal standard: camptothecin

KEY WORDS
plasma; mouse; pharmacokinetics

REFERENCE
Ohdo,S.; Makinosumi,T.; Ishizaki,T.; Yukawa,E.; Higuchi,S.; Nakano,S.; Ogawa,N. Cell cycle-dependent chronotoxicity of irinotecan hydrochloride in mice, *J.Pharmacol.Exp.Ther.*, **1997**, *283*, 1383–1388.

SAMPLE
Matrix: blood
Sample preparation: Determination of lactone form. 1 mL Plasma + 100 μL 25 ng/mL IS in MeOH:10 mM HCl 40:60 + 800 mg solid NaCl, extract with 7.5 mL MeCN:n-butyl chloride 20:80 for 5 min, centrifuge at 4000 g for 2 min. Rotate quickly by hand to break the gels, centrifuge at 4000 g for 5 min. Mix the organic layer with 50 μL DMSO, dry under a gentle stream of nitrogen at 60° to approximately 50 μL. Reconstitute the residue in 100 μL MeOH and 100 μL perchloric acid:water 1:500, vortex for 5 s, inject a 100 μL aliquot. Total determination. Mix 250 μL plasma with 500 μL cold (-20°) MeOH:perchloric acid:water 20:1:20, centrifuge at 24000 g for 5 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100 RP-18
Column: 100 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:buffer 40:60 adjusted to pH 5.5 with HCl (A, lactone form determination) or MeOH:buffer 35:65 adjusted to pH 5.5 with HCl (B, total determination). (Buffer was 100 mM ammonium acetate containing 10 mM tetrabutylammonium sulfate.)
Column temperature: 50
Flow rate: 1
Injection volume: 100
Detector: F ex 355 em 515

CHROMATOGRAM
Retention time: 4.9 (A), 8.3 (B)
Internal standard: camptothecin (6.5, A)
Limit of quantitation: 200 pg/mL (lactone form), 2.0 ng/mL (total)

OTHER SUBSTANCES
Extracted: active metabolite
Noninterfering: acetaminophen, alizapride, codeine, dexamethasone, domperidone, metoclopramide, morphine, ranitidine

KEY WORDS
plasma; pharmacokinetics

REFERENCE
de Bruijn,P.; Verweij,J.; Loos,W.J.; Nooter,K.; Stoter,G.; Sparreboom,A. Determination of irinotecan (CPT-11) and its active metabolite SN-38 in human plasma by reversed-phase high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.B*, **1997**, *698*, 277–285.

SAMPLE
Matrix: blood

Sample preparation: Add 1 mL MeCN:MeOH 50:50 to 500 μL plasma at -20°, vortex for 10 s. Centrifuge at 10000 g at 4° for 3 min, mix 70 μL supernatant with 70 μL mobile phase at 0°, vortex, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 3 C18 (Chrompack, Netherlands)
Column: 150 × 4.6 3.5 μm Zorbax SB C18
Mobile phase: MeCN:100mM pH 6.4 ammonium acetate:triethylamine 15.6:80:0.1 containing 5 mM tetrabutylammonium phosphate
Flow rate: 1.5
Injection volume: 20
Detector: F ex 375 em 460

CHROMATOGRAM
Retention time: 5.7 (carboxylate form), 11.1 (lactone form)
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites (F ex 385 em 525)
Noninterfering: lurtotecan, topotecan

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Herben,V.M.M.; Mazee,D.; van Gortel-van Zomeren,D.M.; Zeedijk,S.; Schellens,J.H.M.; ten Bokkel Huinink,W.W.; Beijnen,J.H. Sensitive determination of the carboxylate and lactone forms of the novel antitumor drug irinotecan and its active metabolite in plasma by HPLC, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1541–1558.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond Elut C18 SPE cartridge with 1 mL MeOH and 1 mL water at 0.5 mL/s. 100 μL Plasma + 50 μL 1 μg/mL camptothecin in 10 mM HCl + 850 μL 10 mM HCl, mix, add to the SPE cartridge at 2.4 mL/min, wash with 1.5 mL water at 6 mL/min, wash with 1 mL 10 mM HCl at 6 mL/min, elute with 1.5 mL acidic MeOH at 2.4 mL/min. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 250 μL mobile phase, inject an aliquot. (Prepare IS solution by sonicating 1 mg camptothecin in 25 mL MeOH at room temperature for 20 min, dilute to 10 μg/mL with water, dilute to 1 μg/mL with 10 mM HCl. Prepare acidic MeOH by mixing 100 μL concentrated HCl with 100 mL MeOH.)

HPLC VARIABLES
Guard column: 22 × 3.5 10 μm Nucleosil C18
Column: 300 × 3.9 10 μm Nucleosil octadecylsilane
Mobile phase: MeCN:100 mM KH_2PO_4 34:66 containing 3 mM sodium heptanesulfonate, adjusted to pH 4 with 1 M HCl
Flow rate: 1
Detector: F ex 380 em 500

CHROMATOGRAM
Retention time: 5.5
Internal standard: camptothecin (9)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE

Barilero,I.; Gandia,D.; Armand,J.-P.; Mathieu-Boué,A.; Ré,M.; Chabot,G.G. Simultaneous determination of the camptothecin analogue CPT-11 and its active metabolite SN-38 by high-performance liquid chromatography: application to plasma pharmacokinetic studies in cancer patients, *J.Chromatogr.*, **1992**, *575*, 275–280.

SAMPLE

Matrix: blood

Sample preparation: Evaporate 50 μL 1 μg/mL camptothecin in acetone into the bottom of a tube under a stream of nitrogen, add 50 μL plasma, add 100 μL ice-cold MeCN:MeOH 50:50, vortex for 5 s, centrifuge at 8000 g briefly. Remove a 100 μL aliquot of the supernatant and add it to 70 μL 75 mM pH 6.4 ammonium acetate buffer, vortex briefly, inject a 5-20 μL aliquot.

HPLC VARIABLES

Guard column: Guard-Pak Nova-Pak C18

Column: 100 × 5 4 μm Nova-Pak Radial-Pak C18

Mobile phase: MeCN:75 mM pH 6.4 ammonium acetate buffer 22:78 containing 5 mM tetrabutylammonium phosphate (PIC A)

Flow rate: 1.5

Injection volume: 5-20

Detector: F ex 355 em 515

CHROMATOGRAM

Retention time: 4.2 (carboxylate form), 8.2 (lactone form)

Internal standard: camptothecin (6.5 (carboxylate form), 10.5 (lactone form))

Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; pharmacokinetics; carboxylate form is the inactive form

REFERENCE

Rivory,L.P.; Robert,J. Reversed-phase high-performance liquid chromatographic method for the simultaneous quantitation of the carboxylate and lactone forms of the camptothecin derivative irinotecan, CPT-11 and its metabolite SN-38 in plasma, *J.Chromatogr.B*, **1994**, *661*, 133–141.

SAMPLE

Matrix: blood

Sample preparation: 200 μL Plasma + 50 μL 1.25 μg/mL camptothecin in MeOH:0.5 M HCl 97.5:2.5 + 750 μL MeOH, vortex for 10 s, centrifuge at 20° at 10000 g for 5 min. Remove the supernatant and evaporate it to dryness under reduced pressure, reconstitute with 400 μL mobile phase adjusted to pH 2.0, vortex for 10 s, centrifuge at 20° at 10000 g for 5 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 15 × 3.2 TSK guardgel ODS-120T

Column: 150 × 4.6 5 μm TSK gel ODS-80Ts

Mobile phase: MeCN:50 mM Na_2HPO_4 28:72 containing 5 mM sodium 1-heptanesulfonate, adjusted to pH 3.0 with orthophosphoric acid (Prepared by dissolving 17.9 g $Na_2HPO_4.12H_2O$ and 1.1 g sodium 1-heptanesulfonate hydrate in 1 L water and adding 388 mL MeCN, adjust pH to 3.0 with orthophosphoric acid.)

Column temperature: 30

Flow rate: 1

Injection volume: 100

Detector: F ex 380 em 556 or ex 370 em 430

CHROMATOGRAM

Retention time: 5.4

Internal standard: camptothecin (8.8)

Limit of quantitation: 30 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Sumiyoshi,H.; Fujiwara,Y.; Ohune,T.; Yamaoka,N.; Tamura,K.; Yamakido,M. High-performance liquid chromatographic determination of irinotecan (CPT-11) and its active metabolite (SN-38) in human plasma, *J.Chromatogr.B*, **1995**, *670*, 309–316.

SAMPLE
Matrix: blood, feces, urine
Sample preparation: Plasma, urine. Thaw frozen plasma or urine sample in a waterbath, vortex, add 250 μL plasma or 250 μL plasma:diluted urine 50:50 to 500 μL MeOH:5% (w/v) aqueous perchloric acid 50:50. Mix for 5 min, centrifuge at 24000 g for 5 min, dilute the upper aqueous layer from plasma and urine extracts 2-fold and 10-fold, respectively, with mobile phase, inject a 100-200 μL aliquot. Feces. Homogenize feces with 5 volumes of 5% (w/v) perchloric acid in water using five 1 min bursts from an Ystral X1020 tissue homogenizer at 20500 r.p.m. Centrifuge at 24000 g for 10 min, dilute with one volume of drug-free human plasma, and process further as described for the urine sample.

HPLC VARIABLES
Guard column: 4 × 4 LiChroCart endcapped RP-18 Merck
Column: 100 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:100 mM ammonium acetate containing 10 mM tetrabutylammonium sulphate 30:70, pH adjusted to 5.3 with HCl
Column temperature: 50
Flow rate: 1.0
Injection volume: 100-200
Detector: F ex 355 em 515

CHROMATOGRAM
Retention time: 16.1
Limit of quantitation: 10 ng/mL (plasma), 100 ng/mL (feces, urine)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
pharmacokinetics; plasma

REFERENCE
Sparreboom,A.; de Bruijn,P.; de Jonge,M.J.A.; Loos,W.J.; Stoter,G.; Verweij,J.; Nooter,K. Liquid chromatographic determination of irinotecan and three major metabolites in human plasma, urine and feces, *J.Chromatogr.B*, **1998**, *712*, 225–235.

SAMPLE
Matrix: blood, tissue
Sample preparation: Condition an Analytichem C18 SPE cartridge with 1.5 mL MeOH and 1.5 mL water. Homogenize (PTFE homogenizer) tissue with four volumes ice-cold 150 mM KCl, centrifuge at 2° at 9000 g for 20 min. Dilute plasma, serum, or tissue homogenate supernatant 10-fold with 100 mM HCl, add to the SPE cartridge, elute the contents of the SPE cartridge on to the column with the mobile phase.

HPLC VARIABLES
Guard column: RP-18
Column: 250 × 4 LiChrosorb RP-18
Mobile phase: MeCN:EtOH:0.8% ammonium carbonate 50:25:25
Column temperature: 50
Flow rate: 1

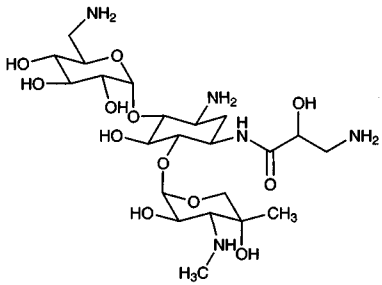
Detector: F ex 373 em 428

CHROMATOGRAM
Retention time: 6.6
Limit of detection: 1 ng

KEY WORDS
mouse; plasma; serum; liver; epithelium; pharmacokinetics; SPE

REFERENCE
Kaneda,N.; Nagata,H.; Furuta,T.; Yokokura,T. Metabolism and pharmacokinetics of the camptothecin analogue CPT-11 in the mouse, *Cancer Res.*, **1990**, *50*, 1715–1720.

Isepamicin



Molecular formula: $C_{22}H_{43}N_5O_{12}$
Molecular weight: 569.61
CAS Registry No.: 58152-03-7, 67814-76-0 (sulfate)
Merck Index: 5121

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL plasma with 0.5-5.0 μg IS and 2 mL EtOH. Add 7 mL dichloromethane and 1 mL water, mix, centrifuge. Inject an aliquot of the aqueous supernatant onto column A, elute to waste with mobile phase A, after 4 min elute the contents of column A onto column B with mobile phase B, elute column B with mobile phase B. Mix the effluent from column B with o-phthalaldehyde at 0.2 mL/min and monitor.

HPLC VARIABLES
Column: A 3.9 × 4.0 10 μm Guard Pak Cyano (Waters); B 150 × 4.6 5 μm Shandon Hypersil C18
Mobile phase: A 17 mM acetic acid containing 10 mM hexanesulfonic acid; B 17 mM acetic acid containing 10 mM hexanesulfonic acid, 100 mM sodium acetate, and 3.53 M MeOH
Detector: F ex 338 em 450 (cut-off filter) following post-column reaction. The column effluent mixed with o-phthalaldehyde pumped at 0.2 mL/min and the mixture flowed to the detector.

CHROMATOGRAM
Retention time: 7.4
Internal standard: dibekacin (9.5)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: aspirin, caffeine, chlorpheniramine, gentamicin, neomycin, netilmicin, sisomicin

KEY WORDS
plasma; pharmacokinetics; comparison with RIA and microbiological assay; post-column reaction; column-switching

REFERENCE
Lin,C.-.; Veals,J.; Korduba,C.; Hilbert,M.J.; Nomeir,A. Analysis of isepamicin in human plasma by radioimmunoassay, microbiologic assay, and high-performance liquid chromatography, *Ther.Drug Monit.*, **1997**, *19*, 675–681.

SAMPLE
Matrix: blood

Sample preparation: Dry blood on gauze, soak gauze in 500 μL 500 mM Na_2HPO_4 at 35° for 30 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 5 μm μBondapak C18
Mobile phase: 16 mM Sodium sulfate containing 5 mM 1-heptanesulfonic acid (PIC B-7)
Flow rate: 0.6
Injection volume: 50
Detector: F ex 360 em 440 following post-column reaction. The column effluent was mixed with reagent pumped at 0.3 mL/min and flowed through a 5 m × 0.25 mm i.d. coil of PTFE tubing to the detector. (Prepare reagent by dissolving 300 mg o-phthalaldehyde in 500 mL MeOH, add 1.25 mL β-mercaptoethanol, add 500 mL 400 mM pH 10.4 potassium borate buffer.)

CHROMATOGRAM
Retention time: 12
Limit of detection: 100 ng/mL

KEY WORDS
post-column reaction; dried blood

REFERENCE
Shoshihara,M.; Kase,K.; Yoshizawa,E.; Takao,M.; Fujimoto,T. Column liquid chromatographic determination of isepamicin in nasal cavity using gauze, *J.Chromatogr.*, **1990**, *529*, 473–478.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Plasma + 20 μL 10 mg/mL gentamicin C1a in water + 50 μL buffer, vortex for 15 s, add 200 μL MeCN, vortex for 20 s, centrifuge at 2000 g for 5 min. Filter (Millex-HV4) the supernatant. Heat 200 μL filtrate and 20 μL 250 mg/mL 1-fluoro-2,4-dinitrobenzene in MeCN at 80° for 1 h, cool, inject a 50 μL aliquot. (Buffer was 3.81 g disodium tetraborate decahydrate in water, adjust pH to 10 with NaOH, make up to 100 mL with water.)

HPLC VARIABLES
Guard column: 25 × 4 10 μm LiChroCART RP 18
Column: 250 × 4 5 μm LiChrosorb RP 18
Mobile phase: MeCN:water 70:30 containing 1 mL/L acetic acid
Flow rate: 2
Injection volume: 50
Detector: UV 365

CHROMATOGRAM
Retention time: 13.0
Internal standard: gentamicin C1a (10.0)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Noninterfering: ampicillin, aspirin, captopril, cefazolin, cefotaxime, ceftazidime, ceftriaxone, cephalosporins, chlorpromazine, diazepam, heparin, propranolol, sulfamethoxazole, sulpiride, trimethoprim, verapamil

KEY WORDS
plasma; guinea pig; human; derivatization

REFERENCE
Dionisotti,S.; Bamonte,F.; Scaglione,F.; Ongini,E. Simple measurement of isepamicin, a new aminoglycoside antibiotic, in guinea pig and human plasma, using high-performance liquid chromatography with ultraviolet detection, *Ther.Drug Monit.*, **1991**, *13*, 73–78.

SAMPLE
Matrix: blood, dialysate, urine

Sample preparation: Plasma. Condition a 3 mL Baker cyanopropylsilane CN SPE cartridge with 2 mL MeOH, 2 mL water, and 2 mL buffer. 1 mL Plasma + 100 μL 100 μg/mL dibekacin in water, vortex for 15 s, add 1 mL buffer, vortex for 15 s, centrifuge at 3100 g at 4° for 7 min, add to SPE cartridge, wash with 500 μL water, wash with 250 μL mobile phase, elute to dryness. Elute with 250 μL mobile phase, inject an aliquot of the eluate. Urine, dialysate. Dilute 1:100 with water, add 100 μL 100 μg/mL dibekacin per 1 mL of sample, mix well, inject a 100 μL aliquot. (Buffer was 0.94 g sodium hexanesulfonate in 300 mL water, add 500 μL glacial acetic acid, dilute to 500 mL with water.)

HPLC VARIABLES

Guard column: 10 × 4.6 5 μm Hypersil C18
Column: 150 × 4.6 5 μm Hypersil C18
Mobile phase: MeOH:buffer 10:90 (Buffer was 3.76 g sodium hexanesulfonate + 28.4 g sodium sulfate in 2 L water, acidify to pH 3.4 with 2 mL glacial acetic acid.)
Column temperature: 25
Flow rate: 1.1
Injection volume: 100
Detector: F ex 338 em 418 (bandpass filter) following post-column reaction. The column effluent mixed with the reagent pumped at 0.4 mL/min and the mixture flowed through a 3 m × 0.05 mm i.d. knitted PTFE reaction coil at 25° to the detector (Derivatizing reagent was 0.4 g o-phthalaldehyde in 3 mL MeOH added to 390 mL buffer, add 2 mL β-mercaptoethanol, make up to 500 mL with water, store at 4°. Buffer was 1 M pH 10.4 borate from equal volumes of 1 M KOH and boric acid.)

CHROMATOGRAM

Retention time: 6.7
Internal standard: dibekacin (17)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES

Simultaneous: kanamycin, gentamicin, tobramycin, netilmicin

KEY WORDS

post-column reaction; SPE; plasma

REFERENCE

Maloney,J.A.; Awni,W.M. High-performance liquid chromatographic determination of isepamicin in plasma, urine and dialysate, *J.Chromatogr.*, **1990**, *526*, 487–496.

SAMPLE

Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + 0.5-5 μg dibekacin + 2 mL EtOH, mix, add 7 mL dichloromethane, add 1 mL water, centrifuge, inject an aliquot of the aqueous supernatant on to column A and elute to waste with mobile phase A, after 4 min elute the contents of column A on to column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 3.9 × 4 10 μm Guard Pak with Cyano insert; B 150 × 4.6 5 μm Hypersil C18
Mobile phase: A 17 mM Acetic acid containing 10 mM hexanesulfonate; B MeOH:buffer 14.3: 85.7 (Mobile phase contained 100 mM sodium acetate, 17 mM acetic acid, 10 mM hexanesulfonate, and 3.53 M MeOH.)
Detector: F ex 338 (band-pass filter) em 418-700 and 450 cut-off filters following post-column reaction with o-phthalaldehyde derivatizing reagent pumped at 0.2 mL/min.

CHROMATOGRAM

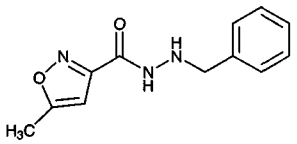
Internal standard: dibekacin
Limit of quantitation: 100 ng/mL

KEY WORDS

plasma; pharmacokinetics; post-column reaction; column-switching

REFERENCE
Lin,C.-C.; Radwanski,E.; Korduba,C.; Cayen,M.; Affrime,M. Pharmacokinetics of intravenously administered isepamicin in men, *Antimicrob.Agents Chemother.*, **1995**, *39*, 2774–2778.

Isocarboxazid



Molecular formula: $C_{12}H_{13}N_3O_2$
Molecular weight: 231.25
CAS Registry No.: 59-63-2
Merck Index: 5172
Lednicer No.: 1 233

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 200 µL 1.5 M NaOH + 10 µL 200 µg/mL IS in 100 mM HCl, vortex, add 5 mL hexane:ethyl acetate 80:20, shake mechanically at low speed for 10 min, centrifuge at 10° at 1100 g for 10 min. Remove the organic layer and add it to 500 µL 2 M HCl, shake mechanically for 5 min, centrifuge at 10° at 1100 g for 5 min. Remove the aqueous phase and add it to 200 µL saturated K_2HPO_4, vortex, inject a 50 µL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 µm LC-18 (Supelco)
Mobile phase: MeOH:5 mM octanesulfonic acid 50:50
Flow rate: 1
Injection volume: 50
Detector: UV 230

CHROMATOGRAM
Retention time: 6.4
Internal standard: 5-methyl-3-isoxazolecarboxylic acid 2-(2-propyl-1-phenyl)hydrazide (Ro 5-1226) (15.5)
Limit of quantitation: 100 ng/mL

KEY WORDS
plasma; dog; pharmacokinetics

REFERENCE
Powell,M.L.; Town,C.; Henderson,L.; Buck,C. Determination of marplan in human plasma using high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *529*, 237–244.

SAMPLE
Matrix: solutions

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

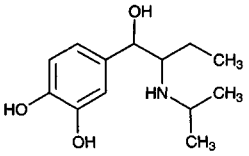
OTHER SUBSTANCES

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

REFERENCE

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

Isoetharine



Molecular formula: $C_{13}H_{21}NO_3$
Molecular weight: 239.31
CAS Registry No.: 530-08-5, 2576-92-3 (HCl), 7279-75-6 (mesylate)
Merck Index: 5185
Lednicer No.: 2 9

SAMPLE

Matrix: blood
Sample preparation: 25-150 μL Plasma + 100 μL 50 ng/mL colterol mesylate in 25 mM sulfuric acid + 2 mL 2% boric acid + 2 g ammonium sulfate + 10 mL 0.5% di(2-ethylhexyl)phosphoric acid in benzene (Caution! Benzene is a carcinogen!), shake, centrifuge. Remove the organic phase and add it to 130 μL 25 mM sulfuric acid, shake, centrifuge, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:buffer 12.5:87.5 (Buffer was 100 mM sodium sulfate adjusted to pH 2.8 phosphoric acid then adjusted to pH 3.0 with NaOH.)
Flow rate: 1.2
Injection volume: 100
Detector: E, Bioanalytical Systems LC-4, TL-5 glassy carbon electrode +0.60 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 9.3
Internal standard: colterol mesylate (7.5)
Limit of quantitation: 0.5 ng/mL

KEY WORDS

plasma; rat; pharmacokinetics

REFERENCE

Park,G.B.; Koss,R.F.; Utter,J.; Mayes,B.A.; Edelson,J. Determination of isoetharine in plasma by reversed-phase chromatography with amperometric detection, *J.Pharm.Sci.*, **1982**, *71*, 932–934.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Chirex 3020 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 55:35:10 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1
Injection volume: 20
Detector: UV 282

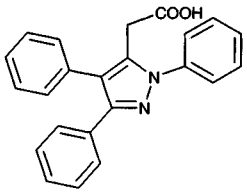
KEY WORDS

chiral; α = 1.21 for enantiomers

REFERENCE

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

Isofezolac



Molecular formula: $C_{23}H_{18}N_2O_2$
Molecular weight: 354.41
CAS Registry No.: 50270-33-2
Merck Index: 5188

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 1 mL 100 mM pH 4.4 citrate buffer + 100 μL 40 μg/mL IS in MeOH + 15 mL diethyl ether, agitate, centrifuge at 3000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 0.5-3 mL mobile phase, inject a 20 μL aliquot. Urine. 100 μL Urine + 1 mL 100 mM pH 4.4 citrate buffer + 100 μL 40 μg/mL IS in MeOH + 100 μL enzyme solution containing 100000 U/mL β-glucuronidase and 1000000 U/mL arylsulfatase (I.B.F.), heat at 37° for 16 h, add 15 mL diethyl ether, agitate, centrifuge at 3000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 1-2 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm LiChrosorb RP 8
Mobile phase: MeCN:water:200 mM pH 3 phosphate buffer 65:15:20
Flow rate: 1.5
Injection volume: 20
Detector: F ex 273 em 335 or UV 265

CHROMATOGRAM

Retention time: 2.7
Internal standard: 1-phenyl-3,4-di-p-chlorophenylpyrazole-5-acetic acid (4.5)
Limit of detection: 10 ng/mL (F)

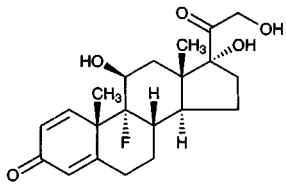
KEY WORDS

plasma; pharmacokinetics

REFERENCE

Bannier,A.; Brazier,J.L. Determination of isofezolac in biological fluids by reversed-phase liquid column chromatography, *J.Chromatogr.*, **1980**, *182*, 369–377.

Isoflupredone



Molecular formula: $C_{21}H_{27}FO_5$
Molecular weight: 378.44
CAS Registry No.: 338-95-4, 338-98-7 (acetate)
Merck Index: 5190

SAMPLE

Matrix: bulk, formulations
Sample preparation: Cream. Weigh out cream containing 1 mg diflorasone diacetate, add 30 mL 40 μg/mL isoflupredone acetate in water-saturated chloroform, shake for 30 min, centrifuge at 2000 rpm for 15 min, inject a 10 μL aliquot of the lower chloroform layer. Ointment. Weigh out ointment containing 0.5 mg diflorasone diacetate, add 15 mL 40 μg/mL isoflupredone acetate in water-saturated chloroform, shake for 30 min, centrifuge at 2000 rpm for 15 min, inject a 10 μL aliquot of the lower chloroform layer. Bulk. Dissolve 1.5 mg bulk drug in 50 mL 40 μg/mL isoflupredone acetate in water-saturated chloroform, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 3 μm silica gel (Perkin-Elmer part 0258-1500)
Mobile phase: Butyl chloride:dichloromethane:THF:acetic acid 70:25:2:3 (Butyl chloride and dichloromethane were saturated with water.)
Flow rate: 2.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 24 (isoflupredone acetate)
Internal standard: isoflupredone acetate

OTHER SUBSTANCES
Simultaneous: related compounds, diflorasone diacetate

KEY WORDS
cream; ointment; normal phase; isoflupredone acetate is IS

REFERENCE
Shaw,M.C.; Vanderwielen,A.J. Liquid chromatographic assay for diflorasone diacetate in cream and ointment formulations, *J.Pharm.Sci.*, **1984**, *73*, 1606–1608.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm SI-100 (Brownlee)
Mobile phase: Butyl chloride:THF:MeOH:glacial acetic acid 88:2.5:2.5:2.5 (Butyl chloride was 50% water saturated.)
Injection volume: 20
Detector: UV 254

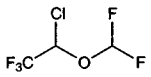
CHROMATOGRAM
Retention time: 16 (isoflupredone), 10 (isoflupredone acetate

OTHER SUBSTANCES
Simultaneous: bromoprednisolone acetate, prednisolone, fluoroprednisone acetate

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.

Isoflurane



Molecular formula: $C_3H_2ClF_5O$
Molecular weight: 184.49
CAS Registry No.: 26675-46-7
Merck Index: 5191

SAMPLE
Matrix: solutions
Sample preparation: Mix 50 μL phosphate buffer containing isoflurane and 50 μL 0.05 mM toluene in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: RCSS Guard-Pak μBondapak C18 precolumn cartridge
Column: 100 × 8 4 μm Nova-Pak C18 Radial Compression Module
Mobile phase: MeOH:water 50:50
Flow rate: 3.5
Injection volume: 20
Detector: UV 203

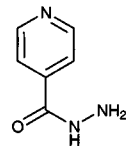
CHROMATOGRAM
Retention time: 5
Internal standard: toluene (12)
Limit of detection: 0.2 mM

OTHER SUBSTANCES
Simultaneous: enflurane, halothane

KEY WORDS
buffer

REFERENCE
Janicki,P.K.; Erskine,W.A.R.; James,M.F.M. High-performance liquid chromatographic method for the direct determination of the volatile anaesthetics halothane, isoflurane and enflurane in water and in physiological buffer solutions, *J.Chromatogr.*, **1990**, *518*, 250–253.

Isoniazid



Molecular formula: $C_6H_7N_3O$
Molecular weight: 137.14
CAS Registry No.: 54-85-3
Merck Index: 5203
Lednicer No.: 1 254

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 100 μL 30μg/mL IS in water, mix, add 2 g ammonium sulfate, add 40 mL water saturated n-butanol:chloroform 30:70, shake for 10 min, centrifuge at 500 g for 10 min. Transfer the organic layer into a tube, add 1 mL 1 M sulfuric acid, shake for 10 min, centrifuge at 500 g for 10 min, inject a 250 μL aliquot of the upper aqueous layer. (Caution! Chloroform is a carcinogen !)

HPLC VARIABLES
Guard column: 10μm μBondapak C18
Column: 115 × 8 5 μm μBondapak C18 radial compression
Mobile phase: EtOH:1 mM dioctyl sulfosuccinate 45:55, adjusted to pH 2.50
Flow rate: 4
Injection volume: 250
Detector: UV 254

CHROMATOGRAM
Retention time: 10.7
Internal standard: 1-benzoyl-2-isonicotinoylhydrazine (8.9)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: acetylisoniazid

KEY WORDS
plasma

REFERENCE
Holdiness,M.R. High pressure liquid chromatographic determination of isoniazid and acetylisoniazid in human plasma, *J.Liq.Chromatogr.*, **1982**, *5*, 707–714.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 50 μL 50 μg/mL phenelzine + 400 μL 10% acetic acid + 7 mL diethyl ether:dichloromethane 2:1, shake, centrifuge at 2059 g for 10 min. Remove the aqueous layer and add it to 600 μL 10% acetic acid, add 300 μL 0.1% salicaldehyde in EtOH, heat at 60° for 30 min, cool to room temperature, add 1 mL 1 M K_2PO_4 (sic), extract with 7 mL diethyl ether, centrifuge at 2059 g for 10 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 buffer, inject a 20 μL aliquot. (Buffer was 5 mM heptanesulfonic acid in MeCN:water: triethylamine 70:30:0.4.)

HPLC VARIABLES
Guard column: 50 × 4.6 30 μm C8
Column: 250 × 4.6 Spherisorb S5 ODS2 C18
Mobile phase: Gradient. MeCN:buffer:water 0:75:25 for 5 min, 15:85:0 for 12 min (step gradient). (Buffer was 5 mM heptanesulfonic acid in MeCN:water:triethylamine 70:30:0.4.)
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 4.68
Internal standard: phenelzine (11.09)
Limit of detection: 250 ng/mL

OTHER SUBSTANCES
Extracted: hydrazine, monoacetylhydrazine

KEY WORDS
derivatization

REFERENCE
Walubo,A.; Smith,P.; Folb,P.I. Comprehensive assay for pyrazinamide, rifampicin and isoniazid with its hydrazine metabolites in human plasma by column liquid chromatography, *J.Chromatogr.B*, **1994**, *658*, 391–396.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Plasma + 150 μL 10% zinc sulfate in water, mix, centrifuge at 1000 g for 1 min. Remove a 250 μL aliquot of the supernatant and add it to 100 μL MeOH, mix, centrifuge, inject a 5 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm LC-CN (Supelco)
Mobile phase: Isopropanol:water 5:95 containing 5 g/L ammonium formate
Flow rate: 1
Injection volume: 5
Detector: E, ESA Model 5100A, Model 5011 analytical cell with first detector +0.6 V and second (monitored) detector +0.8 V, Model 5020 guard cell +1.0 V between pump and injector

CHROMATOGRAM
Retention time: 4.4
Limit of detection: 100 ng/mL

KEY WORDS
rat; plasma

REFERENCE
Hansen,E.B.,Jr.; Dooley,K.L.; Thompson,H.C.,Jr. High-performance liquid chromatographic analysis of the antituberculosis drugs aconiazide and isoniazid, *J.Chromatogr.B*, **1995**, *670*, 259–266.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Plasma + 50 μL 1.5% p-hydroxybenzaldehyde in MeOH + 40 μL 20% trichloroacetic acid in water, vortex thoroughly, centrifuge at 8000 g for 10 min, let stand on ice for 30 min, centrifuge at 8000 g for 10 min, inject a 30 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 20 × 2 30-40 μm Perisorb RP18
Column: 100 × 8 4 μm Radial-Pak Novapak C18
Mobile phase: MeOH:water:20% tetraethylammonium hydroxide:70% perchloric acid 24:76:0.05:0.05, apparent pH 2.3
Flow rate: 2
Injection volume: 30
Detector: UV 350

CHROMATOGRAM
Retention time: 4.4
Limit of detection: <500 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Schall,R.; Müller,F.O.; Duursema,L.; Groenewoud,G.; Hundt,H.K.L.; Middle,M.V.; Mogilnicka,E.M.; Swart,K.J. Relative bioavailability of rifampicin, isoniazid and ethambutol from a combination tablet vs. concomitant administration of a capsule containing rifampicin and a tablet containing isoniazid and ethambutol, *Arzneimittelforschung*, **1995**, *45*, 1236–1239.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 100 μL 30% trichloroacetic acid, mix, centrifuge at 2000 g for 5 min. Remove a 100 μL aliquot of the supernatant and add it to 20 μL 0.1% trans-cinnamaldehyde in MeOH, let stand for 10 min, add 20 μL 1 M KOH, inject an aliquot.

HPLC VARIABLES
Column: 125 × 3.9 4 μm Nova-pak C18
Mobile phase: MeCN:water:triethylamine:acetic acid 40:60:0.2:0.1, pH 5 ± 1
Flow rate: 1.3
Detector: UV 340

CHROMATOGRAM
Retention time: 1.95
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Noninterfering: acepromethazine, adrafinil, allopurinol, alprostadil, altretamine, atenolol (tenormine), baclofen, bendroflumethiazide, benserazide, betamethasone, bisoprolol, bromocriptine, caffeine, captopril, chlorpromazine, clomipramine, clonazepam, cortisone, cyamemazine, difebarbamate, dothiepin (dosulepin), ethosuximide, fenspiride, flumazenil, fluoxetine, fluvoxamine, halofantrine, hydrochlorothiazide, hydroxyzine, ibuprofen, imipramine, levamisol, levodopa, maprotiline, medifoxamine, metopimazine, midazolam, nafronyl (naftidrofuryl), naftazone, naproxen, nicergoline, nitrazepam, nordazepam, nortriptyline, penfluridol, phenobarbital, pimozide, pipamperone, pipotizine, primidone, pyrazinamide, pyridoxine, quinine, rifampin, selegiline (deprenyl), streptomycin, tetrazepam, theophylline, thioproperazide, tiapride, triazolam, trihexyphenidyl, trimeprazine (alimemazine), trimipramine, tropatepine, vigabatrin, zopiclone

KEY WORDS
serum; derivatization

REFERENCE
Sadeg,N.; Pertat,N.; Dutertre,H.; Dumontet,M. Rapid, specific and sensitive method for isoniazid determination in serum, *J.Chromatogr.B*, **1996**, *675*, 113–117.

SAMPLE
Matrix: blood, CSF
Sample preparation: 200-500 μL Plasma or CSF + 50 μL 50 μg/mL phenelzine sulfate + 100 μL 10% (?) aqueous acetic acid + 5 mL n-hexane, shake for 30 min, centrifuge at 1870 g for 10 min. Discard the organic layer. Add 300 μL 0.1% salicaldehyde in EtOH and 400 μL 10% aqueous acetic acid to the aqueous layer, heat at 60° for 30 min, cool, add 1 mL 1 M pH 6.5 K_2HPO_4, shake for 10 s, add 5 mL diethyl ether, shake for 10 min, centrifuge 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 50 μL mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 30 μm C8 (Waters)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water:triethylamine 70:30:0.4 containing 5 mM heptanesulfonic acid, pH adjusted to 6.0 with acetic acid
Flow rate: 1
Injection volume: 25
Detector: UV 320

CHROMATOGRAM
Retention time: 1.6
Internal standard: phenelzine sulfate (3)
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Extracted: hydrazine
Noninterfering: p-aminosalicylic acid, pyrazinamide, rifampin

KEY WORDS
plasma; rabbit; derivatization

REFERENCE
Walubo,A.; Chan,K.; Wong,C.L. Simultaneous assay for isoniazid and hydrazine metabolite in plasma and cerebrospinal fluid in the rabbit, *J.Chromatogr.*, **1991**, *567*, 261–266.

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

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

CHROMATOGRAM
Retention time: 3.10

Internal standard: heptanophenone (19.2)
Limit of quantitation: 2500 ng/mL

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

KEY WORDS
plasma; serum; column-switching

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

SAMPLE
Matrix: blood, CSF, urine
Sample preparation: Centrifuge 1.5 mL whole blood, CSF, or urine at 3000 g for 3 min, add 500 μL of the supernatant to 500 μL 10% trichloroacetic acid, centrifuge at 10000 g for 1 min. Remove a 200 μL aliquot and add it to 20 μL water, add 40 μL 1% trans-cinnamaldehyde in MeOH, let stand at room temperature for 10 min, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Partisil 5 C8
Mobile phase: Gradient. A was 50 mM KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 60:40 for 1 min, to 30:70 over 9 min, maintain at 30:70 for 4.5 min, re-equilibrate at initial conditions for 4 min.
Column temperature: 50
Flow rate: 1
Injection volume: 20
Detector: UV 340

CHROMATOGRAM
Retention time: 7
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: hydrazine

KEY WORDS
whole blood; derivatization

REFERENCE
Seifart,H.I.; Gent,W.L.; Parkin,D.P.; van Jaarsveld,P.P.; Donald,P.R. High-performance liquid chromatographic determination of isoniazid, acetylisoniazid and hydrazine in biological fluids, *J.Chromatogr.B*, **1995**, *674*, 269–275.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a 3 mL CN-bonded maxi-clean cartridge (Alltech) with 5 mL MeOH and 2 mL 1% aqueous acetic acid. 2 mL Plasma or urine + 1 mL isopropanol:chloroform 50:50 + 1 μg rifampin, shake for 30 s, add to the SPE cartridge with rinses, collect the eluate, inject an aliquot of the eluate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Excalibar C18-CN (Alltech)
Mobile phase: MeOH:5 mM tetra-n-butylammonium hydroxide 80:20 adjusted to pH 3.0 with phosphoric acid
Flow rate: 1.5

Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 2.7
Internal standard: rifampin (4.3)
Limit of detection: 250 ng/mL (urine), 200 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: pyrazinamide

KEY WORDS
plasma; SPE

REFERENCE
Gaitonde,C.D.; Pathak,P.V. Rapid liquid chromatographic method for the estimation of isoniazid and pyrazinamide in plasma and urine, *J.Chromatogr.*, **1990**, *532*, 418–423.

SAMPLE
Matrix: milk
Sample preparation: Condition a 3 mL 500 mg C18 SPE cartridge (J.T. Baker) with 6 mL MeOH and 6 mL water. 80 mL milk + 20 mL 20% trichloroacetic acid in water, mix, let stand at room temperature for 5 min, centrifuge at 5500 g for 15 min. Filter (0.45 μm) 75 mL of the liquid, add 1 mL 1% cinnamaldehyde in MeOH to the filtrate, mix for a few s, let stand at room temperature for 15 min, add to the SPE cartridge at 5 mL/min, dry for 10 min, wash with 3 mL mobile phase at 0.2 mL/min, wash with 5 mL n-hexane at 0.3 mL/min, dry for 10 min, elute with 6 mL MeOH. Evaporate the eluate under reduced pressure at 35-40°, reconstitute the residue with 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100-CN
Column: 250 × 4 5 μm LiChrospher 100-CN
Mobile phase: MeOH:water 40:60 containing 0.41 g/L sodium acetate trihydrate and 10 mL/L glacial acetic acid
Flow rate: 1
Injection volume: 100
Detector: UV 330

CHROMATOGRAM
Retention time: 7.4
Limit of detection: 0.1 ng/mL

KEY WORDS
cow; SPE; derivatization

REFERENCE
Defilippi,A.; Piancone,G.; Costa Laia,R.; Balla,S.; Tibaldi,G.P. High-performance liquid chromatography with UV detection and diode-array UV confirmation of isonicotinic acid hydrazide in cattle milk, *J.Chromatogr.B*, **1994**, *656*, 466–471.

SAMPLE
Matrix: milk
Sample preparation: Condition a 3 mL 500 mg 40 μm phenyl SPE cartridge (J.T. Baker) with 6 mL MeOH and 6 mL water. 80 mL Milk + 20 mL 20% trichloroacetic acid in water, let stand at room temperature for 5 min, centrifuge at 5500 g for 15 min, filter (0.45 μm cellulose acetate) a 75 mL aliquot. Add the filtrate to 1 mL 1% cinnamaldehyde in MeOH, mix for a few s, let stand at room temperature for 15 min. Add 70 mL of the sample to the SPE cartridge at 3 mL/min, wash with 3 mL MeOH:water 40:60 at 3 mL/min, dry under vacuum for 1 min, wash with 3 mL MeOH:water 46:54 at 3 mL/min, dry under vacuum for 1 min, wash with 3 mL n-hexane at 3 mL/min, dry under vacuum for 1 min, elute with 6 mL MeOH. Evaporate the eluate under vacuum, reconstitute in 233 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrosphere 100 RP18
Column: 250 × 4 5 μm LiChrosphere 100 RP18
Mobile phase: MeCN:MeOH:buffer 41:13:46 (Buffer was 1% ammonium acetate adjusted to pH 5.5 with acetic acid.)
Flow rate: 1
Injection volume: 100
Detector: UV 330

CHROMATOGRAM
Retention time: 4.02
Limit of detection: 0.05 ng/mL

KEY WORDS
cow; SPE; derivatization

REFERENCE
Defilippi,A.; Piancone,G.; Costa Laia,R.; Tibaldi,G.P. An HPLC screening method for the detection of isonicotinic acid hydrazide in cattle milk, *Chromatographia*, **1995**, *40*, 170–174.

SAMPLE
Matrix: reaction mixtures
Sample preparation: Centrifuge, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Microsorb C8
Mobile phase: MeOH:0.4 g/L $(NH_4)H_2PO_4$ + 0.1% triethylamine (pH 10.0) 10:90
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 5.3
Limit of detection: 10000 ng/mL

REFERENCE
Lunn,G.; Sansone,E.B. Reductive destruction of dacarbazine, procarbazine hydrochloride, isoniazid, and iproniazid, *Am.J.Hosp.Pharm.*, **1987**, *44*, 2519–2524.

SAMPLE
Matrix: solution
Sample preparation: Mix 1 mL pH 7.4 phosphate buffer or hepatocyte solution with 3 mL ethyl acetate:n-butanol 2:1, 300 μL 500 mM tetra-n-butylammonium hydroxide, and 100 μL 80 μg/mL IS in water. Shake the mixture for 20 min, centrifuge at 850 g for 15 min, remove 2.5 mL of the upper organic layer, mix with 1 mL 0.2% hydrobromic acid, shake for 20 min, centrifuge at 850 g for 15 min, discard the upper organic layer, inject a 5 μL aliquot of the aqueous solution.

HPLC VARIABLES
Column: 250 × 4.6 5 μm TSK-gel ODS-80Ts
Mobile phase: MeOH:67 mM KH_2PO_4 4:96
Column temperature: 37
Flow rate: 0.8
Injection volume: 5
Detector: UV 265

CHROMATOGRAM
Retention time: 11.42
Internal standard: 6-methylnicotinic acid (8)
Limit of quantitation: 2 μg/mL

OTHER SUBSTANCES
Simultaneous: acetylisoniazid, isonicotinic acid

KEY WORDS
rat; hepatocytes

REFERENCE
Ono,Y.; Noda,A.; Zaima,Y.; Jitsufuchi,N.; Eto,S.; Noda,H. Determination of isonicotinic acid in the presence of isoniazid and acetylisoniazid. Studies on isonicotinic acid formation from isoniazid in isolated rat hepatocytes, *J.Chromatogr.B*, **1996**, *677*, 339–343.

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: 300 × 3.9 5 μm μBondapak C18
Mobile phase: MeOH:50 mM pH 6.0 KH_2PO_4 3:97
Flow rate: 3
Detector: UV 254

CHROMATOGRAM
Retention time: 3.5

REFERENCE
Koga,H. High-performance liquid chromatography measurement of antimicrobial concentrations in polymorphonuclear leukocytes, *Antimicrob.Agents Chemother.*, **1987**, *31*, 1904–1908.

SAMPLE
Matrix: solutions

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

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, imino-

stilbene, imipramine, indomethacin, isocarbostyril, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 12 μm Dynamax C18 (Rainin)
Mobile phase: pH 7.5 sodium phosphate buffer
Flow rate: 2
Detector: UV 266

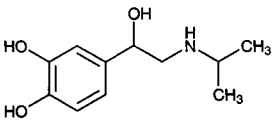
CHROMATOGRAM
Retention time: 5.4

OTHER SUBSTANCES
Simultaneous: metabolites, acetylisoniazid

REFERENCE

Hickman,D.; Palamanda,J.R.; Unadkat,J.D.; Sim,E. Enzyme kinetic properties of human recombinant arylamine N-acetyltransferase 2 allotypic variants expressed in *Escherichia coli*, *Biochem.Pharmacol.*, **1995**, *50*, 697–703.

Isoproterenol



Molecular formula: $C_{11}H_{17}NO_3$
Molecular weight: 211.26
CAS Registry No.: 7683-59-2, 51-30-9 (HCl), 6700-39-6 (sulfate dihydrate), 299-95-6 (sulfate)
Merck Index: 5236

SAMPLE
Matrix: blood

stilbene, imipramine, indomethacin, isocarbostyril, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 12 μm Dynamax C18 (Rainin)
Mobile phase: pH 7.5 sodium phosphate buffer
Flow rate: 2
Detector: UV 266

CHROMATOGRAM

Retention time: 5.4

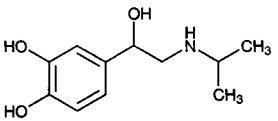
OTHER SUBSTANCES

Simultaneous: metabolites, acetylisoniazid

REFERENCE

Hickman,D.; Palamanda,J.R.; Unadkat,J.D.; Sim,E. Enzyme kinetic properties of human recombinant arylamine N-acetyltransferase 2 allotypic variants expressed in *Escherichia coli*, *Biochem.Pharmacol.*, **1995**, *50*, 697–703.

Isoproterenol



Molecular formula: $C_{11}H_{17}NO_3$
Molecular weight: 211.26
CAS Registry No.: 7683-59-2, 51-30-9 (HCl), 6700-39-6 (sulfate dihydrate), 299-95-6 (sulfate)
Merck Index: 5236

SAMPLE

Matrix: blood

Sample preparation: Plasma. Prepare a SPE column by adding 500 μL of a 20% suspension of 19-40 μm Toyopak SP (strong cation-exchange sulfopropyl resin, Na^+ (Toyo Soda)) in water to a 35 × 6 column, wash with two 1 mL portions of 2 M LiOH, wash with two 5 mL portions of water, wash with two 1 mL portions of EtOH:12 M HCl 90:10, wash with two 5 mL portions of water, wash with three 1 mL portions of buffer. 500 μL Plasma + 500 μL buffer, mix, add to the SPE column, wash with two 5 mL portions of water, wash with 1 mL MeCN:water 50:50, elute with 300 μL 600 μM potassium ferricyanide in 600 mM KCl:MeCN 50:50, add 50 μL reagent to the eluate, heat at 37° for 40 min, cool in ice-water, inject a 100 μL aliquot. Urine. 10 μL Urine + 1 mL MeCN:500 mM KCl 60:40 + 10 μL 75 mM potassium hexacyanoferrate(III) + 100 μL reagent, heat at 37° for 40 min, inject a 100 μL aliquot (J. Chromatogr. 1986, 380, 229). (Prepare buffer by mixing 8 volumes 250 mM LiOH in 200 mM phosphoric acid with 1 volume 200 mM phosphoric acid, pH 5.8. Prepare reagent by dissolving 212 mg 1,2-diphenylethylenediamine in 10 mL 100 mM HCl, pH 6.7.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm TSK-gel ODS-120T (Toyo Soda)
Mobile phase: MeCN:MeOH:50 mM pH 7.0 Tris-HCl buffer 50:10:40 (Wash with MeCN:MeOH:water 50:10:40 for 15 min at the end of each day.)
Flow rate: 1
Injection volume: 100
Detector: F ex 345 em 485 (plasma), F ex 350 em 480 (urine)

CHROMATOGRAM

Retention time: 8
Internal standard: isoproterenol

OTHER SUBSTANCES

Extracted: dopamine, epinephrine, norepinephrine

KEY WORDS

derivatization; plasma; SPE; isoproterenol is IS

REFERENCE

Mitsui,A.; Nohta,H.; Ohkura,Y. High-performance liquid chromatography of plasma catecholamines using 1,2-diphenylethylenediamine as precolumn fluorescence derivatization reagent, *J.Chromatogr.*, **1985**, *344*, 61–70.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 250 μL 1 ng/mL α-methylnorepinephrine + 1 mL buffer + 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol, shake for 2 min, centrifuge at 20° at 1000 g for 5 min, freeze in acetone/dry ice. Remove the organic phase and add it to 2 mL 1-octanol and 200 μL 80 mM acetic acid, shake, centrifuge at 20° at 1000 g for 5 min, freeze in acetone/dry ice. Discard the organic phase, thaw the aqueous phase and add it to 1 mL 10 mM HCl, 1 mL buffer, and 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol, shake for 2 min, centrifuge at 20° at 1000 g for 5 min, freeze in acetone/dry ice. Remove the organic phase and add it to 2 mL 2 M pH 8.6 ammonia/ammonium chloride buffer containing 13.4 mM EDTA, shake, freeze in dry ice/acetone. Remove the organic layer and add it to 2 mL 1-octanol and 150 μL 80 mM acetic acid, shake, centrifuge at 20° at 1000 g for 5 min, freeze in dry ice/acetone, discard the organic layer. Thaw the aqueous layer and add it to 250 μL MeCN, 50 μL 1.75 M pH 7.05 bicine, and 100 μL 100 mM 1,2-diphenylethylenediamine in 100 mM HCl, add 20 μL 20 mM potassium ferricyanide in water, heat at 37° in the dark for 1 h, keep at 20° in the dark, inject a 100 μL aliquot. (Buffer was 2 M pH 8.6 ammonia/ammonium chloride buffer containing 8.9 mM diphenylborate-ethanolamine complex and 13.4 mM EDTA. Stir buffer with 45 g/L activated alumina for 2 h before use. Wash 1-octanol with 80 mM acetic acid. Recrystallize 1,2-diphenylethylenediamine from toluene:light petroleum (bp 60-80°) 10:90, dry overnight at 60°.)

HPLC VARIABLES

Column: 100 × 4.6 3 μm Cp MicroSpher C18 (Chrompack)
Mobile phase: MeCN:MeOH:50 mM pH 7.0 sodium acetate buffer 40:8:50
Flow rate: 1
Injection volume: 100

Detector: F ex 350 em 480

CHROMATOGRAM
Retention time: 8
Internal standard: α-methylnorepinephrine (3)

OTHER SUBSTANCES
Extracted: dihydroxybenzylamine, dopamine, epinephrine, norepinephrine

KEY WORDS
plasma; derivatization; comparison with electrochemical detection

REFERENCE
van der Hoorn,F.A.J.; Boomsma,F.; Man in 't Veld,A.J.; Schalekamp,M.A.D.H. Determination of catecholamines in human plasma by high-performance liquid chromatography: comparison between a new method with fluorescence detection and an established method with electrochemical detection, *J.Chromatogr.*, **1989**, *487*, 17–28.

SAMPLE
Matrix: blood
Sample preparation: Plasma. 1 mL Plasma + 1 mL buffer + 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol, shake for 2 min, centrifuge at 1000 g for 5 min, freeze in dry ice/acetone. Remove the organic phase and add it to 2 mL 1-octanol (saturated with 80 mM acetic acid) and 200 μL 80 mM acetic acid, shake, centrifuge at 1000 g for 5 min. Freeze the aqueous layer and remove the organic layer. Add 1 mL 10 mM HCl, 1 mL buffer, and 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol to the aqueous phase. Shake, centrifuge, freeze, remove the organic layer and add it to 2 mL 2 M pH 8.6 ammonia-ammonium chloride buffer containing 13.4 mM EDTA (but no complex). Freeze, remove the organic layer and add it to 2 mL 1-octanol and 150 μL 80 mM acetic acid, shake, centrifuge at 1000 g for 5 min. Freeze, remove the organic layer and add the aqueous layer to 200 μL MeCN, 50 μL 1.75 M pH 6.95 bicine buffer containing 1% EDTA, 100 μL 100 mM 1,2-diphenylethylenediamine in 100 mM HCl, and 20 μL 20 mM potassium ferricyanide in water. Heat at 37° in the dark for 1 h, inject a 50 μL aliquot (keep it in the dark in the autosampler). Urine. 100 μL Urine + 1 mL 10 mM HCl + 1 mL buffer + 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol, shake for 2 min, centrifuge at 1000 g for 5 min, freeze in dry ice/acetone. Remove the organic phase and add it to 2 mL 1-octanol (saturated with 80 mM acetic acid) and 200 μL 80 mM acetic acid, shake, centrifuge at 1000 g for 5 min. Freeze the aqueous layer and remove the organic layer. Add 1 mL 10 mM HCl, 1 mL buffer, and 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol to the aqueous phase. Shake, centrifuge, freeze, remove the organic layer and add it to 2 mL 1-octanol and 150 μL 80 mM acetic acid, shake, centrifuge at 1000 g for 5 min. Freeze, remove the organic layer and add the aqueous layer to 200 μL MeCN, 50 μL 1.75 M pH 6.95 bicine buffer containing 1% EDTA, 100 μL 100 mM 1,2-diphenylethylenediamine in 100 mM HCl, and 20 μL 20 mM potassium ferricyanide in water. Heat at 37° in the dark for 1 h, inject a 20 μL aliquot (keep it in the dark in the autosampler). (Buffer was a 2 M pH 8.6 ammonia-ammonium chloride buffer containing 8.9 mM diphenyl borate-ethanolamine complex and 13.4 mM EDTA.)

HPLC VARIABLES
Column: 100 × 4.6 3 μm Spherisorb ODS2
Mobile phase: Gradient. A was MeCN:MeOH:50 mM pH 7.0 sodium acetate buffer 20:20:60. B was MeCN:MeOH:50 mM pH 7.0 sodium acetate buffer 60:10:30. A:B 52:48 for 6 min, go to 0:100 over 0.1 min, stay at 0:100 for another 10 min. Equilibrate at initial conditions for 4 min before next sample.
Flow rate: 1
Injection volume: 20-50
Detector: F ex 350 em 480

CHROMATOGRAM
Retention time: 9
Internal standard: isoproterenol

OTHER SUBSTANCES
Simultaneous: dobutamine, epinephrine, dopamine, epinine, norepinephrine, metabolites
Interfering: α-methyldopa

KEY WORDS
plasma; isoproterenol is IS; derivatization

REFERENCE
Alberts,G.; Boomsma,F.; Man in 't Veld,A.J.; Schalekamp,M.A.D.H. Simultaneous determination of catecholamines and dobutamine in human plasma and urine by high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.*, **1992**, *583*, 236–240.

SAMPLE
Matrix: blood
Sample preparation: Mix plasma and N-methyldopamine, add to a TOYOPAK SP strong cationic exchange SPE cartridge (Tosoh), elute with MeCN:600 mM KCl 50:50 containing 0.6 mM potassium hexacyanoferrate (III), derivatize eluate with 1,2-diphenylethylenediamine, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Nucleosil 5C18
Mobile phase: MeOH:50 mM Tris-HCl buffer 80:20, adjusted to pH 7.0
Flow rate: 1
Detector: F

KEY WORDS
plasma; guinea pig; SPE; derivatization; pharmacokinetics

REFERENCE
Ohtani,H.; Yamamoto,K.; Sawada,Y.; Iga,T. Antibronchospasmic, tachycardiac, and hypokalaemic effects of L-isoproterenol in guinea-pigs, *Biopharm.Drug Dispos.*, **1995**, *16*, 745–753.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Toyopak IC-SP S sulfopropyl resin, H^+ form, SPE cartridge (Tosoh) with 10 mL water and 2 mL 200 mM pH 5.0 sodium phosphate buffer. Plasma. 700 μL Plasma + 50 μL 7 μM 3,4-dihydroxyphenylpropanoic acid + 350 μL 2 M perchloric acid, mix, centrifuge at 4° at 1000 g for 15 min. Remove a 700 μL aliquot of the supernatant and adjust the pH to 1.5-2.0 with about 150 μL 2 M potassium carbonate, centrifuge at 4° at 1000 g for 5 min, add the supernatant to the SPE cartridge, wash with 10 mL water, elute with 300 μL MeOH:2 M sodium perchlorate 7:93, filter (cellulose acetate membrane), inject a 100 μL aliquot of the filtrate. Urine. Collect human urine for 24 h in the presence of 10 mL 6 M HCl. 500 μL Urine + 25 μL 800 μM 3,4-dihydroxyphenylpropanoic acid + 500 μL 1 M perchloric acid, mix, centrifuge at 4° at 1000 g for 15 min. Remove a 700 μL aliquot of the supernatant and adjust the pH to 1.5-2.0 with about 130 μL 2 M potassium carbonate, centrifuge at 4° at 1000 g for 5 min, add the supernatant to the SPE cartridge, wash with 1.5 mL water, wash with 500 μL EtOH:water 50:50, wash with 5 mL water, elute with 500 μL 1.5 M KCl in MeOH:100 mM HCl 7:93, filter (cellulose acetate membrane), inject a 100 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm TSK-gel ODS-80TM (Tosoh)
Mobile phase: MeOH:buffer 7:93 (Buffer was 30 mM pH 2.5 citrate buffer containing 0.4 mM sodium octanesulfonate.)
Flow rate: 0.8
Injection volume: 100
Detector: F ex 350 em 480 following post-column reaction. The column effluent mixed with reagent A pumped at 0.3 mL/min and the mixture flowed through a 3 m × 0.5 mm ID stainless steel coil at 90°. The effluent from this coil mixed with reagent B pumped at 0.3 mL/min and the mixture flowed through a 10 m × 0.5 mm ID stainless steel coil at 90° and through a 1 m × 0.5 mm ID stainless steel cooling coil to the detector (Anal. Sci. 1991, 7, 257). (Reagent A was 10 mM sodium periodate containing 3 mM potassium ferricyanide. Reagent B was 30 mM

meso-1,2-diphenylethylenediamine in EtOH:water 70:30 containing 130 mM sodium methylate.)

CHROMATOGRAM
Retention time: 60
Internal standard: isoproterenol

OTHER SUBSTANCES
Extracted: dopamine, epinephrine, levodopa, metanephrine, 3-methoxytyramine, norepinephrine, normetanephrine

KEY WORDS
post-column reaction; plasma; SPE; isoproterenol is IS

REFERENCE
Jeon,H.-K.; Nohta,H.; Ohkura,Y. High-performance liquid chromatographic determination of catecholamines and their precursor and metabolites in human urine and plasma by postcolumn derivatization involving chemical oxidation followed by fluorescence reaction, *Anal.Biochem.*, **1992**, *200*, 332–338.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Dissolve powdered tablets in 10 mM HCl, filter if necessary, inject an aliquot. Injections, solutions. Dilute with 10 mM HCl, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Partisil-5 ODS-3
Mobile phase: MeOH:buffer 30:70 (Buffer was 10 mM sodium 1-octanesulfonate in 0.2% acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 16.5
Limit of detection: 53 ng

OTHER SUBSTANCES
Simultaneous: norepinephrine, epinephrine, levonordefrin, phenylephrine, metaraminol, impurities

KEY WORDS
tablets; injections; ophthalmic solutions; inhalation solutions

REFERENCE
Smela,M.J.,Jr.; Stromberg,R. Liquid chromatographic determination of six sympathomimetic drugs in dosage forms, *J.Assoc.Off.Anal.Chem.*, **1991**, *74*, 289–291.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Grind tablets, weigh out a portion, dissolve in 50 mL mobile phase, sonicate, filter (No. 4 sintered glass plate), dilute, inject an aliquot. Capsules. Dissolve 10 capsules (without opening) in 100 mL mobile phase, sonicate, inject an aliquot. Injections, ampules, sprays. Dilute, inject an aliquot.

HPLC VARIABLES
Column: 120 × 4.6 Spherisorb C18 ODS-2
Mobile phase: Isopropanol:buffer 5:95 (Buffer was 100 mM sodium dodecyl sulfate containing 25 mM Na_2HPO_4, pH adjusted to 3.0 with HCl.)
Flow rate: 1
Injection volume: 20

Detector: UV 280

CHROMATOGRAM
Retention time: 6
Limit of detection: 16 ng/mL

OTHER SUBSTANCES
Simultaneous: carbidopa, dopamine, epinephrine, hydrochlorothiazide, 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: perfusate
Sample preparation: 30 μL Perfusate (artificial CSF) + 10 μL 200 mM perchloric acid. Mix a 25 μL aliquot with 12.5 μL reagent, let stand for 2 min, inject an aliquot. (Prepare a stock solution by dissolving 27 mg o-phthalaldehyde in 1 mL MeOH, add 5 μL β-mercaptoethanol, add 9 mL 100 mM pH 9.3 sodium tetraborate containing 10 μM EDTA. This solution is good for 5 days in a sealed amber bottle at room temperature. Prepare the working reagent by diluting 1 mL of the stock solution with 3 mL 100 mM pH 9.3 sodium tetraborate containing 10 μM EDTA, allow to stand for 24 h before use.)

HPLC VARIABLES
Column: two columns 150 × 4.6 5 μm M.S. Gel C18 (ESA)
Mobile phase: MeOH:buffer 8:92 adjusted to pH 3.0 with phosphoric acid (Buffer was 54 mM NaH_2PO_4 containing 1.24 mM sodium heptanesulfonate.)
Column temperature: 33
Flow rate: 1.2
Detector: E, ESA Coulochem Electrode Array System Model 5500, detector temp 33°, oxidation potential 70 mV

CHROMATOGRAM
Retention time: 8.90
Limit of quantitation: 0.5 ng/mL

OTHER SUBSTANCES
Extracted: apomorphine, dopamine, hydralazine, methoxamine, morphine, norepinephrine, phenylephrine

KEY WORDS
rat; derivatization

REFERENCE
Acworth,I.N.; Yu,J.; Ryan,E.; Gariepy,K.C.; Gamache,P.; Hull,K.; Maher,T. Simultaneous measurement of monoamine, amino acid, and drug levels, using high performance liquid chromatography and coulometric array technology: application to in vivo microdialysis perfusate analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 685–705.

SAMPLE
Matrix: solutions
Sample preparation: Dilute with 5% dextrose, inject a 20 μL aliquot.

HPLC VARIABLES
Column: Waters microparticulate C18
Mobile phase: MeOH:350 mM acetic acid and 5 mM sodium heptanesulfonate 35:65

Flow rate: 1.6-2.0
Injection volume: 20
Detector: F ex 285 em 315

CHROMATOGRAM
Retention time: 4.33

OTHER SUBSTANCES
Simultaneous: theophylline, terbutaline
Interfering: methyldopate

REFERENCE
Williams,D.A.; Fung,E.Y.Y.; Newton,D.W. Ion-pair high-performance liquid chromatography of terbutaline and catecholamines with aminophylline in intravenous solutions, *J.Pharm.Sci.*, **1982**, *71*, 956–958.

SAMPLE
Matrix: solutions
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: Acetic acid:triethylamine:water 1.5:0.5:98
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 0.71

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

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 cellulose tris(3,5-dimethylphenylcarbamate)
Mobile phase: Hexane:isopropanol:trichloroacetic acid 80:15:5
Flow rate: 0.5
Detector: UV

CHROMATOGRAM
Retention time: k' 1.12 (of first (+) enantiomer)

KEY WORDS
chiral; α 1.28

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: 1 mL Saline solution + 100 μL ice-cold 5 M acetic acid + 10 μL 270 mM disodium EDTA, stir, inject an aliquot.

HPLC VARIABLES
Guard column: Guard-Pak CN (Waters)

Column: 150 × 3.9 5 μm Nova-Pak C18
Mobile phase: MeCN:buffer 9:91 adjusted to pH 3.6 with 8.7 M phosphoric acid (Buffer was 70 mM Na_2HPO_4 containing 5 mM sodium heptanesulfonate and 0.1 mM disodium EDTA.) (Recirculate mobile phase.)
Flow rate: 1
Detector: E, Waters Model 410, glassy carbon working electrode + 0.825 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 4.2
Limit of detection: 0.1 pmole

OTHER SUBSTANCES
Simultaneous: 3-O-methylisoprenaline

KEY WORDS
saline

REFERENCE
Bryan,L.J.; O'Donnell,S.R. Analysis of the O-methylated metabolites of isoprenaline, adrenaline and noradrenaline in physiological salt solutions by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1989**, *487*, 29–39.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Partisil ODS-3
Mobile phase: MeOH:buffer 30:70 (Buffer was 10 mM octanesulfonic acid in 0.2% acetic acid.)
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 16.5

OTHER SUBSTANCES
Simultaneous: epinephrine, levonordefrin, metaraminol, phenylephrine

REFERENCE
Phenomenex Catalog, **1994**, p. 1.077.

SAMPLE
Matrix: solutions

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

OTHER SUBSTANCES

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

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Chirex 3020 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 55:35:10 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1
Injection volume: 20
Detector: UV 282

KEY WORDS

chiral; α = 1.21 for enantiomers

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

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 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: 22 (+), 25 (-)

KEY WORDS
chiral

REFERENCE
Oi,N.; Kitahara,H.; Aoki,F. Direct enantiomer separations by high-performance liquid chromatography with chiral urea derivatives as stationary phases, *J.Chromatogr.A*, **1995**, *694*, 129–134.

SAMPLE
Matrix: solutions
Sample preparation: Swab surface with mobile phase, shake swab with mobile phase for 20 min, filter (0.20 μm PDVF membrane), inject a 50 μL aliquot of the filtrate.

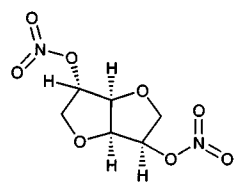
HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil C18
Mobile phase: MeOH:buffer 10:90 (Buffer was 50 mM KH_2PO_4 containing 5 mM sodium 1-pentanesulfonate and 100 μM disodium EDTA, pH adjusted to 3.6 with phosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: E, Bioanalytical Systems, thin-layer glassy carbon electrode +0.65 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 6.9
Limit of detection: 0.1 ng/mL

KEY WORDS
surface contamination

REFERENCE
Elrod,L.,Jr.; Schmit,J.L.; Morley,J.A. Determination of isoproterenol sulfate on surfaces using high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.A*, **1996**, *723*, 235–241.

Isosorbide dinitrate



Molecular formula: $C_6H_8N_2O_8$
Molecular weight: 236.14
CAS Registry No.: 87-33-2
Merck Index: 5245

SAMPLE
Matrix: blood

Sample preparation: 3 mL Plasma + 30 μL 1 μg/mL nitroglycerin in n-hexane + 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 NH_2
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: 3.3
Internal standard: nitroglycerin (5.0)
Limit of detection: 0.25-0.5 ng/mL
Limit of quantitation: 0.56 ng/mL

OTHER SUBSTANCES
Simultaneous: isosorbide mononitrate

KEY WORDS
plasma

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: bulk, formulations
Sample preparation: Prepare a 500 μg/mL aqueous solution, filter, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 200 × 2.1 5 μm Hypersil ODS
Mobile phase: MeOH:water 20:80
Flow rate: 0.4 for 3 min, to 0.6 over 0.5 min, maintain at 0.6 for 9.5 min, return to 0.4 over 0.5 min
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 10.55

OTHER SUBSTANCES
Simultaneous: isosorbide mononitrate
Noninterfering: lactose

REFERENCE
Azcona,T.; Martin-Gonzalez,A.; Zamorano,P.; Pascual,C.; Grau,C.; Garcia de Mirasierra,M. New methods for the assay of 5-isosorbide mononitrate and its validation, *J.Pharm.Biomed.Anal.*, **1991**, *9*, 725–729.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve in water.

HPLC VARIABLES
Column: 300 × 3.9 μBondapack phenyl
Mobile phase: MeCN:THF:water 26:10:64

Flow rate: 2
Injection volume: 10-40
Detector: UV 218

CHROMATOGRAM
Retention time: 6
Internal standard: isosorbide dinitrate

OTHER SUBSTANCES
Simultaneous: nitroglycerin, isosorbide dinitrate is IS

KEY WORDS
injections

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: formulations
Sample preparation: Powder tablets, weigh out a portion equivalent to 1 mg isosorbide dinitrate, add to 10 mL 75 μg/mL nitroglycerin in MeOH, sonicate for 2 min, shake mechanically for 30 min, filter, inject an aliquot

HPLC VARIABLES
Guard column: 40 × 4.6 μBondapak C18/Corasil
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water 40:60
Flow rate: 1
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 9.5
Internal standard: nitroglycerin (14)

OTHER SUBSTANCES
Simultaneous: pentaerythritol tetranitrate, erythrityl tetranitrate

KEY WORDS
tablets

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: 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 5 mg/mL nitroglycerin in MeOH, make up to 100 mL with buffer, filter (0.45 μm), inject a 20 μL aliquot. If the sample clumps when the buffer is added, agitate with a stirring rod and sonicate. (Buffer was MeOH: 200 mM 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 ammonium acetate buffer:water 55:10:35
Flow rate: 1

Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6
Internal standard: nitroglycerin (8)

OTHER SUBSTANCES
Simultaneous: isosorbide mononitrate, saccharin, pentaerythritol tetranitrate

KEY WORDS
tablets; capsules; stability-indicating

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
Sample preparation: Inject directly.

HPLC VARIABLES
Column: 250 × 4.6 LiChrosorb 10 RP 8
Mobile phase: MeOH:water 50:50
Flow rate: 2
Injection volume: 10
Detector: UV 214

CHROMATOGRAM
Retention time: 3.8

OTHER SUBSTANCES
Simultaneous: nitroglycerin

KEY WORDS
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

HPLC VARIABLES
Column: C18
Mobile phase: MeOH:water 50:50
Flow rate: 1.3
Detector: UV 220

CHROMATOGRAM
Internal standard: isosorbide dinitrate

OTHER SUBSTANCES
Simultaneous: nitroglycerin

KEY WORDS
injections; 5% dextrose; isosorbide dinitrate is IS

REFERENCE
Pramar,Y.; Das Gupta,V.; Gardner,S.N.; Yau,B. Stabilities of dobutamine, dopamine, nitroglycerin and sodium nitroprusside in disposable plastic syringes, *J.Clin.Pharm.Ther.*, **1991**, *16*, 203–207.

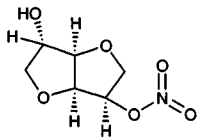
SAMPLE
Matrix: solutions
Sample preparation: 500 μL Buffer solution + 500 μL 1% trifluoroacetic acid, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 YMC R-ODS-1 5-ST
Mobile phase: MeCN:water 45:55 containing 0.05% trifluoroacetic acid
Flow rate: 1.1
Injection volume: 5
Detector: UV 230

KEY WORDS
buffer

REFERENCE
Fukuyama,S.; Hirasawa,Y.; Cox,D.; Koda,S.; Kita,Y. Acceleration of nitric oxide (NO) release from FK409, a spontaneous NO releaser, in the presence of sulfhydryl-bearing compounds, *Pharm.Res.*, **1995**, *12*, 1948–1952.

Isosorbide mononitrate



Molecular formula: $C_6H_9NO_6$
Molecular weight: 191.14
CAS Registry No.: 16051-77-7
Merck Index: 5245

SAMPLE
Matrix: blood
Sample preparation: 3 mL Plasma + 30 μL 1 μg/mL nitroglycerin in n-hexane + 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 NH_2
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: 5.8 (2-isomer), 8.4 (5-isomer)
Internal standard: nitroglycerin (5.0)
Limit of detection: 1-1.2 ng/mL (5-isomer), 0.5-0.8 ng/mL (2-isomer)
Limit of quantitation: 1.66 ng/mL (5-isomer), 0.86 ng/mL (2-isomer)

OTHER SUBSTANCES
Simultaneous: isosorbide dinitrate

KEY WORDS
plasma

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: bulk, formulations
Sample preparation: Prepare a 500 μg/mL aqueous solution, filter, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 200 × 2.1 5 μm Hypersil ODS
Mobile phase: MeOH:water 20:80
Flow rate: 0.4 for 3 min, to 0.6 over 0.5 min, maintain at 0.6 for 9.5 min, return to 0.4 over 0.5 min
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 3.06 (2 isomer), 3.49 (5 isomer)

OTHER SUBSTANCES
Simultaneous: isosorbide dinitrate
Noninterfering: lactose

REFERENCE
Azcona,T.; Martin-Gonzalez,A.; Zamorano,P.; Pascual,C.; Grau,C.; Garcia de Mirasierra,M. New methods for the assay of 5-isosorbide mononitrate and its validation, *J.Pharm.Biomed.Anal.*, **1991**, *9*, 725–729.

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 5 mg/mL nitroglycerin in MeOH, make up to 100 mL with buffer, filter (0.45 μm), inject a 20 μL aliquot. If the sample clumps when the buffer is added, agitate with a stirring rod and sonicate. (Buffer was MeOH: 200 mM 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: A MeOH:200 mM ammonium acetate buffer:water 55:10:35; B MeOH:200 mM ammonium acetate buffer:water 20:10:70
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 3.1 (mobile phase A), 7.20 (2-isomer, mobile phase B), 8.55 (5-isomer, mobile phase B), 46.2 (dinitrate, mobile phase B)
Internal standard: nitroglycerin (8, mobile phase A)

OTHER SUBSTANCES
Simultaneous: saccharin, isosorbide dinitrate, pentaerythritol tetranitrate

KEY WORDS
tablets; capsules

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: solutions

HPLC VARIABLES
Guard column: 10 mm long reversed-phase pellicular (Chrompack)
Column: 250 × 4.6 10 μm LiChrosorb RP-8
Mobile phase: MeCN:MeOH:buffer 16:8:76 (Buffer was 10 mL triethylamine in 760 mL water adjusted to pH 6.8 with acetic acid.)
Flow rate: 2
Injection volume: 175
Detector: UV 230

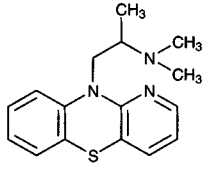
CHROMATOGRAM
Retention time: 3.6 (5 isomer)

OTHER SUBSTANCES
Simultaneous: acenocoumarol, acetaminophen, alizapride, alpiropride, amisulpride, aspirin, caffeine, carbamazepine, clonazepam, codeine, metoclopramide, nitrazepam, nitrofurantoin, theophylline
Noninterfering: amitriptyline, cisplatin, furosemide, indomethacin, isosorbide dinitrate, orphenadrine, propranolol

KEY WORDS
plasma; SPE

REFERENCE
de Jong,A.P.; Wittebrood,A.J.; du Châtinier,W.M.; Bron,J. Liquid chromatographic analysis of alizapride and metoclopramide in human plasma and urine using solid-phase extraction, *J.Chromatogr.*, **1987**, *419*, 233–242.

Isothipendyl



Molecular formula: $C_{16}H_{19}N_3S$
Molecular weight: 285.41
CAS Registry No.: 482-15-5, 1225-60-1 (HCl)
Merck Index: 5248
Lednicer No.: 1 430

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

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

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 248.8

CHROMATOGRAM
Retention time: 13.467

KEY WORDS
whole blood

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

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

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

CHROMATOGRAM
Retention time: 4.4

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, 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, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, qui-

nine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 16.22

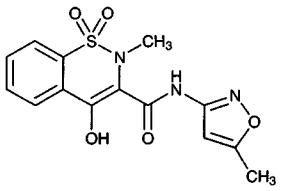
OTHER SUBSTANCES

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, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE

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

Isoxicam



Molecular formula: $C_{14}H_{13}N_3O_5S$
Molecular weight: 335.34
CAS Registry No.: 34552-84-6
Merck Index: 5258
Lednicer No.: 2 394

SAMPLE

Matrix: bile, blood, urine
Sample preparation: Plasma. 500 μL Plasma + 250 μL 1 M HCl + 5 mL diethyl ether, shake at 240 rpm on an orbital shaker for 6 min, centrifuge at 900 g at 4° for 10 min. Remove the organic phase and evaporate it to dryness at 37° under nitrogen. Reconstitute the residue in 250 μL MeCN:water 1:1, vortex for 1 min, inject a 50 μL aliquot. Urine, bile. 500 μL Urine or bile + 250 μL 1 M HCl + 5 mL diethyl ether, shake at 240 rpm on an orbital shaker for 6 min, centrifuge at 900 g at 4° for 10 min. Remove the organic phase, wash it with 2 mL pH 4.9 citric acid-phosphate buffer, and evaporate it to dryness at 37° under nitrogen. Reconstitute the residue in 250 μL MeCN:water 1:1, vortex for 1 min, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: Techsil C10 CN guard column

Column: 200 × 3.9 10 μm Techsil C10 CN (HPLC Technology)
Mobile phase: MeCN:water 22:78 (10:90 for bile analyses) containing 50 mM NaH_2PO_4, final pH 3.5
Flow rate: 2.5
Injection volume: 50
Detector: UV 365

CHROMATOGRAM
Retention time: 7.6, 17 (for bile)
Internal standard: isoxicam

OTHER SUBSTANCES
Simultaneous: piroxicam, 5-hydroxypiroxicam

KEY WORDS
plasma; isoxicam is IS

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: 50 μL Serum + 20 μL 100 mM pH 4.8 citrate buffer + 5 μL 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 μL mobile phase, mix for 10 s, centrifuge, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 7 μm 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: 2.74
Internal standard: isoxicam

OTHER SUBSTANCES
Extracted: piroxicam

KEY WORDS
serum; isoxicam is IS

REFERENCE
Migulla,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
Sample preparation: 1 mL Plasma + 100 μL 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: 9.75
Internal standard: isoxicam

OTHER SUBSTANCES
Extracted: piroxicam
Simultaneous: droxicam

KEY WORDS
protect from light; plasma; isoxicam is IS

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, tissue
Sample preparation: 1 mL Plasma or tissue + 700 mg potassium carbonate + 1 mL THF + 500 μL 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 μL THF, vortex for 5 s, filter (0.5 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 3 μm 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: 8.0
Internal standard: isoxicam
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: piroxicam

KEY WORDS
plasma; rat; skin; muscle; isoxicam is IS

REFERENCE
Cerretani,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, tissue
Sample preparation: 1 mL Plasma or tissue + 700 mg potassium carbonate + 1 mL THF + 500 μL 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 μL THF, vortex for 5 s, filter (0.5 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 3 μm 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: 8.0
Internal standard: isoxicam
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: piroxicam

KEY WORDS
plasma; rat; skin; muscle; isoxicam is IS

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, urine
Sample preparation: Plasma. 500 μL Plasma + 500 μL water + 100 μL THF + 250 μL 200 mM citric acid + 1 mL 2 μg/mL piroxicam in toluene, shake for 10 min at moderate speed, centrifuge at 2000 g for 5 min. Remove 600 μL of the toluene layer and evaporate it to dryness under a stream of air at 67°, reconstitute the residue in 300 μL THF, inject a 30 μL aliquot. Urine. 1 mL Urine + 100 μL THF + 100 μL 1 M HCl + 3 mL 600 ng/mL PD 79,703 in toluene, shake for 10 min, centrifuge at 2000 g for 5 min. Remove 2 mL of the toluene layer and evaporate it to dryness under a stream of air at 67°, reconstitute the residue in 300 μL THF, inject a 30 μL aliquot.

HPLC VARIABLES
Guard column: 40 × 2 Corasil C18
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: THF:water:glacial acetic acid 45:54:1 containing 5 mM 1-heptanesulfonic acid (plasma) or MeCN:water:glacial acetic acid 50:49:1 containing 5 mM 1-heptanesulfonic acid (urine)
Flow rate: 1.5
Injection volume: 30
Detector: UV 320

CHROMATOGRAM
Retention time: 8.1 (plasma), 6 (urine)
Internal standard: piroxicam (4.1), 4-hydroxy-2-methyl-2H-1,2-benzothiazine-3-carboxanilide 1,1-dioxide (PD 79,703) (14)
Limit of detection: 70 ng/mL (urine), 120 ng/mL (plasma)

KEY WORDS
plasma

REFERENCE
Daftsios,A.C.; Johnson,E.L.; Keeley,F.J.; Gryczko,C.; Rawski,V. High-performance liquid chromatographic analysis of isoxicam in human plasma and urine, *J.Chromatogr.*, **1984**, *305*, 145–151.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 100 μL Plasma + 50 μL 100 μg/mL piroxicam in MeCN + 300 μL MeCN, vortex vigorously for 1 min, centrifuge at 2000 g for 2 min, inject a 20 μL aliquot of the supernatant. Urine. 1 mL Urine + 50 μL 40 μg/mL diazepam in MeCN + 100 μL saturated KH_2PO_4 adjusted to pH 2.4 with orthophosphoric acid + 3 mL dichloromethane, vortex for 2 min, centrifuge at 2000 g for 2 min. Remove the organic layer and evaporate it to dryness

under a stream of nitrogen at 50°, reconstitute the residue in 50 μL MeCN, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm LiChrosorb RP18 ODS
Mobile phase: MeCN:buffer 50:50 (plasma) or 45:55 (urine) (Buffer was 50 mM KH_2PO_4 adjusted to pH 3.0 with orthophosphoric acid.)
Flow rate: 2
Injection volume: 20
Detector: UV 325

CHROMATOGRAM
Retention time: 3.1 (plasma), 3.8 (urine)
Internal standard: piroxicam (2.4), diazepam (5.8)
Limit of detection: 20 ng/mL (urine), 200 ng/mL (plasma)

OTHER SUBSTANCES
Noninterfering: allopurinol, cephalexin, chlorothiazide, digoxin, doxepin, furosemide, hydralazine, hydrochlorothiazide, imipramine, labetalol, mepenzolate, methyldopa, metoprolol, minoxidil, naproxen, prazosin, propranolol, sulfamethoxazole, sulfinpyrazone, trifluoperazine, trimethoprim

KEY WORDS
plasma

REFERENCE
Bury,R.W. Liquid chromatographic assay of isoxicam in human plasma and urine, *J.Chromatogr.*, **1985**, *337*, 156–159.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Plasma or urine + 1.1 mL 100 mM NaOH + 250 μL 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 μL 50 mM TRIS, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 10 μm μBondapak CN (plasma) or 300 × 3.9 10 μm μBondapak 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: 6 (plasma), 7 (urine)
Internal standard: isoxicam

OTHER SUBSTANCES
Simultaneous: 5'-hydroxypiroxicam, piroxicam

KEY WORDS
plasma; isoxicam is IS; (see J.Chromatogr. 1984; 305; 145)

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'-hydroxypiroxicam in human plasma and urine, *J.Chromatogr.*, **1986**, *382*, 382–388.

SAMPLE
Matrix: microsomal incubations

Sample preparation: Condition a 6 mL Bond Elut C18 SPE cartridge with 5 mL MeOH and 5 mL water. Filter 30 mL microsomal incubation, add filtrate to SPE cartridge, elute with MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm C18 (Alltech)
Mobile phase: Gradient. MeCN:200 mM pH 6.5 ammonium acetate 10:90 for 5 min, to 20:80 over 5 min, maintain at 20:80 for 10 min, to 50:50 over 10 min.
Flow rate: 1
Detector: UV 254, UV 362

CHROMATOGRAM

Retention time: 35

OTHER SUBSTANCES

Extracted: metabolites

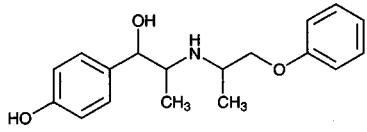
KEY WORDS

rabbit; liver; SPE

REFERENCE

Woolf,T.F.; Black,A.; Chang,T. In vitro metabolism of isoxicam by horseradish peroxidase, *Xenobiotica*, **1989**, *19*, 1369–1377.

Isoxsuprine



Molecular formula: $C_{18}H_{23}NO_3$
Molecular weight: 301.39
CAS Registry No.: 395-28-8, 579-56-6 (HCl)
Merck Index: 5259
Lednicer No.: 1 69

SAMPLE

Matrix: blood
Sample preparation: Dilute blood with an equal volume of water. 1 mL Plasma or 900 μL diluted blood + 0.9-1 mL buffer + 5 mL freshly distilled ethyl acetate, vortex for 1 min, centrifuge at 1750 g for 7 min. Remove the organic layer and evaporate it almost to dryness under a stream of nitrogen at 57°, evaporate the final 500 μL at room temperature, reconstitute the residue in 100 μL MeCN, vortex for 15 s, inject the whole amount. (Buffer was 26.5 g sodium carbonate and 21 g sodium bicarbonate in 500 mL water, pH 9.48.)

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak phenyl (plasma) or 200 × 4.6 5 μm Spheri-5 RP-18 (blood)
Mobile phase: MeCN:0.05% orthophosphoric acid 17:83 (plasma) or 63:37 (blood)
Flow rate: 2
Injection volume: 100
Detector: F ex 200 no emission filter or UV 254

CHROMATOGRAM

Retention time: 15.1 (plasma), 16.3 (blood)
Internal standard: isoxsuprine hydrochloride

OTHER SUBSTANCES

Extracted: ritodrine
Simultaneous: fenoterol

Sample preparation: Condition a 6 mL Bond Elut C18 SPE cartridge with 5 mL MeOH and 5 mL water. Filter 30 mL microsomal incubation, add filtrate to SPE cartridge, elute with MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm C18 (Alltech)
Mobile phase: Gradient. MeCN:200 mM pH 6.5 ammonium acetate 10:90 for 5 min, to 20:80 over 5 min, maintain at 20:80 for 10 min, to 50:50 over 10 min.
Flow rate: 1
Detector: UV 254, UV 362

CHROMATOGRAM

Retention time: 35

OTHER SUBSTANCES

Extracted: metabolites

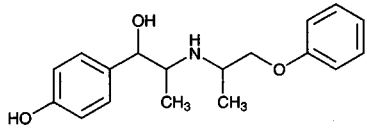
KEY WORDS

rabbit; liver; SPE

REFERENCE

Woolf,T.F.; Black,A.; Chang,T. In vitro metabolism of isoxicam by horseradish peroxidase, *Xenobiotica*, **1989**, *19*, 1369–1377.

Isoxsuprine



Molecular formula: $C_{18}H_{23}NO_3$
Molecular weight: 301.39
CAS Registry No.: 395-28-8, 579-56-6 (HCl)
Merck Index: 5259
Lednicer No.: 1 69

SAMPLE

Matrix: blood
Sample preparation: Dilute blood with an equal volume of water. 1 mL Plasma or 900 μL diluted blood + 0.9-1 mL buffer + 5 mL freshly distilled ethyl acetate, vortex for 1 min, centrifuge at 1750 g for 7 min. Remove the organic layer and evaporate it almost to dryness under a stream of nitrogen at 57°, evaporate the final 500 μL at room temperature, reconstitute the residue in 100 μL MeCN, vortex for 15 s, inject the whole amount. (Buffer was 26.5 g sodium carbonate and 21 g sodium bicarbonate in 500 mL water, pH 9.48.)

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak phenyl (plasma) or 200 × 4.6 5 μm Spheri-5 RP-18 (blood)
Mobile phase: MeCN:0.05% orthophosphoric acid 17:83 (plasma) or 63:37 (blood)
Flow rate: 2
Injection volume: 100
Detector: F ex 200 no emission filter or UV 254

CHROMATOGRAM

Retention time: 15.1 (plasma), 16.3 (blood)
Internal standard: isoxsuprine hydrochloride

OTHER SUBSTANCES

Extracted: ritodrine
Simultaneous: fenoterol

Noninterfering: acetaminophen, albuterol, betamethasone, bupivacaine, caffeine, chloral hydrate, dexamethasone, diazepam, lignocaine, meperidine, metoclopramide, morphine, nitrazepam, terbutaline

KEY WORDS
isoxsuprine is IS; plasma; whole blood

REFERENCE
Gross,A.S.; Brown,K.F.; Baird-Lambert,J.A.; Nation,R.L. Determination of ritodrine in blood and plasma by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1987**, *416*, 400–408.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + nylidrin + 500 μL buffer, mix briefly, add 50-75 mg resin, mix at 10-25 rpm for 30 min, discard the supernatant, wash twice with 1 mL buffer, add 500 μL 50 mg/mL KOH in MeOH:water 50:50, mix for 30 min, inject a 20 μL aliquot of the eluate. (Buffer was 100 mM citric acid:200 mM Na_2HPO_4 29:71, pH 6.5 (McIlvaine buffer) Wash 20-50 mesh Dowex HCR-S resin twice with water and allow it to equilibrate in buffer).

HPLC VARIABLES
Guard column: 25 × 4.6 5 μm Spherisorb ODS-I
Column: 250 × 4.6 5 μm Spherisorb ODS-I
Mobile phase: MeCN:MeOH:buffer 30:18:52 containing 1.8 mM octanesulfonic acid (Buffer was 30 mM KH_2PO_4 adjusted to pH 3.0 with concentrated orthophosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: E, Gynotek M20, glassy carbon working electrode 950 mV, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 8.5
Internal standard: nylidrin (11)
Limit of detection: 1 ng/mL (from 2 mL plasma)
Limit of quantitation: 5 ng/mL

KEY WORDS
horse; plasma; pharmacokinetics; SPE

REFERENCE
Hashem,A.; Lubczyk,B. Determination of isoxsuprine in equine plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1991**, *563*, 216–223.

SAMPLE
Matrix: blood
Sample preparation: Condition a Baker 500 mg C18 SPE cartridge with 3 mL MeOH and 3 mL water. 1 mL Serum + 714 units β-glucuronidase (E. coli, Sigma), heat at 37° overnight, add 2 mL water, add to the SPE cartridge at 0.15 mL/s, wash with 3 mL water, elute with 2 mL MeOH:triethylamine 99:1. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 5 μm LC 8 DB (Supelco)
Mobile phase: Gradient. MeOH:buffer 5:95 for 1 min, to 95:5 over 20 min, maintain at 95:5 for 5 min, re-equilibrate at initial conditions for 10 min. (Buffer was 100 mM sodium acetate containing 0.1% triethylamine adjusted to pH 3.4 with 85% phosphoric acid.)
Flow rate: 1.2
Injection volume: 20
Detector: UV 276

CHROMATOGRAM
Limit of detection: 400 ng/mL

KEY WORDS
serum; horse; SPE; pharmacokinetics

REFERENCE
Pompa,G.; Caloni,F.; Montana,M.; Pasqualucci,C. Prolonged presence of isoxsuprine in equine serum after oral administration, *Xenobiotica*, **1994**, *24*, 339–346.

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

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

CHROMATOGRAM
Retention time: 1.6

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, 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, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

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

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

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

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

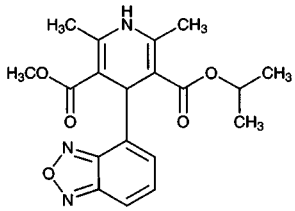
SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Chirex 3014 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 55:35:10 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1
Injection volume: 20
Detector: UV 276

KEY WORDS
chiral; α = 1.40 for enantiomers

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

Isradipine



Molecular formula: $C_{19}H_{21}N_3O_5$
Molecular weight: 371.39
CAS Registry No.: 75695-93-1
Merck Index: 5260
Lednicer No.: 4 107

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 4 ng/mL IS in water + 100 μL 2 M NaOH + 6 mL dichloromethane, mix on a rotary mixer for 20 min, centrifuge at 1500 g for 5 min (if emulsions form stir with a class rod and re-centrifuge). Remove the organic layer and evaporate it under a stream of nitrogen at 40°, add 500 μL dichloromethane, vortex, evaporate under a stream of nitrogen at 40°, reconstitute in 100 μL mobile phase, vortex for 30 s, allow to stand for 10 min, vortex, inject a 75 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-pak C18
Mobile phase: MeOH:10 mM dibutylamine phosphate (Waters D-4) 50:50, pH 2.8-3.0
Column temperature: 48
Flow rate: 1
Injection volume: 75
Detector: UV 325

CHROMATOGRAM
Retention time: 12
Internal standard: PY 108-068 (diethyl ester of isradipine) (13)
Limit of quantitation: 0.5 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
plasma

REFERENCE

Boutagy,J.; Rumble,F.; Dunagan,F. Determination of isradipine and the oxidative pyridine metabolite in human plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *487*, 483–488.

SAMPLE

Matrix: blood, urine

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

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

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

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

Column temperature: 30

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

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 22.352

KEY WORDS

whole blood

REFERENCE

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

SAMPLE

Matrix: cells, culture medium

Sample preparation: Cells. To 100 μL containing 40 × 10^6 cell suspension add 100 μL of a methanolic solution of p-phenoxyphenol and benzoylated spermine so as to give concentrations of 10.63 μm and 2815 nM, respectively. Vortex for 1 min, sonicate for 3 min, add 200 μL MeOH, vortex for 1 min, centrifuge at 2000 g for 5 min, inject a 50 μL aliquot of the supernatant. Culture medium. To 5 mL culture medium add 200 μL of a methanolic solution of p-phenoxyphenol and benzoylated spermine so as to give concentrations of 10.63 μm and 2815 nM, respectively. Adjust the pH to 8.0 with 1 M NaOH, add 5 mL chloroform (Caution! Chloroform is a carcinogen!), mix at 30 rpm for 30 min, centrifuge at 1000 g for 5 min, remove the chloroform layer. Adjust the aqueous phase to pH 3.0 with 1 M HCl, add 1 mL 100 mM pH 3 citrate buffer and 1 mL 37 mM tetrabutylammonium hydrogen sulfate. Extract the aqueous phase with 5 mL chloroform at 30 rpm for 30 min, centrifuge at 1000 g for 5 min. Combine the chloroform layers and evaporate them to dryness under a stream of nitrogen, dissolve the residue in 300 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 5 μm Chromspher C8 (Chrompack)

Mobile phase: MeOH:THF:buffer 43.6:1:55.4 (Buffer was water containing 160 mM NaH_2PO_4 and 17 mM tetrabutylammonium hydrogen sulfate, pH 3.0.)

Flow rate: 1

Injection volume: 50

Detector: UV 240

CHROMATOGRAM
Retention time: 37
Internal standard: p-phenoxyphenol (19), benzoylated spermine (33)
Limit of detection: 110 pmol/10^6 (cells), 210 nM (culture medium)
Limit of quantitation: 350 pmol/10^6 (cells), 700 nM (culture medium)

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE
Bidouil,S.; Dubois,J.; Hanocq,M. Isocratic high-performance liquid chromatographic method for the separation of isradipine and its main metabolites. Application to in vitro metabolization by h3A4/OR cells, *J.Chromatogr.B*, **1997**, *693*, 359–366.

SAMPLE
Matrix: formulations
Sample preparation: Shake bottle by hand, dilute a 1 mL aliquot with MeOH:95% EtOH 1:1 to an expected isradipine concentration of 100 μg/mL, filter (0.22 μm), inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spheri-5 ODS (Applied Biosystems)
Mobile phase: MeOH:THF:water 42:20:38
Flow rate: 1
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
suspensions; stability-indicating

REFERENCE
MacDonald,J.L.; Johnson,C.E.; Jacobson,P. Stability of isradipine in an extemporaneously compounded oral liquid, *Am.J.Hosp.Pharm.*, **1994**, *51*, 2409–2411.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 100 μM solution in buffer, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 column containing riboflavin binding proteins (Prepare as follows. Add riboflavin to saturate protein of egg yolk, homogenize with 3 volumes buffer, centrifuge, add the supernatant to a 500 × 30 column of DEAE-cellulose (Whatman) equilibrated with buffer, wash extensively with buffer to remove bound protein, elute riboflavin binding proteins (RFBP) with buffer containing 200 mM NaCl (RFBP has intense yellow color, absorption at 455 nm). Purify RFBP on a Sephadex G-100 column with 50 mM pH 7.5 Tris-HCl buffer as eluent, remove the bound riboflavin by extensive dialysis at pH 3.0. Add 4.5 g N,N-disuccinylimidyl carbonate to 3 g Nucleosil 5NH2 slurried in MeCN, filter, wash with MeCN, wash with 50 mM pH 7.5 phosphate buffer. Suspend 300 mg RFBP in 50 mM phosphate buffer, add the activated silica, mix gently for 2 h using a rotary evaporator, filter, wash with sterile water, wash with isopropanol:water 1:2, pack in a 100 × 4.6 column.) (Buffer was 100 mM pH 5.3 sodium acetate.)
Mobile phase: 50 mM pH 5.5 KH_2PO_4
Flow rate: 0.8
Injection volume: 20
Detector: UV

CHROMATOGRAM
Retention time: k' 8.57

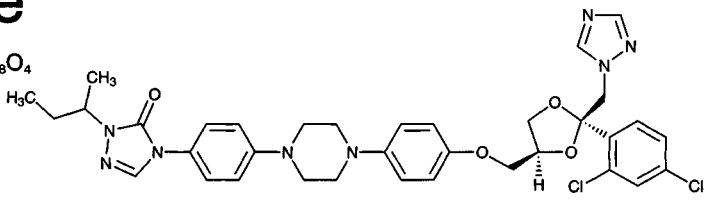
OTHER SUBSTANCES
Simultaneous: flurbiprofen, ketoprofen, nimodipine, suprofen

KEY WORDS
chiral; α = 1.28

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.

Itraconazole

Molecular formula: $C_{35}H_{38}Cl_2N_8O_4$
Molecular weight: 705.64
CAS Registry No.: 84625-61-6
Merck Index: 5262



SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 1 mg/mL IS in MeCN, add 500 mg KCl, vortex, centrifuge at 1500 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher RP8
Mobile phase: MeCN:water 55:45
Flow rate: 1.5
Injection volume: 40
Detector: UV 263

CHROMATOGRAM
Retention time: 10-11
Internal standard: R51012 (14-15) (Janssen, France)
Limit of detection: 20 μg/mL
Limit of quantitation: 40 μg/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE
Cociglio,M.; Hillaire-Buys,D.; Alric,R. Prevalidation statistical design to assess analytical methods. Example of a quick liquid chromatographic assay of itraconazole in serum, *J.Chromatogr.B*, **1997**, *698*, 225–233.

SAMPLE
Matrix: blood
Sample preparation: 250 μL serum + IS + 50 μL 0.3 N barium hydroxide + 50 μL 0.4 N zinc sulfate + 1 mL MeCN, vortex, centrifuge at 3521 g for 15 min, evaporate the supernatant to dryness under a stream of nitrogen, reconstitute with 250 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: 7.5 × 4.6 5 μm Alltech Alltima C18
Column: 250 × 4.6 5 μm Alltech Alltima C18
Mobile phase: MeCN:MeOH:50 mM pH 6.7 phosphate buffer 47:8:45
Column temperature: 37
Flow rate: 1
Detector: UV 263

CHROMATOGRAM
Internal standard: saperconazole
Limit of detection: 10 ng/mL
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; pharmacokinetics

REFERENCE
Christensen,K.J.; Gubbins,P.O.; Gurley,B.J.; Bowman,J.L.; Buice,R.G. Relative bioavailability of itraconazole from an extemporaneously prepared suspension and from the marketed capsules, *Am.J.Health-Syst.Pharm.*, **1998**, *55*, 261–265.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elut SPE cartridge (No. 607101) with 1 mL MeOH and 1 mL water. 1 mL Serum + 100 μL 1 μg/mL IS in MeOH, mix, add to the SPE cartridge, wash with 1-2 mL water, wash with 1-2 mL MeOH:water 50:50, elute with 400 μL MeOH:triethylamine:concentrated orthophosphoric acid 99.7:0.3:0.3, inject a 20 μL aliquot of the eluate.

HPLC VARIABLES
Column: 100 × 4.6 MPLC RP-18 Spheri-5
Mobile phase: MeCN:water 60:40 containing 20 mM triethylamine, pH adjusted to 2.3 with phosphoric acid
Flow rate: 1
Injection volume: 20
Detector: F ex 260 em 365

CHROMATOGRAM
Retention time: 3
Internal standard: cis-4-[4-[4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-5-methyl-2-(3-methylbutyl)-3H-1,2,4-triazol-3-one (R 51 012) (4)
Limit of quantitation: 4 ng/mL

OTHER SUBSTANCES
Noninterfering: amphotericin B, cefaclor, imipenem, flucytosine, gentamycin, ketoconazole, netilmicin, salicylic acid, sulfamethoxazole, tienamycin, tobramycin, trimethoprim

KEY WORDS
serum; SPE

REFERENCE
Allenmark,S.; Edebo,A.; Lindgren,K. Determination of itraconazole in serum with high-performance liquid chromatography and fluorescence detection [letter], *J.Chromatogr.*, **1990**, *532*, 203–206.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma or serum + 300 μL 250 nM IS in MeOH, vortex for 1 min, centrifuge at 2000 g for 2 min, inject a 250 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 50 × 4.6 20 μm Ultrasphere ODS
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:water:diethylamine 60:40:0.05 (At the end of each day flush column with MeOH:DMSO 90:10.)
Flow rate: 1.5
Injection volume: 250
Detector: UV 261

CHROMATOGRAM

Retention time: 6.1
Internal standard: cis-4-[4-[4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-5-methyl-2-(3-methylbutyl)-3H-1,2,4-triazol-3-one (R51012) (8.5)
Limit of detection: 8 nM

OTHER SUBSTANCES

Noninterfering: amoxicillin, amphotericin B, ampicillin, cimetidine, diazepam, erythromycin, gentamycin, griseofulvin, ketoconazole, miconazole, nystatin, penicillin G, prednisolone, sulfamethoxazole, trimethoprim, zidovudine

KEY WORDS

plasma; serum

REFERENCE

Badcock,N.R. Micro-scale method for itraconazole in plasma by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *525*, 478–483.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 1 mL 50 mM sodium borate + 100 μL 10 μg/mL IS in MeOH + 200 μL MeOH, extract twice with 4 mL aliquots of heptane:isoamyl alcohol 95:5 in a rotary mixer. Combine the organic phases and add them to 2 mL 1 M sulfuric acid, extract. Discard the organic phase and add 600 μL concentrated ammonium hydroxide to the aqueous phase. Extract the aqueous phase twice with 2.5 mL portions of heptane:isoamyl alcohol 95:5. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL MeCN:water 60:40, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: C18
Column: 100 × 4.5 3 μm Hypersil octadecylsilane
Mobile phase: MeCN:water 40:60 containing 0.03% diethylamine adjusted to pH 7.8 with dilute orthophosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: k' 7.4
Internal standard: cis-4-[4-[4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-5-methyl-2-(3-methylbutyl)-3H-1,2,4-triazol-3-one (R51012) (k' 10.8)
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, hydroxyitraconazole

KEY WORDS

serum

REFERENCE
Law,D.; Moore,C.B.; Denning,D.W. Bioassay for serum itraconazole concentrations using hydroxyitraconazole standards, *Antimicrob.Agents Chemother.*, **1994**, *38*, 1561–1566.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 1.5 mL MeOH, shake for 5 min, centrifuge. Remove the supernatant and evaporate it to dryness, reconstitute the residue in 100 μL MeOH, inject a 20 μL aliquot. Alternatively, condition a 100 mg Bakerbond C-18 SPE cartridge with 2 mL MeOH and 2 mL water. 1 mL Plasma + 1 mL water, add to the SPE cartridge, wash with 2 mL water containing 100 μL MeCN, elute with 2 mL MeOH or MeCN. Evaporate the eluate to dryness, reconstitute the residue in 100 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3 5 μm Separon SGX C-18 (Tessek, Prague)
Mobile phase: MeOH:water:triethylamine 72:28:0.05
Flow rate: 1.1
Injection volume: 20
Detector: UV (wavelength not specified)

CHROMATOGRAM
Retention time: 9
Internal standard: R 51012 (13)
Limit of detection: 10 ng/mL (?)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
SPE; plasma; serum

REFERENCE
Brandsteterova,E.; Kubalec,P.; Rády,A.; Krcméry,V. Determination of itraconazole and its metabolites in plasma using SPE-HPLC, *Pharmazie*, **1995**, *50*, 597–599.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond-Elut C18 SPE cartridge with 2 mL 95% EtOH and 2 mL MeCN:water 15:85. 200 μL Plasma or whole blood + 50 μL 100 μM testosterone propionate in MeOH + 3 mL MeCN:water 15:85, vortex for 30 s, add to the SPE cartridge, wash with 9 mL MeCN:water 30:70, dry, elute with 200 μL 95% EtOH, inject a 10 μL aliquot of the eluate.

HPLC VARIABLES
Column: 50 × 4.6 5 μm Supelcosil LC-8DB
Mobile phase: MeOH:buffer 72.5:27.5 (Buffer was 25 mM K_2HPO_4 adjusted to pH 3 with 670 mM phosphoric acid.)
Flow rate: 1
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 2.55
Internal standard: testosterone propionate (3.60)

OTHER SUBSTANCES
Extracted: 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, mephenytoin, mephobarbital, meth-

arbital, methsuximide, methylsuccinimide, nortriptyline, paramethadione, phenacemide, phenobarbital, phensuximide, phenylpropanolamine, phenytoin, primidone, procainamide, protriptyline, quinidine, theophylline, tobramycin, trimethadione, valproic acid, vancomycin
Interfering: clotrimazole

KEY WORDS
plasma; SPE; whole blood

REFERENCE
Rifai,N.; Sakamoto,M.; Law,T.; Platt,O.; Mikati,M.; Armsby,C.C.; Brugnara,C. HPLC measurement, blood distribution, and pharmacokinetics of oral clotrimazole, potentially useful antisickling agent, *Clin.Chem.*, **1995**, *41*, 387–391.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond-Elut C18 SPE cartridge with 2 mL 95% EtOH and 2 mL MeCN:water 15:85. 100 μL Plasma + 50 μL 4 μg/mL + 3 mL MeCN:water 15:85, vortex for 30 s, add to the SPE cartridge, wash with 9 mL MeCN:water 40:60, dry the SPE cartridge, elute with 500 μL dichloromethane:MeOH 50:50. Evaporate the eluate to dryness, reconstitute the residue in 100 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 50 × 4.6 5 μm Supelcosil LC-1
Mobile phase: MeCN:MeOH:25 mM pH 6.3 K_2HPO_4 30:30:40
Flow rate: 2
Injection volume: 10
Detector: UV 263

CHROMATOGRAM
Retention time: 2.1
Internal standard: R051012 (Jannsen) (2.8)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, amitriptyline, aspirin, barbituric acid, brompheniramine, caffeine, carbamazepine epoxide, carbamazepine, chloramphenicol, chlorpheniramine, clonazepam, clotrimazole, desipramine, desmethyldoxepin, digitoxin, digoxin, disopyramide, doxepin, ethosuximide, felbamate, gentamicin, ibuprofen, imipramine, lidocaine, maprotiline, mephenytoin, mephobarbital, metharbital, methsuximide, methylsuccinimide, nortriptyline, paramethadione, phenacemide, phenobarbital, phensuximide, phenylpropanolamine, phenytoin, primidone, protriptyline, theophylline, tobramycin, trimethadione, vancomycin

KEY WORDS
SPE; plasma

REFERENCE
Rifai,N.; Sakamoto,M.; Platt,O.; Brugnara,C. A high-performance liquid chromatographic assay for the determination of itraconazole concentration using solid-phase extraction and small sample volume, *Ther.Drug Monit.*, **1995**, *17*, 522–525.

SAMPLE
Matrix: blood, tissue
Sample preparation: Tissue. Homogenize 25 mg tissue with 1 mL MeOH. Add 50 μL 2.5 μg/mL IS in MeOH, vortex. Centrifuge the sample at 833 g for 15 min. Evaporate the supernatant to dryness under a stream of nitrogen. Reconstitute the residue in 300 μL MeOH, inject an aliquot. Plasma. Add 50 μL 12.5 μg/mL IS to 100 μL plasma, vortex. Add 300 μL MeOH, vortex for 1 min. Centrifuge the sample at 833 g for 5 min. Inject a 190 μL aliquot.

HPLC VARIABLES
Guard column: Novapak Guard-Pak

Column: 100 × 8 4 μm Novapak C18
Mobile phase: MeCN:diethylamine:water 58:0.05:42, adjusted to pH 2.45 with 85% phosphoric acid
Flow rate: 1.5
Injection volume: 190
Detector: F ex 260 em 365

CHROMATOGRAM
Retention time: 14.10
Internal standard: R51012 (Janssen Research Diagnostics, USA) (18.5)
Limit of detection: 5 ng/mL
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; serum; liver; bird

REFERENCE
Cox,S.K.; Orosz,S.; Burnette,J.; Frazier,D. Microassay for determination of itraconazole and hydroxyitraconazole in plasma and tissue biopsies, *J.Chromatogr.B*, **1997**, *702*, 175–180.

SAMPLE
Matrix: blood, tissue
Sample preparation: Homogenize (Ultra-Turrax) tissue in water 1:4. 1-2 mL Plasma or homogenate + 100 μL 2-10 μg/mL IS in MeOH + 50 mM pH 7.8 phosphate buffer, extract twice with 4 mL heptane:isoamyl alcohol 98.5:1.5 for 10 min. Combine the organic layers and add them to 3 mL 50 mM sulfuric acid, extract, centrifuge at 1000 g. Remove the aqueous phase and adjust the pH to 9 with concentrated ammonia, extract twice with 2 mL heptane:isoamyl alcohol 98.5:1.5. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 55°, reconstitute the residue in 100 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.1 5 μm RSiL C18HL octadecyl (Alltech)
Mobile phase: MeCN:water 60:40 containing 0.05% diethylamine
Flow rate: 0.5
Injection volume: 40
Detector: UV 263

CHROMATOGRAM
Retention time: 4.3
Internal standard: cis-4-[4-[4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-5-methyl-2-(3-methylbutyl)-3H-1,2,4-triazol-3-one (R 51 012) (5.8)
Limit of detection: 1 ng/mL

KEY WORDS
plasma; human; rat; pharmacokinetics

REFERENCE
Woestenborghs,R.; Lorreyne,W.; Heykants,J. Determination of itraconazole in plasma and animal tissues by high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *413*, 332–337.

SAMPLE
Matrix: blood, tissue
Sample preparation: Tissue. Homogenize (Baxter Scientific disposable tissue grinder) esophageal tissue with 20 volumes of cold phosphate-buffered saline (pH 7.8). 250 μL Homogenate + 1 mL MeCN + 1 μL 1 mg/mL IS, vortex for 1 min, centrifuge at 1000 g for 5 min. Remove the supernatant and dry in air for 30 min, reconstitute in 100 μL mobile phase, inject an aliquot. Plasma. 250 μL Plasma + 1 mL MeCN + 1 μL 1 mg/mL IS, vortex for 1 min, centrifuge at

1000 g for 5 min. Remove the supernatant and dry in air for 30 min, reconstitute in 100 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Nova-pak C18
Mobile phase: MeCN:10 mM K_2HPO_4 60:40, final pH adjusted to 7.8 with 85% phosphoric acid
Flow rate: 1.3
Detector: UV 263

CHROMATOGRAM
Retention time: 4.35
Internal standard: R51012 (Research Diagnostics Inc.) (6.00) Remarks
Limit of detection: 10 ng/g, 5 ng/mL

OTHER SUBSTANCES
Noninterfering: ampicillin, cefazolin, cefoperazone, ceftriaxone, cefuroxime, clindamycin, fluconazole, folic acid, minocycline, nafcillin, norfloxacin, rifampin, tetracycline, vancomycin, zalcitabine, zidovudine

KEY WORDS
plasma

REFERENCE
Darouiche,R.O.; Setoodeh,A.; Anaissie,E.J. Potential use of a simplified method for determination of itraconazole levels in plasma and esophageal tissue by using high-performance liquid chromatography, *Antimicrob.Agents Chemother.*, **1995**, *39*, 757–759.

SAMPLE
Matrix: formulations
Sample preparation: 250 μL Syrup + 1.5 mL DMF, make up to 10 mL with mobile phase. 250 μL Solution + 500 μL 1 mg/mL IS, make up to 10 mL with mobile phase, inject a 5 μL aliquot.

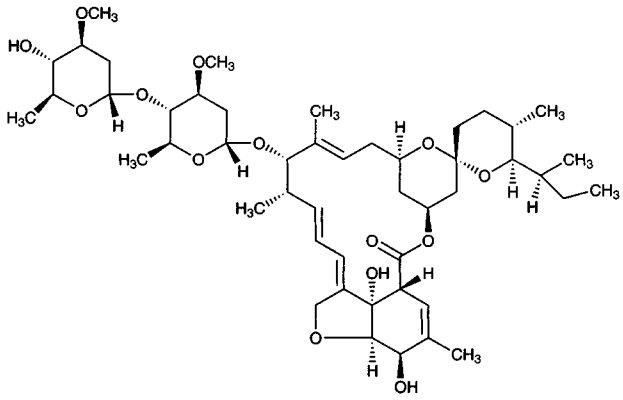
HPLC VARIABLES
Column: 250 × 4.6 5 μm Spheri-5 ODS
Mobile phase: MeCN:water:diethylamine 60:40:0.05
Flow rate: 1
Injection volume: 5
Detector: UV 263

CHROMATOGRAM
Retention time: 7
Internal standard: R51012 (Janssen) (11)

KEY WORDS
syrup; stability-indicating; suspensions

REFERENCE
Jacobson,P.A.; Johnson,C.E.; Walters,J.R. Stability of itraconazole in an extemporaneously compounded oral liquid, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 189–191.

Ivermectin



Molecular formula: $C_{48}H_{74}O_{14}$ (B1a)
Molecular weight: 875.11 (B1a)
CAS Registry No.: 70288-86-7, 70161-11-4 (B1a), 70209-81-3 (B1b)
Merck Index: 5264

SAMPLE

Matrix: blood

Sample preparation: Mix 5 mL Serum with 5.0 mL MeOH:buffer 20:80. Pass through an immunoaffinity column (80 × 7 mm, 1.0 mL bed volume, IgG + CNBr-activated Sepharose 4B, Pharmacia, Sweden) at 1.2 mL/min. Wash with 20 mL MeOH:buffer 10:90 and 5 mL MeOH:water 10:90. Elute with 5 mL MeOH. Evaporate the eluate to dryness at 55°. Add 1 mL MeOH to the residue, vortex for 15 s, inject an aliquot. (Buffer was prepared by dissolving 200 mg KH_2PO_4, 2.9 g $Na_2HPO_4.12\ H_2O$, 200 mg KCl, and 18.8 g NaCl in 900 mL water, adjusting to pH 7.4 with 2 M NaOH, and making up to 1 L with water.)

HPLC VARIABLES

Column: 220 × 4.6 5 μm Spheri-5 RP-18
Mobile phase: MeOH:water 95:5
Flow rate: 1.0
Injection volume: 10
Detector: UV 245

CHROMATOGRAM

Retention time: 6.5
Limit of detection: 200 pg/mL
Limit of quantitation: 2 ng/mL

KEY WORDS

serum; sheep; immunoaffinity; SPE

REFERENCE

Li,J.; Zhang,S. Immunoaffinity column cleanup and liquid chromatographic method for determining ivermectin in sheep serum, *J.AOAC Int.*, **1996**, *79*, 1300–1302.

SAMPLE

Matrix: blood

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 4 mL MeCN:water 50:50. Condition a Sep-Pak silica SPE cartridge with 4 mL MeCN and 4 mL dichloromethane. 5 mL Serum + 5 mL MeCN:water 50:50, add to the C18 SPE cartridge, wash with 4 mL MeCN:water 50:50, blow out excess solvent, elute the C18 SPE cartridge onto the silica SPE cartridge with 4 mL MeCN:dichloromethane 10:90, discard the C18 cartridge and elute the silica cartridge with 4 mL MeCN. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 100 × 8 4 μm Nova-Pak radial PAK
Mobile phase: MeCN:MeOH:water 45:45:10
Flow rate: 1
Detector: UV 245

CHROMATOGRAM
Retention time: 4
Limit of detection: 2 ppb

KEY WORDS
cow; serum; SPE

REFERENCE
Oehler,D.D.; Miller,J.A. Liquid chromatographic determination of ivermectin in bovine serum, *J.Assoc.Off.Anal.Chem.*, **1989**, *72*, 59–59.

SAMPLE
Matrix: blood
Sample preparation: Condition a 500 mg Sep-Pak C18 SPE cartridge with 4 mL MeCN, 5 mL chloroform, 4 mL MeCN, and 4 mL water. 1 mL Plasma + 500 μL MeCN + 500 μL 1 ng/mL IS in MeCN, mix for 15 s, centrifuge at 2500 g for 10 min, add the supernatant to the SPE cartridge, dry under vacuum for 15 min, elute with 5 mL chloroform. Evaporate the eluate to dryness under a stream of nitrogen at <50°, reconstitute with 100 μL N-methylimidazole:MeCN 1:1, add 150 μL trifluoroacetic anhydride:MeCN 1:2, let stand for <30 s, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax C8
Mobile phase: MeCN:THF:water 40:40:20
Column temperature: 30
Flow rate: 1
Injection volume: 100
Detector: F ex 365 em 475

CHROMATOGRAM
Retention time: 18 (ivermectin B_{1a})
Internal standard: avermectin B_{1a} (12.5)
Limit of detection: 20 pg/mL

KEY WORDS
plasma; SPE; cow; derivatization

REFERENCE
de Montigny,P.; Shim,J.S.K.; Pivnichny,J.V. Liquid chromatographic determination of ivermectin in animal plasma with trifluoroacetic anhydride and *N*-methylimidazole as the derivatization reagent, *J.Pharm.Biomed.Anal.*, **1990**, *8*, 507–511.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL C18 SPE cartridge (J.T. Baker) with 4 mL MeCN, 5 mL chloroform, 4 mL MeCN, and 4 mL water. 1 mL Plasma + 50 μL 86-285 ng/mL IS in MeCN, vortex for 15 s, add 1 mL MeCN, mix, centrifuge at 1130 g for 15 min, add the supernatant to the SPE cartridge. Reconstitute the residue in 3.5 mL MeCN:water 1:2, vortex for 15 s, centrifuge at 1130 g for 15 min, add the supernatant to the SPE cartridge. Wash the SPE cartridge with 4 mL MeCN:water 1:2, dry under vacuum for 1 h, elute with 5 mL MeCN:chloroform 50:50. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute with two 100 μL portions of MeCN, evaporate to dryness under a stream of nitrogen at room temperature. Reconstitute with 100 μL N-methylimidazole:MeCN 1:1, add 150 μL trifluoroacetic anhydride:MeCN 1:2, let stand for 1.7 min, inject a 150 μL aliquot.

HPLC VARIABLES
Column: 100 × 2 3 μm MOS-Hypersil-2
Mobile phase: Gradient. MeCN:water from 72:28 to 92:8 over 15 min.
Flow rate: 0.3
Injection volume: 150
Detector: F ex 365 em 475

CHROMATOGRAM
Retention time: 22.5
Internal standard: ivermectin monosaccharide (20)
Limit of detection: 10 pg/mL

KEY WORDS
plasma; SPE; dog; narrow bore; derivatization

REFERENCE
Rabel,S.R.; Stobaugh,J.F.; Heinig,R.; Bostick,J.M. Improvements in detection sensitivity for the determination of ivermectin in plasma using chromatographic techniques and laser-induced fluorescence detection with automated derivatization, *J.Chromatogr.*, **1993**, *617*, 79–86.

SAMPLE
Matrix: blood, tissue
Sample preparation: Whole blood, serum. Condition a 1 mL Bond Elut C18 SPE cartridge with 2 mL MTBE, 2 mL MeCN, and 2 mL MeCN:water 50:50. 500 μL Whole blood or serum + 20 μL IS solution + 50 μL 200 mM zinc sulfate solution + 500 μL MeCN, vortex, centrifuge for 5 min, add to the SPE cartridge, wash with 2 mL MeCN:water 50:50, elute with 2 mL MTBE. Evaporate the eluate and dissolve the residue in 150 μL mobile phase, inject a 50 μL aliquot. Muscle. Condition a 3 mL Bond Elut C18 SPE cartridge with 4 mL MTBE, 4 mL MeCN, and 4 mL MeCN:water 50:50. Weigh out 1 g muscle, add 50 μL/g IS solution, add 6 mL MeCN:water 50:50, homogenize (Vertis 45), rinse blades and jar with 2 mL MeCN:water 50:50, add 200 mL (sic) 200 mM zinc sulfate, mix, centrifuge, add the supernatant to the SPE cartridge, wash with 4 mL MeCN:water 50:50, elute with 4 mL MTBE. Evaporate the eluate and dissolve the residue in 150 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 70 × 4.6 3 μm Ultrasphere XL ODS
Mobile phase: MeCN:MeOH:water 49:33:18
Column temperature: 56
Flow rate: 1
Injection volume: 50
Detector: UV 245

CHROMATOGRAM
Retention time: 5.6
Internal standard: dehydroivermectin (13.4) (Prepare by evaporating 1 mL 278 μg/mL ivermectin in MeCN into a tube. Add 200 μL 1-methylimidazole, add 300 μL acetic anhydride, add 900 μL DMF, mix well, heat at 60° for 15 min, add 4 mL MeCN, pass through a silica SPE cartridge, Evaporate the eluate to 500 μL, make up to 20 mL with MeCN, use this solution.)
Limit of detection: 2 ng/g (tissue), 2 ng/mL (whole blood, serum)

KEY WORDS
whole blood; serum; muscle; human; cow; SPE

REFERENCE
Dickinson,C.M. Improved high-performance liquid chromatographic method for quantitation of ivermectin in whole blood, serum or muscle tissue, *J.Chromatogr.*, **1990**, *528*, 250–257.

SAMPLE
Matrix: feces
Sample preparation: Stir 5 g feces with 25 mL MeOH for 25 min, centrifuge at 1500 g for 15 min, concentrate the supernatant to 7 mL under reduced pressure at 80°, centrifuge at 1500 g for 15 min, add procaine to a concentration of 50 ppm, make up to 10 mL with MeOH, filter (0.50 μm PTFE), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 300 × 9 Bondclone 10C18 (Phenomenex)
Mobile phase: MeCN:MeOH:water 47:33:20
Flow rate: 1

Injection volume: 20
Detector: UV 245

CHROMATOGRAM
Retention time: 12.6
Internal standard: procaine (7.3)
Limit of detection: 20 ng/g

KEY WORDS
cow

REFERENCE
Bernal,J.L.; Del Nozal,M.J.; Salas,M.; Galante,E.; Lumaret,J.P. HPLC determination of ivermectin in cattle dung following subcutaneous injection, *J.Liq.Chromatogr.*, **1994**, *17*, 2429–2444.

SAMPLE
Matrix: milk
Sample preparation: Prepare a SPE cartridge by adding 2 g 40 μm Bondesil C18 18% load endcapped (Varian) to a 25 mL syringe barrel fitted with a 20 μm frit, wash with 5 mL petroleum ether, 5 mL acetone, and two 5 mL aliquots of MeOH, aspirate with full vacuum for <5 s (A). Condition a 500 mg Bond Elut LRC silica SPE cartridge with 3 mL hexane:ethyl acetate 60:40 (B). Condition a 500 mg Bond Elut LRC silica SPE cartridge with 4 mL chloroform (C). 25 mL Milk + 200 μL 500 ng/mL abamectin (avermectins) in MeOH, mix, add 5 mL to the SPE cartridge (A), mix milk with C18 material, let stand for 2 min, wash spatula with water, wash with two 5 mL portions of water, elute with 10 mL ethyl acetate, allow eluate to pass through a 5 cm layer of anhydrous sodium sulfate. Evaporate the eluate to dryness under a stream of nitrogen below 50°, add 2 mL hexane:ethyl acetate 60:40 to the oily residue, vortex, sonicate for 1 min, add mixture to SPE cartridge (B), rinse in with 1 mL hexane:ethyl acetate 60:40, wash with 5 mL hexane:ethyl acetate 60:40, elute with 5 mL MeOH:ethyl acetate 50:50. Evaporate the eluate to dryness under a stream of nitrogen below 60° (this residue should have no moisture in it), reconstitute the residue in 100 μL reagent, vortex gently for a few s, heat at 95° for 1 h, cool, add 1 mL chloroform, vortex, add to SPE cartridge (C), wash in with three 1 mL portions of chloroform, elute with 2 mL chloroform. Collect all the eluate and evaporate it to dryness under a stream of nitrogen below 60°, reconstitute in 500 μL MeOH, inject a 50 μL aliquot. (Prepare reagent by sequentially mixing 900 μL DMF, 300 μL acetic anhydride, and 200 μL N-methylimidazole just before use.)

HPLC VARIABLES
Guard column: Newguard RP-18 (Brownlee)
Column: 250 × 4.6 5 μm Econosil C18
Mobile phase: MeOH:THF:water 85:15:5
Flow rate: 1
Injection volume: 50
Detector: F ex 364 em 455

CHROMATOGRAM
Retention time: 15
Internal standard: abamectin (avermectins) (10.5)
Limit of detection: <1 ppb

KEY WORDS
cow; SPE; MSPD; derivatization; silylate glassware

REFERENCE
Schenck,F.J. Isolation and quantification of ivermectin in bovine milk by matrix solid phase dispersion (MSPD) extraction and liquid chromatographic determination, *J.Liq.Chromatogr.*, **1995**, *18*, 349–362.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX

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

OTHER SUBSTANCES

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

REFERENCE

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

SAMPLE

Matrix: tissue
Sample preparation: Condition a 3 mL 500 mg Isolute C8 end-capped SPE cartridge (International Sorbent Technologies) with 5 mL MeCN and 5 mL MeCN:water 1:2 containing 0.1% triethylamine. Condition a 3 mL 500 mg Isolute silica SPE cartridge (International Sorbent Technologies) with 5 mL ethyl acetate:hexane 40:60. Homogenize (Tissuemizer) 5 g tissue in 25 mL MeCN, centrifuge at 3000 rpm for 5 min, decant supernatant into 50 mL water and 75

μL triethylamine. mix, pass through the C8 SPE cartridge at 2 mL/min, discard eluate, dry column under vacuum for 5 min, elute with 5 mL MeCN. Dry the eluate under a stream of nitrogen at 50-55°, resolubilize dry residues in 5 mL of ethyl acetate:hexane 40:60, vortex briefly, pass through the silica SPE cartridge at 2 mL/min. Rinse the reservoir with 5 mL ethyl acetate:hexane 40:60, add the rinse to the SPE cartridge. Dry the SPE cartridge for 5 min, elute with 5 mL MeOH:ethyl acetate 50:50, dry the eluate under a stream of nitrogen at 50-55°. Reconstitute the extract with 200 μL of fresh methylimidazole:MeCN 50:50, vortex briefly, add 300 μL of fresh trifluoroacetic anhydride:MeCN 1:2, vortex briefly, dry under a stream of nitrogen at 50-55° for 15 min. Add 500 μL MeOH:ammonium acetate:molecular sieves 4:1:1), vortex briefly, dry under a stream of nitrogen at 50-55°. Add 1 mL MeCN, vortex thoroughly, filter (0.45 μm), inject a 50 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 5 μm Hypersil ODS (C18)
Mobile phase: MeCN-water 90:10
Column temperature: 65
Flow rate: 1.0
Injection volume: 50
Detector: F ex 272 em 465

CHROMATOGRAM
Retention time: 11 (B1b), 12.5 (B1a)
Limit of detection: 0.25 ppb

OTHER SUBSTANCES
Extracted: doramectin

KEY WORDS
SPE; derivatization; salmon; muscle

REFERENCE
Rupp,H.S.; Turnipseed,S.B.; Walker,C.C.; Roybal,J.E.; Long,A.R. Determination of ivermectin in salmon muscle tissue by liquid chromatography with fluorescence detection, *J.AOAC Int.*, **1998**, *81*, 549–553.

SAMPLE
Matrix: tissue
Sample preparation: Condition a C8 Bond-Elut SPE cartridge with 5 mL MeCN and 5 mL MeCN:water 30:70 containing 0.1% triethylamine. Mix 5 g muscle with 15 mL MeCN in a high speed blender for 1 min. Centrifuge at 3000 rpm for 5 min. Dilute 15 mL of the supernatant with 35 mL water, add 50 μL triethylamine, add to the SPE cartridge, elute with 5 mL MeCN, evaporate the eluate to dryness under a stream of nitrogen at 60°. Dissolve the residue in 1 mL MeOH, filter (0.45 μm Millipore filter). Transfer a 500 μL aliquot to a silanized 3 mL reaction vial, evaporate to dryness. Add 100 μL derivatization reagent, heat at 95° for 1 h. Cool to room temperature, add 1 mL chloroform, vortex, add quantitatively to a Sep-Pak silica SPE cartridge with 3-4 mL chloroform. Elute with 9 mL chloroform, evaporate the combined chloroform eluates to dryness. Dissolve the residue in 500 μL MeOH, filter (0.45 μm Millipore filter), inject a 50 μL aliquot. (Caution! Chloroform is a carcinogen! Derivatization reagent was 1-methylimidazole:acetic anhydride:DMF 2:6:9, freshly prepared.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Shandon ODS Hypersil C18
Mobile phase: MeOH:water 97:3
Column temperature: 30
Flow rate: 1
Injection volume: 50
Detector: F ex 364 em 470

CHROMATOGRAM
Retention time: 14
Limit of detection: 500-1000 ng/kg

KEY WORDS
pig; muscle; SPE; derivatization

REFERENCE
Nordlander,I.; Johnsson,H. Determination of ivermectin residues in swine tissues--an improved clean-up procedure using solid-phase extraction, *Food Addit.Contam.*, **1990**, *7*, 79–82.

SAMPLE
Matrix: tissue
Sample preparation: Add 15 mL MeOH to 5 g ' homogenized liver, shake thoroughly by hand and again using a shaking apparatus at medium speed for 1 h. Adjust the volume to 20 mL with MeOH, shake, centrifuge at 2000 g for 5 min. Mix 10 mL supernatant with 40 mL phosphate buffer A, add to immunoaffinity column (column preparation described in detail in paper) at 1.2 mL/min, wash with 40 mL MeOH:phosphate buffer B 10:90 and 10 mL MeOH:water 20:80. Elute with 3 mL MeOH, evaporate the eluate to less than 1 mL on a rotary evaporator at 55°, vortex with 5 mL ethyl acetate for 15 s. Collect the organic layer, evaporate to dryness at 55°, redissolve the residue in 1 mL mobile phase by vortexing for 15 s, filter (0.45 μm filter), inject a 100 μL aliquot of the filtrate. (Phosphate buffer A was 200 mg KH_2PO_4, 2900 mg Na_2HPO_4, 200 mg KCl, and 8800 mg NaCl in 900 mL water adjusted to pH 7.4 with 2 M NaOH and diluted to 1 L with water. Phosphate buffer B was 200 mg KH_2PO_4, 2900 mg Na_2HPO_4, 200 mg KCl, and 29.3 g NaCl in 900 mL water adjusted to pH 7.4 with 2 M NaOH and diluted to 1 L with water.)

HPLC VARIABLES
Column: 220 × 4.6 5 μm Brownlee C18
Mobile phase: MeCN:MeOH:water 45:45:10
Flow rate: 1
Injection volume: 100
Detector: UV 245

CHROMATOGRAM
Retention time: 15
Limit of detection: 2 mg/g
Limit of quantitation: 5 mg/g

KEY WORDS
immunoaffinity; liver; pig; SPE

REFERENCE
Li,J.S.; Li,X.W.; Hu,H.B. Immunoaffinity column cleanup procedure for analysis of ivermectin in swine liver, *J.Chromatogr.B*, **1997**, *696*, 166–171.

SAMPLE
Matrix: tissue
Sample preparation: Condition a Bond-Elut C8 SPE cartridge (Analytichem International) with 5 mL MeCN and 5 mL MeCN:water 30:70 containing 0.1% triethylamine. Mix 5 g muscle with 15 mL MeCN in a high speed blender for 1 min, centrifuge at 3000 rpm for 5 min. Dilute 15 mL supernatant with 35 mL water, add 50 μL triethylamine, add the entire mixture to the SPE cartridge, elute with 5 mL MeCN, evaporate the eluate to dryness under a stream of nitrogen at 60°. Dissolve the residue in 1 mL MeOH and filter (0.45 μm). Evaporate a 500 μL aliquot of the filtrate to dryness in a silanized vial, add 100 μL derivatization reagent (1-methyl-imidazole:acetic anhydride:dimethylformamide 2:6:9, freshly prepared), heat in an oven at 95° for 1 h. Cool to room temperature, add 1 mL chloroform and vortex briefly. (Caution! Chloroform is a carcinogen!) Transfer quantitatively to a Sep-Pak silica SPE cartridge with 3-4 mL chloroform, elute with 9 mL chloroform, evaporate the combined chloroform eluates to dryness. Dissolve the residue in 500 μL MeOH, filter (0.45 μm), inject a 50 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil ODS C18 (Shandon)
Mobile phase: MeOH:water 97:3
Column temperature: 30
Flow rate: 1
Injection volume: 50
Detector: F ex 364 em 470

CHROMATOGRAM
Retention time: 14 (dihydroavermectin B1A)
Limit of quantitation: 500-1000 ng/g

KEY WORDS
derivatization; muscle; SPE; pig

REFERENCE
Nordlander,I.; Johnsson,H. Determination of ivermectin residues in swine tissues--an improved clean-up procedure using solid-phase extraction, *Food Addit.Contam.*, **1990**, *7*, 79–82.

SAMPLE
Matrix: tissue
Sample preparation: Condition a Bond Elut C8 SPE cartridge with 5 mL MeCN and 5 mL MeCN:water:triethylamine 30:70:0.1. Homogenize (Silverson) 5 g frozen minced tissue with 15 mL MeCN at full speed for 1 min, centrifuge at 4° at 2000 g for 10 min. Remove a 13 mL aliquot of the supernatant and add it to 35 mL water and 50 μL triethylamine, elute with 5 mL MeCN. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute with 200 μL 1-methylimidazole:MeCN 1:1, add 300 μL trifluoroacetic anhydride:MeCN 1:2, mix, store cold, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Partisil 5 ODS-3
Mobile phase: MeOH:water 96:4
Flow rate: 1.8
Detector: F ex 364 em 470

CHROMATOGRAM
Retention time: 6 (ivermectin B_{1a})
Limit of detection: 1 ng/g

KEY WORDS
SPE; salmon; derivatization; brain; gill; kidney; liver; muscle; skin; spleen

REFERENCE
Kennedy,D.G.; Cannavan,A.; Hewitt,S.A.; Rice,D.A.; Blanchflower,W.J. Determination of ivermectin residues in the tissues of Atlantic salmon (*Salmo salar*) using HPLC with fluorescence detection, *Food Addit.Contam.*, **1993**, *10*, 579–584.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 6 mL 500 mg Bond Elut C18 SPE cartridge with 5 mL MeCN and 5 mL MeCN:water:triethylamine 30:70:0.1. Homogenize (Polytron) 5 g tissue and 15 mL MeCN for 20 s, rinse probe with 5 mL MeCN, shake mechanically at high speed for 5 min, centrifuge at 2000 g for 5 min. Re-extract the solid with 10 mL MeCN. Add the supernatants to the alumina column. Combine the eluates, add 70 mL water, add 100 μL triethylamine, mix, add to the C18 SPE cartridge, pull air through the SPE cartridge for 3 min, elute with 5 mL MeCN. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL freshly prepared reagent, vortex for 15 s, heat at 95-100° for 45 min, cool, add 1 mL chloroform, vortex, add to a 2.8 mL 500 mg Bond Elut silica SPE cartridge, elute with three 3 mL aliquots of chloroform. Combine the eluates and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 1 mL MeOH, filter, inject a 40 μL aliquot. (Prepare alumina column as follows. Shake 94 g Brockman Activity I neutral alumina (Fisher) and 6 mL water for 45 min, add 4.5 g alumina to an 8 mL column with a frit. Reagent was 200 μL 1-methylimidazole, 600 μL acetic anhydride, and 900 μL DMF.)

HPLC VARIABLES
Guard column: 30 × 4.6 RP-18 (Brownlee)
Column: 250 × 4.6 RP-18 OD-224 (Brownlee)
Mobile phase: MeOH:water 97:3
Flow rate: 1.8

Injection volume: 40
Detector: F ex 365 em 425

CHROMATOGRAM
Retention time: 9.3
Limit of detection: 2 ppb

KEY WORDS
SPE; cow; pig; sheep; fish; liver; muscle; derivatization

REFERENCE
Salisbury,C.D.C. Modified method for the determination of ivermectin residues in animal tissues, *J.AOAC Int.*, **1993**, *76*, 1149–1151.

SAMPLE
Matrix: tissue
Sample preparation: Condition a Bond Elut C18 SPE cartridge with 4 mL MeCN and 4 mL MeCN:water 10:90. 4 g Minced meat or liver + 40 mL MeCN + 3.5 mL water, vortex for 2 min, centrifuge at 2000 rpm for 10 min, remove supernatant, repeat extraction with 20 mL MeCN and 3.5 mL water. Combine the supernatants and evaporate them to 6 mL under reduced pressure (all the MeCN should be removed), add 6 mL water to the residue, add to the SPE cartridge, pull air through the cartridge for 10 min, elute with 5 mL MeCN. Evaporate the eluate under a stream of nitrogen, add 150 μL trifluoroacetic anhydride:MeCN 1:2 to the residue, add 100 μL N-methylimidazole:MeCN 50:50, shake, store in the dark, inject a 10-50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:water 95:5 (At the end of the day flush column with 30 mL MeOH.)
Flow rate: 1.5
Injection volume: 10-50
Detector: F ex 364 em 470

CHROMATOGRAM
Retention time: 11
Limit of quantitation: 5 ng/g

KEY WORDS
meat; liver; cow; pig; muscle; SPE; derivatization

REFERENCE
Degroodt,J.M.; Wyhowski de Bukanski,B.; Srebrnik,S. Determination of ivermectin residues in meat and liver by HPLC and fluorometric detection, *J.Liq.Chromatogr.*, **1994**, *17*, 1419–1426.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 3 mL Bakerbond C8 SPE cartridge with 5 mL MeCN and 5 mL MeCN:water:triethylamine 30:70:0.1. Prepare a 55 mm column of 70-230 mesh Kiselgel 60 (Merck) in a Pasteur pipette, condition with 3 mL hexane:isopropanol 60:40. Homogenize (Polytron) 5 g blended tissue with 12 mL MeCN, rinse homogenizer with 3 mL MeCN, centrifuge the mixture at 4000 rpm for 10 min. Remove the supernatant and make up to 50 mL with water, add 50 μL triethylamine, shake, add to the SPE cartridge, elute with 5 mL MeCN at 1 drop/s. Evaporate the eluate to 300 μL under a stream of nitrogen at 40°, transfer to a smaller vial with MeCN, evaporate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL 1-methylimidazole:MeCN 50:50, cool in an ice bath, add 150 μL trifluoroacetic anhydride:MeCN 1:2, shake at room temperature for 1 min, add to the column, rinse the vial with 500 μL hexane:isopropanol 60:40, add the rinse to the column, elute with 1 mL hexane:isopropanol 60:40. Evaporate the eluate to dryness under a stream of nitrogen with heating, reconstitute with 250 μL MeCN, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 5 μm LiChrospher 100 RP-18

Column: 125 × 4 5 μm LiChrospher 60 RP-select B
Mobile phase: MeOH:water 95:5
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: F ex 365 em 465

CHROMATOGRAM
Retention time: 3.5
Limit of detection: 1 ppb
Limit of quantitation: 2.5 ppb

KEY WORDS
derivatization; muscle; liver; pig; cow; SPE

REFERENCE
Guggisberg,D.; Sievi,M.; Koch,H. Methode zur quantitativen Bestimmung von Ivermectin in Fleisch und Leber mit HPLC und Vorsäulenderivatisation [Method for the quantitative determination of ivermectin in meat and liver by HPLC and pre-column derivatization], *Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene*, **1994**, *85*, 395–405.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 6 mL 500 mg Bakerbond C18 SPE cartridge with three 5 mL portions of MeOH, 5 mL MeCN, and three 5 mL portions of MeCN:water:triethylamine 30:70:0.1. Condition a Waters silica SPE cartridge with 8 mL chloroform. Homogenize (Ultraturrax) 5 g minced tissue with 15 mL MeCN at high speed for 3 min, rinse blade with 2 mL MeCN, sonicate for 15 min, centrifuge at 3000 rpm for 5 min, filter (paper), extract the residue again with 10 mL MeCN, wash the filter with 3 mL MeCN. Combine the organic layers and add 70 mL water and 100 μL triethylamine, stir thoroughly, add to the C18 SPE cartridge, wash with two 5 mL portions of MeCN:water 50:50, elute with 7 mL MTBE at 2 mL/min. Store the eluate overnight at -20°, remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 3 mL MeOH, add 100 μL water, add 3 mL hexane, vortex, remove the hexane layer, repeat the hexane wash. Extract the combined hexane layers with 1 mL MeOH. Combine the MeOH layers and evaporate them to dryness under a stream of nitrogen at 50°, heat in a vacuum oven at 50° for 30 min, reconstitute the residue in 150 μL 1-methylimidazole:acetic anhydride:DMF 2:3:9 (freshly prepared), vortex for 30 s, heat at 100° for 1 h, cool, add 1 mL chloroform, vortex, add to the silica SPE cartridge, elute with three 3 mL portions of chloroform. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 400 μL MeOH, vortex, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelcosil LC-18
Column: 150 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeOH:water 95:5
Flow rate: 1.8
Injection volume: 20
Detector: F ex 360 em 470

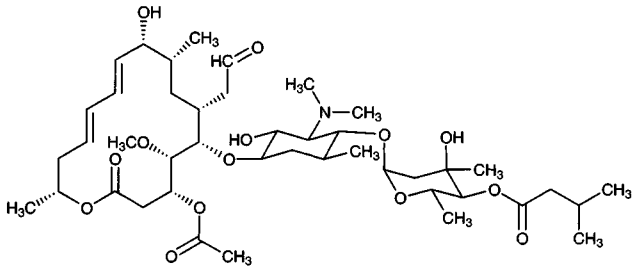
CHROMATOGRAM
Retention time: 7
Limit of detection: 2 ng/g

KEY WORDS
derivatization; SPE; liver; muscle; fat; guinea pig; cow; pig; horse; sheep; pharmacokinetics

REFERENCE
Dusi,G.; Curatolo,M.; Fierro,A.; Faggionato,E. Determination of the antiparasitic drug ivermectin in liver, muscle and fat tissue samples from swine, cattle, horses and sheep using HPLC with fluorescence detection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1607–1616.

Josamycin

Molecular formula: $C_{42}H_{69}NO_{15}$
Molecular weight: 828.01
CAS Registry No.: 16846-24-5, 51016-68-3 (propionate)
Merck Index: 5280



SAMPLE

Matrix: blood, urine

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

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 231.1

CHROMATOGRAM

Retention time: 16.763

KEY WORDS

whole blood

REFERENCE

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

SAMPLE

Matrix: bulk

HPLC VARIABLES

Column: 250 × 4.6 8 µm 1000 Å PLRP-S (Polymer Labs., UK)
Mobile phase: MeCN:buffer:water 52:20:28 (Prepare buffer by mixing 200 mM K_3PO_4 with 200 mM K_2HPO_4 to obtain a pH of 10.0.)
Column temperature: 60
Flow rate: 1
Injection volume: 20
Detector: UV 232

CHROMATOGRAM

Retention time: 20 (josamycin propionate)

OTHER SUBSTANCES
Simultaneous: josamycin, leucomycin A4 propionate, josamycin 2',9-dipropionate, josamycin 3",9-dipropionate, platenomycin A1 propionate

REFERENCE
Roets,E.; Lepoudre,X.; Van Rompaey,V.; Velghe,G.; Liu,L.; Hoogmartens,J. Liquid chromatography of josamycin propionate on poly(styrene-divinylbenzene), *J.Chromatogr.A*, **1998**, *812*, 303–308.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 1 mL 100 mg Bond-Elut diol SPE cartridge with 1 mL chloroform (Caution! Chloroform is a carcinogen!). Mix 2 g minced muscle tissue with 800 μL water. Stir, vortex for 1 min at maximum speed, let stand for 15 min. Add 2 mL pH 8 buffer, mix briefly, add 10 mL chloroform. Stir at 100 rpm for 15 min, centrifuge at 4000 g for 10 min, discard the aqueous layer, filter the chloroform layer through glass wool. Add the filtrate to the SPE cartridge, wash with 500 μL chloroform, dry under vacuum, elute with three 200 μL portions of MeOH:100 mM ammonium acetate 50:50, inject a 200 μL aliquot of the eluate. (Buffer was 33.46 g K_2HPO_4 and 1.046 g KH_2PO_4 in 1 L water.)

HPLC VARIABLES
Guard column: 4 × 4 5 μm C18
Column: 125 × 4 5 μm Lichrospher RP18
Mobile phase: Gradient. A was MeCN. B was MeOH. C was 0.1% trifluoroacetic acid in water. A:B:C from 20:20:60 to 25:55:20 in 10 (?) min
Flow rate: 0.5
Injection volume: 200
Detector: MS, HP Model 5989 A, desolvation chamber 60°, source 280° and 300° in negative and positive chemical ionization mode, respectively, with methane as reagent, quadrupole 100°, particle beam nebulizer helium 345 kPa, scan m/z 735.4-828.5 in NCI and 828.5-769.4 in PCI

CHROMATOGRAM
Retention time: 7.2
Limit of detection: 50 μg/kg

OTHER SUBSTANCES
Extracted: erythromycin, spiramycin, tilmicosin, tylosin

KEY WORDS
muscle; cow; SPE

REFERENCE
Delépine,B.; Hurtaud-Pessel,D.; Sanders,P. Multiresidue method for confirmation of macrolide antibiotics in bovine muscle by liquid chromatography/mass spectrometry, *J.AOAC Int.*, **1996**, *79*, 397–404.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 500 mg Bond Elut SCX SPE cartridge (Varian) with 5 mL MeOH and 10 mL 100 mM pH 4.4 KH_2PO_4 buffer. Homogenize 5 g tissue with 100 mL MeOH: 0.3% metaphosphoric acid 30:70 at high speed for 2 min, filter through 2 mm Hyflo Super-Cel coated on a suction funnel (when filtering liver or kidney add several grams of Hyflo Super-Cel to the homogenized solution before filtration). Evaporate the filtrate to ca. 20 mL under reduced pressure at 45°, add to the SPE cartridge, wash with 10 mL distilled water and 5 mL 100 mM pH 8.9 K_2HPO_4 buffer, elute with 10 mL MeOH, evaporate the eluate to dryness under reduced pressure at 45°, dissolve the residue in 1 mL MeCN:50 mM pH 4.5 NaH_2PO_4 buffer 30:70, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Puresil 5C18 (Waters)
Mobile phase: Gradient. A:B from 60:40 to 0:100 over 16 min. A was buffer. B was MeCN:buffer 40:60 (Buffer was 2.5 g KH_2PO_4 dihydrate and 0.65 mL 85% phosphoric acid dissolved in 1 L distilled water, pH 2.5.)

Column temperature: 35
Flow rate: 1
Injection volume: 10
Detector: UV 232 for 9 min, UV 287 for 2 min, UV 232 for 4 min

CHROMATOGRAM
Retention time: 13.73
Limit of detection: 50 ng/g

OTHER SUBSTANCES
Extracted: leucomycin (kitasamycin), mirosamicin, spiramycin, tylosin

KEY WORDS
meat; SPE

REFERENCE
Horie,M.; Saito,K.; Ishii,R.; Yoshida,T.; Haramaki,Y.; Nakazawa,H. Simultaneous determination of five macrolide antibiotics in meat by high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *812*, 295–302.

SAMPLE
Matrix: tissue
Sample preparation: Blend (Virtis model 45 with U-shaped blades) 2.5 g tissue with 20 mL MeCN:10 mM pH 6.0 phosphate buffer 65:35 for 10 min, centrifuge at 5° at 8500 g for 5 min. Remove the supernatant and adjust the volume to 25 mL with MeCN:10 mM pH 6.0 phosphate buffer 65:35. Remove a 1.5 mL aliquot and add it to 5 mL isooctane, shake for 10 min, centrifuge at 5° at 3000 g for 5 min, discard the organic layer. Add 500 μL reagent to the aqueous layer, mix, heat at 90° for 2 h, cool, inject a 100 μL aliquot. (Prepare reagent by dissolving 1 g cyclohexa-1,3-dione and 25 g ammonium acetate in 60 mL water and 8 mL concentrated HCl, make up to 100 mL with water. Store at 5°, discard after 1 month.)

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100 RP-18 end capped
Column: 125 × 4 5 μm LiChrospher 100 RP-18 end capped
Mobile phase: MeCN:MeOH:10 mmole pH 6.0 phosphate buffer 45:5:50
Column temperature: 45
Flow rate: 1.5
Injection volume: 100
Detector: F ex 375 em 450

CHROMATOGRAM
Retention time: 10.3
Limit of detection: 25 ng/g

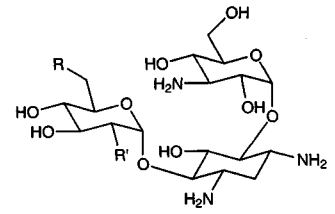
OTHER SUBSTANCES
Simultaneous: spiramycin, tylosin
Noninterfering: acetaldehyde, benzaldehyde, erythromycin, formaldehyde

KEY WORDS
pig; muscle; liver; kidney; fat; derivatization

REFERENCE
Leroy,P.; Decolin,D.; Nicolas,A.; Archimbault,P. Determination of josamycin residues in porcine tissues using high-performance liquid chromatography with pre-column derivatization and spectrofluorometric detection, *Analyst*, **1994**, *119*, 2743–2747.

Kanamycin

Molecular formula: $C_{18}H_{36}N_4O_{11}$ (A)
Molecular weight: 484.51 (A)
CAS Registry No.: 8063-07-8, 25389-94-0 (A sulfate), 59-01-8 (A), 4696-78-8 (B)
Merck Index: 5293



	R	R'
Kanamycin A	NH_2	OH
Kanamycin B	NH_2	NH_2
Kanamycin C	OH	NH_2

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 300 μL Plasma + 30 μL water + 100 μL 2 M perchloric acid, vortex for 2-3 s, centrifuge at 1000 g for 5 min. Remove the supernatant and neutralize it with 1.5 M NaOH, add 300 μL buffer, add 400 μL DMSO, add 100 μL 2% 2,4-dinitrofluorobenzene in EtOH, vortex, heat at 64° for 30 min, add 3 mL toluene, vortex, centrifuge, discard the upper toluene layer, add 3 mL MeCN:toluene 50:50, vortex for 5-10 s. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 30-40°, reconstitute the residue in 1 mL MeCN:water 50:50, inject a 20 μL aliquot. Urine. Dilute urine 100-fold with water. 300 μL Diluted urine + 30 μL water + 300 μL buffer + 400 μL DMSO + 100 μL 2% 2,4-dinitrofluorobenzene in EtOH, vortex, heat at 64° for 30 min, add 3 mL toluene, vortex, centrifuge, discard the upper toluene layer, add 3 mL MeCN:toluene 50:50, vortex for 5-10 s. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 30-40°, reconstitute the residue in 1 mL MeCN:water 50:50, inject a 20 μL aliquot. (Prepare buffer by mixing 80 mL 100 mM Na_2HPO_4 and 20 mL 100 mM NaH_2PO_4, adding 1 g Tris HCl, and adjusting the pH to 7.8 with 6 M HCl.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax SB-C18
Mobile phase: MeOH:water 64:36, adjusted to pH 3.0 with phosphoric acid
Column temperature: 50
Flow rate: 2
Injection volume: 10-20
Detector: UV 350

CHROMATOGRAM

Retention time: 24.0 (kanamycin B)
Internal standard: kanamycin B (24.0)

OTHER SUBSTANCES

Extracted: paromomycin

KEY WORDS

derivatization; plasma; pharmacokinetics; kanamycin B is IS

REFERENCE

Lu,J.; Cwik,M.; Kanyok,T. Determination of paromomycin in human plasma and urine by reversed-phase high-performance liquid chromatography using 2,4-dinitrofluorobenzene derivatization, *J.Chromatogr.B*, **1997**, *695*, 329–335.

SAMPLE

Matrix: bulk
Sample preparation: Prepare a 2 mg/mL solution in 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 μL aliquot of the lower organic phase.

HPLC VARIABLES

Column: 250 × 4.6 5 μm LiChrosorb SI-100

Mobile phase: Chloroform:THF:water 42:56.4:1.6
Flow rate: 1
Injection volume: 20
Detector: UV 350

CHROMATOGRAM
Retention time: 18

KEY WORDS
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: fermentation solutions
Sample preparation: 5 mL Fermentation broth + 5 mL saturated aqueous solution of Tris + 20 mL MeCN, centrifuge at 3000 rpm for 10 min. Remove a 1 mL aliquot of the supernatant and add it to 3 mL 150 mM 2,4-dinitrofluorobenzene in MeOH, heat at 100° under a reflux condenser for 45 min, make up to 4 mL with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 200 × 4.6 10 μm LiChrosorb RP-8
Mobile phase: MeCN:water:acetic acid 55:45:0.15
Flow rate: 1.2
Injection volume: 20
Detector: UV 350

CHROMATOGRAM
Retention time: 11.28 (kanamycin B)

OTHER SUBSTANCES
Extracted: apramycin, tobramycin

KEY WORDS
derivatization

REFERENCE
Harangi,J.; Deák,M.; Nánási,P.; Bacsa,G. Determination of the major factors of fermentation of the nebramycin complex by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1984**, *7*, 83–93.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 3 mg/mL ophthalmic suspension in water with 10 mM sulfuric acid to a tobramycin concentration of 240 μg/mL. Mix 4 mL diluted suspension with 10 mL 10 mg/mL 2,4-dinitrofluorobenzene in EtOH and 10 mL 15 mg/mL tris(hydroxymethyl)aminomethane in water:dimethylsulfoxide 20:80. Heat at 70 ± 2°. for 20 min, allow to cool slightly for 2 min and add 24 mL MeCN. Allow to cool to room temperature, make up to 50 mL with MeCN, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeCN:buffer 55:45 (Prepare mobile phase as follows. Dissolve 2.0 g tris (hydroxymethyl)aminomethane in 960 mL water, add 20 mL 0.5 M sulfuric acid and 1200 mL MeCN.)
Flow rate: 1.5
Injection volume: 30
Detector: UV 365

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: neamine, nebramine, tobramycin

KEY WORDS
derivatization; ophthalmic suspension

REFERENCE
Russ,H.; McCleary,D.; Katimy,R.; Montana,J.L.; Miller,R.B.; Krishnamoorthy,R.; Davis,C.W. Development and validation of a stability-indicating HPLC method for the determination of tobramycin and its related substances in an ophthalmic suspension, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 2165–2181.

SAMPLE
Matrix: reaction mixtures
Sample preparation: 50 μL Buffered reaction mixture + 50 μL isopropanol + 50 μL reagent, heat at 60° for 10 min, centrifuge at 1000 g for 2 min, immediately inject a 50 μL aliquot of the supernatant. (Reagent was 80 mM o-phthalaldehyde and 250 mM thioglycolic acid in 1 M boric acid, pH adjusted to 10.4 with 40% KOH.)

HPLC VARIABLES
Column: 100 × 5 Hypersil ODS
Mobile phase: A was MeOH:water:acetic acid 50:45:5 containing 5 g/L heptanesulfonic acid. B was MeOH:water:acetic acid 75:20:5 containing 5 g/L heptanesulfonic acid. A:B 60:40.
Flow rate: 2
Injection volume: 50
Detector: UV 330

CHROMATOGRAM
Retention time: 19 (kanamycin A)

KEY WORDS
derivatization

REFERENCE
Lovering,A.M.; White,L.O.; Reeves,D.S. Identification of aminoglycoside-acetylating enzymes by high-pressure liquid chromatographic determination of their reaction products, *Antimicrob.Agents Chemother.*, **1984**, *26*, 10–12.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeCN, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere octyl
Mobile phase: MeCN:20 mM buffer 52:48 (Buffer was 2.68 g KH_2PO_4 in 1 L water adjusted to pH 3.0 with phosphoric acid.)
Column temperature: 50
Flow rate: 2
Injection volume: 50
Detector: UV 340

CHROMATOGRAM
Retention time: 12

OTHER SUBSTANCES
Simultaneous: amikacin
Noninterfering: acetaminophen, acetazolamide, N-acetylprocainamide, amobarbital, ampicillin, amitriptyline, caffeine, cefamandole, cefoxime, cefoxitin, cephalothin, clindamycin, chloram-

phenicol, chlordiazepoxide, diazepam, erythromycin, ethosuximide, gentamicin, nitrofurantoin, penicillin G, pentobarbital, phenobarbital, phenytoin, primidone, procainamide, quinidine, salicylic acid, secobarbital, tetracycline, theophylline, tobramycin, vancomycin

REFERENCE

Kabra,P.M.; Bhatnager,P.K.; Nelson,M.A. Liquid chromatographic determination of amikacin in serum with spectrophotometric detection, *J.Chromatogr.*, **1984**, *307*, 224–229.

SAMPLE

Matrix: solutions

Sample preparation: 50 µL Buffer solution + 25 µL 242 mg/mL pH 10.4 Tris buffer + 100 µL MeCN:water 50:50 + 30 µL 250 mg/mL 2,4,6-trinitrobenzenesulfonic acid in MeCN:water 80:20, vortex for 10 s, heat at 70° for 15 min, add 2 mL chloroform, shake horizontally at 180 cycles/min for 5 min, centrifuge at 750 g for 5 min. Remove the lower organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 µL MeCN, vortex, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Ultrasphere octyl
Mobile phase: MeCN:50 mM KH_2PO_4 62:38, pH adjusted to 3.5 with phosphoric acid
Flow rate: 2.5
Injection volume: 20
Detector: UV 340

CHROMATOGRAM

Retention time: 6.4

OTHER SUBSTANCES

Simultaneous: tobramycin

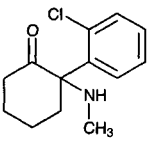
KEY WORDS

derivatization

REFERENCE

Dash,A.K.; Suryanarayanan,R. A liquid-chromatographic method for the determination of tobramycin, *J.Pharm.Biomed.Anal.*, **1991**, *9*, 237–245.

Ketamine



Molecular formula: $C_{13}H_{16}ClNO$
Molecular weight: 237.73
CAS Registry No.: 6740-88-1, 1867-66-9 (HCl)
Merck Index: 5306
Lednicer No.: 1 57

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 20 µL IS + 350 µL 200 mM pH 13 borate buffer, mix. Extract with 5 mL dichloromethane:ethyl acetate 80:20, vortex at 60 rpm for 10 min, centrifuge at 1500g at 15° for 3 min, remove organic phase and extract again with 3 mL dichloromethane:ethyl acetate 80:20. Combine the organic layers and evaporate them to dryness under a stream of nitrogen. Reconstitute the residue in 500 µL dichloromethane:ethyl acetate 80:20, extract with 2 mL 2 M HCl. Remove the aqueous layer and evaporate it to dryness at 45°. Reconstitute the residue in 100 µL mobile phase, inject a 60 µL aliquot.

HPLC VARIABLES

Column: 125 × 4 5 µm Purospher RP-18e (Merck)

Mobile phase: MeCN:30 mM pH 7.2 phosphate buffer 23:77
Column temperature: 20
Flow rate: 1.5
Injection volume: 60
Detector: UV 210

CHROMATOGRAM
Retention time: 16.26
Internal standard: nortilidine (6.29)
Limit of detection: 3 ng
Limit of quantitation: 5 μg/L

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: atropine, buprenorphine, diazepam, dopamide, furosemide, nalbuphine, omeprazole, phenobarbital, phytomenadione, propofol

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Bolze,S.; Boulieu,R. HPLC determination of ketamine, norketamine, and dehydronorketamine in plasma with a high-purity reversed-phase sorbent, *Clin.Chem.*, **1998**, *44*, 560–564.

SAMPLE
Matrix: blood
Sample preparation: Activate two 130 mg Sep-Pak Light C18 SPE cartridges with 1 mL MeOH and 1 mL water. Add 1 mL plasma onto SPE cartridge, wash with 1 mL water, 2 mL 5 mM pH 9.6 ammonium sulfate buffer containing 3% MeCN, and 1 mL 5 mM pH 9.6 ammonium sulfate buffer containing 20% MeCN. Displace washing solution with 200 μL 20 mM pH 2.1 phosphoric acid buffer containing 25% MeCN and elute with 500 μL of the same solution. Mix eluate with 1 mL 40 mM pH 11.5 sodium hydroxide. Add the mixture onto the second SPE cartridge, wash with 2 mL 5 mM pH 9.6 ammonium sulfate buffer containing 3% MeCN and with 1 mL 5 mM pH 9.6 ammonium sulfate buffer containing 20% MeCN. Displace washing solution with 200 μL 20 mM pH 2.1 phosphoric acid buffer containing 25% MeCN and elute with 500 μL of the same solution. Evaporate the eluate in a vacuum centrifuge to about 150 μL, add 12 μL 1 mM sodium hydroxide immediately before injection, inject the whole volume on the column. (Buffers were adjusted with ammonia. SPE flow-rate at all steps was approximately 1.5 mL/min.)

HPLC VARIABLES
Column: 150 × 4.0 5 μm Chiral AGP (Chrom Tech, Sweden)
Mobile phase: MeOH:10 mM pH 7.0 KH_2PO_4 16:84 (Buffer was adjusted with potassium hydroxide.)
Column temperature: 40
Flow rate: 1
Injection volume: 162
Detector: UV 220

CHROMATOGRAM
Retention time: 12 (S), 14 (R)
Limit of detection: 1.7 ng/mL (S), 2.0 ng/mL (R)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolite

KEY WORDS
plasma; SPE; chiral

REFERENCE
Svensson,J.O.; Gustafsson,L.L. Determination of ketamine and norketamine enantiomers in plasma by solid-phase extraction and high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *678*, 373–376.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL bisnortilidine in mobile phase + 100 μL 3 M NaOH, mix, add 5.75 mL ice-cold cyclohexane, agitate at 4° for 15 min, repeat extraction with 3 mL ice-cold cyclohexane. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 500 μL cyclohexane, evaporate to dryness under a stream of nitrogen, reconstitute in 0.2-1 mL mobile phase, mix for 30 s, let stand for 2 min, inject a 50-100 μL aliquot.

HPLC VARIABLES
Column: 100 × 4 AGP (Grom)
Mobile phase: Isopropanol:20 mM pH 7 phosphate buffer 2.5:97.5
Column temperature: 25
Flow rate: 0.5
Injection volume: 50-100
Detector: UV 215

CHROMATOGRAM
Retention time: 22 (S), 26 (R)
Internal standard: bisnortilidine (ethyl trans-2-amino-1-phenyl-3-cyclohexene-1-carboxylatehydrochloride) (one enantiomer only, separated by HPLC) (15)
Limit of detection: 20 ng/mL
Limit of quantitation: 40 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
chiral; plasma; pharmacokinetics

REFERENCE
Geisslinger,G.; Menzel-Soglowek,S.; Kamp,H.-D.; Brune,K. Stereoselective high-performance liquid chromatographic determination of the enantiomers of ketamine and norketamine in plasma, *J.Chromatogr.*, **1991**, *568*, 165–176.

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: 6.2
Limit of detection: 0.5-2.5 ng/mL

OTHER SUBSTANCES
Simultaneous: atipamezole, atropine, butorphanol, flumazenil, medetomidine, midazolam, xylazine

KEY WORDS
plasma; SPE; dog

REFERENCE
Kanazawa,H.; Nagata,Y.; Matsushima,Y.; Takai,N.; Uchiyama,H.; Nishimura,R.; Takeuchi,A. Liquid chromatography-mass spectrometry for the determination of medetomidine and other anaesthetics in plasma, *J.Chromatogr.*, **1993**, *631*, 215–220.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 500 μL MeCN:water 85% phosphoric acid 20:78:2, vortex for 10-15 s, filter (Amicon Centricon-10 microseparation system, 10000 molecular mass cut-off) while centrifuging at 3000 g for 30 min, inject a 20-100 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 100 × 4.6 3 μm Spherisorb phenyl
Mobile phase: MeCN:MeOH:10 mM NaH_4PO_4:85% phosphoric acid 10:30:59.8:0.2
Column temperature: 50
Injection volume: 20-100
Detector: UV 215

CHROMATOGRAM
Retention time: 5.1
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
horse; serum; ultrafiltrate

REFERENCE
Seay,S.S.; Aucoin,D.P.; Tyczkowska,K.L. Rapid high-performance liquid chromatographic method for the determination of ketamine and its metabolite dehydronorketamine in equine serum, *J.Chromatogr.*, **1993**, *620*, 281–287.

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

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 269

CHROMATOGRAM
Retention time: 4.19
Limit of detection: <120 ng/mL

KEY WORDS

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

REFERENCE

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

SAMPLE

Matrix: blood, urine

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

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

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

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

Column temperature: 30

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

Injection volume: 10-30

Detector: UV 202.8

CHROMATOGRAM
Retention time: 9.637

KEY WORDS
whole blood

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

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm YMC GEL, ODS-AM coated with poly-(R)-1-(α-naphthyl)ethyl methacrylamide (Prepare (R)-1-(α-naphthyl)ethyl methacrylamide by reacting methacryl chloride with (R)-1-(α-naphthyl)ethylamine. Prepare poly-(R)-1-(α-naphthyl)ethyl methacrylamide by polymerizing this compound in anhydrous benzene/THF with 2,2'-azobis(isobutyronitrile)(Caution! Benzene is a carcinogen!). Average molecular weight = 2500. Coat 4 g 5 μm YMC GEL, ODS-AM with 0.8 g of this polymer using dichloromethane as a solvent.)
Mobile phase: MeCN:0.5M sodium perchlorate 40:60
Flow rate: 1

CHROMATOGRAM
Retention time: k' 3.18 (α = 1.21)

OTHER SUBSTANCES
Also analyzed: propranolol

KEY WORDS
chiral

REFERENCE
Oi,N.; Hashimoto,S.; Ishizuka,N.; Ohtake,J. Enantiomer separation with poly-(R)-1-(α-naphthyl)-ethyl-methacrylamide coated on ODS silica gel by reversed phase HPLC, *Biomed.Chromatogr.*, **1997**, *11*, 296–297.

SAMPLE
Matrix: solutions

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

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone,

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

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Sumchiral CSP 10 (Sumika Chemical Analysis Service)
Mobile phase: n-Hexane:EtOH:trifluoroacetic acid 200:40:0.6
Flow rate: 1
Detector: UV 230-280

CHROMATOGRAM

Retention time: k' 7.78 (first enantiomer)

KEY WORDS

chiral; α = 1.12

REFERENCE

Oi,N.; Kitahara,H.; Aoki,F. Direct enantiomer separations by high-performance liquid chromatography with chiral urea derivatives as stationary phases, *J.Chromatogr.A*, **1995**, *694*, 129–134.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 CSP-4 (Prepare as follows. Add a solution of 1.07 g L-valyl-L-valyl-L-valine isopropylester (Bunseki Kagaku 1079, 28, 125) in 30 mL dry dioxane (Caution! Dioxane is a carcinogen!) dropwise to a mixture of 2.2 g 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride) in 20 mL dry dioxane stirred at 0°, add 3 g anhydrous sodium carbonate at room temperature, stir, filter, evaporate to give a colorless solid. Dissolve 8.3 g of this solid in 30 mL dry dioxane, add 2 g N-(2-aminoethyl)-3-aminopropyltrimethoxysilane, add 1.5 g anhydrous sodium carbonate, reflux with stirring for 40 h, filter, add 3 g dried 10 μm LiChrosorb Si 100, reflux with slow stirring for 10 h, cool, filter. Wash the solid with dioxane, MeOH, and diethyl ether, dry under reduced pressure (J.Chromatogr. 1984, 292, 427).)
Mobile phase: Hexane:EtOH:trifluoroacetic acid 96:4:0.24
Detector: UV

CHROMATOGRAM

Retention time: k' 7.85 (first enantiomer)

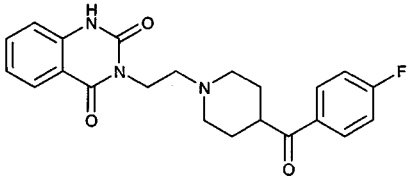
KEY WORDS

chiral; α = 1.09

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.

Ketanserin



Molecular formula: $C_{22}H_{22}FN_3O_3$
Molecular weight: 395.43
CAS Registry No.: 74050-98-9
Merck Index: 5307
Lednicer No.: 3 193

SAMPLE

Matrix: blood
Sample preparation: 25 μL Serum + 50 μL MeOH, vortex for 30 s, centrifuge at 13600 g for 5 min, inject a 25 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeCN:buffer:water 31:50:19 (Buffer was 2% acetic acid adjusted to pH 7.0 with ammonium hydroxide.)
Flow rate: 1
Injection volume: 25
Detector: F ex 225 em no emission filter

CHROMATOGRAM

Retention time: 7.7
Limit of quantitation: 40 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

rat; serum; pharmacokinetics

REFERENCE

Wong,Y.W.; Skinner,M.H. Rapid method for the determination of ketanserin in rat serum by high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.*, **1991**, *571*, 318–323.

SAMPLE

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

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 1.4

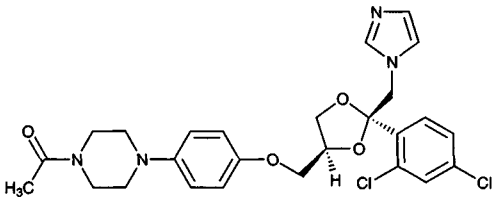
OTHER SUBSTANCES

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

REFERENCE

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

Ketoconazole



Molecular formula: $C_{26}H_{28}Cl_2N_4O_4$
Molecular weight: 531.44
CAS Registry No.: 65277-42-1
Merck Index: 5313
Lednicer No.: 3 132

SAMPLE

Matrix: blood
Sample preparation: 500 μL Plasma + 2 μg clotrimazole + hexane:isoamyl alcohol 98.5:1.5, vortex, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 250 μL MeCN, inject an aliquot.

HPLC VARIABLES

Column: 150 × 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, microsomal incubations
Sample preparation: Vortex 1 mL plasma or microsomal incubation with 200 μL 5 μg/mL diazepam and 100 μL 5 M NaOH solution for 10 s, add 5 mL butan-1-ol:hexane 2:98, vortex for 1 min, centrifuge at 2000 g and 4° for 5 min, evaporate the organic phase to dryness at 40° using a vacuum vortex evaporator, reconstitute the residue in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 5 μm Nova-Pak C18
Mobile phase: MeCN:buffer 35:65 (Buffer was water containing 1% triethylamine, adjusted to pH 3 with orthophosphoric acid.)
Flow rate: 2
Injection volume: 50
Detector: UV 240

CHROMATOGRAM

Retention time: 6.3
Internal standard: diazepam

OTHER SUBSTANCES

Extracted: amitriptyline, nortriptyline
Noninterfering: furafylline, hydroxyamitriptyline, hydroxynortriptyline, quinidine, mephenytoin, triacetyloleandomycin

KEY WORDS

human; liver; rat; plasma

REFERENCE
Ghahramani,P.; Lennard,M.S. Quantitative analysis of amitriptyline and nortriptyline in human plasma and liver microsomal preparations by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *685*, 307–313.

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

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

CHROMATOGRAM
Retention time: 15.738

KEY WORDS
whole blood

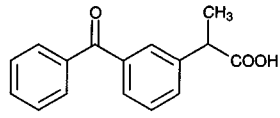
REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 4 5 μm Hibar RP 18 (Merck)
Mobile phase: MeCN:water:diethylamine 48:55:0.02
Flow rate: 1.2
Injection volume: 20, 100
Detector: UV 254

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

Ketoprofen



Molecular formula: $C_{16}H_{14}O_3$
Molecular weight: 254.29
CAS Registry No.: 22071-15-4
Merck Index: 5316
Lednicer No.: 2 64

SAMPLE

Matrix: blood
Sample preparation: Mix 50 μL plasma with 150 μl 25 μg/mL ibufenac in MeCN:water 95:5, vortex for 30 s, centrifuge for 1 min using a Beckman Microfuge (Beckman Instruments, Palo Alto, CA). Inject a 20 gmL aliquot of the supernatant.

HPLC VARIABLES

Column: 33 × 4.6 3 μm C18 (Perkin Elmer, Norwalk, CT)
Mobile phase: MeCN:buffer 40:60 (Prepare mobile phase as follows. Dissolve 4 mL concentrated phosphoric acid in 600 mL water and mix with 400 mL MeCN.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 220

CHROMATOGRAM

Retention time: 1.1
Internal standard: ibufenac (2.6)
Limit of detection: 1 μg/mL
Limit of quantitation: 5 μg/mL

OTHER SUBSTANCES

Simultaneous: dicloxacillin, ibuprofen
Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, amitriptyline, aspirin, aztreonam, barbituric acid, brompheniramine, cafazolin, caffeine, carbamazepine, carbamazepine epoxide, cephalexin, chlorpheniramine, clonazepam, clotrimazole, desipramine, desmethyldoxepin, digitoxin, digoxin, disopyramide, doxepin, ethosuximide, felbamate, gentamicin, imipenem, imipramine, lidocaine, maprotiline, mephenytoin, mephobarbital, metharbital, methsuximide, methylsuccinimide, nortriptyline, paramethadione, phenacemide, phenobarbital, phensuximide, phenylpropanolamine, phenytoin, primidone, protriptyline, sulfamethoxazole, theophylline, tobramycin, trimethadione, trimethoprim, vancomycin.

KEY WORDS

plasma

REFERENCE

Rifai,N.; Lafi,M.; Sakamoto,M.; Law,T. Measurement of plasma ketoprofen by a rapid high-performance liquid chromatography assay, *Ther.Drug Monit.*, **1997**, *19*, 175–178.

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL 100 mg C 18 SPE cartridge (Varian) with 1 mL MeOH and 1 mL 100 mM pH 2.5 phosphate buffer. Mix 100 μL 50 μg/mL 3,4-dimethoxybenzoic acid in MeOH with 1 mL plasma. Vortex with 360 mg ammonium sulfate for 30 min to deproteinate the plasma. Centrifuge at 8000 g at 4°. for 30 min, acidify the supernatant with 3 ml 100 mM sulfuric acid. Add the supernatant to the SPE cartridge, wash twice with 750 μL MeOH:100 mM pH 2.5 phosphate buffer 20:80. Elute with two 500 μL portions of MeOH:50 mM pH 7.4 phosphate buffer 75:25. Inject a 40 μL aliquot.

HPLC VARIABLES

Guard column: Supelco C18

Column: 250 × 4.6 Chirex 3005 [(R)-1-naphtylglycine and 3,5-dinitrobenzoic acid] (Phenomenex, Torrance, CA, USA)
Mobile phase: 20 mM ammonium acetate in MeOH
Flow rate: 1.2
Injection volume: 40
Detector: UV 254

CHROMATOGRAM
Retention time: 14.2 (R-(-)), 16.1 (S-(+))
Internal standard: 3,4-dimethoxybenzoic acid (18.1)
Limit of quantitation: 160 ng/mL

KEY WORDS
SPE; chiral; pharmacokinetics

REFERENCE
Boisvert,J.; Caillé,G.; McGilveray,I.J.; Qureshi,S.A. Quantification of ketoprofen enantiomers in human plasma based on solid-phase extraction and enantioselective column chromatography, *J.Chromatogr.B*, **1997**, *690*, 189–193.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 1 mL 1 M pH 2.0 phosphate buffer + IS, extract with 7 mL diethyl ether, evaporate, reconstitute the residue in 250 μL MeCN:5 mM pH 7.0 phosphate buffer 10:90, inject 50 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 LiChrocart
Column: 125 × 3 5 μm Ecocart packed with LiChrospher 100 RP-18
Mobile phase: MeCN:50 mM pH 7.0 phosphate buffer 16:84
Column temperature: 35
Flow rate: 0.6
Injection volume: 50
Detector: UV 260

CHROMATOGRAM
Retention time: 14.88
Internal standard: naproxen (10.85)
Limit of detection: 30 pg/mL
Limit of quantitation: 100 pg/mL

KEY WORDS
horse; plasma

REFERENCE
Baeyens,W.R.G.; Van Der Weken,G.; Van Overbeke,A.; Corveleyn,S.; Remon,J.P.; Deprez,P. Comparative narrow-bore high-performance liquid chromatographic determination of ketoprofen in horse plasma, *Biomed.Chromatogr.*, **1998**, *12*, 167–169.

SAMPLE
Matrix: blood
Sample preparation: Acidify plasma with an equal volume of 100 mM pH 3.2 phosphoric acid, inject a 20 μL aliquot onto column A and elute to waste with mobile phase A. After 5 min elute the contents of column A onto column B with mobile phase B, monitor the effluent from column B. (Before each run wash column A with MeCN:100 mM pH 3.2 phosphate buffer 20:80 for 3 min, with water for 1 min, with MeOH for 3 min, and with mobile phase A. Equilibrate column B with mobile phase B.)

HPLC VARIABLES
Column: A 10 × 4 5 μm Develosil NH_2-5 (Nomura Chemical Co., Japan) + 10 × 4 Nucleosil 5CN (Macherey-Nagel, Düren, Germany); B 150 × 4.6 Ultron ES-OVM G bonded silica column (Shinwa Kako Co., Japan)

Mobile phase: A 100 mM pH 3.2 phosphate buffer; B MeOH:100 mM pH 3.2 phosphate buffer 20:80
Column temperature: 20
Flow rate: 0.8
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 9.6 (S(+)), 12.0 (R(-))

KEY WORDS
column-switching; chiral; plasma

REFERENCE
Tamai,G.; Edani,M.; Imai,H. Determination of ketoprofen enantiomers in plasma by solid phase extraction and column switching high performance liquid chromatography, *Anal.Sci.*, **1991**, *7*, 29–32.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL tolmetin solution + 500 μL pH 1.8 phosphate buffer, extract with 1-butanol/MTBE. Remove the organic layer and add it to 500 μL pH 6.1 ammonium acetate buffer, mix, inject an aliquot of the aqueous layer.

HPLC VARIABLES
Column: 150 × 4.6 5 μm 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: Condition a 1 mL Bond-Elut C18 SPE cartridge with 1 mL MeOH. 1 mL Serum + 1 mL water + 20 μL saturated ammonium sulfate solution + 60 μL concentrated HCl, vortex for 3 min, add to the SPE cartridge, wash with three 1 mL portions of water, allow to dry for 3 min, elute with five 500 μL portions of MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Spheri-5 cyano
Mobile phase: MeCN:MeOH:water:phosphoric acid 21:22:56.5:0.5
Flow rate: 0.5
Injection volume: 100
Detector: F ex 248 em 335 (filter) following post-column reaction. The column effluent flowed through a knitted 7.9 m × 0.3 mm ID PTFE coil irradiated with an SC3-9 UV lamp (UVP, San Gabriel CA) and cooled with a fan to the detector.

CHROMATOGRAM
Retention time: 6.5
Internal standard: ketoprofen

OTHER SUBSTANCES
Extracted: fenbufen

KEY WORDS
ketoprofen is IS; post-column reaction; post-column photochemical derivatization; serum; SPE

REFERENCE
Siluveru,M.; Stewart,J.T. Determination of fenbufen and its metabolites in serum by reversed-phase high-performance liquid chromatography using solid-phase extraction and on-line post-column ultraviolet irradiation and fluorescence detection, *J.Chromatogr.B*, **1996**, *682*, 89–94.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 200 μL MeCN, vortex for 30 s, centrifuge at 14000 g for 30 s, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Econosphere CN
Mobile phase: MeCN:water:phosphoric acid 4:100:0.02
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Limit of detection: 100 ng/mL

KEY WORDS
comparison with capillary electrophoresis; serum

REFERENCE
Friedberg,M.; Shihabi,Z.K. Ketoprofen analysis in serum by capillary electrophoresis, *J.Chromatogr.B*, **1997**, *695*, 193–198.

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

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

CHROMATOGRAM
Retention time: 19.628

KEY WORDS
whole blood

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

SAMPLE
Matrix: cell suspensions
Sample preparation: Centrifuge cell suspension at 2000 g for 4 min. Remove a 2 mL aliquot of the supernatant and add it to 200 μL 4 mg/mL IS in DMF, mix, add 200 μL 5 M HCl, extract twice with 3 mL portions of toluene. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL dichloromethane, add 20 μL 40 mg/mL 1-hydroxybenzotriazole in dichloromethane:pyridine 99:1, add 300 μL 40 mg/mL 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in dichloromethane, add 300 μL 40 mg/mL (-)-(S)-α-methylbenzylamine in dichloromethane, let stand for 30 min, evaporate to dryness, reconstitute with 500 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 10 mm long Techsphere ODS (HPLC Technology, Macclesfield UK)
Column: 250 × 5 5 μm Techsphere ODS (HPLC Technology, Macclesfield UK)
Mobile phase: MeCN:7.5 mM NaH_2PO_4 50:50, containing 5 mM sodium pentanesulfonate, pH adjusted to 2.8 with phosphoric acid
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 6.60, 7.55 (enantiomers)
Internal standard: phenylacetic acid (k' 2.50)
Limit of detection: 1 μg/mL

KEY WORDS
derivatization; chiral

REFERENCE
Thomason,M.J.; Hung,Y.-F.; Rhys-Williams,W.; Hanlon,G.W.; Lloyd,A.W. Indirect enantiomeric separation of 2-arylpropionic acids and structurally related compounds by reversed phase HPLC, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1765–1774.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 250 μL Microsomal incubation, 150 μL ice-cold MeCN and 2.5 ng ketoprofen, centrifuge. Extract the mixture with 4 mL ethyl acetate, centrifuge at 3000 rpm for 10 min, remove the organic fraction and evaporate it under a gentle stream of nitrogen at 40°. Dissolve the residue in 30 μL MeOH and dilute to 60 μL with water. Inject a 30 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm RP-18 (Kanto Chemical, Tokyo)
Mobile phase: MeCN:water 40:60 containing 0.6% acetic acid
Column temperature: 35
Flow rate: 1
Injection volume: 30
Detector: UV 260

CHROMATOGRAM
Retention time: 18.0

OTHER SUBSTANCES
Extracted: metabolites, indomethacin

KEY WORDS
liver; pharmacokinetics; ketoprofen is IS

REFERENCE
Nakajima,M.; Inoue,T.; Shimada,N.; Tokudome,S.; Yamamoto,T.; Kuroiwa,Y. Cytochrome P450 2C9 catalyzes indomethacin O-demethylation in human liver microsomes, *Drug Metab.Dispos.*, **1998**, *26*, 261–266.

SAMPLE
Matrix: permeate

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 40:60 adjusted to pH 5.5 with NaOH
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 260

OTHER SUBSTANCES
Also analyzed: carbamazepine, fenbufen, indomethacin, α.-naphthoquinone, naproxen, tolmetin

REFERENCE
Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE
Matrix: solutions
Sample preparation: Mix 50 μL of a 0.001-5 mM solution in MeCN with 50 μL 1 mM DNS-APy in MeCN containing 50 mM 2,2'-dipyridyl disulfide and 50 mM triphenylphosphine, let stand at room temperature for 30 min. Remove a 10 μL aliquot and dilute it to 100 μL with MeCN, inject a 2 μL aliquot. (Synthesis of DNS-APy, 1-(5-dimethylamino-1-naphthalenesulfonyl)-(S)-3-aminopyrrolidine, is as follows. Cool a solution of 16.4 g (R)-(+)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane:acetone 95:5 to obtain (3R)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3R)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65°, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry over anhydrous magnesium sulfate, evaporate to obtain (3S)-3-azido-1-(phenylmethyl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3S)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the minimum amount of EtOH to obtain (3S)-(+)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). (3S)-(+)-Aminopyrrolidine dihydrochloride is also reported to be available from Tokyo Kasei. Stir 800 mg (3S)-(+)-3-aminopyrrolidine dihydrochloride and 2 mL triethylamine in 800 mL MeCN at 0-10°, add a solution of 440 mg dansyl chloride in 80 mL MeCN dropwise, stir in the dark for 30 min, evaporate to dryness under reduced pressure, dissolve the residue in 200 mL 5% HCl, wash twice with 40 mL portions of dichloromethane. Adjust the pH of the organic layer to 13-14 with 5% NaOH, extract twice with 10 mL portions of dichloromethane. Combine the organic layers and wash them with 80 mL water. Dry the organic layer over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, dissolve the residue in dichloromethane:MeOH 90:10, chromatograph on silica gel with dichloromethane:MeOH 90:10. Collect the greenish-yellow fluorescent band and evaporate it under reduced pressure to obtain DNS-APy as a greenish-yellow oil.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm TSK gel ODS-80TM (Tosoh)
Mobile phase: MeCN:water 50:50

Flow rate: 1
Injection volume: 2
Detector: F ex 340 em 530

CHROMATOGRAM
Retention time: 37 ((S)-(+)), 40 ((R)-(-))
Limit of detection: 0.1 pmole

OTHER SUBSTANCES
Simultaneous: pranoprofen

KEY WORDS
derivatization; chiral

REFERENCE
Al-Kindy,S.; Santa,T.; Fukushima,T.; Homma,H.; Imai,K. 1-(5-Dimethylamino-1-naphthalenesulphonyl)-(S)-3-aminopyrrolidine (DNS-Apy) as a fluorescence chiral labelling reagent for carboxylic acid enantiomers, *Biomed.Chromatogr.*, **1997**, *11*, 137–142.

SAMPLE
Matrix: urine
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL ethyl acetate and dry by aspiration of air. Evaporate an aliquot of 100 μL 20 μg/mL IS in MeOH to dryness at 37°. Add 1 mL urine, vortex, add 250 μL 1 M pH 5.0 acetate buffer, vortex. Add 250 μL of the mixture to the SPE cartridge, dry by aspiration of air, elute with 3 mL ethyl acetate, evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject a 10-30 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:50 mM pH 5.0 phosphate buffer 42:5
Flow rate: 0.9
Injection volume: 10-30
Detector: UV 230

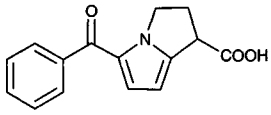
CHROMATOGRAM
Retention time: 6
Internal standard: indomethacin (18.5)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: diclofenac, ibuprofen, felbinac, fenbufen, flurbiprofen, loxoprofen, mefenamic acid, naproxen, piroxicam, sulindac

KEY WORDS
SPE

REFERENCE
Hirai,T.; Matsumoto,S.; Kishi,I. Simultaneous analysis of several non-steroidal anti-inflammatory drugs in human urine by high-performance liquid chromatography with normal solid-phase extraction, *J.Chromatogr.B*, **1997**, *692*, 375–388.

Ketorolac



Molecular formula: $C_{15}H_{13}NO_3$
Molecular weight: 255.27
CAS Registry No.: 74103-06-3, 74103-07-4 (tromethamine)
Merck Index: 5318
Lednicer No.: 4 81

SAMPLE

Matrix: blood
Sample preparation: Dilute 0.1-1 mL plasma to 1 mL with water, add 100 μL 2 μg/mL IS in MeOH:water 90:10, add 100 μL 500 mM pH 3 sodium acetate buffer, add 6 mL hexane:ethyl acetate 70:30, vortex vigorously for 5 min. Centrifuge at 2000 rpm for 2-5 min, place in dry ice/isopropanol or dry ice/MeOH bath to freeze the aqueous layer. Decant the organic layer and evaporate it to dryness under a stream of nitrogen at 38°. Add 500 μL MeOH:water 90:10 and 3 mL hexane, sonicate for 15 s, vortex vigorously for 3 min, let stand for at least 5 min, discard the hexane layer. Evaporate the remaining MeOH/water to dryness under a stream of nitrogen at 38°. Add 100 μL MeOH and 100 μL mobile phase, sonicate for 30 s, vortex for 30 s, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 4 μm Nova Pak C18
Mobile phase: MeCN:0.05% phosphoric acid 34:66
Flow rate: 1
Injection volume: 20
Detector: UV 317

CHROMATOGRAM

Retention time: 5.5
Internal standard: RS-37414-000, (±)-5-p-fluorobenzoyl-1,2-dihydro-3H-pyrrolo[1,2a]-pyrrole-1-carboxylic acid (7)
Limit of quantitation: 10 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Tsina,I.; Chu,F.; Kaloostian,M.; Pettibone,M.; Wu,A. HPLC method for the determination of ketorolac in human plasma, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 957–967.

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 on to 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.Chromatogr.Rel.Technol.*, **1996**, *19*, 89–99.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 100 μL 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 100 μL 2 mg/mL 4-(dimethylamino)pyridine in MeCN, add 100 μL 60 mM trichloroethyl chloroformate in MeCN, add 1 M L-leucinamide in MeCN, let stand for 2 min, add 500 μL 250 mM HCl, extract with chloroform. Remove the organic layer and evaporate it to dryness, reconstitute the residue in mobile phase, inject a 10-100 μL aliquot. (A 7% conversion of S to R is observed during the derivatization procedure. No racemization is observed using a direct procedure with a chiral column.)

HPLC VARIABLES
Column: 100 × 4.6 5 μm Partisil 5 ODS-2
Mobile phase: MeCN:60 mM KH_2PO_4:triethylamine 30:70:0.02
Flow rate: 1
Injection volume: 10-100
Detector: UV 310

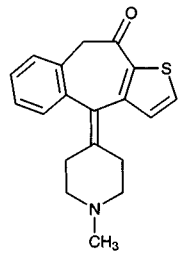
CHROMATOGRAM
Retention time: 10.7 (R), 19.5 (S)
Internal standard: ketorolac

OTHER SUBSTANCES
Extracted: tiaprofenic acid

KEY WORDS
derivatization; chiral; plasma; ketorolac is IS

REFERENCE
Vakily,M.; Jamali,F. Pharmacokinetics of tiaprofenic acid in humans: Lack of stereoselectivity in plasma using both direct and precolumn derivatization methods, *J.Pharm.Sci.*, **1996**, *85*, 638–642.

Ketotifen



Molecular formula: $C_{19}H_{19}NOS$
Molecular weight: 309.43
CAS Registry No.: 34580-13-7, 34580-14-8 (fumarate)
Merck Index: 5319
Lednicer No.: 3 239

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood. Dilute 1 mL plasma with 100 μL 1 M pH 9 phosphate buffer and 100 μL water, add 8 mL diethyl ether, extract. Evaporate the organic layer and dissolve the residue in 400 μL mobile phase. Inject 200 μL aliquot. Tissue. Homogenize the brain with 2

fold the weight of water. Dilute 1500 μL brain homogenate with 500 μL 1 M pH 9 phosphate buffer, add 8 mL diethyl ether, extract. Evaporate the organic layer, dissolve the residue in 400-500 μL mobile phase. Inject a 200 μL aliquot.

HPLC VARIABLES
Column: 250 × 6 Intersil ODS-2
Mobile phase: MeCN:0.018% TFA 20:80 (plasma), MeCN:0.018% TFA 15:85 (tissue)
Column temperature: 40
Flow rate: 0.7
Injection volume: 200
Detector: UV 300

CHROMATOGRAM
Limit of quantitation: 5 ng/mL (plasma), 40 ng/mL (brain)

KEY WORDS
brain; cat; mouse; pharmacokinetics; plasma; rat

REFERENCE
Kato,M.; Nishida,A.; Aga,Y.; Kita,J.; Kudo,Y.; Narita,H.; Endo,T. Pharmacokinetic and pharmacodynamic evaluation of central effect of the novel antiallergic agent betotastine besilate, *Arzneimittelforschung*, **1997**, *47*, 1116–1124.

SAMPLE
Matrix: solutions

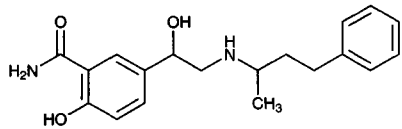
HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 14.72

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, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

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

Labetalol



Molecular formula: $C_{19}H_{24}N_2O_3$
Molecular weight: 328.41
CAS Registry No.: 36894-69-6, 32780-64-6 (HCl)
Merck Index: 5341
Lednicer No.: 3 24; 4 20

fold the weight of water. Dilute 1500 μL brain homogenate with 500 μL 1 M pH 9 phosphate buffer, add 8 mL diethyl ether, extract. Evaporate the organic layer, dissolve the residue in 400-500 μL mobile phase. Inject a 200 μL aliquot.

HPLC VARIABLES
Column: 250 × 6 Intersil ODS-2
Mobile phase: MeCN:0.018% TFA 20:80 (plasma), MeCN:0.018% TFA 15:85 (tissue)
Column temperature: 40
Flow rate: 0.7
Injection volume: 200
Detector: UV 300

CHROMATOGRAM
Limit of quantitation: 5 ng/mL (plasma), 40 ng/mL (brain)

KEY WORDS
brain; cat; mouse; pharmacokinetics; plasma; rat

REFERENCE
Kato,M.; Nishida,A.; Aga,Y.; Kita,J.; Kudo,Y.; Narita,H.; Endo,T. Pharmacokinetic and pharmacodynamic evaluation of central effect of the novel antiallergic agent betotastine besilate, *Arzneimittelforschung*, **1997**, *47*, 1116–1124.

SAMPLE
Matrix: solutions

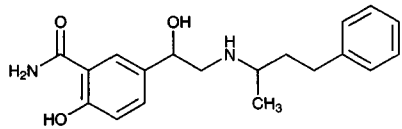
HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 14.72

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, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

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

Labetalol



Molecular formula: $C_{19}H_{24}N_2O_3$
Molecular weight: 328.41
CAS Registry No.: 36894-69-6, 32780-64-6 (HCl)
Merck Index: 5341
Lednicer No.: 3 24; 4 20

SAMPLE
Matrix: amniotic fluid, blood, fetal tracheal fluid
Sample preparation: 50-500 μL Plasma, fetal tracheal fluid, or amniotic fluid + water to 750 μL total volume, add 500 μL pH 9.5 carbonate buffer, add 6 mL ethyl acetate, extract. Remove the organic layer and extract it with 600 μL 10 mM phosphoric acid, inject a 60 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 10 × 3 Chiral AGP (Regis)
Column: 100 × 4 10 μm Chiral AGP (Regis)
Mobile phase: 20 mM phosphate buffer containing 15 mM tetrabutylammonium phosphate, degas with helium for 30 min, adjust pH to 7.10 with phosphoric acid
Flow rate: 0.5
Injection volume: 60
Detector: F ex 230 em 400

CHROMATOGRAM
Retention time: 19 (SR), 23 (SS), 28 (RS), 34 (RR)
Limit of detection: 0.15 ng

KEY WORDS
plasma; sheep; diastereomers; chiral

REFERENCE
Doroudian,A.; Yeleswaram,K.; Rurak,D.W.; Abbott,F.S.; Axelson,J.E. Sensitive high-performance liquid chromatographic method for direct separation of labetalol stereoisomers in biological fluids using an α_1-acid glycoprotein stationary phase, *J.Chromatogr.*, **1993**, *619*, 79–86.

SAMPLE
Matrix: bile, perfusate
Sample preparation: 500 μL Perfusate or 100 μL bile + 1 mL 1 M pH 10.3 carbonate buffer + 5 mL acid-washed diethyl ether, vortex, centrifuge. Remove the organic layer and add it to 125 μL 0.5% phosphoric acid, extract, inject a 10 μL aliquot of the aqueous layer. (Deconjugate 500 μL perfusate with 250 μL 8000 U/mL β-D-glucuronidase/aryl sulfatase in 200 mM pH 4.5 sodium acetate buffer, heat at 40° for 1 h, proceed as above.)

HPLC VARIABLES
Column: 100 × 8 4 μm Novapak phenyl radial compression
Mobile phase: MeCN:water:triethylamine 23:77:1 adjusted to pH 3.6 with concentrated phosphoric acid
Flow rate: 3
Injection volume: 10
Detector: F ex 295 em 360

CHROMATOGRAM
Retention time: 6.9
Internal standard: labetalol

OTHER SUBSTANCES
Extracted: propranolol

KEY WORDS
sheep; liver; labetalol is IS

REFERENCE
Ring,J.A.; Ghabrial,H.; Ching,M.S.; Shulkes,A.; Smallwood,R.A.; Morgan,D.J. Fetal hepatic propranolol metabolism. Studies in the isolated perfused fetal sheep liver, *Drug Metab.Dispos.*, **1995**, *23*, 190–196.

SAMPLE
Matrix: blood

Sample preparation: 1 mL Plasma + 100 μL water + 100 μL 20% sodium metabisulfite (freshly prepared) + 1 mL 1 M pH 10.2 sodium carbonate + 8 mL ether, shake gently for 10 min on a reciprocating shaker, centrifuge at 2000 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, centrifuge for 4 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:buffer 50:50 (Buffer was 10 mM potassium phosphate adjusted to pH 3.4 with 5 M HCl.)
Injection volume: 50
Detector: F ex 310 em 380 (filter)

CHROMATOGRAM
Retention time: 4.8
Internal standard: labetalol

OTHER SUBSTANCES
Extracted: propranolol

KEY WORDS
plasma; labetalol is IS

REFERENCE
Drummer,O.H.; McNeil,J.; Pritchard,E.; Louis,W.J. Combined high-performance liquid chromatographic procedure for measuring 4-hydroxypropranolol and propranolol in plasma: Pharmacokinetic measurements following conventional and slow-release propranolol administration, *J.Pharm.Sci.*, **1981**, *70*, 1030–1032.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 1 mL buffer + 10 mL chloroform:isopropanol 100:2 , shake on a horizontal reciprocal shaker at 30 strokes/min for 10 min, centrifuge at 1000 g for 10 min. Remove 8 mL of the organic phase and evaporate it to dryness under a stream of nitrogen, dissolve the residue in 50 μL MeCN:MeOH:100 mM HCl 30:20:5, inject a 25 μL aliquot. (Buffer was 3.80 g/L sodium bicarbonate and 0.504 g/L sodium carbonate, pH 8.8.)

HPLC VARIABLES
Column: 150 × 6 5 μm Asahipack ODP 50 (octadecyl-bonded polymer gel)
Mobile phase: MeCN:50 mM pH 11.5 diethylamine 84:16 which was 36 mM in NaCl
Flow rate: 0.9
Injection volume: 25
Detector: F ex 340 em 389

CHROMATOGRAM
Retention time: 9.5 (RR-SS), 10.5 (RS-SR)
Limit of detection: 3 ng/mL

KEY WORDS
plasma; rat; pharmacokinetics; diastereomers

REFERENCE
Grellet,J.; Michel-Gueroult,P.; Ducint,D.; Saux,M.C. Sensitive high-performance liquid chromatographic method for the determination of labetalol diastereoisomers in plasma samples without derivatization, *J.Chromatogr.B*, **1994**, *652*, 59–66.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of

the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 229

CHROMATOGRAM

Retention time: 4.58
Limit of detection: <120 ng/mL

KEY WORDS

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

REFERENCE

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

SAMPLE

Matrix: bulk
Sample preparation: 1 mg Labetalol + 3 mg reagent + 100 μL MeCN + 50 μL water + 3 μL triethylamine, vortex, heat at 60° for 1 h. Remove a 150 μL aliquot and add it to 400 μL MeCN, vortex, inject a 5 μL aliquot. (Prepare the reagent, (4S-cis)-2,2-dimethyl-5-isothiocyanato-4-

phenyl-1,3-dioxane (PHEDIT), as follows. Add dropwise 5 g (4S,5S)-(+)-5-amino-2,2-dimethyl-4-phenyl-1,3-dioxane in 150 mL dichloromethane to 5 g 1,1'-thiocarbonyldiimidazole stirred in 150 mL dichloromethane, stir at room temperature for 3 h, wash the reaction mixture three times with 300 mL portions of 5% sodium bicarbonate, wash three times with 300 mL portions of water. Dry the organic layer over anhydrous sodium sulfate for 20 min, evaporate to dryness under reduced pressure, recrystallize from EtOH to give (4S-cis)-2,2-dimethyl-5-isothiocyanato-4-phenyl-1,3-dioxane as a pale yellow solid (mp 105-6°).)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeOH:20 mM pH 4.60 $(NH_4)H_2PO_4$ 63:37
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 19.7 (RS or SR), 21.4 (RS or SR), 23.8 (SS), 30.0 (RR)

KEY WORDS
derivatization; chiral; comparison with other derivatization reagents

REFERENCE
Desai,D.M.; Gal,J. Reversed-phase high-performance liquid chromatographic separation of the stereoisomers of labetalol via derivatization with chiral and non-chiral isothiocyanate reagents, *J.Chromatogr.*, **1992**, *579*, 165–171.

SAMPLE
Matrix: bulk
Sample preparation: 1 mg Labetalol + 3 mg 1-naphthalenemethyl isothiocyanate (Trans World Chemicals, Chevy Chase MD) + 100 μL MeCN + 50 μL water + 3 μL triethylamine, vortex, heat at 60° for 1 h. Remove a 150 μL aliquot and add it to 400 μL MeCN, vortex, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeOH:20 mM pH 4.60 $(NH_4)H_2PO_4$ 70:30
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 10.42 (RS/SR), 14.73 (SS/RR)

KEY WORDS
derivatization; comparison with other derivatization reagents

REFERENCE
Desai,D.M.; Gal,J. Reversed-phase high-performance liquid chromatographic separation of the stereoisomers of labetalol via derivatization with chiral and non-chiral isothiocyanate reagents, *J.Chromatogr.*, **1992**, *579*, 165–171.

SAMPLE
Matrix: formulations, urine
Sample preparation: Urine. Condition a Bond Elut Certify LRC SPE cartridge with 6 mL water. Mix 3.75 mL urine with 750 μL 1 M pH 9.0 borate buffer, centrifuge at 734 g for 5 min. Add a 3 mL aliquot to the SPE cartridge. Wash with 2 mL water, 1 mL 100 mM pH 4.0 acetate buffer, and 2 mL MeOH using vacuum (5 mmHg). Dry the SPE cartridge under vacuum (<150 mmHg) for 5 min. Elute with 2 mL 2% ammonia in chloroform:isopropanol 60:40 using vacuum (2 mmHg) (Caution! Chloroform is a carcinogen!). Evaporate the eluate to dryness under a gentle stream of nitrogen at 60°. Dissolve the residue in 1 mL mobile phase, inject an aliquot. Tablets. Crush and mix tablets to a fine powder, weigh, dissolve in water, shake for 20 min,

filter (Whatman No. 41 filter-paper), wash, make up to a fixed volume. Dilute an aliquot with mobile phase and inject an aliquot.

HPLC VARIABLES
Guard column: μ-Bondapak C18
Column: 250 × 4.6 5 μm Supelcosil ABZ + Plus
Mobile phase: MeCN:water 30:70 containing 5 mM acetate buffer adjusted to pH 4.5 with acetic acid or 1 M KOH
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: E, PAR Model 400, glassy carbon cell + 1.3 V, Ag/AgCl reference electrode, platinum auxiliary electrode in the DC mode with 5-s low-pass filter time constant, current range 20-100 nA

CHROMATOGRAM
Retention time: 5.95
Limit of detection: 5 ng/mL (solutions), 10 ng/mL (urine)
Limit of quantitation: 20 μg/mL (urine)

KEY WORDS
tablets; SPE

REFERENCE
Ceniceros,C.; Maguregui,M.I.; Jiménez,R.M.; Alonso,R.M. Quantitative determination of the β-blocker labetalol in pharmaceuticals and human urine by high-performance liquid chromatography with amperometric detection, *J.Chromatogr.B*, **1998**, *705*, 97–103.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 5 μm Nova-Pak C18
Mobile phase: MeOH:buffer 40:60 (Buffer was pH 4.0 phosphate buffer (ionic strength = 0.1) containing 3.33 mM N,N-dimethyloctylamine, pH readjusted to 4.00 with 85% phosphoric acid.)
Column temperature: 30
Flow rate: 1
Injection volume: 100
Detector: UV 220

CHROMATOGRAM
Retention time: k' 3.67

OTHER SUBSTANCES
Also analyzed: bisoprolol, carvedilol, metipranolol, oxprenolol, talinolol, toliprolol

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

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a solution in mobile phase.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:40 mM pH 4.3 acetate buffer 75:25
Flow rate: 1.5
Injection volume: 20
Detector: UV 278

OTHER SUBSTANCES
Extracted: dextromethorphan

REFERENCE
Abdel-Moety,E.M.; Al-Deeb,O.A.; Khattab,N.A. Determination of dextromethorphan hydrobromide in bulk form and dosage formulations by high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 4127–4134.

SAMPLE
Matrix: 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 308

CHROMATOGRAM
Retention time: 23, 25, 28, 33 (diastereomers)

KEY WORDS
chiral

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

SAMPLE
Matrix: solutions

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

CHROMATOGRAM
Retention time: 7.68 (A), 4.22 (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, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, meth-

yldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
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: Weigh out brain tissue and homogenize in 4 volumes 400 mM perchloric acid using a Tamson motor-driven PTFE/glass homogenizer at 1400 rpm to give a final tissue concentration of 25 mg/mL in the perchloric acid. For each 1 mL of homogenate add 40 μL 1.48 μg/mL propranolol in 200 mM sulfuric acid, centrifuge at 3000 g for 15 min. Remove 1 mL supernatant and add it to 10 μL 10 M NaOH and 350 μL buffer, vortex for 10 s, add 8 mL diethyl ether, shake mechanically for 45 min, centrifuge at 2000 g for 8 min. Remove the organic layer and add it to 200 μL 200 mM sulfuric acid, shake mechanically for 15 min, centrifuge at 2000 g for 8 min. Remove the aqueous layer and heat it at 45° for 1 h to remove traces of ether, inject a 50 μL aliquot. (Buffer was 90 g sodium carbonate and 32 g potassium carbonate in 1 L, pH 9.0.)

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm LC-18-DB (Supelchem)
Column: 250 × 4.6 5 μm LC-18-DB (Supelchem)
Mobile phase: MeCN:50 mM NaH_2PO_4:triethylamine 35:65:0.1, adjusted to pH 3.0 with orthophosphoric acid
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: UV 230

CHROMATOGRAM
Retention time: 5.5
Internal standard: propranolol (7.3)
Limit of detection: 33 ng/mL

OTHER SUBSTANCES
Simultaneous: clenbuterol

KEY WORDS
rat; brain

REFERENCE
Botterblom,M.H.A.; Feenstra,M.G.P.; Erdtsieck-Ernste,E.B.H.W. Determination of propranolol, labetalol and clenbuterol in rat brain by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *613*, 121–126.

SAMPLE
Matrix: urine

Sample preparation: 1 mL Urine + 10 mg β-glucuronidase/arylsulfatase (Helix pomatia, Sigma), heat at 37° overnight, add an equal volume of buffer, centrifuge at 2000 g for 5 min, inject an aliquot of the supernatant onto column A with mobile phase A and elute to waste. After 2.5 min backflush the contents of column A onto column B with mobile phase B, monitor the effluent from column B. Re-equilibrate both columns for 12.5 min before the next injection. (Buffer was 200 mM boric acid adjusted to pH 9.5 with 5 M NaOH.)

HPLC VARIABLES

Column: A 10 × 4.6 5 μm Spherisorb cyanopropyl; B 250 × 4.6 Capcell Pak C18 UG-120 (Shiseido)
Mobile phase: A water; B Gradient. MeCN:buffer from 3:97 to 30:70 over 30 min, to 40:60 over 8 min (Buffer was 3.4 mL/L phosphoric acid adjusted to pH 3.0 with 5 M NaOH.)
Flow rate: A 1.25; B 1
Injection volume: 100
Detector: UV 220

CHROMATOGRAM

Retention time: 14.2
Limit of detection: 250 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, alprenolol, amphetamine, atenolol, bopindolol, codeine, ephedrine, metoprolol, morphine, nadolol, oxprenolol, pindolol, propranolol, timolol

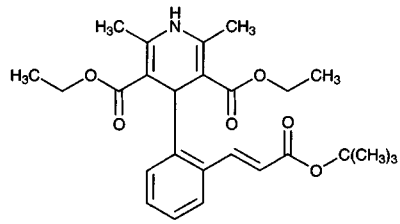
KEY WORDS

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.

Lacidipine



Molecular formula: $C_{26}H_{33}NO_6$
Molecular weight: 455.55
CAS Registry No.: 103890-78-4
Merck Index: 5344
Lednicer No.: 5 82

SAMPLE

Matrix: blood
Sample preparation: Condition a 500 mg Bondelut C18 SPE cartridge with 3 mL MeOH and 2 mL MeCN:water 50:50. 3 mL Plasma + 3 mL MeCN, centrifuge, add the supernatant to the SPE cartridge, wash with 2 mL MeCN:water 50:50, wash with 1.5 mL basic washing solution, wash with 1.5 mL acid washing solution, wash with 2 mL MeCN:water 50:50, elute with two 1.5 mL portions of MeCN. Evaporate the eluate, reconstitute in 100 μL mobile phase, store at 4°, inject an 82 μL aliquot. (Basic washing solution was MeCN:water:33% ammonia 10:88:2. Acid washing solution was MeCN:water:88% orthophosphoric acid 10:89:1.)

HPLC VARIABLES

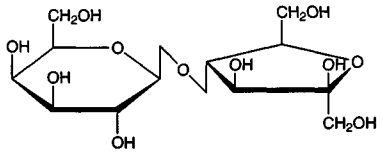
Guard column: 30 × 4.6 30-40 μm RP-8 (Merck)
Column: 60 × 4.6 3 μm Hypersil ODS
Mobile phase: MeCN:MeOH:water 6:66:28
Column temperature: 40
Flow rate: 1
Injection volume: 82
Detector: UV 300 or RIA

CHROMATOGRAM
Retention time: 4.5
Limit of detection: 0.5 ng/mL

KEY WORDS
plasma; human; dog; rat; horse; protect from light; pharmacokinetics; SPE; automation of this procedure reported (see J. Chromatogr. B 1995; 669; 383)

REFERENCE
Pellegatti,M.; Braggio,S.; Sartori,S.; Franceschetti,F.; Bolelli,G.F. Validation of a high-performance liquid chromatographic-radioimmunoassay method for the determination of lacidipine in plasma, *J.Chromatogr.*, **1992**, *573*, 105–111.

Lactulose



Molecular formula: $C_{12}H_{22}O_{11}$
Molecular weight: 342.30
CAS Registry No.: 4618-18-2
Merck Index: 5360

SAMPLE
Matrix: beverages, juice, milk
Sample preparation: Orange juice. Dilute orange juice 100-fold with water, filter (Millipore HV, 0.45 μm), dilute filtrate 10-fold, inject an aliquot. Beverages. Dilute soft drinks 1000-fold with water, inject an aliquot. Milk. Dilute 5 mL milk to 100 mL with mobile phase, filter (Millipore HV, 0.45 μm), dilute filtrate 50-fold, inject an aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 Cation H (Bio-Rad)
Column: 300 × 3.8 9 μm HPX 87-H Aminex (Bio-Rad)
Mobile phase: 10 mM Sulfuric acid
Column temperature: 50
Flow rate: 0.5
Injection volume: 40
Detector: E following post-column reaction, Hewlett-Packard 1049A programmable electrochemical detector, Metrohm detector cell, cuprous oxide working electrode +550 mV, glassy carbon auxiliary electrode, Ag/AgCl (3 M KCl) reference electrode. The column effluent mixed with 200 mM NaOH pumped at 0.4 mL/min, the mixture flowed through a 220 × 0.8 single-bead string reactor packed with 0.6 mm glass beads to the detector. (Prepare cuprous oxide electrode as follows. Stir 300 mg conductive carbon cement (Gerhard Neubauer, Münster), 60 mg cuprous oxide (Fluka), and 300 μL acetone until a thick paste forms as the acetone evaporates. Pack conductive carbon cement into the base of a 3 mm diameter cavity carbon paste electrode base (Metrohm), allow to dry, polish with dry emery paper (grade 2/0, Oakey), remove surface layer with an acetone-soaked tissue, pack the paste into the cavity, allow to dry overnight, polish with dry emery paper (grade 2/0), 3 μm imperial micro finishing film sheet (3M), 0.3 μm imperial micro finishing film sheet (3M), and 0.05 μm alumina particles on a Buehler pad, sonicate for 2 min in water (Anal. Chim. Acta 1995, 300, 5).)

CHROMATOGRAM
Retention time: 9.50
Limit of detection: 2 μM

OTHER SUBSTANCES
Also analyzed: arabinose, cellobiose, dextrose, fructose, fucose, galactitol, galactose, galacturonic acid, lactose, lyxose, maltose, mannitol, mannose, myo-inositol, raffinose, rhamnose, ribose, sorbose, sucrose, xylose

KEY WORDS
orange juice; soft drinks; post-column reaction; fruit

REFERENCE
Huang,X.; Pot,J.J.; Kok,W.T. Determination of sugars by liquid chromatography and amperometric detection with a cuprous oxide modified electrode, *Chromatographia*, **1995**, *40*, 684–689.

SAMPLE
Matrix: blood, urine
Sample preparation: Urine. Dilute urine 1:10 to 1:40 with water, add a 1 mL aliquot to 1 mL 250 μg/mL melibiose, add Amberlite IR-120 H^+ to occupy one third of the volume, inject a 25 μL aliquot of the supernatant. Plasma. 200 μL Plasma + 200 μL 250 μg/mL melibiose, mix, add 200 μL ice cold 35 mg/mL 5-sulfosalicylic acid, let stand on ice for 20 min, centrifuge at 9000 g for 5 min, mix with Amberlite IR-120 H^+:Amberlite IRA 400 Cl^- 40:60, centrifuge, inject a 25 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: Carbopac PA-1 (Dionex)
Column: 250 × 40 Carbopac PA-1 (Dionex)
Mobile phase: 120 mM NaOH containing 0.5 mM zinc acetate (urine) or 160 mM NaOH containing 0.675 mM zinc acetate (plasma) (At the end of each plasma sample wash with 1 M NaOH for 4 min.)
Flow rate: 1
Injection volume: 25
Detector: E, Dionex pulsed electrochemical detector, detection potential -0.01 V (0-0.5 s), oxidation potential +0.75 V (0.51-0.64 s), reduction potential -0.75 V (0.65-0.75 s), integration period 0.05-0.5 s

CHROMATOGRAM
Retention time: 5.9 (plasma), 6.9 (urine)
Internal standard: melibiose (4.0 (plasma), 4.6 (urine))
Limit of detection: 400 ng/mL

OTHER SUBSTANCES
Extracted: mannitol, 3-O-methylglucose, dextrose

KEY WORDS
plasma

REFERENCE
Fleming,S.C.; Kynaston,J.A.; Laker,M.F.; Pearson,A.D.J.; Kapembwa,M.S.; Griffin,G.E. Analysis of multiple sugar probes in urine and plasma by high-performance anion-exchange chromatography with pulsed electrochemical detection. Application in the assessment of intestinal permeability in human immunodeficiency virus infection, *J.Chromatogr.*, **1993**, *640*, 293–297.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: Shodex Sugar SP 0810P and SP 0810
Mobile phase: water
Column temperature: 80
Flow rate: 0.5
Detector: RI

CHROMATOGRAM
Retention time: 19

OTHER SUBSTANCES
Simultaneous: arabinose, dextrose, fructose, galactose, glycerol, lactose, mannitol, pullulan P-10, raffinose, sorbitol, stachyose, sucrose, xylitol

REFERENCE

Majors,R.E. Polymeric liquid chromatography column technology in Japan, *LC.GC*, **1993**, *11*, 778–788.

SAMPLE

Matrix: urine

Sample preparation: Condition a 600 mg Maxi-Clean C18 SPE cartridge with 5 mL MeOH and 5 mL water. Add 2-3 mL urine to the SPE cartridge and pass it through the cartridge. Discard the first 1 mL, collect the residual volume and dilute it 1:1 with water. Dilute a 200 μL aliquot with 1.8 mL water containing 75 μm/mL cellobiose, add 400 g/L Amberlite IRA-400 resin Cl^- form (Fluka). Vortex for 10 s, centrifuge at 3000 g for 2 min. Filter (Micro-spin centrifuge cartridge, Nylon 66, 0.2 μm, Alltech) a 400 μL aliquot of the supernatant while centrifuging at 3000 g for 5 min. Inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: Benson Carbohydrate BC-100 Ca^{2+} (Alltech)
Column: 300 × 6.5 10 μm Alltech 700 CH Carbohydrate (Alltech)
Mobile phase: Water
Column temperature: 85
Flow rate: 0.5
Injection volume: 50
Detector: ELSD, Varex MKIII (Alltech), drift tube temperature 120°, carrier gas flow (air) 41.67 cm^3/s

CHROMATOGRAM

Retention time: 8.88
Internal standard: cellobiose (7.55)
Limit of detection: 820 ng/mL

OTHER SUBSTANCES

Extracted: dextrose, mannitol
Simultaneous: fructose, galactose

KEY WORDS

SPE; pharmacokinetics

REFERENCE

Marsilio,R.; D'Antiga,L.; Zancan,L.; Dussini,N.; Zaccello,F. Simultaneous HPLC determination with light-scattering detection of lactulose and mannitol in studies of intestinal permeability in pediatrics, *Clin.Chem.*, **1998**, *44*, 1685–1691.

SAMPLE

Matrix: urine

Sample preparation: Dilute urine 2.5-20 fold with water, add a 1 mL aliquot to 1 mL water containing 250 μg/mL arabinose and 25 μg/mL cellobiose, add 0.5 g washed Amberlite IR-120 H:Amberlite IRA400 Cl 40:60, vortex, centrifuge, filter (0.2 μm), inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 40 HPIC-AS6 (Dionex)
Mobile phase: 150 mM NaOH
Flow rate: 1
Injection volume: 50
Detector: E, Dionex pulsed electrochemical detector, gold working electrode, detection potential -0.05 V, oxidation potential +0.6 V, reduction potential -0.95 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 6
Internal standard: arabinose (4), cellobiose (9)
Limit of detection: 300 ng/mL

OTHER SUBSTANCES

Extracted: mannitol

REFERENCE

Fleming,S.C.; Kapembwa,M.S.; Laker,M.F.; Levin,G.E.; Griffin,G.E. Rapid and simultaneous determination of lactulose and mannitol in urine, by HPLC with pulsed amperometric detection, for use in studies of intestinal permeability, *Clin.Chem.*, **1990**, *36*, 797–799.

SAMPLE

Matrix: urine
Sample preparation: Centrifuge, dilute 10-20 fold with water, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 6.5 Sugar Pak I cation-exchange in calcium form
Mobile phase: Water containing 1 mL/L of a 50 g/L calcium EDTA solution
Column temperature: 85
Flow rate: 0.5
Injection volume: 20
Detector: RI

CHROMATOGRAM

Retention time: 8

OTHER SUBSTANCES

Extracted: mannitol

REFERENCE

Willems,D.; Cadranel,S.; Jacobs,W. Measurement of urinary sugars by HPLC in the estimation of intestinal permeability: evaluation in pediatric clinical practice, *Clin.Chem.*, **1993**, *39*, 888–890.

SAMPLE

Matrix: urine
Sample preparation: 2 mL Urine + 500 mg washed and mixed Duolite ion-exchange resin (BDH), vortex for 10 s, centrifuge at 3000 g for 10 min, filter (0.2 μm) the supernatant, inject an aliquot.

HPLC VARIABLES

Guard column: Direct-Connect polymeric guard column (Alltech)
Column: 250 × 4.6 5 μm Kromasil NH2 (Alltech)
Mobile phase: MeCN:water 70:30
Flow rate: 1
Injection volume: 10
Detector: RI

CHROMATOGRAM

Retention time: 15
Limit of detection: 50 μM

OTHER SUBSTANCES

Extracted: mannitol, L-rhamnose, urea

REFERENCE

Miki,K.; Butler,R.; Moore,D.; Davidson,G. Rapid and simultaneous quantification of rhamnose, mannitol, and lactulose in urine by HPLC for estimating intestinal permeability in pediatric practice, *Clin.Chem.*, **1996**, *42*, 71–75.

SAMPLE

Matrix: urine
Sample preparation: 10 μL Urine + 200 μL reagent, heat at 65° for 16 h, cool to room temperature, inject a 5 μL aliquot of the clear supernatant. (Prepare reagent by dissolving 5 mg Fmoc-hydrazine in 1 mL MeCN, add 10 μL buffer. Buffer was 1.44 M formic acid containing 600 mM NaOH. Prepare Fmoc-hydrazine as follows. Dissolve 1 g 9-fluorenylmethyl chloroformate in 100 mL EtOH, add this solution dropwise with stirring to 10 mL hydrazine hydrate (Caution!

Hydrazine hydrate is a carcinogen!), stir for 30 min, filter off the precipitate, wash it twice with 20 mL portions of ice-cold EtOH, dry at room temperature.)

HPLC VARIABLES
Guard column: 10 × 4.6 3 μm Spherisorb ODS II
Column: 125 × 4.6 3 μm Spherisorb ODS II
Mobile phase: Gradient. Isopropanol:isobutyl alcohol:water 6:6:88 for 13 min, to 80:0:20 (step gradient), maintain at 80:0:20 for 6 min, re-equilibrate at initial conditions.
Column temperature: 50
Injection volume: 5
Detector: F ex 270 em 315

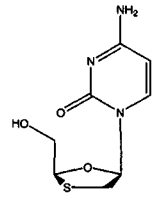
CHROMATOGRAM
Retention time: 5
Limit of detection: 110 nM

OTHER SUBSTANCES
Extracted: 3-O-methyl-D-glucose, rhamnose, xylose

KEY WORDS
derivatization

REFERENCE
Rooyakkers,D.R.; van Eijk,H.M.H.; Deutz,N.E.P. Simple and sensitive multi-sugar-probe gut permeability test by high-performance liquid chromatography with fluorescence labelling, *J.Chromatogr.A*, **1996**, *730*, 99–105.

Lamivudine



Molecular formula: $C_8H_{11}N_3O_3S$
Molecular weight: 229.26
CAS Registry No.: 134678-17-4
Merck Index: 5365
Lednicer No.: 5 99

SAMPLE
Matrix: solutions

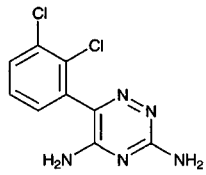
HPLC VARIABLES
Column: 250 × 4.6 Cyclobond I acetyl
Mobile phase: 0.2% Triethylamine in water, adjusted to pH 7.2 with glacial acetic acid
Flow rate: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 6.3 (-), 6.7 (+)

KEY WORDS
chiral

REFERENCE
Coates,J.A.; Cammack,N.; Jenkinson,H.J.; Mutton,I.M.; Pearson,B.A.; Storer,R.; Cameron,J.M.; Penn,C.R. The separated enantiomers of 2'-deoxy-3'-thiacytidine (BCH 189) both inhibit human immunodeficiency virus replication in vitro, *Antimicrob.Agents Chemother.*, **1992**, *36*, 202–205.

Lamotrigine



Molecular formula: $C_9H_7Cl_2N_5$
Molecular weight: 256.09
CAS Registry No.: 84057-84-1 or 84507-84-1 (?)
Merck Index: 5367
Lednicer No.: 4 120

SAMPLE

Matrix: blood
Sample preparation: 200 μL Serum + 100 μL 30 mg/L IS in water + 200 μL 25% saturated ammonium acetate, mix. Add the sample to the reservoir of a primed 4 mm/1 mL Empore C8 SPE disk cartridge suspended in a test tube (16 × 100 mm). Force the liquid then 500 μL water through the disk by centrifuging at 100-120 g for 5 min. Suspend disk cartridge in a tube, elute the drug with 100 μL MeCN and 300 μL water. Combine the eluates, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 2 30 μm Permaphase ETH (DuPont)
Column: 250 × 4.6 Zorbax Stable-Bond CN
Mobile phase: MeCN:MeOH:acetic acid:triethylamine: water 15:12.5:0.1:0.06:72.5 (Connect a 250 × 4.6 column dry packed with 37-53 μm silica gel (Whatman) as a mobile-phase saturating column between the pump and the injector .)
Column temperature: 50
Flow rate: 1.2
Injection volume: 50
Detector: UV 214

CHROMATOGRAM

Retention time: 5.5
Internal standard: cyheptamide (14)
Limit of detection: 15-30 ng/mL

OTHER SUBSTANCES

Extracted: carbamazepine, carbamazepine diol, carbamazepine epoxide, 5-(p-hydroxyphenyl)-5-phenylhydantoin, phenytoin
Simultaneous: acetaminophen, N-acetylprocainamide, amikacin, caffeine, chlordiazepoxide, clonazepam, desmethylchlordiazepoxide, desmethyldiazepam, diazepam, digoxin, disopyramide, erythromycin, ethosuximide, felbamate, flurazepam, gabapentin, gentamicin, lidocaine, methotrexate, nitrazepam, oxazepam, phenylethylmalonamide, phenobarbital, primidone, quinidine, salicylate, temazepam, theophylline, tobramycin, valproic acid, vancomycin

KEY WORDS

serum; SPE

REFERENCE

Lensmeyer,G.L.; Gidal,B.E.; Wiebe,D.A. Optimized high-performance liquid chromatographic method for determination of lamotrigine in serum with concomitant determination of phenytoin, carbamazepine, and carbamazepine epoxide, *Ther.Drug Monit.*, **1997**, *19*, 292–300.

SAMPLE

Matrix: blood
Sample preparation: Mix 550 μL plasma with 100 μL 500 mM monochloroacetic acid in 500 mM pH 2.5 ammonium orthophosphate buffer. Dialyze two 300 μL aliquots against 5 mM pH 7.0 potassium phosphate buffer pumped at 0.25 mL/min for 4 min each using a 15 kD Cuprophan membrane. The dialysate passed through column A and was washed through with 500 μL 1 mM pH 7.0 potassium phosphate buffer. Elute the glucuronide metabolite from column A onto column B with mobile phase. After 30 s remove column A from the circuit, elute column B with mobile phase, elute column A with 200 μL MeCN:water 10:90 and 500 μL 1 mM pH

7.0 potassium phosphate buffer to waste, at the 4 min mark elute lamotrigine and the methylated metabolite from column A onto column B with mobile phase, elute column B with mobile phase, monitor the effluent from column B. (At the end of the process flush the donor channel with 1.5 mL 1 mM pH 7.0 potassium phosphate buffer and flush column A with 500 μL 5 mM pH 7.0 potassium phosphate buffer.)

HPLC VARIABLES

Column: A 70 mg 10 μm Hypersil ODS (Shandon) in a Prelute cartridge; B 150 × 4.6 5 μm Kromasil C8 (Technicol, UK)
Mobile phase: Gradient. A was 50 mM pH 4.15 ammonium phosphate buffer containing 20 mM diethylamine hydrochloride. B was MeCN:500 mM pH 4.15 ammonium phosphate buffer containing 200mM mM diethylamine hydrochloride buffer:water 60:10:30. A:B 86:14 for 3 min, to 69:31 over 0.2 min, maintain at 69:31 for 10.2 min, to 0:100 over 0.1 min, maintain at 0:100 for 1.5 min, return to 86:14 over 0.2 min, re-equilibrate at initial conditions for 3.8 min
Flow rate: 1.5
Detector: UV 270 for 12.5 min, then UV 215

CHROMATOGRAM

Retention time: 9.6
Limit of quantitation: 40 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; column-switching; dialysis

REFERENCE

Cooper,J.D.H.; Shearsby,N.J.; Taylor,J.E.; Fook Sheung,C.T.C. Simultaneous determination of lamotrigine and its glucuronide and methylated metabolites in human plasma by automated sequential trace enrichment of dialysates and gradient high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *702*, 227–233.

SAMPLE

Matrix: blood
Sample preparation: Add 1.5 mL MeCN to 500 μL serum, centrifuge, evaporate the supernatant to dryness, redissolve the residue in 200 μL water. Inject onto column A, wash with MeCN:water 10:90 or MeOH:water 20:80 for 20 min, backflush the contents of column A onto column B with mobile phase, elute with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 25 × 4 25 μm pore diameter 6 nm LiChrospher RP-18 ADS (Merck); B 125 × 4 5 μm endcapped LiChroCART HPLC-cartridge RP-18 (Merck)
Mobile phase: MeCN:50 mM pH 4 phosphate buffer 20:80
Column temperature: 40
Flow rate: 1
Injection volume: 200
Detector: UV 280

CHROMATOGRAM

Retention time: 3.1

OTHER SUBSTANCES

Extracted: oxprenolol

KEY WORDS

serum; column-switching

REFERENCE

Oertel,R.; Richter,K.; Gramatté,T.; Kirch,W. Determination of drugs in biological fluids by high-performance liquid chromatography with on-line sample processing, *J.Chromatogr.A*, **1998**, *797*, 203–209.

SAMPLE
Matrix: blood
Sample preparation: Add an equal volume of 1 μg/mL IS in MeCN to 0.2-1 mL plasma, vortex briefly, add excess sodium carbonate, vortex to form a saturated solution, centrifuge at 6° at 1500 g for 10 min. Remove the MeCN supernatant (top layer) and dilute it by half with water, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100CN
Mobile phase: MeCN:10 mM pH 3.5 ammonium acetate buffer 55:45
Flow rate: 1.5
Injection volume: 30
Detector: UV 280

CHROMATOGRAM
Retention time: 10
Internal standard: 3,5-diamino-6-(2-methoxyphenyl)-1,2,4-triazine (A725C) (12)
Limit of detection: 55 ng/mL

OTHER SUBSTANCES
Noninterfering: carbamazepine, clonazepam, phenobarbital, phenytoin, valproic acid

KEY WORDS
plasma

REFERENCE
Cociglio,M.; Alric,R.; Bouvier,O. Performance analysis of a reversed-phase liquid chromatographc assay of lamotrigine in plasma using solvent-demixing extraction, *J.Chromatogr.*, **1991**, *572*, 269–276.

SAMPLE
Matrix: blood
Sample preparation: 300 μL Plasma + 50 μL 1.7 M perchloric acid, vortex for 20 s, centrifuge at 1500 g for 4 min, add 35 μL 4 M K_2HPO_4, shake gently, allow to settle, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeCN:80 mM KH_2PO_4 25:75
Flow rate: 1.5
Injection volume: 50
Detector: UV 306

CHROMATOGRAM
Retention time: 4.5
Limit of quantitation: 1.2 μM

OTHER SUBSTANCES
Noninterfering: anticonvulsant drugs

KEY WORDS
plasma

REFERENCE
Boutagy,J.; Dell'Anna,M. Simplified and rapid HPLC procedure for analysis of lamotrigine in plasma (Abstract 107), *Ther.Drug Monit.*, **1995**, *17*, 410–410.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 100 μL 10 μg/mL IS in MeOH + 200 μL 2 M NaOH + 1 mL ethyl acetate, vortex for 1 min, centrifuge for 5 min. Remove the organic layer and evap-

orate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm RP-8 (Brownlee/Applied Biosystems)
Mobile phase: MeCN:water:buffer 20:79:1 (The buffer was 20.7 g $NaH_2PO_4.H_2O$ and 14.2 g Na_2HPO_4 in 500 mL water, pH 6.5.)
Flow rate: 1.6
Injection volume: 25
Detector: UV 306

CHROMATOGRAM
Retention time: 7.2
Internal standard: 6-(2-methoxyphenyl)-1,2,4-triazine-3,5-diamine (BWC430C78) (4)
Limit of detection: 1 μM

OTHER SUBSTANCES
Simultaneous: carbamazepine
Noninterfering: N-acetylprocainamide, ethosuximide, phenobarbital, phenytoin, primidone, procainamide, quinidine, theophylline, valproic acid

KEY WORDS
serum

REFERENCE
Fraser,A.D.; MacNeil,W.; Isner,A.F.; Camfield,P.R. Lamotrigine analysis in serum by high-performance liquid chromatography, *Ther.Drug Monit.*, **1995**, *17*, 174–178.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Plasma + 100 μL MeCN, centrifuge, inject a 5 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 4.6 3.5 μm 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 310

CHROMATOGRAM
Retention time: 1.9
Limit of detection: <1 μM

OTHER SUBSTANCES
Extracted: carbamazepine (UV 220), carbamazepine epoxide (UV 220), hydroxycarbamazepine (UV 220), oxcarbazepine (UV 220), phenobarbital (UV 220), phenytoin (UV 220)
Also analyzed: ibuprofen, naproxen, trimethoprim

KEY WORDS
plasma

REFERENCE
Lessing,U.; Vielemeyer,O.; Heilmann,P.; Schöneshöfer,M. Routine determination of serum primidone levels with a fully automated liquid chromatographic method: Comparison with an immuno-assay-technique (Abstract 100), *Ther.Drug Monit.*, **1995**, *17*, 408.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond-Elut SPE cartridge with two 1 mL portions of MeOH and with two 1 mL portions of buffer. 100 μL Serum + 50 μL 6 μg/mL acetanilide in

MeOH:water 50:50 + 800 μL buffer, mix, add to the SPE cartridge, wash with 1 mL buffer, elute with 250 μL MeOH, inject a 40 μL aliquot. (Buffer was 10 mM pH 3.5 phosphate buffer containing 5 mM sodium octanesulfonate.)

HPLC VARIABLES

Column: 125 × 4 4 μm ODS LiChroCART C18
Mobile phase: MeCN:buffer 27:73 (Buffer was 10 mM pH 3.5 phosphate buffer containing 5 mM sodium octanesulfonate.)
Flow rate: 1
Injection volume: 40
Detector: UV 265

CHROMATOGRAM

Retention time: 10.3
Internal standard: acetanilide (3.4)
Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES

Simultaneous: carbamazepine, clonazepam, ethosuximide, phenobarbital, phenytoin, primidone, zonisamide
Noninterfering: valproic acid

KEY WORDS

serum; SPE

REFERENCE

Yamashita,S.; Furuno,K.; Kawasaki,H.; Gomita,Y.; Yoshinaga,H.; Yamatogi,Y.; Ohtahara,S. Simple and rapid analysis of lamotrigine, a novel antiepileptic, in human serum by high-performance liquid chromatography using a solid-phase extraction technique, *J.Chromatogr.B*, **1995**, *670*, 354–357.

SAMPLE

Matrix: blood, formulations, urine
Sample preparation: Tablets. Powder tablets, weigh out amount corresponding to 10.9 mg lamotrigine, add 100 mL MeOH, sonicate for 5 min, centrifuge an aliquot at 3500 g for 15 min. Remove a 5 mL aliquot of the supernatant and make up to 25 mL with mobile phase. Remove a 4 mL aliquot of this solution and add it to 5 mL 5.5 μg/mL in mobile phase, make up to 25 mL with mobile phase, inject a 20 μL aliquot. Plasma. Condition a 3 mL 200 mg Bond Elut C8 SPE cartridge with 1 volume of MeOH and 1 volume of water. 40 μL Plasma + 200 μL 1.1 μg/mL IS in MeOH + 80 μL MeCN, centrifuge at 3500 g for 15 min. Remove the supernatant and evaporate it so as to remove the organic solvents under a stream of nitrogen at 45°, add the residue to the SPE cartridge, wash with 2 volumes of water, elute with 1 volume of 10 mM HCl in MeCN. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot. Urine. Condition a 3 mL 200 mg Bond Elut C8 SPE cartridge with 1 volume of MeOH and 1 volume of water. 100 μL Urine + 200 μL 1.1 μg/mL IS in MeOH + 200 μL MeCN, centrifuge at 3500 g for 15 min. Remove the supernatant and evaporate it so as to remove the organic solvents under a stream of nitrogen at 45°, add the residue to the SPE cartridge, wash with 2 volumes of water, elute with 1 volume of 10 mM HCl in MeCN. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Lichrosorb RP-8
Mobile phase: MeCN:buffer 28:72 (Buffer was 56 mL 1 M acetic acid and 50 mL 1 M NaOH made up to 1 L with water, pH 5.6.)
Flow rate: 1
Injection volume: 20
Detector: UV 306

CHROMATOGRAM

Retention time: 6.5
Internal standard: 5-diamino-6-(2-methoxyphenyl)-1,2,4-triazine (BW725C78) (3.5)
Limit of detection: 1.2 ng (urine), 1.1 ng (plasma)

Limit of quantitation: 3.0 ng (urine), 2.8 ng (plasma)

KEY WORDS
plasma; tablets; SPE

REFERENCE
Papadoyannis,I.N.; Zotou,A.C.; Samanidou,V.F. Solid-phase extraction study and RP-HPLC analysis of lamotrigine in human biological fluids and in antiepileptic tablet formulations, *J.Liq.Chromatogr.*, **1995**, *18*, 2593–2609.

SAMPLE
Matrix: blood, urine
Sample preparation: Whole blood. Condition a 100 mg C18 SPE cartridge (Burdick and Jackson/Baxter) with 2 mL MeOH, 2 mL water, and 1 mL 50 mM pH 1.2 phosphoric acid buffer. 250 µL Whole blood + 750 µL pH 1.2 phosphoric acid buffer containing 15 mM sodium dodecyl sulfate + 50 µL 30 µg/mL IS in MeOH:water 50:50, vortex for 15 s, centrifuge at 13000 g for 7 min, add the supernatant to the SPE cartridge, wash with 300 µL 50 mM phosphoric acid buffer, elute with 1 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen below 40°, reconstitute the residue in 250 µL mobile phase, filter (0.45 µm), inject a 50 µL aliquot. Urine. Acidify urine with 20% acetic acid, filter (0.45 µm). 1 mL Urine + 1 mL 1 M pH 11.0 NaH_2PO_4 + 50 µL 15 µg/mL IS in MeOH:water 50:50, extract twice with 3 mL ethyl acetate:MTBE 50:50. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 250 µL mobile phase, inject an aliquot. (To analyze glucuronide mix 100 µL urine with 400 µL 50 mM pH 1.2 phosphoric acid buffer containing 15 mM sodium dodecyl sulfate, inject a 10-50 µL aliquot (MeCN:buffer 30:70).)

HPLC VARIABLES
Column: 250 × 4.6 5 µm Spherisorb C8
Mobile phase: MeCN:buffer 33:67 (whole blood) or 40:60 (urine) (Buffer was 50 mM pH 2.2 phosphoric acid containing 10 mM sodium dodecyl sulfate.) (After each injection flush with MeCN:buffer 67:33 for 5 min, re-equilibrate for 5 min.)
Column temperature: 40
Flow rate: 1.5
Injection volume: 50
Detector: UV 277

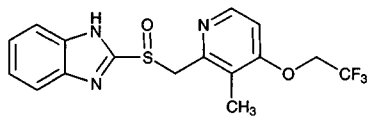
CHROMATOGRAM
Retention time: 19.9 (whole blood), 9.8 (urine)
Internal standard: 3,5-diamino-6-(2-methoxyphenyl)-1,2,4-triazine (BW A725C) (11.9 (whole blood), 7.0 (urine))
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: carbamazepine, cyheptamide, desmethyldiazepam, diazepam, ethosuximide, felbamate, lorazepam, oxazepam, phenobarbital, phenytoin, temazepam, tolybarbital

KEY WORDS
guinea pig; whole blood; SPE; pharmacokinetics

REFERENCE
Sinz,M.W.; Remmel,R.P. Analysis of lamotrigine and lamotrigine 2-N-glucuronide in guinea pig blood and urine by reserved-phase ion-pairing liquid chromatography, *J.Chromatogr.*, **1991**, *571*, 217–230.

Lansoprazole



Molecular formula: $C_{16}H_{14}F_3N_3O_2S$
Molecular weight: 369.37
CAS Registry No.: 103577-45-3
Merck Index: 5373
Lednicer No.: 5 115

SAMPLE

Matrix: blood
Sample preparation: 500 μL Serum + 100 μL 25 μg/mL isobutyl p-hydroxybenzoate in dichloromethane + 3 mL diethyl ether:dichloromethane 70:30, extract, centrifuge, repeat extraction. Add 500 μL diethyl ether:dichloromethane: propylene glycol 70:30:0.5 to the supernatants, evaporate to dryness with a stream of nitrogen, reconstitute in 500 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm TSK gel ODS-120T (Tosoh)
Mobile phase: MeCN:water:n-octylamine 38:62:0.1 adjusted to pH 7 with 85% phosphoric acid
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: UV 285

CHROMATOGRAM

Retention time: 11.5
Internal standard: isobutyl p-hydroxybenzoate (23)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

serum; pharmacokinetics

REFERENCE

Aoki,I.; Okumura,M.; Yashiki,T. High-performance liquid chromatographic determination of lansoprazole and its metabolites in human serum and urine, *J.Chromatogr.*, **1991**, *571*, 283–290.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 20 μg/mL n-butyl p-hydroxybenzoate + 100 μL MeOH, vortex for a few s, add 7 mL MTBE, vortex for 45 s, centrifuge at 4° at 1500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, rinse the tube with 1 mL MTBE, evaporate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL MeOH, vortex for 1 min. Dilute a 100 μL aliquot 10-fold with water, inject 1 mL water then the diluted plasma extract onto column A, wash with 1 mL water, elute the contents of column A onto column B with the mobile phase, elute with the mobile phase and monitor the effluent from column B.

HPLC VARIABLES

Column: A 10 mm long 10 μm Nucleosil CN (SFCC); B 10 mm long 5 μm Nucleosil C18 + 250 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:phosphoric acid:250 mM KH_2PO_4:acetic acid:triethylamine:water 36:10.5:4:0.15:0.15:49.2
Flow rate: 2
Detector: UV 285

CHROMATOGRAM
Retention time: 11
Internal standard: n-butyl p-hydroxybenzoate (23)
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: acebutolol, allopurinol, amiloride, atenolol, phenobarbital, pindolol, prazosin, quinidine, ranitidine, sotalol

KEY WORDS
plasma; column-switching; pharmacokinetics

REFERENCE
Landes,B.D.; Miscoria,G.; Flouvat,B. Determination of lansoprazole and its metabolites in plasma by high-performance liquid chromatography using a loop column, *J.Chromatogr.*, **1992**, *577*, 117–122.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 150 μL 4 μg/mL omeprazole in 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 μL buffer, refrigerate until injection, inject a 100 μL 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 × 4.6 5 μm 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: 13.2
Internal standard: omeprazole (7.6)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

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

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

CHROMATOGRAM
Retention time: 16.613

KEY WORDS
whole blood

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

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in EtOH, inject a 10-20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Chiralpak AD (Daicel)
Mobile phase: Hexane:EtOH 65:35
Column temperature: 35
Flow rate: 1
Injection volume: 10-20
Detector: UV 302

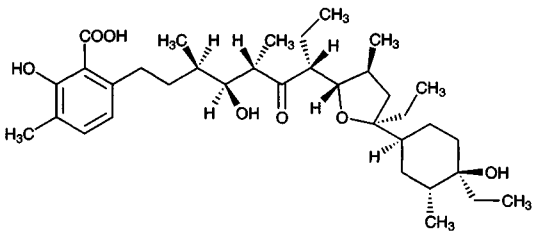
CHROMATOGRAM
Retention time: k' 2.91, α 1.15

OTHER SUBSTANCES
Also analyzed: timoprazole, omeprazole, pantoprazole

KEY WORDS
chiral

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.

Lasalocid A



Molecular formula: $C_{34}H_{54}O_8$
Molecular weight: 590.80
CAS Registry No.: 25999-31-9
Merck Index: 5384

SAMPLE

Matrix: blood

Sample preparation: Cow, dog. 10 mL Whole blood + 1 mL 1 M NaOH, shake vigorously for 10-15 s, let stand at room temperature for 5 (cow) or 20 (dog) min, add 20 mL ethyl acetate, shake vigorously by hand for 50-60 s, centrifuge at 350 g for 10-15 min. Remove 1-10 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 1 mL mobile phase, vortex vigorously for 1 min, inject an aliquot. Chicken. 10 mL Whole blood + 20 mL ethyl acetate, shake vigorously by hand for 10-15 s, shake on a reciprocal shaker at high speed for 5 min, centrifuge at 350 g for 10-15 min. Remove 1-10 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 1 mL mobile phase, vortex vigorously for 1 min, inject an aliquot. Rat, mouse. 1 mL Whole blood + 10 mL ethyl acetate, shake vigorously by hand for 30-40 s, shake on a reciprocal shaker at high speed for 5 min, shake vigorously by hand for 10-15 s, centrifuge at 350 g for 10-15 min. Remove 9 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 1 mL mobile phase, vortex vigorously for 1 min, inject an aliquot.

HPLC VARIABLES

Column: 250 mm long 5 μm Partisil PXS 5/25

Mobile phase: Hexane:THF:MeOH:triethylamine:ammonium hydroxide 81:14:2:2:1 shake for 30-40 s, let stand for 25-30 min, discard the lower phase, add 10 mL/L THF, do not degas or filter. (Place a 10 μm Partisil column before the injector. Flush system with hexane for 30 min at 2 mL/min at the end of each day.)

Flow rate: 0.9

Injection volume: 42-100

Detector: F ex 310 em 430

CHROMATOGRAM

Retention time: 6-8

Limit of detection: <5 ppb

KEY WORDS

whole blood; mouse; rat; cow; dog; chicken; normal phase

REFERENCE

Kaykaty,M.; Weiss,G. Lasalocid determination in animal blood by high-performance liquid chromatography fluorescence detection, *J.Agric.Food Chem.*, **1983**, *31*, 81–84.

SAMPLE

Matrix: feed, premix

Sample preparation: Premix. 2 g Premix + 100 mL acidified MeOH, sonicate at 40° for 2 min, cool, make up to 250 mL with acidified MeOH, mix thoroughly, let stand for 1 h, filter (0.45 μm) an aliquot, dilute the filtrate with acidified MeOH to produce a 4 μg/mL solution, inject a 20 μL aliquot. Feed. Grind feed to pass a 1 mm sieve, add 100 mL acidified MeOH, sonicate at 40° for 2 min, cool, make up to 250 mL with acidified MeOH, mix thoroughly, let stand for 1 h, filter (0.45 μm) an aliquot, inject a 20 μL aliquot of the filtrate. (Acidified MeOH was MeOH:concentrated HCl 99.5:0.5.)

HPLC VARIABLES

Column: 250 or 125 × 4 5 μm C18

Mobile phase: MeOH:buffer 95:5 (Prepare buffer by dissolving 1.36 g KH_2PO_4 in 500 mL water, add 3 mL phosphoric acid, add 10 mL 1,5-dimethylhexylamine, adjust pH to 4.0 with 20% phosphoric acid, make up to 1 L with water.)
Flow rate: 1.2
Injection volume: 20
Detector: F ex 310 em 419

CHROMATOGRAM
Retention time: 7

REFERENCE
Analytical Methods Committee, Determination of lasalocid sodium in poultry feeds and premixes, *Analyst*, **1995**, *120*, 2175–2180.

SAMPLE
Matrix: solutions
Sample preparation: Condition a Mega Bond Elut silica gel SPE cartridge with benzene (Caution! Benzene is a carcinogen!). Evaporate a solution in MeOH to dryness, add 5 mL 5.28 mg/mL 1-bromoacetylpyrene in MeCN, add 5 mL 1.28 mg/mL Kryptofix 222 in MeCN, heat at 50° for 1.5 h, cool. Either inject this solution directly or evaporate it to dryness, dissolve the residue in 5 mL benzene:chloroform 50:50, rinse out the flask with two 5 mL portions of benzene:chloroform 50:50, filter, add the filtrate to the SPE cartridge, elute with two 5 mL portions of benzene:acetone 70:30. Evaporate the eluate to dryness, reconstitute the residue in 10 mL MeCN, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Develosil 5C18
Mobile phase: MeOH:water 97:3
Flow rate: 1
Detector: F ex 360 em 420

CHROMATOGRAM
Retention time: 15
Internal standard: 18,19-dihydrosalinomycin (25), 18,19-dihydro-20-ketosalinomycin (16.5)
Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES
Simultaneous: narasin, monensin, salinomycin

KEY WORDS
derivatization; SPE

REFERENCE
Asukabe,H.; Murata,H.; Harada,K.-I.; Suzuki,M.; Oka,H.; Ikai,Y. Improvement of chemical analysis of antibiotics. XX. Basic study on high-performance liquid chromatographic determination of four polyether antibiotics pre-derivatized with 1-bromoacetylpyrene, *J.Chromatogr.A*, **1993**, *657*, 349–356.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Tissuemizer) 10 g tissue and 25 mL solvent for 1 min, wash blades with 3-4 mL solvent, combine with homogenate, shake for 30 min, centrifuge at 800 g for 15 min, decant. Add 25 mL solvent to residue, mix thoroughly, shake vigorously for 1 min, centrifuge at 800 g for 15 min, add supernatants to a 75 × 20 column of 80-200 mesh alumina (Fisher), rinse container onto column with 25 mL solvent, add 100 mL solvent to the column. Combine all the eluates and add 100 mL 5% NaCl, shake vigorously, let stand 2-3 min, add 30 mL dichloromethane, shake vigorously for 30 s, repeat extraction twice. Combine the dichloromethane layers and evaporate them to dryness under reduced pressure at 48-50°, reconstitute the residue in 1 mL solvent, add to a 75 × 20 column of 25-100 μm Sephadex LH-20, rinse flask with two 3.5 mL portions of solvent and add the rinses to the column, add 10 mL solvent to the column, discard the first 18 mL of eluate, add 10 mL solvent to the column, collect this fraction, evaporate to dryness under a stream of nitrogen at 48-50°, make up to 1 mL with solvent, mix, add 500 μL 9-anthryldiazomethane solution, let stand in the dark for

30 min, evaporate to dryness under a stream of nitrogen at 48-50°, reconstitute in 1 mL hexane. Condition a Baker-10 silica SPE cartridge with 5-10 mL hexane, do not allow to dry. Add the hexane solution to the SPE cartridge, rinse tube with 9 mL hexane, add rinse to the SPE cartridge. Wash SPE cartridge with 10 mL hexane:dichloromethane 50:50, with 10 mL hexane: dichloromethane 20:80, with 10 mL dichloromethane, and with 1 mL MeOH. Elute with 1 mL MeOH, inject a 20 μL aliquot of the eluate. (Solvent was MeOH:water 80:20. Prepare 9-anthryldiazomethane solution as follows. Add 1100 g manganous sulfate tetrahydrate in 1.5 L water and 1170 mL 40% NaOH over 1 h to a hot stirred solution of 960 g potassium permanganate in 6 L water, stir for 1 h, centrifuge, wash solid with water until washings are colorless, dry solid at 100-120°, grind the activated manganese dioxide to a fine powder. Add 8.5 g 85% hydrazine hydrate (Caution! Hydrazine hydrate is a carcinogen!) to 8.8 g 9-anthraldehyde dissolved in 150 mL EtOH, stir at room temperature for 3 h, filter off solid, dry under vacuum, recrystallize from EtOH to give 9-anthraldehyde hydrazone as red-yellow crystals, mp 124-6°. Dissolve 220 mg 9-anthraldehyde hydrazone in 100 mL anhydrous ethyl ether, add 800 mg activated manganese dioxide, add 600 μL EtOH saturated with KOH, stir vigorously for 30 min, filter (glass fiber), wash solid with 20 mL anhydrous ethyl ether, evaporate to reduce volume, make up to 100 mL with anhydrous ethyl ether, store in a dark flask in the dark in a refrigerator. Discard after 30 days (J.Assoc.Off.Anal.Chem. 1985, 68, 1149).)

HPLC VARIABLES
Guard column: pellicular C18 (Alltech)
Column: 200 × 4.6 5 μm RP-C8 (Hewlett-Packard)
Mobile phase: Gradient. A was MeCN. B was MeCN:water 10:90. A:B 20:80 for 9 min, 10:90 for 7 min, 20:80 for 1 min.
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: F ex 365 em 418 (filter)

CHROMATOGRAM
Retention time: 12.6
Limit of detection: 0.15 ppm

OTHER SUBSTANCES
Extracted: monensin, salinomycin

KEY WORDS
cow; liver; SPE; protect from light; derivatization

REFERENCE
Martinez,E.E.; Shimoda,W. Liquid chromatographic determination of multiresidue fluorescent derivatives of ionophore compounds, monensin, salinomycin, narasin, and lasalocid, in beef liver tissue, *J.Assoc.Off.Anal.Chem.*, **1986**, *69*, 637–641.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Polytron) 10 g minced liver and 40 mL MeCN for 15-30 s, scrape down sides and shaft, homogenize for 15-30 s, centrifuge at 5-10° at 2000-2500 rpm for 15-20 min, decant 23 mL supernatant, add 23 mL hexane, shake vigorously for 15-20 s, centrifuge at 1500-2000 rpm. Evaporate 20 mL of the lower MeCN layer to dryness under a stream of nitrogen at 55-65°, add 1 mL water saturated with mobile phase, vortex for 15-20 s, add 2 mL mobile phase, vortex for 15-20 s, centrifuge at 2000-2500 rpm for 15-20 min, recentrifuge if top layer is not clear, inject an aliquot of the top layer.

HPLC VARIABLES
Column: Two Partisil 10 PXS 10/25 columns in series
Mobile phase: THF:MeOH:hexane:mixture 3.75:0.75:20.5:75, do not filter or degas. (Mixture was 150 mL THF + 30 mL MeOH + 10 mL ammonium hydroxide + 810 mL hexane, mix, let stand for 1 h, discard lower layer.) (Place a silica column between pump and injector.)
Flow rate: 2
Injection volume: 50
Detector: F ex 310 em 430

CHROMATOGRAM
Retention time: 7
Limit of detection: 0.24-0.47 ppm

KEY WORDS
normal phase; liver; cow

REFERENCE
Newkirk,D.R.; Barnes,C.J. Liquid chromatographic determination and gas chromatographic-mass spectrometric confirmation of lasalocid sodium in bovine liver: interlaboratory study, *J.Assoc.Off.Anal.Chem.*, **1989**, *72*, 581–584.

SAMPLE
Matrix: tissue
Sample preparation: 10 g Minced tissue + 50 mL MeCN, homogenize for 2 min, sonicate for 5 min, centrifuge at 1860 g for 5 min, repeat extraction. Combine the supernatants and add 30 mL carbon tetrachloride and 20 mL saturated NaCl, shake for 1 min, filter the organic layer through anhydrous sodium sulfate and phase separating paper, evaporate to dryness. Transfer the residue to a Bond-Elut silica SPE cartridge with three 2 mL aliquots of hexane, wash with 5 mL chloroform, elute with 10 mL chloroform:MeOH 95:5. Evaporate the eluate to dryness, transfer to a small vial with three 2 mL portions of hexane, evaporate to dryness with a stream of nitrogen, reconstitute in 500 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 5 × 3 PLRP-S styrene-divinylbenzene copolymer (Polymer Labs)
Column: 250 × 4.6 5 μm PLRP-S styrene-divinylbenzene copolymer (Polymer Labs)
Mobile phase: MeCN:10 mM pH 10.0 disodium tetraborate (borax) 60:40
Flow rate: 1
Injection volume: 10
Detector: F ex 310 em 430

CHROMATOGRAM
Retention time: 5.5
Limit of detection: 2 ng/g

KEY WORDS
chicken; muscle; SPE

REFERENCE
Tarbin,J.A.; Shearer,G. Improved high-performance liquid chromatographic procedure for the determination of lasalocid in chicken tissues and egg using polymeric and porous graphitic carbon columns, *J.Chromatogr.*, **1992**, *579*, 177–183.

SAMPLE
Matrix: tissue, eggs
Sample preparation: 2 g Minced tissue or egg + 25 mL MeCN, homogenize for 2 min, sonicate for 5 min, centrifuge at 1860 g for 5 min, repeat extraction. Combine the supernatants and add 50 mL carbon tetrachloride and 20 mL saturated NaCl, shake for 1 min, filter the organic layer through anhydrous sodium sulfate and phase separating paper, evaporate to dryness. Transfer the residue to a Bond-Elut silica SPE cartridge with three 2 mL aliquots of hexane, wash with 5 mL chloroform, elute with 10 mL chloroform:MeOH 95:5. Evaporate the eluate to dryness, transfer to a small vial with three 2 mL portions of hexane, evaporate to dryness with a stream of nitrogen, reconstitute in 500 μL mobile phase, inject a 25 μL aliquot.

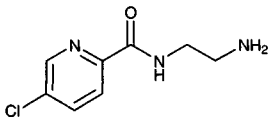
HPLC VARIABLES
Column: 100 × 4.6 7 μm Hypercarb porous graphitic carbon (Shandon)
Mobile phase: MeCN containing 5% 1,1,3,3-tetramethylguanidine
Flow rate: 0.5
Injection volume: 25
Detector: F ex 310 em 420

CHROMATOGRAM
Retention time: 7
Limit of detection: 10 ng/g (eggs), 2 ng/g (tissue)

KEY WORDS
chicken; muscle; SPE

REFERENCE
Tarbin,J.A.; Shearer,G. Improved high-performance liquid chromatographic procedure for the determination of lasalocid in chicken tissues and egg using polymeric and porous graphitic carbon columns, *J.Chromatogr.*, **1992**, *579*, 177–183.

Lazabemide



Molecular formula: $C_8H_{10}ClN_3O$
Molecular weight: 199.64
CAS Registry No.: 103878-84-8, 103878-83-7 (HCl)
Merck Index: 5407

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 50 μL 400 mM NaOH, vortex, add 10 mL MTBE:1-butanol 80:20, shake at 30 rpm on a rotating shaker for 20 min, centrifuge at 2000 g for 5 min. Remove 9 mL of the organic layer and add it to 500 μL 0.17% phosphoric acid, extract for 20 min, centrifuge for 5 min. Remove the aqueous layer and add it to 500 μL buffer, add 500 μL 50 μg/mL fluorescamine in MeCN (prepare fresh each day) with constant vortexing, after 10 min remove the MeCN by evaporation under reduced pressure for exactly 10 min, vortex, inject a 100 μL aliquot. (Prepare buffer by dissolving 30 g Na_2HPO_4 in water, add 24 mL 1 M NaOH, make up to 1 L with water.)

HPLC VARIABLES
Column: 125 × 4 LiChroCART Superspher 60 RP-8e (Merck)
Mobile phase: MeCN:water 32:68 containing 100 mM NaH_2PO_4 and 5 mM Na_2HPO_4, pH 5.9 ± 0.1
Flow rate: 1
Injection volume: 100
Detector: F ex 370 em 485

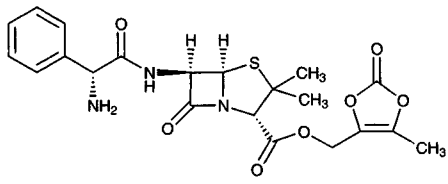
CHROMATOGRAM
Retention time: 3.7
Limit of detection: 0.5 ng/mL
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES
Noninterfering: benserazide, dopamine, levodopa

KEY WORDS
derivatization; plasma; rat; human

REFERENCE
Wyss,R.; Philipp,W. Determination of the monoamine oxidase B inhibitor Ro 19-6327 in plasma by high-performance liquid chromatography using precolumn derivatization with fluorescamine and fluorescence detection, *J.Chromatogr.*, **1990**, *507*, 187–198.

Lenampicillin



Molecular formula: $C_{21}H_{23}N_3O_7S$
Molecular weight: 461.50
CAS Registry No.: 86273-18-9
Merck Index: 5460

SAMPLE
Matrix: blood
Sample preparation: 0.5 mL Plasma + 1 mL MeOH, stir for 5 min, centrifuge at 2400 g for 10 min. Remove 1 mL supernatant, add 2 μg cefazolin, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 5 μm μBondapak C18
Mobile phase: MeOH:67 mM KH_2PO_4 20:80
Flow rate: 1.5
Injection volume: 50
Detector: UV 225

CHROMATOGRAM
Retention time: 9 (measured as ampicillin peak)
Internal standard: cefazolin (14)
Limit of detection: 500 ng/mL

KEY WORDS
plasma

REFERENCE
Marzo,A.; Monti,N.; Ripamonti,M.; Arrigoni Martelli,E.; Picari,M. High-performance liquid chromatographic assay of ampicillin and its prodrug lenampicillin, *J.Chromatogr.*, **1990**, *507*, 235–239.

SAMPLE
Matrix: solutions

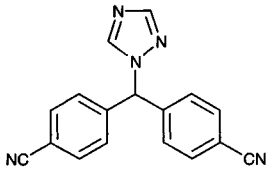
HPLC VARIABLES
Column: 300 × 3.9 5 μm μBondapak C18
Mobile phase: MeOH:67 mM KH_2PO_4 35:65
Flow rate: 1.5
Injection volume: 50
Detector: UV 225

CHROMATOGRAM
Retention time: 20
Internal standard: o-tolylpiperazine (8.5)
Limit of detection: 1500 ng/mL

OTHER SUBSTANCES
Simultaneous: ampicillin

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.

Letrozole



Molecular formula: $C_{17}H_{11}N_5$
Molecular weight: 285.31
CAS Registry No.: 112809-51-5
Merck Index: 5474

SAMPLE
Matrix: blood
Sample preparation: Mix plasma with 50 μL 6.12 μM IS, adjust to pH 13.0, add to an Extrelut SPE cartridge. Elute with 6 mL and 5 mL portions of dichloromethane:diethyl ether 40:60, evaporate the eluates to dryness, dissolve the residue in 500 μL pH 7.0 phosphate buffer, wash with 2 mL hexane, inject a 120 μL aliquot of the aqueous phase.

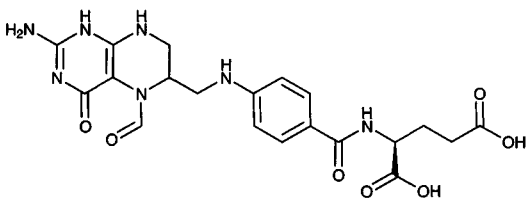
HPLC VARIABLES
Column: 5 μm LiChrospher RP 8
Mobile phase: MeCN:pH 7.0 phosphate buffer 42:58
Flow rate: 1
Injection volume: 120
Detector: UV 234

CHROMATOGRAM
Internal standard: CGS 18320 B (4,4'-[1H-1,3-diazol-1-ylmethylene]bis-benzonitrile)
Limit of quantitation: 8.9 nM

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Sioufi,A.; Sandrenan,N.; Godbillon,J.; Trunet,P.; Czendlik,C.; Howald,H.; Pfister,C.; Ezzet,F. Comparative bioavailability of letrozole under fed and fasting conditions in 1 healthy subjects after a 2.5 mg single oral administration, *Biopharm.Drug Dispos.*, **1997**, *18*, 489–497.

Leucovorin



Molecular formula: $C_{20}H_{23}N_7O_7$
Molecular weight: 473.45
CAS Registry No.: 58-05-9, 1492-18-8 (Ca salt), 6035-45-6 (Ca salt pentahydrate)
Merck Index: 4254

SAMPLE
Matrix: blood
Sample preparation: Mix 20 μL serum with 20 μL 2 M perchloric acid, vortex for a few seconds, centrifuge at 10000 g for 2 min. Inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2 (GL Sciences, Japan)
Mobile phase: MeCN:25 mM pH 7.0 sodium phosphate buffer 8:92 containing 25 mM hydrogen peroxide
Flow rate: 1
Injection volume: 20
Detector: F ex 379 em 457 following post-column reaction. The column effluent flowed through a 5 m × 0.5 mm i.d. stainless-steel reaction coil at 160° and a 3 m × 0.5 mm i.d. stainless-steel coil at 15° to the detector.

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Extracted: methotrexate

KEY WORDS
serum; post-column reaction

REFERENCE
Kubo,H.; Umiguchi,Y.; Fukumoto,M.; Kinoshita,T. Fluorometric determination of methotrexate in serum by high-performance liquid chromatography using in-line oxidation with hydrogen peroxide, *Anal.Sci.*, **1992**, *8*, 789–792.

SAMPLE
Matrix: blood
Sample preparation: Add 2 mg ascorbic acid to each 1 mL blood, centrifuge at 800 g in the cold. 1 mL Plasma + 10 μL 30 μg/mL methotrexate in 1 mg/mL ascorbic acid + 1.5 mL MeOH, vortex, centrifuge at 800 g. Remove the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL water, inject the whole amount.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: Gradient. A was 250 mM pH 5.0 phosphate buffer. B was MeOH:250 mM pH 5.0 phosphate buffer 1:1. A:B 100:0 for 15 min, to 50:50 over 15 min, to 0:100 over 5 min, maintain at 0:100 for 5 min.
Flow rate: 2
Injection volume: 200
Detector: UV 310

CHROMATOGRAM
Retention time: 21
Internal standard: methotrexate (29)

KEY WORDS
plasma

REFERENCE
Wainer,I.W.; Stiffin,R.M. Direct resolution of the stereoisomers of leucovorin and 5-methyltetrahydrofolate using a bovine serum albumin high-performance liquid chromatographic chiral stationary phase coupled to an achiral phenyl column, *J.Chromatogr.*, **1988**, *424*, 158–162.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elut RP-18 SPE cartridge with four 2 mL portions of MeOH and three 2 mL portions of 100 mM pH 4.7 TrisP buffer. Add 1 mg/mL ascorbic acid to plasma. Dilute 500 μL plasma six fold with 9 mg/mL NaCl containing 1 mg/mL ascorbic acid, add 200 μL 8.5 mg/mL phosphoric acid, add to the SPE cartridge, wash with 500 μL 10 mM pH 4.7 TrisP buffer, elute with 1.5 mL eluant. Evaporate the eluate to dryness under a stream of nitrogen at 37° for 30 min, reconstitute the residue in 250 μL 9 mg/mL NaCl containing 1 mg/mL ascorbic acid, inject a 40 μL aliquot onto column A and elute with mobile phase, collect 600 μL effluent (monitor with detector A) in a sample loop and inject it onto column B, elute with mobile phase and monitor the effluent from column B with detector B. (TrisP was tris(hydroxyethyl)methylaminomethane phosphate. Eluant was MeOH:10 mM TrisP buffer 75:25, pH 7 containing 150 μg/mL ascorbic acid.)

HPLC VARIABLES
Column: A 119 × 2 4 μm Superspher RP-8 (Merck) (Condition column with MeOH:water 30:70 every 100-200 injections.); B 150 × 4.5 7 μm chiral protein 2 HSA-BP human serum albumin (Société Française Chromato Colonne)

Mobile phase: 1-Propanol:200 mM Na_2HPO_4 2:98 adjusted to pH 6.2 with 8.5 g/L phosphoric acid
Column temperature: 35 (column B)
Flow rate: A 0.25; B 1
Injection volume: 40
Detector: A UV 313; B E, ESA Coulochem 5100 A, Model 5020 guard cell, Model 5010 analytical cell, E1 0.30 V, E2 0.55 V (monitored), guard cell is placed before second injection valve

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CHROMATOGRAM
Retention time: 6 (l), 10 (d) (after injection onto column B)

KEY WORDS
plasma; narrow bore; column-switching; heart cut; chiral; SPE

REFERENCE
Etienne,M.-C.; Speziale,N.; Milano,G. HPLC of folinic acid diastereoisomers and 5-methyltetrahydrofolate in plasma, *Clin.Chem.*, **1993**, *39*, 82–86.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bakerbond C18 SPE cartridge with 1 mL MeOH and 1 mL 1% acetic acid. 1 mL Plasma + 1 mL 5% acetic acid, add to the SPE cartridge, elute with MeCN:pH 7.0 phosphate buffer (ionic strength 0.1) 20:80, inject a 100 µL aliquot of the eluate onto column A with mobile phase A. Collect the fraction containing leucovorin (about 8 min) in a 250 µL sample loop and inject onto column B with mobile phase B, monitor the effluent from column B. (For achiral determination use only column A and mobile phase A, LOQ 50 ng/mL.)

HPLC VARIABLES
Column: A 5 µm Novapack C18 pre-column + 300 × 4 5 µm Novapack RP-18; B 7 µm Resolvosil BSA chiral pre-column + 150 × 4 7 µm Resolvosil BSA chiral (Macherey-Nagel)
Mobile phase: A Gradient. MeCN:pH 7.0 phosphate buffer (ionic strength 0.1) from 0:100 to 30:70 over 10 min, to 100:0 over 8 min.; B pH 7.0 phosphate buffer (ionic strength 0.1)
Flow rate: A 1; B 0.5
Injection volume: 100
Detector: UV 290

CHROMATOGRAM
Retention time: 19 (S), 22 (R)

KEY WORDS
plasma; SPE; chiral; column-switching; heart-cut

REFERENCE
Vandenbosch,C.; van Belle,S.; de Smet,M.; Taton,G.; Bruynseels,V.; Vandenhoven,G.; Massart,D.L. Determination of leucovorin and 5-fluorouracil in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 77–85.

SAMPLE
Matrix: blood, CSF
Sample preparation: 250 µL Plasma or CSF + 25 µL 10 mg/mL ascorbic acid + 250 µL ice-cold 1.5 M perchloric acid, vortex, let stand in ice-water for 5 min, centrifuge at 4° at 3000 g for 5 min. Remove 350 µL of the supernatant with 50 µL 8 M potassium acetate, keep in ice-water for 2 min, centrifuge at 4° at 3000 g for 2 min, inject a 100 µL aliquot of the supernatant.

HPLC VARIABLES
Column: 200 × 3 5 µm Hypersil ODS glass column
Mobile phase: Gradient. A was 10 mM ammonium formate adjusted to pH 3.5 with HCl. B was MeCN:10 mM ammonium formate 25:75 adjusted to pH 3.5 with HCl. A:B from 85:15 to 5:95 over 21 min, maintain at 5:95 for 1 min, re-equilibrate at initial conditions for 11 min.
Flow rate: 0.4

Injection volume: 100
Detector: UV 305

CHROMATOGRAM
Retention time: 15
Limit of detection: 200 nM

OTHER SUBSTANCES
Extracted: methotrexate, N^5-methyltetrahydrofolate

KEY WORDS
plasma

REFERENCE
van Tellingen,O.; van der Woude,H.R.; Beijnen,J.H.; van Beers,C.J.T.; Nooyen,W.J. Stable and sensitive method for the simultaneous determination of N^5-methyltetrahydrofolate, leucovorin, methotrexate and 7-hydroxymethotrexate in biological fluids, *J.Chromatogr.*, **1989**, *488*, 379–388.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. 500 μL Serum + 250 μg ascorbic acid + 750 μL MeCN, vortex, centrifuge at 400 g for 1 min, add 7 mL chloroform, mix, centrifuge at 400 g for 1 min. 400 μL Aqueous phase + 2 mL mobile phase A, inject the whole amount through a 125 × 4 40 μm silica (Supelco) column on to column A and elute to waste with mobile phase A, backflush the contents of column A on to column B and elute to waste with mobile phase B, collect the fraction containing leucovorin in a 1 mL sample loop, inject this fraction on to column C and elute with mobile phase C, monitor the effluent from column C. Urine. Condition a 1 mL C18 SPE cartridge (Supelco) with 2 mL water, 2 mL MeOH, and 2 mL mobile phase A. Dilute urine with 4 volumes of mobile phase A, add to the SPE cartridge, wash with 2 mL mobile phase A, elute with 2 mL MeCN:water 50:50. Wash the eluate with chloroform, filter (Waters ultrafiltration cartridge) the aqueous layer while centrifuging, dilute 400 μL of the ultrafiltrate with 2 mL mobile phase A, inject the whole amount through a 125 × 4 40 μm silica (Supelco) column on to column A and elute to waste with mobile phase A, backflush the contents of column A on to column B and elute to waste with mobile phase B, collect the fraction containing leucovorin in a 1 mL sample loop, inject this fraction on to column C and elute with mobile phase C, monitor the effluent from column C.

HPLC VARIABLES
Column: A 30 × 4 5 μm C18; B 250 × 2 3 μm C18 (Macherey-Nagel); C 150 × 4.6 7 μm Resolvosil BSA-7
Mobile phase: A 5 mM tetrabutylammonium phosphate (low-UV Pic A) adjusted to pH 6.5 with phosphoric acid; B Isopropanol:buffer 7.5:92.5 adjusted to pH 5 with phosphoric acid (Buffer was 1.5 mM sodium phosphate containing 0.75 mM tetrabutylammonium phosphate (low-UV PIC A).); C 28 mM Phosphate buffer containing 0.6 mM sodium azide (Caution! Sodium azide is toxic! Do not discharge to plumbing system!)
Column temperature: 40 (B and C only, A is ambient)
Flow rate: A 2; B 0.15; C 0.4
Injection volume: 2400
Detector: F ex 308 em 365

CHROMATOGRAM
Retention time: 26 (6S), 30 (6R)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, 5-methyltetrahydrofolate

KEY WORDS
serum; chiral; column-switching; heart-cut; SPE; pharmacokinetics

REFERENCE
Schleyer,E.; Reinhardt,J.; Unterhalt,M.; Hiddemann,W. Highly sensitive coupled-column high-performance liquid chromatographic method for the separation and quantitation of the diastereomers of leucovorin and 5-methyltetrahydrofolate in serum and urine, *J.Chromatogr.B*, **1995**, *669*, 319–330.

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

HPLC VARIABLES
Column: 10 μm μBondapak C18
Mobile phase: MeOH:buffer 21:79 (Buffer was 15 mL 1 M tetrabutylammonium hydroxide in MeOH + 850 mL water, pH adjusted to 7.5 ± 0.1 with 2 M NaH_2PO_4, make up to 875 mL with water.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 4

KEY WORDS
stability-indicating; saline; injections

REFERENCE
Smith,J.A.; Morris,A.; Duafala,M.E.; Bertino,J.R.; Markman,M.; Kleinberg,M. Stability of floxuridine and leucovorin calcium admixtures for intraperitoneal administration, *Am.J.Hosp.Pharm.*, **1989**, *46*, 985–989.

SAMPLE
Matrix: solutions
Sample preparation: Prepare aqueous solutions, stabilize them with ascorbic acid (1 mg/mL water), flush with nitrogen, refrigerate, keep in brown glass vials. Inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 5 μm Lichrospher 60 RP-select B
Column: 125 × 3 3 μm Hypersil BDS
Mobile phase: Gradient. A was MeCN. B was 5 mM monobasic potassium phosphate adjusted to pH 2.3 with phosphoric acid. A:B 7:93 for 5 min, to 13:87 over 15 min, to 21:79 over 6 min, maintain at 21:79 for 1 min, back to 7:93 over 2 min, re-equilibrate at initial conditions for 5 min.
Flow rate: 0.5
Injection volume: 10
Detector: F ex 295 em 355 following post-column photochemical derivatization. The column effluent flowed through a 10 m × 0.3 mm ID Teflon tube irradiated with a 254 nm mercury lamp to the detector.

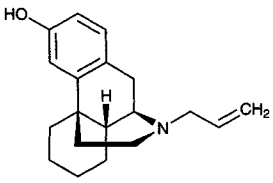
CHROMATOGRAM
Retention time: 14
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites, methotrexate

KEY WORDS
post-column photochemical derivatization; post-column reaction

REFERENCE
Mandl,A.; Lindner,W. Improved detection of leucovorin in mixed folates and antifolates by reversed-phase liquid chromatography and on-line post-column UV irradiation, *Chromatographia*, **1996**, *43*, 327–330.

Levallorphan



Molecular formula: $C_{19}H_{25}NO$
Molecular weight: 283.41
CAS Registry No.: 152-02-3, 71-82-9 (tartrate)
Merck Index: 5485

SAMPLE

Matrix: blood, CSF, urine
Sample preparation: Dilute urine 3:1 or more with water. Mix 1 mL CSF, plasma, or diluted urine with 500 μL saturated sodium carbonate, add 5 mL hexane containing 0.1% n-octylamine. Vortex for 60 s, centrifuge at 2000 g for 10 min. Re-extract aqueous phase with 5 mL hexane containing 0.1% n-octylamine, evaporate the combined hexane extracts to dryness under a stream of nitrogen in a 50° water bath. Reconstitute residue with 150 μL 100 mM hydrochloric acid, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 4.6 5 μm CN
Column: 220 × 4.6 5 μm Brownlee Spheri-5CN (Applied Biosystems, USA)
Mobile phase: MeCN:n-octylamine:water 19:0.05:80.95 adjusted to pH 2.8 with phosphoric acid
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: F ex 230 em 330

CHROMATOGRAM

Retention time: 7.4
Internal standard: levallorphan

OTHER SUBSTANCES

Extracted: dextromethorphan

KEY WORDS

plasma; levallorphan is IS

REFERENCE

Kimiskidis,V.K.; Kazis,A.D.; Niopas,I. Simultaneous determination of dextromethorphan and dextrorphan in human plasma, urine and cerebrospinal fluid by HPLC with fluorescence detection, *J.Liq. Chromatogr.Rel.Technol.*, **1996**, *19*, 1267–1275.

SAMPLE

Matrix: blood, saliva, urine
Sample preparation: Add 100 μL 28% ammonium hydroxide and 5 mL n-butanol:hexane 10:90 to 5 mL urine, 1 mL plasma or 3 mL saliva. Rotate for 30 min and centrifuge at 4500 rpm for 10 min. Remove the upper organic layer and extract it with 300 μL 100 mM HCl by vortexing for 20 min, centrifuge for 5 min. Inject a 40 μL (urine) or 200 μL (plasma, saliva) aliquot of the acidic layer.

HPLC VARIABLES

Column: Zorbax RP-phenyl
Mobile phase: MeCN:10 mM potassium phosphate 50:50 adjusted to pH 4.0 with 8.5% phosphoric acid
Column temperature: 40
Flow rate: 1.0
Injection volume: 40-200
Detector: F ex 280 em 310

CHROMATOGRAM

Retention time: 8.6

Internal standard: levallorphan

OTHER SUBSTANCES
Extracted: dextromethorphan

KEY WORDS
levallorphan is IS; plasma

REFERENCE
Hu,O.Y.-P.; Tang,H.-S.; Lane,H.-Y.; Chang,W.-H.; Hu,T.-M. Novel single-point plasma or saliva dextromethorphan method for determining CYP2D6 activity, *J.Pharmacol.Exp.Ther.*, **1998**, *285*, 955–960.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 500 μL Microsomal incubation + 500 μL MeOH:water:zinc sulfate 50:45:5, centrifuge at 10000 g for 3 min, extract the supernatant twice with 3 mL dichloromethane. Combine dichloromethane extracts, dry under a stream of nitrogen, dissolve the residue in 200 μL MeOH. Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm polymer C18 (Astec, Whippany, NJ)
Mobile phase: MeCN:50 nM (sic) pH 9.0 ammonium carbonate buffer 60:40 (Buffer was adjusted to pH 9.0 with ammonium hydroxide.)
Column temperature: 30
Flow rate: 0.7
Injection volume: 20
Detector: UV (wavelength not given)

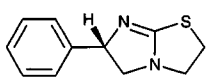
CHROMATOGRAM
Retention time: 11.35
Internal standard: levallorphan

OTHER SUBSTANCES
Extracted: dextromethorphan, dextrorphan

KEY WORDS
liver; levallorphan is IS

REFERENCE
Vielnascher,E.; Spatzenegger,M.; Mayrhofer,A.; Klinger,P.; Jäger,W. Metabolism of dextromethorphan in human liver microsomes: a rapid HPLC assay to monitor cytochrome P450 2D6 activity, *Pharmazie*, **1996**, *51*, 586–588.

Levamisole



Molecular formula: $C_{11}H_{12}N_2S$
Molecular weight: 204.30
CAS Registry No.: 14769-73-4, 16595-80-5 (HCl), 5036-02-2 (racemic), 5086-74-8 (racemic HCl)
Merck Index: 5486
Lednicer No.: 4 217

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the

residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 213.4

CHROMATOGRAM
Retention time: 6.97

KEY WORDS
whole blood

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

SAMPLE
Matrix: cell cultures
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL MeOH and 20 mL water. Add 2 mL cell culture to the SPE cartridge, wash with 20 mL water, elute with 10 mL MeOH:water 80:20, inject a 10 μL portion of the eluate.

HPLC VARIABLES
Column: 100 × 8 4 μm RCM Nova-Pak C18
Mobile phase: MeCN:100 mM ammonium acetate 46:54
Flow rate: 1.3
Injection volume: 10
Detector: UV 245

CHROMATOGRAM
Retention time: 2.2
Limit of quantitation: 5 μg/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
SPE

REFERENCE
Shu,Y.-Z.; Kingston,D.G.I.; Van Tassell,R.L.; Wilkins,T.D. Metabolism of levamisole, an anti-colon cancer drug, by human intestinal bacteria, *Xenobiotica*, **1991**, *21*, 737–750.

SAMPLE
Matrix: milk
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL ethyl acetate, with 10 mL MeOH, and with 10 mL water. Acidify 50 mL milk to pH 4.6 with 6 M HCl, centrifuge at 10° at 4200 g for 15 min, remove the supernatant from the solid and the fat layer. Adjust

the pH of the supernatant to 11.0-11.2 with a few drops of 40% NaOH, add a 25 mL aliquot to the SPE cartridge, elute with 10 mL water-saturated ethyl acetate. Evaporate the eluate to dryness under reduced pressure at 35°, reconstitute the residue in 200 μL mobile phase.

HPLC VARIABLES
Guard column: normal phase universal guard cartridge kit (Whatman)
Column: 250 × 2.1 Spherisorb S5W
Mobile phase: Dichloromethane:MeOH 95:5
Flow rate: 0.2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 12

KEY WORDS
normal phase; SPE; detection with GC for higher sensitivity

REFERENCE
Chappell,C.G.; Creaser,C.S.; Shepherd,M.J. Modified on-column interface for coupled high-performance liquid chromatography-gas chromatography and its application to the determination of levamisole in milk, *J.Chromatogr.*, **1992**, *626*, 223–230.

SAMPLE
Matrix: solutions

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

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone,

naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

SAMPLE

Matrix: solutions
Sample preparation: Inject 10 μL of a solution in RPMI-1640.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil MS C8
Mobile phase: MeCN:water 75:25 containing 0.02% dimethyloctylamine and 0.02% trifluoroacetic acid
Injection volume: 10
Detector: radioactivity

CHROMATOGRAM

Retention time: 9.9

KEY WORDS

tritium labelled

REFERENCE

Ho,N.F.H.; Sims,S.M.; Vidmar,T.J.; Day,J.S.; Barsuhn,C.L.; Thomas,E.M.; Geary,T.G.; Thompson,D.P. Theoretical perspectives on anthelmintic drug discovery: Interplay of transport kinetics, physicochemical properties, and in vitro activity of anthelmintic drugs, *J.Pharm.Sci.*, **1994**, *83*, 1052–1059.

SAMPLE

Matrix: tissue
Sample preparation: Condition a 3 mL Bakerbond Cyano SPE cartridge with 5 mL ethyl acetate:hexane 50:50, do not allow to dry out. Pulverize frozen tissue samples to a fine powder. 3 g Powdered tissue + 2 g anhydrous sodium sulfate + 9 mL ethyl acetate + 500 μL 50% KOH (w/v), homogenize (Silverson) for 1 min, centrifuge at 2000 rpm for 10 min. Remove 6 mL of the ethyl acetate extract and add it to 6 mL hexane, mix, add to the SPE cartridge, wash with 5 mL chloroform:hexane 50:50, dry under vacuum for 10 min, elute with two 2 mL portions of MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 70°, reconstitute the residue in 200 μL, sonicate for 5 min, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 5 μm LiChrospher 60 RP-select B guard column
Column: 125 × 4 5 μm LiChrospher 60 RP-select B
Mobile phase: MeCN:THF:triethylamine:water 35:5:0.2:59.8 containing 100 mM ammonium acetate
Flow rate: 1
Injection volume: 50

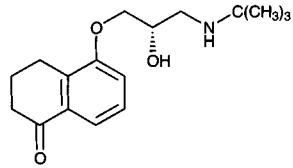
Detector: MS, Hewlett-Packard 5989A, thermospray, vaporizer 15° below take-off temperature, ion source 250°, quadrupole 100°, electron multiplier +200 V with respect to autotune voltage, SIM, m/z 205

CHROMATOGRAM
Retention time: 3.5
Limit of detection: <3 ng/g

KEY WORDS
sheep; liver; kidney; muscle; SPE; LC-MS; thermospray

REFERENCE
Cannavan,A.; Blanchflower,W.J.; Kennedy,D.G. Determination of levamisole in animal tissues using liquid chromatography-thermospray mass spectrometry, *Analyst*, **1995**, *120*, 331–333.

Levobunolol



Molecular formula: $C_{17}H_{25}NO_3$
Molecular weight: 291.39
CAS Registry No.: 47141-42-4, 27912-14-7 (HCl)
Merck Index: 5488
Lednicer No.: 2 110, 215, 280

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 10 μL 2 μg/mL metoprolol in water, 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 μL mobile phase, inject a 10-50 μL aliquot and determine amount of dihydrolevobunolol present (DHL-A). Add MeCN to blood so that the ratio was 1:5. Remove a 1 mL aliquot and add 10 μL 2 μg/mL metoprolol in water, add 1 mL water, adjust pH to 9.8-10.2 with 7-10 drops 100 mM NaOH, add 2 mL benzene, 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 200 μL MeOH, add 5 mg sodium borohydride, let stand at room temperature in a closed tube for 30 min, add 1 mL water, add 300 mg NaCl, extract with 3 mL benzene for 20 min, centrifuge. Remove the organic layer and evaporate it under reduced pressure, dissolve the residue in 50 μL mobile phase, inject a 10-50 μL aliquot and determine the amount of dihydrolevobunolol (DHL-B) that is now present which represents the total amount of levobunolol and dihydrolevobunolol originally present. Determine amount of levobunolol originally present by subtracting DHL-A from DHL-B. Urine. 20-1000 μL Urine + 200 ng metoprolol + 1 mL 200 mM pH 10.2 sodium borate buffer (Sörensen), 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, proceed as described above for blood samples. (Levobunolol itself is not fluorescent so it must be reduced to the fluorescent derivative dihydrolevobunolol. Addition of MeCN to freshly drawn blood stopped enzymatic conversion of levobunolol to dihydrolevobunolol.)

HPLC VARIABLES
Column: 250 × 4.6 10 μm μBondapak 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: 3.8 (dihydrolevobunolol)
Internal standard: metoprolol (4.6)
Limit of detection: 0.5-1 ng/mL

KEY WORDS
human; dog

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.

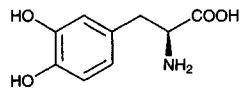
SAMPLE
Matrix: solutions
Sample preparation: Dilute in an appropriate solvent, inject an aliquot.

HPLC VARIABLES
Guard column: RC18 Guardpak (Waters)
Column: 150 × 4.5 5 μm Altex C18
Mobile phase: MeOH:water:phosphoric acid 48:52:0.1, containing 2 g/L sodium heptanesulfonate, pH adjusted to 3.5 with 3 M NaOH
Flow rate: 1.5
Detector: UV 255

CHROMATOGRAM
Retention time: 5-5.3

REFERENCE
Richman,J.B.; Tang-Liu,D.D.-S. A corneal perfusion device for estimating ocular bioavailability in vitro, *J.Pharm.Sci.*, **1990**, *79*, 153–157.

Levodopa



Molecular formula: $C_9H_{11}NO_4$
Molecular weight: 197.19
CAS Registry No.: 59-92-7
Merck Index: 5490

SAMPLE
Matrix: amniotic fluid, blood, CSF, urine
Sample preparation: Plasma. Condition a 100 mg Bond Elut SCX (propylbenzenesulfonic acid, H^+ form) SPE cartridge with 1 mL 50 mM HCl, 1 mL MeOH, 2 mL water, and 1 mL 50 mM HCl. 100 μL Plasma + 100 μL 250 μM norleucine in 100 mM HCl + 10 mg solid sulfosalicylic acid + 800 μL acetone or MeOH, mix, centrifuge, add a 50 μL aliquot to the SPE cartridge, wash with 2 mL water, elute with two 500 μL portions of MeOH:water:triethylamine 40:40:20, dry the eluate under vacuum, add 10 μL MeOH:1 M sodium acetate:triethylamine 40:40:20, dry under vacuum at 70 mTorr, reconstitute with 20 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 20 min, evaporate to dryness under vacuum, reconstitute with 100 μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, inject a 20 μL aliquot. Dried blood. Add 25 μL 250 μM norleucine in 100 mM HCl to a 6 mm filter paper disc containing dried blood, add 100 μL MeCN, let stand for 30 min, centrifuge, remove a 75 μL aliquot of the supernatant, evaporate to dryness under reduced pressure, add 10 μL MeOH:1 M sodium acetate:triethylamine 40:40:20, dry under vacuum at 70 mTorr, reconstitute with 20 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 2 min, evaporate to dryness under vacuum, reconstitute with 50 μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, inject a 20 μL aliquot. Amniotic fluid, CSF. Mix amniotic fluid or CSF with an equal volume of 250 μM norleucine in 100 mM HCl, filter (Centrifree 10000 MW cutoff) while centrifuging at 2200 g. Evaporate a 50 μL aliquot of the

ultrafiltrate to dryness under vacuum, add 10 μL MeOH:1 M sodium acetate:triethylamine 40:40:20, dry under vacuum at 70 mTorr, reconstitute with 20 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 20 min, evaporate to dryness under vacuum, reconstitute with 50 (CSF) or 100 (amniotic fluid) μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, inject a 20 μL aliquot. Urine. Dilute urine with water to a creatinine concentration of 1 mM, mix an aliquot with an equal volume of 250 μM norleucine in 100 mM HCl, filter (Centrifree 10000 MW cutoff) while centrifuging at 2200 g. Evaporate a 50 μL aliquot of the ultrafiltrate to dryness under vacuum, add 10 μL MeOH:1 M sodium acetate:triethylamine 40:40:20, dry under vacuum at 70 mTorr, reconstitute with 20 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 20 min, evaporate to dryness under vacuum, reconstitute with 100 μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 Pico-Tag amino acid column (Waters)
Mobile phase: Gradient. A was MeCN:70 mM pH 6.55 sodium acetate 2.5:97.5. B was MeCN:MeOH:water 45:15:40
Column temperature: 46
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 34.14
Internal standard: norleucine (55.07)

OTHER SUBSTANCES

Extracted: β-alanine, alanine, alloisoleucine, α-aminoadipic acid, 4-aminobenzoic acid, gamma-aminobutyric acid, β-amino-n-butyric acid, gamma-amino-n-butyric acid, 4-aminohippuric acid, β-aminoisobutyric acid, 4-aminophenylacetic acid, α-aminophenylacetic acid, 3-amino-3-phenylpropionic acid, δ-amino-n-valeric acid, ammonia, anserine, arginine, asparagine, aspartic acid, aspartylglucosamine, carnosine, citrulline, cystathionine, cysteic acid, cysteine, cysteine-homocysteine (mixed disulfide), cystine, ethanolamine, ethionine, ethylamine, galactosamine, glucosamine, glutamic acid, glutamine, glutathionine (oxidized), glycine, glycylglycine, glycylhistidine, glycylleucine, glycylphenylalanine, glycyltyrosine, histidine, homoarginine, homocitrulline, homoserine, homocystine, 3-hydroxyanthranilic acid, 3-hydroxykynurenine, hydroxyproline, isoleucine, kynurenine, leucine, lysine, methionine sulfone, methionine, 3-methylhistidine, 1-methylhistidine, ornithine, phenylalanine, phosphoethanolamine, phosphoserine, proline, sarcosine, serine, serotonin, taurine, threonine, tromethamine, tryptophan, tyrosine, valine
Noninterfering: cadaverine, 2-phenylethylamine

KEY WORDS

derivatization; SPE; ultrafiltrate; plasma; dried blood

REFERENCE

Davey,J.F.; Ersser,R.S. Amino acid analysis of physiological fluids by high-performance liquid chromatography with phenylisothiocyanate derivatization and comparison with ion-exchange chromatography, *J.Chromatogr.*, **1990**, *528*, 9–23.

SAMPLE

Matrix: blood
Sample preparation: Mix 450 μL plasma with 50 μL 310 μ g/L iso-homovanilic acid in antioxidant solution. Dialyze this solution using a Carnegie Medicin (Stockholm) microdialysis system. Perfuse 10 mm microdialysis probes with Ringer solution at 2 μ L/min. Collect dialysate for each plasma sample over 20 min in a vial containing 80 μL antioxidant solution, inject a 100 μL aliquot. (Antioxidant solution was 10 mM HCl containing 1 g/L sodium metabisulfite and 0.1 g/L Na_2EDTA.)

HPLC VARIABLES

Guard column: 30 × 4 Bondapak C18/Corasil 37-50 μ m
Column: 250 × 4.8 5 μm ODS (Beckman, CA)

Mobile phase: MeOH:buffer 20:80 (Buffer was 70 mM pH 2.55 NaH_2PO_4 containing 2.08 mM sodium octanesulfonate and 80 μ M EDTA.)
Flow rate: 1
Injection volume: 100
Detector: E , Waters 460 containing an electrochemical cell fitted with a glassy carbon working electrode and an Ag/AgCl reference electrode; the detector potential is + 0.8 V vs the reference electrode

CHROMATOGRAM
Retention time: 3.5
Internal standard: iso-homovanilic acid (10)
Limit of detection: 1.1 nM/L

OTHER SUBSTANCES
Extracted: dihydroxyphenylacetic acid, dopamine, homovanilic acid

KEY WORDS
plasma; pharmacokinetics; dialysate

REFERENCE
Dethy,S.; Laute,M.A.; Van Blercom,N.; Damhaut,P.; Goldman,S.; Hildebrand,J. Microdialysis-HPLC for plasma levodopa and metabolites monitoring in parkinsonian patients, *Clin.Chem.*, **1997**, *43*, 740–744.

SAMPLE
Matrix: blood
Sample preparation: Total levodopa. Add 300 μL 60 mM trichloroacetic acid to 1 mL plasma, place the mixture in an ice bath for 10 min, centrifuge at 5000 g for 10 min, inject a 20 μL aliquot of the supernatant. Unbound levodopa. Add 250 μL plasma to 5 μL 4 M orthophosphoric acid, centrifuge through a membrane Minicent 10 (Bio-Rad) at 8000 g at 25° for 30 min, inject a 20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 45 × 4.6 5 μm C18 (Bio-Rad)
Mobile phase: MeCN:1.4 mM sodium dodecylsulphate:50 mM KH_2PO_4 16:42:42, pH 2.8
Flow rate: 1
Injection volume: 20
Detector: E, ESA Coulochem 5100, analytical cell +50 mV, -300 mV, conditioning cell +300 mV

CHROMATOGRAM
Retention time: 3.7
Limit of detection: 200 pg/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Rizzo,V.; Memmi,M.; Moratti,R.; Melzi,E.; Perucca,E. Concentrations of L-dopa in plasma and plasma ultrafiltrates, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 1043–1046.

SAMPLE
Matrix: blood
Sample preparation: 0.5 mL Plasma + 50 μL 4 M perchloric acid + 50 μL 1 μg/mL dihydroxybenzylamine in 0.1 M perchloric acid, centrifuge at 1500 g for 10 min. Remove 300 μL supernatant and centrifuge it at 1600 g through a 0.2 μm regenerated cellulose filter, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Biophase ODS + 50 × 4.6 Pelliguard LC-18

Column: 250 × 4.6 5 μm Biophase ODS or 250 × 4.6 Phase II ODS (both from Bioanalytical Systems)
Mobile phase: MeOH:buffer 5:95 (Buffer was 20 mM sodium citrate, 100 mM NaH_2PO_4, 0.15 mM, and 1.25 mM heptanesulfonic acid, pH 3.2.)
Column temperature: 28
Flow rate: 1-1.5
Injection volume: 20
Detector: E, Bioanalytical Systems LC-150 in dual-parallel mode, channel 1 700 mV 200 nA f.s. for levodopa and 3-O-methyldopa, channel 2 560 mV 10 nA f.s. for dopamine, carbidopa, and dihydroxyphenylacetic acid, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 5.1
Internal standard: dihydroxybenzylamine (7)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Simultaneous: 3-O-methyldopa, dopamine, carbidopa, dihydroxyphenylacetic acid

KEY WORDS
plasma

REFERENCE
Cedarbaum,J.M.; Williamson,R.; Kutt,H. Simultaneous determination of levodopa, its metabolites and carbidopa in clinical samples, *J.Chromatogr.*, **1987**, *415*, 393–399.

SAMPLE
Matrix: blood
Sample preparation: Prepare a 20 × 5 polypropylene column packed with CM-Sephadex preswollen in water, wash with 5 mL 2 M HCl, wash with 10 mL water, wash with 10 mL 100 mM pH 7 phosphate buffer. Add 1 mL plasma to column, elute with 5.5 mL water, discard first 1 mL. Add next 4.5 mL to 0.5 mL 0.5 M perchloric acid, centrifuge, inject 10 μL aliquot of supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:MeOH:25 mM sodium acetate 4:4:92 containing 0.2 mM 1-octanesulfonic acid and 0.3 mM disodium EDTA, pH was adjusted to pH 3 with acetic acid
Flow rate: 0.9
Injection volume: 10
Detector: E, ESA Coulochem 5100 A, 5010 A analytical cell, first electrode +0.25 V, second electrode -0.30 V

CHROMATOGRAM
Retention time: 7
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Simultaneous: O-methyldopa, carbidopa, dihydroxyphenylacetic acid (DOPAC)

KEY WORDS
plasma

REFERENCE
Betto,P.; Ricciarello,G.; Giambenedetti,M.; Lucarelli,C.; Ruggeri,S.; Stocchi,F. Improved high-performance liquid chromatographic analysis with double detection system for L-dopa, its metabolites and carbidopa in plasma of parkinsonian patients under L-dopa therapy, *J.Chromatogr.*, **1988**, *459*, 341–349.

SAMPLE
Matrix: blood

Sample preparation: 1 mL Plasma + 100 μL 1.25 μg/mL α-ethyldopa in 0.1 M HCl + 100 μL 4 M perchloric acid, vortex, centrifuge at 2000 g for 10 min, inject a 60 μL aliquot of the supernatant (keep sample tray at 6 ± 1°).

HPLC VARIABLES

Guard column: 45 × 4.6 37-40 μm Whatman pellicular-ODS followed by 45 × 4.6 5 μm Ultra sphere-IP C18
Column: 250 × 4.6 5 μm Ultrasphere IP C18
Mobile phase: MeOH:20 mM orthophosphoric acid and 4 mM sodium octanesulfonate 25:75 adjusted to pH 2.8 ± 0.05 with 50% NaOH
Column temperature: 40
Flow rate: 1
Injection volume: 60
Detector: E, BAS LC-4B, 0.75 V vs Ag/AgCl, 5 nA full scale for carbidopa, 20 nA full scale for 3-O-methyldopa and levodopa

CHROMATOGRAM

Retention time: 5.9
Internal standard: α-ethyldopa (15.4)
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES

Extracted: carbidopa, 3-O-methyldopa

KEY WORDS

plasma; stabilize plasma sample immediately with EDTA and 2 mg/mL sodium metabisulfite

REFERENCE

Titus,D.C.; August,T.F.; Yeh,K.C.; Eisenhandler,R.; Bayne,W.F.; Musson,D.G. Simultaneous high-performance liquid chromatographic analysis of carbidopa, levodopa and 3-O-methyldopa in plasma and carbidopa, levodopa and dopamine in urine using electrochemical detection, *J.Chromatogr.*, **1990**, *534*, 87–100.

SAMPLE

Matrix: blood
Sample preparation: 4 mL Plasma + 500 μL 20 mg/mL ascorbic acid solution, vortex for 30 s. 1 mL Aliquot + 75 mg acid washed alumina + 100 μL 1 μg/mL 3,4-dihydroxybenzylamine hydrobromide in buffer, vortex, add 1 mL 1.5 M pH 8.6 TRIS buffer, shake at 230 oscillations/min for 15 min. Allow to settle and discard plasma, wash the alumina twice by shaking with 5 mL water for 10 min. To the washed alumina add 900 μL buffer, vortex for 20 s, allow to settle, inject a 50 μL aliquot of the supernatant. (Buffer was 200 mM phosphoric acid containing 3.3 μm EDTA and 6.7 μm potassium metabisulfite.)

HPLC VARIABLES

Column: 100 × 4.6 3 μm Spherisorb ODS-2
Mobile phase: MeCN:buffer 8:92 (Buffer was pH 2.6 550 mM NaH_2PO_4 containing 1 mM sodium octyl sulfate and 0.7 mM EDTA.)
Flow rate: 1.5
Injection volume: 50
Detector: E, Bioanalytical Systems LC-4B, glassy carbon electrode, Ag/AgCl reference electrode, 0.75 V.

CHROMATOGRAM

Retention time: 3.3
Internal standard: 3,4-dihydroxybenzylamine hydrobromide (4.5)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES

Extracted: carbidopa
Noninterfering: caffeine, ibuprofen, aspirin, nicotine, acetaminophen, theophylline

KEY WORDS

plasma; SPE

REFERENCE
Miller,R.B.; Dehelean,L.; Bélanger,L. Determination of carbidopa and levodopa in human plasma by high-performance liquid chromatography with electrochemical detection, *Chromatographia*, **1993**, *35*, 607–612.

SAMPLE
Matrix: blood
Sample preparation: 0.5 mL Plasma + 25 μL 2 μg/mL 3,4-dihydroxybenzylamine in 0.4 M perchloric acid + 25 μL 70% perchloric acid, vortex 1 min, keep on ice for 1 min, store at -80°. Allow to thaw at +4°, vortex 1 min, centrifuge at 1200 g at +4° for 15 min. Remove 300 μL supernatant and add it to 200 μL 2 M pH 4.5 potassium citrate buffer, centrifuge at 1200 g at +4° for 10 min, inject 50 μL of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb C18
Mobile phase: MeOH:MeCN:buffer 8:4:88 (Buffer was 50 mM pH 3.2 phosphate containing 3.5 mM heptanesulfonic acid and 0.05 mM EDTA.)
Flow rate: 1
Injection volume: 50
Detector: E, ESA Model 5100, Model 5020 guard cell +0.6 V, Model 5010 analytical cell, DET 1 +0.35 V, DET 2 -0.35 V, both DET 1 and DET 2 monitored

CHROMATOGRAM
Retention time: 4.15
Internal standard: 3,4-dihydroxybenzylamine (8.26)
Limit of detection: 19.6 ng/mL

OTHER SUBSTANCES
Simultaneous: 3-O-methyldopa, dopamine, L-DOPA methyl ester

KEY WORDS
plasma; rat; human

REFERENCE
Rondelli,I.; Acerbi,D.; Mariotti,F.; Ventura,P. Simultaneous determination of levodopa methyl ester, levodopa, 3-O-methyldopa and dopamine in plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.B*, **1994**, *653*, 17–23.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 20 μL Plasma + 180 μL isoproterenol in 100 mM perchloric acid containing 10 μM disodium EDTA, centrifuge at 4° at 4000 rpm for 10 min. Filter (0.45 μm cellulose acetate) the supernatant and inject a 10 μL aliquot of the supernatant. Tissue. Homogenize (Polytron PT 10-35) 50 mg brain tissue + 500 μL isoproterenol in 100 mM perchloric acid containing 10 μM disodium EDTA at 15000 rpm for 10 s, centrifuge at 4° at 4000 rpm for 10 min. Filter (0.45 μm cellulose acetate) the supernatant and inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: Supelcosil LC-18-DB
Column: Supelcosil LC-18-DB
Mobile phase: MeOH:10 mM pH 4.4 citrate buffer 10:90 containing 10 μM disodium EDTA and 0.5 mM sodium 1-octanesulfonate
Flow rate: 1
Injection volume: 10
Detector: E, EICOM Co. ECD-100, +0.7 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 4
Internal standard: isoproterenol (45)

KEY WORDS
plasma; rat; brain; pharmacokinetics

REFERENCE

Sato,S.; Koitabashi,T.; Koshiro,A. Pharmacokinetic and pharmacodynamic studies of L-dopa in rats. I. Pharmacokinetic analysis of L-dopa in rat plasma and striatum, *Biol.Pharm.Bull.*, **1994**, *17*, 1616–1621.

SAMPLE

Matrix: blood, urine

Sample preparation: Condition a 150 μL Toyopak IC-SP S (sulfopropyl resin, H^+ form) SPE cartridge (Tosoh) with 10 mL water. Plasma. 700 μL Plasma + 50 μL 700 nM 3,4-dihydroxybenzylamine + 350 μL 2 M perchloric acid, mix, centrifuge at 4° at 1000 g for 15 min. Remove a 700 μL aliquot of the supernatant and add it to 30 μL 2 M potassium carbonate, centrifuge at 4° at 1000 g for 5 min. Add a 500 μL aliquot of the supernatant to the SPE cartridge, wash with 1 mL water, wash with 500 μL EtOH:water 50:50, wash with 5 mL water, elute with 500 μL 2 M sodium perchlorate, filter (0.2 μm), inject a 50 μL aliquot of the filtrate. Urine. Acidify urine collected over 24 h with 10 mL 6 M HCl. 500 μL Urine + 25 μL 10 μM 3,4-dihydroxybenzylamine + 25 μL 40 μM ferulic acid + 500 μL 1 M perchloric acid, mix, centrifuge at 4° at 1000 g for 15 min. Remove a 700 μL aliquot of the supernatant and add it to 30 μL 2 M potassium carbonate, centrifuge at 4° at 1000 g for 5 min, add a 500 μL aliquot of the supernatant to the SPE cartridge, wash with 1.5 mL water, wash with 500 μL EtOH:water 50:50, wash with 5 mL water, elute with 500 μL 2 M sodium perchlorate, filter (0.2 μm), inject a 50 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 150 × 4.6 5 μm TSK-gel ODS-80TM (Tosoh)

Mobile phase: Gradient. A was buffer. B was MeCN:MeOH:buffer 8:12:80, pH 3.1. A:B 100:0 for 4 min, to 60:40 over 8 min, to 0:100 over 2 min, maintain at 0:100 for 16 min, return to initial conditions (step gradient), re-equilibrate for 20 min. Buffer was 60 mM pH 3.1 citric acid containing 32 mM Na_2HPO_4, 1.7 mM sodium hexanesulfonate, and 0.1 mM disodium EDTA (J. Chromatogr. 1989, 467, 237).

Flow rate: 1

Injection volume: 50

Detector: F ex 345 em 480 following post-column reaction. The column effluent passed through a Hitachi 655A electrochemical detector with carbon cloth electrodes; working electrode at +0.68 V versus reference electrode (200 mM equimolar mixture of potassium hexacyanoferrate(II) and potassium hexacyanoferrate(III) containing 200 mM potassium nitrate and 200 mM KOH). The effluent from the electrochemical detector mixed with 20 mM meso-1,2-diphenylethylenediamine in 50 mM HCl pumped at 0.4 mL/min and with 1 M glycine containing 490 mM KOH and 3 mM potassium hexacyanoferrate(III) pumped at 0.4 mL/min. This mixture flowed through a 10 m × 0.47 mm ID coil at 80° to the detector (J. Chromatogr. 1989, 467, 237).

CHROMATOGRAM

Retention time: 9.5

Internal standard: 3,4-dihydroxybenzylamine (12.5)

Limit of detection: 12 nM (urine), 10 nM (plasma)

OTHER SUBSTANCES

Extracted: dopamine, epinephrine, metanephrine, 3-methoxytyramine, norepinephrine

KEY WORDS

post-column reaction; plasma; SPE

REFERENCE

Nohta,H.; Yamaguchi,E.; Ohkura,Y.; Watanabe,H. Measurement of catecholamines, their precursor and metabolites in human urine and plasma by solid-phase extraction followed by high-performance liquid chromatography with fluorescence derivatization, *J.Chromatogr.*, **1989**, *493*, 15–26.

SAMPLE

Matrix: blood, urine

Sample preparation: Condition a Toyopak IC-SP S sulfopropyl resin, H^+ form, SPE cartridge (Tosoh) with 10 mL water and 2 mL 200 mM pH 5.0 sodium phosphate buffer. Plasma. 700 μL Plasma + 30 μL 700 nM isoproterenol + 50 μL 7 μM 3,4-dihydroxyphenylpropanoic acid + 350 μL 2 M perchloric acid, mix, centrifuge at 4° at 1000 g for 15 min. Remove a 700 μL aliquot

of the supernatant and adjust the pH to 1.5-2.0 with about 150 μL 2 M potassium carbonate, centrifuge at 4° at 1000 g for 5 min, add the supernatant to the SPE cartridge, wash with 10 mL water, elute with 300 μL MeOH:2 M sodium perchlorate 7:93, filter (cellulose acetate membrane), inject a 100 μL aliquot of the filtrate. Urine. Collect human urine for 24 h in the presence of 10 mL 6 M HCl. 500 μL Urine + 10 μL 15 μM isoproterenol + 25 μL 800 μM 3,4-dihydroxyphenylpropanoic acid + 500 μL 1 M perchloric acid, mix, centrifuge at 4° at 1000 g for 15 min. Remove a 700 μL aliquot of the supernatant and adjust the pH to 1.5-2.0 with about 130 μL 2 M potassium carbonate, centrifuge at 4° at 1000 g for 5 min, add the supernatant to the SPE cartridge, wash with 1.5 mL water, wash with 500 μL EtOH:water 50:50, wash with 5 mL water, elute with 500 μL 1.5 M KCl in MeOH:100 mM HCl 7:93, filter (cellulose acetate membrane), inject a 100 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 250 × 4.6 5 μm TSK-gel ODS-80TM (Tosoh)

Mobile phase: MeOH:buffer 7:93 (Buffer was 30 mM pH 2.5 citrate buffer containing 0.4 mM sodium octanesulfonate.)

Flow rate: 0.8

Injection volume: 100

Detector: F ex 350 em 480 following post-column reaction. The column effluent mixed with reagent A pumped at 0.3 mL/min and the mixture flowed through a 3 m × 0.5 mm ID stainless steel coil at 90°. The effluent from this coil mixed with reagent B pumped at 0.3 mL/min and the mixture flowed through a 10 m × 0.5 mm ID stainless steel coil at 90° and through a 1 m × 0.5 mm ID stainless steel cooling coil to the detector (Anal. Sci. 1991, 7, 257). (Reagent A was 10 mM sodium periodate containing 3 mM potassium ferricyanide. Reagent B was 30 mM meso-1,2-diphenylethylenediamine in EtOH:water 70:30 containing 130 mM sodium methylate.)

CHROMATOGRAM

Retention time: 23

Internal standard: isoproterenol (60)

Limit of detection: 7-9 nM

OTHER SUBSTANCES

Extracted: dopamine, epinephrine, metanephrine, 3-methoxytyramine, norepinephrine, normetanephrine

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KEY WORDS

post-column reaction; plasma; SPE

REFERENCE

Jeon,H.-K.; Nohta,H.; Ohkura,Y. High-performance liquid chromatographic determination of catecholamines and their precursor and metabolites in human urine and plasma by postcolumn derivatization involving chemical oxidation followed by fluorescence reaction, *Anal.Biochem.*, **1992**, *200*, 332–338.

SAMPLE

Matrix: blood, urine

Sample preparation: 2 mL Plasma or 1 mL urine + dihydroxybenzylamine + 20 mg Sigma WA4 alumina + 200 μL 1 M pH 8.6 Tris-EDTA buffer, mix for 10 min, discard plasma. Wash the alumina three times with 3 mL water and dry it. Add 125 μL 500 mM phosphoric acid, after 1 min inject a 100 μL aliquot. (Ann. Clin. Biochem. 1985, 22, 194-203)

HPLC VARIABLES

Column: 250 × 4.5 5 μm Ultratechsphere

Mobile phase: Per liter 75 mmol citric acid, 58.5 mmol NaH_2PO_4, 0.2 mmol disodium EDTA, and 4.4 mmol heptanesulfonic acid, pH adjusted to 3.4, made up to a final volume of 2 L, add 200 mL MeOH

Flow rate: 1

Injection volume: 100

Detector: E, ESA Coulochem conditioning cell +0.35 V, first electrode +0.05 V, second electrode -0.35 V

CHROMATOGRAM
Retention time: 5.37
Internal standard: dihydroxybenzylamine (10.53)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Simultaneous: norepinephrine, metanephrine, epinephrine, 3-methoxytyrosine, normetanephrine, dihydroxyphenylacetic acid, dopamine

KEY WORDS
plasma

REFERENCE
Dutton,J.; Copeland,L.G.; Playfer,J.R.; Roberts,N.B. Measuring L-dopa in plasma and urine to monitor therapy of elderly patients with Parkinson disease treated with L-dopa and a dopa decarboxylase inhibitor, *Clin.Chem.*, **1993**, *39*, 629–634.

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

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

CHROMATOGRAM
Retention time: 3.575

KEY WORDS
whole blood

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

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets or contents of capsules, weigh out an amount equivalent to about 100 mg levodopa, add 30 mL 0.1 M HCl, sonicate, make up to 50 mL with 0.1 M HCl, mix, filter (0.45 μm), discard first 5 mL filtrate. 5 mL Filtrate + 10 mL 2 mg/mL methyldopa in 0.1 M HCl, make up to 100 mL with mobile phase, mix, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: 3% aqueous acetic acid

Flow rate: 1.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 3
Internal standard: methyldopa (4.5)

KEY WORDS
capsules; tablets

REFERENCE
Ting,S. Liquid chromatographic determination of levodopa and levodopa-carbidopa in solid dosage forms: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1987**, *70*, 987–990.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve in mobile phase, filter, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm μBondapak C18
Mobile phase: MeOH:50 mM ammonium acetate adjusted to pH 4.1 with 0.6 M acetic acid 1:99
Flow rate: 0.9
Detector: E, Coulochem model 5100A, screen electrode +0.3 V, sample electrode +0.6 V and UV 280

CHROMATOGRAM
Retention time: 6.35
Limit of detection: 1000 ng/mL (UV), 20 ng/mL (E)

OTHER SUBSTANCES
Simultaneous: hydroxydopa, carbidopa, methyldopa, methoxytyrosine, methylcarbidopa, impurities

KEY WORDS
stability-indicating; tablets

REFERENCE
Kafil,J.B.; Dhingra,B.S. Stability-indicating method for the determination of levodopa, levodopa-carbidopa and related impurities, *J.Chromatogr.A*, **1994**, *667*, 175–181.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Grind tablets, weigh out a portion, dissolve in 50 mL mobile phase, sonicate, filter (No. 4 sintered glass plate), dilute, inject an aliquot. Capsules. Dissolve 10 capsules (without opening) in 100 mL mobile phase, sonicate, inject an aliquot. Injections, ampules, sprays. Dilute, inject an aliquot.

HPLC VARIABLES
Column: 120 × 4.6 Spherisorb C18 ODS-2
Mobile phase: Isopropanol:buffer 5:95 (Buffer was 100 mM sodium dodecyl sulfate containing 25 mM Na_2HPO_4, pH adjusted to 3.0 with HCl.)
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 3
Limit of detection: 4 ng/mL

OTHER SUBSTANCES
Simultaneous: carbidopa, dopamine, epinephrine, hydrochlorothiazide, isoproterenol, 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: solutions
Sample preparation: Inject an aliquot of a 100 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 150 × 4 5 μm Crownpak CR(+) immobilized crown ether
Mobile phase: MeOH:0.1% pH 1.9 perchloric acid 15:85
Column temperature: 40
Flow rate: 1
Detector: UV 210

CHROMATOGRAM
Retention time: 1.48, 1.70 (enantiomers)

OTHER SUBSTANCES
Simultaneous: baclofen, norephedrine, primaquine

KEY WORDS
chiral; comparison with capillary electrophoresis

REFERENCE
Nishi,H.; Nakamura,K.; Nakai,H.; Sato,T. Separation of enantiomers and isomers of amino compounds by capillary electrophoresis and high-performance liquid chromatography utilizing crown ethers, *J.Chromatogr.A*, **1997**, *757*, 225–235.

SAMPLE
Matrix: tissue
Sample preparation: Prepare a 70 × 5 SPE column of Sephadex G 10 in a Pasteur pipette, wash with 3 mL 20 mM ammonia and 3 mL 10 mM formic acid, let stand for 10 days. Homogenize up to 150 mg rat brain in 1 mL 100 mM perchloric acid, centrifuge at 4000 g at 4° for 15 min, add 500 μL of the supernatant to the SPE column, wash with 2.5 mL 10 mM formic acid, elute with 1 mL 10 mM formic acid followed by 1.5 mL 5 mM Na_2HPO_4, inject an aliquot of the eluate.

HPLC VARIABLES
Column: Nucleosil 5 C18
Mobile phase: pH 5.5 Buffer prepared from 200 mM Na_2HPO_4 and 100 mM citric acid
Flow rate: 0.8
Injection volume: 200
Detector: E, rotating disc electrode, 500 mV

CHROMATOGRAM
Retention time: 4.5
Limit of detection: 0.015 nmole/g

OTHER SUBSTANCES
Extracted: dopamine, uric acid

KEY WORDS
rat; brain; SPE

REFERENCE
Westerink,B.H.C.; Mulder,T.B.A. Determination of picomole amounts of dopamine, noradrenaline, 3,4-dihydroxyphenylacetic acid, homovanillic acid, and 5-hydroxyindolacetic acid in nervous tissue after one-step purification on Sephadex G-10, using high-performance liquid chromatography with a novel type of electrochemical detection, *J.Neurochem.*, **1981**, *36*, 1449–1462.

SAMPLE
Matrix: urine
Sample preparation: 100 μL Urine + 100 μL solution containing 55 mM ascorbic acid and 55 mM disodium EDTA + 25 μL 1.25 μg/mL α-ethyldopa in 0.1 M HCl + 25 mg alumina + 1 mL 2 M pH 8.6 Tris-HCl buffer in a microfilter tube (Centrex, Schleicher & Schuell), vortex 5 min, allow to stand for 10 min, filter off water, wash with 5 mL water, add 5 mL water, centrifuge at 3000 g, vortex with 400 μL 0.2 M perchloric acid containing 11 mM disodium EDTA and 0.4 M sodium metabisulfite, centrifuge at 9000 g for 5 min, inject 50 μL of filtrate. (Stabilize each 10 mL urine sample immediately with 0.5 mL 0.1 M HCl and 1 mL solution containing 55 mM ascorbic acid and 55 mM disodium EDTA.)

HPLC VARIABLES
Guard column: 40 × 4.6 Bio-Sil ODS-10 (Bio-Rad)
Column: 250 × 4.6 5 μm Ultrasphere IP C18
Mobile phase: MeOH:water 22.5:77.5 containing 20 mM citric acid, 20 mM Na_2HPO_4, 4 mM sodium octanesulfonate, and 0.05 mM disodium EDTA, pH adjusted to 2.74 ± 0.01 with 2 M citric acid
Column temperature: 40
Injection volume: 50
Detector: E, BAS LC-4B, 0.54 V vs Ag/AgCl, 50 nA full scale

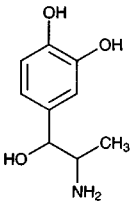
CHROMATOGRAM
Retention time: 6
Internal standard: α-ethyldopa (14)
Limit of quantitation: 250 ng/mL

OTHER SUBSTANCES
Extracted: carbidopa, dopamine

KEY WORDS
SPE

REFERENCE
Titus,D.C.; August,T.F.; Yeh,K.C.; Eisenhandler,R.; Bayne,W.F.; Musson,D.G. Simultaneous high-performance liquid chromatographic analysis of carbidopa, levodopa and 3-O-methyldopa in plasma and carbidopa, levodopa and dopamine in urine using electrochemical detection, *J.Chromatogr.*, **1990**, *534*, 87–100.

Levonordefrin



Molecular formula: $C_9H_{13}NO_3$
Molecular weight: 183.21
CAS Registry No.: 829-74-3, 138-61-4 (racemic HCl)
Merck Index: 6785
Lednicer No.: 1 68

SAMPLE

Matrix: formulations
Sample preparation: Tablets. Dissolve powdered tablets in 10 mM HCl, filter if necessary, inject an aliquot. Injections, solutions. Dilute with 10 mM HCl, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Partisil-5 ODS-3
Mobile phase: MeOH:buffer 30:70 (Buffer was 10 mM sodium 1-octanesulfonate in 0.2% acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 220

CHROMATOGRAM

Retention time: 12
Limit of detection: 36 ng

OTHER SUBSTANCES

Simultaneous: norepinephrine, epinephrine, isoproterenol, phenylephrine, metaraminol, impurities

KEY WORDS

tablets; injections; ophthalmic solutions; inhalation solutions

REFERENCE

Smela,M.J.,Jr.; Stromberg,R. Liquid chromatographic determination of six sympathomimetic drugs in dosage forms, *J.Assoc.Off.Anal.Chem.*, **1991**, *74*, 289–291.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Partisil ODS-3
Mobile phase: MeOH:buffer 30:70 (Buffer was 10 mM octanesulfonic acid in 0.2% acetic acid.)
Flow rate: 1
Detector: UV 220

CHROMATOGRAM

Retention time: 12

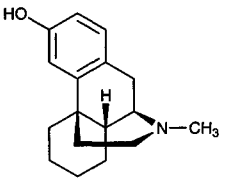
OTHER SUBSTANCES

Simultaneous: epinephrine, isoproterenol, metaraminol, phenylephrine

REFERENCE

Phenomenex Catalog, **1994**, p. 1.077.

Levorphanol



Molecular formula: $C_{17}H_{23}NO$
Molecular weight: 257.38
CAS Registry No.: 77-07-6, 5985-38-6 (tartrate dihydrate), 125-72-4 (tartrate)
Merck Index: 5496
Lednicer No.: 1 293

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 20 ng levallorphan, mix, add 1 mL 1 M pH 9 borate buffer, add 10 mL hexane:ethyl acetate 90:10, shake for 10 min, centrifuge at 2500 rpm for 10 min. Remove 9 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:buffer 30:70 containing 0.1 mM EDTA (Buffer was 10 mM NaCl adjusted to pH 4.8 with 1 M HCl.)
Flow rate: 1
Injection volume: 100
Detector: E, Bioanalytical Systems LC-4B, glassy carbon electrode +1.00 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 6.5
Internal standard: levallorphan (9.5)
Limit of quantitation: 1.25 ng/mL

OTHER SUBSTANCES

Simultaneous: 6-acetylmorphine, diamorphine, oxymorphine, pentazocine
Noninterfering: acetaminophen, 1-α-acetylmethodol, caffeine, codeine, hydrocodone, hydromorphine, meperidine, morphine, oxycodone, propoxyphene

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Lucek,R.; Dixon,R. Quantitation of levorphanol in plasma using high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1985**, *341*, 239–243.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 2.58 μg/mL laudanosine in MeCN + 500 μL 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 μL mobile phase, inject a 300 μL 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 × 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: 6.615
Internal standard: laudanosine (8.603)
Limit of detection: 30 ng/mL

OTHER SUBSTANCES
Extracted: guaifenesin

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, CSF, urine
Sample preparation: Dilute urine 3:1 or more with water. Vortex 1 mL CSF, plasma, or diluted urine with 100 μL 100 ng/mL IS, add 500 μL saturated sodium carbonate, mix, add 5 mL hexane containing 0.1% n-octylamine. Vortex for 60 s, centrifuge at 2000 g for 10 min. Re-extract aqueous phase with 5 mL hexane containing 0.1% n-octylamine, evaporate the combined hexane extracts to dryness under a stream of nitrogen in a 50° water bath. Reconstitute residue with 150 μL 100 mM HCl, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 5 μm CN
Column: 220 × 4.6 5 μm Brownlee Spheri-5CN (Applied Biosystems, USA)
Mobile phase: MeCN:n-octylamine:water 19:0.05:80.95 adjusted to pH 2.8 with phosphoric acid
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: F ex 230 em 330

CHROMATOGRAM
Retention time: 5.3
Internal standard: levallorphan (7.4)
Limit of detection: 1 ng/mL
Limit of quantitation: 1 ng/mL (CSF, plasma), 5 ng/mL (urine)

OTHER SUBSTANCES
Extracted: dextromethorphan

KEY WORDS
plasma

REFERENCE
Kimiskidis,V.K.; Kazis,A.D.; Niopas,I. Simultaneous determination of dextromethorphan and dextrorphan in human plasma, urine and cerebrospinal fluid by HPLC with fluorescence detection, *J.Liq. Chromatogr.Rel.Technol.*, **1996**, *19*, 1267–1275.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 100 μL 1 μg/mL pholcodine in water + 500 μL saturated sodium carbonate, mix, add 4 mL diethyl ether:chloroform:isopropanol 20:9:1, mix on a rotary mixer for 10 min, centrifuge at 2000 g for 10 min. Remove the organic layer and add it to 100 μL 100 mM HCl, mix on a rotary mixer for 10 min, centrifuge at 2000 g for 5 min, inject a 10-50 μL aliquot of the aqueous layer. Urine. 500 μL Urine + 50 μL 50 μg/mL pholcodine in water + 500 μL saturated sodium carbonate, mix, add 4 mL diethyl ether:chloroform:isopropanol 20:9:1, mix on a rotary mixer for 10 min, centrifuge at 2000 g for 10 min. Remove the organic layer and add it to 100 μL 100 mM HCl, mix on a rotary mixer for 10

min, centrifuge at 2000 g for 5 min, inject a 10-50 μL aliquot of the aqueous layer. (If desired, hydrolyse 500 μL plasma or urine with 500 μL 8000 U/mL β-glucuronidase (Helix pomatia, type H-1, Sigma) in 200 mM pH 5 acetate buffer at 37° for 16 h, proceed as above.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb cyano
Mobile phase: MeCN:water:triethylamine 17:82.94:0.06, adjusted to pH 3.0 with orthophosphoric acid
Flow rate: 1
Injection volume: 10-50
Detector: F ex 230 em 330

CHROMATOGRAM
Retention time: 5.1
Internal standard: pholcodine (6.3)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, dextromethorphan
Noninterfering: acetaminophen, clofibrate, codeine, cyclophosphamide, diclofenac, digoxin, doxepin, doxorubicin, estrogens, flucloxacillin, folic acid, furosemide, metformin, metoclopramide, miconazole, minoxidil, morphine, nifedipine, nitroglycerin, norcodeine, norethisterone, oxazepam, oxethazaine, prednisolone, pseudoephedrine, quinine, spironolactone, temazepam, tolbutamide, warfarin

KEY WORDS
plasma

REFERENCE
Chen,Z.R.; Somogyi,A.A.; Bochner,F. Simultaneous determination of dextromethorphan and three metabolites in plasma and urine using high-performance liquid chromatography with application to their disposition in man, *Ther.Drug Monit.*, **1990**, *12*, 97–104.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 600 μL Microsomal incubation + 600 μL saturated sodium carbonate, place on ice, add 150 μL 12.5 μg/mL betaxolol, extract with 5 mL ethyl acetate. Remove the organic layer and add it to 300 μL 0.5% orthophosphoric acid, extract, inject an aliquot of the aqueous layer.

HPLC VARIABLES
Column: 5 mm i.d. 4 μm Nova-Pak phenyl radial-Pak
Mobile phase: MeCN:MeOH:0.05% orthophosphoric acid 24:10:66
Flow rate: 1.6
Detector: F ex 261 em 306

CHROMATOGRAM
Retention time: 5.5
Internal standard: betaxolol
Limit of detection: 60 nM

OTHER SUBSTANCES
Extracted: dextromethorphan

KEY WORDS
rat; liver

REFERENCE
Laslett,T.J.; Alvarez,F.; Nation,R.L.; Evans,A.M.; Scott,S.D.; Stupans,I. Effect of cyclophosphamide administration on the activity and relative content of hepatic P4502D1 in rat, *Xenobiotica*, **1995**, *25*, 1031–1039.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH 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: 5.55

OTHER SUBSTANCES
Simultaneous: buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine, norpipanone, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodeine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, norcodeine, normorphine, pemoline, benzphetamine, diethylpropion, mazindol, tranylcypromine, caffeine, fenethyline, phendimetrazine, methylphenidate, phenelzine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine

Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine

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: 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: 2.755

OTHER SUBSTANCES
Also analyzed: 6-acetylmorphine, amiloride, amphetamine, benzocaine, benzoylecgonine, caffeine, cocaine, codeine, doxylamine, fluoxetine, glutethimide, hexobarbital, hypoxanthine, LSD, meperidine, mephobarbital, methadone, methylphenidate, methyprylon, N-norcodeine, oxazepam, oxycodone, phenylpropanolamine, prilocaine, procaine, terfenadine

REFERENCE
Ascah,T.L. Improved separations of alkaloid drugs and other substances of abuse using Supelcosil LC-ABZ column, *Supelco Reporter*, **1993**, *12(3)*, 18–21.

SAMPLE
Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

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

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

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

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4 5 μm LiChrospher 100 RP-8

Mobile phase: MeCN:0.025% phosphoric acid:buffer 60:25:15 (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 4.26

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, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

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 + 1 mL 100 mM pH 5 acetate buffer + 20 μL β-glucuronidase/β-arylsulfatase (Helix pomatia, Boehringer Mannheim) + 50 μL 600 mM sodium azide in water, heat at 37° for 12 h. Inject a 100 μL aliquot onto column A and elute to waste at 0.5 mL/min, after 3 min elute contents of column A onto column B at 1.4 mL/min, monitor the effluent from column B.

HPLC VARIABLES

Column: A 30 × 4 10 μm LiChrosorb CN; B 250 × 4.6 5 μm Spherisorb phenyl
Mobile phase: MeCN:10 mM KH_2PO_4 50:50, adjusted to pH 4 with phosphoric acid
Flow rate: A 0.5; B 1.4
Injection volume: 100
Detector: UV 200 or F ex 280 em 310

CHROMATOGRAM
Retention time: 9.5
Limit of detection: <50 ng/mL (UV)

OTHER SUBSTANCES
Extracted: metabolites, dextromethorphan

KEY WORDS
column-switching

REFERENCE
Motassim,N.; Decolin,D.; Le Dinh,T.; Nicolas,A.; Siest,G. Direct determination of dextromethorphan and its three metabolites in urine by high-performance liquid chromatography using a precolumn switching system for sample clean-up, *J.Chromatogr.*, **1987**, *422*, 340–345.

SAMPLE
Matrix: urine
Sample preparation: Condition a Bond Elut silica modified with carboxylic acid ion-exchange groups SPE cartridge with 1 mL MeCN:100 mM HCl 40:60 and 1 mL water. Adjust 1 mL urine to pH 5.0-5.5, add β-glucuronidase/arylsulfatase (Helix pomatia (Boehringer Mannheim), heat at 37° for 18 h, add 1 mL to the SPE cartridge, wash with 1 mL water, wash with 500 μL 100 mM HCl, elute with 1 mL MeCN:100 mM HCl 40:60, inject a 20 μL aliquot of the eluate.

HPLC VARIABLES
Column: 250 mm long 5 μm Zorbax phenyl
Mobile phase: MeCN:100 mM KH_2PO_4 45:55, adjusted to pH 4
Flow rate: 1.5
Injection volume: 20
Detector: F ex 280 em 310 or UV 280

CHROMATOGRAM
Retention time: 4.8
Limit of detection: 20 ng/mL (F)

OTHER SUBSTANCES
Extracted: metabolites, dextromethorphan

KEY WORDS
SPE

REFERENCE
Jacqz-Aigrain,E.; Menard,Y.; Popon,M.; Mathieu,H. Dextromethorphan phenotypes determined by high-performance liquid chromatography and fluorescence detection, *J.Chromatogr.*, **1989**, *495*, 361–363.

SAMPLE
Matrix: urine
Sample preparation: Condition a 3 mL 200 mg Bond Elut C18 SPE cartridge with 6 mL MeOH, 6 mL water, and 4 mL 100 mM pH 9.2 sodium carbonate buffer. 750 μL Urine + 750 μL 100 mM pH 5.0 sodium acetate buffer containing 20 μL β-glucuronidase-arylsulfatase (Helix pomatia, 100000 Fisherman units/mL, Boehringer Mannheim) + 50 μL 600 mM sodium azide, heat at 37° for 18 h. 250 μL Hydrolysed urine + 100 μL 10 μg/mL levallorphan tartrate in water + 2 mL 100 mM pH 9.2 sodium carbonate, add to SPE cartridge, wash with 2 mL water, wash with 1 mL MeCN, elute with 3 mL MeOH:MeCN:2% phosphoric acid 50:30:20. Evaporate the eluate to dryness under a stream of nitrogen at 70°, reconstitute the residue in 500 μL mobile phase, inject a 20 μL aliquot. (For low concentrations of dextromethorphan: 500 μL Hydrolysed urine + 100 μL 1 μg/mL levallorphan tartrate in water + 2 mL 100 mM pH 9.2 sodium carbonate, add to SPE cartridge, wash with 2 mL water, wash with 1 mL MeCN, elute with 3 mL MeOH:MeCN:2% phosphoric acid 50:30:20. Evaporate the eluate to dryness under a stream of nitrogen at 70°, reconstitute the residue in 250 μL mobile phase, inject a 120 μL aliquot.)

HPLC VARIABLES
Guard column: 15 × 3.2 RP-2 (Brownlee)
Column: 250 × 4.6 5 μm Zorbax phenyl
Mobile phase: MeCN:MeOH:10 mM pH 2.5 phosphate buffer containing 2.5 mM 1-octanesulfonic acid 27:13:60
Column temperature: 30
Flow rate: 1
Injection volume: 20-120
Detector: F ex 270 em 312

CHROMATOGRAM
Retention time: 7.0
Internal standard: levallorphan tartrate (9.5)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, dextromethorphan

KEY WORDS
SPE

REFERENCE
Wenk,M.; Todesco,L.; Keller,B.; Follath,F. Determination of dextromethorphan and dextrorphan in urine by high-performance liquid chromatography after solid-phase extraction, *J.Pharm.Biomed.Anal.*, **1991**, *9*, 341–344.

SAMPLE
Matrix: urine
Sample preparation: 250 μL Urine + 1.25 μg levallorphan + 250 μL 140 mM pH 5 sodium acetate buffer, mix, add 25 μL β-glucuronidase (glucurase, from bovine liver, 5000 U/mL, Sigma), heat at 37° overnight, add 1.5 mL pH 11.3 glycine buffer, add 6 mL hexane:butanol 90:10, shake vigorously for 10 min, centrifuge at 1500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: Nucleosil 7 C6H5
Mobile phase: MeCN:MeOH:buffer 20:10:70 (Buffer was 10 mM phosphate buffer containing 2.5 mM sodium 1-octanesulfonate, adjusted to pH 2.5 with concentrated phosphoric acid.)
Flow rate: 1.3
Injection volume: 10
Detector: F ex 270 em 312

CHROMATOGRAM
Retention time: 6
Internal standard: levallorphan (9)

OTHER SUBSTANCES
Extracted: dextromethorphan

REFERENCE
Caslavska,J.; Hufschmid,E.; Theurillat,R.; Desiderio,C.; Wolfisberg,H.; Thormann,W. Screening for hydroxylation and acetylation polymorphisms in man via simultaneous analysis of urinary metabolites of mephenytoin, dextromethorphan and caffeine by capillary electrophoretic procedures, *J.Chromatogr.B*, **1994**, *656*, 219–231.

SAMPLE
Matrix: urine
Sample preparation: 250 μL Urine + 5000 U β-glucuronidase in 1 M pH 5.0 sodium acetate buffer, heat at 37° for 18 h, add 500 μL saturated sodium carbonate, add 10 mL hexane:

triethylamine 99.9:0.1. Remove the organic layer, dry, reconstitute the residue in 250 μL mobile phase, inject a 5-50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Selectosil (Phenomenex)
Mobile phase: MeCN:10 mM pH 3.0 potassium phosphate buffer 30:70
Flow rate: 1
Injection volume: 5-50
Detector: F ex 280 em 305

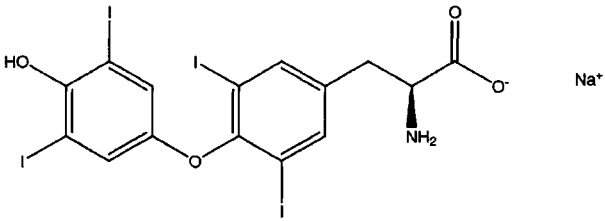
CHROMATOGRAM
Limit of detection: 2 μM

OTHER SUBSTANCES
Extracted: dextromethorphan

REFERENCE
Marinac,J.S.; Foxworth,J.W.; Willsie,S.K. Dextromethorphan polymorphic hepatic oxidation (CYP2D6) in healthy black american adult subjects, *Ther.Drug Monit.*, **1995**, *17*, 120–124.

Levothyroxine sodium

Molecular formula: $C_{15}H_{10}I_4NNaO_4$
Molecular weight: 798.86
CAS Registry No.: 55-03-8, 25416-65-3 (hydrate), 51-48-9 (free acid)
Merck Index: 5497
Lednicer No.: 1 97



SAMPLE
Matrix: blood
Sample preparation: Equilibrate a Sep-Pak silica 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 Sep-Pak 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 μL MeOH, inject.

HPLC VARIABLES
Column: 250 × 4.6 5 μm 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: bulk, 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 μm). (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 ± 0.05 with 6 M HCl) reducing buffer. Deactivating solution was 1:100 phosphoric acid: MeCN.)

HPLC VARIABLES
Column: 300 × 4 μBondapak 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

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: 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: formulations
Sample preparation: Powder tablets, weigh out amount equivalent to about 200 μg sodium levothyroxine, add 10 mL mobile phase, sonicate for 5 min, centrifuge. Filter (0.45 μm, 25 mm Acrodisc CR, Gelman) the supernatant, inject a 200 μL aliquot.

HPLC VARIABLES
Guard column: 40 × 4 40 μm 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 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 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak 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: Condition a 13 mm Empore C18 SPE disk (Baker) with 2.5 mL MeOH and 2.5 mL water at 1.5 mL/min. Dissolve tablet in dissolution medium (?). Pass 40 mL through the SPE disk, wash with 2.5 mL water, dry, add 1 mL MeOH and let it soak in for 3 min, elute at 0.5 mL/min, dilute the eluate to 2 mL with 50 mM pH 2.5 phosphate buffer, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher cyano
Mobile phase: MeCN:water:phosphoric acid 45:55:0.05
Column temperature: 25
Flow rate: 1.6
Injection volume: 20
Detector: UV 225

KEY WORDS
tablets; SPE; comparison with capillary electrophoresis

REFERENCE
Carducci,C.N.; Lucangioli,S.E.; Rodríguez,V.G.; Fernández Otero,G.C. Application of extraction disks in dissolution tests of clenbuterol and levothyroxine tablets by capillary electrophoresis, *J.Chromatogr.A*, **1996**, *730*, 313–319.

SAMPLE
Matrix: solutions
Sample preparation: 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: dextrothyroxine, impurities

KEY WORDS
derivatization; chiral

REFERENCE
Lankmayr,E.P.; Budna,K.W.; Nachtmann,F. Separation of enantiomeric iodinated thyronines by liquid chromatography of diastereomers, *J.Chromatogr.*, **1980**, *198*, 471–479.

SAMPLE
Matrix: solutions
Sample preparation: 20 μL Solution + 40 μL 50 mM pH 8.5 borate buffer + 40 μL 4 mM dabsyl chloride in MeCN, mix, heat at 70° for 15 min, add 100 μL 25 mM pH 6.5 sodium acetate buffer, inject an aliquot.

HPLC VARIABLES
Guard column: 10 × 3 30 μm Chromspher C18 (Chrompack)
Column: 100 × 3 5 μm Chromspher C18 (Chrompack)

Mobile phase: Gradient. A was MeOH:25 mM pH 6.5 sodium acetate buffer 70:30. B was MeOH:25 mM pH 6.5 sodium acetate buffer 90:10. A:B from 100:0 to 0:100 over 15 min, maintain at 0:100 for 5 min.
Column temperature: 35
Flow rate: 0.7
Detector: UV 436

CHROMATOGRAM
Retention time: 14.5
Limit of detection: 0.39 pmole

OTHER SUBSTANCES
Simultaneous: 3,5-diiodothyronine, 3,5-diiodotyrosine, liothyronine, 3-monoiodotyrosine, thyronine, 3,3',5-triiodothyronine, tyrosine

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KEY WORDS
derivatization; comparison with other derivatization procedures

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REFERENCE
Doorn,L.; Jansen,E.H.; Van Leeuwen,F.X. Comparison of high-performance liquid chromatographic detection methods for thyronine and tyrosine residues in toxicological studies of the thyroid, *J.Chromatogr.*, **1991**, *553*, 135–142.

SAMPLE
Matrix: solutions
Sample preparation: 100 μL Solution + 100 μL 500 mM pH 7.7 borate buffer + 100 μL 2.5 mM 9-fluorenylmethyl chloroformate in dry acetone, mix, let stand at room temperature for 45 s, add 200 μL 12 mM 1-adamantamine in MeCN, inject an aliquot.

HPLC VARIABLES
Guard column: 10 × 3 30 μm Chromspher C18 (Chrompack)
Column: 100 × 3 5 μm Chromspher C18 (Chrompack)
Mobile phase: Gradient. A was MeOH:50 mM pH 4.2 sodium acetate buffer 40:60. B was MeCN:MeOH:50 mM pH 4.2 sodium acetate buffer 20:60:20. A:B from 100:0 to 0:100 over 40 min.
Column temperature: 35
Flow rate: 0.7
Detector: UV 260

CHROMATOGRAM
Retention time: 33
Limit of detection: 1.6 pmole

OTHER SUBSTANCES
Simultaneous: 3,5-diiodothyronine, 3,5-diiodotyrosine, liothyronine, 3-monoiodotyrosine, thyronine, 3,3',5-triiodothyronine

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KEY WORDS
derivatization; comparison with other derivatization procedures

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REFERENCE
Doorn,L.; Jansen,E.H.; Van Leeuwen,F.X. Comparison of high-performance liquid chromatographic detection methods for thyronine and tyrosine residues in toxicological studies of the thyroid, *J.Chromatogr.*, **1991**, *553*, 135–142.

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: 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

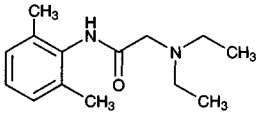
CHROMATOGRAM
Retention time: 17.5

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.

Lidocaine



Molecular formula: $C_{14}H_{22}N_2O$
Molecular weight: 234.34
CAS Registry No.: 137-58-6, 6108-05-0 (HCl monohydrate), 73-78-9 (HCl)
Merck Index: 5505
Lednicer No.: 1 16

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL plasma with 200 µL 2 µg/mL IS in MeOH, add 2 mL water and 2 mL MeCN, vortex gently, set aside for 3 min, centrifuge at 2200 g for 20 min. Separate the clear supernatant, add 500 µL 200 mM NaOH and extract with 6 mL n-hexane by vortexing for 2 min. Centrifuge at 2200 g for 15 min. Evaporate 5 mL of the organic phase to dryness under reduced pressure. Reconstitute the residue in 120 µL mobile phase. Inject a 100 µL aliquot.

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: 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

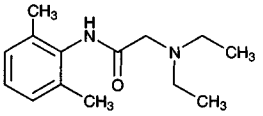
CHROMATOGRAM
Retention time: 17.5

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.

Lidocaine



Molecular formula: $C_{14}H_{22}N_2O$
Molecular weight: 234.34
CAS Registry No.: 137-58-6, 6108-05-0 (HCl monohydrate), 73-78-9 (HCl)
Merck Index: 5505
Lednicer No.: 1 16

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL plasma with 200 µL 2 µg/mL IS in MeOH, add 2 mL water and 2 mL MeCN, vortex gently, set aside for 3 min, centrifuge at 2200 g for 20 min. Separate the clear supernatant, add 500 µL 200 mM NaOH and extract with 6 mL n-hexane by vortexing for 2 min. Centrifuge at 2200 g for 15 min. Evaporate 5 mL of the organic phase to dryness under reduced pressure. Reconstitute the residue in 120 µL mobile phase. Inject a 100 µL aliquot.

HPLC VARIABLES
Guard column: 10 × 3 5 μm AGP bonded silica (ChromTech, Hagersten, Sweden)
Column: 150 × 4 5 micro.m AGP bonded silica (ChromTech, Hagersten, Sweden)
Mobile phase: Isopropanol:buffer 4:96 (Prepare mobile phase by adding 4% isopropanol and 0.6% diethylamine to 8 mM sodium dihydrogen phosphate containing 100 mM NaCl, adjust to pH 7.05 with 50% phosphoric acid.)
Flow rate: 0.9
Injection volume: 100
Detector: UV 214

CHROMATOGRAM
Retention time: 7.5
Internal standard: diazepam (19.21)
Limit of detection: 10 ng/mL
Limit of quantitation: 12.5 ng/mL

OTHER SUBSTANCES
Extracted: bupivacaine

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Abraham,I.; Fawcett,J.P.; Kennedy,J.; Kumar,A.; Ledger,R. Simultaneous analysis of lignocaine and bupivacaine enantiomers in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *703*, 203–208.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL Bond-elut C2 SPE cartridge with 2 mL MeOH and 2 mL water. Apply 1 mL plasma to the cartridge, wash with 1 mL water, wash with 1 mL MeOH: water 50:50, wash with 1 mL MeCN, elute with 2 mL 1 M NaCl:MeOH 5:95. Dry the eluate under vacuum, resuspend in 200 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 10 mm long C18 (Waters)
Column: 250 × 4.6 Supelcosil LC-8-DB
Mobile phase: MeCN:20 mM phosphoric acid containing 200 μL/L triethylamine 10:90
Flow rate: 1.7
Detector: UV 263

CHROMATOGRAM
Retention time: 17.2
Internal standard: tocainide (9.7)
Limit of detection: 100 ng/mL
Limit of quantitation: 200 ng/L

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: acetaminophen, N-acetylprocainamide, amitriptyline, bupivicaine, caffeine, carbamazepine, chloramphenicol, cyclosporin A, desipramine, diazepam, disopyramide, doxepin, ethosuximide, flecainide, fluoxetine, ibuprofen, imipramine, naproxen, norchlordiazepoxide, nordiazepam, nordoxepine, nortriptyline, phenobarbital, phenytoin, primidone, procainamide, quinidine, salicylic acid, theophylline, valproic acid

KEY WORDS
serum; comparison with fluorescence polarization immunoassay; SPE

REFERENCE
O'Neal,C.L.; Poklis,A. Sensitive HPLC for simultaneous quantification of lidocaine and its metabolites monoethylglycinexylidide and glycinexylidide in serum, *Clin.Chem.*, **1996**, *42*, 330–331.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 50 μL 40 μg/mL etidocaine hydrochloride in water + 100 μL 1 M NaOH, vortex for 15 s, add 5 mL diethyl ether, shake on a reciprocating shaker for 10 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 80 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.2 10 μm μBondapak C18
Mobile phase: MeCN:50 mM pH 5.80 Na_2HPO_4 25:75
Flow rate: 0.9
Injection volume: 80
Detector: UV 210

CHROMATOGRAM
Retention time: 4.5
Internal standard: etidocaine (12.0)

OTHER SUBSTANCES
Extracted: 2,6-pipecolylxylidine, bupivacaine, mepivacaine
Noninterfering: metabolites, 2,3-chloroprocaine, theophylline, mexiletine, quinidine, disopyramide, verapamil, phenobarbital, phenytoin, carbamazepine, ethosuximide, digoxin, theobromine, caffeine, furosemide, phenprocoumon, aldactone

KEY WORDS
plasma

REFERENCE
Ha,H.-R.; Funk,B.; Gerber,H.R.; Follath,F. Determination of bupivacaine in plasma by high-performance liquid chromatography, *Anesth.Analg.*, **1984**, *63*, 448–450.

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

HPLC VARIABLES
Column: 150 × 4.6 5 μm GFF-S5-80 internal-surface reversed phase "Pinkerton" (Regis)
Mobile phase: THF:100 mM potassium phosphate 10:90, pH 7.2
Flow rate: 0.8
Injection volume: 10
Detector: UV 220

CHROMATOGRAM
Retention time: 12.9

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: 50 μL Serum + 100 μL 1 M pH 9.0 borate buffer + 1 mL chloroform:EtOH 82.5:17.5, mix, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 50 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 300 × 2 10 μm μBondapak C18

Mobile phase: MeOH:MeCN:buffer 12:16:72 (Buffer was 31 mM sodium acetate adjusted to pH 5.1 with 40% phosphoric acid containing 0.15 mM tetrabutylammonium phosphate.)
Flow rate: 0.3
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 10
Internal standard: lidocaine

OTHER SUBSTANCES
Extracted: caffeine, cocaine, metabolites
Simultaneous: barbital, phenobarbital, flumazepil, mazindol, hexobarbital, nicotine, procaine, cotinine
Noninterfering: amphetamine, desipramine, tetracaine, methadone, reserpine, buspirone, diazepam, haloperidol, chlordiazepoxide, oxazepam, midazolam, clonazepam, chlorpromazine, pentobarbital

KEY WORDS
serum; rat; lidocaine is IS

REFERENCE
Lau,C.E.; Ma,F.; Falk,J.L. Simultaneous determination of cocaine and its metabolites with caffeine in rat serum microsamples by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *532*, 95–103.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 1 M NaOH + 3 mL heptane:ethyl acetate 90:10, shake for 2 min, centrifuge at 1200 g for 10 min. Remove the organic phase and add it to 50 μL 50 mM sulfuric acid, shake for 2 min, centrifuge at 1200 g for 5 min. Remove the aqueous phase and add it to 820 μg sodium acetate, inject a 40 μL aliquot. (The sodium acetate was measured out by adding 50 μL 200 mM sodium acetate in MeOH to the tube and evaporating the MeOH.)

HPLC VARIABLES
Column: 250 × 4 10 μm μBondapak C18
Mobile phase: MeCN:10 mM NaH_2PO_4 5:95, adjusted to pH 2.1
Column temperature: 30
Flow rate: 1
Injection volume: 40
Detector: UV 205

CHROMATOGRAM
Retention time: 13
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: procaine

KEY WORDS
plasma; rabbit

REFERENCE
Le Guévello,P.; Le Corre,P.; Chevanne,P.; Le Verge,R. High-performance liquid chromatographic determination of bupivacaine in plasma samples for biopharmaceutical studies and application to seven other local anaesthetics, *J.Chromatogr.*, **1993**, *622*, 284–290.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 2 M NaOH, mix, add 3 mL n-hexane, shake for 1 min, centrifuge at 3500 rpm for 10 min. Remove the organic layer and evaporate it to dryness

with nitrogen under vacuum, reconstitute the residue in 200 μL mobile phase, vortex, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Spherisorb ODS-2
Mobile phase: MeOH:50 mM pH 5.9 KH_2PO_4 38:62
Flow rate: 1
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 3.8
Internal standard: lidocaine

OTHER SUBSTANCES
Extracted: bupivacaine

KEY WORDS
plasma; lidocaine is IS

REFERENCE
Murillo,I.; Costa,J.; Salvá,P. Determination of bupivacaine in human plasma by HPLC, *J.Liq.Chromatogr.*, **1993**, *16*, 3509–3514.

SAMPLE
Matrix: blood
Sample preparation: Rock 5 mL whole blood + 10 mL water + 8.5 mL Na_2WO_4 in a 50 mL stoppered tube for 1 min, add 6 mL $NiCl_2$, rock for 5 min, add 15 mL dichloromethane:isobutyl alcohol:THF 30:45:25, centrifuge at 2500 g for 15 min. Remove organic phase and repeat the process. Filter all organic phases through a 40-90 μm filter and evaporate to dryness in a 100 mL porcelain dish at a moderate temperature in a sand bath. Take up residue in 500 μL MeCN: water 80:20, inject a 20 μL aliquot. (Na_2WO_4 prepared by mixing 10 g $Na_2WO_4.2H_2O$ in 38 mL of 2 M NaOH and 2.5 g of $NaHCO_3$ and making up to 100 mL. $NiCl_2$ was 17% w/v $NiCl_2$ in water.)

HPLC VARIABLES
Column: 200 × 4.6 5 μm Hypersil C8
Mobile phase: A = MeCN; B = 20 mM n-propylamine adjusted to pH 5 with 85% phosphoric acid. A:B from 15:85 to 20:80 over 5 min to 45:55 over another 15 min to 65:35 over another 5 min
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 16
Limit of detection: 0.40 ppm

OTHER SUBSTANCES
Extracted: buprenorphine, caffeine, cocaine, codeine, diamorphine, ethylmorphine, 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: 200 μL Plasma + 10 μL 15 mg/mL bupivacaine, mix, add 100 μL 2 M NaOH, vortex briefly, add 5 mL anhydrous ethyl ether, vortex for 30 s, rotate for 10 min, centrifuge at 1000 g for 5 min. Remove 4.5 mL ether and add to 250 μL 12.5 mM sulfuric acid, vortex for 30 s, rotate for 10 min, centrifuge for 5 min, inject a 50 μL aliquot of the lower aqueous phase.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Octyl 1B (Keystone)
Mobile phase: MeCN:50 mM Na_2HPO_4 27:73 pH adjusted to 5.8 with 50% phosphoric acid
Flow rate: 1
Injection volume: 50
Detector: UV 210

CHROMATOGRAM
Retention time: 4.90
Internal standard: bupivacaine (9.81)
Limit of detection: 4 ng/mL
Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES
Extracted: prilocaine, o-toluidine

KEY WORDS
plasma; pig; pharmacokinetics

REFERENCE
Klein,J.; Fernandes,D.; Gazarian,M.; Kent,G.; Koren,G. Simultaneous determination of lidocaine, prilocaine and the prilocaine metabolite o-toluidine in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *655*, 83–88.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL 20 μg/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 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 Shimpack CLS-ODS (Shimadzu)
Mobile phase: MeCN:MeOH:0.5 mM phosphoric acid 7.5:3:89.5
Column temperature: 40
Flow rate: 1.5
Injection volume: 20
Detector: UV 210

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: 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: 1.8
Internal standard: protriptyline (4)

OTHER SUBSTANCES

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

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 + 100 μL 10 μg/mL bupivacaine in 25 mM sulfuric acid + 1 mL 1 M NaOH + 5 mL diethyl ether, shake or rotate for 15 min, centrifuge at 1000 rpm for 5 min, freeze at -20°. Remove the organic layer and add it to 250 μL 25 mM sulfuric acid, shake for 15 min, centrifuge at 1000 rpm for 5 min, freeze, discard the organic layer. Thaw the aqueous layer, pass air over the aqueous phase at room temperature to remove traces of ether, adjust pH to 5.0-6.5 by adding 10 μL 1 M NaOH, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 4 μm LiChroCART Superspher 60 RP Select B (Merck)
Column: 125 × 4 4 μm LiChroCART Superspher 60 RP Select B (Merck)
Mobile phase: MeCN:buffer 30:70 (Buffer was 7.0 g/L K_2HPO_4 in water adjusted to pH 5.8 with 1 M NaOH.)

Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: UV 202

CHROMATOGRAM

Retention time: 5
Internal standard: bupivacaine (10)
Limit of quantitation: 100 ng/mL

KEY WORDS

plasma

REFERENCE

Sattler,A.; Krämer,I.; Jage,J.; Vrana,S.; Kleemann,P.P.; Dick,W. Development of a HPLC-system for quantitative measurement of lidocaine and bupivacaine in patients plasma during postoperative epidural pain therapy, *Pharmazie*, **1995**, *50*, 741–744.

SAMPLE

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

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 264

CHROMATOGRAM

Retention time: 4.45
Limit of detection: <120 ng/mL

KEY WORDS

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

vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

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

SAMPLE

Matrix: blood

Sample preparation: Condition a Bond Elut phenyl SPE cartridge with 4 mL MeOH and 4 mL water. Add 1 mL serum or 300-500 μL ultrafiltrate to the SPE cartridge, wash with 5 mL water, wash with 2 mL MeOH:water 5:95, wash with 2 mL EtOH:water 2.5:97.5, wash with 2 mL MeCN:water 10:90, elute with 1 mL MeCN:50 mM pH 2.4 phosphate buffer 25:75, inject a 100 μL aliquot of the eluate.

HPLC VARIABLES

Column: 300 × 4.6 μBondapak

Mobile phase: MeCN:50 mM pH 4.0 KH_2PO_4 25:75

Flow rate: 1.5

Injection volume: 100

Detector: UV 210

CHROMATOGRAM

Internal standard: lidocaine

OTHER SUBSTANCES

Extracted: bupivacaine

KEY WORDS

serum; ultrafiltrate; SPE; lidocaine is IS

REFERENCE

Mazoit,J.X.; Cao,L.S.; Samii,K. Binding of bupivacaine to human serum proteins, isolated albumin and isolated α-1-acid glycopotein. Differences between the two enantiomers are partly due to cooperativity, *J.Pharmacol.Exp.Ther.*, **1996**, *276*, 109–115.

SAMPLE

Matrix: blood, CSF, gastric contents, urine

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

HPLC VARIABLES

Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)

Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.

Column temperature: 50

Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM
Retention time: 8.0
Internal standard: heptanophenone (19)

OTHER SUBSTANCES
Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

KEY WORDS
serum; column-switching

REFERENCE
Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

SAMPLE
Matrix: blood, tissue
Sample preparation: 10 g Whole blood or tissue + 10 mL 100 mM HCl + 100 μL 200 μg/mL procaine in ethyl acetate, homogenize, shake for 10 min, centrifuge at 6000 g for 10 min, add the supernatant to an Extrelut-20 SPE cartridge, let stand for 15 min, pass ammonia gas through the column, elute with 40 mL chloroform. Evaporate the eluate to dryness, reconstitute the residue in 1 mL mobile phase, dilute 10 times with mobile phase, inject a 50 μL aliquot. (Ammonia gas was generated by placing concentrated ammonia under reduced pressure and pulling the evolved ammonia through the column.)

HPLC VARIABLES
Column: 250 × 4.6 6 μm normal phase silica (BST)
Mobile phase: MeCN:100 mM pH 2 KH_2PO_4:water:THF:concentrated phosphoric acid 5.4:90:4.6:1:1
Flow rate: 2
Injection volume: 50
Detector: UV 230

CHROMATOGRAM
Retention time: 5.28
Internal standard: procaine (4.13)
Limit of detection: 250 ng/mL

OTHER SUBSTANCES
Simultaneous: benzocaine, bupivacaine, cocaine, tetracaine

KEY WORDS
whole blood; SPE; brain; liver

REFERENCE
Benkö,A.; Kimura,K. Toxicological analysis of lidocaine in biological materials by using HPLC, *Forensic Sci.Int.*, **1991**, *49*, 65–73.

SAMPLE
Matrix: blood, urine
Sample preparation: 2 mL Whole blood, plasma, or urine + 1 mL saturated sodium carbonate + 10 μL 100 μg/mL etidocaine, add to a 3 mL Extrelut SPE cartridge, elute with 15 mL di-

chloromethane. Evaporate eluate to dryness under a stream of nitrogen at 40°, reconstitute in 100 μL 10 mM HCl, add 3 mL diethyl ether, vortex for 20 s, centrifuge at 2800 g for 5 min, inject a 40 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 5 × 6 μBondapak Guard Pak
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:100 mM ammonium acetate 50:50
Flow rate: 1.5
Injection volume: 40
Detector: UV 230

CHROMATOGRAM
Retention time: 8
Internal standard: etidocaine (14)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: prilocaine, bupivacaine, dibucaine
Also analyzed: procaine, butacaine, tetracaine, p-aminobenzoic acid, articaine, o-toluidine, caffeine, amphetamine, ephedrine, epinephrine, morphine, monoacetylmorphine, diamorphine, ethylmorphine, codeine, acetylcodeine

KEY WORDS
whole blood; plasma; SPE

REFERENCE
Rop,P.P.; Grimaldi,F.; Bresson,M.; Fornaris,M.; Viala,A. Liquid chromatographic analysis of cocaine, benzoylecgonine, local anaesthetic agents and some of their metabolites in biological fluids, *J.Liq.Chromatogr.*, **1993**, *16*, 2797–2811.

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

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

CHROMATOGRAM
Retention time: 9.922

KEY WORDS
whole blood

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

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 10 mg/mL solution in 500 mM sodium bicarbonate solutions, extract a 10 mL aliquot twice with 15 mL portions of dichloromethane. Combine the extracts and add 10 μL phenylisothiocyanate, evaporate to dryness under a stream of air, reconstitute with 10 mL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 70 × 2.1 CO:PELL ODS
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:water:acetic acid 45:54:1
Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 2

OTHER SUBSTANCES
Simultaneous: ephedrine, phenylpropanolamine, pseudoephedrine

KEY WORDS
derivatization

REFERENCE
Noggle,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: bulk
Sample preparation: Prepare a 750 μg/mL solution in 10 mM pH 2.5 orthophosphoric acid, sonicate for 10 min, filter (0.2 μm), inject a 15 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100
Column: 125 × 4 3 μm Spherisorb ODS-1
Mobile phase: Gradient. A was water containing 5 mL/L 85% orthophosphoric acid and 0.56 mL/L hexylamine. B was MeCN:water 90:10 containing 5 mL/L 85% orthophosphoric acid and 0.56 mL/L hexylamine. A:B from 91:9 to 86:14 over 4 min, maintain at 86:14 for 13 min, to 55:45 over 11 min, maintain at 55:45 for 8 min, re-equilibrate at initial conditions for 20 min.
Flow rate: 0.7
Injection volume: 15
Detector: UV 210

CHROMATOGRAM
Retention time: 9.4

OTHER SUBSTANCES
Simultaneous: acetaminophen, acetylcodeine, benzocaine, caffeine, cocaine, codeine, diamorphine, 6-monoacetylmorphine, morphine, noscapine, papaverine, procaine

REFERENCE
Grogg-Sulser,K.; Helmlin,H.-J.; Clerc,J.-T. Qualitative and quantitative determination of illicit heroin street samples by reversed-phase high-performance liquid chromatography: method development by CARTAGO-S, *J.Chromatogr.A*, **1995**, *692*, 121–129.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with MeOH:water 500 mM pH 7 sodium borate 35:65:2, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Whatman PXS ODS-3 C18
Mobile phase: MeCN:buffer 20:80 (Buffer was water glacial acetic acid 93:5 adjusted to pH 3.0 with 1 M NaOH.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

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: perfusate

HPLC VARIABLES
Column: 100 × 8 4 μm Novapak C18
Mobile phase: MeCN:0.092%phosphoric acid + 0.2% triethylamine 26:74
Flow rate: 2
Detector: UV 214

CHROMATOGRAM
Internal standard: lidocaine
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: diphenhydramine, diltiazem, metabolites
Also analyzed: bupivacaine

KEY WORDS
lidocaine is IS; 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: perfusate

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 60 RP-Select B C18
Mobile phase: Gradient. MeCN:buffer 9:91 for 5 min, to 19:81 over 10 min, maintain at 19:81 for 11.5 min, return to initial conditions over 1 min, re-equilibrate for 7.5 min. (Buffer was 6.66 g/L KH_2PO_4 , 150 μL/L phosphoric acid, and 5 mM sodium n-heptanesulfonate, pH 3.5.)
Flow rate: 1

Detector: UV 214

CHROMATOGRAM
Limit of quantitation: 25 ng/mL

KEY WORDS
rat; liver

REFERENCE
Ngo,L.Y.; Tam,Y.K.; Coutts,R.T. Lack of residual effects of diethyl ether, methoxyflurane, and sodium pentobarbital on lidocaine metabolism in a single-pass isolated rat liver perfusion system, *Drug Metab.Dispos.*, **1995**, *23*, 525–528.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 40:60 containing 100 mM KCl
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 262

OTHER SUBSTANCES
Also analyzed: disopyramide, metoprolol

REFERENCE
Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 5 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:water 20:20:60 containing 0.06% sulfuric acid, 0.5% sodium sulfate, and 0.02% sodium heptanesulfonate, pH 2.6
Flow rate: 2
Injection volume: 5
Detector: UV 305

CHROMATOGRAM
Retention time: 2

OTHER SUBSTANCES
Simultaneous: benzocaine, butamben, pramoxine, procaine, tetracaine

REFERENCE
Menon,G.N.; Norris,B.J. Simultaneous determination of tetracaine and its degradation product, p-n-butylaminobenzoic acid, by high-performance liquid chromatography, *J.Pharm.Sci.*, **1981**, *70*, 569–570.

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

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica

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

CHROMATOGRAM
Retention time: 1.4

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

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

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

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 50:1.5:0.5:48
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: butacaine, bupivacaine, benzocaine, tetracaine

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

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 5C18
Mobile phase: MeCN:buffer 35:65 (Buffer was 0.1% phosphoric acid containing 5 mM sodium 1-hexanesulfonate.)
Flow rate: 1
Detector: UV 230

CHROMATOGRAM
Internal standard: ethyl p-hydroxybenzoate

REFERENCE
Cheng,Y.H.; Hosoya,O.; Sugibayashi,K.; Morimoto,Y. Effect of skin surface lipid on the skin permeation of lidocaine from pressure sensitive adhesives, *Biol.Pharm.Bull.*, **1994**, *17*, 1640–1644.

SAMPLE
Matrix: solutions

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

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, lorazepam, lormetazepam, lox-

apine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 8.04 (A), 4.50 (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, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, 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, pemo-

line, 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
details of plasma extraction

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

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 5.37

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

SAMPLE
Matrix: urine
Sample preparation: 500 μL Urine + N-ethylnordiazepam + chlorpheniramine + 100 μL buffer, centrifuge at 11000 g for 30 s, inject a 500 μL aliquot onto column A with mobile phase A, after 0.6 min backflush column A with mobile phase A to waste for 1.6 min, elute column A with 250 μL mobile phase B, with 200 μL mobile phase C, and with 1.15 mL mobile phase D. Elute column A to waste until drugs start to emerge then elute onto column B. Elute column B to waste until drugs started to emerge, then elute onto column C. When all the drugs have emerged from column B remove it from the circuit, elute column C with mobile phase D,

monitor the effluent from column C. Flush column A with 7 mL mobile phase E, with mobile phase D, and mobile phase A. Flush column B with 5 mL mobile phase E then with mobile phase D. (Buffer was 6 M ammonium acetate adjusted to pH 8.0 with 2 M KOH.)

HPLC VARIABLES

Column: A 10 × 2.1 12-20 μm PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 × 3.2 11 μm Aminex A-28 (Bio-Rad); C 25 × 3.2 5 μm C8 (Phenomenex) + 150 × 4.6 5 μm silica (Macherey-Nagel)
Mobile phase: A 0.1% pH 8.0 potassium borate buffer; B 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid; C MeCN:buffer 40:60 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); D MeCN:buffer 33:67 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); E MeCN:buffer 70:30 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)
Column temperature: ambient (column A), 40 (columns B and C)
Flow rate: A 5; B-E 1
Injection volume: 500
Detector: UV 210, UV 235

CHROMATOGRAM

Retention time: k' 2.7
Internal standard: N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)
Limit of detection: 300 ng/mL

OTHER SUBSTANCES

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

KEY WORDS

column-switching

REFERENCE

Binder,S.R.; Regalia,M.; Biaggi-McEachern,M.; Mazhar,M. Automated liquid chromatographic analysis of drugs in urine by on-line sample cleanup and isocratic multi-column separation, *J.Chromatogr.*, **1989**, *473*, 325–341.

SAMPLE

Matrix: urine
Sample preparation: 1 mL Urine + 0.5 mL 1% trichloroacetic acid, centrifuge at 5200 g for 10 min, filter (0.2 μm), inject 20 μL aliquot

HPLC VARIABLES

Column: 250 × 4 Lichrospher 5μm 60 RP-select B
Mobile phase: Gradient. MeCN:50 mM pH 3.2 potassium phosphate buffer from 10:90 to 50:50 over 15 min.
Flow rate: 1.5
Injection volume: 20
Detector: UV 190-370

CHROMATOGRAM

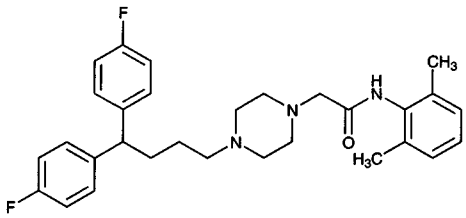
Retention time: 8

OTHER SUBSTANCES

Extracted: morphine, ephedrine, phenylpropanolamine, diphenhydramine, nortriptyline, cocaine, benzoylecgonine, norpropoxyphene, nordiazepam
Also analyzed: amitriptyline, amphetamine, meperidine, codeine, (different gradient)

REFERENCE
Li,S.; Gemperline,P.J.; Briley,K.; Kazmierczak,S. Identification and quantitation of drugs of abuse in urine using the generalized rank annihilation method of curve resolution, *J.Chromatogr.B*, **1994**, *655*, 213–223.

Lidoflazine



Molecular formula: $C_{30}H_{35}F_2N_3O$
Molecular weight: 491.62
CAS Registry No.: 3416-26-0
Merck Index: 5507
Lednicer No.: 1 279

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

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 265

CHROMATOGRAM
Retention time: 10.35
Limit of detection: <120 ng/mL

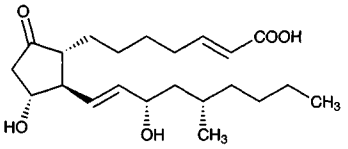
KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride;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

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

REFERENCE

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

Limaprost



Molecular formula: $C_{22}H_{36}O_5$
Molecular weight: 380.52
CAS Registry No.: 74397-12-9, 88852-12-4
Merck Index: 5514

SAMPLE

Matrix: blood

Sample preparation: Condition a Bond Elut Certify C18 SPE cartridge with water, MeCN, and 20 mM citric acid. Add 1 mL plasma to SPE cartridge, wash with 1 mL 20 mM citric acid, wash with 2 mL MeOH:water 10:90, wash with 2 mL cyclohexane, elute with 3 mL 3% ammonia in MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 500 μL MeCN, add 200 μL 10 mM DBD-PZ in MeCN, add 300 μL 10 mM 2,2'-dipyridyl disulfide and 10 mM triphenylphosphine in MeCN, let stand at room temperature for 30 min, inject an aliquot. (DBD-PZ prepared from 123 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN added dropwise to 129 mg piperazine in 20 mL MeCN at room temperature, stir for 30 min, evaporate under reduced pressure, dissolve residue in 50 mL 5% HCl, extract three times with 20 mL ethyl acetate, discard ethyl acetate extracts, adjust pH of aqueous solution to 13-14 with 5% NaOH, extract five times with 50 mL ethyl acetate, combine extracts, wash with 20 mL water, dry over anhydrous sodium sulfate, evaporate under vacuum to give 4-(N,N-dimethylaminosulfonyl)-7-(1-piperazinyl)-2,1,3-benzoxadiazole (DBD-PZ) as orange crystals, mp 121-2° (J. Chromatogr. 1991, 588, 61).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: Gradient. MeCN:water from 35:65 to 60:40 over 1 h
Column temperature: 40
Flow rate: 1
Detector: F ex 440 em 569

CHROMATOGRAM

Retention time: 37.5
Limit of detection: 1.7-5 fmole

OTHER SUBSTANCES

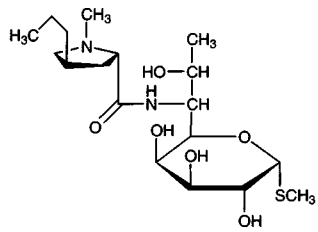
Extracted: alprostadil (prostaglandin E1), dinoprost (prostaglandin F2α), dinoprostone (prostaglandin E2), 6-ketoprostaglandin F1α, prostaglandin F1α, prostaglandin D2, prostaglandin A1, prostaglandin B1

KEY WORDS

plasma; rat; SPE; derivatization

REFERENCE
Toyo'oka,T.; Ishibashi,M.; Terao,T.; Imai,K. Sensitive fluorometric detection of prostaglandins by high performance liquid chromatography after precolumn labelling with 4-(*N,N*-dimethylaminosulphonyl)-7-(1-piperazinyl)-2,1,3-benzoxadiazole (DBD-PZ), *Biomed.Chromatogr.*, **1992**, *6*, 143–148.

Lincomycin



Molecular formula: $C_{18}H_{34}N_2O_6S$
Molecular weight: 406.54
CAS Registry No.: 154-21-2, 7179-49-9 (HCl monohydrate), 859-18-7 (HCl)
Merck Index: 5525

SAMPLE
Matrix: solutions
Sample preparation: Centrifuge and filter cell solutions (0.22 μm), inject an aliquot.

HPLC VARIABLES
Guard column: Guard-PAK C18 (Waters)
Column: 150 × 3.9 5 μm NOVA PAK C18
Mobile phase: MeCN:50 mM pH 6.0 KH_2PO_4 12:88
Flow rate: 1.5
Detector: UV 214

CHROMATOGRAM
Retention time: 3.9

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 250 μg/mL solution of clindamycin in mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 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: k' 0.9

OTHER SUBSTANCES
Simultaneous: benzyl alcohol, clindamycin, pirlimycin

REFERENCE
Theis,D.L. Ion-pairing liquid chromatographic method for the determination of pirlimycin hydrochloride, *J.Chromatogr.*, **1987**, *402*, 335–343.

SAMPLE
Matrix: tissue

Sample preparation: Condition a 3 mL 500 mg Sep-Pak Vac C18 SPE cartridge with 15 mL MeOH and 5 mL water. Homogenize 5 g blended fish tissue with 20 mL 10 mM pH 4.5 KH_2PO_4buffer at 8000 rpm for 2 min. Centrifuge the homogenized sample at 4000 g for 10 min. Decant and collect the supernatant, extract the residue with 20 mL buffer. Filter the combined extracts through glass wool. Add 1 mL 10% sodium tungstate solution and 1 mL 34 mM sulfuric acid, mix. Centrifuge at 4000 rpm for 15 min, filter the supernatant and discard the precipitated protein. Add 1mL 3% 1-pentanesulfonic acid and mix well. Add the fish extract to the SPE cartridge. Wash with 4 mL MeOH:water 10:90 and 2 mL water at 1 mL/min. Elute with 2 mL MeCN:water 50:50. Add 200 μL 1 M KOH and 500 mg NaCl to the eluate. Extract with three 3 mL portions of ethyl acetate by agitating on a Vortex mixer and centrifuging at 800 g for 3-5 min. Combine the extracts and filter through 3 g anhydrous sodium sulfate. Wash sodium sulfate with 2 mL ethyl acetate. Evaporate the filtrate to dryness under vacuum at 35°. Reconstitute the residue in 1 mL mobile phase, filter (0.45 μm). Inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Spherisorb S5 ODS2
Mobile phase: MeCN:20 mM pH 4.5 potassium phosphate buffer containing 20 mM sodium 1-octanesulfonate 22:88
Flow rate: 1.0
Injection volume: 50
Detector: E, ESA Model 5100A, Model 5010 analytical cell, first electrode +0.65 V, second electrode +0.9 V, Model 5020 guard cell +0.95 V

CHROMATOGRAM

Retention time: 22
Limit of detection: 7 ng/g (muscle), 12 ng/g (skin)
Limit of quantitation: 17 ng/g (muscle), 24 ng/g (skin)

KEY WORDS

salmon; muscle; skin; SPE

REFERENCE

Luo,W.; Hansen,E.B.,Jr.; Ang,C.Y.W.; Thompson,H.C.,Jr. Determination of lincomycin residue in salmon tissues by ion-pair reversed-phase liquid chromatography with electrochemical detection, *J.AOAC Int.*, **1996**, *79*, 839–843.

SAMPLE

Matrix: tissue
Sample preparation: Condition a C18 Sep-Pak SPE cartridge with 20 mL MeOH and 40 mL distilled water. Homogenize 5 g chopped kidney with 50 mL MeOH, 5 g sodium sulfate, and 2 mL 1 M NaOH. Centrifuge at 1200 rcf for 10 min, decant the supernatant, repeat the extraction with another 50 mL portion of MeOH. Adjust the MeOH phases to pH 4 with 1 M HCl, filter (Whatman No.4 filter paper), transfer filtrate to a separatory funnel, add 50 mL hexane, shake. Remove the lower MeOH phase and reduce its volume to approximately 5 mL on a rotary evaporator. Transfer concentrated extract to a centrifuge tube with four 2 mL portions of water, add 50 mg sodium tungstate, centrifuge at 1200 rcf for 10 min. Add the supernatant to the SPE cartridge, wash with 4 mL water, elute with 5 mL MeOH, evaporate the eluate to dryness under nitrogen, redissolve the residue in 200 μL mobile phase inject an aliquot.

HPLC VARIABLES

Guard column: 50 × 5 30-35 μm Co Pell ODS
Column: 250 × 5 5 μm Spherisorb ODS
Mobile phase: MeOH:pH 7.0 phosphate buffer 65:35
Flow rate: 1
Injection volume: 200
Detector: UV 214

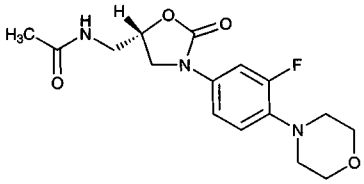
KEY WORDS

cow; pig; kidney; SPE

REFERENCE

Farrington,W.H.H.; Cass,S.D.; Patey,A.L.; Shearer,G. A method for the analysis of lincomycin in porcine and bovine kidneys, *Food Addit.Contam.*, **1987**, *5*, 67–76.

Linezolid



Molecular formula: $C_{16}H_{20}FN_3O_4$
Molecular weight: 337.35
CAS Registry No.: 165800-03-3

SAMPLE
Matrix: solutions

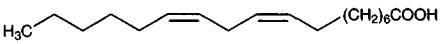
HPLC VARIABLES
Column: Zorbax RX-8
Mobile phase: MeOH:THF:water:trifluoroacetic acid 25:73.7:1.2:0.1
Detector: UV 251

CHROMATOGRAM
Retention time: 7
Internal standard: PNU-101145 (10)
Limit of quantitation: 10 ng/mL

KEY WORDS
linezolid is PNU-100766

REFERENCE
Johnson,R.A.; Haan,D.E.; James,C.A.; Hopkins,N.K. Determination of linezolid, PNU-100766, in human plasma and urine using high-performance liquid chromatography with ultraviolet detection (Abstract 2487), *Pharm.Res.*, **1997**, *14*, S374.

Linoleic acid



Molecular formula: $C_{18}H_{32}O_2$
Molecular weight: 280.45
CAS Registry No.: 60-33-3
Merck Index: 5529

SAMPLE
Matrix: beverages
Sample preparation: Heat 240 mL beer at 60°, cool. Remove a 200 mL aliquot and add it to 10 mL 3 M sulfuric acid, add 100 µL 100 µg/mL n-heptadecanoic acid in MeOH, add 200 mL ether:pentane 50:50, add 60 g NaCl, shake for 30 min, centrifuge at 5000 rpm for 5 min. Remove a 150 mL aliquot of the organic layer and wash it with 150 mL 5% sodium bicarbonate, evaporate to dryness under reduced pressure, reconstitute the residue in 1 mL 6 mM tetramethylammonium hydroxide in DMF, add 1 mL 7.5 mM 9-(chloromethyl)anthracene in DMF, heat at 75° for 20 min, inject a 50 µL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Kaseisorb LC ODS-60-5S C18 (Tokyo Kasei)
Mobile phase: Gradient. MeCN:water 90:10 for 5 min, to 95:5 over 40 min, maintain at 95:5 for 20 min, to 100:0 over 5 min, maintain at 100:0 for 15 min.
Column temperature: 40
Flow rate: 1.1
Injection volume: 50
Detector: F ex 365 em 412

CHROMATOGRAM
Retention time: 54

Limit of detection: 0.2 ng/mL
Limit of quantitation: 0.4 ng/mL

OTHER SUBSTANCES
Extracted: n-heptadecanoic acid, lauric acid, linolenic acid, myristic acid, oleic acid, palmitic acid, palmitoleic acid, stearic acid

KEY WORDS
derivatization; beer

REFERENCE
Kaneda,H.; Kano,Y.; Kamimura,M.; Osawa,T.; Kawakishi,S. Analysis of long-chain fatty acids in beer by HPLC-fluorescence detection method, *J.Agric.Food Chem.*, **1990**, *38*, 1363–1367.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 20 mL chloroform:MeOH 2:1, vortex for 30 s, add 4 mL water, shake for 5 min, centrifuge at 2500 rpm for 5 min, extract the aqueous layer twice more with 10 mL portions of chloroform:MeOH 2:1. Combine the organic layers and dry them over anhydrous sodium sulfate, evaporate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 1 mL dry dichloromethane, add 1 mg p-aminophenol, add 150 μL triethylamine, add at least a 3-fold molar excess of 2-bromo-1-methylpyridinium iodide, heat at 60° for 30 min, cool, concentrate under a stream of nitrogen, add 1 mL 100 mM HCl, add 1 mL ethyl acetate, shake vigorously, centrifuge at 2500 rpm for 5 min, inject an aliquot of the supernatant. (Prepare 2-bromo-1-methylpyridinium iodide by analogy with the preparation of 2-chloro-1-methylpyridinium iodide. Add 15 g methyl iodide to 13.9 g 2-bromopyridine in 3 mL acetone at 0°, stir at room temperature for 3 days. Filter the precipitate and wash it with 50 mL dry ether, dry under reduced pressure to give 2-bromo-1-methylpyridinium iodide (Bull. Chem. Soc. Japan 1977, 50, 1863).)

HPLC VARIABLES
Column: 250 × 4.6 10 μm Nucleosil C-18
Mobile phase: MeOH:water:perchloric acid 88:12:0.1 containing 50 mM sodium perchlorate
Column temperature: 25 ± 0.1
Flow rate: 1.2
Detector: E, 0.75 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 10.5

OTHER SUBSTANCES
Extracted: linolenic acid, oleic acid, palmitic acid, palmitoleic acid, stearic acid

KEY WORDS
derivatization; guinea pig; plasma

REFERENCE
Ikenoya,S.; Hiroshima,O.; Ohmae,M.; Kawabe,K. Electrochemical detector for high performance liquid chromatography. IV. Analysis of fatty acids, bile acids and prostaglandins by derivatization to an electrochemically active form, *Chem.Pharm.Bull.(Tokyo)*, **1980**, *28*, 2941–2947.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 100 μL 100 μg/mL margaric acid in MeOH + 1.4 mL 1/15 M pH 7.0 phosphate buffer, shake, add to a 45 × 12 glass column packed with 1 g Extrelut, let stand for 20 min, elute with 10 mL chloroform. Evaporate the eluate to dryness under reduced pressure and reconstitute the residue with 600 μL benzene (Caution! Benzene is a carcinogen!), add 600 μL 2% oxalyl chloride in benzene, heat at 70° for 30 min, evaporate to dryness under reduced pressure, add 100 μL 40 mM 1-naphthylamine in benzene, add 10 μL 400 μM triethylamine in benzene, heat at 30° for 15 min, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 8-10 μm μBondapak C18
Mobile phase: MeOH:water 81:19
Column temperature: 40
Flow rate: 2
Injection volume: 40
Detector: UV 280

CHROMATOGRAM
Retention time: 12
Internal standard: margaric acid

OTHER SUBSTANCES
Extracted: myristic acid, oleic acid, palmitic acid, palmitoleic acid, stearic acid

KEY WORDS
derivatization; serum

REFERENCE
Ikeda,M.; Shimada,K.; Sakaguchi,T. High-performance liquid chromatographic determination of free fatty acids with 1-naphthylamine, *J.Chromatogr.*, **1983**, *272*, 251–259.

SAMPLE
Matrix: blood
Sample preparation: 10 μL Plasma + 200 μL 500 mM pH 6.5 phosphate buffer + 50 μL 20 μM IS in MeOH + 2 mL n-heptane:chloroform 50:50, vortex for 2 min, centrifuge at 1000 g for 10 min. Remove the lower organic layer and evaporate it to dryness, reconstitute the residue in two 100 μL aliquots of acetone. Evaporate the acetone solution to dryness, add 2-3 mg finely powdered potassium bicarbonate:sodium sulfate 50:50, add 50 μL 800 μM dibenzo-18-crown-6 in acetone, add 50 μL 2 mM 4-bromomethyl-7-acetoxycoumarin in acetone, heat at 50° in the dark for 30 min, inject a 50 μL aliquot. (Prepare 4-bromomethyl-7-acetoxycoumarin as follows. Reflux 50 g 7-hydroxy-4-methylcoumarin (β-methylumbelliferone) and 100 mL acetic anhydride for 1 h, cool, pour into 500 mL cold water, filter, dry the solid, recrystallize from EtOH to give 4-methyl-7-acetoxycoumarin. Reflux 10 g 4-methyl-7-acetoxycoumarin, 9 g N-bromosuccinimide, a little 2,2'-(azobis(2-methylpropionitrile) (α,α'-azobisisobutyronitrile, Eastman), and 100 mL carbon tetrachloride for 20 h, cool, evaporate under reduced pressure to remove the solvent, wash the residue with water, filter, dry, recrystallize from ethyl acetate/cyclohexane to give 4-bromomethyl-7-acetoxycoumarin (mp 184-185°). (J. Chromatogr. 1982, 234, 121).)

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrosorb RP-18
Mobile phase: Gradient. MeCN:MeOH:water from 35:35:30 to 0:90:10 over 70 min (convex gradient).
Column temperature: 40
Flow rate: 1.2
Injection volume: 50
Detector: F ex 365 em 460 following post-column reaction. The column effluent mixed with 200 mM NaOH in MeOH:water 80:20 pumped at 0.4 mL/min and the mixture flowed through a 3.5 m × 0.5 mm ID stainless steel coil at 50° to the detector.

CHROMATOGRAM
Retention time: 42
Internal standard: margaric acid (57)
Limit of quantitation: 5 pmole

OTHER SUBSTANCES
Extracted: arachidonic acid, capric acid, caproic acid, caprylic acid, heptanoic acid, lauric acid, linoleic acid, linolenic acid, myristic acid, myristoleic acid, nonanoic acid, oleic acid, palmitic acid, palmitoleic acid, stearic acid, tridecanoic acid, undecanoic acid

KEY WORDS
derivatization; post-column reaction; plasma

REFERENCE
Tsuchiya,H.; Hayashi,T.; Sato,M.; Tatsumi,M.; Takagi,N. Simultaneous separation and sensitive determination of free fatty acids in blood plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *309*, 43–52.

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-Pak silica SPE cartridge with chloroform. 500 μL Serum + 1 mL 20 μM tridecanoic acid in chloroform containing 9.53 μM 2-heptenoic acid + 100 μL 500 mM sulfuric acid + 5 mL chloroform + 20 mL MeCN, shake slowly, add 1.5 g anhydrous calcium chloride, invert slowly, let stand for 30 min, filter (No 5A paper) the upper layer. Add 500 μL 45 mM dansyl semipiperazide in chloroform and 150 mg dicyclohexylcarbodiimide to the filtrate, let stand for 30 min, remove the solvent by evaporation under reduced pressure, dissolve the residue in the minimum amount of chloroform, add to the SPE cartridge, elute with two 1 mL portions of chloroform. Evaporate the eluate to dryness under reduced pressure, reconstitute with the minimum amount of MeOH, inject an aliquot. (Prepare dansyl semipiperazide as follows. Add 8 g dansyl chloride to 50 g piperazine dissolved in 500 mL acetone with stirring at room temperature over 30 min, stir overnight, evaporate to dryness under reduced pressure. Dissolve the residue in 300 mL chloroform, filter (5A paper), wash the filtrate three times with 5% sodium bicarbonate, wash with water. Extract the organic layer three times with 100 mL portions of 1 M HCl, combine the extracts and wash them repeatedly with chloroform, make alkaline with a slight excess of powdered sodium carbonate, extract with benzene (Caution! Benzene is a carcinogen!). Wash the benzene layer twice with 5% sodium bicarbonate, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure. Dissolve the residue in the minimum amount of chloroform, chromatograph on a 200 × 50 column of Wako Gel C-200 silica gel with chloroform. When the first eluting yellow band reaches the bottom change the eluent to MeOH, continue eluting until all the yellow band is collected. Evaporate the eluate to dryness to obtain dansyl semipiperazide, store in the dark at 5°. Determine the concentration of dansyl semipiperazide by spectrophotometry, extinction coefficient at 340 nm in MeOH or EtOH is 4300 $M^{-1}cm^{-1}$.)

HPLC VARIABLES
Column: 250 × 4.6 Hitachi C18 3056
Mobile phase: Gradient. MeCN:water 45:55 for 43 min, 60:40 for 32 min, 75:25 for 28 min, 85:15 for 100 min, 100:0 for rest of run (step gradient).
Flow rate: 0.8
Detector: F ex 350 em 530

CHROMATOGRAM
Retention time: 145
Internal standard: tridecanoic acid (139), 2-heptenoic acid (77)

OTHER SUBSTANCES
Extracted: acetic acid, arachidic acid, arachidonic acid, butyric acid, capric acid, caproic acid, caprylic acid, docosahexaenoic acid, eicosatrienoic acid, eicosenoic acid, elaidic acid, erucic acid, isobutyric acid, isocaproic acid, isovaleric acid, lactic acid, lauric acid, linoleic acid, linolenic acid, margaric acid, myristic acid, myristoleic acid, nonanoic acid, palmitic acid, palmitoleic acid, pentadecanoic acid, petroselenic acid, propionic acid, stearic acid, undecanoic acid, vaccenic acid, valeric acid

KEY WORDS
derivatization; serum; SPE

REFERENCE
Yanagisawa,I.; Yamane,M.; Urayama,T. Simultaneous separation and sensitive determination of free fatty acids in blood plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *345*, 229–240.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 3 mL 3.9 μg/mL margaric acid in chloroform:n-heptane:MeOH 28:21:1, vortex for 2 min, centrifuge at 2000 g for 20 min. Remove a 2 mL aliquot of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute with 50

μL 2 mg/mL 9-anthracenemethanol (9-(hydroxymethyl)anthracene) in dichloromethane, add 50 μL reagent solution, add 10 μL triethylamine, sonicate for 15 s, vortex for 15 s, heat at 50° for 30 min, evaporate to dryness under a stream of nitrogen, reconstitute with 1 mL mobile phase, inject an aliquot. (Prepare the reagent, 2-bromo-1-methylpyridinium iodide, as follows. Reflux 5 mL 2-bromopyridine and 7 mL iodomethane in 20 mL dry diethyl ether for 1 h, wash the precipitate of 2-bromo-1-methylpyridinium iodide with ether. The reagent solution was a 20 mg/mL suspension of 2-bromo-1-methylpyridinium iodide in dichloromethane.)

HPLC VARIABLES

Column: 250 × 4.5 3 μm Spherisorb C8
Mobile phase: Gradient. MeCN:water 93:7 for 12 min, 86:14 for 5 min, 100:0 for 23 min (step gradient?).
Flow rate: 1
Injection volume: 20
Detector: F ex 360 em 420

CHROMATOGRAM

Retention time: 15
Internal standard: margaric acid (23)
Limit of detection: 50 ng

OTHER SUBSTANCES

Extracted: arachidic acid, arachidonic acid, behenic acid, lauric acid, lignoceric acid, linolenic acid, myristic acid, oleic acid, palmitic acid, palmitoleic acid, stearic acid

KEY WORDS

derivatization; plasma

REFERENCE

Baty,J.D.; Pazouki,S.; Dolphin,J. Analysis of fatty acids as their anthrylmethyl esters by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1987**, *395*, 403–411.

SAMPLE

Matrix: blood
Sample preparation: 15 μL Plasma + 485 μL 6.2 μM IS in water, mix, add 1 mL MeOH, vortex, add 3 mL 50 mg/mL BHT in chloroform, vortex for 1 min, centrifuge at 2000 g for 10 min, remove the organic layer, extract the aqueous layer with 4 mL chloroform:MeOH 75:25. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute with 1 mL chloroform, add to a Bond Elut aminopropyl SPE cartridge, elute with diethyl ether: acetic acid 98:2. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute with acetone, evaporate to dryness (?), add 50 μL 1 mg/mL 4-bromomethyl-7-acetoxycoumarin in acetone, add 50 μL 800 μM dibenzo-18-crown-6 in acetone, add 2-3 mg of a finely powdered mixture of potassium bicarbonate and sodium sulfate (50:50 ?), shake at 50° for 30 min, inject a 5-50 μL aliquot. (Prepare 4-bromomethyl-7-acetoxycoumarin as follows. Reflux 50 g 7-hydroxy-4-methylcoumarin (β-methylumbelliferone) and 100 mL acetic anhydride for 1 h, cool, pour into 500 mL cold water, filter, dry the solid, recrystallize from EtOH to give 4-methyl-7-acetoxycoumarin. Reflux 10 g 4-methyl-7-acetoxycoumarin, 9 g N-bromosuccinimide, a little 2,2'-(azobis(2-methylpropionitrile) (α,α'-azobisisobutyronitrile, Eastman), and 100 mL carbon tetrachloride for 20 h, cool, evaporate under reduced pressure to remove the solvent, wash the residue with water, filter, dry, recrystallize from ethyl acetate/cyclohexane to give 4-bromomethyl-7-acetoxycoumarin (mp 184-185°) (J. Chromatogr. 1982, 234, 121).)

HPLC VARIABLES

Column: 100 × 5 5 μm Nova Pak radial compression
Mobile phase: Gradient. A was MeCN:MeOH:water 35:35:30. B was MeOH:water 90:10. A:B from 100:0 to 0:100 over 70 min (convex gradient).
Flow rate: 2
Injection volume: 5-50
Detector: F ex 365 em 460 following post-column reaction. The column effluent mixed with 200 mM NaOH in MeOH:water 80:20 pumped at 0.4 mL/min and the mixture flowed through a 9 m × 0.23 mm ID stainless steel coil at 80° to the detector.

CHROMATOGRAM
Retention time: 50
Internal standard: margaric acid (62)
Limit of detection: 10 fmole

OTHER SUBSTANCES
Extracted: arachidonic acid, eicosatrienoic acid, lauric acid, linolenic acid, myristic acid, myristoleic acid, oleic acid, palmitic acid, palmitoleic acid, stearic acid

KEY WORDS
derivatization; post-column reaction; rat; plasma; SPE

REFERENCE
Kelly,R.A.; O'Hara,D.S.; Kelley,V. High-performance liquid chromatographic separation of femtomolar quantities of endogenous carboxylic acids, including arachidonic acid metabolites, as 4-bromomethyl-7-acetoxycoumarin derivatives, *J.Chromatogr.*, **1987**, *416*, 247–254.

SAMPLE
Matrix: blood
Sample preparation: 25 μL Serum + 25 μL 80 μM margaric acid in EtOH, mix, add 100 μL 20 mM 2-nitrophenylhydrazine hydrochloride in EtOH:40 mM HCl 25:75, add 200 μL 125 mM 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in EtOH containing 6.5% pyridine, heat at 60° for 20 min, add 2 mL 33.3 mM pH 6.4 phosphate buffer:500 mM HCl 3.8:0.4, add 1.5 mL n-hexane, vortex for 30 s, centrifuge at 1500 g for 5 min. Remove the n-hexane layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 50 μL MeOH, inject a 5-10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm YMC-C8 (Yamamura, Kyoto)
Mobile phase: MeCN:water 85:15 adjusted to pH 4.5 with MeCN:100 mM HCl 85:15
Column temperature: 30
Flow rate: 1.2
Injection volume: 5-10
Detector: UV 400, UV 230

CHROMATOGRAM
Retention time: 7.6
Internal standard: margaric acid (11.2)
Limit of detection: 0.4-1 pmole (UV 400), 100-200 fmole (UV 230)

OTHER SUBSTANCES
Extracted: arachidonic acid, capric acid, dihomo-gamma-linolenic acid, docosahexaenoic acid, eicosapentaenoic acid, lauric acid, linolenic acid, myristic acid, myristoleic acid, oleic acid, palmitic acid, palmitoleic acid, stearic acid

KEY WORDS
derivatization; serum

REFERENCE
Miwa,H.; Yamamoto,M.; Nishida,T.; Nunoi,K.; Kikuchi,M. High-performance liquid chromatographic analysis of serum long-chain fatty acids by direct derivatization method, *J.Chromatogr.*, **1987**, *416*, 237–245.

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL rat platelets suspended in pH 7.2 Tyrode-HEPES buffer containing 2 mM calcium chloride and 2.5 U/mL thrombin with 50 μL 2 μM margaric acid in MeOH, filter (0.45 μm), add 10 μL 20% HCl to the filtrate, extract with 2 mL chloroform:MeOH 50:50, centrifuge at 880 g for 10 min, remove the organic layer, extract with 1 mL chloroform, centrifuge at 800 g for 10 min. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 50 μL DMF, add 50 μL 12 mM mono-

dansylcadaverine in DMF, add 2 μL diethyl cyanophosphonate (diethylphosphorocyanidate), stir for 10 s, let stand at room temperature for 15 min, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 TSKgel ODS-80TM (Tosoh)
Mobile phase: Gradient. A was MeOH:200 mM pH 7.8 Tris-HCl buffer 50:50. B was MeCN. A:B from 50:50 to 10:90 over 1 h.
Column temperature: 40
Flow rate: 1
Detector: F ex 340 em 518

CHROMATOGRAM

Retention time: 32
Internal standard: margaric acid (42)
Limit of detection: 0.1 pmole

OTHER SUBSTANCES

Extracted: lauric acid, linolenic acid, myristic acid, myristoleic acid, oleic acid, palmitic acid, palmitoleic acid, stearic acid

KEY WORDS

derivatization; rat; platelets

REFERENCE

Lee,Y.M.; Nakamura,H.; Nakajima,T. Rapid determination by high-performance liquid chromatography of free fatty acids released from rat platelets after derivatization with monodansylcadaverine, *J.Chromatogr.*, **1990**, *515*, 467–473.

SAMPLE

Matrix: blood
Sample preparation: 50 μL Plasma + 10 μL IS solution + 450 μL micelle solution, vortex for 10 s, add 25 μL 28 mg/mL 9-bromomethylacridine in acetone, mix. Remove a 50 μL aliquot and heat it to 60° for 6 min, inject the whole amount through a 2 μm stainless steel filter of 8 sq mm area onto column A, wash to waste with 400 μL mobile phase A, back flush the contents of column A onto column B with the mobile phase B, monitor the effluent from column B. After each injection backflush the stainless steel filter with 1 mL buffer. (Micelle solution was 25 mM Arkopal N-130 (a polyoxyethylene(13)nonylphenol, Hoechst Holland, Amsterdam) in 10 mM pH 7.0 phosphate buffer containing 6 mM tetrakis(decyl)ammonium bromide. Synthesize 9-bromomethylacridine as follows. Heat 10 g diphenylamine, 10 mL glacial acetic acid, and 40 g anhydrous zinc chloride to 220°, evaporate excess acetic acid with stirring, heat at 220-230° for 6 h, digest with hot 10% sulfuric acid, make strongly alkaline with 25% ammonia to dissolve the zinc chloride. Extract the insoluble residue with toluene. Extract the organic layer with 10% sulfuric acid, make the aqueous layer alkaline with aqueous ammonia. Collect the yellow precipitate that separates and recrystallize it twice from petroleum ether to give 9-methyl acridine as pale yellow needles (Chromatographia 1989, 28, 267). Reflux 560 mg 9-methylacridine, 445 mg N-bromosuccinimide, and 10 mg benzoyl peroxide in 30 mL carbon tetrachloride for more than 2 h, cool, chromatograph on silica gel with benzene:ethyl acetate 30:1 (Caution! Benzene is a carcinogen!) to obtain 9-bromomethylacridine as yellow crystals (mp 147-151°) (Anal. Lett. 1987, 20, 1581).)

HPLC VARIABLES

Column: A 10 × 2.1 40 μm Chromsep C18 (Chrompack); B 100 × 3 5 μm Chromspher C18 (Chrompack)
Mobile phase: A 10 mM pH 7.0 phosphate buffer; B Gradient. MeOH:water 75:25 for 3 min, to 100:0 over 12 min (concave gradient).
Injection volume: 50
Detector: UV 254, F ex 362 em 418

CHROMATOGRAM

Retention time: 8.9
Internal standard: heptadecanoic acid (11.8)
Limit of detection: 300 nM

OTHER SUBSTANCES
Extracted: arachidonic acid, linolenic acid, myristic acid, oleic acid, palmitic acid, palmitoleic acid, stearic acid

KEY WORDS
derivatization; column-switching; plasma

REFERENCE
van der Horst,F.A.L.; Post,M.H.; Holthuis,J.J.M.; Brinkman,U.A.T. Automated high-performance liquid chromatographic determination of plasma free fatty acids using on-line derivatization with 9-bromomethylacridine based on micellar phase-transfer catalysis, *J.Chromatogr.*, **1990**, *500*, 443–452.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 10 μL 100 μM margaric acid in DMF + 200 μL 500 mM pH 6.5 phosphate buffer, mix, add 2 mL chloroform:n-heptane 50:50, vortex for 1 min, centrifuge at 1500 g for 5 min. Remove a 1.5 mL aliquot of the organic layer and evaporate it to dryness, reconstitute the residue in 200 μL DMF. Remove a 100 μL aliquot and add it to 50 μL 1 M 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide in water, 50 μL 10% pyridine in water, and 100 μL 30 mM reagent in DMF, mix well, let stand at room temperature for 45 min, inject a 20 μL aliquot. (Synthesis of the reagent, 2-(4-hydrazinocarbonylphenyl)-4,5-diphenylimidazole, is as follows. Add 3.15 g benzil and 2.46 g methyl 4-formylbenzoate (terephthalaldehydic acid methyl ester) to 10 g ammonium acetate in 30 mL acetic acid, stir at 80° for 9 h, cool to room temperature, pour into cold water, filter. Wash the precipitate with water and recrystallize it from EtOH to give 4-(4,5-diphenyl-1H-imidazol-2-yl)benzoic acid methyl ester as pale yellow crystals (mp 245-248°). Reflux 1.47 g 4-(4,5-diphenyl-1H-imidazol-2-yl)benzoic acid methyl ester and 15 mL 80% hydrazine hydrate (Caution! Hydrazine hydrate is a carcinogen!) in 100 mL EtOH for 4 h, cool, to room temperature, pour into cold water, filter. Wash the precipitate with water and recrystallize it from EtOH:benzene 50:50 (Caution! Benzene is a carcinogen!) to give 2-(4-hydrazinocarbonylphenyl)-4,5-diphenylimidazole as a colorless powder (mp >300°).)

HPLC VARIABLES
Column: 150 × 6 5 μm Shim-pack CLC-ODS
Mobile phase: Gradient. MeOH:water 90:10 for 5 min, to 100:0 over 25 min, maintain at 100:0 for 5 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 335 em 455

CHROMATOGRAM
Retention time: 20.8
Internal standard: margaric acid (26)
Limit of detection: 7-57 fmole

OTHER SUBSTANCES
Extracted: lauric acid, linolenic acid, myristic acid, oleic acid, palmitic acid, palmitoleic acid, stearic acid
Interfering: arachidonic acid

KEY WORDS
derivatization; serum

REFERENCE
Nakashima,K.; Taguchi,Y.; Kuroda,N.; Akiyama,S.; Duan,G. 2-(4-Hydrazinocarbonylphenyl)-4,5-diphenylimidazole as a versatile fluorescent derivatization reagent for the high-performance liquid chromatographic analysis of free fatty acids, *J.Chromatogr.*, **1993**, *619*, 1–8.

SAMPLE
Matrix: blood

Sample preparation: 100 μL Serum + 20 μL 5 mM IS in isopropanol, mix, add 500 μL isopropanol:n-heptane:2 M phosphoric acid 40:10:1, mix, let stand at room temperature for 5-10 min, add 200 μL n-heptane, add 300 μL water, vortex thoroughly, centrifuge at 1000 g for 5 min. Remove a 200 μL aliquot of the upper organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 6 μL reagent, 500 μL MeCN, and ca. 1 mg potassium bicarbonate, flush the tube with nitrogen, close the PTFE-lined cap tightly, heat at 85° with vigorous stirring for 45 min (weigh vial before and after heating to check for leakage), cool, remove stir bar, centrifuge, inject a 10-25 μL aliquot of the supernatant. (Reagent was 50 mM p-bromophenacyl bromide in MeCN containing 5 mM 18-crown-6, store protected from light.)

HPLC VARIABLES

Guard column: 4 × 4 5 μm CN
Column: 25 × 4 3 μm Spherisorb C6
Mobile phase: MeCN:water 77:23
Column temperature: 30
Flow rate: 1.3
Injection volume: 10-25
Detector: UV 254

CHROMATOGRAM

Retention time: 10.3
Internal standard: heptadecanoic acid (margaric acid) (14.7)
Limit of detection: 800 nM

OTHER SUBSTANCES

Extracted: arachidonic acid, docosahexaenoic acid, eicosapentaenoic acid, elaidic acid, lauric acid, linolenic acid, myristic acid, myristoleic acid, oleic acid, palmitic acid, palmitoleic acid, stearic

KEY WORDS

serum; derivatization

REFERENCE

Puttmann,M.; Krug,H.; von Ochsenstein,E.; Kattermann,R. Fast HPLC determination of serum free fatty acids in the picomole range, *Clin.Chem.*, **1993**, *39*, 825–832.

SAMPLE

Matrix: blood

Sample preparation: 10 μL Serum + 44 μL MeOH + 1 μL pyridine, sonicate for 5 min, add 25 μL 100 mM reagent in DMF, add 20 μL 400 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide in MeOH, let stand at 25° for 2 h, centrifuge, inject an aliquot. (Reagent was 2-(5-hydrazinocarbonyl-2-furyl)-5,6-dimethoxybenzothiazole which was synthesized as follows. Pass dry hydrogen chloride into a mixture of 12.6 g methyl 2-furoate, 4.5 g paraformaldehyde, and 3.4 g anhydrous zinc chloride in 50 mL dry chloroform for 3 h while holding the reaction temperature at 30°. After cooling pour the contents of the flask into 100 mL cold water, remove the chloroform layer, extract the aqueous layer with chloroform (cf Coll. Czech. Chem. Commun. 1960, 25, 1058). Combine the chloroform layers, neutralize, dry over anhydrous calcium chloride, evaporate, distil to give 5-chloromethyl furyl-2-carboxylic acid methyl ester (bp 108°/4 mm Hg). Reflux 10 g 5-chloromethyl furyl-2-carboxylic acid methyl ester and 25 g silver carbonate in 100 mL THF:water 70:30 for 5 h, filter through Celite, concentrate the filtrate under reduced pressure, chromatograph the product on silica gel with chloroform to give 5-hydroxymethyl furyl-2-carboxylic acid methyl ester as a light yellow oil. Add a solution of 2.9 g 5-hydroxymethyl furyl-2-carboxylic acid methyl ester in 30 mL dichloromethane to 12 g pyridinium chlorochromate in 100 mL, dichloromethane, stir at room temperature for 4 h, evaporate to dryness under reduced pressure, chromatograph on silica with dichloromethane to give 5-formyl furyl-2-carboxylic acid methyl ester as a light yellow powder. Add 10 mL concentrated nitric acid dropwise to 20 g 4-bromoveratrole in 60 mL acetic acid while keeping the temperature at 10-30° with occasional cooling, when the addition is complete pour the reaction mixture into ice-water. Collect the precipitate and dissolve it in 500 mL hot EtOH, add activated charcoal, filter, add 40 mL water to the filtrate to give 4,5-dimethoxy-2-nitrobromobenzene as a light yellow crystalline solid (mp 121-122°). Prepare sodium sulfide by melting together 5 g sodium sulfide nonahydrate and 700 mg sulfur, add this mixture to 5 g 4,5-dimethoxy-2-nitrobromobenzene in 50 mL EtOH:water 95:5, reflux for 30 min, pour into ice-water, collect the

solid, recrystallize from dichloromethane to give di(4,5-dimethoxy-2- nitrophenyl)sulfide as yellow needles (mp 231-232°). Add 15 mL concentrated HCl dropwise to 1.5 g di(4,5-dimethoxy-2-nitrophenyl)sulfide and 4.5 g tin powder stirred at 40-50° in 150 mL EtOH, reflux for 1 h, cool to room temperature, filter, add 1.17 g 5-formyl furyl-2-carboxylic acid methyl ester to the filtrate, reflux for 1 h, cool, filter, chromatograph the solid on silica gel with dichloromethane, recrystallize from EtOH to give 5-(5',6'-dimethoxybenzothiazolyl)-N-furan-2-carboxylic acid methyl ester as a yellow powder (mp 192-202°). Add 2 mL hydrazine hydrate (Caution! Hydrazine hydrate is a carcinogen!) to 800 mg 5-(5',6'-dimethoxybenzothiazolyl)-N-furan-2-carboxylic acid methyl ester in 20 mL EtOH, reflux for 30 min, collect the solid, wash with MeOH, dry under vacuum over phosphorus pentoxide to give 2-(5-hydrazinocarbonyl-2-furyl)-5,6-dimethoxybenzothiazole as a light yellow solid (mp 226-228°).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Wakosil-II 5C18 HG
Mobile phase: Gradient. MeCN:water from 70:30 to 75:25 over 25 min, to 100:0 over 15 min, maintain at 100:0.
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 363 em 452

CHROMATOGRAM

Retention time: 25
Limit of detection: 50 fmole

OTHER SUBSTANCES

Extracted: alprostadil, arachidonic acid, dinoprost, dinoprostone, lauric acid, linolenic acid, margaric acid, myristic acid, myristoleic acid, oleic acid, palmitic acid, palmitoleic acid, prostaglandin $F_{1\alpha}$, stearic acid

KEY WORDS

derivatization; serum

REFERENCE

Saito,M.; Ushijima,T.; Sasamoto,K.; Ohkura,Y.; Ueno,K. 2-(5-Hydrazinocarbonyl-2-furyl)-5,6-dimethoxybenzothiazole as a precolumn fluorescence derivatization reagent for carboxylic acids in high-performance liquid chromatography and its application to the assay of fatty acids in human serum, *Anal.Sci.*, **1995**, *11*, 103–107.

SAMPLE

Matrix: blood
Sample preparation: 50 μL Serum + 100 μL isopropanol:heptane:0.5 M sulfuric acid 80:20:2, extract. Remove 50 μL of the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 50 μL 15 mM reagent in DMSO, 50 μL 100 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide in MeOH, 50 μL 1% pyridine in MeOH, and 50 μL 100 μM nonadecanoic acid, heat at 37° for 1 h, inject a 10 μL aliquot. (Reagent was 2-(5-hydrazinocarbonyl-2-oxazolyl)-5,6-dimethoxybenzothiazole which was synthesized as follows. Add 10 mL concentrated nitric acid dropwise to 20 g 4-bromoveratrole in 60 mL acetic acid while keeping the temperature at 10-30° with occasional cooling, when the addition is complete pour the reaction mixture into ice-water. Collect the precipitate and dissolve it in 500 mL hot EtOH, add activated charcoal, filter, add 40 mL water to the filtrate to give 4,5-dimethoxy-2-nitrobromobenzene as a light yellow crystalline solid (mp 121-122°). Prepare sodium sulfide by melting together 5 g sodium sulfide nonahydrate and 700 mg sulfur, add this mixture to 5 g 4,5-dimethoxy-2-nitrobromobenzene in 50 mL EtOH:water 95:5, reflux for 30 min, pour into ice-water, collect the solid, recrystallize from dichloromethane to give di(4,5-dimethoxy-2-nitrophenyl)sulfide as yellow needles (mp 231-232°) (Anal. Sci. 1995, 11, 103). Add ethyl oxalyl chloride in ether to a solution of diazomethane in ether at 0° to give ethyl diazopyruvate (Caution! Diazo compounds are explosive and toxic!) (cf. Buehler,C.A.; Pearson,D.E. Survey of Organic Syntheses, Wiley, New York, 1970, p. 179). Heat 100 mg ethyl diazopyruvate, a few mg copper(II) acetylacetonate, and 400 μL chloroacetonitrile in benzene at 60° overnight (Caution! Benzene is a carcinogen!), cool, add to sodium bicarbonate solution, extract with ether, dry the organic layer, evaporate, chromatograph on silica with petroleum ether:ethyl acetate 90:10, distil the product at 90°/12 mm Hg to give ethyl 2-chloromethyl-5-oxazolecarboxylate as

an oil in 18% yield (US Patent 4 603 209 (July 29, 1986)). Reflux 5.0 g ethyl 2-chloromethyl-5-oxazolecarboxylate and 11.7 g NaI in 80 mL acetone for 1 h, partition the reaction mixture between ethyl acetate and water. Wash the organic layer with water and dry it over anhydrous sodium sulfate, evaporate to give ethyl 2-iodomethyl-5-oxazolecarboxylate as a reddish-brown oil. Reflux 7.4 g ethyl 2-iodomethyl-5-oxazolecarboxylate and 21.5 g silver carbonate in 100 mL THF:water 70:30 for 4 h, filter through Celite, evaporate under reduced pressure, chromatograph on silica gel using benzene:ethyl acetate 95:5 to give ethyl 2-hydroxymethyl-5-oxazolecarboxylate (mp 60.5-62°). Stir 2.04 g oxalyl chloride in 15 mL dichloromethane at -50° under nitrogen, add 1.54 g DMSO in 3 mL dichloromethane, after 5 min add 1.4 g ethyl 2-hydroxymethyl-5-oxazolecarboxylate in 6 mL dichloromethane, stir for 15 min at -50°, add 5.7 mL triethylamine, allow to warm to room temperature, dilute with dichloromethane, wash with water, dry over anhydrous sodium sulfate, concentrate under reduced pressure, chromatograph on silica gel using benzene:ethyl acetate 95:5 to give ethyl 2-carboxaldehyde-5-oxazolecarboxylate (mp 71.5-73°). Add 11.3 mL concentrated HCl to 750 mg di(4,5-dimethoxy-2-nitrophenyl)sulfide stirred in 100 mL EtOH, add 3.3 g tin powder at 40-45°, stir for 1 h at 40-45°, dilute with 100 mL water, pass hydrogen sulfide gas through this solution (Caution! Hydrogen sulfide is highly toxic!), filter, concentrate the filtrate under reduced pressure to give 4,5-dimethoxy-2-aminothiophenol. Take up this compound in 30 mL EtOH:acetic acid 2:1 and add 750 mg ethyl 2-carboxaldehyde-5-oxazolecarboxylate, reflux for 1 h, collect the precipitate and recrystallize it from EtOH to give 2-(5-ethoxycarbonyl-2-oxazolyl)-5,6-dimethoxybenzothiazole as yellow needles (mp 200-201°). Add 381 mg 2-(5-ethoxycarbonyl-2-oxazolyl)-5,6-dimethoxybenzothiazole to 20 mL EtOH containing 3 mL DMF and 5 mL hydrazine hydrate, reflux for 1 h, collect the precipitate and wash it with EtOH, dry under vacuum to give 2-(5-hydrazinocarbonyl-2-oxazolyl)-5,6-dimethoxybenzothiazole as a yellow powder (mp 255.5-280° (d)).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Wakosil-II 5C18 HG
Mobile phase: Gradient. MeCN:water from 70:30 to 100:0 over 20 min.
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 369 em 451

CHROMATOGRAM

Retention time: 10
Internal standard: nonadecanoic acid (19)

OTHER SUBSTANCES

Extracted: lauric acid, linolenic acid, margaric acid, myristic acid, myristoleic acid, oleic acid, palmitic acid, palmitoleic acid, stearic acid
Interfering: arachidonic acid

KEY WORDS

derivatization; serum

REFERENCE

Saito,M.; Ushijima,T.; Sasamoto,K.; Ohkura,Y.; Ueno,K. 2-(5-Hydrazinocarbonyl-2-oxazolyl)-5,6-dimethoxybenzothiazole as a precolumn fluorescence derivatization reagent for carboxylic acids in high-performance liquid chromatography and its application to the assay of fatty acids in human serum, *J.Chromatogr.B*, **1995**, *674*, 167–175.

SAMPLE

Matrix: blood
Sample preparation: 10 μL Serum or plasma + 10 μL 100 μM pentadecanoic acid in DMF + 200 μL 500 mM pH 6.5 phosphate buffer, mix, add 2 mL n-heptane:chloroform 50:50, vortex thoroughly, centrifuge at 1500 g for 10 min. Remove 750 μL of the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 375 μL DMF, add 25 μL 4 M 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride in pyridine:water 40:60, add 100 μL 5 mM DBD-ProCZ in DMF, let stand at room temperature in the dark for 1.5 h, inject an aliquot. (Synthesis of DBD-ProCZ is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three

times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene form EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ tlc plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei. Add 100 mg DBD-F in 10 mL MeCN to 47 mg proline in 20 mL 250 mM pH 11.5 sodium carbonate solution, stir at room temperature for 30 min, wash with ethyl acetate, adjust the pH of the aqueous layer to 1-2 with 2 M HCl, extract three times with 30 mL ethyl acetate. Combine the extracts and evaporate them under reduced pressure, recrystallize from benzene/ethyl acetate to give 4-(N,N-dimethylaminosulfonyl)-7-(2- carboxypyrrolidin-1-yl)-2,1,3-benzoxadiazole (DBD-Pro) as yellow needles (mp 187-9° d) (Analyst 1989, 114, 1233). Suspend 55 mg (S)-(-)-DBD-Pro in 55 mL anhydrous diethyl ether at 0°, add 110 mg phosphorus pentachloride, stir at 5° for 1 h, filter quickly, evaporate to dryness under reduced pressure, dry under vacuum over phosphorus pentoxide for 12 h to give 4-(N,N-dimethylaminosulfonyl)-7-(2-chloroformylpyrrolidin-1-yl)-2,1,3-benzoxadiazole (DBD-Pro-Cl) as yellow crystals (mp 116-17°) (Analyst 1993, 118, 759). DBD-Pro-Cl is also available from Tokyo Kasei (TCI America, Portland OR). Add 130 mg DBD-Pro-Cl dissolved in 25 mL anhydrous benzene dropwise to 100 mL MeOH containing 70 mg hydrazine hydrate, stir for 30 min at room temperature, evaporate under reduced pressure, recrystallize from ethyl acetate:MeOH 90:10 to give 4-(2-carbazolyl-pyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole (DBD-ProCZ) as orange crystals (mp 107-109°) (Anal.Proc. 1994, 31, 265).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-80A
Mobile phase: MeCN:water 55:45
Column temperature: 40
Flow rate: 1
Detector: F ex 450 em 550

CHROMATOGRAM

Retention time: 118
Internal standard: pentadecanoic acid (105)

OTHER SUBSTANCES

Extracted: arachidonic acid, linolenic acid, myristic acid, palmitoleic acid

KEY WORDS

rat; human; derivatization; serum; plasma

REFERENCE

Toyo'oka,T.; Takahashi,M.; Suzuki,A.; Ishii,Y. Determination of free fatty acids in blood, tagged with 4-(2-carbazoylpyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole, by high-pressure liquid chromatography with fluorescence detection, *Biomed.Chromatogr.*, **1995**, *9*, 162–170.

SAMPLE

Matrix: blood

Sample preparation: Dilute serum 20 times with water. 100 μL Diluted serum + 100 μL 1 M 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide in MeOH + 100 μL pyridine:MeOH 20:80 + 100 μL 15 mM reagent in DMF, heat at 37° for 20 min, inject a 20 μL aliquot. (Prepare reagent as follows. Stir 20 g 4-bromoveratrole in 60 mL acetic acid and add 10 mL concentrated nitric acid dropwise, keep at 10-30° with occasional cooling, pour in to ice-water, filter. Take up the precipitate in 500 mL hot EtOH and add activated charcoal, filter, add 40 mL water to the filtrate, collect 4-bromo-5-nitroveratrole as light yellow crystals (mp 121-2°). Melt 5 g sodium sulfide nonahydrate and 0.7 g sulfur together, add this mixture to 5 g 4-bromo-5-nitroveratrole in 50 mL 95% EtOH, reflux for 30 min, pour into ice-water, filter, recrystallize the product from dichloromethane to give the nitro disulfide as yellow needles (mp 231-2°). Add 8 g tin powder to 2 g of the nitro disulfide in 300 mL EtOH, add 30 mL concentrated HCl dropwise, basify with 4 M NaOH, filter, dilute the filtrate with 200 mL water. Extract the diluted filtrate twice with 100 mL portions of benzene (Caution! Benzene is a carcinogen!). Combine the extracts and evaporate them under reduced pressure, take up the residue in 10 mL benzene and add 2 mL 10% hydrogen peroxide, stir for 30 min, recrystallize the precipitate from EtOH to give 2,2'-dithiobis(1-amino-4,5-dimethoxybenzene) (mp 155-6°) (Anal.Chim.Acta 1994, 291, 189). Add a mixture of 3.7 g 2,2'-dithiobis(1-amino-4,5-dimethoxybenzene) and 1.2 g tri-n-butylphosphine in 40 mL 800 mM disodium hydrogen phosphite in MeOH to 1.2 g 4-carboxybenzaldehyde in 20 mL MeOH, stir at 37° for 2 h, filter the precipitate. Wash the precipitate with MeOH:water 70:30 and dry it under reduced pressure. Dissolve in 50 mL MeOH and treat with ethereal diazomethane (e.g., prepared as Anal. Chem. 1960, 32, 1412), evaporate to dryness under reduced pressure, chromatograph the residue on a 250 × 35 column of 130 g 70-230 mesh silica gel 60 (Merck) with n-hexane:ethyl acetate:chloroform 50:25:25, collect the main fraction and evaporate to obtain the product, 5,6-dimethoxy-2-(4-carbomethoxyphenyl)benzothiazole, as pale yellow needles (mp 223-224°). Add 100 mL aqueous 45% hydrazine hydrate (Caution! Hydrazine hydrate is a carcinogen and may explode on distillation!) to 500 mg of the product in 50 mL EtOH, reflux for 1 h, collect the precipitate and recrystallize it from EtOH to give the reagent, 5,6-dimethoxy-2-(4-hydrazinocarbonylphenyl)benzothiazole, as colorless needles (mp 252-254°).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm TSK gel ODS 120T (Tosoh)

Mobile phase: Gradient. MeCN:water 40:60 for 15 min, to 70:30 over 20 min, maintain at 70:30 for 30 min, to 0:100 over 25 min, maintain at 0:100 for 20 min.

Flow rate: 1

Injection volume: 20

Detector: F ex 365 em 447

CHROMATOGRAM

Retention time: 69

Limit of detection: 1-2 fmole

OTHER SUBSTANCES

Extracted: arachidic acid, capric acid, caprylic acid, docosahexaenoic acid, lauric acid, linolenic acid, margaric acid, myristic acid, myristoleic acid, oleic acid, palmitic acid, palmitoleic acid, stearic acid

Interfering: arachidonic acid

KEY WORDS

serum; derivatization

REFERENCE

Yamaguchi,M.; Hara,S.; Obata,K. 5,6-Dimethoxy-2-(4'-hydrazinocarbonylphenyl)benzothiazole as a highly sensitive and stable fluorescence derivatization reagent for carboxylic acids in high performance liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 2991–3006.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 200 μL ethyl acetate + 100 μL dilute HCl (pH 3.0), vortex for 5 min. Remove the supernatant and add it to 50 μL 4 mM 4-aminomethyl-6,7-dimethoxycoumarin in DMF containing 25 mM 1-hydroxybenzotriazole, add 40 μL 500 mM pH 7.0 phosphate buffer, add 100 μL 2 M 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide in water, add 10 μL 1 mM nonadecanoic acid in MeOH, vortex at 25° for 5 min, inject a 10 μL aliquot of the organic layer. (Preparation of 4-aminomethyl-6,7-dimethoxycoumarin is as follows. Heat 1 g 4-bromomethyl-6,7-dimethoxycoumarin and 1.3 g potassium phthalimide in 40 mL DMF at 80° for 1 h, cool, add 100 mL chloroform, wash with 50 mL 100 mM NaOH, wash with 100 mL water. Dry the organic layer over anhydrous sodium sulfate, concentrate to a small volume, add ether, collect 4-phthalimidylmethyl-6,7-dimethoxycoumarin as a crystalline solid (mp 240.5-242°). Reflux 950 mg 4-phthalimidylmethyl-6,7-dimethoxycoumarin and 520 mg hydrazine hydrate in 40 mL THF:MeOH 50:50 for 1.5 h (Caution! Hydrazine hydrate is a carcinogen and explodes on distillation in air!), filter, evaporate the filtrate to dryness, add 20 mL dilute HCl, evaporate to dryness, recrystallize from EtOH:ether 50:50 to give 4-aminomethyl-6,7-dimethoxycoumarin hydrochloride as slightly yellow crystals (mp 240-242.5°). Dissolve 370 mg 4-aminomethyl-6,7-dimethoxycoumarin hydrochloride in 20 mL 5% NaOH, extract 5 times with 20 mL portions of ethyl acetate, evaporate to dryness under reduced pressure, chromatograph on silica with chloroform:MeOH 98:2 to give 4-aminomethyl-6,7-dimethoxycoumarin as a slightly yellow powder (mp 137-147°).)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Wakosil-II 5C18
Mobile phase: Gradient. MeCN:water from 40:60 to 100:0 over 35 min, maintain at 100:0.
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 348 em 429

CHROMATOGRAM
Retention time: 27
Internal standard: nonadecanoic acid (42)
Limit of detection: 20-50 fmole

OTHER SUBSTANCES
Extracted: arachidonic acid, lauric acid, linolenic acid, margaric acid, myristic acid, myristoleic acid, oleic acid, palmitic acid, palmitoleic acid, stearic acid

KEY WORDS
derivatization; serum

REFERENCE
Sasamoto,K.; Ushijima,T.; Saito,M.; Ohkura,Y. Precolumn fluorescence derivatization of carboxylic acids using 4-aminomethyl-6,7-dimethoxycoumarin in a two-phase medium, *Anal.Sci.*, **1996**, *12*, 189–193.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 10 μmoles linoleic acid, 20 μmoles α-bromo-2'-acetonaphthone, and 40 μmoles N,N-diisopropylethylamine in 1 mL DMF, heat at 60° for 10 min, inject an aliquot.

HPLC VARIABLES
Column: 914 × 1.8 Corasil C18
Mobile phase: MeOH:water 85:15
Flow rate: 0.2
Detector: UV 254

CHROMATOGRAM
Retention time: 55

OTHER SUBSTANCES
Simultaneous: arachidonic acid, dihomolinolenic acid, linolenic acid, oleic acid

KEY WORDS
derivatization

REFERENCE
Cooper,M.J.; Anders,M.W. Determination of long chain fatty acids as 2-naphthacyl esters by high pressure liquid chromatography and mass spectrometry, *Anal.Chem.*, **1974**, *46*, 1849–1852.

SAMPLE
Matrix: bulk
Sample preparation: Add 3-5 equivalents of anhydrous potassium carbonate to the acid, add 0.5-1.5 mL MeCN containing an excess of α,p-dibromoacetophenone:dicyclohexyl-18-crown-6 10:1, reflux with vigorous stirring for 45 min, evaporate to dryness, take up in chloroform, filter, dilute the filtrate with chloroform, inject an aliquot.

HPLC VARIABLES
Column: 250 × 5 5 μm HI-EFF Micropart C18 (Applied Science Laboratories)
Mobile phase: MeOH:water 90:10
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Retention time: 18

OTHER SUBSTANCES
Simultaneous: elaidic acid, linoleaidic acid, linolenic acid, stearic acid
Also analyzed: oleic acid, palmitelaidic acid, palmitoleic acid, petroselinic acid

KEY WORDS
derivatization

REFERENCE
Pei,P.T.-S.; Kossa,W.C.; Ramachandran,S.; Henly,R.S. High pressure reverse phase liquid chromatography of fatty acid *p*-bromophenacyl esters, *Lipids*, **1976**, *11*, 814–816.

SAMPLE
Matrix: bulk
Sample preparation: Mix 10 μmoles fatty acid, 40 μmoles N,N-diisopropylethylamine or lithium carbonate, and 10-20 μmoles 2-bromo-4'-chloroacetophenone (p-chlorophenacyl bromide) in 1 mL DMF, heat at 65° for 15 min, inject an aliquot.

HPLC VARIABLES
Column: two 300 × 3.9 μBondapak C18 columns in series
Mobile phase: Gradient. MeCN:water from 40:60 to 100:0 (Waters convex curve 5) over 3 h.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 95

OTHER SUBSTANCES
Simultaneous: acetic acid, arachidic acid, arachidonic acid, behenic acid, butyric acid, capric acid, caproic acid, caprylic acid, elaidic acid, lactic acid, lauric acid, lignoceric acid, linolenic acid, myristic acid, nervonic acid, oleic acid, palmitic acid, palmitoleic acid, propionic acid, stearic acid, vaccenic acid

KEY WORDS
derivatization

REFERENCE

Jordi,H.C. Separation of long and short chain fatty acids as naphthacyl and substituted phenacyl esters by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1978**, *1*, 215–230.

SAMPLE

Matrix: bulk
Sample preparation: 10-50 mg Linoleic acid + 4 mL hydrazine hydrate:THF:EtOH:cyclopentene 1:3:3:1 (Caution! Hydrazine is carcinogenic and may explode when distilled!), heat at 50° under nitrogen in a tightly closed tube for 2 h, add 5 mL acetone, heat at 50° for 30 min, cool. Remove a 100 μL aliquot and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 1 mL MeOH, evaporate to dryness under a stream of nitrogen, repeat, reconstitute in 100 μL MeOH, inject a 1-5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil RP-18 ODS
Mobile phase: MeOH:water 85:15 (Wash with 30 mL MeOH after each use.)
Flow rate: 1
Injection volume: 1-5
Detector: UV 229

CHROMATOGRAM

Retention time: 17
Limit of detection: 50 ng

OTHER SUBSTANCES

Simultaneous: elaidic acid, linolenic acid, oleic acid, palmitic acid, palmitoleic acid, stearic acid

KEY WORDS

derivatization

REFERENCE

Agrawal,V.P.; Schulte,E. High-performance liquid chromatography of fatty acid isopropylidene hydrazides and its application in lipid analysis, *Anal.Biochem.*, **1983**, *131*, 356–359.

SAMPLE

Matrix: bulk
Sample preparation: Dissolve up to 1 mg fatty acid in 1 mL dichloromethane, add 1 mL of a solution containing 100 μmoles tetrabutylammonium hydrogen sulfate and 200 μmoles NaOH, add 20 μL pentafluorobenzyl bromide, shake vigorously at room temperature for 30 min. Remove the dichloromethane layer and evaporate it to dryness, reconstitute with hexane, add to a 20 × 10 silica Sep-Pak SPE cartridge, elute with hexane:dichloromethane 85:15, inject an aliquot.

HPLC VARIABLES

Column: 300 × 7.8 μPorasil
Mobile phase: Hexane:dichloromethane 85:15, half saturated with water
Flow rate: 4
Detector: UV 254

CHROMATOGRAM

Retention time: 15

OTHER SUBSTANCES

Simultaneous: linolenic acid, oleic acid, stearic acid

KEY WORDS

derivatization; SPE; normal phase; semi-preparative

REFERENCE

Netting,A.G.; Duffield,A.M. Pentafluorobenzyl esters as derivatives for the semi-preparative high-performance liquid chromatography of fatty acids, *J.Chromatogr.*, **1983**, *257*, 174–179.

SAMPLE
Matrix: bulk
Sample preparation: 100 μg Fatty acid mixture + 25 μL 10 mg/mL α-bromoacetophenone in acetone + 25 μL 10 mg/mL triethylamine in acetone, heat in a boiling water bath for 15 min, add 35 μL 2 mg/mL acetic acid in acetone, heat for 5 min, evaporate to dryness under a stream of nitrogen at 40°, reconstitute with 100 μL MeCN, inject a 5-10 μL aliquot.

HPLC VARIABLES
Guard column: octadecyl
Column: 250 × 4.5 5 μm octadecyl-bonded spherical silica (IBM)
Mobile phase: Gradient. MeCN:water 80:20 for 25 min, to 85:15 over 15 min
Flow rate: 2
Injection volume: 5-10
Detector: UV 242, UV 254

CHROMATOGRAM
Retention time: 21
Limit of quantitation: 10 ng

OTHER SUBSTANCES
Simultaneous: arachidonic acid, all-cis-delta^{8,11,14}-eicosatrienoic acid, cis-delta^{11}-eicosenoic acid, elaidic acid, heptadecanoic acid, trans-delta^{9}-hexadecenoic acid, lauric acid, linolenic acid, myristic acid, myristoleic acid, oleic acid, palmitic acid, pentadecanoic acid, cis-delta^{10}-pentadecenoic acid, stearic acid, tridecanoic acid

KEY WORDS
derivatization

REFERENCE
Wood,R.; Lee,T. High-performance liquid chromatography of fatty acids: Quantitative analysis of saturated, monoenoic, polyenoic and geometrical isomers, *J.Chromatogr.*, **1983**, *254*, 237–246.

SAMPLE
Matrix: bulk
Sample preparation: React 4 mg fatty acid, 3 mg dicyclohexylcarbodiimide, and 120 μL 40 mg/mL dansyl ethanolamine in chloroform in the dark at room temperature overnight, add water, filter, inject an aliquot of the organic layer of the filtrate. (Prepare dansyl ethanolamine by adding dansyl chloride to a large excess of stirred ethanolamine. Collect the precipitate of dansyl ethanolamine and recrystallize it from MeOH.)

HPLC VARIABLES
Column: 250 × 4.65 μm Ultrasphere ODS
Mobile phase: MeCN:MeOH:20 mM silver nitrate in water 45:45:10
Flow rate: 2
Detector: F ex 360 em 420

CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Simultaneous: eicosadienoic acid, elaidic acid, linolenic acid, margaric acid, oleic acid, palmitic acid, palmitoleic acid

KEY WORDS
derivatization

REFERENCE
Ryan,P.J.; Honeyman,T.W. Determination of fatty acids by high-performance liquid chromatography of Dns-ethanolamine derivatives, *J.Chromatogr.*, **1984**, *312*, 461–466.

SAMPLE
Matrix: bulk
Sample preparation: Mix 5-10 mg fatty acid with 1 mL 5% triethylamine in acetone and 1.5 mL acetone containing 1 equivalent of p-phenylazophenacyl bromide, shake for 30 min, purify by TLC on silica gel G with benzene (Caution! Benzene is a carcinogen!), scrape off the appropriate band and elute it with benzene, evaporate the eluate to dryness under a stream of nitrogen, reconstitute with mobile phase, inject an aliquot. (Prepare p-phenylazophenacyl bromide as follows. React equimolar quantities of p-aminoacetophenone and nitrosobenzene in glacial acetic acid at room temperature, recrystallize the crude product from EtOH to give p-phenylazoacetophenone as orange crystals (mp 114.5-116°) (Anal. Chem. 1954, 26, 1228). React 3.5 g bromine in 25 mL chloroform with 5 g p-phenylazoacetophenone in 50 mL chloroform at room temperature to obtain p-phenylazophenacyl bromide as orange red prisms after recrystallization from petroleum ether (mp 103°) (J. Chem. Soc. Japan, Pure Chem. Sect. 1951, 72, 152; Chem. Abs. 1952, 46, 3447i).)

HPLC VARIABLES
Guard column: ODS-5S (Bio-Rad)
Column: 250 × 4 5 μm Bio-Sil ODS (Bio-Rad)
Mobile phase: MeCN:water 99:1
Flow rate: 1.5
Injection volume: 20
Detector: UV 330

CHROMATOGRAM
Retention time: 6.5

OTHER SUBSTANCES
Simultaneous: linolenic acid, oleic acid

KEY WORDS
derivatization

REFERENCE
Vioque,E.; Maza,M.P.; Millán,F. High-performance liquid chromatography of fatty acids as their p-phenylazophenacyl esters, *J.Chromatogr.*, **1985**, *331*, 187–192.

SAMPLE
Matrix: butter, oil, margarine, solutions
Sample preparation: Butter, oil, margarine. Dissolve 20-40 mg in 5 mL chloroform. Remove a 100 μL aliquot and add it to 1.5 mL 25 mM tetraethylammonium carbonate in MeOH, heat in a capped tube at 70° for 5 min, remove the cap, heat at 70° for 30 min, evaporate to dryness under a stream of nitrogen, reconstitute with 1 mL DMF. Remove a 100 μL aliquot and add it to 200 μL 5 mM 9-bromomethylacridine in DMF, let stand at room temperature for at least 10 min, inject an aliquot. Solutions. Mix 100 μL of a solution in DMF with 100 μL 5 mM 9-bromomethylacridine in DMF in 100 μL 2.5 mM tetraethylammonium carbonate in DMF, let stand at room temperature for 10 min, inject a 10 μL aliquot. (Prepare tetraethylammonium carbonate by adding dry ice to an aqueous solution of tetraethylammonium hydroxide, evaporate to dryness under reduced pressure at 56° over phosphorus pentoxide to give tetraethylammonium carbonate as a white hygroscopic powder (mp 294-288° d). Prepare 9-bromomethylacridine as follows. Reflux 560 mg 9-methylacridine, 445 mg N-bromosuccinimide, and 10 mg benzoyl peroxide in 30 mL carbon tetrachloride for more than 2 h, cool, chromatograph on a column of silica gel with benzene:ethyl acetate 30:1 to obtain 9-bromomethylacridine as yellow crystals (mp 147-151°).)

HPLC VARIABLES
Column: 150 × 4.6 TSK-gel ODS 120A (Toyo Soda)
Mobile phase: Gradient. MeOH:water from 90:10 to 97:3 over 32 min (JASCO concave curve 2), maintain at 97:3 for 30 min.
Flow rate: 0.8
Injection volume: 10
Detector: F ex 365 em 425, UV 252

CHROMATOGRAM
Retention time: 36

OTHER SUBSTANCES
Extracted: arachidonic acid, capric acid, caprylic acid, docosahexaenoic acid, eicosapentaenoic acid, eicosatrienoic acid, lauric acid, linolenic acid, margaric acid, myristic acid, oleic acid, palmitic acid, palmitoleic acid, stearic acid

KEY WORDS
derivatization

REFERENCE
Akasaka,K.; Suzuki,T.; Ohrui,H.; Meguro,H.; Shindo,Y.; Takahashi,H. 9-Bromomethylacridine a novel fluorescent labeling reagent of carboxylic group for HPLC, *Anal.Lett.*, **1987**, *20*, 1581–1594.

SAMPLE
Matrix: enzyme incubations
Sample preparation: 50 μL Enzyme incubation + 200 μL n-heptane:isopropanol:1 M sulfuric acid 20:80:2, add 120 μL heptane, add 70 μL water, add 5 nmoles margaric acid, vortex for 20 s. Remove a 30-50 μL aliquot of the heptane layer and evaporate it to dryness under reduced pressure, add 50 μL 500 μg/mL 9-anthryldiazomethane in MeOH:ethyl acetate 90:10 (prepare immediately before use), let stand at room temperature for 15 min, inject a 3-10 μL aliquot. (9-Anthryldiazomethane is available from Funakoshi Co., Tokyo or it may be synthesized as follows. Stir 8.8 g 9-anthraldehyde and 8.5 g 80% hydrazine hydrate in 150 mL EtOH at room temperature for 3 h, filter off the solid 9-anthraldehyde hydrazone and dry under vacuum (mp 124-6°) (Bull. Chem. Soc. Jpn. 1967, 40, 691). Dissolve 220 mg 9-anthraldehyde hydrazone in 100 mL anhydrous ether, add 800 mg activated manganese dioxide, follow the reaction by reverse-phase HPLC using MeCN at 0.4 mL/min and UV 254. At the end of the reaction filter off the manganese and wash it with 20 mL ether, evaporate the filtrate to obtain 9-anthryldiazomethane (mp 64-6°) (Anal.Biochem. 1980, 107, 116 and 1983, 132 456). Prepare activated manganese dioxide as follows. Stir a solution of 20 g potassium permanganate in 250 mL water at room temperature, add 10 g activated carbon (Nuchar C-190 or C-190N), stir for 16 h, filter (Buchner funnel), wash 4 times with 50 mL portions of water, dry in air, dry in an oven at 105-110° for 8-24 h (J.Org.Chem. 1970, 35, 3971).)

HPLC VARIABLES
Column: 50 × 4 Superspher RP-18 (Merck)
Mobile phase: MeCN:water 95:5
Column temperature: 20
Flow rate: 1
Injection volume: 3-10
Detector: UV 254

CHROMATOGRAM
Retention time: 6
Internal standard: margaric acid (15)

OTHER SUBSTANCES
Extracted: arachidonic acid, linolenic acid, oleic acid, palmitic acid, stearic acid

KEY WORDS
derivatization

REFERENCE
Tojo,H.; Ono,T.; Okamoto,M. Reverse-phase high-performance liquid chromatographic assay of phospholipases: application of spectrophotometric detection to rat phospholipase A_2 isozymes, *J.Lipid Res.*, **1993**, *34*, 837–844.

SAMPLE
Matrix: fat, oil

Sample preparation: Prepare a 0.5-1 mg/mL solution of fat or oil in chloroform containing 0.005% BHT. Remove a 100 μL aliquot and add it to 40 nmole margaric acid, evaporate to dryness under a stream of nitrogen at room temperature, add 100 μL 2.5 M KOH:EtOH 20:80, heat at 90° for 10 min, cool to room temperature, add 400 μL reagent solution, add 200 μL 20 mM 2-nitrophenylhydrazine hydrochloride in 250 mM HCl, heat at 60° for 20 min, add 100 μL 15% KOH in MeOH:water 80:20, heat at 60° for 15 min, cool, inject a 1-5 μL aliquot. (Prepare reagent by mixing equal volumes of 3% pyridine in EtOH and 250 mM 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in EtOH.)

HPLC VARIABLES
Guard column: 30 × 4.6 ODS
Column: 250 × 4.6 5 μm YMC-C8 (Yamamura Chemical Institute, Kyoto)
Mobile phase: MeCN:water 85:15 adjusted to pH 4.5 with 100 mM HCl in MeCN
Column temperature: 30
Flow rate: 1.2
Injection volume: 1-5
Detector: UV 230

CHROMATOGRAM
Retention time: 8
Internal standard: margaric acid (12)
Limit of quantitation: 2.5 pmole

OTHER SUBSTANCES
Extracted: arachidonic acid, capric acid, docsahexenoic acid, eicosapentaenoic acid, eicosatrienoic acid, lauric acid, linolenic acid, myristic acid, myristoleic acid, oleic acid, palmitic acid, palmitoleic acid, stearic acid

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KEY WORDS
derivatization

REFERENCE
Miwa,H.; Yamamoto,M. Improved method of determination of biologically important $C_{10:0}$-$C_{22:6}$ fatty acids as their 2-nitrophenylhydrazides by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *351*, 275–282.

SAMPLE
Matrix: fat, oil
Sample preparation: Dissolve 1 mg fat or oil in 200 μL 2 mM margaric acid in EtOH, add 100 μL 400 mM KOH:EtOH 50:50, heat at 80° for 20 min, add 200 μL 20 mM 2-nitrophenylhydrazine hydrochloride in 300 mM HCl:EtOH 50:50, add 200 μL 250 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride in EtOH:pyridine 97:3, heat at 80° for 5 min, add 200 μL 10% KOH in MeOH:water 50:50, heat at 80° for 5 min, cool, inject a 5-10 μL aliquot.

HPLC VARIABLES
Guard column: J'sphere ODS-M 80 (Yamamura Chemical Laboratories, Kyoto)
Column: 250 × 4.6 4 μm J'sphere ODS-M 80 (Yamamura Chemical Laboratories, Kyoto)
Mobile phase: MeCN:water 86:14, adjusted to pH 4-5 with 100 mM HCl
Column temperature: 50
Flow rate: 2
Injection volume: 5-10
Detector: UV 400

CHROMATOGRAM
Retention time: 5.8
Internal standard: margaric acid (9.3

OTHER SUBSTANCES
Extracted: arachidic acid, arachidonic acid, capric acid, caprylic acid, dihomo-gamma-linolenic acid, docosadienoic acid, docosahexaenoic acid, docosatetraenoic acid, docosatrienoic acid, eicosadienoic acid, eicosapentaenoic acid, eicosatrienoic acid, eicosenoic (omega-12) acid, eicosenoic (omega-9) acid, eicosenoic (omega-15) acid, elaidic acid, erucic acid, lauric acid, linoelaidic acid, α-linolenic acid, gamma-linolenic acid, myristic acid, myristoleic acid, octadecatetraenoic acid, oleic acid, palmitic acid, palmitoleic acid, stearic acid

KEY WORDS
derivatization

REFERENCE
Miwa,H.; Yamamoto,M. Rapid liquid chromatographic determination of fatty acids as 2-nitrophenylhydrazine derivatives, *J.AOAC Int.*, **1996**, *79*, 493–497.

SAMPLE
Matrix: food
Sample preparation: Saponify margarine with KOH/EtOH/water, slowly acidify with concentrated HCl, extract with diethyl ether. Allow the ether to evaporate in a hood overnight. Dissolve 300 mg of the residue in 1 mL MeCN, add 3-4 drops THF, mix, make up to 5 mL with MeCN (add additional THF if the solution is cloudy), inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb RP-8
Mobile phase: MeCN:THF:water 67:3:30 containing 0.1% acetic acid (Place an 88.5 × 4.6 37-75 μm Porasil A column between pump and injector.)
Flow rate: 1.3
Injection volume: 25
Detector: RI

CHROMATOGRAM
Retention time: 21

OTHER SUBSTANCES
Extracted: elaidic acid, linoledaic acid, linolenic acid, myristic acid, oleic acid, palmitic acid, stearic acid

KEY WORDS
margarine; protect from light

REFERENCE
Bailie,A.G.,Jr.; Wilson,T.D.; O'Brien,R.K.; Beebe,J.M.; Stuart,J.D.; McCosh-Lilie,E.; Hill,D.W. HPLC analysis of underivatized fatty acids in margarines, *J.Chromatogr.Sci.*, **1982**, *20*, 466–470.

SAMPLE
Matrix: food
Sample preparation: Dissolve 20-40 mg oil, butter, or margarine in 5 mL chloroform. Remove a 100 μL aliquot and add it to 1.5 mL 25 mM tetraethylammonium carbonate in MeOH, heat in a capped tube at 70° for 5 min, heat at 70° for 30 min in an open tube, evaporate to dryness under a stream of nitrogen, reconstitute with 1 mL DMF. Remove a 100 μL aliquot and add it to 200 μL 5 mM 9-bromomethylacridine in DMF, let stand at room temperature for at least 10 min, inject a 10 μL aliquot. (Synthesize 9-bromomethylacridine as follows. Heat 10 g diphenylamine, 10 mL glacial acetic acid, and 40 g anhydrous zinc chloride to 220°, evaporate excess acetic acid with stirring, heat at 220-230° for 6 h, digest with hot 10% sulfuric acid, make strongly alkaline with 25% ammonia to dissolve the zinc chloride. Extract the insoluble residue with toluene. Extract the organic layer with 10% sulfuric acid, make the aqueous layer alkaline with aqueous ammonia. Collect the yellow precipitate that separates and recrystallize it twice from petroleum ether to give 9-methyl acridine as pale yellow needles (Chromatographia 1989, 28, 267). Reflux 560 mg 9-methylacridine, 445 mg N-bromosuccinimide, and 10 mg benzoyl peroxide in 30 mL carbon tetrachloride for more than 2 h, cool, chromatograph on silica gel with benzene:ethyl acetate 30:1 (Caution! Benzene is a carcinogen!) to obtain 9-bromomethyl-

acridine as yellow crystals (mp 147-151°). Prepare tetraethylammonium carbonate by adding dry ice to an aqueous solution of tetraethylammonium hydroxide, evaporate to dryness under reduced pressure over phosphorus pentoxide at 56° to obtain tetraethylammonium carbonate as a white hygroscopic powder (decomposes 284-288°).)

HPLC VARIABLES

Column: 150 × 4.6 TSK-gel ODS 120A (Toyo Soda)
Mobile phase: Gradient. MeOH:water from 90:10 to 97:3 over 32 min (JASCO concave curve 2), maintain at 97:3 for 30 min.
Flow rate: 0.8
Injection volume: 10
Detector: UV 252, F ex 365 em 425

CHROMATOGRAM

Retention time: 36

OTHER SUBSTANCES

Extracted: arachidonic acid, capric acid, caprylic acid, docosahexaenoic acid, eicosapentaenoic acid, eicosatrienoic acid, heptadecanoic acid, lauric acid, linoleic acid, linolenic acid, myristic acid, oleic acid, palmitic acid, palmitoleic acid, stearic acid

KEY WORDS

derivatization; oil; butter; margarine

REFERENCE

Akasaka,K.; Suzuki,T.; Ohrui,H.; Meguro,H.; Shindo,Y.; Takahashi,H. 9-Bromomethylacridine a novel fluorescent labeling reagent of carboxylic group for HPLC, *Anal.Lett.*, **1987**, *20*, 1581–1594.

SAMPLE

Matrix: food
Sample preparation: Orange juice. 10 mL Orange juice + 70 mL water + 8 g NaCl, mix until homogeneous, add 50 mL dichloromethane, shake for 1 min, centrifuge at 400 g for 5 min, repeat extraction twice more. Combine the organic layers and evaporate them to dryness under reduced pressure at 40°, dissolve the residue in five 3 mL portions of MeCN. Combine the MeCN extracts and evaporate them to 1 mL under a stream of nitrogen at 40°, filter (0.45 μm), inject an aliquot. Butter, margarine. Warm 1 g butter of margarine at 40° until it melts, add 10 mL MeCN, vortex for 5 min, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: Gradient. MeCN:buffer from 79:21 to 87:13 over 10 min, to 99:1 over 5 min, maintain at 99:1 for 5 min, return to initial conditions over 2 min, re-equilibrate for 6 min. (Buffer was water adjusted to pH 4.0 with 5% orthophosphoric acid.)
Flow rate: 0.8
Injection volume: 10
Detector: UV 651 following post-column extraction. The column effluent mixed with 2 μg/mL methylene blue in 20 mM Na_2HPO_4 pumped at 2 mL/min and the mixture flowed through a 45 cm × 0.5 mm ID stainless steel coil. The effluent from this coil mixed with chloroform pumped at 1 mL/min and the mixture flowed through a 50 cm × 1 mm ID stainless steel coil to a CTFE polymer phase separator fitted with a polyethylene filter disc from a SPE cartridge (construction details in paper). The organic phase flowed downwards to the detector at 0.5 mL/min and the aqueous phase flowed to waste. Every 2-3 injections rinse detector cell with 2-3 mL MeCN.

CHROMATOGRAM

Retention time: 9.7
Limit of detection: 39 ng

OTHER SUBSTANCES

Extracted: capric acid, caprylic acid, trans-elaidic acid, lauric acid, linoledaidic acid, linolenic acid, myristic acid, cis-oleic acid, palmitic acid, palmitoleic acid, stearic acid

KEY WORDS
post-column extraction; butter; margarine; orange juice

REFERENCE
Lawrence,J.F.; Charbonneau,C.F. Direct, sensitive and selective detection of free fatty acids by high-performance liquid chromatography with post-column ion-pair extraction and absorbance detection, *J.Chromatogr.*, **1988**, *445*, 189–197.

SAMPLE
Matrix: milk, milk products
Sample preparation: Free fatty acids. Measure out 100 μL milk, 20 mg butter, 20 mg cheese, 50 mg condensed milk, 50 mg ice cream, or 50 mg yogurt, add 100 μL water, add 200 μL 100 μM 2-ethylbutyric acid in EtOH containing 100 μM margaric acid, add 200 μL 20 mM 2-nitrophenylhydrazine hydrochloride in EtOH:100 mM HCl 50:50, add 200 μL 250 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride in EtOH:pyridine 97:3, heat at 80° for 5 min, add 200 μL 10% KOH in MeOH:water 50:50, heat at 80° for 5 min, cool, add 4 mL 33 mM pH 6.4 phosphate buffer:500 mM HCl 7:1, extract with 5 mL n-hexane. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 200 μL MeOH, filter (0.45 μm), inject a 10-20 μL aliquot of the filtrate. Total fatty acids. Measure out 10 μL milk, 1 mg butter, 1 mg cheese, 2 mg condensed milk, 2 mg ice cream, or 10 mg yogurt, add 200 μL 2 mM 2-ethylbutyric acid in EtOH containing 1 mM margaric acid, add 100 μL EtOH:400 mM KOH 50:50, heat at 80° for 20 min, add 200 μL 20 mM 2-nitrophenylhydrazine hydrochloride in EtOH:300 mM HCl 50:50, add 200 μL 250 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride in EtOH:pyridine 97:3, heat at 80° for 5 min, add 200 μL 10% KOH in MeOH:water 50:50, heat at 80° for 5 min, cool, add 4 mL 33 mM pH 6.4 phosphate buffer:500 mM HCl 7:1, extract with 5 mL n-hexane. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 200 μL MeOH, filter (0.45 μm), inject a 2-10 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 10 × 4 5 μm BBC-4-C8 (Yamamura Chemical Labs)
Column: 250 × 6 5 μm YMC-FA C8 (Yamamura Chemical Labs)
Mobile phase: MeCN:MeOH:water 75:11:14, adjusted to pH 4.5 with 100 mM HCl
Column temperature: 35
Flow rate: 1.2
Injection volume: 2-10
Detector: UV 400

CHROMATOGRAM
Retention time: 13.5
Internal standard: 2-ethylbutyric acid (17.5 (A)), margaric acid (19.5 (B))
Limit of detection: 0.5-2 pmole

OTHER SUBSTANCES
Extracted: arachidic acid, arachidonic acid, capric acid, caprylic acid, docosadienoic acid, docosahexaenoic acid, docosatetraenoic acid, docosatrienoic acid, eicoatrienoic acid, eicosadienoic acid, eicosaenoic acid, eicosapentaenoic acid, elaidic acid, erucic acid, lauric acid, linoelaidic acid, linolenic acid, myristic acid, myristoleic acid, oleic acid, palmitic acid, palmitoleic acid, stearic acid

KEY WORDS
derivatization; milk; butter; cheese; condensed milk; ice cream; yogurt

REFERENCE
Miwa,H.; Yamamoto,M. Liquid chromatographic determination of free and total fatty acids in milk and milk products as their 2-nitrophenylhydrazides, *J.Chromatogr.*, **1990**, *523*, 235–246.

SAMPLE
Matrix: oil
Sample preparation: Mix oil with mobile phase, centrifuge, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: MeCN:EtOH 90:10
Flow rate: 1.1
Injection volume: 20
Detector: E, Jasco Model EC-840, glassy carbon working electrode -415 mV, saturated calomel reference electrode, stainless steel auxiliary electrode following post-column reaction. The column effluent mixed with 76 mM lithium perchlorate in MeCN:EtOH 90:10 containing 6 mM 2-methyl-1,4-naphthoquinone (menadione, vitamin K_3) pumped at 1.1 mL/min and the mixture flowed through a 50 cm × 0.5 mm ID coil to the detector.

CHROMATOGRAM
Retention time: 5.5
Limit of detection: 20 pmole

OTHER SUBSTANCES
Extracted: oleic acid, palmitic acid, stearic acid

KEY WORDS
post-column reaction; camellia oil; corn oil; rapeseed oil; olive oil; soy bean oil

REFERENCE
Fuse,T.; Kusu,F.; Takamura,K. Determination of higher fatty acids in oils by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.A*, **1997**, *764*, 177–182.

SAMPLE
Matrix: solutions
Sample preparation: Mix 100 μg fatty acid, 10 μL 12 mg/mL phenacyl bromide in acetone, and 10 μL 10 mg/mL triethylamine in acetone, let stand at room temperature overnight, inject an aliquot.

HPLC VARIABLES
Column: 900 × 6.4 10 μm μBondapak C18
Mobile phase: Gradient. MeCN:water 67:33 for 125 min, 74:26 for 50 min, 80:20 for 40 min, 97:3 for 35 min (step gradients).
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 120
Limit of detection: 100 ng

OTHER SUBSTANCES
Simultaneous: arachidic acid, arachidonic acid, behenic acid, eicosadienoic acid, eicosaenoic acid, eicosatrienoic acid, elaidic acid, erucic acid, heneicosanoic acid, heptadecanoic acid, lauric acid, lignoceric acid, linolelaidic acid, linolenic acid, myristic acid, myristoleic acid, nervonic acid, nonadecanoic acid, oleic acid, palmitic acid, palmitoleic acid, pentadecanoic acid, petroselinic acid, stearic acid, vaccenic acid

KEY WORDS
derivatization

REFERENCE
Borch,R.F. Separation of long chain fatty acids as phenacyl esters by high pressure liquid chromatography, *Anal.Chem.*, **1975**, *47*, 2437–2439.

SAMPLE
Matrix: solutions
Sample preparation: Neutralize a solution of the acid in MeOH or water with KOH in MeOH (phenolphthalein endpoint). Evaporate to dryness under reduced pressure, dissolve in 0.5-1.5

mL MeCN containing α,p-dibromoacetophenone:18-crown-6 10:1, stir at 80° for 15 min, cool, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 Corasil II C9
Mobile phase: MeOH:water 62.5:37.5, after 132 min 75:25
Column temperature: 40
Flow rate: 0.3
Detector: UV 254

CHROMATOGRAM
Retention time: 100

OTHER SUBSTANCES
Simultaneous: linolenic acid, oleic acid, stearic acid

KEY WORDS
derivatization

REFERENCE
Durst,H.D.; Milano,M.; Kikta,E.J.,Jr.; Connelly,S.A.; Grushka,E. Phenacyl esters of fatty acids via crown ether catalysts for enhanced ultraviolet detection in liquid chromatography, *Anal.Chem.*, **1975**, *47*, 1797–1801.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve sample in 2 mL carbon tetrachloride, add 1.4 g triphenylphosphine, polymer supported, heat at 80° with gentle shaking for 5 min, cool to room temperature, add 8 mL 62.5 mg/mL p-methoxyaniline in ethyl acetate, heat at 80° for 10 min, inject a 2 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: Gradient. MeCN:water from 0:100 to 100:0 over 40 min (Waters curve 2).
Flow rate: 1
Injection volume: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 24.5

OTHER SUBSTANCES
Simultaneous: arachidonic acid, decanoic acid, docosanoic acid, 4,7,10,13,16,19-docoshexaenoic acid, dodecanoic acid, eicosanoic acid, erucic acid, heptadecanoic acid, hexadecanoic acid, hexanoic acid, linolenic acid, nervonic acid, octanoic acid, oleic acid, palmitoleic acid, pentadecanoic acid, stearic acid, tetracosanoic acid, tetradecanoic acid

KEY WORDS
derivatization

REFERENCE
Hoffman,N.E.; Liao,J.C. High pressure liquid chromatography of p-methoxyanilides of fatty acids, *Anal.Chem.*, **1976**, *48*, 1104–1106.

SAMPLE
Matrix: solutions
Sample preparation: Shake 200-400 μL of a solution of fatty acids in benzene with an equal volume of 2% oxalyl chloride in benzene (Caution! Benzene is a carcinogen!), heat at 70° for 30 min, evaporate to dryness under a stream of nitrogen, add 100 μL 7.76 mM 9-aminophenanthrene in benzene, add 100 μL 0.1% triethylamine in benzene, heat at 70° for 45 min. (Purify 9-aminophenanthrene by dissolving 50 mg in 30 mL EtOH, filter, add hydrochloric acid satu-

rated ether to the filtrate until precipitate no longer appears. Filter off the precipitate and wash it with ether, dry in a desiccator under vacuum, dissolve in hot water, basify with ammonia, filter to obtain pure 9-aminophenanthrene as white crystals (mp 134°).)

HPLC VARIABLES
Column: 300 × 4 8-10 μm μBondapak C18
Mobile phase: MeCN:MeOH:water 27:53:20
Column temperature: 40
Flow rate: 2
Detector: F ex 303 em 376

CHROMATOGRAM
Retention time: 14.5
Internal standard: margaric (26)
Limit of detection: 10 pmole

OTHER SUBSTANCES
Simultaneous: arachidonic acid, myristic acid, oleic acid, palmitic acid, palmitoleic acid, stearic acid

KEY WORDS
derivatization; protect from light; paper contains some details for analysis of fatty acids in serum

REFERENCE
Ikeda,M.; Shimada,K.; Sakaguchi,T.; Matsumoto,U. Fluorometric high-performance liquid chromatography of 9-aminophenanthrene-derivatized free fatty acids, *J.Chromatogr.*, **1984**, *305*, 261–270.

SAMPLE
Matrix: solutions
Sample preparation: Mix 100 μL of a solution in EtOH, EtOH/water, or water with 400 μL reagent solution and 200 μL 20 mM 2-nitrophenylhydrazine hydrochloride in water, heat at 60° for 20 min, add 100 μL 15% KOH in MeOH:water 80:20, heat at 60° for 15 min, cool, inject a 1-2 μL aliquot. (Prepare reagent by mixing equal volumes of 3% pyridine in EtOH and 250 mM 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in EtOH.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm YMC-C8 (Yamamuta Chemical Research, Kyoto)
Mobile phase: MeOH:water 86:14 (B) adjusted to pH 4.5 with 100 mM HCl
Column temperature: 50
Flow rate: 1.2
Injection volume: 1-2
Detector: UV 230, UV 400

CHROMATOGRAM
Retention time: 8.5
Limit of detection: 2.5-5 pmole (UV 230), 10-15 pmole (UV 400)

OTHER SUBSTANCES
Simultaneous: capric acid, lauric acid, myristic acid, linolenic acid, palmitoleic acid, palmitic acid, oleic acid, stearic acid

KEY WORDS
derivatization

REFERENCE
Miwa,H.; Hiyama,C.; Yamamoto,M. High-performance liquid chromatography of short- and long-chain fatty acids as 2-nitrophenylhydrazides, *J.Chromatogr.*, **1985**, *321*, 165–174.

SAMPLE
Matrix: solutions

Sample preparation: Add 500 μL of a solution in MeCN to 100 mg finely powdered potassium carbonate, add 250 μL 3.8 mM 18-crown-6 in MeCN, add 250 μL 0.8 mM reagent in MeCN, heat at 80° in the dark for 20 min, cool, inject a 5 μL aliquot. (Synthesize the reagent, 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone, as follows. Stir 483 g veratrole in 1.45 L acetic acid at 15° for 1 h, add 683 g concentrated nitric acid (d 1.05) over 1 h (maintain the temperature below 40° by cooling and regulating the rate of addition of the nitric acid). Continue stirring and add 2.127 L fuming nitric acid (d 1.50) over 1 h while maintaining the temperature below 30°, let stand for 2 h, pour into a large volume of cold water, filter, wash the solid with water until the washings are neutral, recrystallize from EtOH to give 4,5-dinitroveratrole (mp 129.5-130.5°) (J. Am. Chem. Soc. 1946, 68, 1536). Reflux 5 g 4,5-dinitroveratrole in 200 mL benzene (Caution! Benzene is a carcinogen!), add 100 g 60 mesh iron powder and 20 mL concentrated HCl in small portions over 1 h, reflux for 4 h, add 10 mL water, reflux for 2 h, cool, make alkaline with 2.5 M NaOH, extract several times with 200 mL portions of benzene. Combine the organic layers and evaporate them to dryness, add 10 mL concentrated HCl, recrystallize from EtOH to give 1,2-diamino-4,5-dimethoxybenzene monohydrochloride as very slightly pink needles (mp 240°) (Anal. Chim. Acta 1982, 134, 39). Heat 2.5 mmoles 1,2-diamino-4,5-dimethoxybenzene hydrochloride and 2.4 mmoles pyruvic acid in 30 mL 500 mM HCl on a boiling water bath for 2 h, cool with ice-water, filter. Wash the precipitate with water and dry it under vacuum, recrystallize from MeOH:water 90:10 to give 6,7-dimethoxy-3-methyl-2(1H)-quinoxalinone as yellow needles (mp 255°) (Chem. Pharm. Bull. 1985, 33, 3493). Treat 1 g 6,7-dimethoxy-3-methyl-2(1H)-quinoxalinone dissolved in 50 mL anhydrous MeOH with a solution of diazomethane in ether, evaporate to dryness under reduced pressure, dissolve the residue in 5 mL ethyl acetate, chromatograph on a 250 × 35 column filled with 130 g 70-230 mesh silica gel 60 (Merck) using n-hexane:ethyl acetate 25:75 to give 6,7-dimethoxy-1,3-dimethyl-2(1H)-quinoxalinone as yellow needles (mp 170-171°). Dissolve 350 mg 6,7-dimethoxy-1,3-dimethyl-2(1H)-quinoxalinone in 3 mL acetic acid, add 350 mg anhydrous sodium acetate, add 2 mL 1.5 M bromine in acetic acid, heat at 100° for 15 min, cool, add 10 mL ether, filter, wash the solid 2 or 3 times with small portions of ether. Combine the filtrate and washings and evaporate them to dryness, dissolve the residue in 5 mL ethyl acetate, chromatograph on a 250 × 35 column filled with 130 g 70-230 mesh silica gel 60 (Merck) using ether, evaporate the main fraction to dryness, recrystallize the residue from n-hexane:ethyl acetate 50:50 to give 3-bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone as yellow needles (mp 161-163°).)

HPLC VARIABLES

Column: 100 × 4 10 μm Radial-Pak C18 (Waters)
Mobile phase: Gradient. MeOH:water from 57:43 to 100:0 over 20 min, maintain at 100:0 for 12 min
Flow rate: 2
Injection volume: 5
Detector: F ex 370 em 450

CHROMATOGRAM

Retention time: 22.4
Limit of detection: 0.3-1 fmole

OTHER SUBSTANCES

Simultaneous: p-aminobenzoic acid, arachidic acid, benzoic acid, butyric acid, capric acid, caproic acid, caprylic acid, deoxyuridine, glucuronic acid, imidazole-4-acetic acid, lauric acid, margaric acid, 1-methyl-4-imidazoleacetic acid, myristic acid, myristoleic acid, oleic acid, palmitic acid, propionic acid, salicylic acid, stearic acid, thymidine, uridine, valeric acid
Interfering: arachidonic acid, linolenic acid, palmitoleic acid

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KEY WORDS

derivatization

REFERENCE

Yamaguchi,M.; Hara,S.; Matsunaga,R.; Nakamura,M.; Ohkura,Y. 3-Bromomethyl-6,7-dimethoxy-1-methyl-2(1H)-quinoxalinone as a new fluorescence derivatization reagent for carboxylic acids in high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *346*, 227–236.

SAMPLE

Matrix: solutions

Sample preparation: Mix 100 μL of a 0.01-10 μg/mL solution in MeOH with 100 μL 1 mg/mL 1-pyrenyldiazomethane in ethyl acetate, let stand at room temperature for 1.5 h, inject a 5 μL aliquot. (Synthesis of 1-pyrenyldiazomethane is as follows. Suspend 5 g 1-pyrenecarboxaldehyde in 80 mL EtOH, add 3.4 g hydrazine monohydrate (Caution! Hydrazine monohydrate is a carcinogen!), stir at room temperature for 3 h, filter off the product and wash it with 50 mL cold EtOH, recrystallize from EtOH to obtain 1-pyrenecarboxaldehyde hydrazone as yellow crystals (mp 186-194° d). Add 6.55 g activated manganese dioxide to 2 g 1-pyrenecarboxaldehyde hydrazone in 300 mL diethyl ether, sonicate at room temperature for about 80 min (monitor by HPLC), filter, wash the solid with a little ether, evaporate the filtrate to obtain 1-pyrenyldiazomethane as red crystals. Prepare activated manganese dioxide as follows. Stir a solution of 20 g potassium permanganate in 250 mL water at room temperature, add 10 g activated carbon (Nuchar C-190 or C-190N), stir for 16 h, filter (Buchner funnel), wash 4 times with 50 mL portions of water, dry in air, dry in an oven at 105-110° for 8-24 h (J.Org.Chem. 1970, 35, 3971). 1-Pyrenyldiazomethane is also available from Molecular Probes, Eugene OR.)

HPLC VARIABLES

Column: 150 × 4 5 μm TSK-GEL-120A ODS (TOSOH)
Mobile phase: Gradient. MeCN:water 85:15 for 30 min, to 100:0 over 30 min.
Flow rate: 1
Injection volume: 5
Detector: F ex 340 em 395

CHROMATOGRAM

Retention time: 20
Limit of detection: 20-30 fmole

OTHER SUBSTANCES

Simultaneous: arachidic acid, linolenic acid, myristic acid, oleic acid, palmitic acid, palmitoleic acid, stearic acid

KEY WORDS

derivatization

REFERENCE

Nimura,N.; Kinoshita,T.; Yoshida,T.; Uetake,A.; Nakai,C. 1-Pyrenyldiazomethane as a fluorescent labeling reagent for liquid chromatographic determination of carboxylic acids, *Anal.Chem.*, **1988**, *60*, 2067–2070.

SAMPLE

Matrix: solutions
Sample preparation: Mix 100 μL of a 0.01-10 μg/mL solution with 100 μL of a 9-anthryldiazomethane solution, vortex, let stand at room temperature for 1 h, inject a 5 μL aliquot. (Prepare 9-anthryldiazomethane solution as follows. Stir 8.8 g 9-anthraldehyde and 8.5 g 80% hydrazine hydrate in 150 mL EtOH at room temperature for 3 h, filter off the solid 9-anthraldehyde hydrazone and dry under vacuum (mp 124-6°) (Bull. Chem. Soc. Jpn. 1967, 40, 691). Add 500 μL 69 mM quinuclidine in ethyl acetate and 500 μL 6.9 mM N-chlorosuccinimide in ethyl acetate to 500 μL 6.9 mM 9-anthraldehyde hydrazone in ethyl acetate, vortex, let stand for 30 min, use immediately.)

HPLC VARIABLES

Column: 150 × 4 5 μm TSK-GEL-120T (Tosoh)
Mobile phase: Gradient. MeCN:water 84:0 for 30 min, to 100:0 over 25 min, maintain at 100:0 for 15 min
Column temperature: 50
Flow rate: 1
Injection volume: 5
Detector: F ex 255 or 365 em 412

CHROMATOGRAM

Retention time: 26
Limit of quantitation: 125 fmole

OTHER SUBSTANCES

Simultaneous: arachidic acid, linolenic acid, myristic acid, oleic acid, palmitic acid, palmitoleic acid, stearic acid

KEY WORDS

derivatization

REFERENCE

Yoshida,T.; Uetake,A.; Yamaguchi,H.; Nimura,N.; Kinoshita,T. New preparation method for 9-anthryldiazomethane (ADAM) as a fluorescent labeling reagent for fatty acids and derivatives, *Anal.Biochem.*, **1988**, *173*, 70–74.

SAMPLE

Matrix: solutions

Sample preparation: Mix 100 μL of a 0.3 μM solution in MeCN with 50 μL 100 μM tetraethylammonium carbonate in MeCN, add 50 μL freshly-prepared 200 μM reagent in MeCN, vortex for 10 s, let stand at room temperature for 10 min, inject a 1 μL aliquot. (Prepare tetraethylammonium carbonate by adding dry ice to an aqueous solution of tetraethylammonium hydroxide, evaporate to dryness under reduced pressure at 56° over phosphorus pentoxide to give tetraethylammonium carbonate as a white hygroscopic powder (mp 294-288° d) (Anal. Lett. 1987, 20, 1581). The reagent was 2,3-(anthracenedicarboximido)ethyl trifluoromethanesulfonate, prepared as follows. Add 11.7 g benzoyl chloride dropwise over 30 min to 10 g 1,2,4-trimethylbenzene and 11.7 g aluminum trichloride in 10 mL dichloromethane stirred at 0°, stir at room temperature for 6 h, pour into a mixture of 25 mL concentrated HCl and 50 g ice, remove the organic layer, extract the aqueous layer with dichloromethane. Combine the organic layers and wash them with 5% sodium bicarbonate, dry over anhydrous magnesium sulfate, evaporate to dryness under reduced pressure, distil through a Vigreux column to give 2,4,5-trimethylbenzophenone (bp 130°/0.15 mm Hg). Reflux 12.5 g 2,4,5-trimethylbenzophenone in 75 mL 20% nitric acid for 5 days, cool, decant the aqueous layer, wash the solid with 75 mL water. Dissolve the solid in 125 mL 10% NaOH, reflux this solution while stirring it mechanically, add 35 g potassium permanganate in portions (Caution! Frothing may occur!) over 40 min, reflux for 3 h, allow to cool somewhat, filter. Add the solid that is collected to water, reflux for 6 h, filter while hot. Combine the filtrates and evaporate them to half volume under reduced pressure, cool, acidify slowly with concentrated HCl, filter, dry the solid in air to give benzophenone-2,4,5-tricarboxylic acid as white crystals (mp 281-283°). Stir 2.1 g benzophenone-2,4,5-tricarboxylic acid in 21 g concentrated sulfuric acid at 120° for 3 h, pour onto 30 g of ice, filter, wash the solid with water, dry in air to give anthraquinone-2,3-dicarboxylic acid as a pale yellow solid (mp 342°). Add 1 g anthraquinone-2,3-dicarboxylic acid to 50 mL 20% ammonium hydroxide then add 3.75 g activated zinc dust, reflux, as soon as the blood-red color disappears filter while hot. Add the solid that is collected it to 50 mL 20% ammonium hydroxide, reflux for 2 h, filter while hot. Combine the filtrates and cool them to 0°, acidify to pH 1 with 6 M HCl, let stand at room temperature for 1 day, filter to give 2,3-anthracenedicarboxylic acid as a bright yellow solid (mp 345°) (J. Org. Chem. 1991, 56, 6243). Reflux 2,3-anthracenedicarboxylic acid in acetic anhydride for 2 h to give 2,3-anthracenedicarboxylic anhydride. Vigorously reflux 200 mg 2,3-anthracenedicarboxylic anhydride, 200 mg 2-aminoethanol, 60 mL dry toluene, and 30 mL butanol under a Dean-Stark trap for 1.5 h, evaporate the solvent under reduced pressure until crystallization starts, dissolve these crystals by warming, cool to obtain crystals, recrystallize from toluene/butanol to give N-(hydroxyethyl)-2,3- anthracenedicarboximide as orange crystals (mp 292-294°). Suspend 200 mg N-(hydroxyethyl)-2,3-anthracenedicarboximide in 50 mL dichloromethane and 1 mL pyridine, add this mixture to a solution of 400 mg trifluoromethanesulfonic anhydride in 30 mL dichloromethane at such a rate as to keep the temperature below -5°, stir for 3 h below -5°, add 200 mL cold water. Remove the organic layer and dry it over anhydrous magnesium sulfate, evaporate to dryness under reduced pressure, recrystallize from dichloromethane at -20° to give 2,3-(anthracenedicarboximido)ethyl trifluoromethanesulfonate as pale yellow crystals (mp >300°).)

HPLC VARIABLES

Column: 100 × 4.6 3 μm Develosil ODS-K3
Mobile phase: MeCN:MeOH:water 22.5:67.5:10
Flow rate: 0.8
Injection volume: 1
Detector: F ex 298 em 456

CHROMATOGRAM
Retention time: 14
Limit of detection: 1.4-3.8 pmole

OTHER SUBSTANCES
Simultaneous: arachidonic acid, capric acid, caprylic acid, cis-4,7,10,13,16,19-docosahexaenoic acid, cis-11,14-eicosadienoic acid, cis-5,8,11,14,17-eicosapentaenoic acid, cis-8,11,14-eicosatrienoic acid, gondoic acid, heptadecanoic acid, lauric acid, linolenic acid, myristic acid, myristoleic acid, oleic acid, palmitic acid, palmitoleic acid, stearic acid

KEY WORDS
derivatization

REFERENCE
Akasaka,K.; Ohrui,H.; Meguro,H. Determination of carboxylic acids by high-performance liquid chromatography with 2-(2,3-anthracenedicarboximido)ethyl trifluoromethanesulfonate as highly sensitive fluorescent labelling reagent, *Analyst*, **1993**, *118*, 765–768.

SAMPLE
Matrix: solutions
Sample preparation: Mix 25 μL of an aqueous solution of fatty acids with 100 μL DMF:pyridine 93:7, add 50 μL 5 mM MPIB-hydrazide in DMF, add 100 μL 4 M (sic) 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, heat at 40° for 20 min, inject a 10 μL aliquot. (Synthesis of MPIB-hydrazide, 4-(1-methylphenanthro[9,10-d]imidazol-2-yl)benzohydrazide, is as follows. Stir 1 g 9,10-diaminophenanthrene and 800 mg methyl 4-formylbenzoate (terephthaldehydic acid methyl ester) in 200 mL EtOH at room temperature for 1 h, add 5 mL MeOH saturated with HCl, reflux under an inert gas for 2 h, cool, concentrate to 50 mL under reduced pressure, chromatograph the precipitate on a 200 × 35 column of 70-230 mesh silica gel (ca. 100 g; Merck) with chloroform, recrystallize from MeOH to give methyl 4-(phenanthro[9,10-d]imidazol- 2-yl)benzoate as colorless needles (mp 312-315°). Dissolve 500 mg methyl 4-(phenanthro[9,10-d]imidazol-2-yl)benzoate in 100 mL anhydrous MeOH, treat with a solution of diazomethane in ether, evaporate to dryness under reduced pressure, dissolve the residue in 20 mL chloroform, chromatograph on a 200 × 60 column of about 250 g 100 mesh silica gel with chloroform to give methyl 4-(1-methylphenanthro[9,10-d]imidazol-2-yl)benzoate as colorless needles (mp 199-201°). Dissolve 2 g methyl 4-(1-methylphenanthro[9,10-d]imidazol-2-yl)benzoate in 100 mL aqueous hydrazine hydrate (45%) (Caution! Hydrazine hydrate is a carcinogen and explodes on distillation in air!), heat at 100° for 1 h, recrystallize the precipitate from 95% EtOH to give MPIB-hydrazide (4-(1-methylphenanthro[9,10-d]imidazol-2-yl)-benzohydrazide) (mp 291-293°).)

HPLC VARIABLES
Column: 250 × 4.6 5 μm TSKgel ODS-80Ts (Tosoh)
Mobile phase: MeOH:water 95:5
Flow rate: 1
Injection volume: 10
Detector: F ex 360 em 460, F ex 325 (10 mW He-Cd laser) em 460

CHROMATOGRAM
Retention time: 8.0
Limit of detection: 2.2-12.5 fmole (F), 0.4-2.3 fmole (laser F)

OTHER SUBSTANCES
Simultaneous: arachidic acid, arachidonic acid, margaric acid, oleic acid, palmitic acid, stearic acid

KEY WORDS
derivatization

REFERENCE
Iwata,T.; Hirose,T.; Nakamura,M.; Yamaguchi,M. 4-(1-Methylphenanthro[9,10-*d*]imidazol-2-yl)benzohydrazide as derivatization reagent for carboxylic acids in high-performance liquid chromatography with conventional and laser-induced fluorescence detection, *Analyst*, **1994**, *119*, 1747–1751.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 0.17-1.7 mg/mL solution in MeOH, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. A was MeOH containing 0.05% acetic acid. B was water containing 0.05% acetic acid. A:B 85:15 to 100:0 over 40 min, maintain at 100:0.
Flow rate: 1
Injection volume: 30
Detector: evaporative light scattering (ELSD, MK VIII, Varex), drift tube 75°, nitrogen flow 1 L/min, nitrogen pressure 22 psi or UV 205

CHROMATOGRAM
Retention time: 22.36

OTHER SUBSTANCES
Simultaneous: ricinoleic acid, fatty acids

REFERENCE
Lin,J.-T.; McKeon,T.A.; Stafford,A.E. Gradient reversed-phase high-performance liquid chromatography of saturated, unsaturated and oxygenated free fatty acids and their methyl esters, *J.Chromatogr.A*, **1995**, *699*, 85–91.

SAMPLE
Matrix: tissue
Sample preparation: 50 mg Fat + 1 mL 25% KOH in 96% EtOH, heat in a boiling water bath for 1 h, cool, adjust pH to 2 with 3 M HCl, extract three times with 2 mL portions of n-hexane: diethyl ether 50:50. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue with 25 μL 10 mg/mL α-bromoacetophenone in acetone and 25 μL 10 mg/mL triethylamine in acetone, heat in a boiling water bath for 5 min, add 40 μL 2 mg/mL acetic acid in acetone, heat for 5 min, evaporate to dryness under a stream of nitrogen at 40°, reconstitute with 100 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4 7 μm Separon SGX C18 (Tessek, Prague)
Column: 250 × 4 5 μm Separon SGX C18 (Tessek, Prague)
Mobile phase: Gradient. MeCN:MeOH:water from 40.5:40:19.5 to 0:81.5:18.5 over 25 min, to 0:90:10 over 45 min, to 0:100:0 over 20 min.
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: UV 242

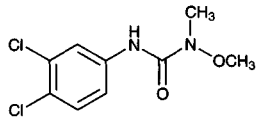
CHROMATOGRAM
Retention time: 31
Limit of detection: 0.8-1.2 ng

OTHER SUBSTANCES
Extracted: arachidonic acid, capric acid, caproic acid, caprylic acid, docosahexaenoic acid, eicosadienoic acid, eicosatrienoic acid, eicosenoic acid, elaidic acid, erucic acid, heptadecanoic acid, lauric acid, linoelaidic acid, linoleic acid, linolenic acid, myristic acid, myristoleic acid, oleic acid, palmitic acid, palmitoleic acid, pentadecanoic acid, stearic acid

KEY WORDS
derivatization; rat; fat

REFERENCE
Hanis,T.; Smrz,M.; Klir,P.; Macek,K.; Klima,J.; Base,J.; Deyl,Z. Determination of fatty acids as phenacyl esters in rat adipose tissue and blood vessel walls by high-performance liquid chromatography, *J.Chromatogr.*, **1988**, *452*, 443–457.

Linuron



Molecular formula: $C_9H_{10}Cl2N_2O_2$
Molecular weight: 249.10
CAS Registry No.: 330-55-2
Merck Index: 5534

SAMPLE

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

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 209.9

CHROMATOGRAM

Retention time: 21.253

KEY WORDS

whole blood

REFERENCE

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

SAMPLE

Matrix: bulk
Sample preparation: Dissolve in 1 mL 2 M NaOH, heat at 75° for 45 min, cool, add 2 drops methyl isobutyl ketone, add 2 drops 0.1% dansyl chloride in acetone, shake well, heat at 65° for 30 min, cool, acidify with 10% HCl, add 300 µL benzene (Caution! Benzene is a carcinogen!), extract, inject a 1-10 µL aliquot.

HPLC VARIABLES

Column: 1000 × 2.4 Zipax coated with 0.5% β,β'-oxydipropionitrile
Mobile phase: Hexane:MeOH 95:5
Flow rate: 0.78
Injection volume: 1-10
Detector: F ex Turner filter no. 811 em Turner filter no. 817

CHROMATOGRAM

Retention time: 5

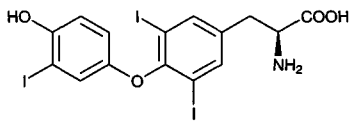
OTHER SUBSTANCES

Simultaneous: chloropropham, propham

KEY WORDS
derivatization; normal phase

REFERENCE
Frei,R.W.; Lawrence,J.F. Fluorigenic labelling in high-speed liquid chromatography, *J.Chromatogr.*, **1973**, *83*, 321–330.

Liothyronine



Molecular formula: $C_{15}H_{12}I_3NO_4$
Molecular weight: 650.98
CAS Registry No.: 6893-02-3, 66-06-1 (Na salt)
Merck Index: 5535
Lednicer No.: 1 97

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 1 mg compound in 1 mL EtOH and 10 μL 1 M KOH in EtOH: water 50:50 (freshly prepared). Remove a 10 μL aliquot and evaporate it to dryness under <0.05 Torr at 45° for 30 min, add 50 μL 80 mM 4-dimethylaminopyridine in dry MeCN, add 5 μL EtOH, vortex thoroughly, add 50 μL trimethylacetic anhydride, vortex for 10 s, heat at 65-70° for 50 min, add 100 μL EtOH, heat at 65-70° for 10 min, evaporate to dryness, add 100 μL toluene, add 100 μL 100 mM pH 6 phosphate buffer, vortex, centrifuge. Remove the organic layer and evaporate it to dryness, add 100 μL MeCN, sonicate for 2 min, add 100 μL pH 2.1 phosphate buffer, mix, inject an aliquot. (Pass MeCN through an aluminum oxide column before use.)

HPLC VARIABLES
Column: 150 × 4.6 Supelcosil LC-8
Mobile phase: Gradient. MeCN:10 mM KH_2PO_4 adjusted to pH 2.1 with phosphoric acid from 30:70 to 87:13 over 10 min.
Flow rate: 2
Detector: UV 214

CHROMATOGRAM
Retention time: 10.7

OTHER SUBSTANCES
Simultaneous: 3,5-diiodothyronine, thyroxine

KEY WORDS
derivatization

REFERENCE
Joppich,M.; Joppich-Kuhn,R.; Sentissi,A.; Giese,R.W. Single-step, quantitative derivatization of amino, carboxyl, and hydroxyl groups in iodothyronine amino acids with ethanolic pivalic anhydride containing 4-dimethylaminopyridine, *Anal.Biochem.*, **1986**, *153*, 159–165.

SAMPLE
Matrix: bulk, 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 μm). (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 ± 0.05 with 6 M HCl) reducing buffer. Deactivating solution was 1:100 phosphoric acid: MeCN.)

HPLC VARIABLES
Column: 300 × 4 μBondapak 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: 13

OTHER SUBSTANCES
Simultaneous: levothyroxine, L-3,3',5'-triiodothyronine

KEY WORDS
tablets

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: 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: 12.5
Internal standard: 3,3',5'-triiodothyronine

OTHER SUBSTANCES
Simultaneous: levothyroxine

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: formulations
Sample preparation: Powder tablets, weigh out amount equivalent to about 200 μg sodium levothyroxine, add 10 mL mobile phase, sonicate for 5 min, centrifuge. Filter (0.45 μm, 25 mm Acrodisc CR, Gelman) the supernatant, inject a 200 μL aliquot.

HPLC VARIABLES
Guard column: 40 × 4 40 μm 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: 3

OTHER SUBSTANCES
Simultaneous: levothyroxine, 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: solution
Sample preparation: Inject a 10 μL aliquot of a solution in MeOH:water 50:50.

HPLC VARIABLES
Column: 125 × 4 5 μm Nucleosil C8
Mobile phase: Gradient. A was MeCN:water 5:95 containing 0.05% acetic acid. B was MeCN:water 80:20 containing 0.05% acetic acid. A:B from 100:0 to 70:30 over 12 min, to 45:55 over 35 min, to 0:100 over 35 min
Flow rate: 1
Injection volume: 10
Detector: UV 231; MS, HP Model 5987, thermospray, tip temperature 250°, negative ion mode or filament mode, source 320°, m/z 200-900

CHROMATOGRAM
Retention time: 25

OTHER SUBSTANCES
Simultaneous: degradation products, levothyroxine

REFERENCE
Andre,M.; Domanig,R.; Riemer,E.; Moser,H.; Groeppelin,A. Identification of the thermal degradation products of G-triiodothyronine sodium (liothyronine sodium) by reversed-phase high-performance liquid chromatography with photodiode-array UV and mass spectrometric detection, *J.Chromatogr.A*, **1996**, *725*, 287–294.

SAMPLE
Matrix: solution
Sample preparation: Inject a 10 μL aliquot of a solution in MeOH:water 50:50

HPLC VARIABLES
Column: 100 × 4.6 5 μm Nucleosil C18
Mobile phase: Gradient. A was 50 mM pH 3.0 triethylammonium phosphate. B was MeCN. A:B from 95:5 to 30:70 over 70 min.
Flow rate: 1
Injection volume: 10
Detector: UV 231

CHROMATOGRAM
Retention time: 23

OTHER SUBSTANCES
Simultaneous: degradation products, levothyroxine

REFERENCE
Andre,M.; Domanig,R.; Riemer,E.; Moser,H.; Groeppelin,A. Identification of the thermal degradation products of G-triiodothyronine sodium (liothyronine sodium) by reversed-phase high-performance liquid chromatography with photodiode-array UV and mass spectrometric detection, *J.Chromatogr.A*, **1996**, *725*, 287–294.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 45 × 4.6 10 μm, 300 Å Nucleosil C8 (Bio-Rad)
Column: 250 × 4.6 5 μm, 300 Å Nucleosil C8 (Bio-Rad)
Mobile phase: Gradient. A was 0.1% pH 2.0 trifluoroacetic acid. B was MeCN:0.1% trifluoroacetic acid 90:10. A:B maintain at 80:20 (initial equilibration), from 80:20 to 20:80 over 40 min, from 20:80 to 0:100 over 10 min, from 0:100 to 80:20 over 10 min (re-equilibration)
Flow rate: 0.5
Detector: UV 280

CHROMATOGRAM
Retention time: 37

OTHER SUBSTANCES
Simultaneous: levothyroxine

REFERENCE
de la Vieja,A.; Calero,M.; Santisteban,P.; Lamas,L. Identification and quantitation of iodotyrosines and iodothyronines in proteins using high-performance liquid chromatography by photodiode-array ultraviolet-visible detection, *J.Chromatogr.B*, **1997**, *688*, 143–149.

SAMPLE
Matrix: solutions
Sample preparation: Take up 1.5 mg liothyronine 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 30 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: 250 × 3.2 7 μm LiChrosorb RP-18
Mobile phase: MeOH:buffer 60:40 (Buffer was 85 mM pH 6.4 phosphate-citrate buffer obtained by mixing 200 mM pH 8.0 phosphate buffer with 100 mM pH 2.2 citrate buffer.)
Flow rate: 1
Injection volume: 30
Detector: UV 230

CHROMATOGRAM
Retention time: 13

OTHER SUBSTANCES
Simultaneous: dextrothyroxine, levothyroxine, D-isomer, impurities

KEY WORDS
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: solutions
Sample preparation: 100 μL Solution + 100 μL 500 mM pH 7.7 borate buffer + 100 μL 2.5 mM 9-fluorenylmethyl chloroformate in dry acetone, mix, let stand at room temperature for 45 s, add 200 μL 12 mM 1-adamantamine in MeCN, inject an aliquot.

HPLC VARIABLES
Guard column: 10 × 3 30 μm Chromspher C18 (Chrompack)
Column: 100 × 3 5 μm Chromspher C18 (Chrompack)
Mobile phase: Gradient. A was MeOH:50 mM pH 4.2 sodium acetate buffer 40:60. B was MeCN:MeOH:50 mM pH 4.2 sodium acetate buffer 20:60:20. A:B from 100:0 to 0:100 over 40 min.
Column temperature: 35
Flow rate: 0.7
Detector: UV 260

CHROMATOGRAM
Retention time: 30
Limit of detection: 3.5 pmole

OTHER SUBSTANCES
Simultaneous: 3,5-diiodothyronine, 3,5-diiodotyrosine, levothyroxine, 3-monoiodotyrosine, thyronine, 3,3',5-triiodothyronine

KEY WORDS
derivatization; comparison with other derivatization procedures

REFERENCE
Doorn,L.; Jansen,E.H.; Van Leeuwen,F.X. Comparison of high-performance liquid chromatographic detection methods for thyronine and tyrosine residues in toxicological studies of the thyroid, *J.Chromatogr.*, **1991**, *553*, 135–142.

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: 6.6

OTHER SUBSTANCES
Simultaneous: degradation products, levothyroxine

REFERENCE
Won,C.M. Kinetics of degradation of levothyroxine in aqueous solution and in solid state, *Pharm.Res.*, **1992**, *9*, 131–137.

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

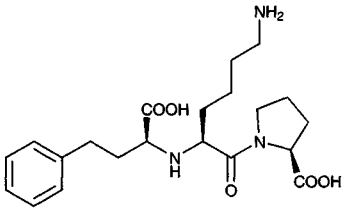
CHROMATOGRAM
Retention time: 17

OTHER SUBSTANCES
Extracted: diiodothyronine (T2), levothyroxine (T4)

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.

Lisinopril



Molecular formula: $C_{21}H_{31}N_3O_5$
Molecular weight: 405.48
CAS Registry No.: 76547-98-3 (anhydrous), 83915-83-7 (dihydrate)
Merck Index: 5540
Lednicer No.: 4 83

SAMPLE
Matrix: formulations
Sample preparation: Add MeOH:100 mM pH 2.8 phosphate buffer 25:75 to powdered capsules or tablets so as to give a lisinopril concentration of ca. 1.8 mg/mL, stir for 15 min, inject a 20 μL aliquot.

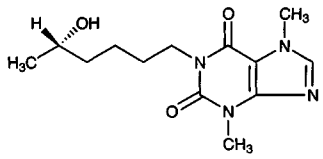
HPLC VARIABLES
Column: 250 × 4.5 5 μm Hypersil ODS
Mobile phase: MeCN:MeOH:20 mM pH 2.5 sodium heptanesulfonate 35.15:1.85:63
Flow rate: 1
Injection volume: 20
Detector: UV 215

CHROMATOGRAM
Retention time: 5.0

KEY WORDS
capsules; tablets

REFERENCE
Bonazzi,D.; Gotti,R.; Andrisano,V.; Cavrini,V. Analysis of ACE inhibitors in pharmaceutical dosage forms by derivative UV spectroscopy and liquid chromatography (HPLC), *J.Pharm.Biomed.Anal.*, **1997**, *16*, 431–438.

Lisofylline



Molecular formula: $C_{13}H_{20}N_4O_3$
Molecular weight: 280.33
CAS Registry No.: 100324-81-0

SAMPLE

Matrix: microsomal incubations
Sample preparation: Add 6 mL ice-cold dichloromethane to 500 µL microsomal incubation, add 100 µL 10 µg/mL CT-2410 R, shake on a reciprocal shaker for 10 min, centrifuge at 3000 g for 10 min, evaporate the organic layer to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 µL mobile phase, inject 30-60 µL aliquot.

HPLC VARIABLES

Guard column: Opti-Guard (Optimize Technologies, Inc.)
Column: 100 × 4.6 3 µm Microsorb-MV C18
Mobile phase: MeOH:buffer 35:65 (Buffer was 25 mM ammonium phosphate containing 0.25% acetic acid, pH adjusted to 4.5 with ammonium hydroxide.)
Flow rate: 0.7
Injection volume: 30-60
Detector: UV 273

CHROMATOGRAM

Retention time: 13.5
Internal standard: CT-2410 R (20.0)

OTHER SUBSTANCES

Extracted: pentoxifylline

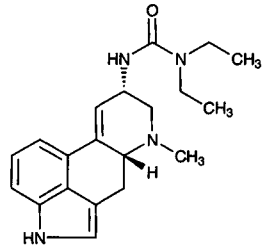
KEY WORDS

human; liver

REFERENCE

Lee,S.H.; Slattery,J.T. Cytochrome P450 isozymes involved in lisofylline metabolism to pentoxifylline in human liver microsomes, *Drug Metab.Dispos.*, **1997**, *25*, 1354–1358.

Lisuride



Molecular formula: $C_{20}H_{26}N_4O$
Molecular weight: 338.45
CAS Registry No.: 18016-80-3, 19875-60-6 (maleate)
Merck Index: 5541

SAMPLE

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

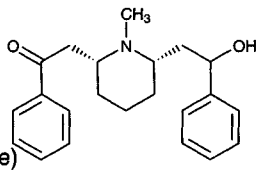
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85: 15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 209.9

CHROMATOGRAM
Retention time: 4.53

KEY WORDS
whole blood

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

Lobeline



Molecular formula: $C_{22}H_{27}NO_2$
Molecular weight: 337.46
CAS Registry No.: 90-69-7, 134-65-6 (±), 134-63-4 (HCl), 134-64-5 (sulfate)
Merck Index: 5580

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

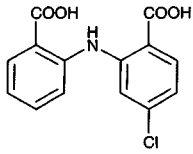
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85: 15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.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

CHROMATOGRAM
Retention time: 14.625

KEY WORDS
whole blood

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

Lobenzarit



Molecular formula: $C_{14}H_{10}ClNO_4$
Molecular weight: 291.69
CAS Registry No.: 63329-53-3, 64808-48-6 (Na salt)
Merck Index: 5581
Lednicer No.: 4 43

SAMPLE
Matrix: blood
Sample preparation: Add 50 μL 35 μ g/mL IS in MeOH to 200 μL plasma, shake, add 300 μL MeCN, shake. Vortex for 2 min, centrifuge at 25° at 460 g for 10 min, inject a 20 μL aliquot of supernatant.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChroCART
Column: 125 × 4 5 μm Lichrospher100 RP-18
Mobile phase: MeCN:water:glacial acetic acid 50:50:0.2
Flow rate: 1.0
Injection volume: 20 RT 4.48
Detector: UV 308

CHROMATOGRAM
Internal standard: diphenylamine (9.20)
Limit of quantitation: 2 μ g/L

KEY WORDS
plasma

REFERENCE
Castillo,B.; Alberto,N.; Nuevas,L.; Peris,J.E. Determination of lobenzarit disodium in human plasma by high-performance liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1063–1072.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 50 μL 200 mM HCl + 50 μL water, mix, add 50 μL 100 μg/mL diphenylamine in MeCN, add 550 μL MeCN, centrifuge at 4° at 1400 g for 10 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax phenyl
Mobile phase: MeCN:water:glacial acetic acid 50:50:0.2
Flow rate: 1
Injection volume: 50
Detector: UV 308

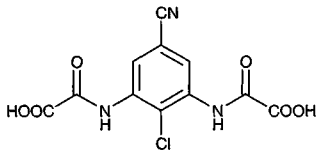
CHROMATOGRAM
Retention time: 10
Internal standard: diphenylamine (18.8)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Simultaneous: acetaminophen, aspirin, flufenamic acid, hydrochlorothiazide, indomethacin, ketoprofen, meclofenamic acid, mefenamic acid, naproxen, phenylbutazone, piroxicam, sulindac, tolmetin
Noninterfering: fenoprofen, ibuprofen

KEY WORDS
plasma; dog; pharmacokinetics

REFERENCE
Schwende,F.J.; Turner,S.W. High-performance liquid chromatographic (HPLC) determination of lobenzarit in plasma and its application to a bioavailability study in beagle dogs, *Pharm.Res.*, **1991**, *8*, 523–526.

Lodoxamide



Molecular formula: $C_{11}H_6ClN_3O_6$
Molecular weight: 311.64
CAS Registry No.: 53882-12-5, 63610-09-3 (tromethamine salt)
Merck Index: 5585
Lednicer No.: 3 57

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 10 μL 115 μg/mL p-nitrocinnamic acid in MeOH:water 50:50 + 100 μL concentrated HCl, vortex for 30 s, heat on a steam bath for 1 min, cool to room temperature, add 5 mL ethyl acetate, vortex for 45 s, centrifuge at 860 g for 10 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH:water 50:50 containing 50 mM tris(hydroxymethyl)aminomethane adjusted to pH 6, if necessary centrifuge at 1239 g for 5 min, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeOH:50 mM tris(hydroxymethyl)aminomethane 10:90 adjusted to pH 6 with concentrated phosphoric acid
Flow rate: 1
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 10
Internal standard: p-nitrocinnamic acid (19)
Limit of detection: 20 ng/mL

KEY WORDS
plasma

REFERENCE
Honigberg,I.L.; Stewart,J.T.; Ewing,B.J.; Taylor,W.D. Determination of lodoxamide in plasma using ion-pairing and reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *276*, 213–217.

SAMPLE
Matrix: formulations
Sample preparation: Spray aerosol (weigh can before and after) into 10 mL 8 mM pH 7.0 phosphate buffer and 5 mL 22 mM salicylic acid in MeOH:8 mM pH 7.0 phosphate buffer 10:90, wash container with 75 mL water, inject an aliquot of this mixture.

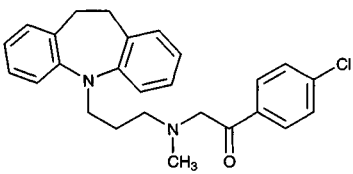
HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeOH:8 mM pH 7.0 phosphate buffer 5:95
Flow rate: 1.2
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Internal standard: salicylic acid (10)

KEY WORDS
aerosol

REFERENCE
Havel,H.A.; Beaubien,L.J.; Haaland,P.D. Analysis of the variance components in a pharmaceutical aerosol product: lodoxamide tromethamine, *J.Pharm.Sci.*, **1985**, *74*, 978–982.

Lofepramine



Molecular formula: $C_{26}H_{27}ClN_2O$
Molecular weight: 418.97
CAS Registry No.: 23047-25-8, 26786-32-3 (HCl)
Merck Index: 5587
Lednicer No.: 4 201

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

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

CHROMATOGRAM
Retention time: 1.4

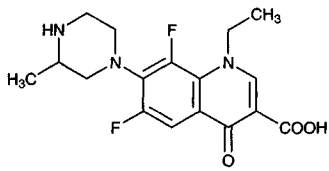
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, 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, loxapine, maprotiline, mecamylamine, meclo-

phenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

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

Lomefloxacin



Molecular formula: $C_{17}H_{19}F_2N_3O_3$
Molecular weight: 351.35
CAS Registry No.: 98079-51-7, 98079-52-8 (HCl)
Merck Index: 5592
Lednicer No.: 5 125

SAMPLE

Matrix: blood
Sample preparation: Mix 1 mL serum with 20 µL 20 µg/mL IS in MeOH and 100 µL 20 mM NaOH. Vortex for 1 min with 4 mL dichloromethane:diethylether 80:20, centrifuge at 2000 g for 10 min, remove 3 mL organic phase. Repeat the same extraction procedure twice. Combine organic layers, evaporate to dryness under a stream of nitrogen. Add 100 µL 20 mM NaOH to the residue, shake for 5 min, inject a 20 µL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 10 µm Vydac AXGU
Column: 250 × 4.6 5 µm Supelcosil LC-SAX
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 10:90
Flow rate: 1.2
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 4.3
Internal standard: 2-[4-(2'-furoyl)phenyl]propionic acid (3.5)
Limit of detection: 50 ng/mL
Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES

Extracted: felbinac, fenbufen

KEY WORDS
plasma

REFERENCE
Carlucci,G.; Mazzeo,P.; Palumbo,G. Simultaneous determination of lomefloxacin, fenbufen and felbinac in human plasma using high performance liquid chromatography, *Chromatographia*, **1996**, *43*, 261–264.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 250 μL 10% trichloroacetic acid, vortex for 10 s, centrifuge at >700 g for 10 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 8 μBondapak 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 288 em 475

CHROMATOGRAM
Retention time: 8.0
Limit of detection: 500 ng/mL

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: 500 μL Plasma + 100 μL 2 μg/mL IS + 400 μL 200 mM pH 7.0 sodium phosphate buffer, vortex for 5 s, add 5 mL chloroform:isoamyl alcohol 95:5, rotate for 5 min, centrifuge at 500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 500 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm 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: 6.2
Internal standard: E-1608 (G.D.Searle) (12.6)
Limit of quantitation: 10 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Healy,D.P.; Schoenle,J.R.; Stotka,J.; Polk,R.E. Lack of interaction between lomefloxacin and caffeine in normal volunteers, *Antimicrob.Agents Chemother.*, **1991**, *35*, 660–664.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 25 μL 100 μg/mL acebutolol in MeOH:water 10:90 + 100 μL 70 mM pH 7 phosphate buffer + 4 mL chloroform:isopentyl alcohol:diethyl ether 71.25:

3.75:25, vortex for 30 s, centrifuge at 1800 g for 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 100 μL chloroform:triethylamine 100:1, add 100 μL 1% (S)-(+)-1-(1-naphthyl)ethyl isocyanate in chloroform, after 1 min add 50 μL 2% ethylchloroformate in chloroform, after 30 s add 50 μL 2.5% ethanolamine in chloroform, inject a 20-125 μL aliquot.

HPLC VARIABLES
Column: 100 × 8 4 μm Nova-Pak silica Radial Pak
Mobile phase: Hexane:chloroform:MeOH 64.5:33:2.5
Flow rate: 2
Injection volume: 20-125
Detector: F ex 245 em 420 for 12 min then ex 280 em 470

CHROMATOGRAM
Retention time: 21 ((S)-(-)), 22 ((R)-(+))
Internal standard: acebutolol (8.5, 9.5 enantiomers)
Limit of quantitation: 10 ng/mL

KEY WORDS
plasma; derivatization; chiral; normal phase

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

SAMPLE
Matrix: blood, urine
Sample preparation: Adjust pH of 500 μL plasma or urine to 7, extract with 5 mL chloroform:isoamyl alcohol 95:5. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 500 μL mobile phase, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:50 mM citric acid containing 1% 1 M ammonium acetate 22:78
Flow rate: 1.3
Injection volume: 30
Detector: F ex 280 em 455

CHROMATOGRAM
Retention time: 6.5
Internal standard: KK-123 (Searle) (12.5)
Limit of quantitation: 3 μg/mL (urine), 20 ng/mL (plasma)

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Hooper,W.D.; Dickinson,R.G.; Eadie,M.J. Effect of food on absorption of lomefloxacin, *Antimicrob.Agents Chemother.*, **1990**, *34*, 1797–1799.

SAMPLE
Matrix: cell suspensions
Sample preparation: Centrifuge an erythrocyte suspension at 15000 g for 3 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Guard column: Perisorb RP-18 (P.J. Cobert)
Column: 150 × 4.6 5 μm MOS Hypersil C8
Mobile phase: MeCN:THF:100 mM phosphoric acid:triethylamine:water 50:10:10:0.03:30
Flow rate: 1.5
Detector: F ex 286 em 418

CHROMATOGRAM
Limit of detection: <1 μg/mL

KEY WORDS
protect from light; erythrocytes

REFERENCE
Knaub,S.R.; Chang,M.F.; Lunte,C.E.; Topp,E.M.; Riley,C.M. Automated analytical systems for drug development studies. Part IV. A microdialysis system to study the partitioning of lomefloxacin across an erythrocyte membrane in vitro, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 121–129.

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, 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: erythrocytes
Sample preparation: Centrifuge erythrocyte suspension at 15000 g for 3 min. inject an aliquot of the supernatant

HPLC VARIABLES
Guard column: Perisorb RP-18 (P.J. Cobert)
Column: 150 × 4.6 5 μm MOS Hypersil C8
Mobile phase: MeCN:THF:100 mM phosphoric acid:triethylamine:water 30:10:10:0.03:50
Flow rate: 1.5
Injection volume: 20
Detector: F ex 286 em 418 (cut-off filter)

KEY WORDS
stability-indicating

REFERENCE
Knaub,S.R.; Priston,M.J.; Morton,M.D.; Slechta,J.D.; Vander Velde,D.G.; Riley,C.M. A ^{19}F NMR study of lomefloxacin in human erythrocytes and its interaction with hemoglobin, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1225–1233.

SAMPLE
Matrix: urine
Sample preparation: 50 μL Urine + 100 μL IS + 3.85 mL water, mix, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm 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

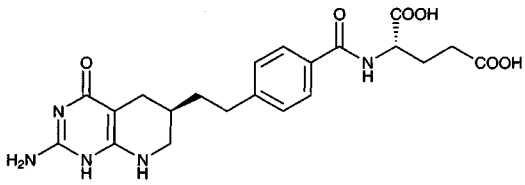
CHROMATOGRAM
Retention time: 3.6
Internal standard: KK-123 (G.D.Searle) (6.3)
Limit of quantitation: 1 μg/mL

OTHER SUBSTANCES
Extracted: ciprofloxacin

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.

Lometrexol



Molecular formula: $C_{21}H_{23}N_5O_6$
Molecular weight: 441.44
CAS Registry No.: 106400-81-8, 120408-07-3 (disodium salt)
Lednicer No.: 5 151

SAMPLE
Matrix: blood
Sample preparation: Condition a 10 mm Bond-Elut C8 SPE cartridge with 5 mL MeOH and 5 mL buffer. Dilute plasma with an equal volume of 100 ng/mL IS in buffer, vortex, centrifuge at 4° at 1000 g for 20 min, add a 2 mL aliquot of the supernatant to the SPE cartridge, wash with 5 mL buffer, elute with 1.5 mL MeCN:buffer 20:80. Centrifuge the eluate at 4° at 1000 g for 5 min, evaporate the supernatant to dryness under reduced pressure, reconstitute with 20 μL 13% formic acid in water, add 100 μL of a freshly prepared 200 μg/mL suspension of activated manganese dioxide (Sigma) in water, vortex, heat at 37° for 1.5 h, cool to 4°, add 30 μL 5 M NaOH:1% pH 5 ammonium carbonate 50:50, cool on ice, centrifuge at room temperature at 13000 g for 10 min, inject a 100 μL aliquot of the supernatant. (Buffer was 1% aqueous formic acid adjusted to pH 3.7 with 5 M NaOH.)

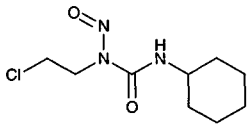
HPLC VARIABLES
Column: 150 × 4.6 3 μm Apex II C18 (Jones Chromatography)
Mobile phase: MeCN:buffer 12:88 containing 171 μg/mL tetramethylammonium hydrogen sulfate (Buffer was 1% aqueous acetic acid adjusted to pH 5 with strong ammonia. Wash column with MeOH for 10 min between experiments.)
Flow rate: 1
Injection volume: 100
Detector: F ex 325 em 450

CHROMATOGRAM
Retention time: 4.2
Internal standard: C10-desmethylene lometrexol (5.3)
Limit of quantitation: 10 ng/mL

KEY WORDS
derivatization; plasma; SPE; analytical procedures that do not involve derivatization are also given in the paper.; pharmacokinetics

REFERENCE
Wedge,S.R.; Laohavinij,S.; Taylor,G.A.; Newell,D.R. Measurement of 5,10-dideaza-5,6,7,8-tetrahydrofolate (lometrexol) in human plasma and urine by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *663*, 327–335.

Lomustine



Molecular formula: $C_9H_{16}ClN_3O_2$
Molecular weight: 233.70
CAS Registry No.: 13010-47-4
Merck Index: 5594
Lednicer No.: 2 12

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg CBA Bond Elut SPE cartridge with 1 mL MeOH and 1 mL water. Centrifuge blood at 5000 g for 2-3 min, freeze plasma in dry ice/hexane within 1 min. Thaw within 3 min by immersion in a 50° water bath. 1 mL Thawed plasma + 500 μL 2.5 μg/mL IS in 100 mM citric acid, vortex for 5 s, centrifuge for 5 min, add a 1 mL aliquot of the supernatant to the SPE cartridge, wash with 1 mL water, elute with 200 μL MeOH into a vial containing 50 μL 100 mM acetic acid, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 125 × 5 5 μm Spherisorb ODS
Mobile phase: MeCN:50 mM ammonium acetate 30:70 adjusted to pH 4.4 with glacial acetic acid
Flow rate: 1
Injection volume: 25
Detector: UV 230

CHROMATOGRAM
Retention time: 35.6
Internal standard: 1-methyl-3-isobutyl-8-vinyl-2,6-dioxopurine (S10338) (7.2)

OTHER SUBSTANCES
Extracted: carmustine, fotemustine

KEY WORDS
plasma; SPE

REFERENCE
Gordon,B.H.; Richards,R.P.; Hiley,M.P.; Gray,A.J.; Ings,R.M.; Campbell,D.B. A new method for the measurement of nitrosoureas in plasma: an h.p.l.c. procedure for the measurement of fotemustine kinetics, *Xenobiotica*, **1989**, *19*, 329–339.

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

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 229.9

CHROMATOGRAM
Retention time: 22.982

KEY WORDS
whole blood

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

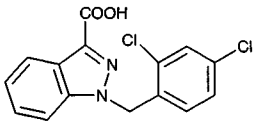
SAMPLE
Matrix: reaction mixtures
Sample preparation: If necessary, remove oxidizing power of solution by adding sodium metabisulfite, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 4.6 5 μm Microsorb C8
Column: 250 × 4.6 5 μm Microsorb C8
Mobile phase: MeOH:0.4 g/L $(NH_4)H_2PO_4$ (pH 4.7) 75:25
Flow rate: 1
Injection volume: 20
Detector: UV 228

CHROMATOGRAM
Retention time: 6.4
Limit of detection: 500 ng/mL

REFERENCE
Lunn,G.; Rhodes,S.W.; Sansone,E.B.; Schmuff,N.R. Photolytic destruction and polymeric resin decontamination of aqueous solutions of pharmaceuticals, *J.Pharm.Sci.*, **1994**, *83*, 1289–1293.

Lonidamine



Molecular formula: $C_{15}H_{10}Cl_2N_2O_2$
Molecular weight: 321.16
CAS Registry No.: 50264-69-2
Merck Index: 5598

SAMPLE
Matrix: blood
Sample preparation: Condition an AASP C8 SPE cartridge (Varian) with 500 μL MeOH and 500 μL 10 mM HCl. 10 μL Serum + 990 μL 10 mM HCl containing IS, add to the SPE cartridge, wash with 500 μL 10 mM HCl, elute the contents of the SPE cartridge on to the analytical column with the mobile phase for 1 min.

HPLC VARIABLES
Column: 150 × 4 4.5 μm MicroPak SP-C18-5

Mobile phase: Gradient. MeCN:100 mM pH 3.5 acetate buffer 40:60 for 2.5 min, to 60:40 over 1 min, to 70:30 over 6.5 min, return to initial conditions over 5 min, re-equilibrate for 10 min.
Flow rate: 1
Injection volume: 10
Detector: UV 300

CHROMATOGRAM
Retention time: 8
Internal standard: 1-[4-chlorobenzyl]-1H-indazole-3-carboxylic acid (AF1312/TS) (6.5)
Limit of quantitation: 420 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; SPE

REFERENCE
Bottalico,C.; Micelli,G.; Guerrieri,A.; Palmisano,F.; Lorusso,V.; De Lena,M. An on-line semi-automated solid-phase extraction procedure for high-performance liquid chromatographic determination of lonidamine in serum, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1349–1353.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + 50 μL 100 μg/mL IS in MeOH + 400 μL 1 M HCl, vortex for 15 s, add 5 mL anhydrous diethyl ether, shake on a reciprocal shaker for 10 min, centrifuge at 800 g for 10 min, repeat extraction with 3 mL anhydrous diethyl ether. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 45°, reconstitute the residue in 200 μL MeCN, inject a 20 μL aliquot.

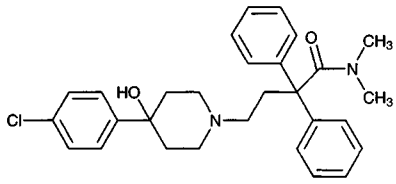
HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS
Mobile phase: MeCN:100 mM pH 3.50 acetate buffer 50:50
Flow rate: 2
Injection volume: 20
Detector: UV 300

CHROMATOGRAM
Retention time: 5.5
Internal standard: 1-(4-chlorobenzyl)-1H-indazole-3-carboxylic acid (AF 1312/TS) (4)
Limit of detection: 200 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Leclaire,R.; Besner,J.-G.; Band,P.; Mailhot,S.; Gervais,P.; De Sanctis,A.; Deschamps,M.; Liverani,L. High-performance liquid chromatography of lonidamine in human plasma and urine, *J.Chromatogr.*, **1983**, *277*, 427–432.

Loperamide



Molecular formula: $C_{29}H_{33}ClN_2O_2$
Molecular weight: 477.05
CAS Registry No.: 53179-11-6, 34552-83-5 (HCl)
Merck Index: 5601
Lednicer No.: 2 334

SAMPLE

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

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 260

CHROMATOGRAM

Retention time: 8.50
Limit of detection: <120 ng/mL

KEY WORDS

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

imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

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

SAMPLE

Matrix: blood, cell incubations, cell lysates, feces, urine

Sample preparation: Urine, plasma. Inject an aliquot directly. Feces. Extract with MeOH, evaporate extract to dryness under a stream of nitrogen, reconstitute in DMSO, inject an aliquot. Cell incubates. Centrifuge, inject an aliquot of the supernatant. Cell lysates. Dilute with an equal volume of DMSO, centrifuge, inject an aliquot of the supernatant.

HPLC VARIABLES

Column: 300 × 4.6 5 μm Hypersil C18

Mobile phase: Gradient. A was 100 mM ammonium acetate containing 0.2% diethylamine, pH 7.88. B was MeCN:MeOH:1 M ammonium acetate containing 2% diethylamine (pH 7.88) 65: 25:10 (?). A:B from 100:0 to 65:35 over 2 min, to 35:65 over 30 min.

Flow rate: 1

Detector: UV 230 or radioactivity

CHROMATOGRAM

Retention time: 41

OTHER SUBSTANCES

Extracted: loperamide oxide (prodrug)

KEY WORDS

plasma; prodrug; pharmacokinetics

REFERENCE

Lavrijsen,K.; Van Dyck,D.; Van Houdt,J.; Hendrickx,J.; Monbaliu,J.; Woestenborghs,R.; Meuldermans,W.; Heykants,K. Reduction of the prodrug loperamide oxide and its active drug loperamide in the gut of rats, dogs, and humans, *Drug Metab.Dispos.*, **1995**, *23*, 354–362.

SAMPLE

Matrix: formulations

Sample preparation: Capsules, tablets. Remove contents of capsules and powder tablets. Weigh out an amount equivalent to about 10 mg loperamide hydrochloride, add 80 mL chloroform, shake for 15 min, make up to 100 mL with chloroform, filter and discard first 10-20 mL of filtrate. 5 mL Filtrate + 1 mL 400 μg/mL cyclizine hydrochloride in chloroform, make up to 25 mL with chloroform, inject an aliquot. Syrups. Add a quantity of syrup corresponding to about 10 mg loperamide hydrochloride to 30 mL water, extract four times with 20 mL portions of chloroform and filter each extract through glass wool, combine the extracts and make up to 100 mL with chloroform. Remove a 5 mL aliquot and add it to 1 mL 400 μg/mL cyclizine hydrochloride in chloroform, make up to 25 mL with chloroform, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Perkin-Elmer Analytical silica

Mobile phase: Chloroform:MeOH:ammonia 95.5:4.5:0.05

Flow rate: 2

Injection volume: 50

Detector: UV 254

CHROMATOGRAM

Retention time: 6

Internal standard: cyclizine (4)

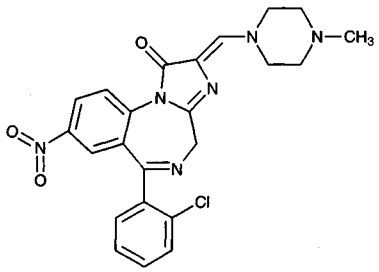
OTHER SUBSTANCES
Noninterfering: propylene glycol

KEY WORDS
capsules; tablets; syrups; normal phase

REFERENCE
Leung,C.P.; Au-Yeung,C.Y. High-performance liquid chromatographic determination of loperamide hydrochloride in pharmaceutical preparations, *J.Chromatogr.*, **1988**, *449*, 341–344.

Loprazolam

Molecular formula: $C_{23}H_{21}ClN_6O_3$
Molecular weight: 464.91
CAS Registry No.: 61197-73-7
Merck Index: 5604



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

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 329

CHROMATOGRAM
Retention time: 5.53
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine;

prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

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

SAMPLE

Matrix: blood, urine

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

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

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

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

Column temperature: 30

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

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 13.387

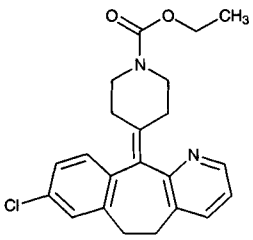
KEY WORDS

whole blood

REFERENCE

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

Loratadine



Molecular formula: $C_{22}H_{23}ClN_2O_2$
Molecular weight: 382.89
CAS Registry No.: 79794-75-5
Merck Index: 5608

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

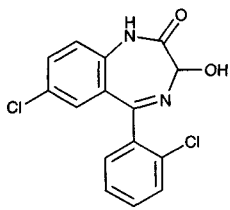
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.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

CHROMATOGRAM
Retention time: 22.943

KEY WORDS
whole blood

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

Lorazepam



Molecular formula: $C_{15}H_{10}Cl_2N_2O_2$
Molecular weight: 321.16
CAS Registry No.: 846-49-1
Merck Index: 5609
Lednicer No.: 1 368

SAMPLE
Matrix: blood
Sample preparation: 500 µL Serum + 20 µL 20 µg/mL IS + 200 µL 1 M potassium carbonate + 3 mL chloroform, mix for 2 min, centrifuge at 1200 g for 5 min, aspirate aqueous phase.

Evaporate the organic phase under a stream of nitrogen at 40°. Dissolve the residue in 100 μL mobile phase, inject a 20 μL aliquot. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES

Column: 100 × 4.6 2 μm TSK gel Super-ODS (A) or 100 × 4.6 5 μm Hypersil ODS-C18 (B)
Mobile phase: MeCN:5 mM pH 6 NaH_2PO_4 45:55
Flow rate: 0.65
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 15.0 (A), 50.1 (B)
Internal standard: diazepam (29.8 (A), 77.5 (B))
Limit of quantitation: 5 ng/mL (A)

OTHER SUBSTANCES

Extracted: bromazepam, chlordiazepoxide, clonazepam, estazolam, etizolam, flutazolam, haloxazolam, nitrazepam, oxazolam, triazolam
Simultaneous: alprazolam
Noninterfering: barbital, carbamazepine, cloxazolam, ethosuximide, hexobarbital, mexazolam, oxazepam, pentobarbital, phenobarbital, phenytoin, primidone, trimethadione

KEY WORDS

serum

REFERENCE

Tanaka,E.; Terada,M.; Misawa,.; Wakasugi,C. Simultaneous determination of twelve benzodiazepines in human serum using a new reversed-phase chromatographic column on a 2-μm porous microspherical silica gel, *J.Chromatogr.B*, **1996**, *682*, 173–178.

SAMPLE

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

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 228.7

CHROMATOGRAM

Retention time: 17.175

KEY WORDS

whole blood

REFERENCE

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

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm cyano
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 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: solutions
Sample preparation: Dilute a 2 mg/mL sample 1:100 with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: Bakerbond C18
Mobile phase: MeCN:80 mM pH 2.0 orthophosphoric acid in water 46:54
Flow rate: 1.2
Detector: UV 240

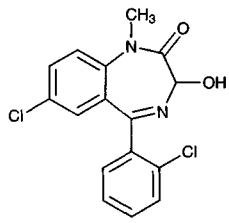
CHROMATOGRAM
Retention time: 6.6

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
stability-indicating

REFERENCE
Stiles,M.L.; Allen,L.V.,Jr.; Prince,S.J. Stability of deferoxamine mesylate, floxuridine, fluorouracil, hydromorphone hydrochloride, lorazepam, and midazolam hydrochloride in polypropylene infusion-pump syringes, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 1583–1588.

Lormetazepam



Molecular formula: $C_{16}H_{12}Cl2N_2O_2$
Molecular weight: 335.19
CAS Registry No.: 848-75-9
Merck Index: 5611
Lednicer No.: 3 196

SAMPLE

Matrix: solutions

HPLC VARIABLES

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

OTHER SUBSTANCES

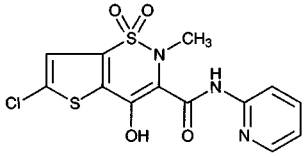
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine,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

thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

Lornoxicam



Molecular formula: $C_{13}H_{10}ClN_3O_4S_2$
Molecular weight: 371.83
CAS Registry No.: 70374-39-9
Merck Index: 5612

SAMPLE

Matrix: blood
Sample preparation: Mix plasma with 800 μL 5 mM pH 4 phosphate buffer and 400 μL 1 μg/mL tenoxicam in water. Centrifuge at 2500 rpm for 5 min. Add the supernatant to Extrelut-1 cartridge (Merck, Darmstadt, Germany). Elute with 10 mL dichloromethane, dry eluate under a stream of nitrogen at 35°, dissolve the residue in 300 μL mobile phase, centrifuge and inject 100 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:50 mM pH 6 phosphate buffer 50:50
Column temperature: 35
Flow rate: 1.3
Injection volume: 100
Detector: UV 371

CHROMATOGRAM

Retention time: 4.4
Internal standard: tenoxicam (2.2)
Limit of detection: 10 ng/mL
Limit of quantitation: 20 ng/mL

KEY WORDS

pharmacokinetics; plasma

REFERENCE

Bareggi,S.R.; Gambaro,V.; Valenti,M.; Benvenuti,C. Absorption of oral lornoxicam in healthy volunteers using a granular formulation in comparison with standard tablets, *Arzneimittelforschung*, **1997**, *47*, 755–757.

SAMPLE

Matrix: blood, synovial fluid
Sample preparation: Plasma. 500 μL Plasma + 100 μL 4 μg/mL IS in 200 mM NaOH + 500 μL 500 mM pH 4 phosphate buffer, mix, homogenize, centrifuge at 900 g. Add 1 mL of the mixture to an Extrelut-1 SPE cartridge. Elute with two 5 mL portions of dichloromethane. Evaporate the eluate under a stream of nitrogen at 35°. Dissolve the residue in 120 μL mobile phase by vortexing, inject an aliquot. Plasma, synovial fluid. Condition a 100 mg C18 SPE cartridge (Phenomenex) with 1 mL MeOH, 1 mL water, and 500 μL 500 mM pH 2 phosphate buffer. 500 μL Plasma or synovial fluid + 100 μL 2 μg/mL IS in 200 mM NaOH + 500 μL 500 mM pH 2 phosphate buffer. Mix, homogenize, centrifuge at 2500 rpm. Add 1 mL of the clean supernatant to the SPE cartridge, wash with 1 mL water, dry with 2 mL air, elute with 1.25 mL MeCN:25% ammonia 90:10. Evaporate the eluate under reduced pressure at 35°. Reconstitute the residue in 100 μL MeOH:100 mM pH 8 phosphate buffer 50:50, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:100 mM pH 6 sodium dihydrogen phosphate buffer 50:50
Flow rate: 1.5
Injection volume: 50-100
Detector: UV 372

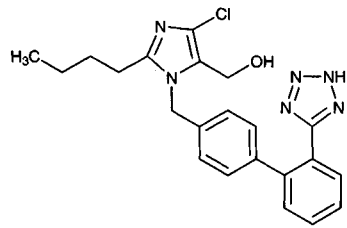
CHROMATOGRAM
Retention time: 5.8-6.1 (Extrelut), 6.7 (C18 SPE)
Internal standard: tenoxicam (2.6-2.8)
Limit of detection: 800 pg
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics; mouse; rat; rabbit; dog; monkey; human; SPE

REFERENCE
Radhofer-Welte,S.; Dittrich,P. Determination of the novel non-steroidal anti-inflammatory drug lornoxicam and its main metabolite in plasma and synovial fluid, *J.Chromatogr.B*, **1998**, *707*, 151–159.

Losartan



Molecular formula: $C_{22}H_{23}ClN_6O$
Molecular weight: 422.92
CAS Registry No.: 124750-99-8
Merck Index: 5613
Lednicer No.: 5 73

SAMPLE
Matrix: bile, blood, tissue, urine
Sample preparation: Plasma, urine. Condition a Varian Bond Elut non-endcapped CN SPE cartridge with two 1 mL portions of MeOH and two 1 mL portions of water. Vortex 500 μL plasma + 300 ng IS + 500 μL 200 mM HCl or 500 μL urine + 300 ng IS + 500 μL water + 200 μL 1.0 M HCl and add to the SPE cartridge. Wash with 500 μL water, with 500 μL MeOH: water 10:90, and with 50 μL MeOH, elute with five 200 μL portions of pH 8 MeOH:ammonia 99:1. Evaporate the combined eluates to dryness. Reconstitute the residue in 100 μL mobile phase adjusted to pH 1.9 with 85% phosphoric acid, inject an 80 μL aliquot. Elute with mobile phase A. Whole blood. Freeze at -30°, centrifuge at 2000 g for 10 min at -10°, dilute 500 μL supernatant with 500 μL water, add 300 ng IS and 200 μL 1 M HCl, vortex, extract with 7 mL MTBE by shaking at low speed for 30 min, centrifuge at 2000 g at -10° for 15 min. Freeze the aqueous layer in a dry ice-acetone bath and discard it, evaporate the organic layer to dryness. Reconstitute the residue in 120 μL mobile phase adjusted to pH 3.1 with 85% phosphoric acid (if necessary centrifuge at 0° at 2000 g for 5 min), inject a 90 μL aliquot. Elute with mobile phase B. Bile. Mix 100 parts bile with 20 parts 1.0 M HCl, centrifuge at 5000 g for 15 min at 15°, dilute 120 μL of the supernatant (=100 μL bile) with 500 μL water, add IS and 100 μL 1 M HCl, vortex, extract with 7 mL MTBE by shaking for 30 min, centrifuge at 2000 g for 15 min at -10°. Freeze the aqueous layer in a dry ice-acetone bath and discard it, evaporate the organic layer to dryness. Reconstitute the residue in 120 μL mobile phase adjusted to pH 3.1 by 85% phosphoric acid, inject a 90 μL aliquot. Elute with mobile phase B. Liver, kidney, heart, lung, small intestine. Homogenize (Ultra Turrax T25) tissue in 2 volumes of pH 7.4 phosphate buffer at highest speed and filter through cotton gauze with a Potter S with 10-20 hubs at 450 g. (All steps were carried out on ice.) 1 mL Homogenate (for li ver 300 μL) + IS + 400 μL 500 mM HCL (for liver 600 μL 100 mM HCl), vortex, extract with 7 mL MTBE by shaking at low speed for 30 min, centrifuge at 2000 g for 15 min at 4°, freeze in dry ice/acetone. Remove the organic layer and evaporate it to dryness. Reconstitute the residue in

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:100 mM pH 6 sodium dihydrogen phosphate buffer 50:50
Flow rate: 1.5
Injection volume: 50-100
Detector: UV 372

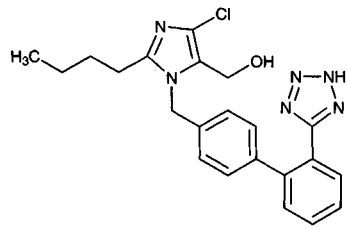
CHROMATOGRAM
Retention time: 5.8-6.1 (Extrelut), 6.7 (C18 SPE)
Internal standard: tenoxicam (2.6-2.8)
Limit of detection: 800 pg
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics; mouse; rat; rabbit; dog; monkey; human; SPE

REFERENCE
Radhofer-Welte,S.; Dittrich,P. Determination of the novel non-steroidal anti-inflammatory drug lornoxicam and its main metabolite in plasma and synovial fluid, *J.Chromatogr.B*, **1998**, *707*, 151–159.

Losartan



Molecular formula: $C_{22}H_{23}ClN_6O$
Molecular weight: 422.92
CAS Registry No.: 124750-99-8
Merck Index: 5613
Lednicer No.: 5 73

SAMPLE
Matrix: bile, blood, tissue, urine
Sample preparation: Plasma, urine. Condition a Varian Bond Elut non-endcapped CN SPE cartridge with two 1 mL portions of MeOH and two 1 mL portions of water. Vortex 500 μL plasma + 300 ng IS + 500 μL 200 mM HCl or 500 μL urine + 300 ng IS + 500 μL water + 200 μL 1.0 M HCl and add to the SPE cartridge. Wash with 500 μL water, with 500 μL MeOH: water 10:90, and with 50 μL MeOH, elute with five 200 μL portions of pH 8 MeOH:ammonia 99:1. Evaporate the combined eluates to dryness. Reconstitute the residue in 100 μL mobile phase adjusted to pH 1.9 with 85% phosphoric acid, inject an 80 μL aliquot. Elute with mobile phase A. Whole blood. Freeze at -30°, centrifuge at 2000 g for 10 min at -10°, dilute 500 μL supernatant with 500 μL water, add 300 ng IS and 200 μL 1 M HCl, vortex, extract with 7 mL MTBE by shaking at low speed for 30 min, centrifuge at 2000 g at -10° for 15 min. Freeze the aqueous layer in a dry ice-acetone bath and discard it, evaporate the organic layer to dryness. Reconstitute the residue in 120 μL mobile phase adjusted to pH 3.1 with 85% phosphoric acid (if necessary centrifuge at 0° at 2000 g for 5 min), inject a 90 μL aliquot. Elute with mobile phase B. Bile. Mix 100 parts bile with 20 parts 1.0 M HCl, centrifuge at 5000 g for 15 min at 15°, dilute 120 μL of the supernatant (=100 μL bile) with 500 μL water, add IS and 100 μL 1 M HCl, vortex, extract with 7 mL MTBE by shaking for 30 min, centrifuge at 2000 g for 15 min at -10°. Freeze the aqueous layer in a dry ice-acetone bath and discard it, evaporate the organic layer to dryness. Reconstitute the residue in 120 μL mobile phase adjusted to pH 3.1 by 85% phosphoric acid, inject a 90 μL aliquot. Elute with mobile phase B. Liver, kidney, heart, lung, small intestine. Homogenize (Ultra Turrax T25) tissue in 2 volumes of pH 7.4 phosphate buffer at highest speed and filter through cotton gauze with a Potter S with 10-20 hubs at 450 g. (All steps were carried out on ice.) 1 mL Homogenate (for li ver 300 μL) + IS + 400 μL 500 mM HCL (for liver 600 μL 100 mM HCl), vortex, extract with 7 mL MTBE by shaking at low speed for 30 min, centrifuge at 2000 g for 15 min at 4°, freeze in dry ice/acetone. Remove the organic layer and evaporate it to dryness. Reconstitute the residue in

120 μL mobile phase adjusted to pH 3.1 with 85% phosphoric acid (if necessary centrifuge at 15° at 4000 g for 30 min), inject an 80 μL aliquot. Elute with mobile phase C. Brain. Homogenize (Ultra Turrax T25) tissue in 2 volumes of pH 7.4 phosphate buffer at highest speed. 1 mL Homogenate + IS + 400 μL 1 M HCL + (?) mL MeCN:MeOH 1:2, let stand on ice for 1 h, centrifuge at 2000 g for 15 min at 0°. Freeze in dry ice/acetone, remove the organic layer, and evaporate it to dryness. Reconstitute the residue in 120 μL mobile phase adjusted to pH 3.1 with 85% phosphoric acid (if necessary centrifuge at 0° at 2000 g for 5 min), inject a 90 μL aliquot. Elute with mobile phase C.

HPLC VARIABLES
Guard column: 30 × 4.6 3 μm Ultremex 5 CN
Column: 250 × 4.6 3 μm Ultremex 3 CN
Mobile phase: MeCN:MeOH:THF:4.5 mM sodium dihydrogen phosphate buffer:85% phosphoric acid 21:5:4:69.9:0.1 (A), MeCN:THF:7.5 mM sodium dihydrogen phosphate buffer:85% phosphoric acid 26:5:68.9:0.1 (B), MeCN:THF:7.5 mM sodium dihydrogen phosphate buffer:85% phosphoric acid 29:3:67.9:0.1 (C)
Flow rate: 0.6
Injection volume: 80-90
Detector: UV 254

CHROMATOGRAM
Retention time: 17-18 (A), 14-15 (B,C)
Internal standard: L-158.854 (DuPont Merck Co.,USA)(20-21 (A), 18-19 (B,C))
Limit of quantitation: 5 ng/mL (plasma), 10 ng/mL (urine), 12.5 ng/mL (blood and bile), 10-15 ng/100 mg (tissue)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
SPE; plasma; urine; whole blood; rat; brain; liver; kidney; heart; lung; spleen; small intestine

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REFERENCE
Soldner,A.; Spahn-Langguth,H.; Mutschler,E. HPLC assays to simultaneously determine the angiotensin-AT1 antagonist losartan as well as its main and active metabolite EXP 3174 in biological material of humans and rats, *J.Pharm.Biomed.Anal.*, **1998**, *16*, 863–873.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 200 ng IS, adjust pH to 9.5 with 1 M KOH, make up to 2.5 mL with tetrabutylammonium hydrogen sulfate in water so that the final concentration of tetrabutylammonium hydrogen sulfate is 10 mM, add 10 mL dichloromethane, extract for 1 h, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 100 μL mobile phase, inject an 80 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax C8
Mobile phase: MeCN:50 mM pH 4.0 ammonium acetate buffer 35:65
Flow rate: 1
Injection volume: 80
Detector: UV 254

CHROMATOGRAM
Retention time: 14
Internal standard: 4-chloro-1-[((1H-tetrazoyl-5-yl)biphenyl-4-yl)methyl]-5-methal-2-n-propyl imidazole
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Noninterfering: metabolites

KEY WORDS
dog; plasma; pharmacokinetics

REFERENCE
Christ,D.D.; Wong,P.C.; Wong,Y.N.; Hart,S.D.; Quon,C.Y.; Lam,G.N. The pharmacokinetics and pharmacodynamics of the angiotensin II receptor antagonist losartan potassium (DuP 753/MK 954) in the dog, *J.Pharmacol.Exp.Ther.*, **1994**, *268*, 1199–1205.

SAMPLE
Matrix: blood
Sample preparation: 150 μL Plasma + 150 μL mobile phase A, mix, centrifuge at 3000 rpm for 2 min, inject a 250 μL aliquot of the supernatant on to column A and elute to waste with mobile phase A, after 5 min backflush the contents of column A on to column B with mobile phase B, monitor the effluent from column B. Re-equilibrate column A with mobile phase A and column B with mobile phase B for 5 min before next injection.

HPLC VARIABLES
Column: A 20 × 3.9 25-40 μm LiChroprep RP-8; B 10 × 4 Nova-Pak C8 + 250 × 4.6 5 μm Inertsil ODS-2
Mobile phase: A MeCN:50 mM phosphoric acid 5:95; B MeCN:50 mM pH 3.5 ammonium acetate
Flow rate: 1
Injection volume: 250
Detector: UV 254

CHROMATOGRAM
Retention time: 24.5
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
column-switching; plasma; rat; pharmacokinetics

REFERENCE
Lee,H.; Shim,H.O.; Lee,H.S. Simultaneous determination of losartan and active metabolite EXP3174 in rat plasma by HPLC with column switching, *Chromatographia*, **1996**, *42*, 39–42.

SAMPLE
Matrix: blood, dialysate, urine
Sample preparation: Blood. 150 μL Plasma + 150 μL MeCN, vortex for 15 s, centrifuge at 13000 g for 10 min, inject a 10 μL aliquot of the supernatant. Dialysate. Inject a 10 μL aliquot of the dialysate. Urine. 50 μL Urine + 450 μL water, vortex for 10 s, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 40-50 μm C18 (Alltech)
Column: 150 × 3.2 3 μm Hypersil Phenyl
Mobile phase: Gradient. MeCN:25 mM pH 2.2 potassium phosphate 35:65 for 5 min, to 60:40 over 4 min, maintain at 60:40 for 3 min, return to initial conditions over 1 min (plasma) or MeCN:25 mM pH 2.2 potassium phosphate 40:60 (dialysate, urine)
Flow rate: 0.75
Injection volume: 10
Detector: F ex 250 em 375

CHROMATOGRAM
Retention time: 7.3 (plasma), 5.5 (urine, dialysate)
Limit of detection: 1 ng/mL
Limit of quantitation: 10 ng/mL (plasma, dialysate), 50 ng/mL (urine)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Farthing,D.; Sica,D.; Fakhry,I.; Pedro,A.; Gehr,T.W.B. Simple high-performance liquid chromatographic method for determination of losartan and E-3174 metabolite in human plasma, urine and dialysate, *J.Chromatogr.B*, **1997**, *704*, 374–378.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 125 μL 1 M phosphoric acid + 100 μL IS solution + 10 mL MTBE, shake at 60 rpm for 20 min, centrifuge at 2060 g for 5 min, freeze in dry ice/acetone. Remove the organic layer and add it to 200 μL 50 mM NaOH, shake at 60 rpm for 15 min, centrifuge at 2060 g for 5 min, freeze in dry ice/acetone. Discard the organic layer and allow the aqueous layer to thaw, add 75 μL 200 mM phosphoric acid to the aqueous layer, add 6 mL hexane, vortex for 2 min, centrifuge, freeze in dry ice acetone. Discard the hexane layer and allow the aqueous layer to thaw, remove residual hexane using a stream of nitrogen, add 75 μL isopropanol, inject a 110 μL aliquot. Urine. 500 μL Urine + 500 μL water + 50 μL 1 M phosphoric acid + 100 μL IS solution + 10 mL MTBE:hexane 80:20, shake at 60 rpm for 20 min, centrifuge at 2060 g for 5 min, freeze in dry ice/acetone. Remove the organic layer and add it to 200 μL 50 mM NaOH, shake at 60 rpm for 15 min, centrifuge at 2060 g for 5 min, freeze in dry ice/acetone. Discard the organic layer and allow the aqueous layer to thaw, add 75 μL 200 mM phosphoric acid to the aqueous layer, add 6 mL hexane, vortex for 2 min, centrifuge, freeze in dry ice acetone. Discard the hexane layer and allow the aqueous layer to thaw, remove residual hexane using a stream of nitrogen, add 75 μL isopropanol, inject a 65 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultremex CN
Mobile phase: MeCN:buffer 23:77 (Buffer was 100 mL 200 mM NaH_2PO_4 and 2 mL 85% phosphoric acid in 2 L water, pH adjusted to 2.5 with 2 M NaOH.)
Flow rate: 1
Injection volume: 110
Detector: UV 254

CHROMATOGRAM
Retention time: 13
Internal standard: 2-n-butyl-4-(2-chlorophenyl)-5-carboxy-1-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole (L-158,854, Merck) (15)
Limit of quantitation: 20 ng/mL (urine), 5 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Furtek,C.I.; Lo,M.-W. Simultaneous determination of a novel angiotensin II receptor blocking agent, losartan, and its metabolite in human plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *573*, 295–301.

SAMPLE
Matrix: blood, urine
Sample preparation: Add IS to plasma or urine, buffer with 100 mM pH 2.8 citrate buffer, add to a Bond Elut C8 or NH2/C18 SPE cartridge, elute with MeOH. Evaporate the eluate, reconstitute with EtOH:water 50:50, inject a 25-50 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 New Guard RP18 (Biosystem Inc.)
Column: 250 × 4.6 Capcell Pak C18-ODS (Shiseido)

Mobile phase: Gradient. MeCN:25 mM pH 2.8 phosphate buffer from 35:65 to 50:50 over 10 min, to 65:35 over 10 min
Flow rate: 1
Injection volume: 25-50
Detector: UV 254

CHROMATOGRAM
Internal standard: 2-n-propyl-4-chloro-1-[2'-(tetrazol-5-yl)-1,1'-biphenyl-4-ylmethyl]-1H-imidazole-5-aldehyde (Dup 167)
Limit of quantitation: 20 ng/mL (urine), 10 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Ohtawa,M.; Takayama,F.; Saitoh,K.; Yoshinaga,T.; Nakashima,M. Pharmacokinetics and biochemical efficacy after single and multiple oral administration of losartan, an orally active nonpeptide angiotensin II receptor antagonist, in humans, *Br.J.Clin.Pharmacol.*, **1993**, *35*, 290–297.

SAMPLE
Matrix: formulations
Sample preparation: Stir ten 50 mg tablets with 1 L 10 mM pH 8 NaH_2PO_4 for 30 min, filter (0.45 μm PVDF), inject a 4 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Lichrosorb 10 RP-8 [C8]
Mobile phase: MeCN:10 mM pH 2.5 NaH_2PO_4 40:60
Column temperature: 35
Flow rate: 1
Injection volume: 4
Detector: UV 230

CHROMATOGRAM
Retention time: 11
Internal standard: butyl paraben (17.5)

KEY WORDS
tablets; comparison with capillary electrophoresis and packed column SFC

REFERENCE
Williams,R.C.; Alasandro,M.S.; Fasone,V.L.; Boucher,R.J.; Edwards,J.F. Comparison of liquid chromatography, capillary electrophoresis and super-critical fluid chromatography in the determination of Losartan Potassium drug substance in Cozaar tablets, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 1539–1546.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Condition a 3 mL BondElut C18 SPE cartridge with 5 mL MeOH and 5-10 mL 0.2% trifluoroacetic acid. 0.5-1 mL Microsomal incubation + 2 mL 0.2% trifluoroacetic acid, add to the SPE cartridge, wash with 2 mL 0.2% trifluoroacetic acid, elute with 4-5 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 35-45° or under reduced pressure, reconstitute the residue in 100-200 μL MeCN:MeOH:2 mM ammonium acetate:trifluoroacetic acid 20:40:40:0.1, inject a 25-50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.5 Zorbax C8
Mobile phase: Gradient. A was MeCN:MeOH:100 mM ammonium acetate:acetic acid 15:30:54:1. B was MeCN:MeOH:2 mM ammonium acetate:trifluoroacetic acid 20:40:40:0.1. A:B 100:0 for 20 min, to 0:100 over 10 min, maintain at 0:100 for 10 min.

Flow rate: 1
Injection volume: 25-50
Detector: UV 250, UV 280, or MS, SCIEX API III tandem MS, nebulizer interface 400°, positive-ion detection, orifice 65 V, m/z 423

CHROMATOGRAM
Retention time: 30

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver; SPE

REFERENCE
Stearns,R.A.; Chakravarty,P.K.; Chen,R.; Chiu,S.-H.L. Biotransformation of losartan to its active carboxylic acid metabolite in human liver microsomes: Role of cytochrome P4502C and 3A sub-family members, *Drug Metab.Dispos.*, **1995**, *23*, 207–215.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 500 μL Microsomal incubation + 50 μL 43% aqueous phosphoric acid + 1 mL dichloromethane, vortex, centrifuge. Remove a 700 μL aliquot of 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: 300 × 3.9 3 μm NovaPak C18
Mobile phase: MeCN:50 mM pH 7.4 ammonium acetate 26:74
Flow rate: 1
Injection volume: 20
Detector: UV 254

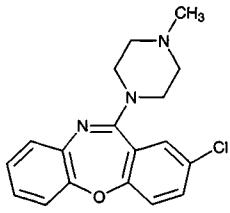
CHROMATOGRAM
Retention time: 19.2

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver

REFERENCE
Yun,C.-H.; Lee,H.S.; Lee,H.; Rho,J.K.; Jeong,H.G.; Guengerich,F.P. Oxidation of the angiotensin II receptor antagonist losartan (DuP 753) in human liver microsomes: role of cytochrome P4503A(4) in formation of the active metabolite EXP3174, *Drug Metab.Dispos.*, **1995**, *23*, 285–289.

Loxapine



Molecular formula: $C_{18}H_{18}ClN_3O$
Molecular weight: 327.81
CAS Registry No.: 1977-10-2, 27833-64-3 (succinate)
Merck Index: 5617
Lednicer No.: 2 427

SAMPLE

Matrix: blood
Sample preparation: 500 μL Plasma or 1 mL diluted red blood cells (red blood cells:water 50:50), add 6 mL ethyl acetate, vortex for 2 min, centrifuge at 2000 g for 5 min. Remove the organic layer, add 250 μL 100 mM HCl, vortex for 2 min, centrifuge at 2000 g for 5 min. Remove a 200 μL volume of the acid layer, evaporate to dryness at 40°. Reconstitute the residue in 200 μL mobile phase and inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Hypersil ODS
Column: 250 × 4.6 5 μm Kromasil Ultrabase C 18
Mobile phase: MeCN:buffer 48:52 (Buffer was 716 mg disodium hydrogen phosphate and 2 g cetrimide in 1 L water, adjusted to pH 7.0 with phosphoric acid.)
Column temperature: 50
Flow rate: 1.5
Injection volume: 100
Detector: UV 254

CHROMATOGRAM

Retention time: 20.0
Internal standard: loxapine

OTHER SUBSTANCES

Extracted: amitriptyline, clonazepam, clorazepate, clozapine, droperidol
Noninterfering: clomipramine, diazepam, hydroxyzine

KEY WORDS

plasma; red blood cells; loxapine is IS

REFERENCE

Guitton,C.; Kinowski,J.-M.; Aznar,R.; Bressolle,F. Determination of clozapine and its major metabolites in human plasma and red blood cells by high-performance liquid chromatography with ultraviolet absorbance detection, *J.Chromatogr.B*, **1997**, *690*, 211–222.

SAMPLE

Matrix: blood
Sample preparation: Add 200 μL 330 mM NaOH to 1 mL plasma, vortex, add 6 mL hexane:isoamyl alcohol 98.5:1.5, shake for 30 min, centrifuge at 4000 rpm for 5 min. Collect the organic layer, add 150 μL 100 mM HCl, vortex for 1 min. Collect the acidic aqueous phase and inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 C8 (Bischoff, Germany)
Column: 125 × 4.6 5 μm Ecotube Nucleosil C8 (Bischoff, Germany)
Mobile phase: MeCN:Pic B5:water:diethylamine 37:2.5:63:0.04 (Pic B5 is a mixture of water, MeOH, 1-pentanesulfonic acid, and acetic acid and is available from Waters.)
Column temperature: 56
Flow rate: 1.7
Injection volume: 50
Detector: UV 245

CHROMATOGRAM
Retention time: 12.7
Internal standard: loxapine

OTHER SUBSTANCES
Extracted: clozapine
Noninterfering: alprazolam, bromazepam, chlordiazepoxide, clorazepate, diazepam, flunitrazepam, haloperidol, lorazepam, nitrazepam, oxazepam, paroxetine, prazepam, temazepam, triazolam

KEY WORDS
plasma; loxapine is IS

REFERENCE
Edno,L.; Combourieu,I.; Cazenave,M.; Tignol,J. Assay for quantitation of clozapine and its metabolite N-desmethylclozapine in human plasma by high-performance liquid chromatography with ultraviolet detection, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 311–318.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 100 μL isopropanol:diethylamine 99.9:0.1 + 250 μL 25% potassium carbonate containing 0.1% diethylamine + 5 mL hexane:isoamyl alcohol 97:3, vortex for 30 s, centrifuge at 500 g for 3 min. Remove the organic layer and add it to 100 μL 250 mM HCl, vortex for 30 s, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: 50 × 4.6 40 μm C8 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil C8
Mobile phase: MeCN:water:diethylamine:85% phosphoric acid 53.3:45.1:1:0.4, pH adjusted to 7.2 with NaOH or phosphoric acid
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: k' 7.18
Internal standard: loxapine

OTHER SUBSTANCES
Extracted: amitriptyline, chlordiazepoxide, chlorpromazine, desipramine, desmethldiazepam, desmethylchlordiazepoxide, desmethyldoxepin, diazepam, doxepin, fluphenazine, haloperidol, imipramine, nortriptyline, oxazepam, thiothixene
Noninterfering: molindone, perphenazine, trifluoperazine

KEY WORDS
plasma; loxapine is IS

REFERENCE
Kiel,J.S.; Abramson,R.K.; Morgan,S.L.; Voris,J.C. A rapid high performance liquid chromatographic method for the simultaneous measurement of six tricyclic antidepressants, *J.Liq.Chromatogr.*, **1983**, *6*, 2761–2773.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 100 μL 1 μg/mL 2-hydroxydesmethylimipramine, mix, add 300 μL 2 M pH 9.7 carbonate buffer, mix, add 4 mL heptane:isopentyl alcohol 93:7, shake mechanically for 20 min, centrifuge at 1600 g for 5 min. Remove the organic layer and add it to 250 μL 7 mM orthophosphoric acid, vortex vigorously, centrifuge at 1600 g for 10 min, inject a 100 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb C6

Mobile phase: 105 μM nonylamine in MeCN:5 mM KH_2PO_4 + 14 mM orthophosphoric acid 23:77
Column temperature: 35
Flow rate: 2.2
Injection volume: 100
Detector: UV 210

CHROMATOGRAM
Retention time: 30
Internal standard: 2-hydroxydesmethylimipramine
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, amoxapine
Simultaneous: propranolol, doxepin, desmethylimipramine, haloperidol, protriptyline, imipramine, amitriptyline
Noninterfering: chlorpromazine, clomipramine, maprotiline, nortriptyline, thioridazine, trimipramine, trifluoperazine

KEY WORDS
plasma

REFERENCE
Cheung,S.W.; Tang,S.W.; Remington,G. Simultaneous quantitation of loxapine, amoxapine and their 7- and 8-hydroxy metabolites in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *564*, 213–221.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Serum + 75 μL MeCN containing 4 mg/mL amoxapine + 2 mL 250 mM NaOH + 400 μL isoamyl alcohol, vortex vigorously, let stand for 5 min, add 10 mL heptane, shake vigorously for 1 h, centrifuge at >2000 g for 30 min. Remove the upper heptane layer and add it to 1 mL 100 mM pH 3 glycylglycine buffer, shake vigorously for 1 h, centrifuge at >2000 g for 30 min. Discard the heptane layer, add 1 mL 250 mM NaOH to the aqueous layer, add 5 mL n-pentane, shake for 1 h, centrifuge at 2000 g for 30 min. Remove the organic layer and evaporate it to dryness under educed pressure, reconstitute the residue in 70 μL mobile phase, vortex vigorously, centrifuge at 2000 g for 2-3 min, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 40 μm pellicular silica (CEL Associates, Houston)
Column: 100 × 6 3 μm silica 80 Å (CEL Associates, Houston)
Mobile phase: MeCN:buffer 20:80 containing 21 mM n-nonylamine, pH 7.4-7.8 (Buffer was 25 mM Na_2HPO_4 adjusted to pH 3 with concentrated phosphoric acid.)
Flow rate: 1.6
Injection volume: 50
Detector: UV (wavelength not specified)

CHROMATOGRAM
Retention time: 22.68
Internal standard: amoxapine (9.63)

OTHER SUBSTANCES
Extracted: doxepin
Simultaneous: amitriptyline, chlorpromazine, clomipramine, desipramine, fluoxetine, imipramine, mianserin, nortriptyline, thioridazine, trimipramine
Noninterfering: diazepam

KEY WORDS
serum; loxapine is IS

REFERENCE
Adamczyk,M.; Fishpaugh,J.R.; Harrington,C. Quantitative determination of *E*- and *Z*-doxepin and *E*- and *Z*-desmethyldoxepin by high-performance liquid chromatography, *Ther.Drug Monit.*, **1995**, *17*, 371–376.

SAMPLE

Matrix: blood

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

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

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

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 299

CHROMATOGRAM

Retention time: 7.37

Limit of detection: <120 ng/mL

KEY WORDS

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

REFERENCE

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

SAMPLE

Matrix: blood, tissue

Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM

Retention time: 18.6
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES

Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine

Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

KEY WORDS

serum; whole blood; liver

REFERENCE

McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE

Matrix: blood, urine

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

HPLC VARIABLES

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

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 209.9

CHROMATOGRAM
Retention time: 14.553

KEY WORDS
whole blood

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

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

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

CHROMATOGRAM
Retention time: 1.8

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, 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, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, qui-

nine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm 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: 29.1

OTHER SUBSTANCES

Simultaneous: alprazolam, amitriptyline, desipramine, diltiazem, imipramine, nortriptyline
Noninterfering: clomipramine

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: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere cyano
Mobile phase: MeCN:10 mM pH 2.5 KH_2PO_4 60:40
Flow rate: 2.5
Injection volume: 20-40
Detector: E, Environmental Science Associates Coulochem Model 5100A, Model 5100 guard cell +0.85 V (between pump and injector), Model 5010 analytical cell +0.8 V, preanalytical cell +0.3 V

CHROMATOGRAM

Retention time: 4.6

OTHER SUBSTANCES

Simultaneous: amitriptyline, amoxapine, chlorpromazine, fluphenazine, haloperidol, imipramine, loxapine, mesoridazine, perphenazine, phenylephrine, prochlorperazine, thioridazine, thiothixene, trazodone, triflupromazine, trimeprazine, tripelennamine
Noninterfering: diazepam, diphenhydramine, ethopropazine, fluoxetine, nordiazepam, oxazepam, phenylpropanolamine, pseudoephedrine, trifluoperazine
Interfering: desipramine, desmethyldoxepin, doxepin, nortriptyline, pheniramine, promazine, promethazine

REFERENCE

Hariharan,M.; VanNoord,T.; Kindt,E.K.; Tandon,R. A simple, sensitive liquid chromatographic assay of cis-thiothixene in plasma with coulometric detection, *Ther.Drug Monit.*, **1991**, *13*, 79–85.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Adsorbosphere C18 (PEEK column) (retention times are longer and peaks broader with stainless steel column)
Mobile phase: MeCN:20 mM pH 3.2 KH_2PO_4 23.4:76.6 containing 0.05% nonylamine
Flow rate: 1.2
Detector: UV 214

CHROMATOGRAM
Retention time: 9

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

REFERENCE
Supelco Catalog, **1993,** p. 440.

SAMPLE
Matrix: solutions

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

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone,

oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 13.78 (A), 6.26 (B)

OTHER SUBSTANCES

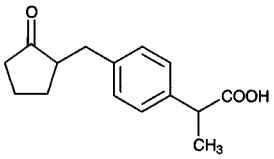
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, 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, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide,

tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
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.

Loxoprofen



Molecular formula: $C_{15}H_{18}O_3$
Molecular weight: 246.31
CAS Registry No.: 68767-14-6
Merck Index: 5619

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 1 M HCl, extract with 5 mL hexane:ethyl acetate 75:25, wash the organic layer with 1 mL 100 mM HCl. Remove a 4 mL aliquot of the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 100 μL 1 mg/mL (1S)-1-(4-dimethylaminonaphthalen-1-yl)ethylamine in dichloromethane:pyridine 10:1, add 20 μL 10 mg/mL 1-hydroxybenzotriazole in dichloromethane:pyridine 10:1, add 100 μL 2 mg/mL N,N'-dicyclohexylcarbodiimide, mix, let stand at room temperature for 1 h, evaporate to dryness, add 500 μL hexane:ethyl acetate 75:25, add 500 μL 100 mM HCl, vortex for 1 min. Remove the organic layer and dry it over anhydrous sodium sulfate, inject a 20 μL aliquot. (Synthesis of (1S)-1-(4-dimethylaminonaphthalen-1-yl)ethylamine is as follows. Mix 9.6 g (S)-(-)-1-(1-naphthyl)ethylamine ((1S)-1-(naphthalen-1-yl)ethylamine) with 37 mL acetic anhydride, let stand at room temperature for 30 min, cool to 12°, add 7.1 mL concentrated nitric acid dropwise, stir at room temperature for 1 h, adjust pH to 9 with 1 M NaOH, extract with ethyl acetate. Dry the organic layer over anhydrous sodium sulfate and evaporate to dryness under reduced pressure, chromatograph on a column of silica gel with ethyl acetate:benzene 10:1 (Caution! Benzene is a carcinogen!). Evaporate the eluate to dryness under reduced pressure to give (1S)-1-(4-nitronaphthalen-1-yl)-N-acetylethylamine as colorless needles. Stir 100 mg 10% PdC and 2.5 g (1S)-1-(4-nitronaphthalen-1-yl)-N-acetylethylamine in 15 mL EtOH, add 1.5 mL hydrazine hydrate in portions (Caution! Hydrazine hydrate is a carcinogen and explodes on distillation in air!), add 100 mg 10% PdC, reflux for 1 h, cool, filter. Evaporate the filtrate to dryness under reduced pressure and chromatograph the residue on a column of silica gel with ethyl acetate, evaporate the eluate to dryness under reduced pressure to give (1S)-1-(4-aminonaphthalen-1-yl)-N-acetylethylamine as a colorless powder. Stir 1.5 g (1S)-1-(4-aminonaphthalen-1-yl)-N-acetylethylamine and 1.7 g sodium bicarbonate in 5 mL water at 0°, add 2 mL dimethyl sulfate (Caution! Dimethyl sulfate is highly toxic!), stir at room temperature for 3 h, distil to remove excess reagent. Concentrate the aqueous layer under reduced pressure, purify the residue by TLC using ethyl acetate (R_f 0.30) to obtain (1S)-1-(4-dimethylaminonaphthalen-1-yl)-N-acetylethylamine as a colorless powder. Reflux 1.2 g (1S)-1-(4-dimethylaminonaphthalen-1-yl)-N-acetylethylamine in 15 mL concentrated HCl for 35 h, adjust the pH to 9 with 2 M NaOH (Caution! Exothermic!), cool, extract with ethyl acetate. Dry the organic layer over anhydrous sodium sulfate and evaporate it to dryness under reduced pressure, purify by TLC using ethyl acetate to obtain (1S)-1-(4-dimethylaminonaphthalen-1-yl)ethylamine as a slightly yellow oil ($[\alpha]_D$ +19.75° (c = 2.0, EtOH).)

HPLC VARIABLES
Column: 300 × 3.9 μPorasil
Mobile phase: n-Hexane:ethyl acetate 68:32
Flow rate: 1.7
Injection volume: 20
Detector: F ex 313 em 420

CHROMATOGRAM
Retention time: 6 (S), 7 (R)
Limit of detection: 1 ng

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
derivatization; normal phase; chiral; rat; plasma; pharmacokinetics

REFERENCE
Nagashima,H.; Tanaka,Y.; Watanabe,H.; Hayashi,R.; Kawada,K. Optical inversion of (2*R*)-to (2*S*)-isomers of 2-[4-(2-oxocyclopentylmethyl)phenyl]propionic acid (loxoprofen), a new anti-inflammatory agent, and its monohydroxy metabolites in the rat, *Chem.Pharm.Bull.*, **1984**, *32*, 251–257.

SAMPLE
Matrix: blood, urine
Sample preparation: Dilute urine nine-fold with water. 500 μL Plasma or diluted urine + 200 μL 2 M HCl + 500 μL 2 μg/mL 1-naphthoic acid, extract with 6 mL benzene (Caution! Benzene is a carcinogen!), centrifuge at 900 g for 10 min, repeat extraction. Combine the upper organic layers and evaporate them to dryness under reduced pressure, reconstitute the residue in 500 μL freshly prepared 250 μg/mL 4-bromomethyl-6,7-methylenedioxycoumarin in MeCN and 500 μL freshly prepared 1.5 mg/mL 18-crown-6 in MeCN (saturated with finely powdered potassium carbonate), sonicate briefly, heat at 40° for 30 min, add 100 μL MeCN:acetic acid 90:10, inject a 5 μL aliquot. (To hydrolyze glucuronides add 500 μL 500 mM NaOH to 500 μL diluted urine, let stand for 30 min, neutralize with 500 mM HCl. Prepare 4-bromomethyl-6,7-methylenedioxycoumarin as follows. Add 250 mmole finely powdered crystalline citric acid stepwise to 67.5 mL concentrated sulfuric acid at 70° (Caution! Carbon monoxide is evolved!), cool in ice, add 250 mmoles finely ground sesamol (3,4-methylenedioxyphenol) keeping the temperature below 5°, add 29 mL concentrated sulfuric acid, stir gently overnight, dilute with 50 mL chilled water, filter. Wash the brown solid with 10 mM sulfuric acid, with 2 M NaOH, and with 2 M HCl. Recrystallize from MeCN to give white prisms, mp 170°. Suspend 10 mL of this solid in 7.5 mL acetic acid, slowly add 7.5 mL acetic acid containing 10 mmoles bromine, reflux for 1 h, cool, chromatograph the solid on a silica gel column, elute with acetone, recrystallize 4-bromomethyl-6,7-methylenedioxycoumarin from MeOH to give yellow prisms, mp 241° (J.Chromatogr. 1989, 478, 149).)

HPLC VARIABLES
Column: 250 × 4.6 Zorbax ODS
Mobile phase: MeCN:water:acetic acid 55:45:1
Flow rate: 1.2 (plasma), 1.5 (urine)
Injection volume: 5
Detector: F ex 355 em 435

CHROMATOGRAM
Retention time: 15 (plasma), 12 (urine)
Internal standard: 1-naphthoic acid (20 (plasma), 14 (urine))
Limit of quantitation: 50 ng/mL (urine), 10 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; derivatization; pharmacokinetics

REFERENCE
Naganuma,H.; Kawahara,Y. High-performance liquid chromatographic determination of loxoprofen and its diastereomeric alcohol metabolites in biological fluids by fluorescence labelling with 4-bromomethyl-6, 7-methylenedioxycoumarin, *J.Chromatogr.*, **1990**, *530*, 387–396.

SAMPLE
Matrix: solutions

Sample preparation: Add 500 ng IS, evaporate to dryness, add 100 μL 200 μg/mL (+)-(R)-1-(1-naphthyl)ethylamine in dichloromethane:pyridine 98:2 + 20 μL 2 mg/mL 1-hydroxybenzotriazole in dichloromethane:pyridine 98:2 + 100 μL 400 μg/mL N,N'-dicyclohexylcarbodiimide in dichloromethane:pyridine 98:2, let stand at room temperature for 1 h, evaporate to dryness, reconstitute in 500 μL ethyl acetate and 500 μL HCl, vortex for 1 min. Remove the organic phase and dry it over anhydrous sodium sulfate, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 6 ERC-Silica-1282 (ERMA CR, Saitama, Japan)
Mobile phase: n-Hexane:ethyl acetate 59:41
Flow rate: 2
Injection volume: 50
Detector: F ex 283 em 330

CHROMATOGRAM

Retention time: 7 (S), 8.5 (R)
Internal standard: (+)-(S)-2-[2-(2-hydroxyprop-2-yl)indan-5-yl]propionic acid (16)

OTHER SUBSTANCES

Simultaneous: metabolites

KEY WORDS

chiral; derivatization; normal phase; plasma is purified on an immobilized antibody column (full preparative details given) before analysis

REFERENCE

Takasaki,W.; Tanaka,Y. Application of antibody-mediated extraction for the stereoselective determination of the active metabolite of loxoprofen in human and rat plasma, *Chirality*, **1992**, *4*, 308–315.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 10 μM solution of the compound in MeCN containing 2 mM L-DBD-APy, 3 mM 2,2'-dipyridyl disulfide, and 3 mM triphenylphosphine, let stand at room temperature for 2 h, inject a 5 μL aliquot. (Synthesis of L-DBD-APy, (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole, is as follows. Cool a solution of 16.4 g (S)-(-)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane: acetone 95:5 to obtain (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65°, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry over anhydrous magnesium sulfate, evaporate to obtain (3R)-3-azido-1-(phenylmethyl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3R)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the minimum amount of EtOH to obtain (3R)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). 3R-(+)-aminopyrrolidine is also reported to be available from Tokyo Kasei. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene form EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-

fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 60F_{254} TLC plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei. Add 100 mg 4-(N,N-dimethylaminosulfonyl)-7- fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to a stirred solution of 200 mg 3R-(+)-aminopyrrolidine in 20 mL MeCN at 0-10°, stir at room temperature for 30 min, remove the MeCN by evaporation under reduced pressure, dissolve the residue in 50 mL 5% HCl, wash 3 times with 50 mL portions of ethyl acetate, adjust the pH of the aqueous solution to 13-14 with 5% NaOH, extract 6 times with 50 mL portions of ethyl acetate. Combine the organic layers and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane to obtain (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as orange crystals (mp 96-98°).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:water:trifluoroacetic acid 40:60:0.1
Column temperature: 40
Flow rate: 1
Injection volume: 5
Detector: F ex 470 em 585

CHROMATOGRAM

Retention time: k' 12.75 (L), k' 15.42 (D)
Limit of detection: 10 fmole

KEY WORDS

derivatization; chiral

REFERENCE

Toyo'oka,T.; Ishibashi,M.; Terao,T. Fluorescent chiral derivatization reagents for carboxylic acid enantiomers in high-performance liquid chromatography, *Analyst*, **1992**, *117*, 727–733.

SAMPLE

Matrix: urine
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL ethyl acetate and dry by aspiration of air. Evaporate an aliquot of 100 μL 20 μg/mL IS in MeOH to dryness at 37°. Add 1 mL urine, vortex, add 250 μL 1 M pH 5.0 acetate buffer, vortex. Add 250 μL of the mixture to the SPE cartridge, dry by aspiration of air, elute with 3 mL ethyl acetate, evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject a 10-30 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:50 mM pH 5.0 phosphate buffer 42:58
Flow rate: 0.9
Injection volume: 10-30
Detector: UV 230

CHROMATOGRAM
Retention time: 5.5
Internal standard: indomethacin (18.5)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: diclofenac, ibuprofen, felbinac, fenbufen, flurbiprofen, ketoprofen, mefenamic acid, naproxen, piroxicam, sulindac

KEY WORDS
SPE

REFERENCE
Hirai,T.; Matsumoto,S.; Kishi,I. Simultaneous analysis of several non-steroidal anti-inflammatory drugs in human urine by high-performance liquid chromatography with normal solid-phase extraction, *J.Chromatogr.B*, **1997**, *692*, 375–388.

SAMPLE
Matrix: urine
Sample preparation: 1 mL Urine + 1 mL 1 M NaOH, mix, let stand at room temperature for 1 h, add 1.5 mL M HCl, add 10 mL mobile phase, shake for 5 min, centrifuge at 1000 g for 5 min. Remove a 1 mL aliquot of the organic layer and evaporate it to dryness under reduced pressure at 40°, reconstitute the residue in 100 µL 2 mg/mL (1S)-1-(4-dimethylaminonaphthalen-1-yl)ethylamine in dichloromethane:pyridine 10:1, add 20 µL 10 mg/mL 1-hydroxybenzotriazole in dichloromethane:pyridine 10:1, add 100 µL 2 mg/mL N,N'-dicyclohexylcarbodiimide, mix, let stand at room temperature for 1 h, evaporate to dryness, add 500 µL mobile phase, add 500 µL 100 mM HCl, vortex for 1 min. Remove the organic layer and dry it over anhydrous sodium sulfate, inject a 20 µL aliquot. (Synthesis of (1S)-1-(4- dimethylaminonaphthalen-1-yl)ethylamine is as follows. Mix 9.6 g (S)-(-)-1-(1-naphthyl)ethylamine ((1S)-1-(naphthalen-1-yl)ethylamine) with 37 mL acetic anhydride, let stand at room temperature for 30 min, cool to 12°, add 7.1 mL concentrated nitric acid dropwise, stir at room temperature for 1 h, adjust pH to 9 with 1 M NaOH, extract with ethyl acetate. Dry the organic layer over anhydrous sodium sulfate and evaporate to dryness under reduced pressure, chromatograph on a column of silica gel with ethyl acetate:benzene 10:1 (Caution! Benzene is a carcinogen!). Evaporate the eluate to dryness under reduced pressure to give (1S)-1-(4-nitro-naphthalen-1-yl)-N-acetylethylamine as colorless needles. Stir 100 mg 10% PdC and 2.5 g (1S)-1-(4-nitro-naphthalen-1-yl)-N-acetylethylamine in 15 mL EtOH, add 1.5 mL hydrazine hydrate in portions (Caution! Hydrazine hydrate is a carcinogen and explodes on distillation in air!), add 100 mg 10% PdC, reflux for 1 h, cool, filter. Evaporate the filtrate to dryness under reduced pressure and chromatograph the residue on a column of silica gel with ethyl acetate, evaporate the eluate to dryness under reduced pressure to give (1S)-1-(4-aminonaphthalen-1-yl)-N-acetylethylamine as a colorless powder. Stir 1.5 g (1S)-1-(4-aminonaphthalen-1-yl)-N-acetylethylamine and 1.7 g sodium bicarbonate in 5 mL water at 0°, add 2 mL dimethyl sulfate (Caution! Dimethyl sulfate is highly toxic!), stir at room temperature for 3 h, distil to remove excess reagent. Concentrate the aqueous layer under reduced pressure, purify the residue by TLC using ethyl acetate (R_f 0.30) to obtain (1S)-1-(4-dimethylaminonaphthalen-1-yl)-N-acetylethylamine as a colorless powder. Reflux 1.2 g (1S)-1-(4-dimethylaminonaphthalen-1-yl)-N-acetylethylamine in 15 mL concentrated HCl for 35 h, adjust the pH to 9 with 2 M NaOH (Caution! Exothermic!), cool, extract with ethyl acetate. Dry the organic layer over anhydrous sodium sulfate and evaporate it to dryness under reduced pressure, purify by TLC using ethyl acetate to obtain (1S)-1-(4-dimethylaminonaphthalen-1-yl)ethylamine as a slightly yellow oil ($[\alpha]_D$ +19.75° (c = 2.0, EtOH) (Chem. Pharm. Bull. 1984, 32, 251).)

HPLC VARIABLES
Column: 300 × 3.9 10 µm µPorasil
Mobile phase: n-Hexane:ethyl acetate 68:32
Flow rate: 1.7
Injection volume: 20
Detector: F ex 313 em 420

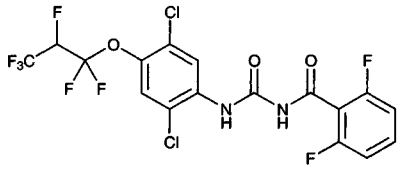
CHROMATOGRAM
Retention time: 6 (S), 7 (R)
Limit of quantitation: 5 ng

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
derivatization; normal phase; chiral

REFERENCE
Nagashima,H.; Tanaka,Y.; Hayashi,R. Column liquid chromatography for the simultaneous determination of the enantiomers of loxoprofen sodium and its metabolites in human urine, *J.Chromatogr.*, **1985**, *345*, 373–379.

Lufenuron



Molecular formula: $C_{17}H_8Cl2F_8N_2O_3$
Molecular weight: 511.15
CAS Registry No.: 103055-07-8
Merck Index: 6524

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

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 209.9

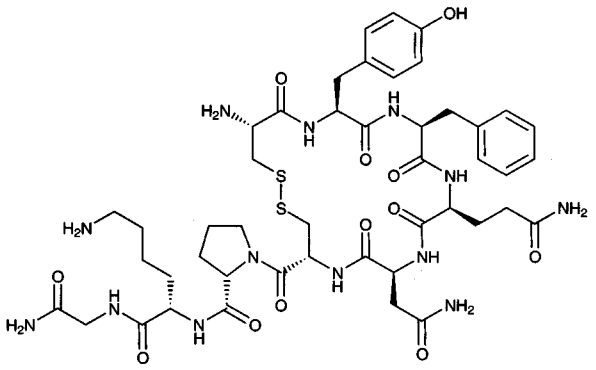
CHROMATOGRAM
Retention time: 27.658

KEY WORDS
whole blood

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

Lypressin

Molecular formula: $C_{46}H_{65}N_{13}O_{12}S_2$
Molecular weight: 1056.23
CAS Registry No.: 50-57-7
Merck Index: 5661



SAMPLE

Matrix: blood, tissue
Sample preparation: Condition a Sep-Pak ODS SPE cartridge with MeOH. Homogenize 500 mg tissue with 6 mL 100 mM pH 7.4 Tris buffer. Acidify a 2 mL aliquot of plasma or tissue homogenate with 200 μL 1 M HCl, add to the SPE cartridge, elute with 3 mL MeOH over 3 min, elute with 2 mL over 1 min. Evaporate the eluate to dryness under a stream of air at 37°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 280 × 5 Dynamax 300-A C8 (Rainin)
Mobile phase: MeCN:water 20:80 containing 0.1% trifluoroacetic acid, 50 mM heptanesulfonic acid and 30 mM triethylamine, pH adjusted to 2.5 with Na_2HPO_4
Flow rate: 1
Injection volume: 20
Detector: UV 200-400

CHROMATOGRAM

Retention time: 6.07
Limit of detection: 1 ng

OTHER SUBSTANCES

Extracted: arginine vasopressin, oxytocin

KEY WORDS

pig; plasma; SPE; heart

REFERENCE

Rao,P.S.; Weinstein,G.S.; Wilson,D.W.; Rujikarn,N.; Tyras,D.H. Isocratic high-performance liquid chromatography-photodiode-array detection method for determination of lysine- and arginine-vasopressins and oxytocin in biological samples, *J.Chromatogr.*, **1991**, *536*, 137–142.

SAMPLE

Matrix: formulations
Sample preparation: Inject a 25 μL aliquot.

HPLC VARIABLES

Column: 250 × 3 10 μm RP 8 (Merck)
Mobile phase: MeCN:pH 7 phosphate buffer 15:80
Flow rate: 1.58
Injection volume: 25
Detector: F ex 390 em 470 following post-column reaction. The column effluent mixed with 300 μg/mL fluorescamine in MeCN pumped at 0.16 mL/min and the mixture flowed through a 4.4 m × 0.25 mm ID coil to the detector.

CHROMATOGRAM

Retention time: 8

Limit of detection: 6.4 ng

KEY WORDS
post-column reaction; injections

REFERENCE
Frei,R.W.; Michel,L.; Santi,W. Post-column fluorescence derivatization of peptides. Problems and potential in high-performance liquid chromatography, *J.Chromatogr.*, **1976**, *16*, 665–677.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil 5 C18
Mobile phase: MeOH:50 mM pH 6.5 ammonium acetate 40:60
Flow rate: 1.2
Detector: UV 206

CHROMATOGRAM
Retention time: 18

KEY WORDS
tritium labelled

REFERENCE
Jánaky,T.; Laczi,F.; László,F.A. Biological half-lives and organ distribution of tritiated 8-lysine-vasopressin and 1-deamino-8-D-arginine-vasopressin in Brattleboro rats, *Ann.N.Y.Acad.Sci.*, **1982**, *394*, 116–127.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 5 Nova-Pak C18
Mobile phase: Gradient. MeCN:10 mM pH 5.0 acetate buffer 15:85 for 10 min, then to 18:82 over 5 min, maintain at 18:82 for 25 min.
Flow rate: 1
Detector: UV 214, UV 280

CHROMATOGRAM
Retention time: 8.7

OTHER SUBSTANCES
Simultaneous: oxytocin, arginine vasopressin

KEY WORDS
hippopotamus

REFERENCE
Rouille,Y.; Chauvet,M.T.; Chauvet,J.; Acher,R.; Hadley,M.E. The distribution of lysine vasopressin (lysipressin) in placental mammals: a reinvestigation of the Hippopotamidae (*Hippopotamus amphibius*) and Tayassuidae (*Tayassu angulatus*) families, *Gen.Comp.Endocrinol.*, **1988**, *71*, 475–483.

Lysozyme

Molecular weight: about 14400
CAS Registry No.: 9001-63-2
Merck Index: 5671

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 2 mg/mL solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 7 μm PolyCAT A WCX polyaspartate (Custom LC, Houston)
Mobile phase: 40 mM calcium acetate buffer containing 8 M urea, pH 5.0
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 19

REFERENCE
Parente,E.S.; Wetlaufer,D.B. Influence of urea on the high-performance cation-exchange chromatography of hen egg white lysozyme, *J.Chromatogr.*, **1984**, *288*, 389–398.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 50 × 4.6 C4 wide pore (300 Å) (Supelco)
Mobile phase: Gradient. A was MeOH:isopropanol:water:trifluoroacetic acid 8:2:90:0.05 containing 2.8 g/L NaCl. B was MeOH:isopropanol:water:trifluoroacetic acid 70:10:20:0.05 containing 4.2 g/L NaCl. A:B from 95:5 to 0:100
Flow rate: 1.5
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: 18

OTHER SUBSTANCES
Simultaneous: b-lactoglobulin A, ribonuclease A

KEY WORDS
post-column reaction; post-column photochemical derivatization

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
Sample preparation: Inject an aliquot of a solution in 50 mM pH 7.5 phosphate buffer.

HPLC VARIABLES
Column: 300 × 7.5 TSKgel-G3000SW (Tosoh)
Mobile phase: 100 mM pH 7.5 Phosphate buffer containing 100 mM sodium sulfate
Flow rate: 0.8
Injection volume: 20
Detector: F ex 370 em 440 following post-column reaction. The column effluent mixed with the oxidant pumped at 0.2 mL/min and this mixture flowed through a 3 m × 0.5 mm ID PTFE coil at 70°. The effluent from this coil mixed with the reagent pumped at 0.2 mL/min and this mixture flowed through a 5 m × 0.5 mm ID PTFE coil at 70° and a 1 m × 0.5 mm ID PTFE cooling coil to the detector. (Prepare oxidant by diluting 10% sodium hypochlorite solution with 50 mM NaH_2PO_4 containing 0.1% Brij-35 and 50 mM Na_2HPO_4 containing 0.1% Brij-35 to a final chlorine concentration of 0.8% and final pH of 7.5 . Prepare reagent by dissolving 8 g sodium nitrite and 40 mg thiamine hydrochloride in 100 mL 50 mM pH 7.5 phosphate buffer, adjust to pH 7.5 with 50 mM Na_2HPO_4 or 50 mM NaH_2PO_4, make up to 200 mL with 50 mM pH 7.5 phosphate buffer. The chlorinated proteins oxidize thiamine to fluorescent thiochrome.)

CHROMATOGRAM
Retention time: 16
Limit of detection: 10 ng

OTHER SUBSTANCES
Simultaneous: bovine serum albumin, cytochrome c, myoglobin, ovalbumin, thyroglobulin

KEY WORDS
post-column reaction

REFERENCE
Yokoyama,T.; Kinoshita,T. High-performance liquid chromatographic determination of proteins by post-column fluorescence derivatization with thiamine reagent, *J.Chromatogr.*, **1990**, *518*, 141–148.

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: 20

OTHER SUBSTANCES
Simultaneous: cytochrome C, insulin, myoglobin, ribonuclease A, trypsin

REFERENCE
Rainin Catalog 1991-2, 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: 10

OTHER SUBSTANCES
Simultaneous: bovine serum albumin, cytochrome C, insulin, myoglobin, ovalbumin, ribonuclease

REFERENCE
Rainin Catalog 1991-2, p. 3.63.

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: 12

OTHER SUBSTANCES
Simultaneous: angiotensin I, angiotensin II, bradykinin, insulin, leucin enkephalin, melittin, methionine enkephalin, oxytocin

REFERENCE
Supelco Catalog, **1992**, p. 104.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 SynChropak C4 (SynChrom)
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.1% trifluoroacetic acid in MeOH. A:B from 95:5 to 0:100 over 30 min.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 22

OTHER SUBSTANCES
Simultaneous: conalbumin, cytochrome C, β-lactoglobulin, ovalbumin

REFERENCE
Scientific Products Chromatography Catalog, Baxter Diagnostics, **1992**, p. 187.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm 300 Å Rexchrom ODS (Regis)
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.1% trifluoroacetic acid in MeCN. A:B from 75:25 to 0:100 over 30 min.
Flow rate: 1.5
Injection volume: 50
Detector: UV 220

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: insulin, myoglobin, ovalbumin, ribonuclease

REFERENCE
Regis Technologies, Inc. Catalog, **1993,** p. 29.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Vydac 228TP
Mobile phase: Gradient. A was 0.25% trifluoroacetic acid in water. B was 0.25% trifluoroacetic acid in MeCN:water 70:30. A:B from 95:5 to 0:100 over 30 min.
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM
Retention time: 17

OTHER SUBSTANCES
Simultaneous: angiotensin I, angiotensin II, bradykinin, eledosin, insulin, myoglobin, neurotensin, ovalbumin, oxytocin

REFERENCE
Supelco Catalog, **1993,** p. 581.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Spherisorb 300Å C18
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B MeCN. A:B from 80:20 to 20:80 over 30 min.
Flow rate: 1.5
Detector: UV 280

CHROMATOGRAM
Retention time: 16.5

OTHER SUBSTANCES
Simultaneous: insulin, myoglobin, ovalbumin, ribonuclease

REFERENCE
Supelco Catalog, **1993,** p. 566.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 75 × 4.6 5 μm Hypersil WP 300-octyl
Mobile phase: Gradient. A was 0.1% trifluoroacetic in water. B was 0.1% trifluoroacetic acid in n-propanol. A:B 100:0 for 2.5 min, to 90:10 over 2.5 min, to 10:90 over 112 min
Flow rate: 1
Detector: UV 225

CHROMATOGRAM
Retention time: 26.5

OTHER SUBSTANCES
Simultaneous: angiotensin II, bombesin, bovine growth hormone, bovine serum albumin, catalase, L-tryptophan

REFERENCE
Supelco Catalog, **1993**, p. 602.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.5 Kromasil-5-C8 (Eka Nobel)
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in MeCN:water 10:90. B was 0.1% trifluoroacetic acid in MeCN:water 90:10. A:B from 100:0 to 75:25 over 8 min, to 25:75 over 12 min
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 12

OTHER SUBSTANCES
Simultaneous: angiotensin I, angiotensin II, bradykinin, insulin, leukin enkephalin, melittin, methionine enkephalin, oxytocin

REFERENCE
Bodman Chromatography Catalog, **1994**, p. 62.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 5 400VHP552 (Vydac)
Mobile phase: Gradient. A was pH 7.21 Tris-HCl. B was pH 7.21 Tris HCl containing 500 mM NaCl. A:B from 100:0 to 0:100 over 5 min
Flow rate: 4

CHROMATOGRAM
Retention time: 2.4

OTHER SUBSTANCES
Simultaneous: α-chymotrypsinogen A, conalbumin, cytochrome, myoglobin

REFERENCE
Bodman Chromatography Catalog, **1994**, p. 193.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 5 mg/mL solution.

HPLC VARIABLES
Column: 250 × 4.6 Dynamax-300A C8 (Rainin)
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.1% trifluoroacetic acid in MeCN. A:B from 85:15 to 45:55 over 30 min
Flow rate: 0.75
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 25

OTHER SUBSTANCES
Simultaneous: insulin, ribonuclease A, bovine serum albumin, ovalbumin

REFERENCE
Rainin Catalog, C1-94, **1994**, p. 7.51.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 50 × 4.6 β-cyclodextrin sulfate immobilized on TSKgel AF-Epoxy TOYOPEARL 650M (Tosoh) (preparation details in paper)
Mobile phase: Gradient. A was 20 mM Tris-HCl buffer containing 60 mM NaCl. B was 20 mM Tris-HCl buffer containing 3 M NaCl. A:B 100:0 for 10 min, to 0:100 over 5 min, maintain at 0:100 for 5 min.
Flow rate: 1
Detector: UV 280

CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Simultaneous: bovine serum albumin, basic fibroblast growth factor

REFERENCE
Ishimura,K.; Fukunaga,K.; Ohta,T.; Nakamura,H.; Irie,T.; Uekama,K. Preparation of β-cyclodextrin sulfate-immobilized hydrophilic vinyl-polymer gel as a selective, high recovery and stable adsorbent for high-performance affinity chromatography of heparin-binding substances, *Chromatographia*, **1995**, *41*, 349–352.

SAMPLE
Matrix: solutions

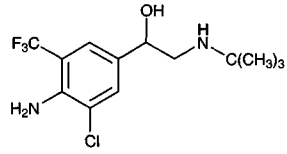
HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax 300 Å SB-C3
Mobile phase: Gradient. A was MeCN:water:trifluoroacetic acid 5:95:0.1. B was MeCN:water:trifluoroacetic acid 5:95:0.085. A:B from 85:15 to 47:53 over 20 min.
Column temperature: 35
Flow rate: 1
Injection volume: 10
Detector: UV 215

CHROMATOGRAM
Retention time: 13

OTHER SUBSTANCES
Simultaneous: angiotensin II, carbonic anhydrase, cytochrome C, insulin, leucine enkephalin, myoglobin, RNAase

REFERENCE
Ricker,R.D.; Sandoval,L.A.; Permar,B.J.; Boyes,B.E. Improved reversed-phase high performance liquid chromatography columns for biopharmaceutical analysis, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 93–105.

Mabuterol



Molecular formula: $C_{13}H_{18}ClF_3N_2O$
Molecular weight: 310.75
CAS Registry No.: 56341-08-3
Merck Index: 5674

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 250 mM NaOH + 100 µL 50 ng/mL IS in water + 5 mL diethyl ether:2-butanol 90:10, shake for 10 min, centrifuge at 1500 g for 10 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 µL mobile phase, vortex for 2 min, store at 4° overnight, warm tyo room temperature, inject a 50 µL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 7 µm LiChrosorb (select B) C18
Column: 125 × 4 7 µm LiChrosorb (select B) C18
Mobile phase: MeCN:buffer 23:77 containing 0.2 mM sodium 1-heptanesulfonate (Buffer was pH 4.0-4.1 phosphate buffer, ionic strength 0.1.)
Injection volume: 50
Detector: E, ESA Coulochem Model 5100A, Model 5011 detector, +0.75 V

CHROMATOGRAM

Retention time: 13.5
Internal standard: 4-amino-3,5-dichloro-α-[[(1,1-dimethylpropyl)amino]methyl]benzenemethanol (NAB 760) (10)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Extracted: clenbuterol

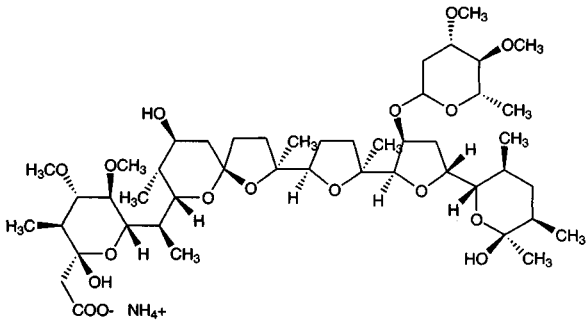
KEY WORDS

horse; plasma

REFERENCE

Qureshi,G.A.; Eriksson,A. Determination of clenbuterol and mabuterol in equine plasma by ion-pair liquid chromatography with electrochemical detection. Chromatographic and electrochemical characteristics, *J.Chromatogr.*, **1988**, *441*, 197–205.

Maduramicin



Molecular formula: $C_{47}H_{83}NO_{17}$
Molecular weight: 934.17
CAS Registry No.: 84878-61-5
Merck Index: 5682

SAMPLE

Matrix: bulk, feed, premix
Sample preparation: Bulk, premix. Dissolve in MeCN to give a maduramicin concentration of 3 μg/mL (sonicate premix). Remove a 1 mL aliquot and mix it with 30 ± 3 mg calcium carbonate, 100 μL 7.5 mg/mL dansyl hydrazine in MeCN, and 100 μL 150 mg/mL trichloroacetic acid in MeCN, shake vigorously by hand for 1 min, centrifuge for 1 min, immediately inject an aliquot. Feed. Add 50 g feed to 250 mL MeCN, shake for 30 min, clarify. Remove a 2 mL aliquot and add it to 600 μL 7.5 mg/mL dansyl hydrazine in MeCN and 600 μL 150 mg/mL trichloroacetic acid in MeCN, shake by hand for 10 s, add to a Florisil Sep-Pak at 1 drop/s, wash with three 5 mL aliquots of MeCN at 1 drop/s, purge with air, elute with MeCN:water 90:10, collect 5 mL eluate, mix eluate, inject an aliquot.

HPLC VARIABLES

Column: 50 × 4.6 5 μm C18 (IBM)
Mobile phase: MeCN:water 75:25 containing 250 mg/L tetrabutylammonium hydrogen sulfate, pH 3.5
Flow rate: 2
Injection volume: 60
Detector: F ex 210 em 320 (cutoff filter)

CHROMATOGRAM

Retention time: 5.8, 7.7 (two possible derivatives are formed)
Limit of quantitation: 3 ppm

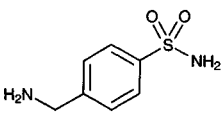
KEY WORDS

derivatization; SPE

REFERENCE

Markantonatos,A. Derivatization and HPLC/fluorescence quantitation of maduramicin ammonium in feed and premixes at levels down to 5 ppm, *J.Liq.Chromatogr.*, **1988**, *11*, 877–890.

Mafenide



Molecular formula: $C_7H_{10}N_2O_2S$
Molecular weight: 186.23
CAS Registry No.: 138-39-6, 130099-9-9 (acetate), 138-37-4 (HCl)
Merck Index: 5683
Lednicer No.: 2 114

SAMPLE

Matrix: formulations
Sample preparation: Shake 25-30 mg cream with 25 mL mobile phase at 40° for 30 min, make up to 50 mL with mobile phase, filter (0.45 μm Nylon), inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb C18
Mobile phase: MeOH:buffer 65:35 containing 0.44 g/L sodium 1-heptanesulfonate, pH 4.2 (Buffer was 992 mL 9.073 g/L KH_2PO_4 and 8 mL 9.48 g/L Na_2HPO_4, pH 5.0.)
Flow rate: 1
Injection volume: 20
Detector: UV 270

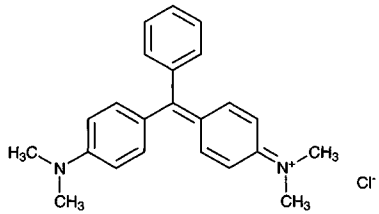
CHROMATOGRAM
Retention time: 2.6
Internal standard: lidocaine (5.3)
Limit of detection: 490 ng/mL
Limit of quantitation: 1.15 μg/mL

KEY WORDS
cream

REFERENCE
Dash,A.K.; Harrison,J.S. Ion-pair chromatographic method for the analysis of mafenide acetate, *J.Chromatogr.A*, **1995**, *708*, 83–88.

Malachite green

Molecular formula: $C_{23}H_{25}ClN_2$
Molecular weight: 364.92
CAS Registry No.: 569-64-2 (chloride)
Merck Index: 5739



SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 200 μL Plasma + 1 mL MeCN, vortex for 20 s, centrifuge for 5 min. Add the supernatant to 2 mL MeOH, evaporate to dryness, reconstitute with 500 μL diluting solvent with sonication, centrifuge for 5 min, filter (0.2 μm), inject a 50 μL aliquot of the filtrate. Muscle. Condition a 6 mL Bakerbond neutral alumina SPE cartridge with 1 column volume MeCN and a 3 mL Bond Elut LRC propylsulfonic acid (PRS) SPE cartridge with 1 column volume MeCN, place the alumina cartridge on top of the PRS cartridge. 5 g Muscle + 1.5 mL 250 mg/mL hydroxylamine hydrochloride in water + 2.5 mL 1 M p-toluenesulfonic acid in water + 5 mL buffer, mix, homogenize (Tekmar SDT Tissuemizer) at medium speed for 30 s, remove contents of container, add 45 mL MeCN to container and shake vigorously for 30 s, add 10 g alumina (activated 80-200 mesh, Alcoa type F-20) to the container and shake vigorously for 30 s. Combine all these mixtures and centrifuge at 4° at 2700 g for 10 min, remove the supernatant, add 45 mL MeCN to the pellet, shake vigorously for 30 s. Combine the supernatants and add them to 100 mL water and 2 mL diethylene glycol, add 50 mL dichloromethane, shake vigorously for 30 s, allow to separate for 15 min, repeat extraction. Combine the organic layers and evaporate to about 10 mL under reduced pressure at 40°, add 15 mL dichloromethane and evaporate to about 10 mL, add 10 mL MeCN and evaporate to about 1 mL, add 2 mL dichloromethane, mix, add 5 mL MeCN, mix, add to the SPE cartridges, rinse container 3 times with MeCN and add the rinses to the SPE cartridges, discard the alumina cartridge. Elute the PRS cartridge with two 1 mL portions of mobile phase then 1 mL 2.5 mg/mL hydroxylamine hydrochloride in MeOH, make up the eluate to 3 mL with mobile phase (if necessary), mix, inject a 50 μL aliquot. (Diluting solvent was MeCN:buffer 60:40 containing 700 μL/L 250 mg/mL hydroxylamine hydrochloride in water. Prepare buffer by dissolving 8.2 g sodium acetate trihydrate and 0.95 g p-toluenesulfonic acid monohydrate in 900 mL water, adjust pH to 4.5 with glacial acetic acid, make up to 1 L with water.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultremex 5 CN

Mobile phase: MeCN:buffer 50:50 (Prepare buffer by dissolving 8.2 g sodium acetate trihydrate and 0.95 g p-toluenesulfonic acid monohydrate in 900 mL water, adjust pH to 4.5 with glacial acetic acid, make up to 1 L with water.)
Flow rate: 1
Injection volume: 50
Detector: UV 618 following post-column reaction. The column effluent passed through a column (Alltech direct-connect rfefillable guard column) packed with lead(IV) oxide:Celite 545-AW (25:75) to the detector.

CHROMATOGRAM
Retention time: 16.2 (malachite green), 6.3 (leucomalachite green)
Limit of detection: 2 ppb (muscle), 10 ppb (plasma)

OTHER SUBSTANCES
Extracted: metabolites, leucomalachite green

KEY WORDS
plasma; muscle; fish; catfish; SPE; post-column reaction

REFERENCE
Plakas,S.M.; El Said,K.R.; Stehly,G.R.; Roybal,J.E. Optimization of a liquid chromatographic method for determination of malachite green and its metabolites in fish tissues, *J.AOAC Int.*, **1995**, *78*, 1388–1393.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 6 mL neutral alumina SPE cartridge (J.T. Baker) and a 2.8 mL 500 mg Bond Elut PRS SPE cartridge with 5 mL MeCN. Place the alumina SPE cartridge on the top of the PRS SPE cartridge using a Bond Elut adapter. Mix 3 mL 250 mg/mL hydroxylamine hydrochloride in water with 5 mL 50 mM p-toluene sulfonic acid, 20 mL 100 mM ammonium acetate adjusted to pH 4.5 with glacial acetic acid, and 20 g fish tissue. Homogenize at 20000 rpm for 1 min (Ultra-Turrax T25 Tissuemizer, Tekmar, USA). Add 90 mL MeCN to the sample and homogenize for 10 s. Shake vigorously by hand for 1 min. Add 20 g basic alumina (Brockman activity I), shake for 1 min. Centrifuge, decant the supernatant. Add 30 mL MeCN to the residue, extract, combine the supernatants. Add 100 mL water, 50 mL dichloromethane, and 20 mL diethylene glycol to the supernatants, shake vigorously, separate the bottom layer. Again add 50 mL dichloromethane, shake for 1 min, combine the separated dichloromethane layers. Concentrate to 2-3 mL under reduced pressure at 65°. Add 2 mL dichloromethane and 5 mL MeCN then add the mixtures to the SPE cartridges. Rinse the flask twice with 5 mL portions of MeCN and add the rinses to the SPE cartridges. Wash with 5 mL MeCN to waste. Remove the alumina cartridge. Wash the PRS cartridge with 2 mL water and with 1 mL MeCN:100 mM ammonium acetate buffer 50:50 adjusted to pH 4.5 with glacial acetic acid. Elute with 2 mL MeCN:100 mM ammonium acetate buffer 50:50 adjusted to pH 4.5 with glacial acetic acid and collect in a tube containing 500 μL 2.5 mg/mL hydroxylamine hydrochloride in water. Inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2.0 pellicular C18
Column: 150 × 4.6 5 μm SynChropak SCD-100 (SynChrom, USA)
Mobile phase: MeCN:buffer 55:45 (Buffer was 400 mg ammonium acetate and 1 mL triethylamine in 400 mL water, adjusted to pH 3.0 with glacial acetic acid, and made up to 450 mL with water)
Flow rate: 2.0
Injection volume: 100
Detector: E, ESA Coulochem Model, oxidative electrochemical cell (EC) +900 mV; UV 588; F ex 265 em 360

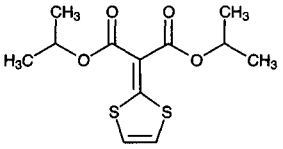
CHROMATOGRAM
Retention time: 5.2
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, gentian violet

KEY WORDS
SPE; catfish; trout,

REFERENCE
Rushing,L.G.; Hansen,E.B.,Jr. Confirmation of malachite green, gentian violet and their leuco analogs in catfish and trout tissue by high-performance liquid chromatography utilizing electrochemistry with ultraviolet-visible diode array detection and fluorescence detection, *J.Chromatogr.B*, **1997**, *700*, 223–231.

Malotilate



Molecular formula: $C_{12}H_{16}O_4S_2$
Molecular weight: 288.39
CAS Registry No.: 59937-28-9
Merck Index: 5751
Lednicer No.: 5 75

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 1 M pH 5.0 acetate buffer + 1 mL water + 5 mL 200 ng/mL IS in chloroform; shake horizontally for 10 min, centrifuge. Remove 4 mL of the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 Nucleosil 5C18
Mobile phase: MeCN:water:acetic acid 60:39:1
Flow rate: 1
Injection volume: 10
Detector: UV 360

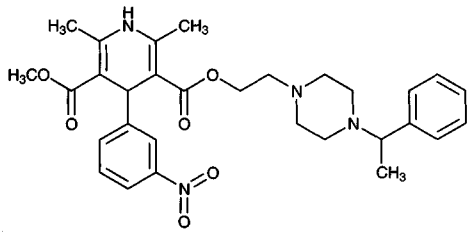
CHROMATOGRAM
Retention time: 8
Internal standard: di-sec-butyl 1,3-dithiol-2-ylidenemalonate (15)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; dog

REFERENCE
Takasugi,N.; Mafune,E.; Yokokawa,S.; Toriyama,K.; Tsuchiya,K.; Sugimoto,T. Determination of malotilate and its metabolites in plasma and urine, *J.Chromatogr.*, **1985**, *342*, 349–358.

Manidipine



Molecular formula: $C_{35}H_{38}N_4O_6$
Molecular weight: 610.71
CAS Registry No.: 120092-68-4
Merck Index: 5786

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 1 mL 20 mM pH 10 Na_2HPO_4, extract twice with 5 mL n-hexane:diethyl ether 2:1. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 200 µL mobile phase A, inject a 150 µL aliquot into column A and elute to waste with mobile phase A. Divert the eluate from column A containing manidipine onto column B. When all the manidipine has passed onto column B elute column B with mobile phase B and monitor the effluent from column B. (Elute column A with mobile phase A to waste.)

HPLC VARIABLES

Column: A 100 × 2.1 3 µm Develosil ODS-3K; B 100 × 2.1 5 µm Develosil ODS-5K
Mobile phase: A MeCN:20 mM KH_2PO_4 46:54 containing 5 mM sodium nonanesulfonate, adjusted to pH 3.0 with 85% orthophosphoric acid; B MeCN:20 mM KH_2PO_4 46:54 adjusted to pH 3.0 with 85% orthophosphoric acid
Column temperature: 40
Flow rate: 0.3
Injection volume: 150
Detector: UV 230

CHROMATOGRAM

Retention time: 20
Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

serum; protect from light; column-switching; heart-cut; pharmacokinetics

REFERENCE

Miyabayashi,T.; Yamashita,K.; Aoki,I.; Motohashi,M.; Yashiki,T.; Yatani,K. Determination of manidipine and its pyridine metabolite in human serum by high-performance liquid chromatography with ultraviolet detection and column switching, *J.Chromatogr.*, **1989**, *494*, 209–217.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 1 mL 20 mM pH 10 Na_2HPO_4, extract twice with 5 mL n-hexane:diethyl ether 2:1. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 200 µL EtOH, inject a 150 µL aliquot. Collect the eluate for each enantiomer, evaporate to dryness under a stream of nitrogen, reconstitute in 200 µL mobile phase A, inject a 150 µL aliquot, and analyze further as in J.Chromatogr. 1989, 494, 209. This resolves the enantiomers from interfering endogenous serum peaks.

HPLC VARIABLES

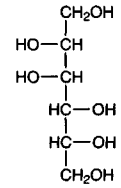
Column: 250 × 4.6 10 µm Chiralcel OJ
Mobile phase: n-Hexane:EtOH:MeOH 80:15:5
Column temperature: 50
Flow rate: 1
Injection volume: 150
Detector: UV 230

CHROMATOGRAM
Retention time: 10 (R-(-)), 14 (S-(+))

KEY WORDS
serum; protect from light; chiral

REFERENCE
Yamaguchi,M.; Yamashita,K.; Aoki,I.; Tabata,T.; Hirai,S.-I.; Yashiki,T. Determination of manidipine enantiomers in human serum using chiral chromatography and column-switching liquid chromatography, *J.Chromatogr.*, **1992**, *575*, 123–129.

Mannitol



Molecular formula: $C_6H_{14}O_6$
Molecular weight: 182.17
CAS Registry No.: 69-65-8
Merck Index: 5788

SAMPLE
Matrix: beverages, juice, milk
Sample preparation: Orange juice. Dilute orange juice 100-fold with water, filter (Millipore HV, 0.45 μm), dilute filtrate 10-fold, inject an aliquot. Beverages. Dilute soft drinks 1000-fold with water, inject an aliquot. Milk. Dilute 5 mL milk to 100 mL with mobile phase, filter (Millipore HV, 0.45 μm), dilute filtrate 50-fold, inject an aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 Cation H (Bio-Rad)
Column: 300 × 3.8 9 μm HPX 87-H Aminex (Bio-Rad)
Mobile phase: 10 mM Sulfuric acid
Column temperature: 50
Flow rate: 0.5
Injection volume: 40
Detector: E following post-column reaction, Hewlett-Packard 1049A programmable electrochemical detector, Metrohm detector cell, cuprous oxide working electrode +550 mV, glassy carbon auxiliary electrode, Ag/AgCl (3 M KCl) reference electrode. The column effluent mixed with 200 mM NaOH pumped at 0.4 mL/min, the mixture flowed through a 220 × 0.8 single-bead string reactor packed with 0.6 mm glass beads to the detector. (Prepare cuprous oxide electrode as follows. Stir 300 mg conductive carbon cement (Gerhard Neubauer, Münster), 60 mg cuprous oxide (Fluka), and 300 μL acetone until a thick paste forms as the acetone evaporates. Pack conductive carbon cement into the base of a 3 mm diameter cavity carbon paste electrode base (Metrohm), allow to dry, polish with dry emery paper (grade 2/0, Oakey), remove surface layer with an acetone-soaked tissue, pack the paste into the cavity, allow to dry overnight, polish with dry emery paper (grade 2/0), 3 μm imperial micro finishing film sheet (3M), 0.3 μm imperial micro finishing film sheet (3M), and 0.05 μm alumina particles on a Buehler pad, sonicate for 2 min in water (Anal. Chim. Acta 1995, 300, 5).)

CHROMATOGRAM
Retention time: 11.65
Limit of detection: 0.8 μM

OTHER SUBSTANCES
Also analyzed: arabinose, cellobiose, dextrose, fructose, fucose, galactitol, galactose, galacturonic acid, lactose, lactulose, lyxose, maltose, mannose, myo-inositol, raffinose, rhamnose, ribose, sorbose, sucrose, xylose

KEY WORDS
orange juice; soft drinks; post-column reaction; fruit

REFERENCE
Huang,X.; Pot,J.J.; Kok,W.T. Determination of sugars by liquid chromatography and amperometric detection with a cuprous oxide modified electrode, *Chromatographia*, **1995**, *40*, 684–689.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 25 μg adonitol + 1 mL EtOH, centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to 500 μL under a stream of nitrogen. Apply the residue to a Bond Elut SCX SPE cartridge (100 mg/mL), elute with 2 mL water. Apply the eluate to a Bond Elut SAX SPE cartridge (100 mg/mL), elute with 2 mL water. Freeze-dry the eluate, reconstitute in 100 μL MeOH:water 50:50, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 10 μm Gelpack GL-C64Z sulfonated styrene-divinylbenzene copolymer with zinc ions (Hitachi-Kasei)
Mobile phase: MeCN:water 80:20
Column temperature: 80
Flow rate: 1
Injection volume: 20
Detector: MS, Hitachi M-1000S quadrupole, negative-ion APCI, drift voltage -25 V, vaporizer 250°, desolvator 399°, m/z 217, $[M + Cl]^-$, SIM The column effluent was mixed with MeOH: chloroform 99:1 pumped at 0.5 mL/min, the combined effluent then flowed to the MS detector.

CHROMATOGRAM
Retention time: 12.5
Internal standard: adonitol (m/z 187) (9)

OTHER SUBSTANCES
Extracted: 1,5-anhydroglucitol, arabinose, arabitol, dextrose, erythritol, fructose, galactose,-myoinositol, sorbitol, xylitol

KEY WORDS
serum; SPE

REFERENCE
Niwa,T.; Tohyama,K.; Kato,Y. Analysis of polyols in uremic serum by liquid chromatography combined with atmospheric pressure chemical ionization mass spectrometry, *J.Chromatogr.*, **1993**, *613*, 9–14.

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

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

CHROMATOGRAM
Retention time: 11.3, 11.5 (pentabenzoyl), 18.7 (hexabenzoyl)
Internal standard: D-glucosamine (9.7)
Limit of detection: 1-5 pmol

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

KEY WORDS
serum; derivatization; fetal bovine serum

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

SAMPLE
Matrix: blood, erythrocytes, tissue
Sample preparation: Homogenize lens tissue in 4 (human) or 1 (rat) mL 2 mg/mL sodium fluoride. Dilute 600 μL frozen and thawed erythrocytes with 400 μL water. Filter (Amicon Centrifree) 1 mL homogenate, plasma, or diluted erythrocytes while centrifuging at 2400 g for 30 min. 200 μL Filtrate + 10 μL 1 mg/mL IS, mix, lyophilize, add 200 μL 100 mg/mL p-nitrobenzoyl chloride in pyridine, heat at 60° for 1 h, add 1 drop of water, add 2 mL chloroform. Wash mixture twice with 2 mL 5% sodium bicarbonate and twice with 3 mL 1 M HCl by vortexing for 1 min and centrifuging for 30 s, inject a 50 μL aliquot of the organic layer.

HPLC VARIABLES
Column: 250 × 4.6 6 μm Zorbax SIL
Mobile phase: Hexane:chloroform:MeCN 10:3:1.9 containing 0.1% water
Column temperature: 35
Flow rate: 1.5
Injection volume: 50
Detector: UV 260

CHROMATOGRAM
Retention time: 18
Internal standard: perseitol (α-mannoheptitol) (26)
Limit of detection: 1-2 ng

OTHER SUBSTANCES
Extracted: fructose, myo-inositol, sorbitol, dextrose

KEY WORDS
plasma; lens; human; rat; normal phase; derivatization

REFERENCE
Petchey,M.; Crabbe,M.J.C. Analysis of carbohydrates in lens, erythrocytes, and plasma by high-performance liquid chromatography of nitrobenzoate derivatives, *J.Chromatogr.*, **1984**, *307*, 180–184.

SAMPLE
Matrix: blood, urine
Sample preparation: Urine. Dilute urine 1:10 to 1:40 with water, add a 1 mL aliquot to 1 mL 250 μg/mL melibiose, add Amberlite IR-120 H^+ to occupy one third of the volume, inject a 25 μL aliquot of the supernatant. Plasma. 200 μL Plasma + 200 μL 250 μg/mL melibiose, mix, add 200 μL ice cold 35 mg/mL 5-sulfosalicylic acid, let stand on ice for 20 min, centrifuge at 9000 g for 5 min, mix with Amberlite IR-120 H^+:Amberlite IRA 400 Cl^- 40:60, centrifuge, inject a 25 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: Carbopac PA-1 (Dionex)
Column: 250 × 40 Carbopac PA-1 (Dionex)
Mobile phase: 120 mM NaOH containing 0.5 mM zinc acetate (urine) or 160 mM NaOH containing 0.675 mM zinc acetate (plasma) (At the end of each plasma sample wash with 1 M NaOH for 4 min.)
Flow rate: 1
Injection volume: 25

Detector: E, Dionex pulsed electrochemical detector, detection potential -0.01 V (0-0.5 s), oxidation potential +0.75 V (0.51-0.64 s), reduction potential -0.75 V (0.65-0.75 s), integration period 0.05-0.5 s

CHROMATOGRAM
Retention time: 2.1 (plasma), 2.2 (urine)
Internal standard: melibiose (4.0 (plasma), 4.6 (urine))

OTHER SUBSTANCES
Extracted: lactulose, 3-O-methylglucose, dextrose

KEY WORDS
plasma

REFERENCE
Fleming,S.C.; Kynaston,J.A.; Laker,M.F.; Pearson,A.D.J.; Kapembwa,M.S.; Griffin,G.E. Analysis of multiple sugar probes in urine and plasma by high-performance anion-exchange chromatography with pulsed electrochemical detection. Application in the assessment of intestinal permeability in human immunodeficiency virus infection, *J.Chromatogr.*, **1993**, *640*, 293–297.

SAMPLE
Matrix: food
Sample preparation: Freeze chewing gum, pulverize. Sonicate 1 g with 80 mL EtOH:water 96:4 at 60° for 20 min, cool, filter, rinse the filter, make up the filtrate to 100 mL. Remove a 5 mL aliquot and evaporate it to dryness under reduced pressure, add 4 mL pyridine, add 500 μL benzoyl chloride, sonicate at 60° for 1 h with swirling every 15 min, add 500 μL MeOH, swirl, let stand for 10 min, add 50 mL water (Z. Lebensm. Unters. Forsch. 1984, 178, 199), shake, add to a Sep-Pak RP-18 SPE cartridge (conditioning of cartridge is not necessary), rinse flask four times with 5 mL portions of water, add the rinses to the SPE cartridge, push 5 mL volumes of air through cartridge 3 times, elute with five 10 mL portions of isooctane:ether:MeCN 60:32:8, make up the volume of the eluate to 50 mL, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3 5 μm LiChrosorb Si 60 (glass column)
Mobile phase: Isooctane:ether:MeCN 150:60:10
Flow rate: 0.9
Injection volume: 10
Detector: UV 230

CHROMATOGRAM
Retention time: 17.5
Limit of detection: 0.1 ppm

OTHER SUBSTANCES
Extracted: dextrose, saccharose, sorbitol, xylitol

KEY WORDS
derivatization; SPE; chewing gum; mayonnaise; normal phase

REFERENCE
Galensa,R. Hochleistungs-flüssigchromatographische Bestimmung von Zuckeralkoholen mit UV-Detektion im ppm-Bereich in Lebensmitteln. I. [High-performance liquid chromatographic determination of sugar alcohols with UV-detection in the ppm-range in food. I.], *Z.Lebensm.Unters.Forsch.*, **1983**, *176*, 417–420.

SAMPLE
Matrix: intestinal mucosal homogenate
Sample preparation: Homogenate mixture + 100 μL 250 mM NaCN, mix, centrifuge at 4° at 34000 g for 10 min, filter (0.45 μm) the supernatant, inject an aliquot of the filtrate.

HPLC VARIABLES
Column: Supelco Gel C610-H

Mobile phase: 0.1% phosphoric acid in water
Flow rate: 0.5
Detector: UV 190

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

HPLC VARIABLES
Column: Shodex Sugar SP 0810P and SP 0810
Mobile phase: water
Column temperature: 80
Flow rate: 0.5
Detector: RI

CHROMATOGRAM
Retention time: 34

OTHER SUBSTANCES
Simultaneous: arabinose, dextrose, fructose, galactose, glycerol, lactose, lactulose, pullulan P-10, raffinose, sorbitol, stachyose, sucrose, xylitol

REFERENCE
Majors,R.E. Polymeric liquid chromatography column technology in Japan, *LC.GC*, **1993**, *11*, 778–788.

SAMPLE
Matrix: urine
Sample preparation: Condition a 600 mg Maxi-Clean C18 SPE cartridge with 5 mL MeOH and 5 mL water. Add 2-3 mL urine to the SPE cartridge and pass it through the cartridge. Discard the first 1 mL, collect the residual volume and dilute it 1:1 with water. Dilute a 200 μL aliquot with 1.8 mL water containing 75 μm/mL cellobiose, add 400 g/L Amberlite IRA-400 resin Cl^- form (Fluka). Vortex for 10 s, centrifuge at 3000 g for 2 min. Filter (Micro-spin centrifuge cartridge, Nylon 66, 0.2 μm, Alltech) a 400 μL aliquot of the supernatant while centrifuging at 3000 g for 5 min. Inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: Benson Carbohydrate BC-100 Ca^{2+} (Alltech)
Column: 300 × 6.5 10 μm Alltech 700 CH Carbohydrate (Alltech)
Mobile phase: water
Column temperature: 85
Flow rate: 0.5
Injection volume: 50
Detector: ELSD, Varex MKIII (Alltech), drift tube temperature 120°, carrier gas flow (air) 41.67 mL/s

CHROMATOGRAM
Retention time: 13.25
Internal standard: cellobiose (7.55)
Limit of detection: 650 μg/L

OTHER SUBSTANCES
Extracted: dextrose, lactulose
Simultaneous: fructose, galactose

KEY WORDS
SPE; pharmacokinetics

REFERENCE
Marsilio,R.; D'Antiga,L.; Zancan,L.; Dussini,N.; Zaccello,F. Simultaneous HPLC determination with light-scattering detection of lactulose and mannitol in studies of intestinal permeability in pediatrics, *Clin.Chem.*, **1998**, *44*, 1685–1691.

SAMPLE
Matrix: urine
Sample preparation: Dilute urine 2.5-20 fold with water, add a 1 mL aliquot to 1 mL water containing 250 μg/mL arabinose and 25 μg/mL cellobiose, add 0.5 g washed Amberlite IR-120 H:Amberlite IRA400 Cl 40:60, vortex, centrifuge, filter (0.2 μm), inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 40 HPIC-AS6 (Dionex)
Mobile phase: 150 mM NaOH
Flow rate: 1
Injection volume: 50
Detector: E, Dionex pulsed electrochemical detector, gold working electrode, detection potential -0.05 V, oxidation potential +0.6 V, reduction potential -0.95 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 3
Internal standard: arabinose (4), cellobiose (9)
Limit of detection: 300 ng/mL

OTHER SUBSTANCES
Extracted: lactulose

REFERENCE
Fleming,S.C.; Kapembwa,M.S.; Laker,M.F.; Levin,G.E.; Griffin,G.E. Rapid and simultaneous determination of lactulose and mannitol in urine, by HPLC with pulsed amperometric detection, for use in studies of intestinal permeability, *Clin.Chem.*, **1990**, *36*, 797–799.

SAMPLE
Matrix: urine
Sample preparation: Centrifuge, dilute 10-20 fold with water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 6.5 Sugar Pak I cation-exchange in calcium form
Mobile phase: Water containing 1 mL/L of a 50 g/L calcium EDTA solution
Column temperature: 85
Flow rate: 0.5
Injection volume: 20
Detector: RI

CHROMATOGRAM
Retention time: 13

OTHER SUBSTANCES
Extracted: lactulose

REFERENCE
Willems,D.; Cadranel,S.; Jacobs,W. Measurement of urinary sugars by HPLC in the estimation of intestinal permeability: evaluation in pediatric clinical practice, *Clin.Chem.*, **1993**, *39*, 888–890.

SAMPLE
Matrix: urine

Sample preparation: 2 mL Urine + 500 mg washed and mixed Duolite ion-exchange resin (BDH), vortex for 10 s, centrifuge at 3000 g for 10 min, filter (0.2 μm) the supernatant, inject an aliquot.

HPLC VARIABLES

Guard column: Direct-Connect polymeric guard column (Alltech)
Column: 250 × 4.6 5 μm Kromasil NH2 (Alltech)
Mobile phase: MeCN:water 70:30
Flow rate: 1
Injection volume: 10
Detector: RI

CHROMATOGRAM

Retention time: 9.4
Limit of detection: 50 μM

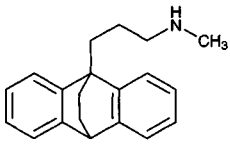
OTHER SUBSTANCES

Extracted: lactulose, L-rhamnose, urea

REFERENCE

Miki,K.; Butler,R.; Moore,D.; Davidson,G. Rapid and simultaneous quantification of rhamnose, mannitol, and lactulose in urine by HPLC for estimating intestinal permeability in pediatric practice, *Clin.Chem.*, **1996**, *42*, 71–75.

Maprotiline



Molecular formula: $C_{20}H_{23}N$
Molecular weight: 277.41
CAS Registry No.: 10262-69-8, 10347-81-6 (HCl)
Merck Index: 5792
Lednicer No.: 2 220

SAMPLE

Matrix: blood
Sample preparation: Add 250 μL 2 M sodium carbonate to 500 μL plasma. Add 100 μL 1 μg/mL IS in MeOH, extract with 10 mL n-hexane. Shake for 30 min and centrifuge at 3000 g for 10 min. Cool in a dry ice-acetone bath. Add 200 μL 0.3% phosphoric acid to upper organic layer. Shake for 10 min and centrifuge at 3000 g for 10 min. Separate the organic layer. Inject a 100 μL aliquot of the acidic aqueous layer.

HPLC VARIABLES

Column: 250 × 4.6 5 μm C18 Symmetry (Waters Millipore, USA)
Mobile phase: MeCN:67 mM potassium phosphate buffer adjusted to pH 3.0 with phosphoric acid 35:65 (After each chromatographic session wash the column with 200 mL MeCN:water 50:50.)
Flow rate: 1.2
Injection volume: 100
Detector: UV 226, UV 254, UV 400

CHROMATOGRAM

Retention time: 10.92
Internal standard: clovoxamine (6.5)
Limit of quantitation: 5 ng/mL (UV 226, UV 400 nm); 7 ng/mL (UV 254)

OTHER SUBSTANCES

Extracted: metabolites, amitriptyline clomipramine desipramine fluoxetine, imipramine, nortriptyline

Simultaneous: amineptine, carbamazepine, chlordiazepoxide, chlorpromazine, clonazepam, clorazepate, clozapine, cyamemazine, desmethylmaproptiline, desmethylvenlafaxine, doxepin, flunitrazepam, fluvoxamine, haloperidol, levomepromazine, lorazepam, loxapine, mianserine, sulpiride, trimipramine, venlafaxine, viloxazine, zolpidem, zopiclone
Noninterfering: diazepam, valproic acid

KEY WORDS
plasma

REFERENCE
Aymard,G.; Livi,P.; Pham,Y.T.; Diquet,B. Sensitive and rapid method for the simultaneous quantification of five antidepressants with their respective metabolites in plasma using high-performance liquid chromatography with diode-array detection, *J.Chromatogr.B*, **1997**, *700*, 183–189.

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL plasma with 400 μL 700 mM pH 9.7 bicarbonate buffer, vortex for 1 min, add 1.5 mL isoamyl alcohol:hexane 7.5:92.5, shake at 300 rpm for 30 min. Centrifuge at 1500 g for 15 min, freeze at -20° overnight, decant the organic layer into a tube containing 200 μL 0.05% orthophosphoric acid. Shake vigorously at 300 rpm for 45 min, centrifuge at 1500 g for 15 min, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: 13 × 4.3 5 μm C4/E butyl-bonded reversed-phase (MetaChem Technologies)
Column: 150 × 4.6 5 μm C4/E butyl-bonded reversed-phase(MetaChem Technologies)
Mobile phase: MeCN:40 mM pH 6.8 sodium phosphate buffer 50:50
Flow rate: 1.5
Injection volume: 100
Detector: F ex 276 em 598

CHROMATOGRAM
Retention time: 10
Internal standard: maprotiline

OTHER SUBSTANCES
Extracted: venlafaxine

KEY WORDS
plasma; maprotiline is IS

REFERENCE
Vu,R.L.; Helmeste,D.; Albers,L.; Reist,C. Rapid determination of venlafaxine and O-desmethylvenlafaxine in human plasma by high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.B*, **1997**, *703*, 195–201.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 800 ng clomipramine in MeOH + 2 mL 1 M NaOH + 5 mL hexane:isoamyl alcohol 99:1, shake mechanically for 15 min, centrifuge at 1686 g for 5 min. Remove the organic phase and add it to 200 μL 0.05% orthophosphoric acid, shake for 15 min, centrifuge for 5 min, inject a 50 μL aliquot of the aqueous phase (J.Liq.Chromatogr. 1981, 4, 849).

HPLC VARIABLES
Guard column: 23 × 3.9 Bondapak/Corasil C 18
Column: 300 × 4.6 10 μm μBondapak C18
Mobile phase: MeCN:buffer 40:60 (Buffer was 13.68 g KH_2PO_4 in 2 L water, adjusted to pH 4.7 with dilute KOH.)
Column temperature: 50
Flow rate: 2
Injection volume: 50

Detector: UV 214

CHROMATOGRAM
Retention time: 5
Internal standard: clomipramine (8.5)
Limit of detection: 3 ng

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: amoxapine, amitriptyline, chlordiazepoxide, chlorpromazine, cimetidine, desipramine, diazepam, doxepin, flurazepam, imipramine, lorazepam, oxazepam, pentobarbital, perphenazine, phenobarbital, phenytoin, prochlorperazine, propoxyphene, secobarbital, thioridazine, trifluoperazine
Noninterfering: acetaminophen, codeine, meperidine
Interfering: nortriptyline

KEY WORDS
plasma

REFERENCE
Wong,S.H.Y.; Waugh,S.W. Determination of the antidepressants maprotiline and amoxapine, and their metabolites, in plasma by liquid chromatography, *Clin.Chem.*, **1983**, *29*, 314–318.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 500 μL 10% potassium carbonate + 3 mL freshly-distilled n-hexane, shake for 30 min, centrifuge. Remove 2 mL of the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 500 μL 1 mg/mL benoxaprofen chloride in dry dichloromethane, heat at 50° for 30 min, inject a 10 μL aliquot. (Prepare benoxaprofen chloride as follows. Dissolve 600 mg benoxaprofen in 50 mL dry toluene, slowly add 5 mL thionyl chloride (freshly distilled from linseed oil), reflux for 30 min, evaporate to dryness, recrystallize from dichloromethane (if necessary) to give benoxaprofen chloride (mp 91.5°).)

HPLC VARIABLES
Column: 250 × 4.6 7 μm Zorbax-sil
Mobile phase: Cyclohexane:dichloromethane:THF 5:1:1
Flow rate: 1
Injection volume: 10
Detector: F ex 312 em 365

CHROMATOGRAM
Retention time: 8.6

OTHER SUBSTANCES
Simultaneous: amphetamine, benzylamine, methamphetamine, α-methylbenzylamine, phenylbutylamine, β-phenylethylamine, tolylethylamine, tranylcypromine
Interfering: procaine

KEY WORDS
derivatization; normal phase; plasma

REFERENCE
Spahn,H.; Weber,H.; Mutschler,E.; Möhrke,W. α-Alkyl-α-arylacetic acid derivatives as fluorescence markers for thin-layer chromatographic and high-performance liquid chromatographic assay of amines and alcohols, *J.Chromatogr.*, **1984**, *310*, 167–178.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 37 μL 2 μg/mL IS in MeOH + 500 μL pH 10 borate buffer + 1.5 mL hexane:isoamyl alcohol 95:5, shake for 10 min. Evaporate the organic layer to

dryness under a stream of nitrogen, reconstitute in 100 μL MeOH, inject a 50 μL aliquot. (The borate buffer was prepared as follows. Prepare a solution of 61.8 g boric acid and 74.6 g KCl in 1 L water. Add 630 mL of this solution to 370 mL 106 g/L sodium carbonate solution. Adjust pH to 10.0 with 6 M NaOH and store at 35-37°.)

HPLC VARIABLES
Column: 250 × 4.6 Zorbax Sil
Mobile phase: MeOH:ammonium hydroxide 998:2
Flow rate: 1.5
Injection volume: 50
Detector: UV 214

CHROMATOGRAM
Retention time: 14
Internal standard: desipramine hydrochloride (12)
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Extracted: desmethylclomipramine, clomipramine, protriptyline, metabolites
Also analyzed: doxepin, desmethyldoxepin, amitriptyline, nortriptyline, imipramine, 2-hydroxyimipramine, 2-hydroxydesipramine
Noninterfering: chlordiazepoxide, diazepam, flurazepam, oxazepam, thioridazine

KEY WORDS
plasma

REFERENCE
Sutfin,T.A.; D'Ambrosio,R.; Jusko,W.J. Liquid-chromatographic determination of eight tri- and tetracyclic antidepressants and their major active metabolites, *Clin.Chem.*, **1984**, *30*, 471–474.

SAMPLE
Matrix: blood
Sample preparation: Inject 200 μL serum onto column A and elute with mobile phase A for 10 min then back-flush column A onto column B with mobile phase B for 4 min. Elute column B with mobile phase B and monitor the effluent. Remove column A from circuit and wash with MeCN:water 60:40 for 6 min then with mobile phase A for 10 min.

HPLC VARIABLES
Column: A 40 × 4 TSKprecolumn PW (Tosoh); B 150 × 4 TSKgel ODS-80TM (Tosoh)
Mobile phase: A 50 mM pH 7.5 potassium phosphate; B MeCN:100 mM pH 2.7 potassium phosphate 32.5:67.5, containing 0.2 g/L sodium 1-heptanesulfonate
Flow rate: 1
Injection volume: 200
Detector: UV 210

CHROMATOGRAM
Retention time: 15
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: amitriptyline, amoxapine, clomipramine, desipramine, doxepin, imipramine, nortriptyline, trimipramine

KEY WORDS
serum; column-switching; use gradient to determine metabolites

REFERENCE
Matsumoto,K.; Kanba,S.; Kubo,H.; Yagi,G.; Iri,H.; Yuki,H. Automated determination of drugs in serum by column-switching high-performance liquid chromatography. IV. Separation of tricyclic and tetracyclic antidepressants and their metabolites, *Clin.Chem.*, **1989**, *35*, 453–456.

SAMPLE

Matrix: blood

Sample preparation: Add 10 μL 20 μg/mL oxaprotiline in MeOH to 990 μL plasma or serum. Inject 100 μL plasma or serum onto column A with mobile phase A and elute to waste, after 15 min elute column A onto column B with mobile phase B for 2 min. Remove column A from circuit and re-equilibrate it with mobile phase A for 5 min. Chromatograph on column B with mobile phase B.

HPLC VARIABLES

Column: A 20 × 4.6 10 μm Hypersil MOS C8; B 20 × 4.6 5 μm Hypersil CPS CN + 250 × 4.6 5 μm Nucleosil 100 CN

Mobile phase: A MeOH:water 5:95; B MeOH:MeCN:10 mM pH 6.8 potassium phosphate buffer 188:578:235

Flow rate: 1.5

Injection volume: 100

Detector: UV 214

CHROMATOGRAM

Retention time: 17.0

Internal standard: oxaprotiline (9.5)

Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Simultaneous: clozapine, fluvoxamine, metoclopramide, fluoxetine, norfluoxetine, imipramine, nortriptyline, desipramine, doxepin, clomipramine, amitriptyline

Noninterfering: haloperidol, spiroperidol, pimozide, fluspirilene, trifluperidol, perazine, chlordiazepoxide, clobazam, diazepam, nordiazepam, flurazepam, lorazepam, nitrazepam, oxazepam, carbamazepine

KEY WORDS

plasma; serum; column-switching

REFERENCE

Härtter,S.; Wetzel,H.; Hiemke,C. Automated determination of fluvoxamine in plasma by column-switching high-performance liquid chromatography, *Clin.Chem.*, **1992**, *38*, 2082–2086.

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 1 mL 0.6 M pH 9.8 carbonate buffer + 40 μL 5 μg/mL maprotiline in 10 mM HCl + 5 mL 200 g/L ethyl acetate in n-heptane, mix by rocking for 10 min, centrifuge at 1500 g for 10 min. Remove organic layer and add it to 150 μL 100 mM HCl, mix 10 min, centrifuge at 1500 g for 10 min. Discard organic layer and evaporate aqueous layer at 45° in a vacuum centrifuge for 1 h. Take up residue in 50 μL 1 M pH 10.3 carbonate buffer and 25 μL 10 mg/mL dansyl chloride in MeCN, vortex, allow to react at room temperature for 45 min, evaporate at 45° in a vacuum centrifuge for 20 min, reconstitute in 125 μL MeCN:water 75:25, vortex, centrifuge for 3-5 min, inject a 25-40 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-18

Mobile phase: MeCN:25 mM KH_2PO_4 75:25 + 500 μL/L orthophosphoric acid + 600 μL/L n-butylamine

Flow rate: 2

Injection volume: 25-40

Detector: F ex 235 em 470 (cut-off)

CHROMATOGRAM

Retention time: 12.8

Internal standard: maprotiline

OTHER SUBSTANCES

Simultaneous: fluoxetine, propranolol, clovoxamine, fluvoxamine, fenfluramine, amoxapine, protriptyline, nortriptyline, norfluoxetine, desipramine, sertraline

Noninterfering: amitriptyline, imipramine, clomipramine, trimipramine, mianserin, chlordiazepoxide, trazodone, cyclobenzaprine, nomifensine, bupropion, metoprolol, atenolol, pindolol, tranylcypromine, moclobemide, thioridazine, citalopram, clozapine, carbamazepine, doxepin, loxapine

KEY WORDS
plasma; maprotiline is IS

REFERENCE
Suckow,R.F.; Zhang,M.F.; Cooper,T.B. Sensitive and selective liquid-chromatographic assay of fluoxetine and norfluoxetine in plasma with fluorescence detection after precolumn derivatization, *Clin.Chem.*, **1992**, *38*, 1756–1761.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL BondElut C18 SPE cartridge with 1 mL 1 M HCl, 1 mL MeOH, 1 mL water, and 1 mL 1% potassium carbonate. 700 μL Serum + 50 μL 5 μg/mL trimipramine in 5% potassium bicarbonate + 700 μL MeCN, vortex, centrifuge at 1500 g for 5 min, add supernatant to SPE cartridge (at ca. 1 mL/min). Wash with 2 mL water and 1 mL MeCN, elute with 250 μL MeOH:35% perchloric acid 20:1 by gravity (10 min) then centrifuge for 20 s to remove rest of eluant, inject a 50 μL aliquot of the eluate.

HPLC VARIABLES
Guard column: 15 mm 7 μm Brownlee RP-8
Column: 150 × 4.6 5 μm Ultrasphere Octyl
Mobile phase: MeCN:water 37.5:62.5 containing 0.5 g/L tetramethylammonium perchlorate and 0.5 mL/L 7% perchloric acid
Flow rate: 1.5
Injection volume: 50
Detector: UV 215

CHROMATOGRAM
Retention time: 8.0
Internal standard: trimipramine (9.6)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: clomipramine, desipramine, doxepin, fluoxetine, fluvoxamine, imipramine, nortriptyline, protriptyline
Interfering: amitriptyline, desmethyltrimipramine

KEY WORDS
serum; SPE

REFERENCE
Gupta,R.N. An improved solid phase extraction procedure for the determination of antidepressants in serum by column liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 2751–2765.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 1 mL 2 M sodium bicarbonate + 6 mL hexane, extract, centrifuge at 2000 g for 10 min. Remove 5 mL of the hexane layer and add it to 150 μL 100 mM phosphoric acid, mix, centrifuge at 2000 g for 10 min, inject a 100 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 250 × 4 4 μm Supersphere Select B (Merck)
Mobile phase: MeCN:pH 5.8 phosphate buffer 25:75 (Buffer was 2 mL 85% phosphoric acid and 4 mL triethylamine in 1 L water.)
Flow rate: 0.3-1
Injection volume: 100

Detector: F ex 275 em 315 following post-column photolysis. The effluent from the column flowed through a Beam Boost photochemical reactor equipped with a 20 m coil and then to the detector.

CHROMATOGRAM
Retention time: 28.1
Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; post-column reaction; pharmacokinetics; post-column photochemical derivatization

REFERENCE
Kuss,H.-J.; Sirch,S.; Zhao,D.Y. Assay for maprotiline in human serum with improved sensitivity and selectivity, *J.Chromatogr.B*, **1994**, *656*, 245–249.

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

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

CHROMATOGRAM
Retention time: 5.0
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, lidocaine, methadone, methaqualone, mexiletine, midazolam, norchlorimipramine, nordoxepin, norfluoxetine, norverapamil, pentazocine, promazine, propafenone, propranolol, protriptyline, quinidine, trazodone, trimipramine
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: nordiazepam, propoxyphene, temazepam, imipramine, nortriptyline, 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: 100 μL Plasma + 100 μL 100 ng/mL desipramine in 2-methoxyethanol containing 75 mM triethylamine + 500 μL 6 M NaOH + 3 mL n-hexane:isoamyl alcohol 95:5, vortex for 5 min, centrifuge at 1000 g for 5 min. Remove a 2 mL aliquot of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL 75 mM triethylamine in 2-methoxyethanol, add 100 μL 1 mM 6-isothiocyanatobenzo[g]phthalazine-1,4(2H,3H)-dione in DMSO, add 10 μL 75 mM triethylamine in 2-methoxyethanol, heat at 80° for 10 min, inject a 20 μL aliquot. (Prepare 6-isothiocyanatobenzo[g]phthalazine-1,4(2H,3H)-dione as follows. Dissolve 2.2 g 2,3-naphthalenedicarboxylic acid in 6.6 mL MeOH:concentrated sulfuric acid 10:1, reflux for 3 h, pour into ice-water, filter. Dissolve the precipitate in 20 mL ethyl acetate and purify on a 250 × 35 column containing 120 g 70-230 mesh silica gel 60 (Merck) using n-hexane:ethyl acetate 50:50 to give dimethyl 2,3-naphthalenedicarboxylate as a brownish crystalline solid (mp 46-49°). Stir 8 mL concentrated nitric acid:concentrated sulfuric acid 3:5 at 4°, add 2.5 g dimethyl 2,3-naphthalenedicarboxylate, stir at 4° for 4-6 h until the reaction is complete. (Check by TLC using Merck Kieselgel 60 F254 with n-hexane:ethyl acetate 50:50.) Pour the reaction mixture into ice-water and extract with 350 mL dichloromethane. Wash the organic layer with water, with 5% sodium bicarbonate, and with water. Evaporate to dryness under reduced pressure, recrystallize from EtOH to give dimethyl 5-nitro-2,3- naphthalenedicarboxylate as yellow needles (mp 146-148°). Dissolve 1.25 g dimethyl 5-nitro-2,3-naphthalenedicarboxylate in 60 mL MeOH, add 500 mg 5% platinum on activated carbon, hydrogenate, filter, evaporate to dryness under reduced pressure, recrystallize from n-hexane/ethyl acetate to give dimethyl 5-amino-2,3-naphthalenedicarboxylate as pale yellow-green crystals (mp 129-131°). Add 2.7 mL triethylamine and 2.7 mL hydrazine hydrate (Caution! Hydrazine hydrate is a carcinogen!) to 1.2 g dimethyl 5-amino-2,3-naphthalenedicarboxylate dissolved in 18 mL MeOH, reflux for 3 h, evaporate to remove the solvent, rinse the residue with EtOH, dry to give 6-aminobenzo[g]phthalazine-1,4(2H,3H)-dione as a yellow powder (mp 280° d). Add 1 mL thiophosgene dropwise to a solution of 1 g 6-aminobenzo[g]phthalazine-1,4(2H,3H)-dione in 20 mL water stirred at room temperature, stir for 1 h, filter, wash the precipitate with MeCN, dry to give 6-isothiocyanatobenzo[g]-phthalazine-1,4(2H,3H)-dione as a pale brownish-yellow powder (mp 360° d) (Anal. Chim. Acta 1995, 302, 61).)

HPLC VARIABLES
Column: 150 × 4.6 5 μm TSKgel ODS-80 (Tosoh)
Mobile phase: MeCN:100 mM pH 3.2 acetate buffer 40:60
Flow rate: 1
Injection volume: 20
Detector: Chemiluminescence following post-column reaction. The column effluent mixed with 20 mM hydrogen peroxide in water pumped at 1 mL/min and with 40 mM potassium ferricyanide in 1.5 M NaOH pumped at 2 mL/min and the mixture flowed to the detector.

CHROMATOGRAM
Retention time: 59.5
Internal standard: desipramine (39.5)
Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES
Simultaneous: amoxapine, metoprolol, nortriptyline, propranolol
Noninterfering: betamethasone, diazepam, phenobarbital, phenytoin, prednisolone, triazolam

KEY WORDS
derivatization; plasma; pharmacokinetics

REFERENCE

Ishida,J.; Horike,N.; Yamaguchi,M. Determination of maprotiline in plasma by high-performance liquid chromatography with chemiluminescence detection, *J.Chromatogr.B*, **1995**, *669*, 390–396.

SAMPLE

Matrix: blood

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

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18

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

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 272

CHROMATOGRAM

Retention time: 8.93

Limit of detection: <120 ng/mL

KEY WORDS

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

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

SAMPLE
Matrix: blood, CSF, urine
Sample preparation: Dilute urine ten-fold. 1 mL Plasma, serum, CSF, or diluted urine + 250 μL 100 mM diethylamine in 4 M HCl + 1 mL 1 μM desmethyldoxepin hydrochloride in water, mix briefly, add 8 mL n-hexane, shake horizontally at 150 rpm for 30 min, centrifuge, discard the hexane layer. Add 250 μL 6 M NaOH to the aqueous layer, add 8 mL hexane, shake for 30 min, centrifuge. Remove the organic layer and evaporate it to 0.5 mL under a stream of nitrogen at 45°, add 100 μL 100 mM HCl to the residue , vortex for 2 min, inject an aliquot of the aqueous layer.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:100 mM pH 2.5 potassium phosphate buffer 30:70
Flow rate: 2
Injection volume: 50
Detector: UV 205

CHROMATOGRAM
Retention time: k' 9.45
Internal standard: desmethyldoxepin (k' 4.56)
Limit of detection: 11 nM

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: amitriptyline, chlordiazepoxide, chlorpromazine, desmethyldiazepam, desmethylimipramine, desmethylmaprotiline, diazepam, doxepin, fluphenazine, haloperidol, imipramine, lorazepam, mianserin, nomifensine, oxazepam, perphenazine, trifluoperazine
Noninterfering: sulpiride, thioridazine
Interfering: nortriptyline

KEY WORDS
plasma; serum

REFERENCE
Salonen,J.S.; Scheinin,M. Determination of maprotiline and N-desmethylmaprotiline from biological fluids by HPLC, *J.Anal.Toxicol.*, **1983**, *7*, 175–177.

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM
Retention time: 6.00
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES
Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine
Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine
Interfering: desipramine

KEY WORDS
serum; whole blood; liver

REFERENCE
McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE
Matrix: blood, tissue, urine
Sample preparation: Serum, urine. 500 μL Serum or urine + 100 μL 2 μg/mL diazepam + 200 μL 20% sodium carbonate + 500 μL water + 3 mL n-hexane:isoamyl alcohol 98.5:1.5, mix for 2 min, centrifuge at 1200 g for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, inject a 10 μL aliquot. Tissue. Homogenize 1 g sample with 9 mL 100 mM HCl and 100 μL 20 μg/mL diazepam, centrifuge at 15000 g for 10 min. Add 500 μL 20% sodium carbonate and 4 mL n-hexane:isoamyl alcohol 98.5:1.5 to 1 mL of the supernatant, mix for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, filter by microconcentrator (Microcon-30, Grace). Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 2 μm TSK gel Super-Octyl (A) or 100 × 4.6 5 μm Hypersil MOS-C8 (B), (Yokogawa, Japan)
Mobile phase: MeOH:20 mM pH 7 KH_2PO_4 60:40
Flow rate: 0.6
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 5.6 (A), 7.8 (B)
Internal standard: diazepam (4.4, A)
Limit of quantitation: 50 ng/mL (serum, urine) (A), 500 ng/mL (tissue) (A)

OTHER SUBSTANCES
Extracted: amitriptyline, clomipramine, dothiepin, doxepin, imipramine, melitracen, mianserin, nortriptyline
Noninterfering: barbital, carbamazepine, ethosuximide, hexobarbital, lofepramine, pentobarbital, phenobarbital, phenytoin, primidone, sulpiride, trimethadione, trimipramine
Interfering: amoxapine, desipramine

KEY WORDS
serum; brain; liver

REFERENCE
Tanaka,E.; Terada,M.; Nakamura,T.; Misawa,S.; Wakasugi,C. Forensic analysis of eleven cyclic antidepressants in human biological samples using a new reversed-phase chromatographic column of 2 μm porous microspherical silica gel, *J.Chromatogr.B*, **1997**, *692*, 405–412.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 0.5-2 mL Plasma + 2 mL pH 10 phosphate buffer + 6 mL diethyl ether:hexane 50:50, shake for 15 min, centrifuge at 3750 g for 5 min. Remove the organic phase and add it to 2 mL 250 mM sulfuric acid, shake for 10 min, centrifuge for 5 min, discard the organic layer. Adjust the pH of the aqueous phase to 9.5-10.5 with 500 mM NaOH containing 1 M K_2HPO_4, add 6 mL diethyl ether:hexane 50:50, shake for 10 min, centrifuge for 10 min. Remove the organic layer and evaporate it to dryness under vacuum and a stream of nitrogen at 45°, reconstitute the residue in 100 μL 100 mM sodium carbonate, add 10 μL 1% dansyl chloride in acetone, vortex for 20-30 s, heat at 45° for 30 min. Evaporate the solvent, reconstitute in 100 μL mobile phase, inject a 10-50 μL aliquot. Urine. 0.5-2 mL Urine + 2 mL pH 10 phosphate buffer + 6 mL diethyl ether:hexane 50:50, shake for 15 min, centrifuge at 3750 g for 5 min. Remove the organic phase and evaporate it to dryness under vacuum and a stream of nitrogen at 45°, reconstitute the residue in 100 μL 100 mM sodium carbonate, add 10 μL 1% dansyl chloride in acetone, vortex for 20-30 s, heat at 45° for 30 min. Evaporate the solvent, reconstitute in 100 μL mobile phase, inject a 10-50 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrosorb RP-18
Mobile phase: MeCN:water 65:35
Flow rate: 2
Injection volume: 10-50
Detector: F ex 248 em 470

CHROMATOGRAM
Retention time: 18.5
Internal standard: maprotiline

OTHER SUBSTANCES
Extracted: metapramine

KEY WORDS
plasma; derivatization; maprotiline is IS

REFERENCE
Sommadossi,J.P.; Lemar,M.; Necciari,J.; Sumirtapura,Y.; Cano,J.P.; Gaillot,J. High-performance liquid chromatographic method for the determination of plasma and urine metapramine after dansylation, *J.Chromatogr.*, **1982**, *228*, 205–213.

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

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

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

CHROMATOGRAM
Retention time: 15.508

KEY WORDS
whole blood

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

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

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

CHROMATOGRAM
Retention time: 2.9

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, 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, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, qui-

nine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

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

SAMPLE

Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: 20 mm long Pelliguard LC-8 40 μm
Column: 150 × 4.6 C8 5 μm (Supelco)
Mobile phase: MeCN:buffer 50:50 (Buffer was obtained by adding 1.2 mL of butylamine to 1 L 10 mM NaH_2PO_4 then adjusting pH to 3 with phosphoric acid.)
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: k' 2.613
Internal standard: clobazam (k' 1.344)

OTHER SUBSTANCES

Simultaneous: diazepam, desipramine, haloperidol, nortriptyline, imipramine, amitriptyline, clomipramine

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: solutions

HPLC VARIABLES

Column: 150 × 4.6 5 μm Adsorbosphere C18 (PEEK column) (retention times are longer and peaks broader with stainless steel column)
Mobile phase: MeCN:20 mM pH 3.2 KH_2PO_4 23.4:76.6 containing 0.05% nonylamine
Flow rate: 1.2
Detector: UV 214

CHROMATOGRAM

Retention time: 13

OTHER SUBSTANCES

Simultaneous: amitriptyline, desmethyldoxepin, desipramine, doxepin, imipramine, loxapine, nortriptyline, trazodone

REFERENCE

Supelco Catalog, **1993**, p. 440.

SAMPLE

Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Econosil C8
Mobile phase: MeCN:buffer 30:70 (Buffer was 20 mM KH_2PO_4 and 14 mM triethylamine adjusted to pH 3.0 with phosphoric acid.)
Injection volume: 20
Detector: UV 210

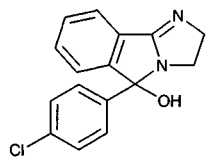
CHROMATOGRAM
Retention time: 10.5
Limit of quantitation: < 1000 ng/mL

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

KEY WORDS
UV spectra given

REFERENCE
Ryan,T.W. Identification and quantification of tricyclic antidepressants by UV-photodiode array detection with multicomponent analysis, *J.Liq.Chromatogr.*, **1993**, *16*, 1545–1560.

Mazindol



Molecular formula: $C_{16}H_{13}ClN_2O$
Molecular weight: 284.74
CAS Registry No.: 22232-71-9
Merck Index: 5801
Lednicer No.: 2 462

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 1.58

OTHER SUBSTANCES
Simultaneous: methylphenidate, phenelzine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodeine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine

Interfering: pemoline, benzphetamine, diethylpropion, tranylcypromine, caffeine, fenethyline, phendimetrazine, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine, norpipanone

REFERENCE

Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

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

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

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

trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 9.71 (A), 5.06 (B)

OTHER SUBSTANCES

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

KEY WORDS

details of plasma extraction

REFERENCE

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

SAMPLE

Matrix: urine

Sample preparation: Condition a 3 mL Bond-Elut strong cation exchange SPE cartridge packed with benzenesulfonic acid material with two 3 mL portions of MeOH, two 3 mL portions of water, and 3 mL 7 mM phosphoric acid (pH 3.5), do not allow column to dry. Adjust pH of 10 mL urine to 3.5 with 5 mL phosphoric acid, add to column, allow column to dry for 30 s, wash with 3 mL dilute phosphoric acid (pH 3.5), wash with 1 mL 1 M acetic acid, wash with 1 mL MeOH, elute with 1 mL 1% ammonium hydroxide in MeOH (pH 10), inject a 20 μL aliquot of the eluate.

HPLC VARIABLES

Column: 300 × 4.5 5 μm μBondapak C18
Mobile phase: MeCN:5 mM pentanesulfonic acid:85% phosphoric acid 25:75:5
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 12
Limit of detection: 25 ng/mL
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES

Simultaneous: metabolites

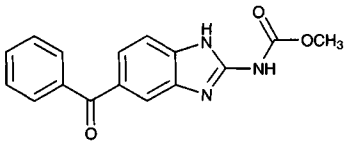
KEY WORDS

horse; SPE; details of GC-MS procedure given

REFERENCE

Moore,C.M.; Tebbett,I.R.; Kalita,S.; Artememko,M. Rapid extraction and detection of mazindol in horse urine, *J.Pharm.Biomed.Anal.*, **1990**, *8*, 445–448.

Mebendazole



Molecular formula: $C_{16}H_{13}N_3O_3$
Molecular weight: 295.30
CAS Registry No.: 31431-39-7
Merck Index: 5807
Lednicer No.: 2 353

SAMPLE

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

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 5
Limit of detection: 20 ng/mL

Sample preparation: Condition a 3 mL Bond-Elut strong cation exchange SPE cartridge packed with benzenesulfonic acid material with two 3 mL portions of MeOH, two 3 mL portions of water, and 3 mL 7 mM phosphoric acid (pH 3.5), do not allow column to dry. Adjust pH of 10 mL urine to 3.5 with 5 mL phosphoric acid, add to column, allow column to dry for 30 s, wash with 3 mL dilute phosphoric acid (pH 3.5), wash with 1 mL 1 M acetic acid, wash with 1 mL MeOH, elute with 1 mL 1% ammonium hydroxide in MeOH (pH 10), inject a 20 μL aliquot of the eluate.

HPLC VARIABLES

Column: 300 × 4.5 5 μm μBondapak C18
Mobile phase: MeCN:5 mM pentanesulfonic acid:85% phosphoric acid 25:75:5
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 12
Limit of detection: 25 ng/mL
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES

Simultaneous: metabolites

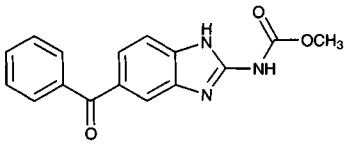
KEY WORDS

horse; SPE; details of GC-MS procedure given

REFERENCE

Moore,C.M.; Tebbett,I.R.; Kalita,S.; Artememko,M. Rapid extraction and detection of mazindol in horse urine, *J.Pharm.Biomed.Anal.*, **1990**, *8*, 445–448.

Mebendazole



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SAMPLE

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

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 5
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: albendazole, oxfendazole, cambendazole, thiabendazole, oxibendazole, fenbendazole, parbendazole

KEY WORDS
plasma; sheep

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

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 40 μL MeOH:DMF 99:1 + 1 mL 50 mM pH 8 borate buffer + 5 mL ethyl acetate, vortex for 20 s, centrifuge at 2500 g for 5 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 50° for about 50 min, reconstitute the residue in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 125 × 4.6 5 μm Hypersil SAS
Mobile phase: MeCN:buffer 30:70 (Buffer was 5 mM phosphoric acid adjusted to pH 5.9 with tetraethylammonium hydroxide.)
Flow rate: 2
Injection volume: 100
Detector: UV 300

CHROMATOGRAM
Retention time: 3.2
Internal standard: mebendazole

OTHER SUBSTANCES
Extracted: fenbendazole

KEY WORDS
plasma; sheep; mebendazole is IS

REFERENCE
Lehr,K.H.; Damm,P. Simultaneous determination of fenbendazole and its two metabolites and two triclabendazole metabolites in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *382*, 355–360.

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL 20 mM KH_2PO_4. Add 1 mL serum to the SPE cartridge, wash with 20 mL water, wash with 500 μL MeOH:water 40:60, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 200 μL MeOH, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil SAS
Mobile phase: MeCN:10 mM KH_2PO_4 20:80 adjusted to pH 3 with orthophosphoric acid
Flow rate: 0.8
Injection volume: 100
Detector: E, ESA Coulochem 5100 A, Model 5020 guard cell +0.70 V, Model 5010 A analytical cell, first electrode +0.60 V, second electrode -0.10 V (monitored)

CHROMATOGRAM
Retention time: 8.13
Limit of detection: 0.25 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; SPE

REFERENCE
Betto,P.; Gianbenedetti,M.; Ponti,F.; Ferretti,R.; Settimj,G.; Gargiulo,M.; Lorenzini,R. Application of a high-performance liquid chromatography coulometric method for the estimation of mebendazole and its metabolites in human sera, *J.Chromatogr.*, **1991**, *563*, 115–123.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Whole blood + 10 μL 10 μg/mL flubendazole + 2 mL 150 mM saline, vortex for 15 s, add 6 mL diethyl ether, vortex for 1.5 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, reconstitute the residue in 100 μL DMSO, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Nova Pak
Mobile phase: MeOH:50 mM $(NH_4)H_2PO_4$ 50:50 adjusted to pH 4.0 with 17.2 M orthophosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 291

CHROMATOGRAM
Retention time: 6.8
Internal standard: flubendazole (8.5)
Limit of detection: 6 ng/mL

OTHER SUBSTANCES
Extracted: UMF-058

KEY WORDS
whole blood; monkey; pharmacokinetics

REFERENCE
Ramanathan,S.; Nair,N.K.; Mansor,S.M.; Navaratnam,V. Determination of a new antifilarial drug, UMF-058, and mebendazole in whole blood by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *615*, 303–307.

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

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

CHROMATOGRAM
Retention time: 8.0
Internal standard: mebendazole

OTHER SUBSTANCES
Extracted: albendazole

KEY WORDS
plasma; mebendazole is IS; SPE

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

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

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

CHROMATOGRAM
Internal standard: mebendazole

OTHER SUBSTANCES
Extracted: albendazole

KEY WORDS
serum; lung; mouse; mebendazole is IS

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

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

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

Injection volume: 10-30
Detector: UV 209.9

CHROMATOGRAM
Retention time: 16.077

KEY WORDS
whole blood

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

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

HPLC VARIABLES
Column: Nucleosil C18
Mobile phase: Gradient. MeCN:0.5% acetic acid 35:65 for 5 min, 70:30 for 5 min, re-equilibrate at initial conditions for 5 min.
Flow rate: 1 for 5 min, 1.5 for 5 min, re-equilibrate at 1
Detector: UV 292

CHROMATOGRAM
Retention time: 7.3
Internal standard: mebendazole

OTHER SUBSTANCES
Extracted: albendazole

KEY WORDS
rat; intestine; SPE; mebendazole is IS

REFERENCE
Villaverde,C.; Alvarez,A.I.; Redondo,P.; Voces,J.; del Estal,J.L.; Prieto,J.G. Small intestinal sulphoxidation of albendazole, *Xenobiotica*, **1995**, *25*, 433–441.

SAMPLE
Matrix: 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, chlor-

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

REFERENCE

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

SAMPLE

Matrix: tissue

Sample preparation: Activate amino propyl SPE cartridge (containing 1 g 40 μm Bakerbond Amino aminopropylsilane (J.T. Baker) for flash chromatography) with 5 mL MeOH. Condition SPE cartridge with 2 mL ethyl acetate and 2 mL ethyl acetate:hexane 4:5. Add 1 mL ethyl acetate:hexane 4:5 to SPE cartridge. Attach SPE reservoir to SPE cartridge. 5 g tissue + 3 mL buffer, vortex for 10 s. Add 10 mL ethyl acetate, vortex for 10 s, shake mechanically at 500 rpm for 10 min, centrifuge at 3800 g for 10 min. Decant the supernatant, add 10 mL ethyl acetate to pellet, vortex for 10 s, shake mechanically at 500 rpm for 10 min, centrifuge at 3800 g for 10 min. Combine the supernatants, add 25 mL hexane and mix well. Centrifuge at 3800 g for 5 min. Add combined centrifuged supernatants into SPE reservoir and aspirate through SPE cartridge at flow rate of ca 2 mL/min. Add 5 mL ethyl acetate:hexane 4:5 to remaining pellet, vortex for 10 s, centrifuge at 3800 g for 5 min. Add centrifuged liquid into SPE reservoir and aspirate through SPE cartridge at ca. 2 mL/min. Remove SPE reservoir, wash SPE cartridge twice with 2 mL isooctane, let dry, dry under a stream of nitrogen for 20 min. Elute twice with 2 mL portions of MeOH. Evaporate eluate to dryness under a stream of nitrogen at 37°. Reconstitute the residue in 1.0 mL mobile phase, vortex for 10 s, centrifuge at 3800 g for 5 min. Inject a 50 μL aliquot of the supernatant. (Prepare buffer as follows. Dissolve 44.5 g Na_2HPO_4. $2H_2O$ in 450 mL water, adjust to pH 7.8 with 5 M NaOH and make up to 500 mL with water.)

HPLC VARIABLES

Guard column: 10 × 2.1 20 μm nonporous nonspherical C18 (Chrompack)
Column: 100 × 3.0 5 μm ChromSpher B porous spherical C18 (Chrompack)
Mobile phase: MeCN:buffer 30:70 (Buffer was 10 mM NaH_2PO_4 adjusted to pH 6.2 with 500 mM NaOH.)
Flow rate: 0.5
Injection volume: 50
Detector: UV 289

CHROMATOGRAM

Retention time: 6
Limit of detection: 1.4 μg/kg
Limit of quantitation: 2.3 μg/kg

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

muscle; eel; SPE

REFERENCE

Hajee,C.A.J.; Haagsma,N. Liquid chromatographic determination of mebendazole and its metabolites, aminomebendazole and hydroxymebendazole, in eel muscle tissue, *J.AOAC Int.*, **1996**, *79*, 645–651.

SAMPLE

Matrix: tissue
Sample preparation: Wash 22 g bulk 40 μm 18% load end-capped C18 material (Analytichem) in a syringe barrel with 100 mL hexane, with 100 mL dichloromethane, and with 100 mL MeOH and dry under vacuum aspiration. Gently blend 2 g C18 material and 0.5 g liver in a glass pestle for 1 min until homogeneous in appearance. Place in a 10 mL syringe barrel plugged with filter paper (Whatman No. 1), cover with filter paper, compress to 4.5 mL, place a 100 μL pipette tip on the barrel to restrict flow, wash with 8 mL hexane, elute with 8 mL MeCN. Pass the eluate through 0.5 g activated alumina (EM Science Type F-20 80-200 mesh) between filter paper in a 10 mL syringe barrel (wash column with 4 mL MeCN just before use). Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH and 400 μL 17 mM phosphoric acid, sonicate for 5-10 min, centrifuge at 17000 g for 5 min, filter the supernatant (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 4 10 μm Micro Pak ODS (Varian)
Mobile phase: MeCN:17 mM phosphoric acid 40:60
Column temperature: 45
Flow rate: 1
Injection volume: 20
Detector: UV 290

CHROMATOGRAM

Retention time: 9
Internal standard: mebendazole

OTHER SUBSTANCES

Extracted: albendazole, thiafendazole, oxfendazole, fenbendazole

KEY WORDS

matrix solid-phase dispersion; liver; mebendazole is IS

REFERENCE

Long,A.R.; Malbrough,M.S.; Hsieh,L.C.; Short,C.R.; Barker,S.A. Matrix solid phase dispersion isolation and liquid chromatographic determination of five benzimidazole anthelmintics in fortified beef liver, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 860–863.

SAMPLE

Matrix: tissue

Sample preparation: Condition a 2.8 mL 500 mg 40 μm 60 Å Bond Elut silica SPE cartridge with 2 mL dichloromethane. 10 g Minced tissue + 5 mL 1 M sodium carbonate + 150 mL ethyl acetate + 1 mL 10 mg/mL BHT in ethyl acetate, blend (Waring) at high speed for 5 min, add 80 g anhydrous sodium sulfate, blend at low speed for 1 min. Decant the organic layer and filter it (No. 41 paper), add 150 mL acetone to material remaining in blender, blend at low speed for 2-3 min, filter, wash solid with 10 mL EtOH. Combine all the filtrates and evaporate them to dryness under vacuum at 30-35° (beware of bumping). Rinse out flask with two 10 mL portions of hexane and two 10 mL portions of 1 M phosphoric acid, combine rinses, shake vigorously for 2 min, allow to separate for 10 min, extract the hexane layer twice more with 10 mL portions of 1 M phosphoric acid. Combine all the aqueous layers and wash them with 10 mL hexane, adjust the pH of the aqueous layer to 8.5 ± 1.0 by slowly adding about 9 mL 10 M KOH while using an ice bath. Extract twice with 50 mL ethyl acetate (2 min shaking), pass ethyl acetate layers through 40 g anhydrous sodium sulfate, wash the sodium sulfate with 25 mL ethyl acetate. Combine the ethyl acetate layers, add 200 μL 10 mg/mL BHT in ethyl acetate, evaporate to dryness under vacuum at 30-35° (beware of bumping). Rinse out flask with three 3 mL portions of dichloromethane, add rinses to the SPE cartridge, wash with 5 mL dichloromethane, elute with 5 mL dichloromethane:MeOH 75:25. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase, vortex, filter (0.2 μm), inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 Brownlee RP-18 Spheri-10 MPLC

Column: 250 × 4.6 5 μm C18 (Alltech)

Mobile phase: MeOH:buffer 53:47 (Buffer was 1.15 g $(NH_4)H_2PO_4$ in 950 mL water, adjust pH to 7.0 with dilute ammonia, make up to 1 L with water.)

Flow rate: 1

Injection volume: 50

Detector: UV 298

CHROMATOGRAM

Retention time: 20

Limit of detection: 100 ppb

OTHER SUBSTANCES

Extracted: oxfendazole, thiabendazole

Simultaneous: chloramphenicol

Noninterfering: amprolium, chlortetracycline, erythromycin, levamisole, morantel, oxytetracycline, phenothiazine, sulfadimethoxine, sulfamethazine, sulfaquinoxaline

KEY WORDS

cow; liver; SPE

REFERENCE

LeVan,L.W.; Barnes,C.J. Liquid chromatographic method for multiresidue determination of benzimidazoles in beef liver and muscle: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1991**, *74*, 487–493.

SAMPLE

Matrix: tissue

Sample preparation: Condition a J.T.Baker 3 mL 500 mg silica SPE cartridge with 6 mL n-hexane:ethyl acetate 2.5:1. 5 g Ground eel muscle tissue + 5 g sodium sulfate + 500 μL 4 M potassium carbonate solution + 10 mL ethyl acetate, vortex for 10 s, shake mechanically at 500 rpm for 10 min, centrifuge at 1300 g for 5 min, decant the supernatant, repeat the extraction with 10 mL ethyl acetate. Combine the extracts, add 50 mL hexane (ratio of n-hexane to ethyl acetate should be 2.5:1), add 5 g anhydrous sodium sulfate, shake, allow to stand until solution becomes transparent, filter (S & S 589.1 paper), add the filtrate to the SPE cartridge, rinse flask with 5 mL n-hexane:ethyl acetate 2.5:1, add the rinse to the SPE cartridge. Dry the SPE cartridge in a stream of nitrogen for 10 min, elute with 3 mL MeOH:acetic acid 97:3. Evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 1 mL mobile phase, inject a 50 μL aliquot.

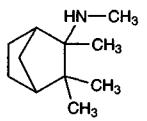
HPLC VARIABLES
Guard column: 10 × 2.1 40 μm ChromSep pellicular reversed-phase (stainless steel)
Column: 100 × 3 5 μm LiChrosorb RP-8 glass column
Mobile phase: MeCN:buffer 30:70, apparent pH 6.7 (Buffer was 50 mM $(NH_4)H_2PO_4$ adjusted to pH 6.2 with 10 M NaOH.)
Flow rate: 0.5
Injection volume: 50
Detector: UV 311

CHROMATOGRAM
Retention time: 8
Limit of detection: 2 ng/g
Limit of quantitation: 10 ng/g

KEY WORDS
eel; muscle; SPE

REFERENCE
Steenbaar,J.G.; Hajee,C.A.J.; Haagsma,N. High-performance liquid chromatographic determination of the anthelmintic mebendazole in eel muscle tissue, *J.Chromatogr.*, **1993**, *615*, 186–190.

Mecamylamine



Molecular formula: $C_{11}H_{21}N$
Molecular weight: 167.29
CAS Registry No.: 60-40-2, 826-39-1 (HCl)
Merck Index: 5814

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

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

CHROMATOGRAM
Retention time: 2.4

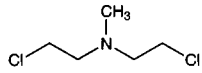
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, 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, hal-

operidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

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

Mechlorethamine



Molecular formula: $C_5H_{11}Cl_2N$
Molecular weight: 156.05
CAS Registry No.: 51-75-2, 55-86-7 (HCl)
Merck Index: 5815

SAMPLE

Matrix: blood
Sample preparation: Condition a 2.4 mL 500 mg Bond Elut phenyl SPE cartridge with 2 mL MeOH and 2 mL water. 1 mL Plasma + 100 μL freshly prepared 100 mg/mL diethyldithiocarbamic acid in 100 mM NaOH, heat at 37° for 30 min, add to the SPE cartridge, wash with 5 mL water, allow to dry in the dark at room temperature for 1 h, elute with 2 mL MeCN. Evaporate the eluate to dryness under reduced pressure at 40°, reconstitute in 200 μL MeOH, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.8 10 μm μBondapak C18
Mobile phase: Gradient. MeCN:5 mM pH 3.0 orthophosphoric acid 30:70 for 4 min, to 100:0 over 9 min, return to initial conditions over 7 min, re-equilibrate for 5 min.
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: UV 276

CHROMATOGRAM

Retention time: 13.1
Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Extracted: galamustine

KEY WORDS
plasma; derivatization; SPE

REFERENCE
Cummings,J.; MacLellan,A.; Smyth,J.F.; Farmer,P.B. Determination of reactive nitrogen mustard anticancer drugs in plasma by high-performance liquid chromatography using derivatization, *Anal.Chem.*, **1991**, *63*, 1514–1519.

SAMPLE
Matrix: formulations
Sample preparation: 0.1 g Ointment + 8 mL chloroform + 1.9 mL isopropanol + 100 μL water, shake until homogeneous, add 10 mL 10 mM HCl, invert twice for 2-3 min with a 10 min interval. Remove the aqueous phase and neutralize it with 1 mL 100 mM NaOH, add 1 mL 100 mg/mL diethyldithiocarbamic acid in 100 mM NaOH, heat at 37° for 1 h. Remove a 1 mL aliquot and add it to 1 mL MeOH, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.8 10 μm μBondapak C18
Mobile phase: Gradient. MeCN:5 mM pH 3.0 orthophosphoric acid 30:70 for 4 min, to 100:0 over 9 min, return to initial conditions over 7 min, re-equilibrate for 5 min.
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: UV 276

CHROMATOGRAM
Retention time: 13.1

OTHER SUBSTANCES
Extracted: degradation products

KEY WORDS
ointment; derivatization; chromatographic procedure as (Anal.Chem. 1991; 63; 1514)

REFERENCE
Cummings,J.; MacLellan,A.; Langdon,S.J.; Smyth,J.F. The long term stability of mechlorethamine hydrochloride (nitrogen mustard) ointment measured by HPLC, *J.Pharm.Pharmacol.*, **1993**, *45*, 6–9.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm cyano
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 200

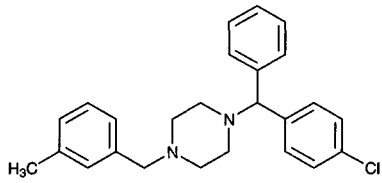
CHROMATOGRAM
Retention time: 2.24

OTHER SUBSTANCES
Simultaneous: granisetron (UV 300), morphine (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.

Meclizine



Molecular formula: $C_{25}H_{27}ClN_2$
Molecular weight: 390.96
CAS Registry No.: 569-65-3, 31884-77-2 (di HCl monohydrate), 1104-22-9 (di HCl)
Merck Index: 5817
Lednicer No.: 1 59

SAMPLE
Matrix: blood
Sample preparation: 4 mL Plasma + 15 mL toluene, shake by hand, centrifuge, discard organic layer, add 3 mL 1 M NaOH to the aqueous layer, add 15 mL diethyl ether, centrifuge. Remove the ether layer and evaporate it to dryness under a stream of nitrogen. Reconstitute in 230 μL MeCN:40 mM sodium dodecyl sulfate + 2.5% glacial acetic acid adjusted to 4.3 with ammonium hydroxide 80:20, inject a 200 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 3.9 Corasil
Column: 150 × 3.9 4 μm Novapak C18
Mobile phase: Gradient. A was 8 mM sodium dodecyl sulfate in water with 0.5% glacial acetic acid, pH adjusted to 4.3 with ammonium hydroxide. B was MeCN:40 mM sodium dodecyl sulfate + 2.5% glacial acetic acid adjusted to 4.3 with ammonium hydroxide 80:20. A:B 22:78 until meclizine eluted then to 0:100 for 8 min
Flow rate: 1.2
Injection volume: 200
Detector: UV 232

CHROMATOGRAM
Retention time: 12
Limit of detection: 1.25 ng/mL

KEY WORDS
plasma; rat; dog

REFERENCE
Chovan,J.P.; Klett,R.P.; Rakieten,N. Comparison of meclizine levels in the plasma of rats and dogs after intranasal, intravenous, and oral administration, *J.Pharm.Sci.*, **1985**, *74*, 1111–1113.

SAMPLE
Matrix: blood, formulations
Sample preparation: Blood. Centrifuge 200 μL fresh blood at 3000 rpm for 10 min. Inject an aliquot of the plasma. Formulations. Completely dissolve 50 mg sample in 20 mL MeOH, sonicate. Filter insoluble material and adjust filtrate to 50 mL with MeOH. Inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 4.6 Cosmosil 5C18 (Nacalai Tesque, Japan)
Mobile phase: MeOH:100 mm pH 6.2 acetate buffer 90:10
Column temperature: 40
Flow rate: 1.5
Injection volume: 100

Detector: UV 232

CHROMATOGRAM
Internal standard: pyrene

KEY WORDS
freeze-dried formulations; plasma; rat; egg albumin; olive oil

REFERENCE
Tsuji,Y.; Kakegawa,H.; Miyataka,H.; Nishiki,M.; Matsumoto,H.; Satoh,T. Pharmaceutical properties of freeze-dried formulations of egg albumin, several drugs and olive oil, *Biol.Pharm.Bull.*, **1996**, *19*, 636–640.

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

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

CHROMATOGRAM
Retention time: 19.955

KEY WORDS
whole blood

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

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 3.9 5 μm Symmetry C8
Mobile phase: MeCN:water:triethylamine:100 mM citrate buffer 40:55:0.05:5
Detector: UV (?)

REFERENCE
Lambropoulos,J.; Spanos,G.A.; Lazaridis,N.V. Method development and validation for the HPLC assay in paroxetine 20 mg strength tablets (Abstract 3391), *Pharm.Res.*, **1997**, *14*, S591.

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: 1.5

OTHER SUBSTANCES

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

REFERENCE

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

SAMPLE

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

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeOH:acetic acid:triethylamine:water 80:1.5:0.5:18
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 1.94

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

SAMPLE
Matrix: solutions

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

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

fasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Vydac 201HS54 C18
Mobile phase: Gradient MeCN:25 mM pH 3.6 phosphate buffer from 20:80 to 70:30 over 20 min
Flow rate: 1.5
Detector: UV 220 (from Vydac Applications Brochure)

CHROMATOGRAM

Retention time: 16

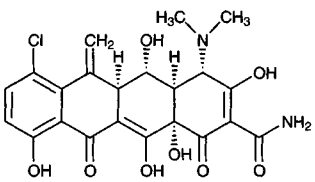
OTHER SUBSTANCES

Simultaneous: chlorcyclizine, tripelennamine, triprolidine, methaphenilene, pyrrobutamine, cyclizine, buclizine

REFERENCE

Vydac HPLC Catalog, 1994-5, p. 26.

Meclocycline



Molecular formula: $C_{22}H_{21}ClN_2O_8$
Molecular weight: 476.87
CAS Registry No.: 2013-58-3, 73816-42-9 (sulfosalicylate)
Merck Index: 5818

SAMPLE

Matrix: formulations
Sample preparation: Weigh out cream containing 5 mg meclocycline, add 20 mL MeOH, add 20 mL 12.5 mM sulfuric acid, sonicate for 1 h, vortex for 2 min until cream is thoroughly dispersed, make up to 50 mL with MeOH, centrifuge at 2000 g for 10 min. Remove a 5 mL aliquot of the supernatant and make it up to 50 mL with mobile phase, filter (0.5 μm, Millipore Fluorophore FHL P04700), inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 3.2 10 μm 201 TP reverse phase (Vydac)
Mobile phase: THF:buffer 15:85 (Prepare buffer by dissolving 0.6 g EDTA in 2 mL MeOH in 15 mL concentrated ammonium hydroxide, add 1.8 L water, adjust pH to 6.6 with glacial acetic acid, make up to 2 L with water. Periodically purge column with 95% EtOH.)
Flow rate: 0.8
Injection volume: 10
Detector: UV 340

CHROMATOGRAM

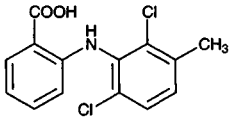
Retention time: 7
Limit of detection: 0.4 ppm

OTHER SUBSTANCES
Simultaneous: methacycline, C-4-epimeclocycline

KEY WORDS
cream

REFERENCE
Depaolis,A.M.; Britt,T.E.; Holman,A.J.; McGonigle,E.J.; Kaplan,G.; Davies,W.C. Determination of meclocycline, a tetracycline analogue, in cream formulations by liquid chromatography, *J.Pharm.Sci.*, **1984**, *73*, 1650–1651.

Meclofenamic acid



Molecular formula: $C_{14}H_{11}Cl_2NO_2$
Molecular weight: 296.15
CAS Registry No.: 644-62-2, 6385-02-0 (Na salt)
Merck Index: 5819
Lednicer No.: 1 110

SAMPLE
Matrix: aqueous humor
Sample preparation: 100 μL Aqueous humor + 500 μL MeCN + 30 μL 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 μL mobile phase by swirl-mixing for 1 min, centrifuge at 3000 g for 20 s, reduce volume to 20-30 μL, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.5 5 μm 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: 11.2
Internal standard: naproxen (3.89)

OTHER SUBSTANCES
Extracted: diclofenac, flurbiprofen, indomethacin
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: 1.5 mL Plasma + 2 mL 150 ng/mL diclofenac sodium in 100 mM pH 4.6 sodium citrate buffer, mix, add 5 mL dichloromethane, rotate for 30 min, centrifuge. Remove 3 mL of the organic layer and evaporate it to dryness, reconstitute the residue in 200 μL eluent, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 3 10 μm Spherisorb ODS
Mobile phase: MeOH:100 mM pH 6.1 sodium phosphate buffer 40:60
Flow rate: 0.8
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 10
Internal standard: diclofenac sodium (5.5)
Limit of detection: 107 ng/mL

OTHER SUBSTANCES
Simultaneous: flunixin, mefenamic acid

KEY WORDS
plasma; horse; pharmacokinetics

REFERENCE
Johansson,I.M.; Eklund,M.-L. Liquid chromatographic determination of meclofenamic acid in equine plasma, *J.Liq.Chromatogr.*, **1984**, *7*, 1609–1626.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 7.5 mL chloroform + 200 μL 6 M HCl + 50 μL 100 μg/mL (?) diclofenac in MeOH, shake for 2 min. Remove the organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 500 μL mobile phase, vortex for 1 min, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Resolve C18 (Waters)
Mobile phase: MeOH:water 60:40, pH adjusted to 3.0 with acetic acid
Column temperature: 35
Flow rate: 1.5
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 5.9
Internal standard: diclofenac (3.6)
Limit of quantitation: 150 ng/mL

KEY WORDS
plasma; dog; pharmacokinetics

REFERENCE
Niazy,E.M.; Khidr,S.H.; El-Sayed,Y.M. High-performance liquid chromatographic determination of meclofenamate in plasma, *J.Liq.Chromatogr.*, **1994**, *17*, 2331–2341.

SAMPLE
Matrix: blood, urine
Sample preparation: Dilute urine with an equal volume of water. 1 mL Plasma or diluted urine + 500 μL 6 M HCl + 10 mL toluene:EtOH 80:20, shake horizontally at slow speed for 15 min,

centrifuge at 2000 rpm for 10 min. Remove 8 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 200 (plasma) or 500 (urine) μL MeOH:water:acetic acid 70:30:0.5, inject a 20 (plasma) or 30 (urine) μL aliquot. (To assay total concentration add 1 mL 2 M pH 5.2 acetate buffer and 25 μL β-glucuronidase (Helix pomatia, Type H-1, 500000 units, Sigma) to 1 mL plasma or diluted urine, heat at 37° for 24 h, add 6 M HCl and proceed as above.)

HPLC VARIABLES

Column: 250 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water 70:30 (plasma) or 64:36 (urine) containing 5 mM tetrabutylammonium phosphate
Flow rate: 0.6
Injection volume: 20-30
Detector: UV 340

CHROMATOGRAM

Retention time: 25 (plasma)
Internal standard: 3,5-dichloroanthranilic acid (11.3)
Limit of detection: 200 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Koup,J.R.; Tucker,E.; Thomas,D.J.; Kinkel,A.W.; Sedman,A.J.; Dyer,R.; Sharoky,M. A single and multiple dose pharmacokinetic and metabolism study of meclofenamate sodium, *Biopharm.Drug Dispos.*, **1990**, *11*, 1–15.

SAMPLE

Matrix: solutions

HPLC VARIABLES

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

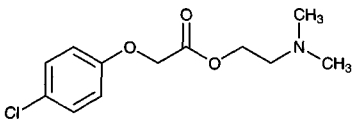
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminos-

tilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

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

Meclofenoxate



Molecular formula: $C_{12}H_{16}ClNO_3$
Molecular weight: 257.72
CAS Registry No.: 51-68-3, 3685-84-5 (HCl)
Merck Index: 5620

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher100RP-18
Column: 125 × 4 5 μm Spherisorb ODS 2
Mobile phase: Gradient. MeCN:buffer from 15:85 to 30:70 over 5 min. (Buffer was 20 mM sodium acetate containing 0.28% triethylamine, adjusted to pH 4.5 with acetic acid.)
Flow rate: 1.5
Detector: UV 280

CHROMATOGRAM
Retention time: k' 4.48

OTHER SUBSTANCES
Simultaneous: 4-chlorophenoxyacetic acid

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

SAMPLE

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

HPLC VARIABLES

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

CHROMATOGRAM

Retention time: 2.4

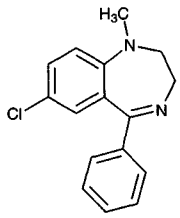
OTHER SUBSTANCES

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

REFERENCE

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

Medazepam



Molecular formula: $C_{16}H_{15}ClN_2$
Molecular weight: 270.76
CAS Registry No.: 2898-12-6, 2898-11-5 (HCl)
Merck Index: 5829
Lednicer No.: 1 368

SAMPLE

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

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 255

CHROMATOGRAM

Retention time: 6.62
Limit of detection: <120 ng/mL

KEY WORDS

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

imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

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

SAMPLE

Matrix: blood, urine

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

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

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

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

Column temperature: 30

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

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 15.83

KEY WORDS

whole blood

REFERENCE

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

SAMPLE

Matrix: solutions

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

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2

Injection volume: 20

Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.0

OTHER SUBSTANCES

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

REFERENCE

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

SAMPLE

Matrix: solutions

HPLC VARIABLES

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

OTHER SUBSTANCES

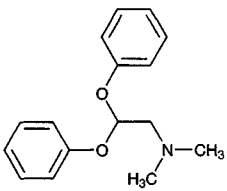
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine,

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

REFERENCE

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

Medifoxamine



Molecular formula: $C_{16}H_{19}NO_2$
Molecular weight: 257.33
CAS Registry No.: 32359-34-5, 16604-45-8 (fumarate)
Merck Index: 5834

SAMPLE

Matrix: blood

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

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 266

CHROMATOGRAM
Retention time: 5.71
Limit of detection: <120 ng/mL

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

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

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + 50 μL 100 (plasma) or 1000 (urine) μg/mL 5,6-benzoquinoline in water + 50 μL 4 M NaOH + 5 mL diethyl ether, shake gently for 10 min, centrifuge at 350 g for 5 min. Remove the organic layer and evaporate it to dryness at 70°, reconstitute the residue in 120 μL mobile phase, inject a 80 μL aliquot.

HPLC VARIABLES
Column: 150 × 5 5 μm Spherisorb silica
Mobile phase: MeOH:60% perchloric acid 100:0.02

Flow rate: 2
Injection volume: 80
Detector: UV 266

CHROMATOGRAM
Retention time: 3
Internal standard: 5,6-benzoquinoline (6)
Limit of detection: 10 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Saleh,S.; Johnston,A.; Chanon,M.; Turner,P. Determination of medifoxamine in plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *496*, 223–227.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 13.833

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: urine
Sample preparation: 1 mL Urine + 1 mL pH 10 borate-NaOH buffer + 50 μL 1 μg/L (sic) 5,6-benzoquinoline in water + 6 mL ethyl acetate:diethyl ether, shake gently for 10 min, centrifuge at 350 g for 5 min. Remove the organic layer and evaporate it to dryness at 70°, reconstitute the residue in 120 μL mobile phase, inject an aliquot. Hydrolyze conjugates as follows. 1 mL Urine + 1 mL buffer + 100 μL 1000 U/mL β-glucuronidase (from Helix pomatia, Sigma) in 0.2% NaCl, shake gently, heat at 37° for 24 h. (Buffer was 681 mg sodium acetate trihydrate in 40 mL water, equilibrate at 37°, adjust pH to 5 with 1 M HCl, make up to 50 mL with water.)

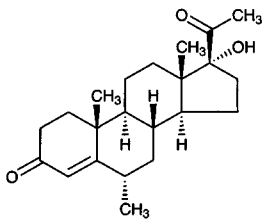
HPLC VARIABLES
Column: 150 × 5 5 μm Hypersil silica 5 ODS
Mobile phase: MeOH:buffer 56:40 (Buffer was 2.375 g Na_2HPO_4 and 0.135 g KH_2PO_4 in 400 mL water, pH 8.)
Flow rate: 2
Detector: UV 266

CHROMATOGRAM
Retention time: 15.6
Internal standard: 5,6-benzoquinoline (5.7)

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE
Saleh,S.; Johnston,A.; Turner,P. Measurement of medifoxamine metabolites in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *528*, 531–536.

Medroxyprogesterone



Molecular formula: $C_{22}H_{32}O_3$
Molecular weight: 344.49
CAS Registry No.: 520-85-4, 71-58-9 (acetate)
Merck Index: 5838
Lednicer No.: 1 180, 2 165

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 1 mL 0.5 μg/mL 16β-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 μL mobile phase, inject a 150 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm 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 (medroxyprogesterone acetate)
Internal standard: 16β-methylprogesterone (9.0)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: progesterone, 17α-hydroxyprogesterone, cortisone, corticosterone, aldosterone, testosterone, androstenedione, estradiol
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: 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 μL MeOH:water 80:20, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm 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 (medroxyprogesterone acetate)

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
plasma

REFERENCE
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, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 241.7

CHROMATOGRAM
Retention time: 24.203

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations

Sample preparation: 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 × 4 μBondapak CN
Mobile phase: MeOH:20 mM KH_2PO_4 30:70
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 7.5 (medroxyprogesterone acetate)
Internal standard: hydrocortisone (2.5)

OTHER SUBSTANCES

Simultaneous: progesterone, benzyl benzoate
Noninterfering: polyethylene glycol 4000, mystristyl-gamma-picolinium chloride, methylcellulose, thimerosal

REFERENCE

Das Gupta,V. Quantitation of hydroxyprogesterone caproate, medroxyprogesterone acetate, and progesterone by reversed-phase high-pressure liquid chromatography, *J.Pharm.Sci.*, **1982**, *71*, 294–297.

SAMPLE

Matrix: formulations
Sample preparation: 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 μm), inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 5 μm Novapak C18
Mobile phase: MeOH:10 mM $(NH_4)_2HPO_4$ 80:20, adjust pH to 7.2 ± 0.1 with 85% phosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 6.7 (medroxyprogesterone acetate)

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: 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 (medroxyprogesterone acetate)

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Waring blender) tissue at full speed for 2 min, lyophilize, grind. Extract with supercritical carbon dioxide at 60° at 400 atmospheres with a 20 cm × 21 μm restrictor for 1 h, collect the extract in 1 mL MeOH cooled to 5°. Evaporate the MeOH to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeCN:MeOH:20 mM ammonium formate 15:15:70, inject an aliquot. Alternatively, vortex 5 g ground tissue with 10 mL 40 mM sodium acetate, adjust pH to 4.2-4.7 with glacial acetic acid, add 100 μL β-glucuronidase (Sigma), heat at 37° for 8 h, add 20 mL MeCN, vortex for 30 s, centrifuge at 5000 rpm for 20 min. Remove a 30 mL aliquot of the supernatant and add it to 8 mL hexane and 2 mL dichloromethane, rotate for 3 min, centrifuge at 2000 rpm for 2 min. Remove a 15 mL aliquot of the middle layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 1 mL dichloromethane, inject an aliquot.

HPLC VARIABLES
Column: 50 × 4.6 5 μm Supelcosil
Mobile phase: Gradient. MeCN:MeOH:20 mM ammonium formate from 2.5:2.5:95 to 47.5:47.5:5 over 19 min.
Flow rate: 1
Injection volume: 20
Detector: UV 245 or MS, Sciex TAGA 6000E tandem triple quadrupole, APCI

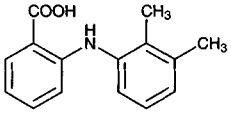
CHROMATOGRAM
Retention time: 13.7
Limit of detection: 100 ppb

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.Chromatogr.Rel.Technol.*, **1996**, *19*, 69–87.

Mefenamic acid



Molecular formula: $C_{15}H_{15}NO_2$
Molecular weight: 241.29
CAS Registry No.: 61-68-7
Merck Index: 5842
Lednicer No.: 1 10

SAMPLE
Matrix: blood

Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 3.12 (medroxyprogesterone acetate)

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Waring blender) tissue at full speed for 2 min, lyophilize, grind. Extract with supercritical carbon dioxide at 60° at 400 atmospheres with a 20 cm × 21 μm restrictor for 1 h, collect the extract in 1 mL MeOH cooled to 5°. Evaporate the MeOH to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeCN:MeOH:20 mM ammonium formate 15:15:70, inject an aliquot. Alternatively, vortex 5 g ground tissue with 10 mL 40 mM sodium acetate, adjust pH to 4.2-4.7 with glacial acetic acid, add 100 μL β-glucuronidase (Sigma), heat at 37° for 8 h, add 20 mL MeCN, vortex for 30 s, centrifuge at 5000 rpm for 20 min. Remove a 30 mL aliquot of the supernatant and add it to 8 mL hexane and 2 mL dichloromethane, rotate for 3 min, centrifuge at 2000 rpm for 2 min. Remove a 15 mL aliquot of the middle layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 1 mL dichloromethane, inject an aliquot.

HPLC VARIABLES
Column: 50 × 4.6 5 μm Supelcosil
Mobile phase: Gradient. MeCN:MeOH:20 mM ammonium formate from 2.5:2.5:95 to 47.5:47.5:5 over 19 min.
Flow rate: 1
Injection volume: 20
Detector: UV 245 or MS, Sciex TAGA 6000E tandem triple quadrupole, APCI

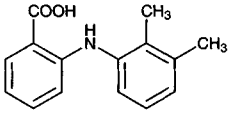
CHROMATOGRAM
Retention time: 13.7
Limit of detection: 100 ppb

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.Chromatogr.Rel.Technol.*, **1996**, *19*, 69–87.

Mefenamic acid



Molecular formula: $C_{15}H_{15}NO_2$
Molecular weight: 241.29
CAS Registry No.: 61-68-7
Merck Index: 5842
Lednicer No.: 1 10

SAMPLE
Matrix: blood

Sample preparation: 1 mL Plasma + 1 mL 1 M hydrochloric acid + 6 mL dichloromethane, shake for 20 min. Centrifuge at 2000 g for 5 min, evaporate organic phase to dryness under a gentle stream of nitrogen at 40°. Reconstitute the residue with 50 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 5 μm Nucleosil C18
Mobile phase: MeOH:water 77:23
Flow rate: 0.8
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 8.6
Internal standard: mefenamic acid

KEY WORDS
plasma; rat; mefenamic acid is IS

REFERENCE
Cerretani,D.; Micheli,L.; Fiaschi,L.; Giorgi,G. High-performance liquid chromatography of flufenamic acid in rat plasma, *J.Chromatogr.B*, **1996**, *678*, 365–368.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 50 μL 1 M HCl, add 4 mL hexane:acetone 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 μL MeCN:20 mM pH 5 phosphate buffer 35:65, inject a 200 μL aliquot onto column A with mobile phase A, collect the eluate containing mefenamic acid in a sample loop and inject it onto column B with mobile phase B. Collect the eluate containing mefenamic acid 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 × 4.6 5 μm YMC ODS A type (Yakamura Chemical); B 70 × 4.6 5 μm YMC ODS A type (Yakamura Chemical); C 150 × 4.6 5 μm TSK gel ODS 80 TM (Tosoh)
Mobile phase: A MeCN:20 mM pH 5 phosphate buffer 35:65; B MeCN:20 mM pH 3.5 phosphate buffer 45:55; C MeCN:20 mM pH 6 phosphate buffer 45:55
Column temperature: 40
Flow rate: 1
Injection volume: 200
Detector: UV 219

CHROMATOGRAM
Retention time: 39
Limit of detection: 0.1 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 μL Plasma + 100 μL 4 μg/mL methylclonazepam in 100 mM HCl + 3 mL diethyl ether, vortex for 30 s, centrifuge at 4° at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of air at room temperature, reconstitute the residue in 100 μL MeOH, inject a ≤10 μL aliquot.

HPLC VARIABLES
Guard column: 10 mm long 10 μm CN (Merck)
Column: 250 × 4 10 μm CN (Merck)
Mobile phase: MeCN:MeOH:water:17 M acetic acid 15:15:69:1
Flow rate: 1.5
Injection volume: ≤10
Detector: UV 290

CHROMATOGRAM
Retention time: 6.9
Internal standard: methylclonazepam (4.8)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Noninterfering: carbamazepine, clonazepam, ketoprofen, phenobarbital, phenytoin, salicylic acid, valproic acid
Interfering: indomethacin

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Poirier,J.-M.; Lebot,M.; Cheymol,G. Rapid and sensitive liquid chromatographic assay of mefenamic acid in plasma, *Ther.Drug Monit.*, **1992**, *14*, 322–326.

SAMPLE
Matrix: blood
Sample preparation: 0.5 mL Plasma + 10 μL 50 μg/mL mefenamic acid + 50 μL 85% phosphoric acid, vortex 10 sec, add 3 mL chloroform, vortex 1 min, centrifuge at 6000 rpm for 5 min. Remove organic layer and evaporate it to dryness at 45° under a stream of nitrogen. Vortex residue with 200 μL mobile phase for 10 s, inject 50 μL aliquot.

HPLC VARIABLES
Guard column: 30-40 μm C18 pellicular
Column: 150 × 3.9 Novapak C18
Mobile phase: MeCN:water 50:50 adjusted to pH 3.5 with glacial acetic acid
Flow rate: 1.5
Injection volume: 50
Detector: UV 278

CHROMATOGRAM
Retention time: 6.3
Internal standard: mefenamic acid
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: diclofenac

KEY WORDS
plasma; dog; mefenamic acid is IS

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: 50 μL Plasma + 250 μL 0.6 μg/mL indomethacin in MeCN + 50 μL MeCN, vortex, centrifuge at 9000 g for 3 min. Remove 250 μL of the supernatant and evaporate it to dryness under vacuum, dissolve the residue in 50 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 Alltech 5 μm C18 bonded-phase silica
Column: 250 × 4.6 Vydac column packed with Merck 5 μm C18 bonded-phase silica
Mobile phase: MeCN:10 mM phosphoric acid 60:40, pH 2.6
Flow rate: 0.9
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 9.2
Internal standard: indomethacin (6.3)
Limit of detection: 80 ng/mL

KEY WORDS
plasma

REFERENCE
Niopas,I.; Mamzoridi,K. Determination of indomethacin and mefenamic acid in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *656*, 447–450.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 220

CHROMATOGRAM
Retention time: 16.48
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; fle-

cainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood

Sample preparation: 500 μL Plasma + 200 μL 2 M HCl + 5 mL dichloromethane, rotate for 10 min, centrifuge at 5000 rpm for 8 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 250 μL plasma mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 4 μm Lichrocart C18 (Merck)
Column: 50 × 4 4 μm Lichrocart C18 (Merck)
Mobile phase: MeCN:100 mM pH 7.4 phosphate buffer:triethylamine 25:75:0.02
Flow rate: 1
Injection volume: 100
Detector: UV 282

CHROMATOGRAM

Internal standard: mefenamic acid

OTHER SUBSTANCES

Extracted: diclofenac
Noninterfering: fluvastatin

KEY WORDS

plasma; mefenamic acid is IS

REFERENCE

Transon,C.; Leemann,T.; Vogt,N.; Dayer,P. In vivo inhibition profile of cytochrome P450TB (CYP2C9) by (±)-fluvastatin, *Clin.Pharmacol.Ther.*, **1995**, *58*, 412–417.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 1 mL Plasma + 50 μL 500 ng/mL indomethacin + 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°. Redissolve 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: UV 235

CHROMATOGRAM
Retention time: 8
Internal standard: indomethacin (5)
Limit of detection: 50-250 ng/mL

OTHER SUBSTANCES
Simultaneous: naproxen, flunixin, thiosalicylic acid, ethacrynic acid, phenylbutazone

KEY WORDS
plasma

REFERENCE
Singh,A.K.; Jang,Y.; Mishra,U.; Granley,K. Simultaneous analysis of flunixin, naproxen, ethacrynic acid, indomethacin, phenylbutazone, mefenamic acid and thiosalicyclic acid in plasma and urine by high-performance liquid chromatography and gas chromatography-mass spectrometry, *J.Chromatogr.*, **1991**, *568*, 351–361.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Add 150 μL MeCN to 250 μL microsomal incubation, vortex vigorously, filter (0.45 μm), inject an aliquot

HPLC VARIABLES
Column: 250 × 5 Spherisorb C8
Mobile phase: Gradient. A was MeCN. B was 50 mM pH 4.5 ammonium acetate buffer. A:B from 20:80 to 60:40 over 20 (?) min.
Detector: UV 280

CHROMATOGRAM
Retention time: 18

OTHER SUBSTANCES
Extracted: metabolites, mefenamic acid 1-O-acyl glucuronide

REFERENCE
McGurk,K.A.; Remmel,R.P.; Hosagrahara,V.P.; Tosh,D.; Burchell,B. Reactivity of mefenamic acid 1-O-acyl glucuronide with proteins in vitro and ex vivo, *Drug Metab.Dispos.*, **1996**, *24*, 842–849.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb S10-ODS2
Mobile phase: MeCN:water:acetic acid 60:35:0.5
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Internal standard: flurbiprofen

REFERENCE
Galia,E.; Nicolaides,E.; Hörter,D.; Löbenberg,R.; Reppas,C.; Dressman,J.B. Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs, *Pharm.Res.*, **1998**, *15*, 698–705.

SAMPLE
Matrix: solutions
Sample preparation: 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: 3.16

OTHER SUBSTANCES
Also analyzed: acetaminophen, caffeine, bucetin (3-hydroxy-p-butyrophenetidine), phenacetin, dipyrone (sulpyrin), aspirin, salicylamide, salicylic acid, ethenzamide (o-ethoxybenzamide), theobromine, theophylline

KEY WORDS
semi-micro; porous polymer

REFERENCE
Matsushima,Y.; Nagata,Y.; Niyomura,M.; Takakusagi,K.; Takai,N. Analysis of antipyretics by semimicro liquid chromatography, *J.Chromatogr.*, **1985**, *332*, 269–273.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 500 μg/mL solution in MeOH:water 50:50, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax C8
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L MeCN:water 20:80. A:B from 100:0 to 0:100 over 30 min. (Purify triethylamine as follows. Wash neutral alumina (Merck) 3 times with 2 bed volumes of pentane, 3 times with 2 bed volumes of dichloromethane, and 3 times with 2 bed volumes of MeOH, allow solvent to evaporate in a fume hood overnight, heat alumina at 130° for 2 h. Prepare a 14 cm column of the washed alumina in a 290 × 22 tube, pass through a head volume of MeOH, pass through triethylamine. When triethylamine starts to elute discard the first 20 mL, use the next 20 mL, discard the column.)
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 27

OTHER SUBSTANCES
Simultaneous: acetophenone, amphetamine, desipramine, ethylmorphine, imipramine, methamphetamine, morphine, phenylbutazone, salicylic acid

KEY WORDS
also details of isocratic elution

REFERENCE
Hill,D.W. Evaluation of alkyl bonded silica and solvent phase modifiers for the efficient elution of basic drugs on HPLC, *J.Liq.Chromatogr.*, **1990**, *13*, 3147–3175.

SAMPLE
Matrix: solutions

Sample preparation: Inject a 50 μL 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 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 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'-trimethylammoniummethylcinnamonitrile 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'- 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' 7.5039
Limit of detection: 2 μg/mL

OTHER SUBSTANCES
Simultaneous: ibuprofen, ketoprofen, naproxen, probenecid, salicylic acid, valproic acid

KEY WORDS
post-column extraction; post-column reaction

REFERENCE
Kim,M.; Stewart,J.T. HPLC post-column ion-pair extraction of acidic drugs using a substituted α-phenylcinnamonitrile quaternary ammonium salt as a new fluorescent ion-pair reagent, *J.Liq.Chromatogr.*, **1990**, *13*, 213–237.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 0.5 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN: water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 25.6

OTHER SUBSTANCES
Simultaneous: acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, diflunisal, doxapram, estrone, fluoxymesterone, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, testosterone, testosterone propionate, tranylcypromine, tripelennamine

KEY WORDS
details for purification of triethylamine in paper

REFERENCE
Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primi-

done, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 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 75:25 containing 1% acetic acid
Flow rate: 1
Injection volume: 10
Detector: UV 230

CHROMATOGRAM

Retention time: 9.36

OTHER SUBSTANCES

Simultaneous: flufenamic 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 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 9.77 (A), 13.08 (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, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL ethyl acetate and dry by aspiration of air. Evaporate an aliquot of 100 μL 20 μg/mL IS in MeOH to dryness at 37°. Add 1 mL urine, vortex, add 250 μL 1 M pH 5.0 acetate buffer, vortex. Add 250 μL of the mixture to the SPE cartridge, dry by aspiration of air, elute with 3 mL ethyl acetate, evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject a 10-30 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:50 mM pH 5.0 phosphate buffer 42:58
Flow rate: 0.9
Injection volume: 10-30
Detector: UV 230

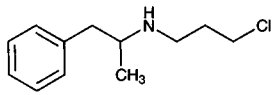
CHROMATOGRAM
Retention time: 33
Internal standard: indomethacin (18.5)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: diclofenac, ibuprofen, felbinac, fenbufen, flurbiprofen, ketoprofen, loxoprofen, naproxen, piroxicam, sulindac

KEY WORDS
SPE

REFERENCE
Hirai,T.; Matsumoto,S.; Kishi,I. Simultaneous analysis of several non-steroidal anti-inflammatory drugs in human urine by high-performance liquid chromatography with normal solid-phase extraction, *J.Chromatogr.B*, **1997**, *692*, 375–388.

Mefenorex



Molecular formula: $C_{12}H_{18}ClN$
Molecular weight: 211.73
CAS Registry No.: 17243-57-1, 5586-87-8 (HCl)
Merck Index: 5843
Lednicer No.: 2 47

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

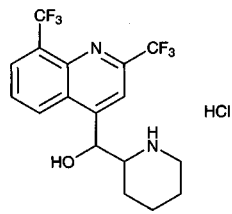
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 207.5

CHROMATOGRAM
Retention time: 11.897

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Mefloquine hydrochloride



Molecular formula: $C_{17}H_{17}ClF_6N_2O$
Molecular weight: 414.78
CAS Registry No.: 51773-92-3, 53230-10-7 (free base)
Merck Index: 5845

SAMPLE
Matrix: blood
Sample preparation: Add 150 μL 100 mM zinc sulfate to 600 μL plasma while vortexing over 15 s, add 700 μL MeCN containing 4 μm WR 184806 and 75 μM sulfadimethoxine while vortexing over 15 s, , let stand for 15 min, centrifuge at 10000 g for 10 min. Remove the supernatant and add it to 2 mL pH 9.0 phosphate buffer, add 2 mL 60 mM tetrabutylammo-

nium hydroxide, add 5 mL MTBE, shake for 10 min, centrifuge at 1200 g for 5 min. Remove the upper organic layer and evaporate it to dryness at 50°, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 3 μm Spherisorb S3-ODS-1
Mobile phase: MeCN:100 mM phosphate buffer 48:52, adjusted to pH 3.5
Flow rate: 0.5
Injection volume: 100
Detector: UV 229

CHROMATOGRAM
Retention time: 16.21
Internal standard: sulfadimethoxine (6.22), 2,8-bis(trifluoromethyl)-4-[1-hydroxy-3-(N-tert-butylamino)propyl]quinoline phosphate (WR 184806) (Walter Reed) (21.00)
Limit of detection: 250 nM

OTHER SUBSTANCES
Extracted: metabolites, pyrimethamine, sulfadoxine

KEY WORDS
plasma

REFERENCE
Bergqvist,Y.; Eckerbom,S.; Larsson,H.; Malekzadeh,M. Reversed-phase liquid chromatographic method for the simultaneous determination of the antimalarial drugs sulfadoxine, pyrimethamine, mefloquine and its major carboxylic metabolite in plasma, *J.Chromatogr.*, **1991**, *571*, 169–177.

SAMPLE
Matrix: blood
Sample preparation: 300 μL Plasma + 75 μL 100 mM zinc sulfate, vortex for 15 s, add 750 μL 3 mM quinine in MeCN, vortex for 15 s, let stand for 15 min, centrifuge at 10000 g for 10 min. Remove the supernatant and add it to 2 mL 0.1% ammonium (?) solution (also specified on p. 222 as 1% ammonia) and 6 mL MTBE, extract for 30 min, centrifuge at 3000 g for 5 min. Remove the organic layer and evaporate it to dryness at 80° under a stream of air. Reconstitute the residue in 150 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 7 μm Hypercarb-S
Mobile phase: MeCN:MeOH:60 mM pH 4.6 acetate buffer 48:20:32 containing 5 mM N-benzyloxycarbonylglycyl-L-proline (L-ZGP), pH adjusted to 4.6 with NaOH
Flow rate: 0.8
Injection volume: 100
Detector: UV 278

CHROMATOGRAM
Retention time: 6 (SR), 7 (RS)
Internal standard: quinine (10)
Limit of detection: 500 nM

KEY WORDS
plasma; chiral

REFERENCE
Bergqvist,Y.; Al Kabbani,J.; Pettersson,C.; Huynh,N.-H. Enantioselective high-performance liquid chromatographic determination of (SR)- and (RS)-mefloquine in plasma using N-benzyloxycarbonyl-glycyl-L-proline as chiral counter ion, *J.Chromatogr.*, **1993**, *620*, 217–224.

SAMPLE
Matrix: blood

Sample preparation: Allow 100 μL whole blood to dry on chromatographic paper (Whatman 31 ET Chroma). Cut paper into small pieces, add to 2 mL ammonia:water 10:90, incubate for 30-60 min at room temperature, sonicate for 30 min at 37°, add 5 mL MTBE, add 2 mL 500 mM pH 9.0 phosphate buffer, add 2 mL 20 μM IS in 60 mM tetrabutylammonium hydroxide, shake for 30 min, centrifuge at 1000 g for 5 min. Remove the organic layer and evaporate it to dryness at 80°, reconstitute the residue in 150 μL mobile phase, inject a 125 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere IP C18
Mobile phase: MeCN:100 mM phosphate buffer:1 M sodium perchlorate 137:173:15 containing 7 mM N,N-dimethyloctylamine, adjust pH to 4.0 with 5 M NaOH
Flow rate: 1.4
Injection volume: 125
Detector: UV 227

CHROMATOGRAM

Retention time: 7.8
Internal standard: 2,8-bis(trifluoromethyl)-4-[1-hydroxy-3-(N-t-butylamino)propyl]quinoline (WR 184,806) (14.6)
Limit of detection: 500 nM

OTHER SUBSTANCES

Simultaneous: metabolites

KEY WORDS

whole blood

REFERENCE

Bergqvist,Y.; Al Kabbani,J.; Krysén,B.; Palme,I.B.; Rombo,L. High-performance liquid chromatographic method for the simultaneous determination of mefloquine and its carboxylic metabolite in 100-μl blood samples dried on paper, *J.Chromatogr.*, **1993**, *615*, 297–302.

SAMPLE

Matrix: blood
Sample preparation: 500 μL Plasma + 500 μL 20% Na_3PO_4 + 2 mL MTBE, vortex for 30 s, centrifuge at 2000 g for 10 min, freeze in dry ice/acetone. Remove the organic phase, repeat the extraction with 2 mL MTBE. Combine the organic phases and evaporate them under a stream of nitrogen, reconstitute the residue in 70 μL mobile phase, inject a 50 μL aliquot onto column A with mobile phase A. Three min after mefloquine had left column A and entered column B (use a detector between column A and column B to ascertain this) backflush contents of column B onto column C with mobile phase B, elute column C with mobile phase B and monitor the effluent for the enantiomers.

HPLC VARIABLES

Column: A 250 × 4.6 5 μm Nucleosil cyanopropyl; B 60 × 4 5 μm Kromasil (Informatiques & Technologies); C 250 × 4.6 5 μm chiral (S)-naphthylurea (S.F.C.C./Shandon)
Mobile phase: A Hexane:isopropanol:MeOH 82:4:14 modified with 125 μL triethylamine in MeOH (1:40); B Hexane:isopropanol:MeOH 50:5:45 modified with 125 μL triethylamine in MeOH (1:40)
Flow rate: A 2; B 1.5
Injection volume: 50
Detector: UV 285

CHROMATOGRAM

Retention time: 36 (+), 38 (-)

KEY WORDS

plasma; chiral; column-switching

REFERENCE

Gimenez,F.; Dumartin,C.; Wainer,I.W.; Farinotti,R. Improved column-switching liquid chromatographic method for the determination of the enantiomers of mefloquine, *J.Chromatogr.*, **1993**, *619*, 161–166.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 50 μL 10 μg/mL quinidine in MeOH + 500 μL 20% Na_3PO_4 + 2 mL MTBE, vortex for 30 s, centrifuge at 2000 g for 10 min, freeze in dry ice/acetone. Remove the organic phase, repeat the extraction with 2 mL MTBE. Combine the organic phases and evaporate them under a stream of nitrogen, reconstitute the residue in 70 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil cyanopropyl
Mobile phase: Hexane:isopropanol:MeOH 82:4:14 modified with 125 μL triethylamine in MeOH (1:40)
Flow rate: 2
Injection volume: 50
Detector: UV 285

CHROMATOGRAM
Retention time: k' 5.35
Internal standard: quinidine (k' 13.65)
Limit of quantitation: 50 ng/mL

KEY WORDS
plasma

REFERENCE
Gimenez,F.; Dumartin,C.; Wainer,I.W.; Farinotti,R. Improved column-switching liquid chromatographic method for the determination of the enantiomers of mefloquine, *J.Chromatogr.*, **1993**, *619*, 161–166.

SAMPLE
Matrix: blood
Sample preparation: Whole blood. 100 μL Whole blood was allowed to dry on Whatman 31 ET Chroma chromatographic paper. Cut paper into small pieces and add to 150 μL 5 μM IS in water, add 2 mL ammonia:water 90:10, incubate at room temperature for 30-60 min, sonicate at 37° for 30 min, add 4 mL 10 mM pH 10.0 borate buffer and 5 mL MTBE, shake for 20 min, centrifuge at 3000 g for 5 min. Remove the organic layer and evaporate it to dryness at 65°, add 125 μL 0.4 mM (-)-1-(9-fluorenyl)ethyl chloroformate in water-free MeCN, add 50 μL 15 mM pH 8.5 borate buffer, vortex, leave at room temperature for 40 min, centrifuge at 3000 g for 10 min, inject a 100 μL aliquot. Plasma. 100 μL Plasma + 25 μL 100 mM zinc sulfate, vortex for 15 s, add 750 μL 1 μM IS in MeCN, vortex for 15 s, let stand for 15 min, centrifuge at 10000 g for 10 min. Remove the supernatant and add it to 4 mL 10 mM pH 10.0 borate buffer and 5 mL MTBE, shake for 20 min, centrifuge at 3000 g for 5 min. Remove the organic layer and evaporate it to dryness at 65°, add 125 μL 0.4 mM (-)-1-(9-fluorenyl)ethyl chloroformate in water-free MeCN, add 50 μL 15 mM pH 8.5 borate buffer, vortex, leave at room temperature for 40 min, centrifuge at 3000 g for 10 min, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere octadecylsilica
Mobile phase: MeCN:water:acetic acid 82:18:0.07
Flow rate: 1
Injection volume: 100
Detector: F ex 263 em 475

CHROMATOGRAM
Retention time: 13 (RS), 15 (SR)
Internal standard: D,L-erythro-α-(2-piperidyl)-2-trifluoromethyl-6,8-dichloro-4-quinolinemethanol (18 and 22)
Limit of detection: 50 nM (SR), 10 nM (RS)

OTHER SUBSTANCES
Noninterfering: chloroquine, quinine, pyrimethamine, sulfadoxine

KEY WORDS
whole blood; plasma; chiral; derivatization

REFERENCE
Bergqvist,Y.; Doverskog,M.; Al Kabbani,J. High-performance liquid chromatographic determination of (*SR*)- and (*RS*)-enantiomers of mefloquine in plasma and capillary blood sampled on paper after derivatization with (-)-1-(9-fluorenyl)ethyl chloroformate, *J.Chromatogr.B*, **1994**, *652*, 73–81.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 222

CHROMATOGRAM
Retention time: 9.58
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine;

amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: 1 mL Plasma or urine + 100 μL 1 M NaOH + 2 mL MTBE, extract by rotating slowly for 15 min, centrifuge at 3000 g for 5 min. Remove the organic phase and add it to 500 μL 500 mM phosphoric acid, extract for 15 min, centrifuge. Remove the aqueous phase and add it to 1 mL 1 M NaOH and 1.5 mL MTBE, extract for 15 min, centrifuge. Remove the organic phase and evaporate it under nitrogen, dissolve the residue in 300 μL mobile phase, inject an aliquot.

HPLC VARIABLES

Guard column: Chiral-AGP guard column
Column: 150 × 4 Chiral-AGP (ChromTech)
Mobile phase: n-Propanol:50 mM pH 4.85 sodium phosphate buffer 10:90
Flow rate: 0.9
Injection volume: 100
Detector: UV 222

CHROMATOGRAM

Retention time: 10 (SR), 13 (RS)
Limit of detection: 100 nM

OTHER SUBSTANCES

Noninterfering: atenolol, cimetidine, cycloguanil, diazepam, flunitrazepam, hydroxychloroquine, proguanil, propranolol, pyrimethamine, ranitidine, sulfadoxine, verapamil

KEY WORDS

plasma; chiral

REFERENCE

Wallén,L.; Ericsson,.; Wikström,I.; Hellgren,U. High-performance liquid chromatographic method for the enantioselective analysis of mefloquine in plasma and urine, *J.Chromatogr.B*, **1994**, *655*, 153–157.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30

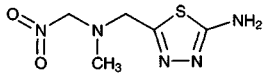
Detector: UV 222.8

CHROMATOGRAM
Retention time: 16.597

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Megazol



Molecular formula: $C_6H_6N_6O_2S$
Molecular weight: 226.21
CAS Registry No.: 19622-55-0

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL plasma with 50 μL 10 μg/mL tinidazole in mobile phase and 50 μL 1 M NaOH. Add 7 mL dichloromethane, mix on a rotary agitator for 20 min and centrifuge at 1636 g for 10 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 45°. Reconstitute the dry residue by vortex agitation with 200 μL mobile phase, inject a 50 μL aliquot.

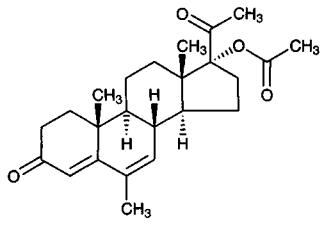
HPLC VARIABLES
Column: 250 × 4 10 μm Kromasil C8 (Bischoff Chromatography, Germany)
Mobile phase: MeCN:MeOH:68 mM pH 3 phosphate buffer 15:20:65
Flow rate: 0.7
Injection volume: 50
Detector: UV 360

CHROMATOGRAM
Retention time: 9.60
Internal standard: tinidazole (6.10)
Limit of quantitation: 2 ng/mL

KEY WORDS
human; rat; plasma; pharmacokinetics

REFERENCE
Enanga,B.; Labat,C.; Boudra,H.; Chauvière,G.; Keita,M.; Bouteille,B.; Dumas,M.; Houin,G. Simple high-performance liquid chromatographic method to analyse megazol in human and rat plasma, *J.Chromatogr.B*, **1997**, *696*, 261–266.

Megestrol acetate



Molecular formula: $C_{24}H_{32}O_4$
Molecular weight: 384.52
CAS Registry No.: 595-33-5
Merck Index: 5849
Lednicer No.: 1 180

SAMPLE

Matrix: blood
Sample preparation: 500 µL Plasma + 500 µL water + 50 µL 3 µg/mL 2,3-diphenyl-1-indenone in MeOH + 7 mL hexane, rotate at 40 rpm for 10 min, centrifuge at 800 g for 7 min. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 30-35°, reconstitute the residue in 100 µL MeOH, vortex thoroughly, inject a 50 µL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 µBondapak C18
Mobile phase: MeCN:MeOH:water:acetic acid 41:23:36:1
Flow rate: 2
Injection volume: 50
Detector: UV 280

CHROMATOGRAM

Retention time: 6-7 (megestrol acetate)
Internal standard: 2,3-diphenyl-1-indenone (12-14)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: megestrol (4-5 min)

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Gaver,R.C.; Movahhed,H.S.; Farmen,R.H.; Pittman,K.A. Liquid chromatographic procedure for the quantitative analysis of megestrol acetate in human plasma, *J.Pharm.Sci.*, **1985**, *74*, 664–667.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 100 µL 14 µg/mL cyproterone acetate + 100 µL MeOH, mix, let stand for 1 h, add to a pre-washed Sep-Pak C18 SPE cartridge, wash with 5 mL water, elute with 2 mL MeOH. Evaporate the eluate to 500 µL under a stream of nitrogen, add 5 mL water, extract with 5 mL dichloromethane. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 µL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 220 × 4.6 5 µm Spheri RP18 (Brownlee)
Mobile phase: MeCN:MeOH:water 30:35:35
Flow rate: 1
Detector: UV 260

CHROMATOGRAM

Retention time: 14 (megestrol acetate)
Internal standard: cyproterone acetate (12)
Limit of detection: 5 ng/mL

KEY WORDS

serum; SPE

REFERENCE
Dikkeschei,L.D.; Wolthers,B.G.; de Ruyter-Buitenhuis,A.W.; Nagel,G.T.; Sleijfer,D.T.; Willemse,P.H.; van der Slik,W. Determination of megestrol acetate and cyproterone acetate in serum of patients with advanced breast cancer by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *529*, 145–154.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 290.2

CHROMATOGRAM
Retention time: 23.815

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone,

debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES

Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: k' 12.88

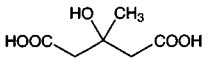
OTHER SUBSTANCES

Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

Meglutol



Molecular formula: $C_6H_{10}O_5$
Molecular weight: 162.14
CAS Registry No.: 503-49-1
Merck Index: 5852

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets, weigh out amount equivalent to 100 mg meglutol, suspend in 10 mL water, make up to 100 mL with MeOH, stir for 10 min, dilute an aliquot 1:10 with water. 200 μL Sample + 150 μL 20 mM tetrahexylammonium bromide in 100 mM pH 7.0 phosphate buffer + 100 μL 4.2 mg/mL 2-bromoacetyl-6-methoxynaphthalene in acetone, stir at 65° for 43 min, add 150 μL 7.5 μg/mL IS in MeCN, sonicate for 1 min, inject a 50 μL aliquot. (Synthesis of 2-bromoacetyl-6-methoxynaphthalene is as follows. Stir equimolar amounts of 2-acetyl-6-methoxynaphthalene (6'-methoxy-2'-acetonaphthone, Aldrich) and methyltriphenylphosphonium tribromide in anhydrous THF under nitrogen at room temperature for 1 h, dilute the reaction mixture with ether, wash with sodium bisulfite solution, wash with water (Phosphorus and Sulfur 1985, 25, 357). [Bromination can also be achieved with phenyltrimethylammonium tribromide over 3 h but the reaction is less selective.] Purify the crude product by column chromatography on silica gel using chloroform:petroleum ether 50:50 to give 2-bromoacetyl-6-methoxynaphthalene (mp 109-112°) (Chromatographia 1992, 33, 13).)

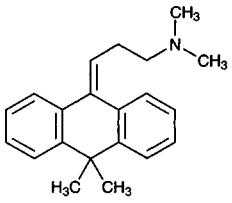
HPLC VARIABLES
Column: 250 × 4.6 Hypersil 5 ODS
Mobile phase: MeCN:MeOH:THF:water 35.75:26:3.25:35
Column temperature: 35
Flow rate: 1.2
Injection volume: 50
Detector: F ex 300 em 460

CHROMATOGRAM
Retention time: 12
Internal standard: n-hexanoic acid 6-methoxynaphthacylester (15) [Prepare by dissolving 2 mmoles n-hexanoic acid and 1 mmole 2-bromoacetyl-6-methoxynaphthalene in 10 mL anhydrous MeCN, add 0.5 mL triethylamine, heat at 60° for 30 min, cool, dilute with 30 mL water, extract three times with 10 mL portions of diethyl ether. Combine the extracts, wash with 5% sodium bicarbonate, wash three times with 10 mL portions of water, dry over anhydrous sodium sulfate, evaporate under reduced pressure, recrystallize from MeOH/water.]

KEY WORDS
tablets; derivatization

REFERENCE
Gatti,R.; Andrisano,V.; Di Pietra,A.M.; Cavrini,V. Analysis of aliphatic dicarboxylic acids in pharmaceuticals and cosmetics by liquid chromatography (HPLC) with fluorescence detection, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 589–595.

Melitracen



Molecular formula: $C_{21}H_{25}N$
Molecular weight: 291.44
CAS Registry No.: 5118-29-6, 10563-70-9 (HCl)
Merck Index: 5866

SAMPLE

Matrix: blood, tissue, urine

Sample preparation: Serum, urine. 500 µL Serum or urine + 100 µL 2 µg/mL diazepam + 200 µL 20% sodium carbonate + 500 µL water + 3 mL n-hexane:isoamyl alcohol 98.5:1.5, mix for 2 min, centrifuge at 1200 g for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 µL mobile phase, inject a 10 µL aliquot. Tissue. Homogenize 1 g sample with 9 mL 100 mM HCl and 100 µL 20 µg/mL diazepam, centrifuge at 15000 g for 10 min. Add 500 µL 20% sodium carbonate and 4 mL n-hexane:isoamyl alcohol 98.5:1.5 to 1 mL of the supernatant, mix for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 µL mobile phase, filter by microconcentrator (Microcon-30, Grace). Inject a 10 µL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 2 µm TSK gel Super-Octyl (A) or 100 × 4.6 5 µm Hypersil MOS-C8 (B), (Yokogawa, Japan)
Mobile phase: MeOH:20 mM pH 7 KH_2PO_4 60:40
Flow rate: 0.6
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 19.1 (A), 33.8 (B)
Internal standard: diazepam (4.4, A)
Limit of quantitation: 50 ng/mL (serum, urine) (A), 500 ng/mL (tissue) (A)

OTHER SUBSTANCES

Extracted: amitriptyline, amoxapine, clomipramine, desipramine, dothiepin, doxepin, imipramine, maprotiline, mianserin, nortriptyline
Noninterfering: barbital, carbamazepine, ethosuximide, hexobarbital, lofepramine, pentobarbital, phenobarbital, phenytoin, primidone, sulpiride, trimethadione, trimipramine

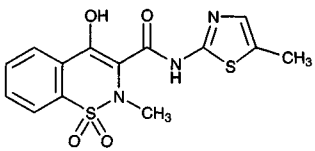
KEY WORDS

serum; brain; liver

REFERENCE

Tanaka,E.; Terada,M.; Nakamura,T.; Misawa,S.; Wakasugi,C. Forensic analysis of eleven cyclic antidepressants in human biological samples using a new reversed-phase chromatographic column of 2 µm porous microspherical silica gel, *J.Chromatogr.B*, **1997**, *692*, 405–412.

Meloxicam



Molecular formula: $C_{14}H_{13}N_3O_4S_2$
Molecular weight: 351.41
CAS Registry No.: 71125-38-7
Merck Index: 5869

SAMPLE

Matrix: blood

Sample preparation: Inject 50 μL plasma onto column A and elute to waste with mobile phase A, after 4 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 Bondapak C18 Corasil; B 125 × 4.6 Hypersil ODS
Mobile phase: A 5 mM sulfuric acid; B MeCN:MeOH:water:glacial acid 5:60:50:2 containing 0.86 g/L heptanesulfonic acid
Detector: UV 355

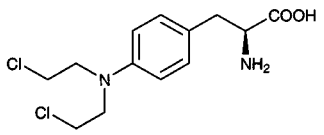
CHROMATOGRAM
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
baboon; dog; human; kidney; liver; mouse; pharmacokinetics; pig; plasma; rat; column-switching

REFERENCE
Busch,U.; Schmid,J.; Heinzel,G.; Schmaus,H.; Baierl,J.; Huber,C.; Roth,W. Pharmacokinetics of meloxicam in animals and the relevance to humans, *Drug Metab.Dispos.*, **1998**, *26*, 576–584.

Melphalan



Molecular formula: $C_{13}H_{18}Cl_2N_2O_2$
Molecular weight: 305.20
CAS Registry No.: 148-82-3
Merck Index: 5871
Lednicer No.: 2 120

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 5 μg dansylproline + 2 mL ice-cold MeOH, vortex for 20 s, freeze in dry ice/acetone for 3 min, centrifuge at 3000 rpm for 3 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: micro C18 (Waters)
Mobile phase: MeOH:water:acetic acid 50:50:1
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 4
Internal standard: dansylproline (8)
Limit of detection: 50 ng/mL

KEY WORDS
plasma; rat; human; pharmacokinetics

REFERENCE
Chang,S.Y.; Alberts,D.S.; Melnick,L.R.; Walson,P.D.; Salmon,S.E. High-pressure liquid chromatographic analysis of melphalan in plasma, *J.Pharm.Sci.*, **1978**, *67*, 679–681.

SAMPLE
Matrix: blood

Sample preparation: 1 mL Plasma + 500 μL 5% trichloroacetic acid, vortex, centrifuge, filter (0.45 μm) the supernatant, inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 2.6 10 μm HCODS/SIL X-C18 (Perkin-Elmer)
Mobile phase: Gradient. MeCN:17.5 mM acetic acid from 12:88 to 80:20 over 14 min (concave gradient).
Column temperature: 50
Flow rate: 1.5
Injection volume: 10
Detector: UV 263

CHROMATOGRAM
Retention time: 12
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; human; rat; pharmacokinetics

REFERENCE
Ahmed,A.E.; Hsu,T.-F. Quantitative analysis of melphalan and its major hydrolysate in patients and animals by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *222*, 453–460.

SAMPLE
Matrix: blood
Sample preparation: Wash Amberlite XAD-2 thoroughly with acetone and then MeOH until absorbance of washings was less than 0.1 a.u. at 254 nm. Prepare a 25 × 5 column of washed Amberlite XAD-2 in a 5 mL pipette and wash it with 10 mL acetone, with 10 mL of MeOH, and with 10 mL of water. 1 mL Plasma + 500 ng IS, mix, add to the column, wash with 10 mL water, elute with 1.5 mL MeOH. Centrifuge the eluate at 12000 g for 3 min (or filter (0.45 μm PTFE) and inject a 200 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS
Mobile phase: MeOH:675 μg/mL sodium dodecyl sulfate 80:20 adjusted to pH 3.0 with concentrated sulfuric acid
Column temperature: 40
Injection volume: 200
Detector: UV 254 or UV 263

CHROMATOGRAM
Retention time: 9
Internal standard: Dns-arginine (Nα(5-dimethylaminonaphthalene)-1-sulfonyl)-L-arginine) (11.5)
Limit of detection: 5 ng/mL

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Bosanquet,A.G.; Gilby,E.D. Measurement of plasma melphalan at therapeutic concentrations using isocratic high-performance liquid chromatography, *J.Chromatogr.*, **1982**, *232*, 345–354.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 44 μL cold concentrated perchloric acid, mix vigorously for 10 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: MeOH:2% acetic acid (pH 3.6) 22:78
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Retention time: 18
Limit of detection: 10 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Davis,T.P.; Peng,Y.-M.; Goodman,G.E.; Alberts,D.S. HPLC, MS, and pharmacokinetics of melphalan, bisantrene and 13-cis retinoic acid, *J.Chromatogr.Sci.*, **1982**, *20*, 511–516.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 250 mM trichloroacetic acid, mix at 4°, let stand at 4° for 30 min, centrifuge at 1500 g for 10 min. Remove a 1 mL aliquot of the supernatant and add it to 200 μL 1 M N-acetylcysteine, 200 μL 2.65 M NaOH, and 200 μL 500 mM pH 11.0 phosphate buffer, mix, heat at 70° for 15 min, add 200 μL 2 M citric acid, filter (0.2 μm), inject a 100 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 4 5 μm Nova-Pak C18 Radial-Pak
Mobile phase: EtOH:36 mM pH 4.25 citrate buffer containing 50 μM octanesulfonic acid 4:96 (The mobile phase flowed through a 100 × 4 column of μBondapak C18 before the injector.)
Injection volume: 100
Detector: F ex 260 em 360

CHROMATOGRAM
Retention time: 13
Limit of quantitation: 5 ng/mL

KEY WORDS
derivatization; plasma; pharmacokinetics

REFERENCE
Ehrsson,H.; Eksborg,S.; Lindfors,A. Quantitative determination of melphalan in plasma by liquid chromatography after derivatization with N-acetylcysteine, *J.Chromatogr.*, **1986**, *380*, 222–228.

SAMPLE
Matrix: blood
Sample preparation: Condition a 2.4 mL 500 mg Bond Elut phenyl SPE cartridge with 2 mL MeOH and 2 mL water. 1 mL Plasma + 100 μL freshly prepared 100 mg/mL diethyldithiocarbamic acid in 100 mM NaOH, heat at 50° for 90 min, add to the SPE cartridge, wash with 5 mL water, allow to dry in the dark at room temperature for 1 h, elute with 2 mL MeCN. Evaporate the eluate to dryness under reduced pressure at 40°, reconstitute in 200 μL MeOH, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.8 10 μm μBondapak C18
Mobile phase: Gradient. MeCN:5 mM pH 3.0 orthophosphoric acid 30:70 for 4 min, to 100:0 over 9 min, return to initial conditions over 7 min, re-equilibrate for 5 min.
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: UV 276

CHROMATOGRAM
Retention time: 14.6 (derivatized), 7.6 (underivatized)
Limit of detection: 2 ng/mL

KEY WORDS
plasma; derivatization; SPE

REFERENCE
Cummings,J.; MacLellan,A.; Smyth,J.F.; Farmer,P.B. Determination of reactive nitrogen mustard anticancer drugs in plasma by high-performance liquid chromatography using derivatization, *Anal.Chem.*, **1991**, *63*, 1514–1519.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 60% perchloric acid, vortex for 30 s, centrifuge at 4° at 2000 g for 10 min. Remove 700 μL of the supernatant and add it to 2 mL chloroform, vortex for 30 s, centrifuge at 4° at 2000 g for 10 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4 5 μm Spheri 5 phenyl
Mobile phase: MeCN:100 mM pH 4.5 sodium phosphate buffer 12:88
Flow rate: 1.2
Injection volume: 50
Detector: E, ESA Coulochem 5100A, ESA 5011 analytical cell, upstream electrode 0.1 V, downstream electrode 0.6 V monitored

CHROMATOGRAM
Retention time: 15.5
Limit of detection: 2 ng/mL
Limit of quantitation: 5 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Silvestro,L.; Viano,I.; Baiocchi,C.; Saini,G.; Marmont,F.; Ferro,R. Quantitation of melphalan in plasma of patients by reversed-phase high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1991**, *563*, 443–450.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 261

CHROMATOGRAM
Retention time: 3.93
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromet azine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, perfusate, tissue

Sample preparation: Plasma, perfusate. 100 μL Plasma or perfusate + 200 μL cold 38 μg/mL dansylarginine in MeOH, vortex at 4° for 30 s, centrifuge at 10000 g for 15 min, inject a 20 μL aliquot of the supernatant. (Keep all samples and reagents on ice.) Tissue. Sonicate (3 mm, Sonics and Materials, Danbury, CT) 100 mg minced tissue and 400 μL 38 μg/mL dansylarginine in MeOH on ice for 1 min, centrifuge at 10000 g for 15 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Alltima phenyl (Alltech)

Mobile phase: MeOH:water:glacial acetic acid 25:75:2, pH 2.7 containing 500 μg/mL 1-octanesulfonic acid

Flow rate: 2

Injection volume: 20

Detector: F ex 265 em 360

CHROMATOGRAM

Retention time: 8.4

Internal standard: dansylarginine (F ex 265 em 575) (12.6)

Limit of quantitation: 7.2 ng (tissue), 1.4 ng (plasma, perfusate)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; human; rat; fat; skin; muscle; pharmacokinetics

REFERENCE
Wu,Z.-Y.; Thompson,M.J.; Roberts,M.S.; Addison,R.S.; Cannell,G.R.; Grabs,A.J.; Smithers,B.M. High-performance liquid chromatographic assay for the measurement of melphalan and its hydrolysis products in perfusate and melphalan in tissues from human and rat isolated limb perfusions, *J.Chromatogr.B*, **1995**, *673*, 267–279.

SAMPLE
Matrix: blood, tissue
Sample preparation: Allow whole blood to clot. Homogenize <500 mg mouse tissue or clotted blood with 500 μL 10% perchloric acid in 2-methoxyethanol:0.1% acetic acid 55:45. Homogenize >500 mg mouse tissue or clotted blood with 1 mL 10% perchloric acid in 2-methoxyethanol: 0.1% acetic acid 55:45. Homogenize 1 g dog tissue with 1 mL 10% perchloric acid in 2-methoxyethanol:0.1% acetic acid 55:45. Centrifuge homogenate at 2000 g and mix 2 volumes of the supernatant with 1 volume 1.0 M KH_2PO_4. Centrifuge, filter (Millipore Pellicon molecular filter with 25000 dalton cut-off) the supernatant under 3.7 atmospheres of nitrogen, inject a 5 μL aliquot of the filtrate.

HPLC VARIABLES
Column: μ Bondapak C18
Mobile phase: Gradient. A:B 85:15 for 1 min, to 15:85 (step gradient), maintain at 15:85. A was 2-methoxyethanol:0.1% acetic acid 30:70. B was 2-methoxyethanol:0.1% acetic acid 55:45.
Flow rate: 1.5
Injection volume: 5
Detector: UV (wavelength not given)

CHROMATOGRAM
Retention time: 9.15
Limit of quantitation: 10 ng/mL

KEY WORDS
whole blood; serum; bile; brain; testes; muscle; kidney; liver; heart; spleen; stomach; intestine; lung; ovary; adrenal gland; pancreas; fat; bladder; dog; mouse; pharmacokinetics

REFERENCE
Furner,R.L.; Mellett,L.B.; Brown,R.K.; Duncan,G. A method for the measurement of L-phenylalanine mustard in the mouse and dog by high-pressure liquid chromatography, *Drug Metab.Dispos.*, **1976**, *4*, 577–583.

SAMPLE
Matrix: blood, tissue
Sample preparation: Vortex up to 3 mL plasma or tissue homogenate with 2 volumes of chilled MeOH at 4° for 20 s, centrifuge at 1500 g for 15 min, repeat the extraction with another volume of MeOH. Combine the supernatants and add them to 4 volumes of ice cold water (i.e., MeOH concentration is 15%). Pass this mixture through a Sep-Pak C18 SPE cartridge, wash with 10 mL ice-cold MeOH:water 15:85, elute with 2 mL MeOH, discard the first 400 μL, collect the rest, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.9 5 μm Spherisorb S5 ODS
Mobile phase: MeOH:water 40:60
Column temperature: 60
Flow rate: 3
Detector: F ex 260 em 350

CHROMATOGRAM
Limit of detection: <5 ng/mL

KEY WORDS
plasma; rat; kidney; liver; SPE

REFERENCE
Egan,C.M.; Jones,C.R.; McCluskey,M. Method for the measurement of melphalan in biological samples by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1981**, *224*, 338–342.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 201.7

CHROMATOGRAM
Retention time: 12.93

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: reaction mixtures
Sample preparation: Mix an aliquot with an equal volume of 20 mM pH 4.4 KH_2PO_4, centrifuge, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Microsorb C8
Mobile phase: MeOH:20 mM pH 4.4 KH_2PO_4 58:42
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7.8
Limit of detection: 2000 ng/mL

REFERENCE
Lunn,G.; Sansone,E.B.; Andrews,A.W.; Hellwig,L.C. Degradation and disposal of some antineoplastic drugs, *J.Pharm.Sci.*, **1989**, *78*, 652–659.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Resolve C18 (Waters)
Mobile phase: MeCN:MeOH:triethylamine:200 mM ammonium acetate 18:2:0.5:79.5 pH adjusted to 3.8 with acetic acid
Flow rate: 1.4
Injection volume: 50
Detector: F ex 265 em 345

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Simultaneous: pentamidine

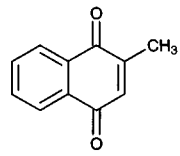
REFERENCE
Yeh,T.-K.; Dalton,J.T.; Au,J.L.-S. High-performance liquid chromatographic determination of pentamidine in plasma, *J.Chromatogr.*, **1993**, *622*, 255–261.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 500 μL aliquot of a solution in 10 mM pH 7.57 potassium phosphate buffer.

HPLC VARIABLES
Column: 100 × 10 15 μm Pharmacia PepRPC C18
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.1% trifluoroacetic acid in MeCN. A:B 100:0 for 4 min, to 80:20 over 32 min, to 0:100 (step gradient).
Flow rate: 5
Injection volume: 500
Detector: UV 260

REFERENCE
Awasthi,S.; Bajpai,K.K.; Piper,J.T.; Singhal,S.S.; Ballatire,A.; Seifert,W.E.,Jr.; Awasthi,Y.C.; Ansari,G.A.S. Interactions of melphalan with glutathione and the role of glutathione *S*-transferase, *Drug Metab.Dispos.*, **1996**, *24*, 371–374.

Menadione



Molecular formula: $C_{11}H_8O_2$
Molecular weight: 172.18
CAS Registry No.: 58-27-5
Merck Index: 5874

SAMPLE
Matrix: blood
Sample preparation: 800 μL Plasma + 100 μL 35 μg/mL carbazole in mobile phase + 6 mL n-hexane, rotate for 45 min, centrifuge at 1080 g for 15 min, freeze at -76° for 30 min. Remove the organic layer and add it to 200 μL MeOH:water 70:30, evaporate under a stream of nitrogen, inject an aliquot.

HPLC VARIABLES
Guard column: 10 μm Bondapak C18

Column: 250 × 4.6 μm Ultrasphere ODS
Mobile phase: MeOH:water 70:30
Flow rate: 0.8
Detector: UV 265

CHROMATOGRAM
Retention time: 8.7
Internal standard: carbazole (12.5)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: menadiol, metabolites

KEY WORDS
plasma; protect from light; rabbit; pharmacokinetics

REFERENCE
Hu,O.Y.-P.; Wu,C.-Y.; Chan,W.-K.; Wu,F.Y.-H. Determination of anticancer drug vitamin K3 in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *666*, 299–305.

SAMPLE
Matrix: feed
Sample preparation: Grind feed to pass 20 mesh sieve. Feed + 100 mL chloroform, shake for 3 min, add 5 mL 25% ammonium hydroxide, shake for 3 min, add 10 g Celite:anhydrous sodium sulfate 3:10 w/w, shake for 20 min, neutralize with acetic acid, shake mechanically for 20 min, centrifuge at 2500 rpm for 10 min. Either dilute chloroform layer with chloroform and inject a 40-100 μL aliquot or evaporate chloroform layer to dryness under reduced pressure at 40°, reconstitute in 1,2-dichloroethane, inject a 40 -100 μL aliquot.

HPLC VARIABLES
Guard column: Guard-Pak Resolve Si (waters)
Column: 250 × 4 5 μm Lichrosorb Si 60
Mobile phase: 1,2-Dichloroethane
Column temperature: 45
Flow rate: 1.8
Injection volume: 40-100
Detector: UV 251

CHROMATOGRAM
Retention time: 3.08
Limit of detection: 2.5 ppm

KEY WORDS
normal phase; protect from light

REFERENCE
Laffi,R.; Marchetti,S.; Marchetti,M. Normal-phase liquid chromatographic determination of menadione in animal feeds, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 826–828.

SAMPLE
Matrix: feed
Sample preparation: Extract 500 mg feed in a 152 × 19 stainless steel tube with supercritical carbon dioxide at 65° at 8000 psi for 20 min, the effluent is passed through a 152 × 6.35 column of silica gel. Elute the silica gel with 10 mL dichloromethane and evaporate the eluate to dryness under reduced pressure, reconstitute in 1 mL MeCN, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:25 mM sodium perchlorate 90:10
Flow rate: 2
Injection volume: 20

Detector: E, polished silver working electrode -0.75 V, calomel reference electrode

CHROMATOGRAM
Retention time: 2.5
Limit of detection: 125 pg

KEY WORDS
SFE

REFERENCE
Schneiderman,M.A.; Sharma,A.K.; Locke,D.C. Determination of menadione in an animal feed using supercritical fluid extraction and HPLC with electrochemical detector, *J.Chromatogr.Sci.*, **1988**, *26*, 458–462.

SAMPLE
Matrix: feed
Sample preparation: 1 g Ground feed + 10 mL MeOH:water 40:60, shake for 30 min on a mechanical shaker, centrifuge at 1000 g for 10 min. Remove 5 mL of the supernatant and add it to 10 mL 5% sodium carbonate and 10 mL n-pentane, shake for 1 min, centrifuge at 1000 g for 1 min, repeat extraction twice. Combine the n-pentane layers and evaporate to dryness under reduced pressure at room temperature, reconstitute the residue in 10 mL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeOH:water 75:25
Flow rate: 0.9
Injection volume: 10
Detector: F ex 325 em 425 following post-column reduction. The effluent from the column flowed through a 20 × 2 column packed with powdered zinc (≤ 38 μm) and a 0.5 μm filter and then to the detector.

CHROMATOGRAM
Retention time: 6
Limit of quantitation: 1 ng

KEY WORDS
post-column reaction; also for menadione sodium bisulfite

REFERENCE
Billedeau,S.M. Fluorimetric determination of vitamin K3 (menadione sodium bisulfite) in synthetic animal feed by high-performance liquid chromatography using a post-column zinc reducer, *J.Chromatogr.*, **1989**, *472*, 371–379.

SAMPLE
Matrix: feed, premix
Sample preparation: 1-10 g Ground feed or premix + 96 mL EtOH:water 40:60, shake for 10 min, add 4 mL 10% tannin solution, shake for 1 min, centrifuge at 2000 g for 5 min, filter through a fine-pore glass filter. Add a 40 mL portion of the filtrate to 50 mL n-hexane and 20 mL 10% sodium carbonate, shake for 1 min, discard the lower layer. Wash the n-hexane layer twice with 100 mL portions of water and dry it with strips of blue-ribbon filter paper. Evaporate an aliquot to dryness under nitrogen with a rotary evaporator, reconstitute in mobile phase, sonicate for 1 min, filter (0.2 μm) (if necessary), inject a 100 μL aliquot. (Use a brown glass separatory funnel.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm ODS-Hypersil
Mobile phase: EtOH:water 60:40
Flow rate: 0.6
Injection volume: 100
Detector: F ex 325 em 425 following post-column reduction. The reagent passed through a debubbler was mixed with the effluent at 0.27 mL/min, the combined solution flowed through a

1.4 m long stainless steel reaction coil to the detector. (Reagent was 0.8 g/L sodium borohydride in EtOH, sonicate for 5 min, stable for 2 h.)

CHROMATOGRAM
Retention time: 12
Limit of quantitation: 20 ng/g

KEY WORDS
protect from light; post-column reaction

REFERENCE
Speek,A.J.; Schrijver,J.; Schreurs,W.H.P. Fluorimetric determination of menadione sodium bisulphite (vitamin K3) in animal feed and premixes by high-performance liquid chromatography with post-column derivatization, *J.Chromatogr.*, **1984**, *301*, 441–447.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out 500 mg ground tablets, extract with water, make up to 50 or 100 mL with water, filter, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 10 C18
Mobile phase: MeOH:1% acetic acid 25:75
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 12.5 (menadione hydrogen sulfite)

OTHER SUBSTANCES
Simultaneous: niacinamide, pyridoxine, riboflavin, thiamine, ascorbic acid

KEY WORDS
tablets; multi-vitamin

REFERENCE
Sadlej-Sosnowska,N.; Blitek,D.; Wilczynska-Wojtulewicz,I. Determination of menadione sodium hydrogen sulphite and nicotinamide in multivitamin formulations by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *357*, 227–232.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in EtOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 5 μm Shim-pack CLC-ODS (Shimadzu)
Mobile phase: MeOH:water 85:15 containing 50 mM sodium perchlorate
Flow rate: 1
Injection volume: 20
Detector: E, EICOM ECD 100, glassy carbon working electrode +0.7 V, Ag/AgCl reference electrode following post-column catalytic reduction. The column effluent flowed through a 10 × 4.6 column packed with 10 μm 5% platinum on alumina catalyst (Toa Electronics) to the detector. (Purge catalyst column with water at 10 mL/min for 5 min before use.)

CHROMATOGRAM
Retention time: 4.9

OTHER SUBSTANCES
Simultaneous: idebenone

KEY WORDS
post-column reaction

REFERENCE
Wakabayashi,H.; Nakajima,M.; Yamato,S.; Shimada,K. Determination of idebenone in rat serum and brain by high-performance liquid chromatography using platinum catalyst reduction and electrochemical detection, *J.Chromatogr.*, **1992**, *573*, 154–157.

Menotropins

CAS Registry No.: 9002-68-0
Merck Index: 4299

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in 100 mM NaH_2PO_4 adjusted to pH 2.1 with orthophosphoric acid, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 Aquapore RP 300 (Kontron)
Mobile phase: Gradient. A was 100 mM NaH_2PO_4 adjusted to pH 2.1 with orthophosphoric acid. B was MeOH. A:B from 90:10 to 35:65 over 180 min.
Flow rate: 1
Injection volume: 100
Detector: UV 225

CHROMATOGRAM
Retention time: 4 (LH), 18 (FSH)

OTHER SUBSTANCES
Simultaneous: adrenocorticotropin hormone and fragments, lipotropic hormone and fragments, melanotropin, endorphins, prolactin, somatropin

REFERENCE
Richter,W.O.; Schwandt,P. Separation of neuropeptides by HPLC: evaluation of different supports, with analytical and preparative applications to human and porcine neurophysins, β-lipotropin, adrenocorticotropic hormone, and β-endorphin, *J.Neurochem.*, **1985**, *44*, 1697–1703.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in 100 mM pH 7.0 sodium phosphate buffer, inject a 25 μL aliquot.

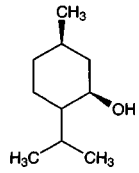
HPLC VARIABLES
Column: 100 × 4.6 6.5 μm 300 Å C1 SynChropak RP-1 + 250 × 10 5 μm 300 Å Vydac C4
Mobile phase: Gradient. A was 100 mM triethylamine phosphate in water adjusted to pH 6.5 with triethylamine. B was a 100 mM triethylamine phosphate in MeCN:water 60:40 adjusted to pH 6.5 with triethylamine. A:B from 80:20 to 0:100 over 60 min.
Flow rate: 0.8
Injection volume: 25
Detector: UV 226

CHROMATOGRAM
Retention time: 32

OTHER SUBSTANCES
Simultaneous: luteinizing hormone, thyroid-stimulating hormone

REFERENCE
Hiyama,J.; Renwick,A.G.C. Separation of human glycoprotein hormones and their subunits by reversed-phase liquid chromatography, *J.Chromatogr.*, **1990**, *529*, 33–41.

Menthol



Molecular formula: $C_{10}H_{20}O$
Molecular weight: 156.27
CAS Registry No.: 1490-04-6, 89-78-1
Merck Index: 5882

SAMPLE
Matrix: formulations
Sample preparation: Powder, granules. Dissolve the powder or granules in a small volume of MeCN, sonicate for 30 min, centrifuge at 2500 rpm for 5 min, dilute 2-fold with water, pass through a Sep-pak C18 SPE cartridge, filter(0.45 μm), inject a 100 μL aliquot. Lotion. Dilute lotion 2-fold with water, filter(0.45 μm), inject a 100 μL aliquot. Plaster. Extract plasters by sonication with MeCN for 30 min., filter (0.45 μm), inject a 100 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 J'sphere ODS-H80 (YMC)
Mobile phase: MeCN:water 60:40
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: UV 210; Polarized photometric detector, SPD-10 AV UV-VIS detector with HN32 polarizers (Polaroid, MA), 530 nm, angle+1.0 rad

CHROMATOGRAM
Retention time: 7.5
Limit of detection: 500 ng

KEY WORDS
chiral; comparison with GC; SPE; polarized photometric detection; powders; granules; lotion; plasters

REFERENCE
Hamasaki,K.; Kato,K.; Watanabe,T.; Yoshimura,Y.; Nakazawa,H.; Yamamoto,A.; Matsunaga,A. Determination of l-menthol in pharmaceutical products by high performance liquid chromatography with polarized photometric detection, *J.Pharm.Biomed.Anal.*, **1998**, *16*, 1275–1280.

SAMPLE
Matrix: formulations
Sample preparation: Shake 2 g powder with 10 mL EtOH for 1 h, directly inject 1 mL of this solution using a syringe-coupled nylon filter (Teknokroma).

HPLC VARIABLES
Column: 125 × 4 5 μm Aluspher RP Select B (E. Merck) (alumina particles bonded with polybutadiene)
Mobile phase: MeOH:water:diammonium phosphate 30:70:0.2 (v:v:w), pH 8.2
Flow rate: 1
Injection volume: 20
Detector: UV 191

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: benzocaine, tyrothricin
Noninterfering: gramicidin

REFERENCE
Caraballo,I.; Fernandez-Arevalo,M.; Holgado,M.-A.; Vela,M.-T.; Rabasco,A.-M. A rapid HPLC method for the quantification of tyrothricin, menthol, and benzocaine in pharmaceutical formulations, *J.Pharm.Sci.*, **1994**, *83*, 1147–1149.

SAMPLE
Matrix: solutions

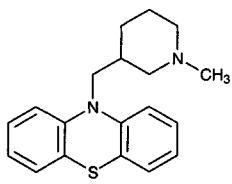
HPLC VARIABLES
Column: 250 × 4.6 Zorbax SAX
Mobile phase: MeOH:buffer 50:50 (Buffer was 180 mM Na_2HPO_4 adjusted to pH 3.00 ± 0.05 with 180 mM orthophosphoric acid. Pass mobile phase through a 250 × 4.6 25-40 μm silica (HPLC Technology) column to saturate it with silica.)
Flow rate: 1
Detector: UV 253

CHROMATOGRAM
Retention time: 2.8

OTHER SUBSTANCES
Simultaneous: cromolyn, minocromil, nedocromil, quinoline yellow, saccharin, salicylic acid
Interfering: acetaminophen, albuterol, aspartame, aspirin, beclomethasone dipropionate, caffeine, isoproterenol, 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.

Mepazine



Molecular formula: $C_{19}H_{22}N_2S$
Molecular weight: 310.46
CAS Registry No.: 60-89-9
Merck Index: 5892

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 4.4

OTHER SUBSTANCES
Simultaneous: benzocaine, tyrothricin
Noninterfering: gramicidin

REFERENCE
Caraballo,I.; Fernandez-Arevalo,M.; Holgado,M.-A.; Vela,M.-T.; Rabasco,A.-M. A rapid HPLC method for the quantification of tyrothricin, menthol, and benzocaine in pharmaceutical formulations, *J.Pharm.Sci.*, **1994**, *83*, 1147–1149.

SAMPLE
Matrix: solutions

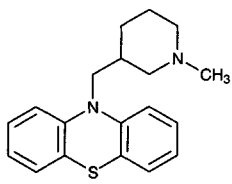
HPLC VARIABLES
Column: 250 × 4.6 Zorbax SAX
Mobile phase: MeOH:buffer 50:50 (Buffer was 180 mM Na_2HPO_4 adjusted to pH 3.00 ± 0.05 with 180 mM orthophosphoric acid. Pass mobile phase through a 250 × 4.6 25-40 μm silica (HPLC Technology) column to saturate it with silica.)
Flow rate: 1
Detector: UV 253

CHROMATOGRAM
Retention time: 2.8

OTHER SUBSTANCES
Simultaneous: cromolyn, minocromil, nedocromil, quinoline yellow, saccharin, salicylic acid
Interfering: acetaminophen, albuterol, aspartame, aspirin, beclomethasone dipropionate, caffeine, isoproterenol, 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.

Mepazine



Molecular formula: $C_{19}H_{22}N_2S$
Molecular weight: 310.46
CAS Registry No.: 60-89-9
Merck Index: 5892

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 4.4

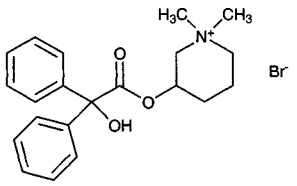
OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Mepenzolate bromide



Molecular formula: $C_{21}H_{26}BrNO_3$
Molecular weight: 420.35
CAS Registry No.: 76-90-4
Merck Index: 5893

SAMPLE

Matrix: urine

Sample preparation: Condition a 500 mg 14 mL 40 μm CCX-2 cation-exchange SPE cartridge (Worldwide Monitoring) with two 2.5 mL aliquots of MeOH, two 2.5 mL aliquots of water, and two 2.5 mL aliquots of 100 mM pH 7.00 phosphate buffer, do not allow to dry. 5 mL Urine + 3 mL 100 mM pH 7.00 phosphate buffer + 5 mL water, centrifuge at 800 g for 5 min, add to the SPE cartridge, wash with 5 mL MeOH, wash with 5 mL water, dry under vacuum for 5 min, elute with 4 mL MeOH:500 mM pH 3.00 ammonium acetate 95:5 (all flow rates were 1-2 mL/min). Evaporate the eluate under a stream of nitrogen at 60°, reconstitute in 100 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.1 10 μm LiChroma (Chromatographic Specialties)
Mobile phase: MeOH:50 mM pH 3.0 ammonium acetate 80:20
Flow rate: 0.8
Injection volume: 10
Detector: MS, Sciex API III triple quadrupole, ion spray interface, split column effluent 95:5 before entering detector, nebulizing gas air at 550 kPa, collision gas argon, curtain gas nitrogen, positive-ion mode, m/z 340 and 130

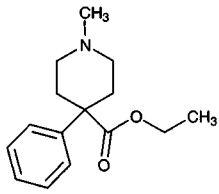
CHROMATOGRAM
Retention time: 2.1
Internal standard: mepenzolate

OTHER SUBSTANCES
Extracted: glycopyrrolate

KEY WORDS
SPE; horse; mepenzolate is IS

REFERENCE
Matassa,L.C.; Woodard,D.; Leavitt,R.K.; Firby,P.; Beaumier,P. Solid-phase extraction techniques for the determination of glycopyrrolate from equine urine by liquid chromatography-tandem mass spectrometry and gas chromatography-mass spectrometry, *J.Chromatogr.*, **1992**, *573*, 43–48.

Meperidine



Molecular formula: $C_{15}H_{21}NO_2$
Molecular weight: 247.34
CAS Registry No.: 57-42-1, 50-13-5 (HCl)
Merck Index: 5894
Lednicer No.: 1 300

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 200 ng doxepin or desipramine + 100 μL 1 M NaOH + 9 mL freshly prepared hexane:isoamyl alcohol 99:1, shake vigorously for 5 min, centrifuge. Remove 8.5 mL of the organic phase and add it to 200 μL 50 mM HCl, shake well for 1 min, centrifuge, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: 300 × 4 μBondapak phenyl
Mobile phase: MeCN:0.01% phosphoric acid containing 0.01% NaCl 35:65, final pH 2.8
Flow rate: 1.5
Injection volume: 50
Detector: UV 210

CHROMATOGRAM
Retention time: 5.4
Internal standard: doxepin (12.2), desipramine (14.2)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: cocaine, dextromoramide, methadone, normeperidine, norpropoxyphene, pentazocine, propoxyphene
Simultaneous: amitriptyline, buprenorphine, chlorpromazine, codeine, desmethyldoxepin, diphenhydramine, ephedrine, imipramine, nortriptyline, oxazepam, oxycodone, pericyazine, pheniramine, propranolol, quinine, thiopropazate, thioridazine

KEY WORDS
serum

REFERENCE
Hackett,L.P.; Dusci,L.J.; Ilett,K.F. The analysis of several nonopiate narcotic analgesics and cocaine in serum using high-performance liquid chromatography, *J.Anal.Toxicol.*, **1987**, *11*, 269–271.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL BondElut C18 SPE cartridge once with 1 M HCl, twice with MeOH, and once with water, remove the liquid completely with suction each time. Add 250 μL IS solution and 250 μL serum to the column at 1 mL/min, wash twice with water and once with MeCN draining the column completely after each wash, elute with 250 μL eluting solution, centrifuge for 20 s to remove last of eluate, inject a 5 μL aliquot of the eluate. (Prepare IS solution by adding 40 μL 1 mg/mL N-pentyl-2,6-pipecoloxylidide (1-pentyl-N-(2,6-dimethylphenyl)-2-piperidinecarboxamide, pentyl-PPX) in MeOH to 10 mL 100 mM NaH_2PO_4. Eluting solution was 2.5 mL 35% perchloric acid in 100 mL MeOH.)

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP-8 (Applied Biosystems)
Column: 150 × 4.6 5 μm Ultrasphere octyl
Mobile phase: MeCN:10 mM KH_2PO_4 25:80, pH 5.2
Flow rate: 1.5
Injection volume: 5
Detector: UV 205

CHROMATOGRAM
Retention time: 4.3
Internal standard: N-pentyl-2,6-pipecoloxylidide (1-pentyl-N-(2,6-dimethylphenyl)-2-piperidinecarboxamide, pentyl-PPX) (14.5)

OTHER SUBSTANCES
Extracted: bupivacaine, mepivacaine, fentanyl
Noninterfering: acetaminophen, codeine, epinephrine, morphine, diazepam

KEY WORDS
serum; SPE

REFERENCE
Gupta,R.N.; Dauphin,A. Column liquid chromatographic determination of bupivacaine in human serum using solid-phase extraction, *J.Chromatogr.B*, **1994**, *658*, 113–119.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 259

CHROMATOGRAM
Retention time: 4.55
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE
Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE
Matrix: blood tissue
Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30

Detector: UV 220

CHROMATOGRAM
Retention time: 4.89
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES
Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, imipramine, loxapine, maprotiline, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine
Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine
Interfering: haloperidol, norpropoxyphene, northiaden

KEY WORDS
serum; whole blood; liver

REFERENCE
McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. 1 mL Serum + 100 μL 1 μg/mL diphenhydramine and 1 μg/mL nordiphenhydramine in 1 mM orthophosphoric acid + 100 μL 1 M NaOH + 5 mL hexane, rotate at 60 rpm for 15 min, centrifuge. Remove 4 mL of the organic layer and add it to 80 μL 1 mM orthophosphoric acid, vortex vigorously for 30 s, inject a 50 μL aliquot of the aqueous phase. Urine. Dilute 1:10 with drug-free urine. 1 mL Diluted urine + 100 μL 1 μg/mL diphenhydramine and 1 μg/mL nordiphenhydramine in 1 mM orthophosphoric acid + 1 mL saturated sodium borate (pH 10.2) + 5 mL hexane, rotate at 60 rpm for 15 min, centrifuge. Remove 4 mL of the organic layer and add it to 80 μL 1 mM orthophosphoric acid, vortex vigorously for 30 s, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelguard LC-CN cyanopropyl (Supelcosil)
Column: 150 × 4.6 5 μm Supelcosil LC-PCN cyanopropyl
Mobile phase: MeCN:MeOH:buffer 55:20:25 (Buffer was 2.6 g K_2HPO_4 in 1 L water, pH adjusted to 7.0 with 900 mM orthophosphoric acid.) (Optimize the separation by adjusting the pH of the mobile phase with a few drops of 1 M NaOH or 900 mM orthophosphoric acid.)
Flow rate: 2.5
Injection volume: 50
Detector: UV 205

CHROMATOGRAM
Retention time: 1.5
Internal standard: diphenhydramine (1.9), nordiphenhydramine (2.9)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, normeperidine

KEY WORDS
serum; pharmacokinetics

REFERENCE
Meatherall,R.C.; Guay,D.R.P.; Chalmers,J.L. Analysis of meperidine and normeperidine in serum and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *338*, 141–149.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 11.77

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm NovaPak phenyl
Mobile phase: MeCN:20 mM pH 6.6 ammonium acetate 80:20
Flow rate: 1.5
Injection volume: 20
Detector: UV 232

CHROMATOGRAM
Retention time: 6.7

OTHER SUBSTANCES
Noninterfering: cefazolin

KEY WORDS
injections; 5% dextrose; stability-indicating

REFERENCE
Lee,D.K.T.; Wong,C.-Y.; Wang,D.-P. Stability of cefazolin sodium and meperidine hydrochloride, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 1608–1610.

SAMPLE
Matrix: solutions

Sample preparation: Weigh 95.6 mg meperidine hydrochloride and 15.0 mg ondansetron hydrochloride in a 10 mL volumetric flask, add 0.9% sodium chloride, shake vigorously for 2 min, add 0.9% sodium chloride to volume. Dilute 1:2.5, 1:7.5, and 1:15, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 220 × 4.6 5 μm underivatized silica column (Brownlee Silica Applied Biosystems, Inc.,San Jose)
Mobile phase: MeOH:10 mM pH 4.0 aqueous monobasic potassium phosphate (adjusted with 10% phosphoric acid) 40:60
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 8.8
Limit of detection: 1730 ng/mL

OTHER SUBSTANCES

Simultaneous: ondansetron

REFERENCE

Venkateshwaran,T.G.; Stewart,J.T.; King,D.T. HPLC determination of morphine-ondansetron and meperidine-ondansetron mixtures in 0.9% sodium chloride injection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1329–1338.

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: 2.05

OTHER SUBSTANCES

Simultaneous: acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodeine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine pemoline, benzphetamine, diethylpropion, mazindol, tranylcypromine, caffeine, fenethyline, phendimetrazine, methylphenidate, phenelzine, fencamfamin, chlorphentermine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine, norpipanone
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: epinephrine, pipradol, phenylpropanolamine, levallorphan, hydroxypethidine, normethadone, dipipanone, diamorphine, pentazocine

REFERENCE

Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve 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: 11.2
Limit of detection: 8.8 ng/mL

OTHER SUBSTANCES
Simultaneous: buprenorphine, nalbuphine, ethylmorphine, morphine, codeine, fentanyl, butorphanol
Noninterfering: thiopentone, succinylcholine, pancuronium, diazepam, atropine, neostigmine
Interfering: tramadol

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: 150 × 4.6 Supelcosil LC-ABZ
Mobile phase: MeCN:25 mM pH 6.9 potassium phosphate buffer 35:65
Flow rate: 1.5
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 4.216

OTHER SUBSTANCES
Also analyzed: 6-acetylmorphine, amiloride, amphetamine, benzocaine, benzoylecgonine, caffeine, cocaine, codeine, doxylamine, fluoxetine, glutethimide, hexobarbital, hypoxanthine, levorphanol, LSD, mephobarbital, methadone, methylphenidate, methyprylon, N-norcodeine, oxazepam, oxycodone, phenylpropanolamine, prilocaine, procaine, terfenadine

REFERENCE
Ascah,T.L. Improved separations of alkaloid drugs and other substances of abuse using Supelcosil LC-ABZ column, *Supelco Reporter*, **1993**, *12(3)*, 18–21.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30

Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 9.21 (A), 4.83 (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, 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, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
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 + 0.5 mL 1% trichloroacetic acid, centrifuge at 5200 g for 10 min, filter (0.2 μm), inject 20 μL aliquot

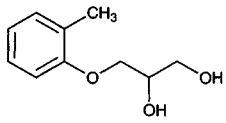
HPLC VARIABLES
Column: 250 × 4 Lichrospher 5μm 60 RP-select B
Mobile phase: Gradient. MeCN:50 mM pH 3.2 potassium phosphate buffer from 10:90 to 75:25 over 7 min, hold at 75:25 for 3 min, return to 10:90 over 5 min, equilibrate at 10:90 for 5 min
Flow rate: 1.5
Injection volume: 20
Detector: UV 190-370

CHROMATOGRAM
Retention time: 6.5

OTHER SUBSTANCES
Extracted: amitriptyline, morphine, codeine, benzoylecgonine, amphetamine, norpropoxyphene, nordiazepam
Also analyzed: phenylpropanolamine, lidocaine, diphenhydramine, nortriptyline, ephedrine (different gradient).
Interfering: cocaine

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.

Mephenesin



Molecular formula: $C_{10}H_{14}O_3$
Molecular weight: 182.22
CAS Registry No.: 59-47-2, 533-06-2 (carbamate)
Merck Index: 5895
Lednicer No.: 1 118

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 272

CHROMATOGRAM
Retention time: 3.53
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclam-

ide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Serum. 2 mL Serum, adjust pH to 14 with 2 M NaOH, extract twice with 6 mL diethyl ether. Combine the organic layers, dry under nitrogen, dissolve the residue in 200 μL MeOH. Inject a 20 μL aliquot. Urine. 5 mL Urine, adjust pH to 5 with 1 mL 2 M pH 5 sodium acetate buffer. Add 100 μL β-glucuronidase (Helix pomatia 98400 U/mL), incubate at 37° overnight, cool, wash with 8 mL diethyl ether, discard the organic layer. Adjust to pH 14 with 25% NaOH, extract twice with 8 mL diethyl ether. Combine the organic layers, dry under nitrogen, reconstitute the residue in 200 μL MeOH. Inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 7 μm LiChrospher 100 RP-18
Column: 250 × 4 7 μm LiChrospher 100 RP-18
Mobile phase: MeCN:1% acetic acid 20:80
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 19
Internal standard: mephenesin

OTHER SUBSTANCES

Extracted: methocarbamol

KEY WORDS

horse; serum; mephenesin is IS

REFERENCE

Koupai-Abyazani,M.R.; Esaw,B.; Laviolette,B. Determination of methocarbamol in equine serum and urine by high-performance liquid chromatography with ultraviolet detection and atmospheric pressure ionization-mass spectrometric confirmation, *J.Anal.Toxicol.*, **1997**, *21*, 301–305.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

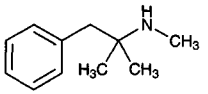
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Mephentermine



Molecular formula: $C_{11}H_{17}N$
Molecular weight: 163.26
CAS Registry No.: 100-92-5, 6190-60-9 (sulfate dihydrate)
Merck Index: 5897
Lednicer No.: 1 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 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 259

CHROMATOGRAM

Retention time: 4.30
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: 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: 2.25

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200

mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

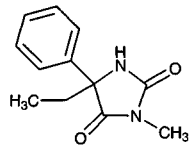
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Mephenytoin



Molecular formula: $C_{12}H_{14}N_2O_2$
Molecular weight: 218.26
CAS Registry No.: 50-12-4
Merck Index: 5898

SAMPLE

Matrix: blood
Sample preparation: Prepare an SPE cartridge by plugging the end of a 1 mL disposable pipette tip with glass wool and adding about 100 mg Chromosorb P/NAW. Add 50 μL plasma then 50 μL 10 μg/mL tolylphenobarbital in 200 mM HCl to the SPE cartridge, let stand for 2 min, elute with 1 mL chloroform:isopropanol 6:1. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject a 15 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Supelcosil-LC-8
Mobile phase: MeCN:water 20:80
Flow rate: 3.3
Injection volume: 15
Detector: UV 208

CHROMATOGRAM

Retention time: 6.02
Internal standard: tolylphenobarbital (7.57)
Limit of detection: 50-100 ng/mL

OTHER SUBSTANCES

Extracted: theophylline, caffeine, barbital, ethosuximide, primidone, carbamazepinediol, phenacemide, methyprylon, nirvanol, phenobarbital, chloramphenicol, butabarbital, carbamazepine epoxide, pentobarbital, amobarbital, carbamazepine, glutethimide, phenytoin, secobarbital, methaqualone
Noninterfering: acetaminophen, amikacin, amitriptyline, clonazepam, cyclosporine, desipramine, diazepam, digoxin, disopyramide, gentamicin, imipramine, lidocaine, methotrexate, N-acetylprocainamide, netilmicin, nortriptyline, procainamide, quinidine, salicylic acid, sulfamethoxazole, tobramycin, trimethoprim, valproic acid, p-hydroxyphenobarbital, vancomycin

KEY WORDS

plasma; SPE

REFERENCE

Svinarov,D.A.; Dotchev,D.C. Simultaneous liquid-chromatographic determination of some bronchodilators, anticonvulsants, chloramphenicol, and hypnotic agents, with Chromosorb P columns used for sample preparation, *Clin.Chem.*, **1989**, *35*, 1615–1618.

SAMPLE

Matrix: blood, milk
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL water and MeOH:water 20:80. Add 5 mL 0.5% pH 6.0 KH_2PO_4 to 1 mL human breast milk or plasma, mix briefly, add the sample to the SPE cartridge, elute with 5 mL MeOH, evaporate the eluate to dryness, dissolve the residue in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Develosil C8-5 (Nomura Chemicals)
Mobile phase: MeCN:0.5% KH_2PO_4 buffer 30:70 (The pH of mobile phase was adjusted to 4.5 with 50% H_3PO_4.)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 13
Internal standard: mephenytoin

OTHER SUBSTANCES
Extracted: phenytoin

KEY WORDS
cord blood plasma; human breast milk; maternal plasma; mephenytoin is IS; human; plasma; SPE

REFERENCE
Shimoyama,R.; Ohkubo,T.; Sugawara,K.; Ogasawara,T.; Ozaki,T.; Kagiya,A.; Saito,Y. Monitoring of phenytoin in human breast milk, maternal plasma and cord blood plasma by solid-phase extraction and liquid chromatography, *J.Pharm.Biomed.Anal.*, **1998**, *17*, 863–869.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb RP18
Mobile phase: EtOH:water 10:90 containing 10 mM α-cyclodextrin and 0.5 mM tri-O-methyl-β-cyclodextrin
Column temperature: 25
Flow rate: 0.95
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 9.1, k' 10.7 (enantiomers)

OTHER SUBSTANCES
Extracted: morsuximide

KEY WORDS
chiral

REFERENCE
Nowakowski,R.; Bielejewska,A.; Duszczyk,K.; Sybilska,D. Chiral discrimination by high-performance liquid chromatography with joint use of two cyclodextrin additives, *J.Chromatogr.A*, **1997**, *782*, 1–11.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in 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: 8

OTHER SUBSTANCES
Simultaneous: phenytoin

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: solutions
Sample preparation: Inject a 6-10 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 Supelguard LC-1 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil LC-1 (Supelco)
Mobile phase: MeOH:MeCN:buffer 17.5:17.5:65 (Buffer was 2.72 g KH_2PO_4 in 1.9 L water, pH adjusted to 6.3 with about 2 mL 1 M NaOH, made up to 2 L.)
Flow rate: 2
Injection volume: 6-10
Detector: UV 204

CHROMATOGRAM
Retention time: 4.65
Internal standard: 5-ethyl-5-p-tolybarbituric acid (tolybarb) (4.80)

OTHER SUBSTANCES
Simultaneous: acetaminophen, acetanilide, N-acetylcysteine, N-acetylprocainamide, amobarbital, ampicillin, aspirin, barbital, butabarbital, butalbital, caffeine, carbamazepine, chloramphenicol, chlorpropamide, codeine, cyheptamide, diazoxide, diflunisal, diphylline, disopyramide, ethchlorvynol, gentisic acid, glutethimide, heptabarbital, hexobarbital, ibuprofen, indomethacin, ketoprofen, mefenamic acid, mephobarbital, methaqualone, methsuximide, methyl salicylate, methyprylon, morphine, naproxen, nirvanol, oxphenylbutazone, pentobarbital, phenacetin, phenobarbital, phensuximide, phenytoin, procainamide, salicylamide, salicylic acid, secobarbital, sulfamethoxazole, sulindac, theophylline, thiopental, tolmetin, trimethoprim, vancomycin
Noninterfering: amikacin, gentamicin, meprobamate, netilmicin, quinidine, tetracycline, tobramycin, valproic acid
Interfering: ethosuximide, cimetidine, primidone, phenylbutazone

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

HPLC VARIABLES
Column: 250 × 4.6 10 μm Chiralcel OJ
Mobile phase: MeOH
Flow rate: 0.5
Detector: UV 254

CHROMATOGRAM
Retention time: 7.51 (R-(-)), 9.11 (S-(+))

OTHER SUBSTANCES
Also analyzed: mephobarbital (flow rate 1 mL/min)

KEY WORDS
chiral

REFERENCE
Aboul-Enein,H.Y.; Serignese,V.; Bojarski,J. Simple chiral liquid chromatographic enantioseparation of some racemic antiepileptic drugs, *J.Liq.Chromatogr.*, **1993**, *16*, 2741–2749.

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: 5.4

OTHER SUBSTANCES
Simultaneous: barbital, carbamazepine, diazepam, ethotoin, methsuximide, phenacemide, phenobarbital, phensuximide

REFERENCE
Vydac HPLC Catalog, 1994-5, **1994**, p. 26.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine,

puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 5.99 (A), 5.40 (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, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine
Sample preparation: 200 μL Urine + 125 μL 200 μg/mL IS, extract twice with 3.0 mL portions of ethyl acetate:diethyl ether 67:33. Dry combined organic phases over 200 mg anhydrous magnesium sulfate for 30 min, centrifuge. Evaporate supernatant under a stream of the nitrogen at 35°. Reconstitute residue in 500 μL MeCN:water 20:80, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax C8
Mobile phase: MeCN:water 20:80
Injection volume: 5
Detector: UV 220

CHROMATOGRAM
Retention time: 21
Internal standard: 5-(4-hydroxyphenyl)-5-phenylhydantoin (12.2)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
metabolism

REFERENCE
Sarich,T.; Kalhorn,T.; Magee,S.; Al-sayegh,F.; Adams,S.; Slattery,J.; Goldstein,J.; Nelson,S.; Wright,J. The effect of omeprazole pretreatment on acetaminophen metabolism in rapid and slow metabolizers of S-mephenytoin, *Clin.Pharmacol.Ther.*, **1997**, *62*, 21–28.

SAMPLE
Matrix: urine
Sample preparation: Condition a Sep-Pak silica SPE cartridge with 2 mL chloroform. Adjust pH of urine to 6 with 100 mM HCl or 100 mM NaOH, centrifuge. Remove a 10 mL aliquot of the supernatant and add it to 15 mL chloroform, shake for 30 s, filter (Whatman 1PS phase separating paper), add the organic filtrate to the SPE cartridge, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 50 μL MeOH, shake for 30 s, inject a 20 μL aliquot.

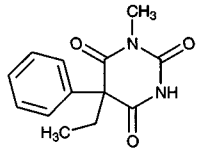
HPLC VARIABLES
Column: 75 × 4.5 3 μm Supelcosil LC-8
Mobile phase: MeOH:100 mM pH 5.0 acetate buffer containing 10 mM β-cyclodextrin 20:80
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 8.3 (S), 9.8 (R)
Limit of detection: 100 ng/mL

KEY WORDS
chiral; SPE

REFERENCE
Róna,K.; Szabó,I. Determination of mephenytoin stereoselective oxidative metabolism in urine by chiral liquid chromatography employing β-cyclodextrin as a mobile phase additive, *J.Chromatogr.*, **1992**, *573*, 173–177.

Mephobarbital



Molecular formula: $C_{13}H_{14}N_2O_3$
Molecular weight: 246.27
CAS Registry No.: 115-38-8
Merck Index: 5899

SAMPLE

Matrix: blood
Sample preparation: 100 μL Serum + 100 μL buffer + 1.5 mL IS in 5% isopropanol in chloroform, vortex for 30 s, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of air at room temperature, reconstitute the residue in 100 μL mobile phase, inject a 6-10 μL aliquot. (Buffer was 13.6 g KH_2PO_4 in 90 mL water, pH adjusted to 6.8 with about 3 mL 10 M NaOH, made up to 100 mL.)

HPLC VARIABLES

Guard column: 20 × 4.6 Supelguard LC-1 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil LC-1 (Supelco)
Mobile phase: MeOH:MeCN:buffer 17.5:17.5:65 (Buffer was 2.72 g KH_2PO_4 in 1.9 L water, pH adjusted to 6.3 with about 2 mL 1 M NaOH, made up to 2 L.)
Flow rate: 2
Injection volume: 6-10
Detector: UV 204

CHROMATOGRAM

Retention time: 5.60
Internal standard: 5-ethyl-5-p-tolybarbituric acid (tolybarb) (4.80)

OTHER SUBSTANCES

Extracted: acetaminophen, amobarbital, barbital, caffeine, carbamazepine, chloramphenicol, ethosuximide, 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, 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
Interfering: ethchlorvynol

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: 500 μL Serum + 600 μL allobarbital in 75 mM pH 6.8 buffer, add 200 units β-glucuronidase (Type VII-A from E. coli), incubate at 37° for 30 min, add 1 mL of the sample to an Extrelut-1 SPE cartridge, after 10 min elute with 2.5 mL MTBE, dry the eluate under a stream of nitrogen, dissolve the residue in 50 μL MeOH:water 1:1, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 25 × 4 4 μm Superspher RP-18e (Merck)

Mobile phase: MeOH:11.2 mM β-cyclodextrin in 20 mM KH_2PO_4 5:95
Flow rate: 0.8
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 19 (R), 21 (S)
Internal standard: allobarbital (16)
Limit of detection: 13 ng/mL

OTHER SUBSTANCES
Simultaneous: phenobarbital, zonisamide

KEY WORDS
serum; 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: 500 μL Serum + 600 μL allobarbital 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 μL MeOH:water 50:50, inject a 10 μL 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 allobarbital 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 × 4 4 μm Superspher RP-18e (E. Merck); B 250 × 4 4 μm 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: A UV 210; B UV 210

CHROMATOGRAM
Retention time: 19 (R), 21 (S) (column B)
Internal standard: allobarbital (17, from column B)
Limit of detection: 12.8 ng/mL (S), 11.6 ng/mL (R)

OTHER SUBSTANCES
Extracted: carbamazepine diol, carbamazepine epoxide, carbamazepine, 5-(p-hydroxyphenyl)-5-phenylhydantoin, zonisamide, phenytoin, phenobarbital, metabolites

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: solutions

HPLC VARIABLES
Column: 250 × 1 5 μm LiChrosorb RP18
Mobile phase: EtOH:water 20:80 containing 20 mM α-cyclodextrin and 0.5 mM tri-O-methyl-α-cyclodextrin
Column temperature: 25
Flow rate: 0.04
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 5.4, k' 6.7 (enantiomers)

OTHER SUBSTANCES
Extracted: glutethimide

KEY WORDS
chiral

REFERENCE
Nowakowski,R.; Bielejewska,A.; Duszczyk,K.; Sybilska,D. Chiral discrimination by high-performance liquid chromatography with joint use of two cyclodextrin additives, *J.Chromatogr.A*, **1997**, *782*, 1–11.

SAMPLE
Matrix: solutions
Sample preparation: Mix 50 μL of a 20-200 μg/mL solution in acetone with 50 μL of a 0.4-1.6 mg/mL solution of 2-bromo-2'-acetonaphthone in acetone, add 5-10 mg cesium carbonate, heat at 30° for 30 min, add 50 μL glacial acetic acid, mix, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak C18
Mobile phase: MeOH:water 80:20
Flow rate: 2
Detector: UV 249

CHROMATOGRAM
Retention time: 3.1
Limit of detection: 1 ng

OTHER SUBSTANCES
Simultaneous: amobarbital, barbital, butobarbital, heptobarbital, pentobarbital, phenobarbital, secobarbital
Interfering: hexobarbital

KEY WORDS
derivatization

REFERENCE
Hulshoff,A.; Roseboom,H.; Renema,J. Improved detectability of barbiturates in high-performance liquid chromatography by pre-column labelling and ultraviolet detection, *J.Chromatogr.*, **1979**, *186*, 535–541.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 OmniPac PAX-500 (Dionex)
Mobile phase: Gradient. A was MeCN:5 mM sodium carbonate 9:81. B was MeCN:20 mM sodium carbonate 20:80. A:B from 100:0 to 0:100 over 10 min.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 10.5

OTHER SUBSTANCES
Simultaneous: allobarbital, amobarbital, barbital, barbituric acid, butabarbital, methabarbital, methohexital, phenobarbital, phenytoin, secobarbital, thiamylal

REFERENCE
Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, *13*, 107–134.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Chiralcel OJ
Mobile phase: MeOH
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 7.38 (S-(+)), 15.76 (R-(-))

OTHER SUBSTANCES
Also analyzed: mephenytoin (flow rate 0.5 mL/min)

KEY WORDS
chiral

REFERENCE
Aboul-Enein,H.Y.; Serignese,V.; Bojarski,J. Simple chiral liquid chromatographic enantioseparation of some racemic antiepileptic drugs, *J.Liq.Chromatogr.*, **1993**, *16*, 2741–2749.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 Supelcosil LC-ABZ
Mobile phase: MeCN:25 mM pH 6.9 potassium phosphate buffer 35:65
Flow rate: 1.5
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 4.128

OTHER SUBSTANCES
Also analyzed: 6-acetylmorphine, amiloride, amphetamine, benzocaine, benzoylecgonine, caffeine, cocaine, codeine, doxylamine, fluoxetine, glutethimide, hexobarbital, hypoxanthine, levorphanol, LSD, meperidine, methadone, methylphenidate, methyprylon, N-norcodeine, oxazepam, oxycodone, phenylpropanolamine, prilocaine, procaine, terfenadine

REFERENCE
Ascah,T.L. Improved separations of alkaloid drugs and other substances of abuse using Supelcosil LC-ABZ column, *Supelco Reporter*, **1993**, *12(3)*, 18–21.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mm KH_2PO_4 + 5 mM 1-decanesulfonic acid 30:70, adjusted to pH 3.2 with 85% phosphoric acid
Flow rate: 1
Injection volume: 10
Detector: UV 214

CHROMATOGRAM
Retention time: 11.5
Internal standard: methyl paraben (7.0)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: allobarbital, barbital, butalbital, aprobarbital, pentobarbital, phenobarbital, secobarbital, talbutal, vinbarbital

KEY WORDS
stability-indicating

REFERENCE
Ibrahim,F.B. Simultaneous determination and separation of several barbiturates and analgesic products by ion-pair high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 2835–2851.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 5 μm LiChroCART ChiraDex (β-cyclodextrin chemically bonded to silica) (Merck)
Mobile phase: MeOH:water 50:50
Column temperature: 35
Flow rate: 0.5
Detector: UV 220

CHROMATOGRAM
Retention time: 13, 14 (enantiomers)

KEY WORDS
chiral

REFERENCE
Cabrera,K.; Lubda,D. Influence of temperature on chiral high-performance liquid chromatographic separations of oxazepam and Prominal on chemically bonded β-cyclodextrin as stationary phase, *J.Chromatogr.A*, **1994**, *666*, 433–438.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in mobile phase at a concentration of 100 μg/mL, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 300 × 2 μBondapak C18

Mobile phase: MeCN:water 30:70 adjusted to pH 3.0 with formic acid

Flow rate: 0.27

Injection volume: 5

Detector: MS, VG TRIO 2000 single quadrupole MS with EI or CI or UV 270

KEY WORDS

mass spectra given

REFERENCE
Ryan,T.W. Identification of barbiturates using high performance liquid chromatography-particle beam EI/CI mass spectrometry, *J.Liq.Chromatogr.*, **1994**, *17*, 867–881.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 62 × 2 packed with chiral packing (Prepare packing by dissolving 3,5-dimethylphenylcarbamate amylose in DMF, coat on Nucleosil 1000-7, dry at 60° for 3 h under reduced pressure.)
Mobile phase: Hexane:isopropanol 90:10
Flow rate: 0.1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 3.80

KEY WORDS
narrow-bore; chiral; α 1.80

REFERENCE
Chankvetadze,B.; Chankvetadze,L.; Sidamonidze,S.; Yashima,E.; Okamoto,Y. Enantioseparation of some chiral pharmaceuticals using narrow-bore liquid chromatography, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 695–699.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of an 8 μg/mL solution.

HPLC VARIABLES
Column: 250 × 4 4 μm Superspher 100 RP-18
Mobile phase: EtOH:buffer containing 25 mM β-cyclodextrin substituted with 2-hydroxy-3-trimethylammoniumpropyl groups (Roquette Frères, Lestrem, France) (Buffer was 1.776 g/L NaH_2PO_4 adjusted to pH 2.5 with orthophosphoric acid.)
Column temperature: 22.5
Flow rate: 0.6
Injection volume: 20
Detector: UV 254

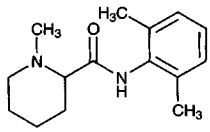
CHROMATOGRAM
Retention time: 16.75 (-), 18.09 (+)

OTHER SUBSTANCES
Interfering: hexobarbital

KEY WORDS
chiral

REFERENCE
Roussel,C.; Favrou,A. Cationic β-cyclodextrin: a new versatile chiral additive for separation of drug enantiomers by high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *704*, 67–74.

Mepivacaine



Molecular formula: $C_{15}H_{22}N_2O$
Molecular weight: 246.35
CAS Registry No.: 96-88-8, 1722-62-9 (HCl)
Merck Index: 5905
Lednicer No.: 1 17

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 25 µL EtOH + 25 µL 17.6 µg/mL ropivacaine in EtOH, mix. Add 5 mL diethyl ether, vortex for 3 min, centrifuge at 2000 g for 10 min at 4°. Add 500 µL 100 mM hydrochloric acid to organic phase, back extract for 3 min. Discard organic phase, add 5 mL n-pentane containing 100 µL isoamyl alcohol, wash for 3 min, discard organic phase again. Add 50 µL 2 M NaOH, extract aqueous phase with 5 mL n-pentane containing 100 µL isoamyl alcohol, centrifuge at 2000 g for 10 min at 4°. Evaporate organic phase to dryness under a gentle stream of nitrogen at 40°. Reconstitute the residue in 100 µL mobile phase, inject a 30 µL aliquot.

HPLC VARIABLES

Guard column: 10 × 4.0 40 µm α1-AGP (J.T.Baker)
Column: 100 × 4.0 5 µm α1-AGP (J.T.Baker)
Mobile phase: 2-Propanol:buffer 6.8:93.2 (Buffer was 3.6 g/L $Na_2HPO_4.12$ H2O adjusted to pH 6.8 with phosphoric acid.)
Column temperature: 30
Flow rate: 1.1
Injection volume: 30
Detector: UV 210

CHROMATOGRAM

Retention time: 4.6 (R-(-)), 5.8 (S-(+))
Internal standard: S-(-)-ropivacaine (10.5)
Limit of detection: 3 ng/mL
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES

Noninterfering: bupivacaine
Interfering: lidocaine, prilocaine

KEY WORDS

plasma; chiral; pharmacokinetics

REFERENCE

Vletter,A.A.; Olieman,W.; Burm,A.G.L.; Groen,K.; van Kleef,J.W. High-performance liquid chromatographic assay of mepivacaine enantiomers in human plasma in the nanogram per milliliter range, *J.Chromatogr.B*, **1996**, *678*, 369–372.

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL Bond-Elut C18 SPE cartridge with 1 mL 100 mM HCl, 1 mL MeOH and 1 mL water. Add 350 µL 100 mM pH 4.5 phosphate buffer and 40 µL 200 µg/mL S-bupivacaine in MeOH to 1 mL serum, vortex. Add the mixture to the SPE cartridge and pass it through the cartridge attached to a vacuum manifold, wash 4 times with 250 µL water, then wash with 500 µL MeCN and allow to air dry for 1 min between each wash. Elute with four 250 µL aliquots of MeOH containing 2% HCl, evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase and inject a 100 µL aliquot.

HPLC VARIABLES

Column: 250 × 4 5 µm Sumichiral OA-4700 (YMC, Wilmington, NC, USA)

Mobile phase: Hexane:dichloroethane:absolute MeOH 85:10:5
Flow rate: 0.8
Injection volume: 100
Detector: UV 220

CHROMATOGRAM
Retention time: 9.3 (R-(+)), 11.4 (S-(-))
Internal standard: S-bupivacaine (7.7)
Limit of detection: 100 ng/mL
Limit of quantitation: 150 ng/mL

KEY WORDS
chiral; SPE; serum

REFERENCE
Siluveru,M.; Stewart,J.T. Stereoselective determination of mepivacaine in human serum using a bush-type chiral stationary phase and solid-phase extraction, *J.Chromatogr.B*, **1997**, *690*, 359–362.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 50 μL 40 μg/mL etidocaine hydrochloride in water + 100 μL 1 M NaOH, vortex for 15 s, add 5 mL diethyl ether, shake on a reciprocating shaker for 10 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 80 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.2 10 μm μBondapak C18
Mobile phase: MeCN:50 mM pH 5.80 Na_2HPO_4 25:75
Flow rate: 0.9
Injection volume: 80
Detector: UV 210

CHROMATOGRAM
Retention time: 3.7
Internal standard: etidocaine (12.0)

OTHER SUBSTANCES
Extracted: 2,6-pipecolylxylidine, bupivacaine, lidocaine
Noninterfering: metabolites, 2,3-chloroprocaine, theophylline, mexiletine, quinidine, disopyramide, verapamil, phenobarbital, phenytoin, carbamazepine, ethosuximide, digoxin, theobromine, caffeine, furosemide, phenprocoumon, aldactone

KEY WORDS
plasma

REFERENCE
Ha,H.-R.; Funk,B.; Gerber,H.R.; Follath,F. Determination of bupivacaine in plasma by high-performance liquid chromatography, *Anesth.Analg.*, **1984**, *63*, 448–450.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 1 M NaOH + 3 mL heptane:ethyl acetate 90:10, shake for 2 min, centrifuge at 1200 g for 10 min. Remove the organic phase and add it to 50 μL 50 mM sulfuric acid, shake for 2 min, centrifuge at 1200 g for 5 min. Remove the aqueous phase and add it to 820 μg sodium acetate, inject a 40 μL aliquot. (The sodium acetate was measured out by adding 50 μL 200 mM sodium acetate in MeOH to the tube and evaporating the MeOH.)

HPLC VARIABLES
Column: 250 × 4 10 μm μBondapak C18
Mobile phase: MeCN:10 mM NaH_2PO_4 7:93, adjusted to pH 2.1

Column temperature: 30
Flow rate: 1
Injection volume: 40
Detector: UV 205

CHROMATOGRAM
Retention time: 15
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: articaine

KEY WORDS
plasma; rabbit

REFERENCE
Le Guévello,P.; Le Corre,P.; Chevanne,P.; Le Verge,R. High-performance liquid chromatographic determination of bupivacaine in plasma samples for biopharmaceutical studies and application to seven other local anaesthetics, *J.Chromatogr.*, **1993**, *622*, 284–290.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL BondElut C18 SPE cartridge once with 1 M HCl, twice with MeOH, and once with water, remove the liquid completely with suction each time. Add 250 μL bupivacaine in 100 mM NaH_2PO_4 and 250 μL serum to the column at 1 mL/min, wash twice with water and once with MeCN draining the column completely after each wash, elute with 250 μL eluting solution, centrifuge for 20 s to remove last of eluate, inject a 5 μL aliquot of the eluate. (Eluting solution was 2.5 mL 35% perchloric acid in 100 mL MeOH.)

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP-8 (Applied Biosystems)
Column: 150 × 4.6 5 μm Ultrasphere octyl
Mobile phase: MeCN:10 mM KH_2PO_4 25:80, pH 5.2
Flow rate: 1.5
Injection volume: 5
Detector: UV 205

CHROMATOGRAM
Retention time: 2.6
Internal standard: bupivacaine (7.2)

OTHER SUBSTANCES
Extracted: bupivacaine, meperidine, fentanyl
Noninterfering: acetaminophen, codeine, epinephrine, morphine, diazepam

KEY WORDS
serum; SPE

REFERENCE
Gupta,R.N.; Dauphin,A. Column liquid chromatographic determination of bupivacaine in human serum using solid-phase extraction, *J.Chromatogr.B*, **1994**, *658*, 113–119.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 264

CHROMATOGRAM
Retention time: 4.54
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE
Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.7

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions
Sample preparation: Inject an aliquot of a 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' 2.32 (first enantiomer)

KEY WORDS
chiral; α = 1.11

REFERENCE
Huynh,N.-H.; Karlsson,A.; Pettersson,C. Enantiomeric separation of basic drugs using N-benzyloxycarbonyl-glyclyl-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

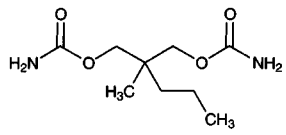
CHROMATOGRAM
Retention time: 7.71 (A), 4.20 (B)

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, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
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.

Meprobamate



Molecular formula: $C_9H_{18}N_2O_4$
Molecular weight: 218.25
CAS Registry No.: 57-53-4
Merck Index: 5908
Lednicer No.: 1 218

SAMPLE
Matrix: blood
Sample preparation: 500 µL Serum + 100 µL 200 mM HCl + 200 µL 100 µg/mL carisoprodol + 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 µL of the organic layer and evaporate it to dryness, dissolve the residue in 50 µL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES
Column: 100 × 3 octyl CP-tm-Spher C8 glass column (Chrompack)
Mobile phase: MeCN:50 mM NaH_2PO_4 35:65 adjusted to pH 2.2 with phosphoric acid
Flow rate: 0.8
Injection volume: 20
Detector: UV 190

CHROMATOGRAM
Retention time: 1.8
Internal standard: carisoprodol (5.5)
Limit of detection: 2000 ng/mL

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 + 100 µL 200 mM HCl + 200 µL 100 µg/mL carisoprodol + 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 µL of the organic layer and evaporate it to dryness, dissolve the residue in 50 µL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES
Column: 100 × 3 octyl CP-tm-Spher C8 glass column (Chrompack)
Mobile phase: MeCN:50 mM NaH_2PO_4 35:65 adjusted to pH 2.2 with phosphoric acid
Flow rate: 0.8
Injection volume: 20
Detector: UV 190

CHROMATOGRAM
Retention time: 1.8
Internal standard: carisoprodol (5.5)
Limit of detection: 2000 ng/mL

KEY WORDS
serum

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, weigh out amount equivalent to 100 mg meprobamate, dissolve in 50 mL mobile phase, sonicate for 10 min, filter, inject an aliquot.

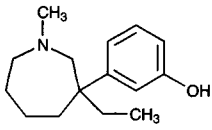
HPLC VARIABLES
Column: 125 × 4 5 μm Lichrospher RP-18
Mobile phase: MeOH:50 mM pH 1.9 phosphoric acid 30:70 containing 1 mM benzoic acid
Column temperature: 35
Flow rate: 0.9
Injection volume: 50
Detector: UV 273

CHROMATOGRAM
Retention time: 13.5

KEY WORDS
tablets; indirect UV detection

REFERENCE
Bechet,I.; Ceccato,A.; Hubert,P.; Herne,P.; Crommen,J. Determination of meprobamate in pharmaceutical dosage forms also containing carbromal by liquid chromatography and indirect photometric detection, *J.Pharm.Biomed.Anal.*, **1992**, *10*, 995–999.

Meptazinol



Molecular formula: $C_{15}H_{23}NO$
Molecular weight: 233.35
CAS Registry No.: 54340-58-8, 59263-76-2 (HCl)
Merck Index: 5910

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 20 μL 2.5 μg/mL IS in MeOH + 100 μL buffer + 3 mL ethyl acetate, vortex for 2 min, centrifuge at 2000 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 μL MeOH, add 50 μL water, inject an aliquot. (Buffer was 1 M Na_2HPO_4 adjusted to pH 9.5 with 5 M NaOH.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb CN
Mobile phase: MeOH:0.5% ammonium acetate 45:55
Flow rate: 2
Detector: F ex 282 em 300

CHROMATOGRAM
Retention time: 5.8
Internal standard: m-(1-cyclopropylmethyl-3-ethylhexahydro-1H-azepin-3-yl)phenol (7.0)
Limit of detection: 3 ng/mL

KEY WORDS
plasma

REFERENCE
Frost,T. Determination of meptazinol in plasma by high-performance liquid chromatography with fluorescence detection, *Analyst*, **1981**, *106*, 999–1000.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 100 μL 40 ng/mL fenethazine in 2 M aqueous Tris + 200 μL MTBE, vortex for 30 s, centrifuge at 9950 g for 2 min, inject a 100 μL aliquot of the organic phase.

HPLC VARIABLES
Column: 125 × 5 5 μm Spherisorb S5W silica
Mobile phase: MeOH:glacial acetic acid:ammonia 996:3:1 (Ammonia was 0.88 g/mL.)
Flow rate: 2
Injection volume: 100
Detector: E, EDT Research LCA 15, glassy carbon electrode +1.2 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 3
Internal standard: fenethazine (4)
Limit of detection: 500 ng/mL

KEY WORDS
plasma; cow; human

REFERENCE
Storey,G.C.A.; Schootstra,R.; Henry,J.A. Measurement of meptazinol in plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1985**, *341*, 223–227.

SAMPLE
Matrix: microsomal incubations, urine
Sample preparation: Microsomal incubations. 2 mL Microsomal incubation + IS + 1 mL 1 M pH 10.0 ammonia/ammonium chloride buffer + 5 mL n-hexane:ethyl acetate 50:50, extract. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50 μL water, inject a 20 μL aliquot. Urine. Untreated or deconjugated (with 500-2000 IU/mL β-glucuronidase/sulfatase from Helix pomatia for 24 h) urine + IS + 1 mL 1 M pH 10.0 ammonia/ammonium chloride buffer + 5 mL n-hexane:ethyl acetate 30:70, stir vigorously for 10 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50 μL water, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4 10 μm LiChrospher 60 CN
Column: 250 × 4 5 μm LiChrospher 100 CN
Mobile phase: MeCN:MeOH:1% triethylammonium acetate buffer (pH 5.5) 15:5:80
Flow rate: 0.5
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 31
Internal standard: 3-(3-ethylhexahydro-1-ethyl-1H-azepin-3-yl)phenol (35)
Limit of quantitation: 392 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rabbit; liver; human; pharmacokinetics; rat

REFERENCE
Rudolphi,C.; Blaschke,G. Determination of the stereoselective aspects in in-vitro and in-vivo metabolism of the analgesic meptazinol by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *663*, 315–326.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 3.6

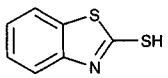
OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Mercaptobenzothiazole



Molecular formula: $C_7H_5NS_2$
Molecular weight: 167.26
CAS Registry No.: 149-30-4
Merck Index: 5916

SAMPLE

Matrix: blood, tissue, urine

Sample preparation: Urine. 1 mL Urine + 4 mL EtOH, centrifuge at 3000 rpm for 10 min. Dilute the supernatant with EtOH, filter (0.2 μm, Acro LC13), inject a 20 μL aliquot. (Hydrolyze conjugates by mixing 100 μL urine with 900 μL 100 mM pH 5 acetate buffer and 33 units sulfatase or 5000 units β-glucuronidase, heat at 37° for 30 min, proceed as above.) Tissue. Homogenize liver or kidney in 3 volumes of cold 100 mM pH 5 acetate buffer containing 2 mM aminooxyacetic acid, add 4 volumes of EtOH, centrifuge at 3000 rpm for 20 min. Filter (0.2 μm, Acro LC13) the supernatant and inject a 20 μL aliquot. Plasma. Add 4 volumes of EtOH to plasma, centrifuge at 3000 rpm for 20 min. Filter (0.2 μm, Acro LC13) the supernatant and inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 30 mm long C18
Column: 250 × 4.6 Partisil 10 ODS 3
Mobile phase: MeCN:water 50:50 adjusted to pH 4.5 with trifluoroacetic acid
Flow rate: 1
Injection volume: 20
Detector: UV 321

CHROMATOGRAM

Retention time: 7.0
Limit of detection: 200 nM

KEY WORDS

rat; hamster; guinea pig; mouse; plasma; liver; kidney

REFERENCE

Elfarra,A.A.; Hwang,I.Y. *In vivo* metabolites of *S*-(2-benzothiazolyl)-L-cysteine as markers of *in vivo* cysteine conjugate β-lyase and thiol glucuronosyl transferase activities, *Drug Metab.Dispos.*, **1990**, *18*, 917–922.

SAMPLE

Matrix: bulk, formulations

Sample preparation: Bulk. Weigh out 100 mg morphine, dissolve in 25 mL MeOH:water:acetic acid 24:72:1, dilute with MeOH:water:acetic acid 24:72:1 to a final concentration of 240 μg/mL morphine, filter (0.45 μm), inject a 20 μL aliquot. Injections. Dilute with MeOH:water:acetic acid 24:72:1 to a final concentration of 240 μg/mL morphine, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:5 mM sodium 1-heptanesulfonate:acetic acid 24:72:1
Flow rate: 1.5
Injection volume: 20
Detector: UV 230

CHROMATOGRAM

Retention time: 16

OTHER SUBSTANCES

Simultaneous: morphine (UV 284), phenol (UV 284), pseudomorphine (UV 230)

KEY WORDS
injections

REFERENCE
Bello,A.C.; Jhangiani,R.K. Liquid chromatographic determination of morphine sulfate and some contaminants in injections and bulk drug material: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 1046–1048.

SAMPLE
Matrix: formulations
Sample preparation: Directly inject a 20 μL aliquot of a 250 μg/mL digoxin injection.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water 29:71
Flow rate: 2
Injection volume: 20
Detector: UV 218

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: digoxin

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

HPLC VARIABLES
Column: 100 × 3 5 μm ChromSpher C18 (Chrompack)
Mobile phase: MeOH:water:acetic acid 45:54:1
Flow rate: 0.4
Injection volume: 75
Detector: UV 321

CHROMATOGRAM
Retention time: 5.5
Limit of detection: 5 μM

REFERENCE
Stijntjes,G.J.; te Koppele,J.M.; Vermeulen,N.P.E. High-performance liquid chromatography-fluorescence assay of pyruvic acid to determine cysteine conjugate β-lyase activity: application to *S*-1,2-dichlorovinyl-L-cysteine and *S*-2-benzothiazolyl-L-cysteine, *Anal.Biochem.*, **1992**, *206*, 334–343.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeCN, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Spheri-10 RP-18
Mobile phase: MeCN:water:acetic acid 30:70:0.03
Flow rate: 1
Injection volume: 50

Detector: UV 328

CHROMATOGRAM
Retention time: 2
Limit of detection: 500 ng

REFERENCE
Gaind,V.S.; Jedrzejczak,K. HPLC determination of rubber septum contaminants in the iodinated intravenous contrast agent (sodium iothalamate), *J.Anal.Toxicol.*, **1993**, *17*, 34–37.

SAMPLE
Matrix: solutions
Sample preparation: Mix 500 μL of a solution in MeOH:water 90:10 with 500 μL 50 μM CY5.4a-IA in MeOH:water 70:30, pass dry nitrogen through the mixture for 1 min, heat at 65° for 1.5 h, inject a 25 μL aliquot. (Some details for the synthesis of CY5.4a-IA are given in the paper.)

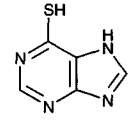
HPLC VARIABLES
Column: 150 × 3.1 5 μm LiChrosorb RP-8
Mobile phase: MeOH:10 mM pH 6.8 phosphate buffer 65:35 containing 1 mM triethylamine
Flow rate: 0.75
Injection volume: 25
Detector: F ex 670 (9.5 mW Lasermax LAS200-670-10 diode laser)

CHROMATOGRAM
Retention time: 7
Limit of detection: 1 nM

KEY WORDS
derivatization

REFERENCE
Mank,A.J.G.; Molenaar,E.J.; Lingeman,H.; Gooijer,C.; Brinkman,U.A.T.; Velthorst,N.H. Visible diode laser induced fluorescence detection in liquid chromatography after precolumn derivatization of thiols, *Anal.Chem.*, **1993**, *65*, 2197–2203.

Mercaptopurine



Molecular formula: $C_5H_4N_4S$
Molecular weight: 152.18
CAS Registry No.: 50-44-2, 6112-76-1 (monohydrate)
Merck Index: 5919

SAMPLE
Matrix: blood
Sample preparation: Condition a 500 mg Bakerbond SPE cartridge packed with LiChrosorb RP-18 with 2 mL MeOH, 2 mL water and 1 mL MeOH. Make up 500 μL plasma to 3 mL with MeOH. Centrifuge at 1100 g for 15 min, add 1.5 mL supernatant to the SPE cartridge, elute at 50 μL/min flow-rate, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 200 × 4 7 μm Lichrosorb RP-18
Mobile phase: MeOH: pH 4.15 phosphate buffer 20:80
Flow rate: 1
Injection volume: 20
Detector: UV 262

CHROMATOGRAM
Retention time: 3.30
Internal standard: mercaptopurine

KEY WORDS
plasma; SPE; mercaptopurine is IS

REFERENCE
Misztal,G.; Paw,B. Determination of fludarabine phosphate in human plasma using reversed phase high-performance liquid chromatography, *Pharmazie*, **1996**, *51*, 733–734.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Blood + 120 μg dithiothreitol (to prevent oxidation of 6-mercaptopurine), centrifuge at 2000 g for 5 min. Cool plasma in ice, add a volume of ice-cold 50% trichloroacetic acid equal to 10% of the plasma volume, mix vigorously, keep on ice for 10 min, centrifuge at 2000 g for 10 min. Remove the supernatant and adjust the pH to 6-7 with 4 M KOH, inject a 460 μL aliquot.

HPLC VARIABLES
Column: Two 250 × 4.6 10 μm Spherisorb 10-ODS columns in series
Mobile phase: 50 mM pH 6.35 potassium phosphate buffer
Flow rate: 1.5
Injection volume: 460
Detector: UV 312

CHROMATOGRAM
Retention time: 9
Limit of detection: 3 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics; dog

REFERENCE
De Abreu,R.A.; van Baal,J.M.; Schouten,T.J.; Schretlen,E.D.A.M.; de Bruyn,C.H.M.M. High-performance liquid chromatographic determination of plasma 6-mercaptopurine in clinically relevant concentrations, *J.Chromatogr.*, **1982**, *227*, 526–533.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 25 μL 4 μg/mL 6-thioguanine in water + 100 μL 400 mM NaOH + 1 mL 0.3% phenylmercuric acetate in ethyl acetate + 3 mL diethyl ether, shake on a tumble mixer for 10 min, centrifuge for 5 min. Remove the organic layer and add it to 500 μL 100 mM HCl, whirlmix for 2 min, centrifuge for 5 min, discard the organic layer, evaporate traces of organic solvent under a stream of nitrogen at room temperature for 15 min, add 10 μL 3 mg/mL dithioerythritol in water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 LiChrosorb 10 RP-18
Mobile phase: Isopropanol:water 3:97 containing 13.80 g/L $NaH_2PO_4.H_2O$, 200 μL/L 85% phosphoric acid, 60 mg/L dithioerythritol, and 500 mg/L sodium octanesulfonate, pH 3.6-3.7
Flow rate: 1.5
Detector: F ex 295 em 380 following post-column reaction. The column effluent mixed with 8 mM potassium chromate in 500 mM HCl pumped at 0.16 mL/min and with air flowing at 0.32 mL/min and the mixture flowed through a single mixing coil. The effluent from this coil mixed with 1.6% sodium metabisulfite pumped at 0.16 mL/min and this mixture flowed through a single mixing coil. The effluent from this coil mixed with 4 M ammonium hydroxide pumped

at 0.23 mL/min and this mixture flowed through a double mixing coil to a debubbler. The liquid effluent from the debubbler flowed to the detector.

CHROMATOGRAM
Retention time: 6
Internal standard: 6-thioguanine (8)
Limit of detection: <2 ng/mL

KEY WORDS
post-column reaction; plasma; pharmacokinetics

REFERENCE
Jonkers,R.E.; Oosterhuis,B.; ten Berge,R.J.M.; van Boxtel,C.J. Analysis of 6-mercaptopurine in human plasma with a high-performance liquid chromatographic method including post-column derivatization and fluorimetric detection, *J.Chromatogr.*, **1982**, *233*, 249–255.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 80 μL 10 μg/mL 6-thioguanine in water + 10 μL 1 M dithiothreitol, vortex for 10 s, add 2 mL MeCN, vortex for 30 s, centrifuge at 2000 g for 5 min. Remove the supernatant and add it to 2 mL dichloromethane, shake on a reciprocating shaker for 5 min, centrifuge at 2000 g for 5 min. Remove 750 μL from the top aqueous layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 150 μL distilled water, vortex for 1 min, inject a 15 μL aliquot.

HPLC VARIABLES
Guard column: 70 × 2.2 30-38 μm Co:Pell ODS
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:acetic acid:water 3.5:0.2:96.3
Flow rate: 1.4
Injection volume: 15
Detector: UV 322

CHROMATOGRAM
Retention time: 4.8
Internal standard: 6-thioguanine (6.5)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: caffeine, cytarabine, 5-fluorouracil, prednisone, theophylline, vinblastine, vincristine

KEY WORDS
plasma; protect from light; monkey; pharmacokinetics; human

REFERENCE
Narang,P.K.; Yeager,R.L.; Chatterji,D.C. Quantitation of 6-mercaptopurine in biologic fluids using high-performance liquid chromatography: a selective and novel procedure, *J.Chromatogr.*, **1982**, *230*, 373–380.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 5 μL 50 μg/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 μBondapak 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: 18.6
Internal standard: 2-ethyl-4-oxoquinazoline (28)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: azathioprine

KEY WORDS
serum; derivatization

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: blood
Sample preparation: 250 μL Plasma + 5 μL 2.5 μg/mL 6-thioguanine + 25 μL 1 M aluminum perchlorate in water, let stand at room temperature for 15 min, chill in ice water for 15 min, centrifuge at 15600 g for 15 min, discard the supernatant. Suspend the precipitate in 500 μL 50 mM aluminum perchlorate in water by stirring to break up the precipitate, vortex for 20 s, centrifuge at 15600 g for 15 min, discard the supernatant. Suspend the precipitate in 150 μL 400 mM perchloric acid, add 5 μL freshly prepared 200 mM aqueous sodium hydrosulfite, mix, let stand at room temperature for 30 min, chill in ice water, centrifuge at 15600 g for 15 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 45 × 2 40 μm ODS
Column: 150 × 4.3 5 μm Ultrasphere ODS
Mobile phase: Water:85% phosphoric acid 99.32:0.68 containing 154.3 mg/L dithiothreitol
Flow rate: 1
Injection volume: 50
Detector: UV 340

CHROMATOGRAM
Retention time: 6.08
Internal standard: 6-thioguanine (4.73)
Limit of quantitation: 3 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, 6-thiouric acid

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Lin,K.T.; Varin,F.; Rivard,G.E.; Leclerc,J.M. Isolation of 6-mercaptopurine in human plasma by aluminum ion complexation for high-performance liquid chromatographic analysis, *J.Chromatogr.*, **1991**, *536*, 349–355.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 800 μL MeOH, stir for 15 s, let stand for 20 min, centrifuge at 5° at 3000 rpm for 10 min. Remove 800 μL of the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 ODS-II (Shimadzu)
Mobile phase: MeOH:water 7:100 containing 0.2% glacial acetic acid and 0.1% sodium 1-heptanesulfonate
Flow rate: 1
Injection volume: 40
Detector: UV 325

CHROMATOGRAM
Retention time: 6.8
Limit of detection: 2 ng/mL

KEY WORDS
plasma; pharmacokinetics; rat

REFERENCE
Takeichi,Y.; Kimura,T. Improvement of aqueous solubility and rectal absorption of 6-mercaptopurine by addition of sodium benzoate, *Biol.Pharm.Bull.*, **1994**, *17*, 1391–1394.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 50 μL 2.8 μg/mL 6-mercaptopurine arabinoside + 2-3 mg mercurial cellulose, vortex for 30 s, centrifuge at 550 g for 5 min. Resuspend the pellet in 2 mL phosphate buffered saline, centrifuge, repeat this process twice. Suspend the pellet in 250 μL 20 mM 2-mercaptoethanol, centrifuge, inject a 100 μL aliquot of the supernatant. (Prepare mercurial cellulose as follows. Add 40 g Sigmacell Type 20 cellulose (20 μm) to 175 mL 40% NaOH at 5°, let stand at 0° for 2 h, add 40 mL allyl glycidyl ether dropwise, add 100 mL water, heat at 70-80° for 2 h. Suspend the cellulose in 4 L water, allow to settle, decant the water, wash several more times, filter, wash with water until the pH of the filtrate is about 8, wash with 1 L 10% acetic acid, wash 1 L with EtOH:water 95:5, wash with 1 L MeOH, wash with 1 L diethyl ether, air dry. Add to 400 mL 10% acetic acid containing 2.3 g mercuric acetate, stir at 60° for 1 h, filter, wash with 2 L 10% acetic acid, wash with 1.5 L water, wash with 300 mL EtOH:water 95:5, wash with 700 mL MeOH, wash with 1 L diethyl ether, air dry in the dark, store in the dark (Anal.Biochem. 1985, 144, 514).)

HPLC VARIABLES
Column: 250 × 4.6 5 μm ODS (Beckman)
Mobile phase: MeCN:10 mM pH 3.0 sodium phosphate buffer 2:98
Flow rate: 1.2
Injection volume: 100
Detector: UV 323

CHROMATOGRAM
Retention time: 7.6
Internal standard: 6-mercaptopurine arabinoside (13.6)
Limit of detection: 0.5 ng/mL

OTHER SUBSTANCES
Noninterfering: aspirin, chloroquine, cyclosporin, diltiazem, nifedipine, prednisolone

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Albertioni,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: Add 1 volume ice-cold 8 M perchloric acid to 20 volumes plasma, mix, keep on ice for 10 min, centrifuge at 10 000 g for 15 min, remove the supernatant. Adjust the pH of the supernatant to 6-7 with 10 volumes ice-cold 4 M K_2KHPO_4, keep on ice for 10 min, centrifuge at 10 000 g for 5 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-18-DB
Mobile phase: Gradient. A was 25 mM KH_2PO_4. B was MeOH:50 mM KH_2PO_4 25:75. A:B from 98:2 to 96:4 over 5 min, to 85:15 over 3 min, to 80:20 over 2 min, to 40:60 over 10 min, maintain at 40:60 over 2 min, to 20:80 over 3 min, maintain at 20:80 for 20 min, return to initial conditions over 3 min, re-equilibrate for 12 min.
Flow rate: 1.25
Injection volume: 100
Detector: UV 320

CHROMATOGRAM
Retention time: 11.6
Limit of detection: 20-50 nM

OTHER SUBSTANCES
Extracted: metabolites, thioguanine (UV 342)

KEY WORDS
plasma

REFERENCE
Keuzenkamp-Jansen,S.W.; De Abreu,R.A.; Bökkerink,J.P.M.; Trijbels,J.M.F. Determination of extracellular and intracellular thiopurines and methylthiopurines by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *672*, 53–61.

SAMPLE
Matrix: blood
Sample preparation: Treat whole blood with EDTA, freeze a 2 mL aliquot at -20°, thaw, add 2 mL PBS, centrifuge at 3000 g for 15 min, suspend the pellet in 200 μL PBS, treat with proteinase K at 70° for 10 min, add to a disposable spin column (Diagen QIAamp Blood Kit, Hilden, Germany), centrifuge for 1 min, wash twice with buffer for 1 min, elute with 200 μL 10 mM pH 9.0 Tris-HCl buffer containing 0.1 mM EDTA, heat at 100° for 5 min, cool in ice. Remove a 100 μl aliquot and add it to 10 μL buffer, add 20 μL 25 μg/mL P_1 nuclease (Boehringer Mannheim) and 12.5 U/mL acid phosphatase (Sigma) in buffer:water 10:90, heat at 42° for 1 h, add 10 μL 400 mM formic acid, add 60 μL MeOH, add 1 μL 5 mM N-[6-(7-amino-4-methylcoumarin-3-acetamido)hexyl]-3'-(2'-pyridyldithio)propionamide (AMCA-HPDP, Pierce) in DMF, inject a 25 μL aliquot. (Buffer was 500 mM pH 4.5 sodium acetate buffer containing 10 mM magnesium chloride.)

HPLC VARIABLES
Guard column: 20 mm long Supelguard (Supelco)
Column: 150 × 4.6 3 μm Supelcosil LC-8
Mobile phase: MeOH:buffer 37:63 (Between analyses wash column with MeOH:buffer 80:20 for 3 min. Buffer was 200 mM formic acid adjusted to pH 4.0 with 10 M NaOH.)
Column temperature: 45
Flow rate: 1
Injection volume: 25
Detector: F ex 345 em 450

CHROMATOGRAM
Retention time: 11.5 (as 2'-deoxy-6-thioguanosine metabolite)
Limit of detection: 60 pmole/g DNA

KEY WORDS
derivatization; whole blood

REFERENCE

Warren,D.J.; Andersen,A.; Slordal,L. Quantitation of 6-thioguanine residues in peripheral blood leukocyte DNA obtained from patients receiving 6-mercaptopurine-based maintenance therapy, *Cancer Res.*, **1995**, *55*, 1670–1674.

SAMPLE

Matrix: blood, CSF, urine

Sample preparation: Blood, CSF. Collect 2 mL blood in tubes containing heparin and 120 μg dithiothreitol (DTT). Mix, centrifuge at 2000 g for 5 min, remove plasma. CSF. Collect 0.5 mL CSF in tubes containing 30 μg DTT. Cool CSF and plasma samples on ice, add freshly prepared ice-cold 50% trichloroacetic acid equal to 10% of sample volume. Urine. Collect 2 mL urine in tubes containing 120 μg DTT, filter (0.22 μm), inject an aliquot.

HPLC VARIABLES

Column: two 250 × 4.6 10μm Nucleosil 10 C18 columns in series

Mobile phase: Gradient. A was 25 mM pH 2.75 phosphoric acid. B was MeOH:water 50:50. C was 100 mM pH 6.6 KH_2PO_4. A:B:C from 100:0:0 to 98:2:0 over 5 min, to 30:3.5:66.5 over 5 min, maintain at 30:3.5:66.5 for 10 min, re-equilibrate at initial conditions for 10 min.

Column temperature: 33

Flow rate: 1.7

Injection volume: 195, 500

Detector: UV 312

CHROMATOGRAM

Retention time: 14

Limit of detection: 20 nM

OTHER SUBSTANCES

Extracted: metabolites, 6-mercaptopurine riboside, thioguanine (UV 342), 6-thioguanosine

KEY WORDS

plasma; goat; pharmacokinetics

REFERENCE

van Baal,J.M.; van Leeuwen,M.B.; Schouten,T.J.; De Abreu,R.A. Sensitive high-performance liquid chromatographic determination of 6-mercaptopurine, 6-thioguanine, 6-mercaptopurine riboside and 6-thioguanosine in biological fluids, *J.Chromatogr.*, **1984**, *336*, 422–428.

SAMPLE

Matrix: enzyme incubations

Sample preparation: To 237 μL enzyme incubation add 850 μL ice-cold 3.5 mM DL-dithiothreitol immediately followed by 500 μL 1.5 M sulfuric acid, place tubes on ice. Equilibrate tubes to room temperature, heat at 100° for 2 h. Cool, add 500 μL 3.4 M NaOH immediately followed by 8 mL toluene:amyl alcohol:phenyl mercury acetate mixture. Shake gently for 10 min, centrifuge at 10° at 900 g for 5 min. Transfer 6 mL toluene to another tube and add 200 μL 100 mM HCl. Vortex for four 20 s periods, centrifuge at 10° at 900 g for 5 min, inject a 50 μL aliquot of the aqueous layer. (Prepare toluene:amyl alcohol:phenyl mercury acetate mixture by adding enough phenyl mercury acetate to toluene containing 170 mM amyl alcohol so as to form a solution containing 1.3 mM phenyl mercury acetate, mix gently for 1 h, store in the dark (J.Chromatogr. 1992, 583, 83).)

HPLC VARIABLES

Guard column: 5 × 4 5 μm Resolve C18

Column: 100 × 8 5μm Resolve C18 radial compression

Mobile phase: MeOH:water 20:80 containing 100 mM triethylamine and 0.5 mM dithiothreitol, pH adjusted to 3.2 with orthophosphoric acid (Add dithiothreitol immediately prior to use.)

Flow rate: 1.5

Injection volume: 50

Detector: UV 303

CHROMATOGRAM

Retention time: 4

OTHER SUBSTANCES
Extracted: methylmercaptopurine

REFERENCE
Lennard,L.; Singleton,H.J. High-performance liquid chromatographic assay of human red blood cell thiopurine methyltransferase activity, *J.Chromatogr.B*, **1994**, *661*, 25–33.

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 × 5 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: 1.7
Internal standard: theophylline (3.5)

OTHER SUBSTANCES
Simultaneous: azathioprine, impurities, degradation products

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.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 40 × 4 C18 Corasil II
Column: 300 × 4 10 μm μBondapak phenyl
Mobile phase: Propanol:6 mM C_{12} DAPS (Fluka) 3:97 (C_{12} DAPS is 3-(dimethyldodecylammonio)propanesulfonate.)
Injection volume: 25
Detector: UV 273

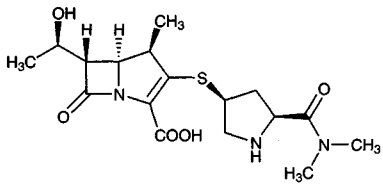
CHROMATOGRAM
Retention time: 3.3

OTHER SUBSTANCES
Simultaneous: albendazole, aminophylline, antipyrine, caffeine, dipropyline, flubendazole, metronidazole, nimorazole, procaine, β-hydroxytheophylline, theophylline, tinidazole
Interfering: albendazole sulfoxide, amyleine, theobromine

KEY WORDS
micellar chromatography

REFERENCE
Habel,D.; Guermouche,S.; Guermouche,M.H. Direct determination of theophylline in human serum by high-performance liquid chromatography using zwitterionic micellar mobile phase. Comparison with an enzyme multiplied immunoassay technique, *Analyst*, **1993**, *118*, 1511–1513.

Meropenem



Molecular formula: $C_{17}H_{25}N_3O_5S$
Molecular weight: 383.45
CAS Registry No.: 96036-03-2
Merck Index: 5960

SAMPLE
Matrix: bile, blood
Sample preparation: Condition a 100 mg Bond Elut C8 SPE cartridge with two 1 mL portions of MeOH and two 1 mL portions of 500 mM KH_2PO_4. 100 µL Plasma or 50 µL bile + 900 µL water, mix, add to the SPE cartridge, wash with 1 mL 50 mM KH_2PO_4, elute with 800 (plasma) or 600 (bile) µL MeOH:50 mM pH 6.0 phosphate buffer 10:90, inject a 100 µL aliquot of the eluate.

HPLC VARIABLES
Column: 100 × 4.6 3 µm Hypersil ODS
Mobile phase: MeOH:buffer 15:85 (Buffer was 50 (plasma) or 57.5 (bile) mM pH 7.0 phosphate buffer.)
Flow rate: 1
Injection volume: 100
Detector: UV 296 (plasma), UV 310 (bile)

CHROMATOGRAM
Retention time: 5-5.5
Limit of detection: 280 ng/mL (bile), 100 ng/mL (plasma)

KEY WORDS
plasma; SPE

REFERENCE
Granai,F.; Smart,H.L.; Triger,D.R. A study of the penetration of meropenem into bile using endoscopic retrograde cholangiography, *J.Antimicrob.Chemother.*, **1992**, *29*, 711–718.

SAMPLE
Matrix: blood
Sample preparation: Dilute 50 µL plasma with 75 µL 50mM pH 7.0 phosphate buffer, inject a 100 µL aliquot onto column A with mobile phase A, elute to waste with mobile phase A, after 4 min backflush the contents of column A onto column B with mobile phase B, monitor the effluent from column B. After 11 min re-equilibrate column A with mobile phase A and column B with mobile phase B for 5 min.

HPLC VARIABLES
Column: 20 × 3.9 25-40 µm LiChroprep RP-8; B 4.0 × 10 Nova -Pak C8 + 150 × 4.6 5 µm Inertsil ODS
Mobile phase: A 50 mM pH 7.0 phosphate buffer; B MeCN:50 mM pH 7.0 phosphate buffer 6:94
Flow rate: 1
Injection volume: 100
Detector: UV 300

CHROMATOGRAM
Retention time: 6.5

Limit of detection: 100 ng/mL

KEY WORDS
rat; plasma; column-switching; pharmacokinetics

REFERENCE
Lee,H.S.; Shim,H.O.; Yu,S.R. High-performance liquid chromatographic determination of meropenem in rat plasma using column-switching, *Chromatographia*, **1996**, *42*, 405–408.

SAMPLE
Matrix: blood
Sample preparation: Dilute serum with an equal volume of water, inject 20 μL diluted serum onto column A, elute to waste with mobile phase A, after 1.1 min elute the contents of column A to column B with mobile phase B, elute with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 50 × 2.1 40 μm Supelclean LC-NH_2 (Supelco); B 150 × 4.6 3 μm C18 (Supelco)
Mobile phase: A MeOH:10 mM pH 7.0 phosphate buffer 5:95; B MeOH:10 mM pH 7.0 phosphate buffer containing 5 mM tetrabutylammonium hydrogen sulfate 30:70
Flow rate: A 0.3; B 1
Injection volume: 20
Detector: UV 298

CHROMATOGRAM
Retention time: 4.2
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Noninterfering: acetaminophen, acyclovir, digoxin, fluconazole, theophylline, vancomycin

KEY WORDS
column-switching; serum

REFERENCE
Bompadre,S.; Ferrante,L.; de Martinis,M.; Leone,L. Determination of meropenem in serum by high-performance liquid chromatography with column switching, *J.Chromatogr.A*, **1998**, *812*, 249–253.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 900 μL water, add to a conditioned Bond Elut C18 SPE cartridge, wash with 1 mL 50 mM KH_2PO_4, elute with 800 μL mobile phase, inject an aliquot of the eluate.

HPLC VARIABLES
Column: 100 mm long 3 μm Hypersil ODS
Mobile phase: MeOH:5 mM tetrabutylammonium dihydrogen phosphate 12:88
Flow rate: 1
Detector: UV 296

CHROMATOGRAM
Retention time: 9
Limit of detection: 200 ng/mL

KEY WORDS
SPE; plasma; pharmacokinetics

REFERENCE
Harrison,M.P.; Haworth,S.J.; Moss,S.R.; Wilkinson,D.M.; Featherstone,A. The disposition and metabolic fate of ^{14}C-meropenem in man, *Xenobiotica*, **1993**, *23*, 1311–1323.

SAMPLE
Matrix: blood
Sample preparation: Dilute plasma with water, prepare using a 1 mL C18 SPE cartridge.

HPLC VARIABLES
Column: 100 × 4.6 3 μm Hypersil C18
Mobile phase: MeOH:5 mM tetrabutylammonium dihydrogen phosphate 15:85
Detector: UV 296

CHROMATOGRAM
Limit of quantitation: 500 ng/mL

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Bedikian,A.; Okamoto,M.P.; Nakahiro,R.K.; Farino,J.; Heseltine,P.N.R.; Appleman,M.D.; Yellin,A.E.; Berne,T.V.; Gill,M.A. Pharmacokinetics of meropenem in patients with intra-abdominal infections, *Antimicrob.Agents Chemother.*, **1994**, *38*, 151–154.

SAMPLE
Matrix: blood, formulations, urine
Sample preparation: Dilute plasma 1:4, urine 1:10, and injections 1:100 with water, filter (0.6 μm), inject a 5-50 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 200 × 4 5 μm Nucleosil C18
Mobile phase: MeOH:10 mM pH 7.4 potassium phosphate buffer 18:82 (plasma, injections) or 25:75 (urine)
Flow rate: 1
Injection volume: 5-50
Detector: UV 296

CHROMATOGRAM
Limit of detection: 5 μg/mL (urine), 500 ng/mL (plasma)

KEY WORDS
plasma; injections; saline; pharmacokinetics

REFERENCE
Burman,L.Å.; Nilsson-Ehle,I.; Hutchison,M.; Haworth,S.J.; Norrby,S.R. Pharmacokinetics of meropenem and its metabolite ICI 213,689 in healthy subjects with known renal metabolism of imipenem, *J.Antimicrob.Chemother.*, **1991**, *27*, 219–224.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Condition a Bond Elut C18 SPE cartridge. Add 100 μL plasma to the SPE cartridge, wash with 50 mM KH_2PO_4, elute with 800 μL eluent, inject an aliquot of the eluate. Urine. Dilute urine 1:10 with water, inject an aliquot directly. (Eluent was MeOH: 5 mM tetrabutylammonium dihydrogen phosphate 12:88.)

HPLC VARIABLES
Column: 100 × 4 3 μm Hypersil ODS
Mobile phase: MeOH:5 mM tetrabutylammonium dihydrogen phosphate 12:88 (plasma) or MeCN:10 mM pH 7.4 phosphate buffer 6:100 (urine)
Detector: UV 296

CHROMATOGRAM
Limit of detection: 60 ng/mL (plasma), 10 μg/mL (urine)

KEY WORDS
SPE; plasma; pharmacokinetics

REFERENCE
Bax,R.P.; Bastain,W.; Featherstone,A.; Wilkinson,D.M.; Hutchison,M. Haworth,S.J. The pharmacokinetics of meropenem in volunteers, *J.Antimicrob.Chemother.*, **1989**, *24*, 311–320.

SAMPLE
Matrix: enzyme incubations
Sample preparation: Add 2 volumes of MeOH, mix well, centrifuge at 3000 g for 15 min, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES
Column: Inertsil ODS-2
Mobile phase: MeOH:5 mM tetrabutylammonium phosphate 30:70
Flow rate: 1
Injection volume: 10
Detector: UV 300

CHROMATOGRAM
Limit of quantitation: 1 μg/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: urine
Sample preparation: Dilute urine 1:5 with water, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 50 mm long 3 μm Hypersil ODS
Mobile phase: MeCN:10 mM pH 7.4 phosphate buffer 6:100
Flow rate: 1
Injection volume: 50
Detector: UV 296

CHROMATOGRAM
Retention time: 5.5
Limit of detection: 600 ng/mL

KEY WORDS
pharmacokinetics

REFERENCE
Harrison,M.P.; Haworth,S.J.; Moss,S.R.; Wilkinson,D.M.; Featherstone,A. The disposition and metabolic fate of ^{14}C-meropenem in man, *Xenobiotica*, **1993**, *23*, 1311–1323.

SAMPLE
Matrix: urine
Sample preparation: Dilute urine with water, prepare using a 1 mL C18 SPE cartridge.

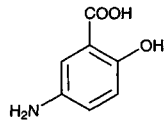
HPLC VARIABLES
Column: 150 × 4.6 5 μm Partisil silica
Mobile phase: 0.1% Phosphoric acid in water
Detector: UV 313

CHROMATOGRAM
Limit of quantitation: 1 μg/mL

KEY WORDS
SPE; pharmacokinetics

REFERENCE
Bedikian,A.; Okamoto,M.P.; Nakahiro,R.K.; Farino,J.; Heseltine,P.N.R.; Appleman,M.D.; Yellin,A.E.; Berne,T.V.; Gill,M.A. Pharmacokinetics of meropenem in patients with intra-abdominal infections, *Antimicrob.Agents Chemother.*, **1994**, *38*, 151–154.

Mesalamine



Molecular formula: $C_7H_7NO_3$
Molecular weight: 153.14
CAS Registry No.: 89-57-6
Merck Index: 5964

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 4 mL MeOH, let stand for 15 min at 4°, centrifuge at 3000 g for 15 min. Remove 1 mL of the supernatant and add it to 1 mL water, mix, inject an aliquot.

HPLC VARIABLES
Column: 40 × 4.6 3 μm Hypersil
Mobile phase: MeOH:water:200 mM pH 6.5 potassium phosphate buffer 40:40:20 containing 4 mM hexadecyltrimethylammonium bromide
Column temperature: 34
Flow rate: 1.7
Injection volume: 20
Detector: F ex 315 em 470

CHROMATOGRAM
Retention time: 2.5

KEY WORDS
plasma

REFERENCE
Tjornelund,J.; Hansen,S.H. Stability of 5-aminosalicylic acid and its metabolites in plasma at -20°C. Formation of N-β-D-glucopyranosyl-5-aminosalicylic acid, *J.Chromatogr.*, **1991**, *570*, 224–228.

SAMPLE
Matrix: blood, feces, urine
Sample preparation: Plasma. 1 mL Plasma + 2 mL MeOH, vortex for 20 s, let stand for 15 min at -20° and 10 min at room temperature, mix, centrifuge at 2000 g for 15 min. Remove the supernatant and add it to 5 mL 1,1,1-trichloroethane, shake for 2 min, centrifuge at 2000 g for 15 min, inject a 20 μL aliquot of the supernatant. Urine. 1 mL Urine + 4 mL MeOH, mix, let stand at -20° for 15 min, centrifuge at 2000 g for 15 min. Dilute an aliquot of the supernatant with an equal volume of water, inject a 20 μL aliquot. Feces. Extract with 500-1000 mL MeOH, centrifuge at 10000 g for 4 min. Dilute an aliquot of the supernatant with an equal volume of water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 40 × 4.6 3 μm Hypersil
Mobile phase: MeOH:water:200 mM pH 6.5 potassium phosphate buffer 40:40:20 containing 4 mM hexadecyltrimethylammonium bromide
Column temperature: 34
Flow rate: 1.7
Injection volume: 20

Detector: F ex 315 em 470

CHROMATOGRAM
Retention time: 2.5
Limit of detection: 4 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
plasma

REFERENCE
Tjornelund,J.; Hansen,S.H. High-performance liquid chromatographic assay of 5-aminosalicylic acid (5-ASA) and its metabolites N-β-D-glucopyranosyl-5-ASA, N-acetyl-5-ASA, N-formyl-5-ASA and N-butyryl-5-ASA in biological fluids, *J.Chromatogr.*, **1991**, *570*, 109–117.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. Add disodium-3,5'-azo-bis-(6-hydroxybenzoate) to serum, treat with proteinase K (0.5 mg/mL protein) for 10 min, add tetrabutylammonium hydrogen sulfate buffered to pH 6.5, add dichloromethane, agitate for 30 min, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in mobile phase, inject an aliquot. Urine. Add 2,4-dihydroxybenzoic acid to urine, react with propionic anhydride for 5 min, add perchloric acid, add diethyl ether, shake for 10 min, freeze. Remove the organic layer and add it to pH 7.4 phosphate buffer, extract, inject an aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: 30 × 4 30-40 μm Perisorb RP-18
Column: 250 × 4 10 μm Nucleosil C18
Mobile phase: MeOH:buffer 30:70 (Buffer was pH 7.4 phosphate buffer containing 20 mM tetrabutylammonium hydrogen sulfate.)
Detector: F ex 312 em 469

CHROMATOGRAM
Retention time: k' 2.4 (propionyl derivative)
Internal standard: disodium-3,5'-azo-bis-(6-hydroxybenzoate), 2,4-dihydroxybenzoic acid
Limit of quantitation: 6.1 μM urine, 0.4 μM (serum)

OTHER SUBSTANCES
Extracted: acetylaminosalicylic acid

KEY WORDS
serum; pharmacokinetics; derivatization

REFERENCE
Ryde,E.M.; Ahnfelt,N.-O. The pharmacokinetics of olsalazine sodium in healthy volunteers after a single i.v. dose and after oral doses with and without food, *Eur.J.Clin.Pharmacol.*, **1988**, *34*, 481–488.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 500 μL Plasma + 500 μL 100 mg/mL (sic) 2,4-dihydroxybenzoic acid in MeOH, mix, centrifuge, inject an aliquot. Urine. 100 μL Urine + 500 μL 600 mg/mL (sic) 2,4-dihydroxybenzoic acid in MeOH, mix, centrifuge, inject an aliquot.

HPLC VARIABLES
Guard column: μBondapak C18 Guard Pak
Column: 250 × 4.1 10 μm Caulab CSC PRP-1
Mobile phase: MeCN:MeOH:50 mM pH 7.9 potassium phosphate buffer 10:10:80
Flow rate: 1

Detector: F ex 315 em 475

CHROMATOGRAM
Retention time: 5.1
Internal standard: 2,4-dihydroxybenzoic acid (3.3)
Limit of quantitation: 1000 ng/mL (urine), 25 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: N-acetyl-5-aminosalicylic acid

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Corey,A.E.; Rose,G.M.; Conklin,J.D. Bioavailability of single and multiple doses of enteric-coated mesalamine and sulphasalazine, *J.Int.Med.Res.*, **1990**, *18*, 441–453.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 205.2

CHROMATOGRAM
Retention time: 4.737

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve in dilute HCl (pH 2), sonicate if necessary, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil-BDS C18
Mobile phase: MeOH:THF:buffer 11:4:85 (Buffer was 8.6 g $NaH_2PO_4.H_2O$, 8.2 g NaCl, 2.2 g sodium 1-heptanesulfonate, and 5.75 mL 85% phosphoric acid in 2 L water, pH 2.0.)
Column temperature: 35
Flow rate: 1.5

Injection volume: 20
Detector: UV 300 or 215

CHROMATOGRAM
Retention time: 3.6

OTHER SUBSTANCES
Simultaneous: impurities, salicylic acid

REFERENCE
Kersten,B.S.; Catalano,T.; Rozenman,Y. Ion-pairing high-performance liquid chromatographic method for the determination of 5-aminosalicylic acid and related impurities in bulk chemical, *J.Chromatogr.*, **1991**, *588*, 187–193.

SAMPLE
Matrix: formulations
Sample preparation: 75 μL Sample + 6 mL mobile phase, vortex for 2 min, add 300 μL 300 μg/mL acetaminophen, make up to 10 mL with mobile phase, mix for 2 min, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 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: 6.0
Internal standard: acetaminophen (3.8)

KEY WORDS
stability-indicating; 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: microsomal incubations
Sample preparation: 250 μL Microsomal incubation + 500 μL MeOH, centrifuge, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 120 × 4.6 5 μm RP-18
Mobile phase: MeCN:200 mM pH 7.5 phosphate buffer:1.25 mM cetyltrimethylammonium bromide 30:5:65
Flow rate: 1
Detector: F ex 315 em 500

KEY WORDS
rat; for 5-aminosalicylic acid-O-sulfate and 5-aminosalicylic acid

REFERENCE
Herzog,R.; Leuschner,J. Experimental studies on the pharmacokinetics and toxicity of 5-aminosalicylic acid-O-sulfate following local and systemic application, *Arzneimittelforschung*, **1995**, *45*, 300–303.

SAMPLE
Matrix: tissue

Sample preparation: Freeze mucosal intestinal biopsy (ca. 5 mg) in liquid nitrogen and crush the sample, allow to warm to room temperature, add 20 μL propionic anhydride, add 100 μL 57.6 μg/mL N-propionyl-4-aminosalicylic acid (purified by HPLC) in mobile phase, add 500 μL 50 mM pH 7.4 phosphate buffer, wash grinding/mixing rod with 500 μL 50 mM pH 7.4 phosphate buffer, sonicate (80 W) by immersing microprobe tip in mixture for 1 min, vortex, let stand at 37° for 1 h, add 500 μL 10% NaCl, add 6 mL MeCN, extract, cool at 4° for 1 h, centrifuge at 1500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of air, reconstitute the residue in 500 μL mobile phase, filter (0.45 μm), inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 5 μm Spherisorb ODS-2
Column: 150 × 4.6 3 μm Spherisorb ODS-2
Mobile phase: MeCN:100 mM acetic acid:triethylamine 8:92:0.2
Flow rate: 1.5
Injection volume: 100
Detector: F ex 315 em 430

CHROMATOGRAM
Retention time: 5.7
Internal standard: N-propionyl-4-aminosalicylic acid (7.3)
Limit of detection: 1 ng

OTHER SUBSTANCES
Extracted: N-acetyl-5-aminosalicylic acid

KEY WORDS
mucosal intestinal biopsy; derivatization

REFERENCE
De Vos,M.; Verdievel,H.; Schoonjans,R.; Beke,R.; De Weerdt,G.A.; Barbier,F. High-performance liquid chromatographic assay for the determination of 5-aminosalicylic acid and acetyl-5-aminosalicylic acid concentrations in endoscopic intestinal biopsy in humans, *J.Chromatogr.*, **1991**, *564*, 296–302.

SAMPLE
Matrix: tissue
Sample preparation: Sonicate tissue in 2 mL 5 ng/mL 3,4-dihydroxybenzylamine in MeOH for two 30 s cycles (W = 60), centrifuge at 1800 g for 10 min. Filter (0.5 μm) the supernatant, evaporate the filtrate to dryness under a stream of nitrogen under reduced pressure, reconstitute with 100 μL mobile phase, vortex, inject a 5 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 40 μm Pelliguard (Supelco)
Column: 250 × 4.6 10 μm Erbasil S C18 (Carlo Erba)
Mobile phase: MeOH:buffer 15:85, pH adjusted to 3 with 100 mM NaOH. (Buffer was 10 mM Na_2HPO_4 containing 0.1 mM EDTA, 100 mM citric acid, and 0.1 mM heptanesulfonic acid.)
Flow rate: 1
Injection volume: 5
Detector: E, ESA Model 5100A, Model 5021 conditioning cell +0.35 V (between column and analytical cell), Model 5011 analytical cell, first electrode +0.05 V, second electrode -0.50 V (monitored)

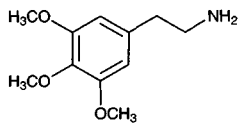
CHROMATOGRAM
Retention time: 3.2
Internal standard: 3,4-dihydroxybenzylamine (4.4)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE

Palumbo,G.; Carlucci,G.; Mazzeo,P.; Frieri,G.; Pimpo,M.T.; Fanini,D. Simultaneous determination of 5-aminosalicylic acid, acetyl-5-aminosalicylic acid and 2,5-dihydroxybenzoic acid in endoscopic intestinal biopsy samples in humans by high-performance liquid chromatography with electrochemical detection, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 175–180.

Mescaline



Molecular formula: $C_{11}H_{17}NO_3$
Molecular weight: 211.26
CAS Registry No.: 54-04-6
Merck Index: 5965

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

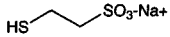
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primi-

done, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Mesna



Molecular formula: $C_2H_5NaO_3S_2$
Molecular weight: 164.18
CAS Registry No.: 19767-45-4, 3375-50-6 (free acid)
Merck Index: 5969

SAMPLE
Matrix: blood
Sample preparation: 50 μL Plasma (within 3 min of collection) + penicillamine + 10 μL 25 mM monobromobimane in MeCN, let stand for 5 min at room temperature, add 20 μL 20% perchloric acid. (For total mesna add 100 μL 20 mM dithiothreitol in 200 mM pH 8.5 Tris/HCl buffer to 50 μL plasma, let stand for 40 min at room temperature, add 50 μL 15% sulfosalicylic acid, centrifuge. Remove 200 μL of the supernatant and wash it three times with ethyl acetate. Remove 60 μL of the aqueous phase and add it to 300 μL 200 mM pH 8.5 Tris/HCl buffer and 10 μL 15 mM monobromobimane in MeCN, let stand at room temperature for 5 min, add 20 μL 20% perchloric acid.)

HPLC VARIABLES
Column: 150 × 4.6 7 μm Nucleosil RP-18
Mobile phase: Gradient. A was MeCN. B was 1% aqueous acetic acid containing 1 g/L octanesulfonic acid. A:B from 5:95 to 8:92 over 2 min, to 10:90 over 13 min (Waters convex), to 30:70 over 20 min (Waters convex), maintain at 30:70 for 6 min, re-equilibrate at initial conditions for 9 min.
Flow rate: 1.4
Detector: F (wavelengths not given)

CHROMATOGRAM
Internal standard: penicillamine
Limit of detection: 10 μM

KEY WORDS
plasma; derivatization; pharmacokinetics

REFERENCE
Stofer-Vogel,B.; Cerny,T.; Borner,M.; Lauterburg,B.H. Oral bioavailability of mesna tablets, *Cancer Chemother.Pharmacol.*, **1993**, *32*, 78–81.

SAMPLE
Matrix: blood, urine

Sample preparation: Plasma. 400 μL Plasma + 400 μL 333 mM sulfuric acid + 400 μL 5% sodium hexametaphosphate, vortex for 5 s, centrifuge at 15000 g for 2 min, inject a 20 μL aliquot of the supernatant. (Process plasma as rapidly as possible after collection.) (For total mesna mix 100 μL plasma, 100 μL EDTA solution, and 100 μL 4% sodium borohydride, heat to 50° for 30 min, cool, add 200 μL 9.7% acetic acid, store in the dark for 3-4 days, inject an aliquot. (EDTA solution was 1% disodium EDTA in a mixture of 50 mL 500 mM Na_2HPO_4 and 22 mL 1 M NaOH.)) Urine. Dilute 1:10 to 1:400 with mobile phase, inject an aliquot. (For total mesna dilute 1:10 to 1:1000 then proceed as above.)

HPLC VARIABLES

Column: 100 × 4.6 3 μm Hypersil ODS
Mobile phase: MeCN:buffer 3:97 (Buffer was 100 mM pH 7.0 phosphate buffer containing 5 mM n-tetrabutylammonium hydroxide.)
Flow rate: 1
Injection volume: 20
Detector: E, Coulochem Model 5100A, Model 5011 analytical cell, detector 1 not used, detector 2 -200 mV

CHROMATOGRAM

Retention time: 4.5
Limit of quantitation: 6.1 μM

KEY WORDS

plasma

REFERENCE

James,C.A.; Rogers,H.J. Estimation of mesna and dimesna in plasma and urine by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1986**, *382*, 394–398.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 100 μL Plasma + 25 μL 330 mM sulfuric acid + 25 μL 5% sodium hexametaphosphate + 10 μL 40 μg/mL p-aminobenzoic acid, make up to 200 μL, vortex for 2 s, centrifuge at 3400 g for 6 min, inject a 50-100 μL aliquot of the supernatant. Urine. Dilute 1:50 with water. 100 μL Diluted urine + 100 μL 150 μg/mL p-aminobenzoic acid in 1.25% sodium hexametaphosphate, vortex for 2 s, inject a 50 μL aliquot. (Reduce dimesna to mesna as follows. 100 μL Plasma or diluted urine + 100 μL 1% EDTA in buffer + 100 μL 1.06 M sodium borohydride in water, mix thoroughly, heat at 50° for 30 min, cool to room temperature, add 200 μL 1.74 M acetic acid, vortex for 20 s, centrifuge for 10 min, inject a 50-100 μL aliquot of the supernatant. Buffer was 50 mL 500 mM Na_2HPO_4 + 22 mL NaOH.)

HPLC VARIABLES

Guard column: Guard PAK C18 (Waters)
Column: 100 × 8 10 μm Resolve C18 (Waters)
Mobile phase: 100 mM Sodium citrate containing 1 mM tetrabutylammonium phosphate and 0.71 mM triethylamine, pH adjusted to 5 with 85% phosphoric acid
Flow rate: 2
Injection volume: 50-100
Detector: E, ESA Coulochem II model 5100, model 5011 analytical cell +450 mV, model 5021 conditioning cell +500 mV

CHROMATOGRAM

Retention time: 6.73
Internal standard: p-aminobenzoic acid (8.87)
Limit of quantitation: 1 μg/mL

OTHER SUBSTANCES

Simultaneous: acetaminophen, 5-fluorouracil, procarbazine, prochlorperazine
Noninterfering: aspirin, bleomycin, carboplatin, carmustine, chlorambucil, cyclophosphamide, cyclosporine A, cytarabine, doxorubicin, etoposide, hydrocortisone, ifosfamide, lomustine, methotrexate, mitomycin, mitoxantrone, teniposide, thiotepa, vincristine

KEY WORDS
plasma; pharmacokinetics

REFERENCE
el-Yazigi,A.; Yusuf,A.; Al-Rawithi,S. Liquid chromatographic analysis of mesna and dimesna in plasma and urine of patients treated with mesna, *Ther.Drug Monit.*, **1995**, *17*, 153–158.

SAMPLE
Matrix: formulations
Sample preparation: Dilute formulation with 15 μg/mL disodium EDTA solution to a mesna concentration of 3.2 μg/mL. Remove a 1 mL aliquot and add it to 300 μL reagent solution, let stand at room temperature for 20 min, add 500 μL 300 mM phosphoric acid solution, add 3 mL 4 μg/mL IS solution, make up to 10 mL with water, inject a 50 μL aliquot. (Prepare the reagent solution by dissolving 3.5 mg methyl 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoate in 10 mL THF, make up to 25 mL with pH 7.5 borate buffer. Prepare methyl 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoate as follows. Dissolve 5 g 6'-methoxy-2'-acetonaphthone in warm glacial acetic acid and add 2.5 g glyoxylic acid, reflux for 24 h, evaporate to dryness under reduced pressure. Take up the residue in chloroform and extract it three times with 5% sodium carbonate solution. Combine the aqueous layers and acidify them with concentrated HCl, collect the product by filtration, recrystallize from MeOH/water or acetic acid to give 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoic acid (mp 167-9°) (Farmaco, Ed. Sci. 1982, 37, 171). Reflux 0.5 g 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoic acid, 2.5 mL MeOH, and 2-3 drops sulfuric acid in 25 mL anhydrous benzene (Caution! Benzene is a carcinogen!) for 1 h, add 20 mL water, wash the organic layer with 10 mL 5% sodium bicarbonate solution, wash the organic layer with 20 mL water. Dry the organic layer over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, purify by flash chromatography on silica gel using ethyl acetate:light petroleum (bp 40-70°) 40:60 to give methyl 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoate as a pale yellow compound (mp 116-120°).)

HPLC VARIABLES
Column: 150 × 4 5 μm Spherisorb RP-8
Mobile phase: MeOH:50 mM pH 3.0 triethylammonium phosphate 53:47
Flow rate: 1
Injection volume: 50
Detector: F ex 310 em 450

CHROMATOGRAM
Retention time: 6
Internal standard: 4-(6-methoxynaphthalen-2-yl)-4-oxobutanoic acid (8)

OTHER SUBSTANCES
Simultaneous: acetylcysteine, cysteamine, cysteine, glutathione, homocysteine
Noninterfering: bacitracin, biotin, calcium pantothenate, cystine, glycine, magnesium oxide, neomycin, starch, threonine, vitamin E, pyridoxine, riboflavin phosphate

KEY WORDS
solutions; derivatization

REFERENCE
Gatti,R.; Cavrini,V.; Roveri,P.; Pinzauti,S. High-performance liquid chromatographic determination of aliphatic thiols with aroylacrylic acids as fluorogenic precolumn derivatization reagents, *J.Chromatogr.*, **1990**, *507*, 451–458.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm cyano
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 2.5

Injection volume: 20
Detector: UV 200

CHROMATOGRAM
Retention time: 1.16

OTHER SUBSTANCES
Simultaneous: diphenhydramine, 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: urine
Sample preparation: Dilute urine 1:3 to 1:39, inject a 50 μL aliquot.

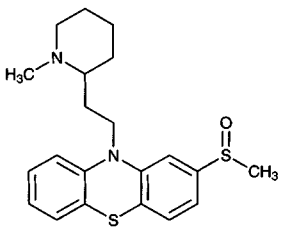
HPLC VARIABLES
Column: 250 × 4 5 μm Hypersil ODS
Mobile phase: MeOH:250 mM pH 7.4 phosphate buffer 5:95 containing 5 mM tetrabutylammonium phosphate
Flow rate: 1
Injection volume: 50
Detector: UV 412 following post-column reaction. The column effluent mixed with the reagent pumped at 0.5 mL/min and the mixture flowed through a 200 × 4 column packed with 100-120 mesh glass beads (dichlorodimethylsilane treated) to the detector. (Prepare reagent by diluting 0.2% 5,5'-dithiobis(2-nitrobenzoic acid) in 250 mM pH 7.4 phosphate buffer containing 10% tripotassium citrate 1:10 with water.)

CHROMATOGRAM
Retention time: 7.5
Limit of detection: 75 ng

KEY WORDS
post-column reaction; comparison with electrochemical detection without derivatization

REFERENCE
Sidau,B.; Shaw,I.C. Determination of sodium 2-mercaptoethanesulphonate by high-performance liquid chromatography using post-column reaction colorimetry or electrochemical detection, *J.Chromatogr.*, **1984**, *311*, 234–238.

Mesoridazine



Molecular formula: $C_{21}H_{26}N_2OS_2$
Molecular weight: 386.58
CAS Registry No.: 5588-33-0, 32672-69-8 (besylate)
Merck Index: 5970
Lednicer No.: 1 389

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 100 μL 1 M HCl, vortex for 30 s, add 4 mL isopropanol, mix for 5 min, centrifuge at 5000 rpm at 0° for 20 min. Remove the supernatant and adjust

Injection volume: 20
Detector: UV 200

CHROMATOGRAM
Retention time: 1.16

OTHER SUBSTANCES
Simultaneous: diphenhydramine, 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: urine
Sample preparation: Dilute urine 1:3 to 1:39, inject a 50 μL aliquot.

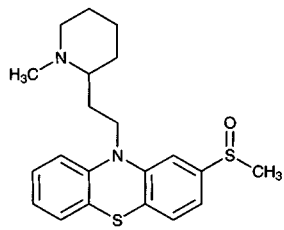
HPLC VARIABLES
Column: 250 × 4 5 μm Hypersil ODS
Mobile phase: MeOH:250 mM pH 7.4 phosphate buffer 5:95 containing 5 mM tetrabutylammonium phosphate
Flow rate: 1
Injection volume: 50
Detector: UV 412 following post-column reaction. The column effluent mixed with the reagent pumped at 0.5 mL/min and the mixture flowed through a 200 × 4 column packed with 100-120 mesh glass beads (dichlorodimethylsilane treated) to the detector. (Prepare reagent by diluting 0.2% 5,5'-dithiobis(2-nitrobenzoic acid) in 250 mM pH 7.4 phosphate buffer containing 10% tripotassium citrate 1:10 with water.)

CHROMATOGRAM
Retention time: 7.5
Limit of detection: 75 ng

KEY WORDS
post-column reaction; comparison with electrochemical detection without derivatization

REFERENCE
Sidau,B.; Shaw,I.C. Determination of sodium 2-mercaptoethanesulphonate by high-performance liquid chromatography using post-column reaction colorimetry or electrochemical detection, *J.Chromatogr.*, **1984**, *311*, 234–238.

Mesoridazine



Molecular formula: $C_{21}H_{26}N_2OS_2$
Molecular weight: 386.58
CAS Registry No.: 5588-33-0, 32672-69-8 (besylate)
Merck Index: 5970
Lednicer No.: 1 389

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 100 μL 1 M HCl, vortex for 30 s, add 4 mL isopropanol, mix for 5 min, centrifuge at 5000 rpm at 0° for 20 min. Remove the supernatant and adjust

the pH to 12.5 with 200 μL 5 M NaOH, mix for 10 s, add 4 mL n-heptane, mix for 10 min, centrifuge at 2500 rpm. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeCN, mix for 2 min, inject a 75-100 μL aliquot.

HPLC VARIABLES
Column: 250 × 3.2 5 μm Spherisorb CN
Mobile phase: MeCN:15 mM pH 6.5 acetate buffer 90:10
Flow rate: 1.6
Injection volume: 75-100
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Internal standard: mesoridazine

OTHER SUBSTANCES
Extracted: chlorpromazine

KEY WORDS
plasma; meosridazine is IS

REFERENCE
Midha,K.K.; Cooper,J.K.; McGilveray,I.J.; Butterfield,A.G.; Hubbard,J.W. High-performance liquid chromatographic assay for nanogram determination of chlorpromazine and its comparison with a radioimmunoassay, *J.Pharm.Sci.*, **1981**, *70*, 1043–1046.

SAMPLE
Matrix: blood
Sample preparation: 10 mL Plasma or whole blood + 1 mL 1 M NaOH, extract twice with 10 mL hexane for 30 min. Remove the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL 100 mM HCl, add 5 mL chloroform, vortex for 1 min, centrifuge. Remove a 4.5 mL aliquot of the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot. (It is implied, but not explicitly stated in the paper, that this extraction procedure works for this compound.)

HPLC VARIABLES
Column: 10 μm Micropak CN (Varian)
Mobile phase: MeCN:20 mM ammonium acetate 90:10
Flow rate: 2.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 23.9
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: acetophenazine, amitriptyline, benztropine, butaperazine, carphenazine, chlorpromazine, fluphenazine, haloperidol, imipramine, nortriptyline, orphenadrine, piperacetazine, promazine, promethazine, thioridazine, thiothixene, trifluoperazine, triflupromazine, trihexyphenidyl, trimeprazine

KEY WORDS
plasma; whole blood

REFERENCE
Curry,S.H.; Brown,E.A.; Hu,O.Y.-P.; Perrin,J.H. Liquid chromatographic assay of phenothiazine, thioxanthene and butyrophenone neuroleptics and antihistamines in blood and plasma with conventional and radial compression columns and UV and electrochemical detection, *J.Chromatogr.*, **1982**, *231*, 361–376.

SAMPLE

Matrix: blood, tissue

Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM

Retention time: 12.46
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES

Simultaneous: amitriptyline, amoxapine, benztropine, 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, tranylcypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine

Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

Interfering: propoxyphene

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: formulations

Sample preparation: Tablets. Powder tablets, weigh out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, filter (0.45 μm) (discard first 10 mL of filtrate), inject a 20 μL aliquot of the filtrate. Syrups, elixirs, injectables. Measure out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak CN
Mobile phase: MeOH:3 mM ammonium acetate 90:10
Flow rate: 1.3
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 5.2

OTHER SUBSTANCES
Also analyzed: chlorpheniramine, cyclizine, doxylamine, pentazocine, promethazine, protriptyline, pyrilamine, pyrimethamine, tripelennamine

KEY WORDS
tablets; syrups; elixirs; injections

REFERENCE
Walker,S.T. Liquid chromatographic determination of organic nitrogenous bases in dosage forms: a progress report, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 539–542.

SAMPLE
Matrix: solutions
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: 5.3

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeOH:acetic acid:triethylamine:water 70:1.5:0.5:28

Flow rate: 1.5

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 5

OTHER SUBSTANCES

Simultaneous: promethazine, acetophenazine, chlorpromazine, thioridazine, prochlorperazine, butaperazine, thiethylperazine

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere cyano

Mobile phase: MeCN:10 mM pH 2.5 KH_2PO_4 60:40

Flow rate: 2.5

Injection volume: 20-40

Detector: E, Environmental Science Associates Coulochem Model 5100A, Model 5100 guard-cell +0.85 V (between pump and injector), Model 5010 analytical cell +0.8 V, preanalytical cell +0.3 V

CHROMATOGRAM

Retention time: 4.0

OTHER SUBSTANCES

Simultaneous: amitriptyline, chlorpromazine, desipramine, doxepin, fluphenazine, haloperidol, imipramine, loxapine, nortriptyline, perphenazine, pheniramine, phenylephrine, prochlorperazine, promazine, promethazine, thioridazine, thiothixene, trifluprom azine, trimeprazine, tripelennamine

Noninterfering: diazepam, diphenhydramine, ethopropazine, fluoxetine, nordiazepam, oxazepam, phenylpropanolamine, pseudoephedrine, trifluoperazine

Interfering: amoxapine, reduced haloperidol, desmethyldoxepin, trazodone

REFERENCE

Hariharan,M.; VanNoord,T.; Kindt,E.K.; Tandon,R. A simple, sensitive liquid chromatographic assay of cis-thiothixene in plasma with coulometric detection, *Ther.Drug Monit.*, **1991**, *13*, 79–85.

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: 12.5

OTHER SUBSTANCES
Simultaneous: promazine, thiothixene, chlorpromazine, trifluoperazine, thioridazine
Also analyzed: amitriptyline, amphetamine, chlordiazepoxide, desalkylflurazepam, desipramine, desmethyldoxepin, diazepam, diethylpropion, doxepin, ephedrine, fenfluramine, flurazepam, imipramine, methamphetamine, norchlordiazepoxide, nordiazepam, nortriptyline, oxazepam, phentermine, phenylpropanolamine, prazepam

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REFERENCE
Rainin Catalog, C1-94, **1994,** p. 7.24.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone,

oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 10.12 (A), 5.02 (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, 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, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetra-

caine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine
Sample preparation: Perform all procedures in subdued light. 10 mL Urine + 10 μL 100 μg/mL IS, lyophilize, extract residue with 3 mL MeOH by shaking for 15 min, repeat extraction twice, combine extracts and evaporate them to dryness under vacuum below 45°, dissolve residue in 2 mL 300 mM pH 7.2 phosphate buffer, extract three times with 3 mL dichloromethane, wash the combined organic layers twice with 2 mL phosphate buffer, twice with 2 mL water, dry over anhydrous sodium sulfate, evaporate to dryness, reconstitute with 100 μL MeCN, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 3 μm Spherisorb cyano
Mobile phase: MeCN:50 mM ammonium acetate:diethylamine 92:8:0.05
Flow rate: 1.1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 10.9
Internal standard: prochlorperazine ring sulfoxide (14)
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
human; rat; dog

REFERENCE
Lin,G.; Hawes,E.M.; McKay,G.; Korchinski,E.D.; Midha,K.K. Metabolism of piperidine-type phenothiazine antipsychotic agents. IV. Thioridazine in dog, man and rat, *Xenobiotica*, **1993**, *23*, 1059–1074.

SAMPLE
Matrix: urine
Sample preparation: Perform all procedures in subdued light. 10 mL Urine + 10 μL 100 μg/mL IS, lyophilize, extract residue with 3 mL MeOH by shaking for 15 min, repeat extraction twice, combine extracts and evaporate them to dryness under vacuum below 45°, dissolve residue in 2 mL 300 mM pH 7.2 phosphate buffer, extract three times with 3 mL dichloromethane, wash the combined organic layers twice with 2 mL phosphate buffer, twice with 2 mL water, dry over anhydrous sodium sulfate, evaporate to dryness, reconstitute with 100 μL MeCN, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Serva Si 100 polyol (Terochem Laboratories)
Mobile phase: MeCN:47 mM pH 7.8 ammonium acetate 83:17
Flow rate: 1
Injection volume: 10
Detector: MS plasmaspray, VG 70SQ, discharge 320 V, ion source 250°, plasmaspray probe tip 200-300

CHROMATOGRAM
Retention time: 5.72

OTHER SUBSTANCES
Simultaneous: metabolites, sulforidazine, thioridazine

KEY WORDS
human; rat; dog

REFERENCE
Lin,G.; Hawes,E.M.; McKay,G.; Korchinski,E.D.; Midha,K.K. Metabolism of piperidine-type phenothiazine antipsychotic agents. IV. Thioridazine in dog, man and rat, *Xenobiotica*, **1993**, *23*, 1059–1074.

SAMPLE
Matrix: urine
Sample preparation: Human urine. 10 mL Urine + 1 mL 10 μg/mL IS, lyophilize, add 3 mL MeOH, shake for 15 min, repeat extraction twice more. Combine the extracts and evaporate them to dryness under reduced pressure below 45°, reconstitute the residue in 2 mL 300 mM pH 7.2 phosphate buffer, extract three times with 3 mL dichloromethane. Combine the organic extracts and wash them twice with 2 mL phosphate buffer, wash twice with 2 mL water, dry over anhydrous sodium sulfate, evaporate to dryness, reconstitute in 100 μL MeCN, inject a 10 μL aliquot. Dog, rat urine. 1 mL Urine + 50 μL 100 μg/mL IS, lyophilize, add 3 mL MeOH, shake for 15 min, repeat extraction twice more. Combine the extracts and evaporate them to dryness under reduced pressure below 45°, reconstitute the residue in 2 mL 300 mM pH 7.2 phosphate buffer, extract three times with 3 mL dichloromethane. Combine the organic extracts and wash them twice with 2 mL phosphate buffer, wash twice with 2 mL water, dry over anhydrous sodium sulfate, evaporate to dryness, reconstitute in 100 μL MeCN, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 3 μm Spherisorb cyano
Mobile phase: MeCN:50 mM ammonium acetate:diethylamine 92:8:0.05
Flow rate: 1
Injection volume: 10
Detector: UV 254

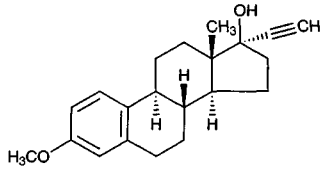
CHROMATOGRAM
Retention time: 11.3
Internal standard: prochlorperazine ring sulfoxide
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
dog; rat; human; protect from light

REFERENCE
Lin,G.; Hawes,E.M.; McKay,G.; Korchinski,E.D.; Midha,K.K. The metabolism of piperidine-type phenothiazine antipsychotic agents. III. Mesoridazine in dog, human and rat, *Xenobiotica*, **1993**, *23*, 37–52.

Mestranol



Molecular formula: $C_{21}H_{26}O_2$
Molecular weight: 310.44
CAS Registry No.: 72-33-3
Merck Index: 5976
Lednicer No.: 1 162

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 or 280

CHROMATOGRAM
Retention time: k' 8.08
Internal standard: BHT (butylated hydroxytoluene) (k' 16.54)

OTHER SUBSTANCES
Simultaneous: ethynodiol diacetate, ethinyl estradiol, degradation products

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: Dissolve 6 tablets in 600 mL dissolution medium (water:isopropanol 97:3), remove 5 mL samples, centrifuge at 1500 rpm for 10 min, inject a 50-200 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm Bondapak C18
Mobile phase: MeCN:water 55:45
Flow rate: 1
Injection volume: 50-200
Detector: UV 200

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: microsomal incubations
Sample preparation: 500 μL Microsomal incubation + 100 μL MeCN + IS, centrifuge at 16000 g for 5 min. Inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 300 × 3.9 μBondapack C18
Mobile phase: MeCN:50 mM pH 6 potassium phosphate buffer 47:53
Flow rate: 1.5
Detector: UV 208

CHROMATOGRAM
Retention time: 23-24
Internal standard: estradiol-3-acetate (13-14)

OTHER SUBSTANCES
Simultaneous: metabolites
Noninterfering: fluconazole, itraconazole, miconazole, α-naphthoflavone, quinidine, sulfaphenazole, troleandomycin
Interfering: ketoconazole

KEY WORDS
liver

REFERENCE
Schmider,J.; Greenblatt,D.J.; von Moltke,L.L.; Karsov,D.; Vena,R.; Friedman,H.L.; Shader,R.I. Biotransformation of mestranol to ethinyl estradiol in vitro: The role of cytochrome P-450 2C9 and metabolic inhibitors, *J.Clin.Pharmacol.*, **1997**, *37*, 193–200.

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 80:1.5:0.5:18
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 1.51

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions
Sample preparation: Extract 15 mL water with dichloromethane, evaporate organic layer, take up residue in 3 mL mobile phase, inject 50 μL 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

OTHER SUBSTANCES
Simultaneous: ethinylestradiol

KEY WORDS
mestranol is IS

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

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: 6

OTHER SUBSTANCES
Simultaneous: estrone, ethinyl estradiol, norethindrone, norethindrone acetate, norgestrel

KEY WORDS
normal phase

REFERENCE
Gazdag,M.; Szepesi,G.; Fábián-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 10 μm Nucleosil C18
Mobile phase: MeCN:THF:water 12.9:22.4:64.7
Flow rate: 1
Detector: UV 240

CHROMATOGRAM
Retention time: 37

OTHER SUBSTANCES
Simultaneous: estrone, ethinyl estradiol, norethindrone, norethindrone acetate, norgestrel

REFERENCE
Gazdag,M.; Szepesi,G.; Szeleczki,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
Guard column: 12 × 6 Zorbax Reliance

Column: 150 × 4.6 Zorbax ODS
Mobile phase: MeCN:pH 3.8 acetate buffer 70:30 containing 0.1 mM tetrabutylammonium phosphate
Flow rate: 0.9
Injection volume: 100
Detector: UV 280

CHROMATOGRAM
Retention time: 4

REFERENCE
Patel,J.U.; Prankerd,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.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a solution in MeOH:water 50:50.

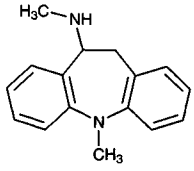
HPLC VARIABLES
Column: 250 × 4 7 μm LichroCART RP-8 (Merck)
Mobile phase: MeCN:MeOH:water 32:37:31
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: fluoxymesterone, medrogestone, norethindrone, 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.

Metapramine



Molecular formula: $C_{16}H_{18}N_2$
Molecular weight: 238.33
CAS Registry No.: 21730-16-5
Merck Index: 5991

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 50 μL 10 μg/mL chlorohaloperidol in MeOH + 50 μL 1 M NaOH + 6 mL distilled ether, shake mechanically for 10 min, centrifuge for 5 min. Remove the organic layer and add it to 100 μL 100 mM HCl, shake for 10 min, centrifuge for 5 min. Remove the aqueous layer, centrifuge for 2 min, inject a 60 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 5 μm Spherisorb C8
Mobile phase: MeCN:MeOH:buffer 13:35:52 (Buffer was 6.5 g/L (?) KH_2PO_4 adjusted to pH 3 with orthophosphoric acid.)
Column temperature: 45

Flow rate: 1.2
Injection volume: 60
Detector: UV 254

CHROMATOGRAM
Retention time: 3.65
Internal standard: chlorohaloperidol (6.83)
Limit of quantitation: 3 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: amitriptyline, bromazepam, chlorpromazine, clobazam, clomipramine, desipramine, diazepam, flunitrazepam, haloperidol, imipramine, indalpine, levomepromazine, lorazepam, maprotiline, mianserin, oxazepam, prochlorperazine, triazolam
Noninterfering: meprobamate

KEY WORDS
plasma

REFERENCE
Rouquette,C.; Hecquet,D.; Pommereau,X.; Gardere,J.J.; Brachet-Liermain,A. Metapramine overdose: report of two cases and analytical determinations, *J.Anal.Toxicol.*, **1985**, *9*, 275–277.

SAMPLE
Matrix: blood
Sample preparation: Evaporate 200 μL 1 μg/mL citalopram in MeOH into a tube, add 2 mL plasma, add 2 mL pH 10 Titrisol buffer (Merck), add 8 mL diethyl ether, shake for 15 min, centrifuge at 2800 g for 5 min. Remove the organic phase and shake it with 100 μL 50 mM phosphoric acid for 15 min, centrifuge at 2800 g for 10 s. Remove the aqueous layer and vortex it with 2 mL diethyl ether for 10 s, centrifuge at 2800 g. Discard the organic layer and inject a 10-50 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:25 mM KH_2PO_4:water 45:55:10
Flow rate: 0.6
Injection volume: 10-50
Detector: UV 254

CHROMATOGRAM
Retention time: 8.6
Internal standard: citalopram (10.8)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites
Noninterfering: diazepam, amitriptyline, clobazam, levomepromazine, norclobazam, triazolam, monodesmethyltrimipramine, flunitrazepam, alimemazine, alprazolam, amineptine, caffeine, carbamazepine, desmethylflunitrazepam, diazepam, dibenzepine, estazolam, ethyl loflazepate, loprazolam, lorazepam, meprobamate, nitrazepam, nordiazepam, nortriptyline, oxazepam, viloxazine
Interfering: indalpine

KEY WORDS
plasma

REFERENCE
Pok Phak,R.; Conquy,T.; Gouezo,F.; Viala,A.; Grimaldi,F. Determination of metapramine, imipramine, trimipramine and their major metabolites in plasma by reversed-phase column liquid chromatography, *J.Chromatogr.*, **1986**, *375*, 339–347.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Plasma + 200 μL MeOH + 2 mL 1 M pH 10.0 phosphate buffer + 6 mL diethyl ether:hexane 50:50, shake for 15 min, centrifuge at 4000 g for 5 min. Remove the organic layer and add it to 2 mL 62.5 mM sulfuric acid, vortex for 5 min, centrifuge at 4000 g for 5 min. Remove the aqueous phase and add it to 1 mL 500 mM NaOH, vortex, add 6 mL hexane:diethyl ether 50:50, shake for 10 min, centrifuge at 4000 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 mM sodium carbonate, add 10 μL 10 mg/mL dansyl chloride in acetone, vortex for 1 min, heat at 45° for 30 min, evaporate under a stream of nitrogen at 50°. Reconstitute the residue in 200 μL MeCN:water 45:55, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.6 5 μm Hypersil ODS

Mobile phase: Gradient. MeCN:water from 45:55 to 65:35 over 10 min, maintain at 65:35 for 20 min

Column temperature: 30

Flow rate: 1.5

Injection volume: 100

Detector: F ex Fluorichrom 7.54 and 7.60 filters, em 3.71 and 4.76 filters

CHROMATOGRAM

Retention time: 28

Internal standard: metapramine

Limit of detection: 1.5 ng/mL

OTHER SUBSTANCES

Extracted: fluvoxamine

Noninterfering: alimemazine, alprazolam, amineptine, amitriptyline, caffeine, clobazam, clomipramine, clorazepate, cyamemazine, diazepam, demethyldiazepam, flunitrazepam, levomepromazine, loprazolam, lorazepam, meprobamate, nitrazepam, oxazepam, triazolam, viloxazine

KEY WORDS

plasma; protect from light; derivatization; metapramine is IS

REFERENCE

Pommery,J.; Lhermitte,M. High performance liquid chromatographic determination of fluvoxamine in human plasma, *Biomed.Chromatogr.*, **1989**, *3*, 177–179.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 269

CHROMATOGRAM

Retention time: 5.08

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromet azine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 0.5-2 mL Plasma + 10-1000 ng maprotiline in water + 2 mL pH 10 phosphate buffer + 6 mL diethyl ether:hexane 50:50, shake for 15 min, centrifuge at 3750 g for 5 min. Remove the organic phase and add it to 2 mL 250 mM sulfuric acid, shake for 10 min, centrifuge for 5 min, discard the organic layer. Adjust the pH of the aqueous phase to 9.5-10.5 with 500 mM NaOH containing 1 M K_2HPO_4, add 6 mL diethyl ether:hexane 50:50, shake for 10 min, centrifuge for 10 min. Remove the organic layer and evaporate it to dryness under vacuum and a stream of nitrogen at 45°, reconstitute the residue in 100 μL 100 mM sodium carbonate, add 10 μL 1% dansyl chloride in acetone, vortex for 20-30 s, heat at 45° for 30 min. Evaporate the solvent, reconstitute in 100 μL mobile phase, inject a 10-50 μL aliquot. Urine. 0.5-2 mL Urine + 10-1000 ng maprotiline in water + 2 mL pH 10 phosphate buffer + 6 mL diethyl ether:hexane 50:50, shake for 15 min, centrifuge at 3750 g for 5 min. Remove the organic phase and evaporate it to dryness under vacuum and a stream of nitrogen at 45°, reconstitute the residue in 100 μL 100 mM sodium carbonate, add 10 μL 1% dansyl chloride in acetone, vortex for 20-30 s, heat at 45° for 30 min. Evaporate the solvent, reconstitute in 100 μL mobile phase, inject a 10-50 μL aliquot.

HPLC VARIABLES

Column: 125 × 4 5 μm LiChrosorb RP-18
Mobile phase: MeCN:water 65:35
Flow rate: 2
Injection volume: 10-50

Detector: F ex 248 em 470

CHROMATOGRAM
Retention time: 16.2
Internal standard: maprotiline (18.5)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; derivatization; pharmacokinetics

REFERENCE
Sommadossi,J.P.; Lemar,M.; Necciari,J.; Sumirtapura,Y.; Cano,J.P.; Gaillot,J. High-performance liquid chromatographic method for the determination of plasma and urine metapramine after dansylation, *J.Chromatogr.*, **1982**, *228*, 205–213.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 1 mL Microsomal incubation + 100 µL 1 M NaOH + 25 µL 4 mM desipramine in MeOH, extract twice with diethyl ether. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 200 µL, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 Lichrosorb RP18
Mobile phase: Gradient. A was MeCN:20 mM sulfuric acid 20:80. B was MeCN:20 mM sulfuric acid 90:10. A:B from 100:0 to 0:100 over 20 min.
Flow rate: 1
Detector: UV 275

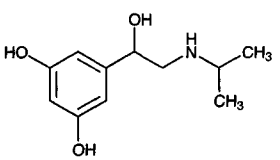
CHROMATOGRAM
Retention time: 10.6
Internal standard: desipramine (13.3)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; liver

REFERENCE
Barret,R.; Jaussaud,P.; Pautet,F.; Guyot,J.L.; Daudon,M. Metabolism of metapramine in vitro by chemical model systems and rat liver microsomes, *Arzneimittelforschung*, **1989**, *39*, 1574–1576.

Metaproterenol



Molecular formula: $C_{11}H_{17}NO_3$
Molecular weight: 211.26
CAS Registry No.: 586-06-1, 5874-97-5 (sulfate)
Merck Index: 5992
Lednicer No.: 1 64

SAMPLE
Matrix: blood

Sample preparation: Condition a Bond-Elut C18 SPE cartridge with MeCN and water. 1 mL Plasma + 100 μL 2 μg/mL terbutaline sulfate + 1 mL 20 mM pH 9.0 Na_2HPO_4, mix, add to the SPE cartridge, wash with 5 column volumes of water, dry the SPE cartridge for 5 min, wash with 3 mL dichloromethane:n-butanol 97:3, elute with two 1 mL portions of 0.09% HCl in MeCN, evaporate the eluate to dryness under a stream of nitrogen at 37°, dissolve the residue in 300 μL mobile phase, inject a 200 μL aliquot. (To hydrolyze 3-O-metaproterenol sulfate mix 1 mL plasma and 200 μL 2 μg/mL terbutaline sulfate, add 1 mL 6% trichloroacetic acid, vortex, centrifuge at 1000 g for 15 min. Remove the supernatant and add it to 200 μL 2 M HCl, heat at 65° for 90 min, cool, adjust pH to 10 with 400 μL 2 M carbonate buffer, proceed as above.)

HPLC VARIABLES

Column: 250 × 4.9 5 μm Spherisorb C8
Mobile phase: MeCN:buffer:water 4:1.5:94.5
Flow rate: 1.8
Injection volume: 200
Detector: F ex 200 em 300 (cut-off filter)

CHROMATOGRAM

Retention time: 6.9
Internal standard: terbutaline (14.8)
Limit of quantitation: 0.5 ng/mL

KEY WORDS

SPE; plasma; pharmacokinetics

REFERENCE

Selinger,K.; Hill,H.M.; Matheou,D.; Dehelean,L. Determination of free and total metaproterenol in human plasma by high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.*, **1989**, *493*, 230–238.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 4.15

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions

Sample preparation: Evaporate an aliquot of a solution in MeCN containing 625 ng drug to dryness under a stream of nitrogen at room temperature, add 200 μL saturated sodium carbonate, add 200 μL 4% (-)-menthyl chloroformate in MeCN, vortex for 30 s, add an excess amount of 4-hydroxy-L-proline, vortex for 30 s, centrifuge for 3 min, inject a 10-25 μL aliquot of the upper layer.

HPLC VARIABLES

Guard column: 50 × 4.6 Pellicular ODS (Whatman)
Column: 100 × 4.6 5 μm Partisil 5 ODS3
Mobile phase: MeOH:water 60:40
Flow rate: 1
Injection volume: 10-25
Detector: F ex 232 em no emission filter

CHROMATOGRAM

Retention time: 19, 21 (enantiomers)

OTHER SUBSTANCES

Simultaneous: sotalol

KEY WORDS

derivatization; chiral

REFERENCE

Mehvar,R. Stereospecific liquid chromatographic analysis of racemic adrenergic drugs utilizing precolumn derivatization with (-)-menthyl chloroformate, *J.Chromatogr.*, **1989**, *493*, 402–408.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Chirex 3020 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 55:35:10 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1
Injection volume: 20
Detector: UV 278

KEY WORDS

chiral; α = 1.24 for enantiomers

REFERENCE

Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 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: 5.41 (A), 3.18 (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, 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, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine
Sample preparation: Condition a Bond Elut C18 SPE cartridge with 3 volumes of MeOH and 2 volumes of water, dry under vacuum. 500 μL Urine + 5 μg terbutaline, add to the SPE cartridge, wash with 5 volumes of water, elute with 200 μL MeOH:50 mM pH 6 potassium phosphate buffer 50:50, add 50 μL 50 mM Na_3PO_4 to the eluate, pass argon through the mixture, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 300 mm long μBondapak phenyl
Mobile phase: MeCN:50 mM pH 5 phosphate buffer 6:94
Flow rate: 2.8
Injection volume: 25
Detector: F ex 280 em 310

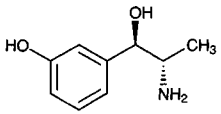
CHROMATOGRAM
Retention time: 2.7

Internal standard: terbutaline (4.1)
Limit of quantitation: 500 ng/mL

KEY WORDS
SPE; protect from light; pharmacokinetics

REFERENCE
MacGregor,T.R.; Nastasi,L.; Farina,P.R.; Keirns,J.J. Isolation and characterization of metaproterenol-3-O-sulfate: a conjugate of metaproterenol in human urine, *Drug Metab.Dispos.*, **1983**, *11*, 568–573.

Metaraminol



Molecular formula: $C_9H_{13}NO_2$
Molecular weight: 167.21
CAS Registry No.: 54-49-9, 33402-03-8 (bitartrate)
Merck Index: 5993

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 950 mg metaraminol bitartrate in 50 mL water. Remove a 2 mL aliquot and add it to 5 mL 100 μg/mL butylparaben in water, make up to 100 mL with water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeOH:water:acetic acid 60:40:1 containing 2.2 g/L dioctyl sodium sulfosuccinate (Dissolve dioctyl sodium sulfosuccinate in MeOH then add water and acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 11.4
Internal standard: butylparaben (8.2)

OTHER SUBSTANCES
Simultaneous: methylparaben, propylparaben

REFERENCE
Martin,C.J.; Saxena,S.J. High-performance liquid chromatographic determination of metaraminol bitartrate in the presence of parabens, *J.Pharm.Sci.*, **1980**, *69*, 1459–1461.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Dissolve powdered tablets in 10 mM HCl, filter if necessary, inject an aliquot. Injections, solutions. Dilute with 10 mM HCl, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Partisil-5 ODS-3
Mobile phase: MeOH:buffer 30:70 (Buffer was 10 mM sodium 1-octanesulfonate in 0.2% acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 22

Limit of detection: 50 ng

OTHER SUBSTANCES

Simultaneous: norepinephrine, epinephrine, levonordefrin, isoproterenol, phenylephrine, impurities

KEY WORDS

tablets; injections; ophthalmic solutions; inhalation solutions

REFERENCE

Smela,M.J.,Jr.; Stromberg,R. Liquid chromatographic determination of six sympathomimetic drugs in dosage forms, *J.Assoc.Off.Anal.Chem.*, **1991**, *74*, 289–291.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.7

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions

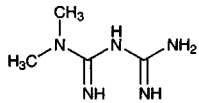
HPLC VARIABLES
Column: 250 × 4.6 5 μm Partisil ODS-3
Mobile phase: MeOH:buffer 30:70 (Buffer was 10 mM octanesulfonic acid in 0.2% acetic acid.)
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 21.5

OTHER SUBSTANCES
Simultaneous: epinephrine, isoproterenol, levonordefrin, phenylephrine

REFERENCE
Phenomenex Catalog, **1994**, p. 1.077.

Metformin



Molecular formula: $C_4H_{11}N_5$
Molecular weight: 129.17
CAS Registry No.: 657-24-9
Merck Index: 6001

SAMPLE
Matrix: blood
Sample preparation: Add 10 μL 60% perchloric acid to 250 μL plasma. Vortex for 1 min, centrifuge at 12800 g for 3 min. Inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 5 μm Spherisorb CN
Column: 250 × 4.6 5 μm Supelcosil LC-CN
Mobile phase: MeCN:buffer 40:60 (Buffer was 10 mM KH_2PO_4 adjusted to pH 3.5 with glacial acetic acid.)
Flow rate: 1
Injection volume: 50
Detector: UV 234

CHROMATOGRAM
Retention time: 5.94
Limit of detection: 15 ng/mL
Limit of quantitation: 60 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Yuen,K.H.; Peh,K.K. Simple high-performance liquid chromatographic method for the determination of metformin in human plasma, *J.Chromatogr.B*, **1998**, *710*, 243–246.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Plasma + 50 μL 100 g/L trichloroacetic acid in water, mix, centrifuge, inject a 20-50 μL aliquot of the supernatant

HPLC VARIABLES
Column: 250 × 4.6 10 μm Whatman SCX
Mobile phase: 100 mM pH 4.42 $(NH_4)H_2PO_4$
Flow rate: 3
Injection volume: 20-50
Detector: UV 232

CHROMATOGRAM
Retention time: 5
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Noninterfering: glibornuride, gliclazide, glipizide, glyburide (glibenclamide)

KEY WORDS
plasma

REFERENCE
Lacroix,C.; Danger,P.; Wojciechowski,F. Microdosage de la metformine plasmatique et intra-érythrocytaire par chromatographie en phase liquide [Microassay of plasma and erythrocyte metformin by high performance liquid chromatography], *Ann.Biol.Clin.(Paris)*, **1991**, *49*, 98–101.

SAMPLE
Matrix: blood
Sample preparation: Condition an Amprep 100 mg C8 SPE cartridge (Amersham) with 2 mL MeOH and 1 mL water, do not allow to run dry. 500 μL Plasma + 1 μg phenformin, add to the SPE cartridge, wash with 2 mL diethyl ether, elute with 500 μL MeCN:10 mM pH 3.5 KH_2PO_4 70:30. Centrifuge for 5 min or filter (Millipore HV) the eluate, inject an aliquot.

HPLC VARIABLES
Guard column: Guard-pak C18 (Waters)
Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: MeCN:10 mM KH_2PO_4 40:60 adjusted to pH 7 with diethylamine
Flow rate: 1.35
Injection volume: 50
Detector: UV 236

CHROMATOGRAM
Retention time: 2.8
Internal standard: phenformin (5.6)
Limit of detection: 50 ng/mL

KEY WORDS
plasma; SPE; pharmacokinetics; human; rat

REFERENCE
Huupponen,R.; Ojala-Karlsson,P.; Rouru,J.; Koulu,M. Determination of metformin in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *583*, 270–273.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of

the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 231

CHROMATOGRAM

Retention time: 3.41
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 500 μL Plasma + 200 μL 30 μg/mL 1-propylbiguanide sulfate in 1.2 mM trichloroacetic acid, vortex for 5 s, let stand for 10 min, centrifuge at 5000 g for 5 min, inject a 100 μL aliquot of the supernatant. Urine. Dilute urine with water, if necessary. 500

μL Urine + 200 μL 30 μg/mL 1-propylbiguanide sulfate in water, vortex for 5 s, let stand for 10 min, centrifuge at 5000 g for 5 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4.6 Partisil-10 SCX
Mobile phase: 30 mM $(NH_4)H_2PO_4$ adjusted to pH 2.4 with orthophosphoric acid
Column temperature: 50
Flow rate: 3
Injection volume: 100
Detector: UV 230

CHROMATOGRAM

Retention time: 8
Internal standard: 1-propylbiguanide sulfate (Synthesis of 1-propylbiguanide is as follows. Heat 9 g propylamine, 7.5 g cyanoguanidine, 11.6 g copper sulfate pentahydrate, 75 mL water in a sealed tube at 100° for 12 h, cool, dilute with 350 mL water, heat to 80°, pass a stream, of hydrogen sulfide (Caution! Highly toxic!) through the solution to precipitate copper salts, filter. Evaporate the filtrate under reduced pressure at 100°. Recrystallize the product from hot EtOH and dry it at 100°, mp 194-6°.) (10)
Limit of detection: 50-100 ng/mL

OTHER SUBSTANCES

Noninterfering: acetaminophen, albuterol, allopurinol, amiloride, amitriptyline, ampicillin, amobarbital, aspirin, caffeine, carbamazepine, chlorpropamide, chlorothiazide, clonidine, danthron, dextropropoxyphene, diazepam, digoxin, dioctyl sodium sulfosuccinate, doxepin, ephedrine, ethylestranol, furosemide, glyburide (glibenclamide), hydrochlorothiazide, insulin, isosorbide dinitrate, methyclothiazide, methyldopa, metoprolol, nitrazepam, nitroglycerin, nortriptyline, oxazepam, phenylbutazone, phenytoin, pindolol, prazosin, prochlorperazine, propoxyphene, propranolol, quinine, sodium chromoglycate, sulfamethoxazole, theophylline, thyroxine, tolbutamide, trimethoprim, verapamil, vitamin B, warfarin

KEY WORDS

plasma

REFERENCE

Charles,B.G.; Jacobsen,N.W.; Ravenscroft,P.J. Rapid liquid-chromatographic determination of metformin in plasma and urine, *Clin.Chem.*, **1981**, *27*, 434–436.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 250 μL water + 1 mL bromothymol blue solution + 50 μL 2 μg/mL propyl biguanide in water, vortex briefly, adjust pH to 7.6-7.8 with a few drops of 0.1% orthophosphoric acid, add 5 mL diethyl ether:dichloromethane 2:1, vortex for 1 min, centrifuge at 2000 g for 5 min. Remove 4 mL of the upper organic phase and add it to 100 μL 0.1% tetrabutylammonium hydroxide adjusted to pH 7 with 0.1% orthophosphoric acid, vortex for 1 min, centrifuge at 2000 g for 5 min. Discard the organic phase, heat the aqueous phase at 70-90° for 10 min, inject a 25 μL aliquot of the aqueous phase. Urine. 20 μL Urine + 2 mL bromothymol blue solution + 100 μL 20 μg/mL propyl biguanide in water, vortex briefly, adjust pH to 7.6-7.8 with a few drops of 0.1% orthophosphoric acid, add 5 mL diethyl ether: dichloromethane 2:1, vortex for 1 min, centrifuge at 2000 g for 5 min. Remove 4 mL of the upper organic phase and add it to 100 μL 1% tetrabutylammonium hydroxide adjusted to pH 7 with 0.1% orthophosphoric acid, vortex for 1 min, centrifuge at 2000 g for 5 min. Discard the organic phase, heat the aqueous phase at 70-90° for 10 min, inject a 25 μL aliquot of the aqueous phase. (Bromothymol blue solution was 1.48 g bromothymol blue in 20 mL 200 mM NaOH, make up to 200 mL with water, let stand at room temperature with occasional mixing for 2 days before use.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Spherisorb ODS 2
Mobile phase: MeCN:buffer 8:92 adjusted to pH 4.0 with orthophosphoric acid (Buffer was 50 mM K_2HPO_4 containing 3 mM heptanesulfonic acid.)
Flow rate: 1
Injection volume: 25

Detector: UV 234

CHROMATOGRAM

Retention time: 3.3

Internal standard: propyl biguanide (Synthesis of 1-propylbiguanide is as follows. Heat 9 g propylamine, 7.5 g cyanoguanidine, 11.6 g copper sulfate pentahydrate, 75 mL water in a sealed tube at 100° for 12 h, cool, dilute with 350 mL water, heat to 80°, pass a stream, of hydrogen sulfide (Caution! Highly toxic!) through the solution to precipitate copper salts, filter. Evaporate the filtrate under reduced pressure at 100°. Recrystallize the product from hot EtOH and dry it at 100°, mp 194-6° (Clin.Chem. 1981, 27, 434).) (7.80)

Limit of detection: 10 ng/mL

KEY WORDS

plasma

REFERENCE

Keal,J.; Somogyi,A. Rapid and sensitive high-performance liquid chromatographic assay for metformin in plasma and urine using ion-pair extraction techniques, *J.Chromatogr.*, **1986**, *378*, 503–508.

SAMPLE

Matrix: blood, urine

Sample preparation: Blood. Add plasma or whole blood to MeCN containing propylbiguanide, wash the supernatant with dichloromethane, inject an aliquot. Urine. Mix urine with MeCN containing propylbiguanide, inject an aliquot.

HPLC VARIABLES

Column: 250 × 3.6 5 μm Si

Mobile phase: MeCN:water 20:80 containing 10 mM $(NH_4)_2HPO_4$, pH adjusted to 7.5 with phosphoric acid

Detector: UV 235

CHROMATOGRAM

Internal standard: propylbiguanide

Limit of detection: 4 μg/mL (urine), 10 ng/mL (plasma, whole blood)

KEY WORDS

plasma; whole blood; pharmacokinetics

REFERENCE

Sambol,N.C.; Chiang,J.; Lin,.T.; Goodman,A.M.; Liu,C.Y.; Benet,L.Z.; Cogan,M.G. Kidney function and age are both predictors of pharmacokinetics of metformin, *J.Clin.Pharmacol.*, **1995**, *35*, 1094–1102.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 233.4

CHROMATOGRAM
Retention time: 2.803

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 5.68 (A), 3.25 (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, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine
Sample preparation: Mix 2 mL urine with 1 mL 20% NaOH and saturate the mixture with NaCl, add 1 mL MeCN, add 10 mg p-nitrobenzoyl chloride, let stand at room temperature for 1 h, add 10 mg p-nitrobenzoyl chloride, let stand for 1 h, inject a 10 μL aliquot of the upper MeCN phase.

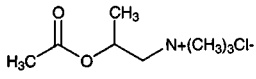
HPLC VARIABLES
Column: 914 × 2.2 37-50 μm Bondapak phenyl/Corasil
Mobile phase: MeOH:water 40:60
Flow rate: 1
Injection volume: 10
Detector: UV 280

CHROMATOGRAM
Retention time: 6
Limit of detection: 200 ng/mL (using more urine and less MeCN)

KEY WORDS
deerivatization

REFERENCE
Ross,M.S.F. Determination of metformin in biological fluids by derivatization followed by high-performance liquid chromatography, *J.Chromatogr.*, **1977**, *133*, 408–411.

Methacholine chloride



Molecular formula: $C_8H_{18}ClNO_2$
Molecular weight: 195.69
CAS Registry No.: 62-51-1
Merck Index: 6003

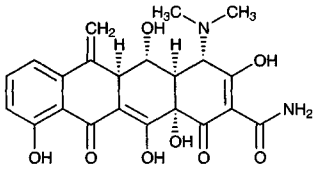
SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in saline, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:buffer 25:75 (Buffer was 20 mL Low-UV PIC B-7 (Waters) diluted with 480 mL water (10 mM 1-heptanesulfonic acid).)
Flow rate: 1
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 9.6
Limit of detection: 80 μg/mL

REFERENCE
Woodman,T.F.; Johnson,B.; Marwaha,R.K. Determination of methacholine chloride by ion-pair high-pressure liquid chromatography, *J.Liq.Chromatogr.*, **1982**, *5*, 1341–1348.

Methacycline



Molecular formula: $C_{22}H_{22}N_2O_8$
Molecular weight: 442.43
Merck Index: 6007
Lednicer No.: 2 227

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

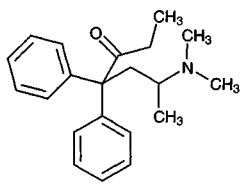
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 242.9

CHROMATOGRAM
Retention time: 11.493

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Methadone

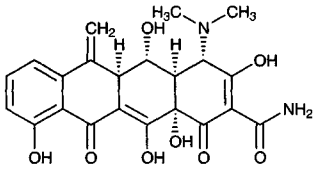


Molecular formula: $C_{21}H_{27}NO$
Molecular weight: 309.45
CAS Registry No.: 76-99-3, 1095-90-5 (HCl)
Merck Index: 6008

SAMPLE
Matrix: blood

REFERENCE
Woodman,T.F.; Johnson,B.; Marwaha,R.K. Determination of methacholine chloride by ion-pair high-pressure liquid chromatography, *J.Liq.Chromatogr.*, **1982**, *5*, 1341–1348.

Methacycline



Molecular formula: $C_{22}H_{22}N_2O_8$
Molecular weight: 442.43
Merck Index: 6007
Lednicer No.: 2 227

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

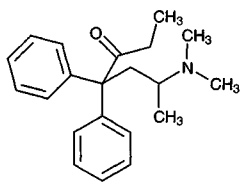
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 242.9

CHROMATOGRAM
Retention time: 11.493

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Methadone



Molecular formula: $C_{21}H_{27}NO$
Molecular weight: 309.45
CAS Registry No.: 76-99-3, 1095-90-5 (HCl)
Merck Index: 6008

SAMPLE
Matrix: blood

Sample preparation: 2 mL Plasma + 50 μL 2 μg/mL methadone in MeOH + 500 μL 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 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 10 μm 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

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CHROMATOGRAM
Retention time: 16
Internal standard: methadone

OTHER SUBSTANCES
Simultaneous: oxycodone, fentanyl, meperidine, phenoperidine

KEY WORDS
plasma; methadone is IS

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: blood
Sample preparation: 1 mL Serum + 200 ng doxepin or desipramine + 100 μL 1 M NaOH + 9 mL freshly prepared hexane:isoamyl alcohol 99:1, shake vigorously for 5 min, centrifuge. Remove 8.5 mL of the organic phase and add it to 200 μL 50 mM HCl, shake well for 1 min, centrifuge, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: 300 × 4 μBondapak phenyl
Mobile phase: MeCN:0.01% phosphoric acid containing 0.01% NaCl 35:65, final pH 2.8
Flow rate: 1.5
Injection volume: 50
Detector: UV 210

CHROMATOGRAM
Retention time: 20
Internal standard: doxepin (12.2), desipramine (14.2)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: cocaine, dextromoramide, meperidine, normeperidine, norpropoxyphene, pentazocine, propoxyphene
Simultaneous: amitriptyline, buprenorphine, chlorpromazine, codeine, desmethyldoxepin, diphenhydramine, ephedrine, imipramine, nortriptyline, oxazepam, oxycodone, pericyazine, pheniramine, propranolol, quinine, thiopropazate, thioridazine

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KEY WORDS
serum

REFERENCE

Hackett,L.P.; Dusci,L.J.; Ilett,K.F. The analysis of several nonopiate narcotic analgesics and cocaine in serum using high-performance liquid chromatography, *J.Anal.Toxicol.*, **1987**, *11*, 269–271.

SAMPLE

Matrix: blood
Sample preparation: 900 μL Plasma + 100 μL 10 μg/mL difenoxin in mobile phase, add to a C18 Sep Pak SPE cartridge at 1 mL/min, wash with 4 mL MeCN:buffer 10:90 at 1 mL/min, elute with 4 mL MeCN:buffer 40:60 at 5 mL/min, inject a 100 μL aliquot of the eluate. (Buffer was 0.08% diethylamine in water adjusted to pH 2.3 with orthophosphoric acid.)

HPLC VARIABLES

Guard column: C18
Column: 150 × 4.6 30 μm Ultracarb ODS (Phenomenex)
Mobile phase: MeCN:buffer 25:75 (Buffer was 0.08% diethylamine in water adjusted to pH 2.3 with orthophosphoric acid.)
Column temperature: 28
Flow rate: 1.5
Injection volume: 100
Detector: UV 210

CHROMATOGRAM

Retention time: 18
Internal standard: difenoxin (32)
Limit of detection: 0.25 ng

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; SPE; rat

REFERENCE

Pierce,T.L.; Murray,A.G.W.; Hope,W. Determination of methadone and its metabolites by high performance liquid chromatography following solid-phase extraction in rat plasma, *J.Chromatogr.Sci.*, **1992**, *30*, 443–447.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 1 mL 160 nM dextropropoxyphene in 1 M sodium carbonate + 6 mL n-hexane, shake horizontally for 15 min, centrifuge at 1300 g for 5 min, freeze in dry ice/acetone for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject a 10-80 μL aliquot.

HPLC VARIABLES

Guard column: reversed-phase (Chrompack)
Column: 100 × 3 5 μm Spherisorb CN + 100 × 4 Chiralcel-AGP (in series)
Mobile phase: MeCN:10 mM pH 5.0 sodium phosphate buffer:dimethyloctylamine 10:90:0.05
Flow rate: 0.9
Injection volume: 10-80
Detector: UV 200

CHROMATOGRAM

Retention time: 18.5 (R-(-)), 21.1 (S-(+))
Internal standard: dextropropoxyphene (15.2)
Limit of quantitation: 1.7 ng/mL

OTHER SUBSTANCES

Noninterfering: benzodiazepines, carbamazepine, ketobemidone, morphine, piroxicam, tenoxicam, valproic acid

KEY WORDS
serum; chiral; pharmacokinetics

REFERENCE
Kristensen,K.; Angelo,H.R.; Blemmer,T. Enantioselective high-performance liquid chromatographic method for the determination of methadone in serum using an AGP and a CN column as chiral and analytical column, respectively, *J.Chromatogr.A*, **1994**, *666*, 283–287.

SAMPLE
Matrix: blood
Sample preparation: Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL 400 ng/mL protriptyline in water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μL 0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300 μL 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: LC-8-DB (Supelco)
Column: 150 × 4.6 LC-8-DB (Supelco)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)
Flow rate: 2
Injection volume: 100
Detector: UV 228

CHROMATOGRAM
Retention time: 5.4
Internal standard: protriptyline (4)

OTHER SUBSTANCES
Extracted: acetazolamide, chlordiazepoxide, chlorimipramine, chlorpromazine, desipramine, dextromethorphan, diazepam, diphenhydramine, doxepin, encainide, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, ibuprofen, imipramine, lidocaine, maprotiline, methaqualone, mexiletine, midazolam, norchlorimipramine, nordoxepin, nortriptyline, norverapamil, pentazocine, promazine, propafenone, 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, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin
Interfering: amitriptyline, nordiazepam, norfluoxetine, propoxyphene, 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 or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 261

CHROMATOGRAM

Retention time: 7.53
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood

Sample preparation: Condition a 3 mL Bond Elut Certify SPE cartridge with 2 mL MeOH and 2 mL 100 mM pH 6.0 phosphate buffer, do not allow to dry. 1 mL Blood + 6 mL 100 mM pH 6.0 phosphate buffer, vortex, sonicate, centrifuge, add the supernatant to the SPE cartridge, wash with water, wash with 1 mM pH 3.3 acetic acid, dry by suction, wash with 2 mL acetone: chloroform 50:50, elute with 3 mL ethyl acetate:ammonia 98:2. Evaporate the eluate under a stream of nitrogen at 40°, reconstitute the residue in 50 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pack C18
Mobile phase: MeOH:50 mM ammonium acetate 75:25 (Mix column effluent with 50 mM ammonium acetate pumped at 0.5 mL/min.)
Flow rate: 0.6
Injection volume: 10
Detector: MS, Finnigan MAT TSQ 700 tandem quadrupole, MAT TSP-2 interface, thermospray, selective reaction monitoring m/z 310-265, collision offset -10 V, repeller 100 V, vaporizer 130°, source 200°, filament on 200 μA, argon 2.5 mTorr, multiplier 1500 V, dynode 15 kV, scan time 1.20 s, MSMSC factor 10

CHROMATOGRAM

Retention time: 7.45
Limit of detection: 50 pg

OTHER SUBSTANCES

Extracted: benperidol, dextromoramide, droperidol, haloperidol, penfluridol, pimozide, pipamperidone, propoxyphene (dextropropoxyphene)

KEY WORDS

SPE; LC/MS

REFERENCE

Verweij,A.M.; Hordijk,M.L.; Lipman,P.J. Quantitative liquid chromatographic thermospray-tandem mass spectrometric analysis of some analgesics and tranquilizers of the methadone, butyrophenone, or diphenylbutylpiperidine groups in whole blood, *J.Anal.Toxicol.*, **1995**, *19*, 65–68.

SAMPLE

Matrix: blood, CSF
Sample preparation: 200 μL Serum, plasma, or CSF + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, elute the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine hydrochloride and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 30 × 2.1 40 μm preparative grade C18 (Analytichem); B 250 × 4.6 10 μm Partisil C8
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 15 min, maintain at 30:70 for 4 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 280 for 5 min then UV 254

CHROMATOGRAM

Retention time: 8.10
Internal standard: heptanophenone (19.2)

OTHER SUBSTANCES

Extracted: acetazolamide, ampicillin, bromazepam, caffeine, carbamazepine, chloramphenicol, chlorothiazide, diazepam, droperidol, ethionamide, furosemide, isoniazid, penicillin G, phenobarbital, phenytoin, prazepam, propoxyphene, pyrazinamide, rifampin, trimeprazine, trimethoprim

KEY WORDS
plasma; serum; column-switching

REFERENCE
Seifart,H.I.; Kruger,P.B.; Parkin,D.P.; van Jaarsveld,P.P.; Donald,P.R. Therapeutic monitoring of antituberculosis drugs by direct in-line extraction on a high-performance liquid chromatography system, *J.Chromatogr.*, **1993**, *619*, 285–290.

SAMPLE
Matrix: blood, 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: 9.8
Internal standard: SKF-525-A (11.2)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: norpropoxyphene, propoxyphene, diazepam, N-desmethyldiazepam

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: blood, tissue
Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18

Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM
Retention time: 11.16
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES
Simultaneous: amitriptyline, amoxapine, benztropine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine
Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine
Interfering: brompheniramine

KEY WORDS
serum; whole blood; liver

REFERENCE
McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. Mix 100 μL serum with 100 μL. 10 μg/mL estazolam in EtOH. Add 300μL 10% anhydrous sodium carbonate in water, 4 mL hexane, and 500 μL 2-propanol. Vortex for 2 min. Centrifuge at 1500 g for 10 min and evaporate the upper organic layer to dryness under a stream of nitrogen at room temperature. Add 100 μL mobile phase, vortex for 30 s, centrifuge at 3000 g or filter (0.22 μm). Inject a 50 μL aliquot. Urine. Mix 100 μL urine with 50 μL. 10 μg/mL estazolam in EtOH. Add 300μL 10% anhydrous sodium carbonate in water and 4 mL hexane. Vortex for 2 min. Centrifuge at 1500 g for 10 min and evaporate the upper organic layer to dryness under a stream of nitrogen at room temperature. Add 100 μL mobile phase, vortex for 30 s. Inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 3.2 5 μm Cyclobond I-200 RSP
Column: 250 × 4.6 5 μm Cyclobond I-200 RSP
Mobile phase: MeCN:buffer:water 19:8:73 (Prepare buffer by adding 1 mL triethylamine to 70 mL water, adjusting the pH to 4.5 with glacial acetic acid, and making up to 100 mL with water.)
Flow rate: 0.4
Injection volume: 50
Detector: UV 210

CHROMATOGRAM
Retention time: 15.5 (R-(-)), 17 (S-(+))
Internal standard: estazolam (22)
Limit of detection: 1 ng/mL
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: amphetamine, benzoylecgonine, caffeine, clonazepam, cocaine, codeine, dextropropoxyphene, diamorphine, diazepam, dionine, lorazepam, morphine, monoacetylmorphine, nalorphine, narcotine, nitrazepam, oxazepam, theophylline

Noninterfering: aspirin, barbiturates, clomipramine, imipramine, phenytoin, salicylic acid, valproic acid

KEY WORDS
serum; chiral

REFERENCE
Pham-Huy,C.; Chikhi-Chorfi,N.; Galons,H.; Sadeg,N.; Laqueille,X.; Aymard,N.; Massicot,F.; Warner,J.-M.; Claude,J.-R. Enantioselective high-performance liquid chromatography determination of methadone enantiomers and its major metabolite in human biological fluids using a new derivatized cyclodextrin-bonded phase, *J.Chromatogr.B*, **1997**, *700*, 155–163.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 15.753

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject 75-100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelco
Mobile phase: EtOH:MeCN:t-butylamine 98:2:0.05 (Prepared from 1 gal EtOH + 77 mL MeCN + 1.9 mL t-butylamine.)
Flow rate: 2
Injection volume: 75-100
Detector: UV 254

CHROMATOGRAM
Retention time: 2.9
Internal standard: promazine (5.2)

OTHER SUBSTANCES

Simultaneous: N-acetylprocainamide, amitriptyline, amoxapine, amphetamine, buprion, chlordiazepoxide, chlorpheniramine, chlorpromazine, cocaine, codeine, demoxepam, desipramine, desmethylchlordiazepoxide, desmethyldisopyramide, desmethyldoxepin, dextropropoxyphene, diazepam, disopyramide, doxepin, hydroxyamoxapine (7- and 8-), 2-hydroxydesipramine, 2-hydroxyimipramine, 10-hydroxynortriptyline, iminostilbene, imipramine, iprindole, maprotiline, meperidine, mianserin, morphine, nortriptyline, norzimeldine, oxapam, oxaprotiline, perphenazine, procainamide, prochlorperazine, prolixin, promethazine, propoxyphene, protriptyline, pyrilamine, quinidine, thioridazine, trifluoperazine, trimeprazine, trimipramine, zimeldine
Noninterfering: thiopropazine
Interfering: chlorimipramine, fluphenazine, loxepin, phentermine, triflupromazine

KEY WORDS

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: 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: 2.68

OTHER SUBSTANCES

Simultaneous: codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodeine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine, pemoline, benzphetamine, diethylpropion, mazindol, tranylcypromine, caffeine, fenethyline, phendimetrazine, methylphenidate, phenelzine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, phentermine, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine, norpipanone, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, thebaine, norlevorphanol, benzylmorphine, ethylmorphine, morphine-N-oxide

REFERENCE

Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.9

OTHER SUBSTANCES
Simultaneous: metabolites
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Guard column: Pelliguard LC-CN (Supelco)
Column: 150 × 4.6 5 μm Supelcosil LC-PCN
Mobile phase: MeCN:MeOH:10 mM pH 7.0 phosphate buffer 58:14:28
Flow rate: 1.2
Injection volume: 40

Detector: UV 254

CHROMATOGRAM
Retention time: 11.0
Internal standard: N-propionylprocainamide (6)

OTHER SUBSTANCES
Simultaneous: amitriptyline, atropine, butalbital, chlorpromazine, desipramine, desmethylmaprotiline, doxepin, imipramine, maprotiline, norpropoxyphene, nortriptyline, phenylpropanolamine, procainamide, prochlorperazine, promethazine, propranolol, protriptyline, quinidine, trifluoperazine, trimeprazine, trimipramine
Noninterfering: acetaminophen, allopurinol, amikacin, amoxapine, amytal, bretylium, caffeine, carbamazepine, carisoprodol, chloramphenicol, chlordiazepoxide, chlorpropamide, clonazepam, codeine, diazepam, disopyramide, droperidol, ethinamate, ethinamate, ethosuximide, fluphenazine, flurazepam, furosemide, gentamicin, haloperidol, hydrochlorothiazide, hydroxyzine, ibuprofen, kanamycin, lidocaine, loxapine, meperidine, mephobarbital, meprobamate, methaqualone, methotrexate, morphine, nafcillin, naloxone, neomycin, perphenazine, phenacetin, phenobarbital, phenytoin, prazepam, primidone, procaine, propoxyphene, reserpine, salicylamide, salicylic acid, secobarbital, spironolactone, theophylline, thiopental, thioridazine, tobramycin, valproic acid, verapamil

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: 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: 16

OTHER SUBSTANCES
Simultaneous: cocaine, codeine, 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: 7.650

OTHER SUBSTANCES
Also analyzed: 6-acetylmorphine, amiloride, amphetamine, benzocaine, benzoylecgonine, caffeine, cocaine, codeine, doxylamine, fluoxetine, glutethimide, hexobarbital, hypoxanthine, levorphanol, LSD, meperidine, mephobarbital, methylphenidate, methyprylon, N-norcodeine, oxazepam, oxycodone, phenylpropanolamine, prilocaine, procaine, terfenadine

REFERENCE

Ascah,T.L. Improved separations of alkaloid drugs and other substances of abuse using Supelcosil LC-ABZ column, *Supelco Reporter*, **1993**, *12(3)*, 18–21.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 16.58 (A), 8.43 (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, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine
Sample preparation: 500 μL Urine + N-ethylnordiazepam + chlorpheniramine + 100 μL buffer, centrifuge at 11000 g for 30 s, inject a 500 μL aliquot onto column A with mobile phase A, after

0.6 min backflush column A with mobile phase A to waste for 1.6 min, elute column A with 250 μL mobile phase B, with 200 μL mobile phase C, and with 1.15 mL mobile phase D. Elute column A to waste until drugs start to emerge then elute onto column B. Elute column B to waste until drugs started to emerge, then elute onto column C. When all the drugs have emerged from column B remove it from the circuit, elute column C with mobile phase D, monitor the effluent from column C. Flush column A with 7 mL mobile phase E, with mobile phase D, and mobile phase A. Flush column B with 5 mL mobile phase E then with mobile phase D. (Buffer was 6 M ammonium acetate adjusted to pH 8.0 with 2 M KOH.)

HPLC VARIABLES

Column: A 10 × 2.1 12-20 μm PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 × 3.2 11 μm Aminex A-28 (Bio-Rad); C 25 × 3.2 5 μm C8 (Phenomenex) + 150 × 4.6 5 μm silica (Macherey-Nagel)

Mobile phase: A 0.1% pH 8.0 potassium borate buffer; B 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid; C MeCN:buffer 40:60 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); D MeCN:buffer 33:67 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); E MeCN:buffer 70:30 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)

Column temperature: ambient (column A), 40 (columns B and C)

Flow rate: A 5; B-E 1

Injection volume: 500

Detector: UV 210, UV 235

CHROMATOGRAM

Retention time: k' 3.8

Internal standard: N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)

Limit of detection: 300 ng/mL

OTHER SUBSTANCES

Extracted: caffeine, cotinine, benzoylecgonine, secobarbital, oxazepam, phenobarbital, nordiazepam, diazepam, phenylpropanolamine, phentermine, amphetamine, phenmetrazine, lidocaine, ephedrine, pentazocine, methamphetamine, desipramine, nortriptyline, diphenhydramine, imipramine, flurazepam, amitriptyline, morphine, codeine, hydromorphone, hydrocodone

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KEY WORDS

column-switching

REFERENCE

Binder,S.R.; Regalia,M.; Biaggi-McEachern,M.; Mazhar,M. Automated liquid chromatographic analysis of drugs in urine by on-line sample cleanup and isocratic multi-column separation, *J.Chromatogr.*, **1989**, *473*, 325–341.

SAMPLE

Matrix: urine

Sample preparation: Extract 1 mL urine.

HPLC VARIABLES

Guard column: Lichrospher 100 RP-18

Column: CHIRAL-AGP (Chrom-Tech)

Mobile phase: MeCN:10 mM pH 5.0 sodium phosphate buffer:dimethyloctylamine 10:90:0.05

Flow rate: 0.7

Detector: UV 200

CHROMATOGRAM

Retention time: 14.3 (R), 17.9 (S)

Internal standard: imipramine (26.9)

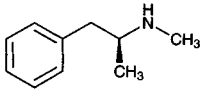
OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS
chiral

REFERENCE
Kristensen,K.; Angelo,H.R. A stereoselective HPLC method for the determination of methadone and is main metabolite in urine (using an AGP column) (Abstract 39), *Ther.Drug Monit.*, **1995**, *17*, 393–393.

Methamphetamine



Molecular formula: $C_{10}H_{15}N$
Molecular weight: 149.24
CAS Registry No.: 537-46-2
Merck Index: 6015
Lednicer No.: 1 37

SAMPLE
Matrix: blood
Sample preparation: Inject a 5 μL aliquot of serum directly.

HPLC VARIABLES
Column: 100 × 4.6 5-10 μm Silicalite (by sieving Silicalite, 3M Co.(?))
Mobile phase: MeNC:20 mM pH 6.9 phosphate buffer 10:90
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 3.79

OTHER SUBSTANCES
Extracted: ethosuximide, sulfamethoxazole, primidone

KEY WORDS
serum

REFERENCE
Ambrose,D.L.; Fntz,J.S. High-performance liquid chromatographic determination of drugs and metabolites in human serum and urine using direct injection and a unique molecular sieve, *J.Chromatogr.B*, **1998**, *709*, 89–96.

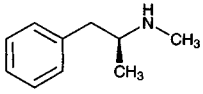
SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 100 mM NaOH + 3 mL n-hexane, shake for 20 min, centrifuge for 10 min. Remove 2 mL of the organic layer and evaporate it to dryness using a vacuum centrifuge, reconstitute the residue in 500 μL 100 μg/mL (S)-(+)-benoxaprofen chloride in dried dichloromethane, let stand at room temperature for 30 min, inject a 10 μL aliquot. (Synthesis of benoxaprofen chloride is as follows. Dissolve 600 mg benoxaprofen in 50 mL toluene, slowly add 5 mL freshly-distilled thionyl chloride, reflux for 30 min, evaporate to dryness, recrystallize benoxaprofen chloride from dichloromethane.)

HPLC VARIABLES
Column: 250 × 4.6 7 μm Zorbax-Sil
Mobile phase: Cyclohexane:dichloromethane:THF 50:10:10
Flow rate: 1
Injection volume: 10
Detector: F ex 312 em 365

KEY WORDS
chiral

REFERENCE
Kristensen,K.; Angelo,H.R. A stereoselective HPLC method for the determination of methadone and is main metabolite in urine (using an AGP column) (Abstract 39), *Ther.Drug Monit.*, **1995**, *17*, 393–393.

Methamphetamine



Molecular formula: $C_{10}H_{15}N$
Molecular weight: 149.24
CAS Registry No.: 537-46-2
Merck Index: 6015
Lednicer No.: 1 37

SAMPLE
Matrix: blood
Sample preparation: Inject a 5 μL aliquot of serum directly.

HPLC VARIABLES
Column: 100 × 4.6 5-10 μm Silicalite (by sieving Silicalite, 3M Co.(?))
Mobile phase: MeNC:20 mM pH 6.9 phosphate buffer 10:90
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 3.79

OTHER SUBSTANCES
Extracted: ethosuximide, sulfamethoxazole, primidone

KEY WORDS
serum

REFERENCE
Ambrose,D.L.; Fntz,J.S. High-performance liquid chromatographic determination of drugs and metabolites in human serum and urine using direct injection and a unique molecular sieve, *J.Chromatogr.B*, **1998**, *709*, 89–96.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 100 mM NaOH + 3 mL n-hexane, shake for 20 min, centrifuge for 10 min. Remove 2 mL of the organic layer and evaporate it to dryness using a vacuum centrifuge, reconstitute the residue in 500 μL 100 μg/mL (S)-(+)-benoxaprofen chloride in dried dichloromethane, let stand at room temperature for 30 min, inject a 10 μL aliquot. (Synthesis of benoxaprofen chloride is as follows. Dissolve 600 mg benoxaprofen in 50 mL toluene, slowly add 5 mL freshly-distilled thionyl chloride, reflux for 30 min, evaporate to dryness, recrystallize benoxaprofen chloride from dichloromethane.)

HPLC VARIABLES
Column: 250 × 4.6 7 μm Zorbax-Sil
Mobile phase: Cyclohexane:dichloromethane:THF 50:10:10
Flow rate: 1
Injection volume: 10
Detector: F ex 312 em 365

CHROMATOGRAM
Retention time: 10.5 (R-(-)), 11.5 (S-(+))

OTHER SUBSTANCES
Extracted: amphetamine
Interfering: tranylcypromine

KEY WORDS
plasma; derivatization; normal phase; chiral

REFERENCE
Weber,H.; Spahn,H.; Mutschler,E.; Möhrke,W. Activated α-alkyl-α-arylacetic acid enantiomers for stereoselective thin-layer chromatographic and high-performance liquid chromatographic determination of chiral amines, *J.Chromatogr.*, **1984**, *307*, 145–153.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 1 mL acetone, vortex for 2 min, centrifuge at 2000 g for 10 min. Remove a 500 μL aliquot of the supernatant and add it to 500 μL reagent, add 20 μL 0.5% triethylamine in MeOH, vortex, heat at 80° for 30 min, cool to room temperature, inject a 20 μL aliquot. (Prepare reagent by mixing 2 mL 2.5 mM N-(4-aminobutyl)-N-ethylisoluminol in MeOH with 2 mL 2.5 mM N,N'-disuccinimidyl carbonate in MeCN, let stand for 2 h.)

HPLC VARIABLES
Guard column: 30 × 4.6 TSKm Guardgel ODS-80TM (Toyo Soda)
Column: 150 × 6 5 μm Shimpack CLC C18 (Shimadzu)
Mobile phase: MeOH:water 54:46 containing 30 mM sodium 1-octanesulfonate
Flow rate: 1
Injection volume: 20
Detector: Chemiluminescence following post-column reaction. The column effluent mixed with 15 mM potassium ferricyanide in 2.5 M NaOH pumped at 1 mL/min and this mixture flowed through a 200 mm × 0.5 mm ID stainless steel coil. The effluent from this coil mixed with 300 mM hydrogen peroxide containing 10 mM β-cyclodextrin pumped at 1 mL/min and this mixture flowed through a 100 mm × 0.5 mm ID stainless steel coil to the detector.

CHROMATOGRAM
Retention time: 30
Limit of detection: 20 pM

KEY WORDS
derivatization; serum

REFERENCE
Nakashima,K.; Suetsugu,K.; Akiyama,S.; Yoshida,M. High-performance liquid chromatography-chemiluminescence determination of methamphetamine in human serum using N-(4-aminobutyl)-N-ethylisoluminol as a chemiluminogen, *J.Chromatogr.*, **1990**, *530*, 154–159.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 50 μL 100 ng/mL aniline sulfate in water + 200 μL 20 mM pH 10.6 carbonate buffer + 2 mL ethyl acetate, shake for 15 min, centrifuge at 1200 g for 5 min. Remove the organic layer and add it to 200 μL 50 mM HCl, shake for 15 min, centrifuge at 1200 g for 5 min. Remove the aqueous layer and add it to 40 μL 250 mM NaOH, add 50 μL 330 mM pH 7.8 phosphate buffer, add 250 μL MeCN, add 25 μL 1 mM (-)-1-(9-fluorenyl)ethyl chloroformate in acetone, let stand overnight at room temperature, add 30 μL 100 mM glycine in water, add 750 μL n-pentane, vortex for 2 min, centrifuge at 1200 g for 5 min. Remove the organic layer and evaporate it to dryness under vacuum, reconstitute the residue in MeCN: water 50:50, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: Direct-Connect column prefilter (Alltech)

Column: 150 × 4.6 3 μm Adsorbosphere HS C18 (Alltech)
Mobile phase: MeCN:THF:20 mM pH 3.6 acetate buffer 39:15:46
Flow rate: 1
Injection volume: 100
Detector: F ex 265 em 330

CHROMATOGRAM
Retention time: 27.7 (R), 29.0 (S)
Internal standard: aniline (21.0)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: amphetamine

KEY WORDS
serum; rat; chiral; derivatization

REFERENCE
Hutchaleelaha,A.; Walters,A.; Chow,H.-H.; Mayersohn,M. Sensitive enantiomer-specific high-performance liquid chromatographic analysis of methamphetamine and amphetamine from serum using precolumn fluorescent derivatization, *J.Chromatogr.B*, **1994**, *658*, 103–112.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 500 mM pH 11 borate buffer, mix, add 2.5 mL diethyl ether, vortex for 5 min, centrifuge at 1200 g for 5 min, remove organic layer, repeat extraction. Combine the organic layers and add them to 200 μL 100 mM HCl, vortex for 2 min, centrifuge at 1200 g for 5 min. Remove the aqueous phase and add it to 150 μL 1 M pH 8 borate buffer and 100 μL 4 mM 9-fluorenylmethyl chloroformate in MeCN, shake, allow to react at 50° for 5 min, add 20 μL 20 mM proline in water, allow to react at 50° for 2 min, inject a 200 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak phenyl
Mobile phase: MeCN:50 mM pH 6.0 sodium phosphate buffer 50:50
Flow rate: 1
Injection volume: 200
Detector: F ex 260 em 315

CHROMATOGRAM
Retention time: 15
Limit of quantitation: 0.5 ng/mL

OTHER SUBSTANCES
Extracted: amphetamine, desmethyldeprenyl

KEY WORDS
plasma

REFERENCE
La Croix,R.; Pianezzola,E.; Strolin Benedetti,M. Sensitive high-performance liquid chromatographic method for the determination of the three main metabolites of selegiline (L-deprenyl) in human plasma, *J.Chromatogr.B*, **1994**, *656*, 251–258.

SAMPLE
Matrix: blood, urine
Sample preparation: Adjust pH of 10 mL plasma or 20 mL urine to 11.4 with 5 M NaOH, add to a column containing 1.5 g Amberlite XAD-2, wash with 10 mL water, elute with 20 (plasma) or 40 (urine) mL chloroform:isopropanol 75:25, add 100 μL 6 M HCl in EtOH to the eluate, evaporate to dryness under reduced pressure, reconstitute with 1 mL 8% sodium bicarbonate,

add 1 mL 0.5% sodium 1,2-naphthoquinone-4-sulfonate, heat at 70° for 20 min, add an equal volume of chloroform, vortex for 1 min, inject a 50 μL aliquot of the organic layer.

HPLC VARIABLES
Column: 150 × 5 Partisil 5
Mobile phase: Hexane:chloroform:ethyl acetate:EtOH 50:25:35:1
Column temperature: 20
Flow rate: 2.5
Injection volume: 50
Detector: UV 248

CHROMATOGRAM
Retention time: 3
Internal standard: phenylethylamine (6)
Limit of detection: 2 ng

OTHER SUBSTANCES
Extracted: amphetamine, hydroxyamphetamine, norephedrine

KEY WORDS
derivatization; plasma; normal phase; SPE; comparison with other derivatization reagents and with ion-pair chromatography

REFERENCE
Farrell,B.M.; Jefferies,T.M. An investigation of high-performance liquid chromatographic methods for the analysis of amphetamines, *J.Chromatogr.*, **1983**, *272*, 111–128.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 206.4

CHROMATOGRAM
Retention time: 8.433

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Mix a 1 mg/mL solution in 1 M sodium carbonate with 2 mL 5 mg/mL 8-quinolinesulfonyl chloride in acetone, heat at 65° for 20 min, cool, extract twice with 30 mL portions of chloroform. Combine the extracts and dry them over anhydrous magnesium sulfate, evaporate to dryness under a stream of air, reconstitute, inject an aliquot.

HPLC VARIABLES
Guard column: 70 × 2.1 Co:Pell ODS
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water:acetic acid 40:59:1
Flow rate: 1.5
Detector: UV 254, UV 280

CHROMATOGRAM
Retention time: 25

OTHER SUBSTANCES
Simultaneous: amphetamine, ephedrine, phenmetrazine, phentermine, phenylpropanolamine, pseudoephedrine

KEY WORDS
derivatization

REFERENCE
Noggle,F.T.,Jr.; Clark,C.R. Liquid chromatographic determination of primary and secondary amines as 8-quinolinesulfonyl chloride derivatives, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 687–691.

SAMPLE
Matrix: bulk
Sample preparation: Prepare an aqueous solution. Adjust pH of 500 μL aqueous solution to 12 with 1 M NaOH, add 200 μL (+)-1-(9-fluorenyl)ethyl chloroformate:dichloromethane 1:100, let stand for 20 min, add 500 μL dichloromethane, shake for 30 min. Remove the organic phase and wash it twice with 500 μL aliquots of water, evaporate the organic phase to dryness under a stream of nitrogen, reconstitute the residue in 3 mL MeOH, filter (0.45 μm), inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 3.9 5 μm 5C18-AR (Waters)
Mobile phase: MeCN:50 mM pH 6.0 phosphate buffer 65:35
Flow rate: 1
Injection volume: 25
Detector: F ex 295 em 315

CHROMATOGRAM
Retention time: 44 ((S)-(+)), 47 ((R)-(-))
Limit of quantitation: 16.7 ng/mL

OTHER SUBSTANCES
Extracted: ephedrine, pseudoephedrine

KEY WORDS
derivatization; chiral

REFERENCE
Chen,Y.-P.; Hsu,M.-C.; Chien,C.S. Analysis of forensic samples using precolumn derivatization with (+)-1-(9-fluorenyl)ethyl chloroformate and liquid chromatography with fluorimetric detection, *J.Chromatogr.A*, **1994**, *672*, 135–140.

SAMPLE
Matrix: bulk

Sample preparation: Dissolve 10 μmole compound (as free base or hydrochloride) in 500 μL MeCN, add 250 μL 5% sodium carbonate (for hydrochlorides only), add 500 μL 100 mM reagent in MeCN, vortex for 1 min, heat at 60° for 2 h, add 100 μmole L-proline, heat at 60° for 30 min. Remove a 100 μL aliquot and dilute it with mobile phase, neutralize with acetic acid, inject a 10 μL aliquot. Prepare the reagent ((R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate) as follows. Add 0.7 mL carbon disulfide to 6 mL (1R,2R)-(-)-1,2-diaminocyclohexane, 12 mL water, and 12 mL EtOH, heat the oil bath to 80°, add 2.8 mL carbon disulfide dropwise (making sure that the product does not start to precipitate), when addition is complete reflux for 1 h, acidify with 500 μL 5 M HCl, reflux for 12 h, cool, filter, wash the solid with a little cold EtOH to give trans-4,5-tetramethyleneimidazolidine-2-thione as a white fluffy solid (mp 148-150°) (Tetrahedron 1993, 49, 4419). Stir 7.97 g 3,5-dinitrobenzoyl chloride in 30 mL dichloroethane at 50°, add a solution of 6 g trans-4,5-tetramethyleneimidazolidine-2-thione in 120 mL dichloroethane containing a catalytic amount of 4-(dimethylamino)pyridine over 15 min, reflux for 2 h, remove the crystals of (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate by filtration, evaporate the filtrate to dryness and dissolve the residue in 60 mL dichloroethane, reflux for 16 h to obtain more (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate (mp >250°, $[\alpha]_{546}$ = -133° (c = 1) in MeCN).

HPLC VARIABLES

Column: 125 × 4 5 μm Lichrospher 60 RP Select B
Mobile phase: MeCN:20 mM ammonium acetate 55:45
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: k' 14.33, k' 15.90 (enantiomers)

OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, atenolol, carazolol, carvedilol, formoterol, 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: Powder tablet and add 50 mg to 50 mL MeCN:20 mM pH 3.8 phosphate buffer 3:97, sonicate for 5 min, filter (0.5 μm), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: Supelguard pre-column containing 5 μm Suplex pKb100 (Supelco)
Column: 150 × 4.6 5 μm Suplex pKb100 (Supelco)
Mobile phase: Gradient. MeCN:20 mM pH 3.8 phosphate buffer at 3:97 for 3 min, to 15:85 over 5 min, stay at 15:85 for 4 min, re-equilibrate for 8 min.
Flow rate: 1.5
Injection volume: 20
Detector: UV 220 for 5 min then UV 280

CHROMATOGRAM

Retention time: 4.3
Limit of quantitation: 10 μg/mL

OTHER SUBSTANCES

Simultaneous: ephedrine, amphetamine, caffeine, 3,4-methylenedioxyamphetamine, N-methyl-3,4-methylenedioxyamphetamine, N-ethyl-3,4-methylenedioxyamphetamine

KEY WORDS
tablets

REFERENCE
Longo,M.; Martines,C.; Rolandi,L.; Cavallaro,A. Simple and fast determination of some phenethylamines in illicit tablets by base-activated reversed phase HPLC, *J.Liq.Chromatogr.*, **1994**, *17*, 649–658.

SAMPLE
Matrix: solutions
Sample preparation: Mix 1 mL 20-300 μg/mL amine solution in water with 2 mL 50 mg/mL 4-nitrobenzoyl chloride in THF (freshly prepared) and 1 mL 1 M NaOH, heat at 65° for 1 h, cool, adjust pH to 12 with 1 M NaOH, extract with two 10 mL portions of chloroform. Combine the extracts and wash them with two 20 mL portions of 10% potassium carbonate, wash with water, dry over anhydrous magnesium sulfate. Evaporate to dryness under a stream of air, reconstitute the residue in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water 35:65
Flow rate: 1.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Simultaneous: amphetamine, benzylamine, α-methylbenzylamine, n-propylamphetamine

KEY WORDS
derivatization

REFERENCE
Clark,R.C.; Teague,J.D.; Wells,M.M.; Ellis,J.H. Gas and high-pressure liquid chromatographic properties of some 4-nitrobenzamides of amphetamines and related arylalkylamines, *Anal.Chem.*, **1977**, *49*, 912–915.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 4.05

OTHER SUBSTANCES
Simultaneous: dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine, pemoline, benzphetamine, diethylpropion, mazindol, tranylcypromine, caffeine, fenethyline, phendimetrazine, methylphenidate, phenelzine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, mephentermine, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine, norpipanone, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodine

Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: dimethylamphetamine, mescaline, norpethidine, hydrocodone

REFERENCE

Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE

Matrix: solutions
Sample preparation: Mix 1 mL of an aqueous solution with 1 mL 100 mM nickel sulfate in water, 1 mL 20% aqueous ammonia, and 5 mL chloroform:carbon disulfide 98:2, shake vigorously for 1 min, wash the organic layer with three 2 mL portions of water, filter (phase-separation paper). Evaporate the filtrate to dryness under a stream of nitrogen, reconstitute with 1 mL mobile phase, inject a 10 μL aliquot. (Copper may also be used with electrochemical detection or UV detection at 270 nm.)

HPLC VARIABLES

Guard column: 30 × 4 40 μm LiChrosorb RP-18
Column: 250 × 4 7 μm LiChrosorb RP-18
Mobile phase: MeOH:20 mM pH 5.8 sodium acetate buffer 80:20 containing 5 mM lithium perchlorate
Flow rate: 1.5
Injection volume: 10
Detector: UV 325, E, Merck-Clevenot E 230, Model LCC 231 thin-layer electrolytic cell with a glassy carbon electrode at +0.7 V, standard calomel reference electrode

CHROMATOGRAM

Retention time: 8.49
Limit of detection: 1 fmole (E), 1 nmole (UV)

OTHER SUBSTANCES

Simultaneous: ephedrine
Also analyzed: acebutolol, alprenolol, flecainide, propranolol

KEY WORDS

derivatization; complexation

REFERENCE

Leroy,P.; Nicolas,A. Determination of secondary amino drugs as their metal dithiocarbamate complexes by reversed-phase high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1984**, *317*, 513–521.

SAMPLE

Matrix: solutions
Sample preparation: 2 mL THF + 1 mL 33.5 mM reagent in THF (freshly prepared) + 1 mL 1 mg/mL amphetamine in water + 700 μL 10% sodium bicarbonate in water, heat at 65° for 1 h, cool, extract three times with 10 mL aliquots of chloroform. Combine the extracts and wash them with 10 mL water, dry over anhydrous magnesium sulfate, evaporate to dryness, reconstitute with 2.5 mL mobile phase, inject a 5 μL aliquot. (Prepare reagent (1-[(4-nitrophenyl)sulfonyl]prolyl chloride) as follows. Mix 40-45 mmoles L-(-)-proline, 40 mL THF, and 200 mL 10% potassium carbonate, add 37-43 mmoles 4-nitrobenzenesulfonyl chloride in 40 mL THF dropwise, heat at 50° and maintain at pH 8 or above for 3 h, cool, acidify to pH 2, extract with chloroform. Extract the organic layers with potassium carbonate in water. Acidify the aqueous layer and extract it with chloroform. Dry the chloroform layer and evaporate it to dryness, recrystallize the resulting 1-[(4-nitrophenyl)sulfonyl]proline from petroleum ether and benzene (Caution! Benzene is a carcinogen!). Stir 15 mmoles 1-[(4-nitrophenyl)sulfonyl]proline in 100 mL benzene and add 75 mmoles thionyl chloride in 50 mL benzene dropwise, heat at 35-40° until the reaction is complete (about 48 h; monitor by IR), evaporate to dryness, recrystallize from n-heptane to give 1-[(4-nitrophenyl)sulfonyl]prolyl chloride (Anal.Chem. 1984, 56, 958) (mp 110-110.5°).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Zorbax ODS

Mobile phase: MeOH:water 60:40
Flow rate: 1.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 12 (S), 14 (R)

KEY WORDS
derivatization; chiral

REFERENCE
Barksdale,J.M.; Clark,C.R. Liquid chromatographic determination of the enantiomeric composition of amphetamine and related drugs by diastereomeric derivatization, *J.Chromatogr.Sci.*, **1985**, *23*, 176–180.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.7

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, 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, thenyldi-

amine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 15:1.5:0.5:83
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 13

OTHER SUBSTANCES

Simultaneous: phenylpropanolamine, ephedrine, hydroxyamphetamine, amphetamine, phentermine, mephentermine

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions
Sample preparation: 100 μL 100 μM Solution + 300 μL buffer + 500 μL 1 mM dansyl chloride in acetone, mix, heat at 45° in the dark for 1 h, dilute with MeCN:water 50:50, inject a 20 μL aliquot. (Prepare buffer by adjusting 10 mM sodium bicarbonate to pH 9.0 with NaOH.)

HPLC VARIABLES

Guard column: 30 × 4.6 Spheri-5 RP-18
Column: 250 × 4.6 Inertsil ODS-2
Mobile phase: MeCN:water 70:30 containing 1 mM imidazole, pH adjusted to 7.0 with nitric acid
Flow rate: 1
Injection volume: 20
Detector: Chemiluminescence following post-column reaction. The column effluent mixed with the reagent pumped at 1 mL/min and the mixture flowed through a 300 mm × 0.25 mm ID coil to the detector. (Prepare the reagent by dissolving 112 mg bis(2,4,6-trichlorophenyl) oxalate in 500 mL MeCN, add 8.6 mL 30% hydrogen peroxide, sonicate.), F ex 343 em 530

CHROMATOGRAM

Retention time: 18.5
Limit of detection: 4 fmole (chemiluminescence), 50 fmole (F)

OTHER SUBSTANCES

Simultaneous: benzylamine, ephedrine, N-isopropylbenzylamine, N-methylphenethylamine, phenylbutylamine, phenylethylamine, phenylpropanolamine, phenylpropylamine

KEY WORDS

derivatization; post-column reaction; comparison with other derivatization reagents

REFERENCE

Hayakawa,K.; Hasegawa,K.; Imaizumi,N.; Wong,O.S.; Miyazaki,M. Determination of amphetamine-related compounds by high-performance liquid chromatography with chemiluminescence and fluorescence detections, *J.Chromatogr.*, **1989**, *464*, 343–352.

SAMPLE

Matrix: solutions

Sample preparation: 100 μL 100 μM Solution + 400 μL buffer + 500 μL 80 mM 4-fluoro-7-nitrobenzoxadiazole in EtOH, mix, heat at 60° in the dark for 1 min, dilute with MeCN:water 50:50, inject a 20 μL aliquot. (Prepare buffer by adjusting 100 mM boric acid to pH 8.0 with NaOH.)

HPLC VARIABLES

Guard column: 30 × 4.6 Spheri-5 RP-18

Column: 250 × 4.6 Inertsil ODS-2

Mobile phase: MeCN:water 60:40 containing 1 mM imidazole, pH adjusted to 7.0 with nitric acid

Flow rate: 1

Injection volume: 20

Detector: Chemiluminescence following post-column reaction. The column effluent mixed with the reagent pumped at 1 mL/min and the mixture flowed through a 300 mm × 0.25 mm ID coil to the detector. (Prepare the reagent by dissolving 112 mg bis(2,4,6-trichlorophenyl) oxalate in 500 mL MeCN, add 8.6 mL 30% hydrogen peroxide, sonicate.), F ex 470 em 530

CHROMATOGRAM

Retention time: 17

Limit of detection: 20 nmole (chemiluminescence), 30 nmole (F)

OTHER SUBSTANCES

Simultaneous: benzylamine, ephedrine, N-isopropylbenzylamine, N-methylphenethylamine, phenylbutylamine, phenylethylamine, phenylpropanolamine, phenylpropylamine

KEY WORDS

derivatization; post-column reaction; comparison with other derivatization reagents

REFERENCE

Hayakawa,K.; Hasegawa,K.; Imaizumi,N.; Wong,O.S.; Miyazaki,M. Determination of amphetamine-related compounds by high-performance liquid chromatography with chemiluminescence and fluorescence detections, *J.Chromatogr.*, **1989**, *464*, 343–352.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 500 μg/mL solution in MeOH:water 50:50, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Zorbax C8

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L MeCN:water 20:80. A:B from 100:0 to 0:100 over 30 min. (Purify triethylamine as follows. Wash neutral alumina (Merck) 3 times with 2 bed volumes of pentane, 3 times with 2 bed volumes of dichloromethane, and 3 times with 2 bed volumes of MeOH, allow solvent to evaporate in a fume hood overnight, heat alumina at 130° for 2 h. Prepare a 14 cm column of the washed alumina in a 290 × 22 tube, pass through a head volume of MeOH, pass through triethylamine. When triethylamine starts to elute discard the first 20 mL, use the next 20 mL, discard the column.)

Flow rate: 2

Injection volume: 5

Detector: UV 210

CHROMATOGRAM

Retention time: 10

OTHER SUBSTANCES
Simultaneous: acetophenone, amphetamine, desipramine, ethylmorphine, imipramine, mefenamic acid, morphine, phenylbutazone, salicylic acid

KEY WORDS
also details of isocratic elution

REFERENCE
Hill,D.W. Evaluation of alkyl bonded silica and solvent phase modifiers for the efficient elution of basic drugs on HPLC, *J.Liq.Chromatogr.*, **1990**, *13*, 3147–3175.

SAMPLE
Matrix: solutions
Sample preparation: Mix 50 μL of a 200 ppm solution in MeCN:500 mM pH 9.0 borate buffer 50:50 with 25 mg reagent, after 1 min elute with 1 mL hexane:THF 75:25, inject a 5 μL aliquot. (Reagent was dinitrophenyl carbamate benzotriazole polymeric reagent, synthesized as follows. (Caution! Chloroform, dichloromethane, dioxane, and hydrazine are carcinogenic in experimental animals! DMF may be carcinogenic! 3,5-dinitrobenzoyl chloride and aluminum chloride are corrosive! Nitrobenzene is toxic!) 10 g Dried macroporous polystyrene (Xe-305, Rohm and Haas) + 10 g 3-nitro-4-chlorobenzyl alcohol + 10 g anhydrous aluminum chloride + 50 mL nitrobenzene, heat at 65-70° for 3 days, cool, filter, wash polymer with three 50 mL portions of 1 M HCl in dioxane, with three 50 mL portions of DMF,with three 50 mL portions of MeOH, and with three 50 mL portions of dichloromethane, dry under vacuum at 100°. Reflux 19 g of this polymer in 60 mL hydrazine hydrate:ethylene glycol monoethyl ether 40:60 for 20 h, cool to room temperature, filter off the polymer and wash it thoroughly with water. Suspend the polymer in 100 mL concentrated HCl:dioxane 50:50, reflux for 20 h, filter the polymer and wash it with five 100 mL portions of water, with three 100 mL portions of MeOH, and with three 50 mL portions of ether, dry under vacuum at 80°. Functionalization was 1.17 mmoles/g (Eur.J.Biochem. 1975, 59, 55). Dissolve 3,5-dinitrobenzoyl chloride in the minimum amount of glacial acetic acid, add an equimolar amount of sodium azide, stir for 30 min, dilute with water, filter to obtain 3,5-dinitrobenzoyl azide (Caution! Azides are toxic and potentially explosive!) (J. Liq. Chromatogr. 1986,9, 443). Heat 71 mg 3,5-dinitrobenzoyl azide in 15 mL toluene (dried over calcium hydride) at ??? for 30 min, cool using an ice bath, add 200 mg polymer, allow to warm to room temperature with stirring for 1 h, filter, wash the polymer with four 10 mL portions of warm (40°) dichloromethane, dry under high vacuum for 1 h.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm LC-(R)-naphthylurea (Supelco)
Mobile phase: Hexane:EtOH:MeCN 93:7:0.5
Flow rate: 2
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 8.7, 10.7 (enantiomers)

OTHER SUBSTANCES
Simultaneous: amphetamine

KEY WORDS
derivatization; chiral

REFERENCE
Bourque,A.J.; Krull,I.S. Immobilized isocyanates for derivatization of amines for chiral recognition in liquid chromatography with UV detection, *J.Pharm.Biomed.Anal.*, **1993**, *11*, 495–503.

SAMPLE
Matrix: solutions

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: 7

OTHER SUBSTANCES
Simultaneous: diethylpropion, phenylpropanolamine, ephedrine, amphetamine, phentermine, fenfluramine
Also analyzed: amitriptyline, chlordiazepoxide, chlorpromazine, desalkylflurazepam, desipramine, desmethyldoxepin, diazepam, doxepin, flurazepam, imipramine, mesoridazine, norchlordiazepoxide, nordiazepam, nortriptyline, oxazepam, prazepam, promazine, thioridazine, thiothixene, trifluoperazine

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REFERENCE
Rainin Catalog, C1-94, **1994**, p. 7.24.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepi-

nephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4 5 μm Chiradex (immobilized β-cyclodextrin) (Merck)
Mobile phase: MeOH:100 mM pH 7 ammonium acetate 5:95
Column temperature: 20
Flow rate: 0.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 30.28 (S(+)), 31.93 (R(-))

KEY WORDS

chiral

REFERENCE

Rizzi,A.M.; Hirz,R.; Cladrowa-Runge,S.; Jonsson,H. Enantiomeric separation of amphetamine, methamphetamine and ring substituted amphetamines by means of a β-cyclodextrin-chiral stationary phase, *Chromatographia*, **1994**, *39*, 131–137.

SAMPLE

Matrix: solutions
Sample preparation: 1 mL Solution + 500 μL 0.5% sodium 1,2-naphthoquinone-4-sulfonate in water + 500 μL buffer, let stand for 10 min, extract three times with 2 mL aliquots of n-hexane: ethyl acetate 50:50. Combine the organic layers and evaporate them to dryness at 80°, reconstitute with 2 mL MeCN:water 50:50, inject a 50 μL aliquot. (Buffer was 4% sodium bicarbonate adjusted to pH 10 with 10% NaOH.)

HPLC VARIABLES

Column: 250 × 4 5 μm Hypersil ODS C18
Mobile phase: Gradient. MeCN:0.5% propylamine hydrochloride in water from 40:60 to 50:50 over 2.5 min, to 70:30 over 1 min, maintain at 70:30 for 4.5 min.
Flow rate: 1
Injection volume: 50
Detector: UV 280

CHROMATOGRAM

Retention time: 7

OTHER SUBSTANCES
Extracted: amphetamine

KEY WORDS
derivatization

REFERENCE
Herráez-Hernández,R.; Campíns-Falcó,P.; Sevillano-Cabeza,A. On-line derivatization into precolumns for the determination of drugs by liquid chromatography and column switching: Determination of amphetamines in urine, *Anal.Chem.*, **1996**, *68*, 734–739.

SAMPLE
Matrix: solutions
Sample preparation: 500 μL Solution + 250 μL buffer + 250 μL 20 mM 9-fluorenylmethyl chloroformate in MeCN, mix, add 1 mL MeCN, inject a 50 μL aliquot. (Prepare buffer by adjusting the pH of 4% sodium bicarbonate to 10 with 10% NaOH.)

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 100 RP 18
Mobile phase: Gradient. MeCN:water from 40:60 to 50:50 over 2.5 min, to 70:30 over 2.5 min, to 100:0 over 5 min.
Flow rate: 1.5
Injection volume: 50
Detector: F ex 264 em 313

CHROMATOGRAM
Retention time: 8.8

OTHER SUBSTANCES
Extracted: amphetamine

KEY WORDS
derivatization

REFERENCE
Herráez-Hernández,R.; Campíns-Falcó,P.; Sevillano-Cabeza,A. On-line derivatization into precolumns for the determination of drugs by liquid chromatography and column switching: Determination of amphetamines in urine, *Anal.Chem.*, **1996**, *68*, 734–739.

SAMPLE
Matrix: urine
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH, 3 mL MeCN: 10 mM ammonium acetate 40:60 adjusted to pH 3 with acetic acid, and 5 mL water. 5 mL Urine + 5 mL 500 mM ammonium acetate, adjusted to pH 9.5 with ammonia, mix, add to the SPE cartridge, wash with 20 mL 5 mM pH 9.5 ammonium acetate, wash with 0.5 mL water. Elute with 2 mL MeCN:10 mM ammonium acetate 40:60 adjusted to pH 3 with acetic acid, inject a 50 μL aliquot of the eluate.

HPLC VARIABLES
Column: 150 × 4.6 L-column ODS (Chemical Inspection & Testing Institute, Tokyo)
Mobile phase: Gradient. MeCN:100 mM ammonium acetate 0:100 for 1 min, to 40:60 over 20 min.
Flow rate: 1
Injection volume: 50
Detector: UV 210; MS Shimadzu model QP-1100EX thermospray, vaporizer temperature from 170 to 150° over 20 min. SIM, m/z 150

CHROMATOGRAM
Retention time: 15
Limit of detection: 2–40 ng/mL

OTHER SUBSTANCES
Extracted: 6-acetylmorphine, amphetamine, benzoylecgonine, cocaine, ephedrine, methylephedrine, morphine, morphine-3-glucuronide, morphine-6-glucuronide

KEY WORDS
SPE

REFERENCE
Tatsuno,M.; Nishikawa,M.; Katagi,M.; Tsuchihashi,H. Simultaneous determination of illicit drugs in human urine by liquid chromatography-mass spectrometry, *J.Anal.Toxicol.*, **1996**, *20*, 281–286.

SAMPLE
Matrix: urine
Sample preparation: 500 μL Urine + N-ethylnordiazepam + chlorpheniramine + 100 μL buffer, centrifuge at 11000 g for 30 s, inject a 500 μL aliquot onto column A with mobile phase A, after 0.6 min backflush column A with mobile phase A to waste for 1.6 min, elute column A with 250 μL mobile phase B, with 200 μL mobile phase C, and with 1.15 mL mobile phase D. Elute column A to waste until drugs start to emerge then elute onto column B. Elute column B to waste until drugs started to emerge, then elute onto column C. When all the drugs have emerged from column B remove it from the circuit, elute column C with mobile phase D, monitor the effluent from column C. Flush column A with 7 mL mobile phase E, with mobile phase D, and mobile phase A. Flush column B with 5 mL mobile phase E then with mobile phase D. (Buffer was 6 M ammonium acetate adjusted to pH 8.0 with 2 M KOH.)

HPLC VARIABLES
Column: A 10 × 2.1 12-20 μm PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 × 3.2 11 μm Aminex A-28 (Bio-Rad); C 25 × 3.2 5 μm C8 (Phenomenex) + 150 × 4.6 5 μm silica (Macherey-Nagel)
Mobile phase: A 0.1% pH 8.0 potassium borate buffer; B 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid; C MeCN:buffer 40:60 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); D MeCN:buffer 33:67 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); E MeCN:buffer 70:30 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)
Column temperature: ambient (column A), 40 (columns B and C)
Flow rate: A 5; B-E 1
Injection volume: 500
Detector: UV 210, UV 235

CHROMATOGRAM
Retention time: k' 3.1
Internal standard: N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)
Limit of detection: 300 ng/mL

OTHER SUBSTANCES
Extracted: methadone, imipramine, flurazepam, amitriptyline, morphine, codeine, hydromorphone, hydrocodone, caffeine, cotinine, benzoylecgonine, secobarbital, oxazepam, phenobarbital, nordiazepam, diazepam, phenylpropanolamine, phentermine, amphetamine, phenmetrazine, lidocaine, ephedrine
Interfering: pentazocine, desipramine, nortriptyline, diphenhydramine

KEY WORDS
column-switching

REFERENCE
Binder,S.R.; Regalia,M.; Biaggi-McEachern,M.; Mazhar,M. Automated liquid chromatographic analysis of drugs in urine by on-line sample cleanup and isocratic multi-column separation, *J.Chromatogr.*, **1989**, *473*, 325–341.

SAMPLE
Matrix: urine
Sample preparation: 500 μL Urine + 100 μL 25 μg/mL N-n-propylaniline + 6 mL pH 10.0 carbonate buffer + 15 mL water, add mixture to an Extrelut SPE cartridge, let stand for 20 min, elute with 40 mL hexane:ethyl acetate 90:10. Add the eluate to 3 mL 100 mM sulfuric acid and 500 mg NaCl, stir for 20 min, centrifuge at 1000 g for 5 min. Remove the lower layer and add it to 3 mL 2.5 M NaOH and 20 μL benzoyl chloride, stir vigorously for 30 min. Extract the mixture with 1.5 mL chloroform. Wash the chloroform layer twice with 5 mL water and evaporate it to dryness at 40°, reconstitute the residue in 200 μL hexane:isopropanol 90:10, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Chiralcel OB + 250 × 4.6 Chiralcel OJ
Mobile phase: Hexane:isopropanol 90:10
Column temperature: 48
Flow rate: 1-1.4
Detector: UV 220

CHROMATOGRAM
Retention time: 13 (d), 15 (l)
Internal standard: N-n-propylaniline (11)
Limit of detection: 25 ng

OTHER SUBSTANCES
Extracted: amphetamine

KEY WORDS
rat; SPE; derivatization; chiral

REFERENCE
Nagai,T.; Kamiyama,S. Assay of the optical isomers of methamphetamine and amphetamine in rat urine using high-performance liquid chromatography with chiral cellulose-based columns, *J.Chromatogr.*, **1990**, *525*, 203–209.

SAMPLE
Matrix: urine
Sample preparation: 200-500 μL Rat urine + 200-500 μL pH 3.8 acetate buffer + 25 μL 40 μg/mL β-glucuronidase and 20 μg/mL arylsulfatase (Merck), heat at 37° for 24 h, add 100 μL 25 μg/mL 3-methoxytyramine in water, add 100 μL water, adjust pH to 9.0 with 1.9 M sodium carbonate, add to an Extrelut SPE cartridge, let stand for 20 min, elute with 6 mL ethyl acetate. Add the eluate to 1 mL 100 mM sulfuric acid, extract. Add the aqueous layer to 3 mL 2.5 M NaOH, add 25 μL benzoyl chloride, extract with 5 mL ethyl acetate. Wash the ethyl acetate layer with water, evaporate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL EtOH, 3.5 mL 50 mM pH 8.0 Tris/HCl buffer, and 35 μL esterase (Type 1 porcine liver, Sigma). Heat at 25° for 45 min, add to an activated Sep-Pak C18 SPE cartridge, wash with 5 mL water, elute with 5 mL acetone. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL hexane:EtOH 89:11, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Chiralcel OB + 250 × 4.6 Chiralcel OJ
Mobile phase: n-Hexane:EtOH 89:11
Column temperature: 48
Flow rate: 1.4
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 10 (D), 11.5 (L)
Internal standard: 3-methoxytyramine (54)

OTHER SUBSTANCES
Extracted: metabolites, amphetamine

KEY WORDS
rat; SPE; derivatization; chiral

REFERENCE
Nagai,T.; Kamiyama,S. Simultaneous HPLC analysis of optical isomers of methamphetamine and its metabolites, and stereoselective metabolism of racemic methamphetamine in rat urine, *J.Anal.Toxicol.*, **1991**, *15*, 299–304.

SAMPLE
Matrix: urine
Sample preparation: Adjust pH of 3 mL urine to 11 with 10 M KOH, add to an Extrelut 3 column, let stand for 10 min, elute with 15 mL n-hexane into a tube containing one drop of 3 M HCl. Evaporate the eluate to dryness under a stream of nitrogen at 35°. Add 1.5 mL 8% sodium bicarbonate in water and 1 mL 0.5% sodium naphthoquinone-4-sulfonate in water to the residue, heat at 70° for 20 min, cool, extract with 5 mL carbon tetrachloride. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm Lichrospher 100 RP8
Column: 250 × 4 5 μm Lichrospher 100 RP8
Mobile phase: MeCN:buffer 55:45 (Buffer was 1.361 g KH_2PO_4 in 950 mL, add 1.3 mL methanesulfonic acid, adjust pH to 3 with 5 M KOH, make up to 1 L with water.)
Flow rate: 1
Injection volume: 20
Detector: UV 460

CHROMATOGRAM
Retention time: 7.8
Limit of detection: 60 ng/mL

OTHER SUBSTANCES
Extracted: amphetamine
Noninterfering: acetaminophen, aspirin, amitriptyline, buprenorphine, caffeine, carbamazepine, chlorpromazine, desipramine, dextromethorphan, doxepin, ephedrine, fenfluramine, imipramine, lidocaine, loxapine, meperidine, methadone, methaqualone, naloxone, naltrexone, nicotine, orphenadrine, oxycodone, papaverine, pentazocine, phendimetrazine, phenmetrazine, phentermine, phenylpropanolamine, phenytoin, primidone, procaine, promethazine, propoxyphene, propyphenazone, theobromine, theophylline, trazodone, trifluprom azine, trimethoprim, trimipramine

KEY WORDS
SPE; derivatization

REFERENCE
Ferrara,S.D.; Tedeschi,L.; Frison,G.; Castagna,F. Solid-phase extraction and HPLC-UV confirmation of drugs of abuse in urine, *J.Anal.Toxicol.*, **1992**, *16*, 217–222.

SAMPLE
Matrix: urine
Sample preparation: Condition a 100 mg Adsorbex SCX cation-exchange SPE cartridge (Merck) with 2 mL MeOH, 1 mL water, and 1 mL 17 mM KH_2PO_4, do not allow to dry. Centrifuge urine at 2000 g for 5 min. 1 mL Urine + 500 μL 50 mM KH_2PO_4, sonicate for 1 min, add to the SPE cartridge, rinse vial with 50 μL 50 mM KH_2PO_4 and add to cartridge, dry cartridge for 1 min, wash with three 500 μL portions of 17 mM KH_2PO_4, wash with 1 mL MeOH, dry under vacuum for 1 min, elute with four 500 μL portions of MeOH:7.3% HCl (97.5:2.5) at a flow rate of 0.5 mL/min, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 125 × 4 3 μm Spherisorb ODS-1
Mobile phase: Gradient. A was water containing 5 mL (8.5 g) 85% orthophosphoric acid and 280 μL (0.22 g) hexylamine per liter. B was MeCN containing 100 mL water, 5 mL (8.5 g) 85% orthophosphoric acid, and 280 μL (0.22 g) hexylamine per liter. A:B 94.5:5.5 for 10.6 min, then to 61:39 over 11 min.
Column temperature: 40
Flow rate: 0.8
Injection volume: 10
Detector: UV 198

CHROMATOGRAM

Retention time: 9
Limit of detection: 30 ng/mL

OTHER SUBSTANCES

Extracted: 3,4-methylenedioxyamphetamine, amphetamine, 4-methoxyamphetamine, phentermine, 3,4-methylenedioxymethamphetamine, 5-methoxy-3,4-methylenedioxyamphetamine, 3,4,5-trimethoxyamphetamine, 3,4-methylenedioxyethylamphetamine, 2,5-dimethoxyamphetamine, 4-bromo-2,5-dimethoxyphenylethylamine, 2,5-dimethoxy-4-methylamphetamine, 4-bromo-2,5-dimethoxyamphetamine, 2,5-dimethoxy-4-ethylamphetamine, mescaline, methoxamine

KEY WORDS

SPE

REFERENCE

Helmlin,H.-J.; Brenneisen,R. Determination of psychotropic phenylalkylamine derivatives in biological matrices by high-performance liquid chromatography with photodiode-array detection, *J.Chromatogr.*, **1992**, *593*, 87–94.

SAMPLE

Matrix: urine
Sample preparation: 5 mL Urine + 4.5 mL MeCN + 500 μL 1 M KOH, centrifuge at 2500 rpm for 10 min, filter (45 μm) the supernatant. Inject on to column A at 180 μL/min 25 μL 50 mM KOH in MeCN:water 20:80, 50 μL filtrate, 25 μL 50 mM KOH in MeCN:water 20:80, and 200 μL MeCN:water 20:80, backflush the contents of column A on to column B with mobile phase, after 18 s remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Wash column A with 400 μL MeCN.

HPLC VARIABLES

Column: A 20 × 2 polymeric reagent; B 250 × 4.6 5 μm Supelcosil LC-18-DB (with a guard column) (Prepare polymeric reagent as follows. Prepare a porous rigid resin using a divinylbenzene:ethylstyrene:styrene 24:6:70 mixture with trimethylsilyl modified silica (102 Å average pore size, 1.08 mL/g pore volume, 366 m^2/g surface area, 16-20 μm irregular particle shape, IMPAQ RG 1020 Si silica, PQ Co., Valley Forge PA). Further preparation details are not given but a typical procedure given in the cited reference is as follows. Aerate a mixture of 10 g modified silica in 100 mL water with nitrogen for 15 min, add 10 mL styrene:80% divinylbenzene:t-butyl peroxybenzoate 49:49:2 (remove preservative by passing through a butylcatechol remover (Scientific Polymer, Ontario NY), shake vigorously at room temperature for 4 h, add 150 mL 0.75% polyvinyl alcohol, shake for 4 h, heat at 120° for 24 h while shaking on a Parr instrument, cool to room temperature, filter, wash with 100 mL water, wash with 50 mL MeOH. Add the solid to 500 mL 3 M NaOH in MeOH:water 40:60, shake at room temperature for 14 h (to dissolve the silica), filter, wash with water until the washings are neutral, wash with 100 mL MeOH, dry at 60°. The polymer has similar properties to the template silica (US Pat. 4 933 372 (1990)). Soxhlet extract the resin with dioxane for 8 h (Caution! Dioxane is a carcinogen!). Add 25 g aluminum trichloride in 300 mL dry nitrobenzene to 50 g resin and 100 g 4-chloro-3-nitrobenzoyl chloride, stir mechanically at 60° for 5 h, pour into a mixture of 150 mL DMF, 100 mL concentrated HCl, and 150 g ice, filter. Wash the solid with 300 mL portions of DMF:water 75:25 until the washings are colorless, wash with warm (60°) DMF, wash with six 300 mL portions of dichloromethane:MeOH 2:1. Stir the product in 130 mL 40% benzyltrimethylammonium hydroxide in water, 130 mL water, and 260 mL dioxane at 90° for 8 h, filter, repeat the process. Wash the product with four portions of warm (60°) dioxane. Stir the solid

with 30 mL acetic acid for 15 min, filter. Wash the solid with dioxane until the washings are neutral, wash with six 300 mL portions of dichloromethane:MeOH 2:1 to give a nitrobenzophenol-substituted polymer (J. Org. Chem. 1984, 49, 924). Heat 4 g 9-fluoreneacetic acid, 3.9 mL oxalyl chloride, 30 mL benzene (dried over anhydrous sodium sulfate, Caution! Benzene is a carcinogen!), and 3 drops of triethylamine at 55° for 1 h, evaporate under reduced pressure to remove oxalyl chloride, dissolve the product in 35 mL dichloromethane to give a 120 mg/mL solution of 9-fluoreneacetyl chloride, dilute to obtain a 2 mM solution. Stir 1.3 g nitrobenzophenol-substituted polymer, 4.2 mL 2 mM 9-fluoreneacetyl chloride solution, 300 μL triethylamine, and 20 mL dichloromethane at room temperature for 1 h, filter, wash with three 20 mL portions of MeCN to obtain the reagent, polymer-bound nitrobenzophenol 9-fluoreneacetate (J. Chromatogr. 1992, 609, 103).)

Mobile phase: Gradient. MeCN:water 50:50 for 3.5 min, to 70:30 over 12 min, maintain at 70:30 for 2.5 min, return to initial conditions over 1 min. (Place a 100 × 4.6 30-40 μm silica column before the injector.)

Column temperature: 60 (column A only)

Injection volume: 25-50

Detector: F ex 254 em 305-395

CHROMATOGRAM

Retention time: 13.6

Limit of quantitation: 25 ng/mL

KEY WORDS

derivatization; column-switching

REFERENCE

Bourque,A.J.; Krull,I.S.; Feibush,B. Automated HPLC analyses of drugs of abuse via direct injection of biological fluids followed by simultaneous solid-phase extraction and derivatization with fluorescence detection, *Biomed.Chromatogr.*, **1994**, *8*, 53–62.

SAMPLE

Matrix: urine

Sample preparation: Condition a 100 mg Bond-Elut C18 SPE cartridge with 500 μL MeOH and 500 μL water. Adjust pH of urine to 10, centrifuge at 1500 g. 2 mL Supernatant + 100 μL 75 μg/mL β-phenylethylamine hydrochloride in water, add to the SPE cartridge, wash with 2.5 mL water, elute with 2 mL MeOH, evaporate the eluate to dryness. Reconstitute in water, add 500 μL 8% sodium bicarbonate, add 500 μL 0.5% 1,2-naphthoquinone-4-sulfonic acid sodium salt, make up to 1.5 mL with water, heat at 70° for 20 min, cool, add an equal volume of chloroform, shake for 2 min, centrifuge at 1500 g for 5 min. Remove the organic layer and dry it over anhydrous sodium sulfate, filter (0.45 μm), inject a 25 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 125 × 4 5 μm LiChrospher Si-60

Mobile phase: EtOH:chloroform:ethyl acetate:n-hexane 1:22:32:45

Flow rate: 2

Injection volume: 25

Detector: UV 280

CHROMATOGRAM

Retention time: 2.6

Internal standard: β-phenylethylamine hydrochloride (4.9)

OTHER SUBSTANCES

Extracted: amphetamine

KEY WORDS

SPE; normal phase; derivatization

REFERENCE

Campins Falcó,P.; Molins Legua,C.; Herráez Hernandez,R.; Sevillano Cabeza,A. Improved amphetamine and methamphetamine determination in urine by normal-phase high-performance liquid chromatography with sodium 1,2-naphthoquinone 4-sulphonate as derivatizing agent and solid-phase extraction for sample clean-up, *J.Chromatogr.B*, **1995**, *663*, 235–245.

SAMPLE
Matrix: urine
Sample preparation: Condition a Bond Elut C18 SPE cartridge with 1 mL MeOH and 1 mL 50 mM pH 11 phosphate buffer. 500 μL Urine + 500 μL 2000 U/mL β-glucuronidase with sulfatase activity (Type H-1, Sigma) in 100 mM pH 5 acetate buffer, heat at 37° overnight, add 500 mg NaCl, add 500 μL 50 mM pH 11 potassium phosphate buffer, adjust pH to 11 with ammonium hydroxide, mix. Add the mixture to the SPE cartridge, wash with 1 mL 50 mM pH 11 potassium phosphate buffer, wash with 1 mL freshly prepared MeOH:water 30:70, wash with 1 mL MeCN, elute with 1 mL freshly prepared MeCN:acetic acid 98:2, elute with 1 mL MeCN:HCl 98:2. Combine the eluates and evaporate them to dryness under a stream of air at room temperature, reconstitute the residue in mobile phase (?), inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: phenyl
Column: Microsorb phenyl
Mobile phase: MeCN:MeOH:50 mM pH 3 potassium phosphate 5:10:85
Flow rate: 1
Injection volume: 10
Detector: UV 215

CHROMATOGRAM
Retention time: 15.6
Internal standard: methamphetamine

OTHER SUBSTANCES
Extracted: amphetamine

KEY WORDS
SPE; rat; methamphetamine in IS

REFERENCE
Law,M.Y.L.; Moody,D.E. Simultaneous quantitation of amphetamine and 4'-hydroxyamphetamine by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 2029–2043.

SAMPLE
Matrix: urine
Sample preparation: Condition an Extra-Sep C18 SPE cartridge (Teknokroma) with 1 mL MeOH and 1 mL buffer. Adjust pH of 2 mL urine to ca. 10 with 100 μL concentrated ammonium hydroxide, add 5 μg β-phenylethylamine, add to the SPE cartridge, wash with 5 mL water, wash with 1 mL MeCN, elute with 2 mL MeOH. Add 100 μL EtOH:concentrated HCl 6:1 to the eluate, evaporate to dryness. Reconstitute with 1 mL buffer and 1 mL 0.5% 1,2-naphthoquinone-4-sulfonic acid sodium salt, let stand at room temperature for 10 min, add 2 mL n-hexane:ethyl acetate 50:50, shake for 2 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 500 μL MeCN:water 50:50, inject a 50 μL aliquot. (Buffer was 1% aqueous sodium bicarbonate adjusted to pH 10 with 5 M NaOH.)

HPLC VARIABLES
Column: 250 × 4 5 μm Hypersil ODS-C18
Mobile phase: Gradient. MeCN:0.5% propylamine in water from 40:60 to 50:50 over 2.5 min, to 70:30 over 1 min, maintain at 70:30.
Flow rate: 1
Injection volume: 50
Detector: UV 280

CHROMATOGRAM
Retention time: 6.3
Internal standard: β-phenylethylamine (4.1)
Limit of detection: 2 ng/mL
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: amphetamine

KEY WORDS
SPE; derivatization

REFERENCE
Molíns Legua,C.; Campíns Falcó,P.; Sevillano Cabeza,A. Amphetamine and methamphetamine determination in urine by reversed-phase high-performance liquid chromatography with sodium 1,2-naphthoquinone 4-sulfonate as derivatizing agent and solid-phase extraction for sample clean-up, *J.Chromatogr.B*, **1995**, *672*, 81–88.

SAMPLE
Matrix: urine
Sample preparation: 100-300 μL Urine + 100 μL 1.5 M NaOH + 5 μg IS, make up to 1 mL with water, add to an Extrelut 1 SPE cartridge, let stand for 20 min, elute with 6 mL benzene (Caution! Benzene is a carcinogen!). Add the eluate to 1 mL 100 mM sulfuric acid, extract. Remove the aqueous layer and add it to 3 mL 1.5 M NaOH, add 5 μL benzoyl chloride, vortex vigorously, extract twice with 3 mL portions of n-hexane. Combine the organic layers and wash them twice with 3 mL portions of water, evaporate the organic to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: Chiralcel OB-H
Mobile phase: n-Hexane:isopropanol 90:10
Column temperature: 55
Flow rate: 1
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 6.5 (D), 10 (L)
Internal standard: l-p-methoxyamphetamine (12)
Limit of detection: 30 ng

OTHER SUBSTANCES
Extracted: amphetamine, ethylamphetamine

KEY WORDS
rat; derivatization; SPE; chiral

REFERENCE
Nagai,T.; Kamiyama,S.; Matsushima,K. Analysis of time-lapse changes of d- and l-enantiomers of racemic ethylamphetamine and stereoselective metabolism in rat urine by HPLC determination, *J.Anal.Toxicol.*, **1995**, *19*, 225–228.

SAMPLE
Matrix: urine
Sample preparation: Dilute urine 20-fold or more. 500 μL Diluted urine + 50 μL 500 ng/mL (+)-2,5-dimethoxyamphetamine hydrochloride + 100 μL 500 mM NaOH + 2 mL benzene (Caution! Benzene is a carcinogen!), shake for 15 min, centrifuge at 1200 g for 5 min. Remove 1.8 mL of the organic phase and add it to 220 μL 50 mM HCl, shake for 15 min, centrifuge at 1200 g for 5 min. Remove 200 μL of the aqueous phase and add it to 40 μL 250 mM NaOH, add 50 μL 330 mM pH 7.8 phosphate buffer, add 250 μL MeCN, add 25 μL 3 mM (-)-1-(9-fluorenyl)ethyl chloroformate, let stand at room temperature for 24 h, add 30 μL 100 mM glycine in water, let stand for 30 min, add 750 μL pentane, vortex for 2 min, centrifuge at 1200 g for 5 min. Remove the organic layer and evaporate it to dryness in a centrifugal evaporator at room temperature, reconstitute the residue in 300 μL MeCN:water 50:50, inject a 100 μL aliuot.

HPLC VARIABLES
Column: 150 × 4.6 3 μm Adsorbosphere HS C18

Mobile phase: MeCN:THF:20 mM pH 3.6 sodium acetate buffer 25:21:54
Flow rate: 1
Injection volume: 100
Detector: F ex 265 em 330

CHROMATOGRAM
Retention time: 42 (L), 44 (D)
Internal standard: (+)-2,5-dimethoxyamphetamine (33)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: amphetamine

KEY WORDS
rat; derivatization; chiral

REFERENCE
Sukbuntherng,J.; Hutchaleelaha,A.; Chow,H.-H.; Mayersohn,M. Separation and quantitation of the enantiomers of methamphetamine and its metabolites in urine by HPLC: Precolumn derivatization and fluorescence detection, *J.Anal.Toxicol.*, **1995**, *19*, 139–147.

SAMPLE
Matrix: urine
Sample preparation: Condition a 200 mg Bond Elut C18 SPE cartridge with 1 mL MeOH and 1 mL 1% pH 10 sodium bicarbonate buffer. 2 mL Urine + 400 μL 8% pH 10 sodium bicarbonate buffer, mix, centrifuge at 1500 g for 2 min, add a 2 mL aliquot of the supernatant to the SPE cartridge, wash with 3 mL water, pass 500 μL 2% sodium 1,2-naphthoquinone 4-sulfonate through the cartridge, pass 500 μL 1% pH 10 sodium bicarbonate buffer through the cartridge, let stand at room temperature for 15 min, wash with 3 mL water, elute with 1 mL MeCN:water 50:50, inject a 20 μL aliquot of the eluate.

HPLC VARIABLES
Column: 250 × 4 5 μm Hypersil ODS
Mobile phase: Gradient. MeCN:buffer from 40:60 to 50:50 over 2.5 min, to 70:30 over 0.5 min, maintain at 70:30 for 1.5 min, to 85:15 over 1 min, maintain at 85:15 for 1.5 min. (Buffer was 5 mL/L propylamine in water.)
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 5.6
Internal standard: β-phenylethylamine (3.6)
Limit of detection: 400 ng/mL

OTHER SUBSTANCES
Extracted: amphetamine

KEY WORDS
derivatization; SPE

REFERENCE
Campíns-Falcó,P.; Sevillano-Cabeza,A.; Molíns-Legua,C.; Kohlmann,M. Amphetamine and methamphetamine determination in urine by reversed-phase high-performance liquid chromatography with simultaneous sample clean-up and derivatization with 1,2-naphthoquinone 4-sulphonate on solid-phase cartridges, *J.Chromatogr.B*, **1996**, *687*, 239–246.

SAMPLE
Matrix: urine
Sample preparation: Inject 15 μL urine, inject a mixture of 5 μL 20 mM 9-fluorenylmethyl chloroformate in MeCN and 45 μL water, and inject 10 μL buffer on to column A and elute to

waste with mobile phase A. After 2.8 min backflush the contents of column A on to column B with mobile phase B and start the gradient, monitor the effluent from column B. At the end of the run condition column A with 1 mL mobile phase A. (Buffer was 4% sodium bicarbonate adjusted to pH 10 with 10% NaOH.)

HPLC VARIABLES
Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 125 × 4 5 μm LiChrospher 100 PR-C18
Mobile phase: A water; B Gradient. MeCN:water from 40:60 to 70:30 over 15 min. to 100:0 over 5 min.
Flow rate: A 0.35; B 1
Injection volume: 15
Detector: F ex 264 em 313

CHROMATOGRAM
Retention time: 17.8
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: amphetamine, ephedrine, norephedrine, 3-phenylpropylamine, pseudoephedrine

KEY WORDS
column-switching; derivatization; on-column derivatization

REFERENCE
Herráez-Hernández,R.; Campíns-Falcó,P.; Sevillano-Cabeza,A. Determination of amphetamine and related compounds in urine using on-line derivatization in octadecyl silica columns with 9-fluorenylmethyl chloroformate and liquid chromatography, *J.Chromatogr.B*, **1996**, *679*, 69–78.

SAMPLE
Matrix: urine
Sample preparation: Inject 50 μL urine on to column A and elute to waste with mobile phase A, after 2 min inject a mixture of 25 μL 0.5% sodium 1,2-naphthoquinone-4-sulfonate in water and 25 μL buffer on to column A, stop the flow of mobile phase A, after 10 min start pump A, after 5 min backflush the contents of column A on to column B with mobile phase B and start the gradient, monitor the effluent from column B. After each run flush column A with ethyl acetate for 1 min, n-hexane for 1 min, and ethyl acetate for 1 min, re-equilibrate with mobile phase A. (Buffer was 4% sodium bicarbonate adjusted to pH 10 with 10% NaOH.)

HPLC VARIABLES
Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 250 × 4 5 μm Hypersil ODS C18
Mobile phase: A water; B Gradient. MeCN:0.5% propylamine hydrochloride in water from 40:60 to 50:50 over 2.5 min, to 70:30 over 1 min, maintain at 70:30 for 4.5 min.
Flow rate: 1
Injection volume: 50
Detector: UV 280

CHROMATOGRAM
Retention time: 7
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Extracted: amphetamine

KEY WORDS
column-switching; derivatization; on-column derivatization

REFERENCE
Herráez-Hernández,R.; Campíns-Falcó,P.; Sevillano-Cabeza,A. On-line derivatization into precolumns for the determination of drugs by liquid chromatography and column switching: Determination of amphetamines in urine, *Anal.Chem.*, **1996**, *68*, 734–739.

SAMPLE
Matrix: urine
Sample preparation: Condition a Bond Elut SCX SPE cartridge with 10 mL MeOH:aqueous ammonia 98:2, 10 mL MeOH, and 30 mL water. 10 mL Urine + 10 mL 50 mM pH 7.8 potassium phosphate buffer, mix, add to the SPE cartridge, wash with 2 mL water, wash with 10 mL MeOH, elute with 3 mL MeOH:2% aqueous ammonia 98:2. Add 30 μL glacial acetic acid and 30 μL 1 mg/mL N-ethylaniline in MeOH to the eluate, inject a 5-50 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 ULTRON ES-PhCD β-cyclodextrin phenylcarbamate-bonded silica (Shinwa Chemical Industries, Kyoto)
Mobile phase: MeCN:MeOH:buffer 10:30:60 (Buffer was 50 mM pH 6.0 potassium phosphate for UV detection or 100 mM pH 6.0 ammonium acetate for MS detection.)
Column temperature: 25
Flow rate: 1
Injection volume: 5-50
Detector: UV 220, MS, Shimadzu LCMS-QP1100EX, thermospray, positive ion mode, filament off, vaporizer 230°, ion source 300°, m/z 150

CHROMATOGRAM
Retention time: 12 (D, UV), 15 (L, UV), 15 (D, MS), 17.5 (L, MS)
Internal standard: N-ethylaniline (17, UV only)
Limit of detection: 50 ng/mL (D, UV detection), 100 ng/mL (L, UV detection), 10 ng/mL (D, scan MS), 20 ng/mL (L, scan MS), 0.8 ng/mL (D, SIM MS), 1.0 ng/mL (L, SIM MS)

OTHER SUBSTANCES
Extracted: metabolites, amphetamine, p-hydroxymethamphetamine

KEY WORDS
SPE; chiral

REFERENCE
Katagi,M.; Nishioka,H.; Nakajima,K.; Tsuchihashi,H.; Fujima,H.; Wada,H.; Nakamura,K.; Makino,K. Direct high-performance liquid chromatographic and high-performance liquid chromatographic-thermospray-mass spectrometric determination of enantiomers of methamphetamine and its main metabolites amphetamine and p-hydroxymethamphetamine in human urine, *J.Chromatogr.B*, **1996**, *676*, 35–43.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 20 μL 100 μM 1-phenylethylamine in water + 400 μL concentrated HCl, heat at 80° for 1 h, cool, neutralize with 600 μL 25% ammonia, add 5 mL 10% sodium carbonate solution, add 2 mL 500 mM pH 10.5 sodium borate buffer, add 2 mL chloroform:isopropanol 75:25, vortex for 1 min, centrifuge at 12.5° at 1500 g for 10 min, repeat the extraction. Combine the organic layers and remove a 100 μL aliquot, add 10 μL acetic acid to the aliquot, evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 50 μL carbonate buffer, add 50 μL 10 mM fluorescein-4-isothiocyanate in EtOH, mix, heat in the dark at 80° for 15 min, inject a 20 μL aliquot. (Prepare carbonate buffer by adjusting the pH of 200 mM sodium bicarbonate to 9.0 with 200 mM sodium carbonate.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Daisopak SP-120-5-ODS (Daiso, Osaka)
Mobile phase: Gradient. MeCN:20 mM pH 7.9 sodium phosphate buffer 20:80 for 16 min then 24:76 (step-gradient).
Flow rate: 0.8
Injection volume: 20
Detector: F ex 496 em 518

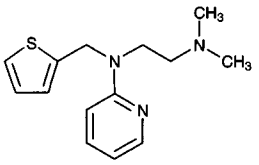
CHROMATOGRAM
Retention time: 35.2
Internal standard: 1-phenylethylamine (26.6)
Limit of detection: 5.5 nM

OTHER SUBSTANCES
Extracted: metabolites, amphetamine, norepinephrine

KEY WORDS
derivatization

REFERENCE
Al-Dirbashi,O.; Kuroda,N.; Akiyama,S.; Nakashima,K. High-performance liquid chromatography of methamphetamine and its related compounds in human urine following derivatization with fluorescein isothiocyanate, *J.Chromatogr.B*, **1997**, *695*, 251–258.

Methapyrilene



Molecular formula: $C_{14}H_{19}N_3S$
Molecular weight: 261.39
CAS Registry No.: 91-80-5, 33032-12-1 (fumarate), 135-23-9 (HCl)
Merck Index: 6027
Lednicer No.: 1 54

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 4.6

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone,

phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

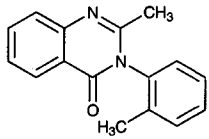
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenyl-

butazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Methaqualone



Molecular formula: $C_{16}H_{14}N_2O$
Molecular weight: 250.30
CAS Registry No.: 72-44-6, 340-56-7 (HCl)
Merck Index: 6028
Lednicer No.: 1 353

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 226

CHROMATOGRAM

Retention time: 4.19
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine;

prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

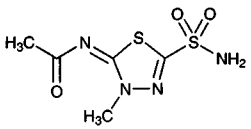
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, me-

phesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Methazolamide



Molecular formula: $C_5H_6N_4O_3S_2$
Molecular weight: 236.28
CAS Registry No.: 554-57-4
Merck Index: 6031
Lednicer No.: 1 250

SAMPLE

Matrix: blood, urine
Sample preparation: To 500 μL whole blood, plasma, or urine add 20 μL 1 mg/mL acetazolamide solution and 2.5 mL 50% ammonium sulfamate, vortex for 30 s. (Place the tube containing whole blood in boiling water for 30 s and then quickly in cold water.) Add 5 mL ethyl acetate, vortex, centrifuge at 3000 g for 10 min, transfer the organic layer to 5 mL pH 8.0 phosphate buffer, vortex, centrifuge at 3000 g for 10 min, transfer the organic layer to 500 μL pH 10.0 glycine buffer, vortex for 30 s, centrifuge at 3000 g for 5 min. Aspirate and discard the organic layer, add 500 μL ether to the remaining glycine buffer layer, vortex for 1 min, discard the ether phase. Vent the aqueous layer for about 30 min and inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Altima C18 (Alltech)
Mobile phase: MeCN:100 mM pH 4.0 sodium acetate 20:80
Flow rate: 1
Injection volume: 20
Detector: UV 285

CHROMATOGRAM

Retention time: 7.18
Internal standard: acetazolamide (4.55)

KEY WORDS

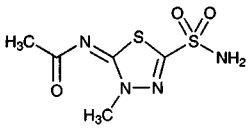
plasma; whole blood

phesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Methazolamide



Molecular formula: $C_5H_6N_4O_3S_2$
Molecular weight: 236.28
CAS Registry No.: 554-57-4
Merck Index: 6031
Lednicer No.: 1 250

SAMPLE

Matrix: blood, urine
Sample preparation: To 500 μL whole blood, plasma, or urine add 20 μL 1 mg/mL acetazolamide solution and 2.5 mL 50% ammonium sulfamate, vortex for 30 s. (Place the tube containing whole blood in boiling water for 30 s and then quickly in cold water.) Add 5 mL ethyl acetate, vortex, centrifuge at 3000 g for 10 min, transfer the organic layer to 5 mL pH 8.0 phosphate buffer, vortex, centrifuge at 3000 g for 10 min, transfer the organic layer to 500 μL pH 10.0 glycine buffer, vortex for 30 s, centrifuge at 3000 g for 5 min. Aspirate and discard the organic layer, add 500 μL ether to the remaining glycine buffer layer, vortex for 1 min, discard the ether phase. Vent the aqueous layer for about 30 min and inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Altima C18 (Alltech)
Mobile phase: MeCN:100 mM pH 4.0 sodium acetate 20:80
Flow rate: 1
Injection volume: 20
Detector: UV 285

CHROMATOGRAM

Retention time: 7.18
Internal standard: acetazolamide (4.55)

KEY WORDS

plasma; whole blood

REFERENCE
Iyer,G.R.; Taft,D.R. Determination of methazolamide concentrations in human biological fluids using high performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1998**, *16*, 1021–1027.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 RP-18 (Merck)
Mobile phase: MeOH:water:acetic acid 50:149:1
Flow rate: 0.7
Detector: UV 300

OTHER SUBSTANCES
Simultaneous: glutathione conjugate

REFERENCE
Kishida,K.; Akaki,Y.; Sasabe,T.; Yamamoto,C.; Manabe,R. Glutathione conjugation of methazolamide and subsequent reactions in the ciliary body in vitro, *J.Pharm.Sci.*, **1990**, *79*, 638–642.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

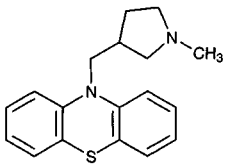
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone,

oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Methdilazine



Molecular formula: $C_{18}H_{20}N_2S$
Molecular weight: 296.44
CAS Registry No.: 1982-37-2, 1229-35-2 (HCl)
Merck Index: 6034
Lednicer No.: 1 387

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: 6.1

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl,

isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 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: 15.16 (A), 6.66 (B)

OTHER SUBSTANCES

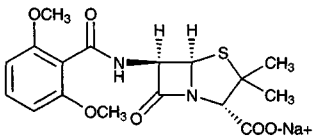
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, 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, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine,

phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
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.

Methicillin sodium



Molecular formula: $C_{17}H_{19}N_2NaO_6S$
Molecular weight: 402.40
CAS Registry No.: 132-92-3, 7246-14-2 (monohydrate), 61-32-5 (free acid)
Merck Index: 6047
Lednicer No.: 1 412

SAMPLE
Matrix: blood
Sample preparation: 400 μL Serum + 400 μL MeCN, vortex for 10 s, shake slowly for 15 min, centrifuge at 3000 g for 10 min. Remove the supernatant and add it to 4 mL dichloromethane, vortex for 10 s, shake for 15 min, centrifuge at 3000 g for 10 min, inject a 50 μL aliquot of the upper aqueous layer.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:water:200 mM ammonium acetate 28:62:10, pH 5.6
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 4.0
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: cloxacillin, dicloxacillin, nafcillin, oxacillin
Noninterfering: amdinocillin (mecillinam), amikacin, amoxicillin, ampicillin, carbenicillin, cefamandole, cefazolin, ceforanide, cefatoxamine, cefoxitin, cephalexin, cephaloridine, cephalothin, cephradine, cepharin, chloramphenicol, clindamycin, co-trimoxazole, fluorocytosine, gentamicin, metronidazole, moxalactam, penicillin, piperacillin, sulfamethoxazole, theophylline, ticarcillin, tobramycin, trimethoprim, vancomycin

KEY WORDS
serum

REFERENCE
Rudrik,J.T.; Bawdon,R.E. Determination of penicillinase-resistant penicillins in serum using high-pressure liquid chromatography, *J.Liq.Chromatogr.*, **1981**, *4*, 1525–1545.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 2 mL MeCN, vortex for 1 min, centrifuge at 3000 g for 10 min. Remove the supernatant and add it to 5 mL dichloromethane, vortex, centrifuge at 3000 g for 10 min, inject a 15 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: 300 mm long μBondapak C18
Mobile phase: MeCN:100 mM pH 6.1 sodium phosphate buffer 25:75
Flow rate: 2.5
Injection volume: 15
Detector: UV 229

CHROMATOGRAM
Internal standard: methicillin
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Extracted: piperacillin, mezlocillin

KEY WORDS
serum; pharmacokinetics; methicillin is IS

REFERENCE
Martens,M.G.; Faro,S.; Feldman,S.; Cotton,D.B.; Dorman,K.; Riddle,G.D. Pharmacokinetics of the acyclureidopenicillins piperacillin and mezlocillin in the postpartum patient, *Antimicrob.Agents Chemother.*, **1987**, *31*, 2015–2017.

SAMPLE
Matrix: blood
Sample preparation: Condition a 55 × 5 100-200 mesh AG 50W-X8 (H^+) column (Bio-Rad) with 10 mL MeCN:water 50:50. 600 μL Serum + 600 μL MeCN, vortex for 1 min, centrifuge at 2000 g for 5 min, add a 1 mL aliquot of the supernatant to the column, discard the first 200 μL effluent, collect the rest of the effluent. Remove a 450 μL aliquot and add it to 50 μL 10% sodium carbonate solution, heat at 60° for 1 h (to hydrolyse the β-lactam ring), cool in an ice bath. Remove a 100 μL aliquot and add it to 15 μL 200 mM pH 6.0 phosphate buffer, add 35 μL 80 mM 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole in MeCN, heat at 60° for 10 min, cool in an ice bath, add 30 μL 1 M HCl, inject a 5-10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 ODS-80TM (Tosoh)
Mobile phase: MeOH:100 mM pH 3.0 phosphate buffer 40:60
Flow rate: 1
Injection volume: 5-10
Detector: F ex 470 em 530

CHROMATOGRAM
Retention time: 12
Limit of detection: 85 ng/mL

OTHER SUBSTANCES
Extracted: penicillin G, piperacillin

KEY WORDS
derivatization; serum; SPE

REFERENCE
Iwaki,K.; Okumura,N.; Yamazaki,M.; Nimura,N.; Kinoshita,T. Precolumn derivatization technique for high-performance liquid chromatographic determination of penicillins with fluorescence detection, *J.Chromatogr.*, **1990**, *504*, 359–367.

SAMPLE

Matrix: fermentation solutions

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 μL reagent, heat at 50° for 50 min, cool in an ice bath, inject a 20 μL aliquot. (Prepare reagent by dissolving 4.125 g imidazole in 2.5 mL water, add 1 mL HCl, add 500 μL 110 mM mercury(II) chloride, add 1.5 mL HCl. Recrystallize imidazole twice from isopropanol.)

HPLC VARIABLES

Guard column: 10 × 4 5 μm Spherisorb C18

Column: 20 × 4.6 5 μm 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 NaH_2PO_4 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: 12

Limit of detection: 1 μg/mL

OTHER SUBSTANCES

Extracted: penicillin G, penicillin V, 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: Blend tablets and capsules with water in a high-speed blender for 5 min, filter, dilute with mobile phase, inject a 20 μL aliquot. Dilute oral suspensions and injections with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 70 mm long Co:Pell ODS

Column: 300 × 4.6 10 μm Chromegabond C18 (E.S. Industries)

Mobile phase: MeCN:MeOH:10 mM KH_2PO_4 19:11:70

Flow rate: 1

Injection volume: 20

Detector: UV 225

CHROMATOGRAM

Retention time: 5.2

Limit of detection: 629 ng/mL

OTHER SUBSTANCES

Simultaneous: amoxicillin, ampicillin, cloxacillin, dicloxacillin, 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: 50 g Milk + 2 drops penicillinase (Difco Laboratories), let stand 1 h at 37°, add 50 MeCN, shake vigorously for 1 min, centrifuge at 9000 g for 10 min, decant, add 5 g NaCl, swirl to dissolve, add 100 mL dichloromethane, shake for 1 min, centrifuge at 1000 g for 10 min. Remove top aqueous layer and extract organic layer with 25 mL 10% NaCl by shaking and centrifuging as before. Combine aqueous layers, add 1 mL 0.3% mercuric chloride in water, let stand 30 min, add 1 mL 2 M HCl, extract with three 50 mL portions of dichloromethane by shaking each portion for 1 min and centrifuging at 1000 g for 10 min, filter dichloromethane extracts through 30 g anhydrous sodium sulfate, evaporate to dryness under reduced pressure at 35°, if water remains add 5-10 mL MeOH to flask and complete evaporation. Dissolve residue in 1 mL 10% acetic acid, add 0.5 mL 0.08% dansyl hydrazine in 10% acetic acid, let stand 90 min to overnight in the dark, transfer reaction mixture to a separatory funnel with three 25 mL portions of dichloromethane, add 5 mL 2 M HCl, shake for 1 min, wash organic layer with 5 mL 5% $NaHCO_3$ solution, filter through 10-20 g anhydrous sodium sulfate. Extract acid aqueous layer again with 25 mL dichloromethane. Combine dichloromethane layers and evaporate to dryness at 35° under reduced pressure. Dissolve residue in 2 mL IS solution, inject a 20 μL aliquot. (Prepare IS solution by dissolving 10 μL benzaldehyde in 100 mL dichloromethane, evaporate 1 mL to dryness under reduced pressure, dissolve residue in 1 mL 10% acetic acid, add 0.5 mL 0.08% dansyl hydrazine in 10% acetic acid, let stand 90 min to overnight in the dark, transfer reaction mixture to a separatory funnel with three 25 mL portions of dichloromethane, add 5 mL 2 M HCl, shake for 1 min, wash organic layer with 5 mL 5% $NaHCO_3$ solution, filter through 10-20 g anhydrous sodium sulfate. Extract acid aqueous layer again with 25 mL dichloromethane. Combine dichloromethane layers and evaporate to dryness at 35° under reduced pressure. Dissolve residue in 100 mL MeCN then dilute an aliquot 1:4 with MeCN.)

HPLC VARIABLES

Column: 250 × 4 10 μm Lichrosorb RP-18
Mobile phase: MeCN:water 58:42
Flow rate: 1
Injection volume: 20
Detector: F ex 254 em 500 filter

CHROMATOGRAM

Retention time: 5.23
Internal standard: benzaldehyde (derivatized) (12.18)
Limit of detection: 5 ng/g

OTHER SUBSTANCES

Extracted: penicillin V, phenethicillin, oxacillin, cloxacillin, dicloxacillin, nafcillin
Interfering: penicillin G

KEY WORDS

derivatization

REFERENCE

Munns,R.K.; Shimoda,W.; Roybal,J.E.; Vieira,C. Multiresidue method for determination of eight neutral β-lactam penicillins in milk by fluorescence-liquid chromatography, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 968–971.

SAMPLE

Matrix: milk

Sample preparation: Add 2 volumes MeCN to milk, stand 5 min, decant aqueous portion, suction filter, extract with an equal volume of 1:1 methylene chloride:hexane, centrifuge aqueous phase at 3000 rpm for 10 min. Dilute 3:1 with 20 mM sodium acetate buffer and filter (0.2 μm nylon). Inject 50 μL onto column with mobile phase A, run mobile phase A for 30 min and elute to waste. After 30 min switch to mobile phase B and elute through detector.

HPLC VARIABLES

Column: 100 × 8 Radial-Pak 10 μm μBondapak C18

Mobile phase: A 20 mM sodium acetate buffer; B Gradient. MeCN:MeOH:20 mM sodium acetate buffer from 15:10:75 to 30:0:70 over 15 min and hold at 30:0:70
Flow rate: A 3; B 2
Injection volume: 50
Detector: E, Waters 464 pulsed electrochemical detector using a thin layer cell with a Ag/AgCl reference electrode. E1 = 1300 mV for 0.166 s, E2 = 1500 mV for 0.166 s, E3 = -200 mV for 0.333 s.

CHROMATOGRAM
Retention time: 7.2
Limit of detection: 0.3 ppm

OTHER SUBSTANCES
Simultaneous: penicillin V, ampicillin, penicillin G, oxacillin, cloxacillin, nafcillin, dicloxacillin.

REFERENCE
Kirchmann,E.; Earley,R.L.; Welch,L.E. The electrochemical detection of penicillins in milk, *J.Liq.Chromatogr.*, **1994**, *17*, 1755–1772.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of an aqueous solution.

HPLC VARIABLES
Column: 250 × 4.6 10 μm μBondapak C18
Mobile phase: Gradient. A was 1.2 mM triethylamine and 42 mM acetic acid. B was MeCN: water 24:76 containing 1.2 mM triethylamine and 42 mM acetic acid. A:B from 75:25 to 60:40 using Waters Model 660 curve select-9 over 15 min then stay at 60:40.
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 22

OTHER SUBSTANCES
Simultaneous: ampicillin, cefoperazone
Noninterfering: gentamicin, kanamycin, tobramycin
Interfering: penicillin G

REFERENCE
Dokladalova,J.; Quercia,G.T.; Stankewich,J.P. High-performance liquid chromatographic determination of cefoperazone in human serum and urine, *J.Chromatogr.*, **1983**, *276*, 129–137.

SAMPLE
Matrix: 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: 13

OTHER SUBSTANCES
Simultaneous: dicloxacillin, penicillin G, penicillin V, cloxacillin, nafcillin, carbenicillin

REFERENCE
Moats,W.A. Effect of the silica support of bonded reversed-phase columns on chromatography of some antibiotic compounds, *J.Chromatogr.*, **1986**, *366*, 69–78.

SAMPLE
Matrix: solutions
Sample preparation: React the antibiotic, triethylamine, and 1-(2,5-dihydroxyphenyl)-2-bromoethanone in a 1:2:4 molar ratio in DMF at 45° for 2 h (use dibenzo-18-crown-6 to make the sodium salt soluble), inject a 10 μL aliquot. (Preparation of 1-(2,5-dihydroxyphenyl)-2-bromoethanone is as follows. Stir 27.6 g 1,4-dimethoxybenzene and 28 mL bromoacetyl bromide at 0°, add 53.4 g aluminum bromide over 10 min (an exothermic reactions ensues), let stand at room temperature for 12 h, add 100 mL 48% HBr, add 100 g ice, stir for 1 h, extract twice with 200 mL portions of diethyl ether. Combine the extracts and wash them 3 times with 200 mL portions of water, dry over 40 g anhydrous magnesium sulfate, evaporate to dryness, recrystallize the product 3 times from EtOH to yield 1-(2,5-dihydroxyphenyl)-2- bromoethanone monobromoacetate (mp 105-107°). Dissolve 11 g 1-(2,5-dihydroxyphenyl)-2-bromoethanone monobromoacetate in 200 mL warm dry MeOH saturated with HBr, stir for 18 h, add 200 mL water, cool to -10°. Collect the yellow solid and dry it under vacuum at 50° for 48 h, recrystallize from toluene:heptane 50:50 then toluene to obtain 1-(2,5-dihydroxyphenyl)-2-bromoethanone as yellow needles (mp 117-119°).)

HPLC VARIABLES
Column: 250 × 4 7 μm RP-18 LiChrocart (Merck)
Mobile phase: MeOH:100 mM pH 6.5 sodium acetate 58:42
Flow rate: 1
Injection volume: 10
Detector: E, Bioanalytical Systems Model LC4B, glassy carbon electrode 0.8 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 10.7

OTHER SUBSTANCES
Extracted: carbenicillin, cephapirin, cloxacillin, dicloxacillin, hetacillin, nafcillin, oxacillin, penicillin G

KEY WORDS
derivatization

REFERENCE
Munns,R.K.; Roybal,J.E.; Shimoda,W.; Hurlbut,J.A. 1-(4-Hydroxyphenyl)-, 1-(2,4-dihydroxyphenyl)- and 1-(2,5-dihydroxyphenyl)-2-bromoethanones: new labels for determination of carboxylic acids by high-performance liquid chromatography with electrochemical and ultraviolet detection, *J.Chromatogr.*, **1988**, *442*, 209–218.

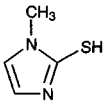
SAMPLE
Matrix: solutions
Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate 22:78
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 220

OTHER SUBSTANCES
Also analyzed: penicillin G, cefoperazone, cephalothin

REFERENCE
Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.

Methimazole



Molecular formula: $C_4H_6N_2S$
Molecular weight: 114.17
CAS Registry No.: 60-56-0
Merck Index: 6049
Lednicer No.: 1 240

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 4 mL buffer + 200 μL 100 mg/mL 2,6-dichloroquinone-4-chloroimide in MeOH, vortex for 1 min, let stand at room temperature for 5 min, add 2 mL chloroform, vortex for 5 min, centrifuge at 1000 g for 10 min, filter (Whatman 1PS phase-separating paper) the lower organic layer, evaporate the filtrate to dryness, reconstitute with 50 μL chloroform, inject a 20 μL aliquot. (Prepare buffer by dissolving 3.1 g boric acid, 3.75 g KCl, and 100 g NaCl in water, add 40 mL 100 mM NaOH, make up to 1 L with water, adjust pH to 8 with 6 M NaOH.)

HPLC VARIABLES
Column: 300 × 3.9 μBondapak-NH2
Mobile phase: chloroform
Flow rate: 1.5
Injection volume: 20
Detector: UV 405

CHROMATOGRAM
Retention time: 5
Limit of detection: 5 ng/mL

KEY WORDS
derivatization; plasma; pharmacokinetics

REFERENCE
Meulemans,A.; Manuel,C.; Ferriere,C.; Vulpillat,M. Determination of methimazole in plasma by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1980**, *3*, 287–298.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 300 μL 160 ng/mL p-hydroxyanisole in MeOH, vortex for 1 min, let stand for 30 min at room temperature, centrifuge at 10000 g for 10 min, inject a 10-20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 8 10 μm radial Pak C18 (Waters)
Mobile phase: MeOH:10 mM ammonium phosphate containing 1 mM disodium EDTA 8:92, pH adjusted to 4.00 with phosphoric acid
Flow rate: 3
Injection volume: 10-20
Detector: E, Bioanalytical Systems, glassy carbon working electrode +0.70 V, Ag/AgCl reference electrode, detector temp 20

CHROMATOGRAM
Retention time: 4

Internal standard: p-hydroxyanisole (28)
Limit of detection: 10 ng/mL

KEY WORDS
serum; pharmacokinetics

REFERENCE
Tatsuhara,T.; Tabuchi,F.; Unate,M.; Okamura,Y.; Shigemasa,C.; Abe,K.; Mashiba,H. Determination of thiamazole in serum by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1985**, *339*, 149–156.

SAMPLE
Matrix: blood
Sample preparation: 500 µL Plasma + 100 mg disodium EDTA + 3 mL ethyl acetate, vortex for 3 min, centrifuge at 5000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 200 µL MeOH, inject a 10 µL aliquot.

HPLC VARIABLES
Guard column: 25 × 3 LiChroprep RP-18 (Merck)
Column: 250 × 4 10 µm LiChrosorb RP-18
Mobile phase: Gradient. MeOH:25 mM pH 3 phosphate buffer from 10:90 to 70:30 over 30 min
Flow rate: 1
Injection volume: 10
Detector: UV 258

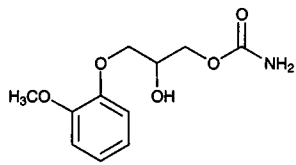
CHROMATOGRAM
Retention time: 6.95
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Extracted: methylthiouracil (UV 276), propylthiouracil (UV 276), phenylthiouracil (UV 276), thiouracil (UV 276)

KEY WORDS
cow; plasma

REFERENCE
Moretti,G.; Betto,P.; Cammarata,P.; Fracassi,F.; Giambenedetti,M.; Borghese,A. Determination of thyreostatic residues in cattle plasma by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1993**, *616*, 291–296.

Methocarbamol



Molecular formula: $C_{11}H_{15}NO_5$
Molecular weight: 241.24
CAS Registry No.: 532-03-6
Merck Index: 6060
Lednicer No.: 1 118

SAMPLE
Matrix: blood
Sample preparation: 200 µL Plasma + 100 µL 200 µg/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 µL of water, inject a 50 µL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 3 μm Microsorb C18
Mobile phase: MeOH:100 mM KH_2PO_4:water 35:10:55
Column temperature: 40
Flow rate: 0.8
Injection volume: 50
Detector: UV 272

CHROMATOGRAM
Retention time: 5.2
Internal standard: mephenesin (10.5)

OTHER SUBSTANCES
Simultaneous: guaifenesin
Noninterfering: acetaminophen, ibuprofen

KEY WORDS
plasma

REFERENCE
Weng,N.; 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: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 223

CHROMATOGRAM
Retention time: 3.01
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; almino-

profen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Serum. 2 mL Serum + 20 μg IS, adjust pH to 14 with 2 M NaOH, extract twice with 6 mL diethyl ether. Combine the organic layers, dry under nitrogen, dissolve the residue in 200 μL MeOH. Inject a 20 μL aliquot. Urine. 5 mL Urine + 75-1000 μg IS, adjust pH to 5 with 1 mL 2 M pH 5 sodium acetate buffer. Add 100 μL β-glucuronidase (Helix pomatia 98400 U/mL), incubate at 37° overnight, cool, wash with 8 mL diethyl ether, discard the organic layer. Adjust the aqueous layer to pH 14 with 25% NaOH, extract twice with 8 mL diethyl ether. Combine the organic layers, dry under nitrogen, reconstitute the residue in 200 μL MeOH. Inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 7 μm LiChrospher 100 RP-18
Column: 250 × 4 7 μm LiChrospher 100 RP-18
Mobile phase: MeCN:1% acetic acid 20:80
Flow rate: 1
Injection volume: 20
Detector: UV 280; MS, API III LC-MS-MS (PE Sciex, Canada), triple quadrupole, nebulizer 400° and 75 psi, auxiliary nitrogen gas, nitrogen-curtain gas, positive mode ionization, CID technique, collision gas argon, m/z 242

CHROMATOGRAM

Retention time: 11.8
Internal standard: mephenesin (19)
Limit of detection: 700 ng/mL (urine), 200 ng/mL (serum)
Limit of quantitation: 1.09 μg/mL (urine), 550 ng/mL (serum)

OTHER SUBSTANCES

Extracted: degradation products

KEY WORDS

horse; serum; pharmacokinetics

REFERENCE

Koupai-Abyazani,M.R.; Esaw,B.; Laviolette,B. Determination of methocarbamol in equine serum and urine by high-performance liquid chromatography with ultraviolet detection and atmospheric pressure ionization-mass spectrometric confirmation, *J.Anal.Toxicol.*, **1997**, *21*, 301–305.

SAMPLE
Matrix: blood, urine
Sample preparation: Dilute urine 1:100. Plasma or diluted urine + 10 μg/mL (R)-(-)-flecainide acetate in water + 1 mL 1 M NaOH + 5 mL ethyl acetate, shake mechanically for 20 min, centrifuge at 1000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 1 mL distilled ethyl acetate, add 80 μL 0.2% (S)-(+)-1-(1-naphthyl)ethyl isocyanate in ethyl acetate, vortex for 5 s, heat at 85° for 12 h, evaporate, reconstitute in 200 μL ethyl acetate, inject a 30-150 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Partisil 5 silica
Mobile phase: Hexane:isopropanol 95:5
Flow rate: 1.6
Injection volume: 30-150
Detector: UV 280

CHROMATOGRAM
Retention time: 33 (S), 41 (R)
Internal standard: (R)-(-)-flecainide (12)
Limit of detection: 10 ng/mL
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Simultaneous: guaifenesin

KEY WORDS
rat; human; plasma; normal phase; chiral; derivatization; pharmacokinetics

REFERENCE
Alessi-Severini,S.; Coutts,R.T.; Jamali,F.; Pasutto,F.M. High-performance liquid chromatographic analysis of methocarbamol enantiomers in biological fluids, *J.Chromatogr.*, **1992**, *582*, 173–179.

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 218, 269

CHROMATOGRAM
Retention time: 2.0
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, 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, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions
Sample preparation: 450 μL Buffer solution + 50 μL 4 mg/mL acetanilide, cool in ice, inject a 10 μL aliquot.

HPLC VARIABLES
Column: Perkin-Elmer 3 × 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: 4.7
Internal standard: acetanilide (2.1)

OTHER SUBSTANCES
Simultaneous: guaifenesin, degradation products

KEY WORDS
buffers; stability indicating

REFERENCE
Pouli,N.; Antoniadou-Vyzas,A.; Foscolos,G.B. Methocarbamol degradation in aqueous solution, *J.Pharm.Sci.*, **1994**, *83*, 499–501.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Chirex 3020 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 60:35:5 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1
Injection volume: 20
Detector: UV 274

KEY WORDS
chiral; α = 1.09 for enantiomers

REFERENCE
Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

SAMPLE
Matrix: solutions

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: 5.02 (A), 3.94 (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, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

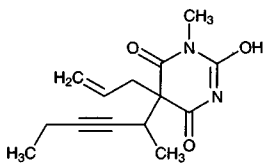
KEY WORDS

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.

Methohexital



Molecular formula: $C_{14}H_{18}N_2O_3$
Molecular weight: 262.31
CAS Registry No.: 18652-93-2, 151-83-7, 309-36-4 (Na salt)
Merck Index: 6061
Lednicer No.: 1 269

SAMPLE

Matrix: blood
Sample preparation: Mix 250 µL plasma with 400 µL MeCN, vortex, centrifuge. Inject a 20 µL aliquot of the clear supernatant.

HPLC VARIABLES

Column: C18 reverse phase
Mobile phase: MeOH:0.5% tetrabutylammonium phosphate in water 50:50
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Limit of detection: 100 ng/mL

KEY WORDS
sheep; plasma; methohexital is IS

REFERENCE
Upton,R.N.; Huang,Y.F.; Grant,C.; Gray,E.C.; Ludbrook,G.L. Myocardial pharmacokinetics of thiopental in sheep after short-term administration: Relationship to thiopental-induced reductions in myocardial contractility, *J.Pharm.Sci.*, **1996**, *85*, 863–867.

SAMPLE
Matrix: blood
Sample preparation: Buffer serum to pH 5.6 with 100 mM acetate buffer, add thiopental, extract with hexane. Remove the hexane and extract it with 250 mM NaOH. Neutralize the aqueous layer with phosphoric acid and inject an aliquot.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:0.1 mM pH 4.2 phosphate buffer 43:57
Flow rate: 1
Detector: UV 195

CHROMATOGRAM
Internal standard: thiopental
Limit of detection: 10 ng/mL

KEY WORDS
serum; pharmacokinetics

REFERENCE
Hudson,R.J.; Stanski,D.R.; Burch,P.G. Pharmacokinetics of methohexital and thiopental in surgical patients, *Anesthesiology*, **1983**, *59*, 215–219.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma or hemolyzed (frozen and thawed) whole blood + 500 μL 2 μg/mL hexobarbital in water + 500 μL 250 mM HCl + 40 mg NaCl + 3 mL toluene, rotate, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of air at 50 ± 5°, reconstitute the residue in 200 μL MeCN and 200 μL 50 mM NaH_2PO_4, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4 Perisorb RP-18 (Merck)
Column: 250 × 4 7 μm LiChroCart RP-18 (Merck)
Mobile phase: MeCN:50 mM pH 4.6 NaH_2PO_4 50:50
Flow rate: 1
Injection volume: 20
Detector: UV 195

CHROMATOGRAM
Retention time: 7.3
Internal standard: hexobarbital (4.8)
Limit of quantitation: 31 ng/mL

OTHER SUBSTANCES
Extracted: thiopental
Simultaneous: barbital, caffeine, indomethacin, pentobarbital, phenobarbital
Noninterfering: aspirin, salicylic acid

KEY WORDS
plasma; whole blood; pharmacokinetics

REFERENCE
Bjorkman,S.; Idvall,J. A high-performance liquid chromatographic method for methohexital and thiopental in plasma or whole blood, *J.Chromatogr.*, **1984**, *307*, 481–487.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond-Elut C8 SPE cartridge with 2 volumes of MeOH, 2 volumes of water, and 1 volume of 100 mM pH 5.59 Sörensen's phosphate buffer. 500 μL Plasma + 10 μL 1 mg/mL sodium secobarbital in EtOH, add to the SPE cartridge, wash with 2 volumes of 100 mM pH 5.59 Sörensen's phosphate buffer, wash with 1 volume of water, elute with 500 μL MeOH. Evaporate the eluate to dryness under vacuum, reconstitute in 50 μL MeOH, inject an aliquot.

HPLC VARIABLES
Guard column: 10 μm Guard-Pak C18 (Waters)
Column: 100 × 8 10 μm Radial-Pak C8 (Waters)
Mobile phase: MeOH:THF:100 mM pH 7.72 Sörensen's phosphate buffer 28:16:52
Flow rate: 2.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 7.98
Internal standard: secobarbital (6.39)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Extracted: pentobarbital, thiopental
Noninterfering: ketamine

KEY WORDS
plasma; dog; pharmacokinetics; SPE

REFERENCE
Avram,M.J.; Krejcie,T.C. Determination of sodium pentobarbital and either sodium methohexital or sodium thiopental in plasma by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1987**, *414*, 484–491.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a solution in EtOH.

HPLC VARIABLES
Column: 250 × 4.6 Hypersil
Mobile phase: Isooctane:acetic acid:isopropanol 100:1.5:1
Flow rate: 2
Injection volume: 20
Detector: UV 250

CHROMATOGRAM
Retention time: k' 1.48

OTHER SUBSTANCES
Simultaneous: allobarbital, amobarbital, aprobarbital, barbital, brallobarbital, butabarbital, butalbital, butobarbital, cyclobarbital, cyclopentobarbital, enallylpropymal, heptabarbital, hexethal, hexobarbital, ibomal, idobutal, metharbital, methylphenobarbital, nealbarbital, pentobarbital, phenobarbital, phenylmethylbarbituric acid, probarbital, secobarbital, sigmodal, talbutal, vinbarbital

KEY WORDS
normal phase

REFERENCE
Gill,R.; Stead,A.H.; Moffat,A.C. Identification of drugs by the effective combination of gas-liquid, high-performance liquid and thin-layer chromatographic techniques, *J.Chromatogr.*, **1981**, *204*, 275–284.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeCN:water 25:75, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm C18 (Alltech)
Mobile phase: MeCN:4 mM potassium phosphate buffer 50:50, pH 4.0
Flow rate: 1.2
Injection volume: 100
Detector: UV 290

CHROMATOGRAM
Retention time: 6.4

OTHER SUBSTANCES
Simultaneous: amobarbital, heptabarbital, hexobarbital, pentobarbital, phenobarbital, secobarbital, thiamylal, thiopental

REFERENCE
Ebling,W.F.; Mills-Williams,L.; Harapat,S.R.; Stanski,D.R. High-performance liquid chromatographic method for determining thiopental concentrations in twelve rat tissues: application to physiologic modeling of disposition of barbiturate, *J.Chromatogr.*, **1989**, *490*, 339–353.

SAMPLE
Matrix: solutions

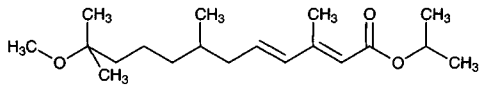
HPLC VARIABLES
Column: 250 × 4 OmniPac PAX-500 (Dionex)
Mobile phase: Gradient. A was MeCN:5 mM sodium carbonate 9:81. B was MeCN:20 mM sodium carbonate 20:80. A:B from 100:0 to 0:100 over 10 min.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 11.5

OTHER SUBSTANCES
Simultaneous: allobarbital, amobarbital, barbital, barbituric acid, butabarbital, mephobarbital, methabarbital, phenobarbital, phenytoin, secobarbital, thiamylal

REFERENCE
Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, *13*, 107–134.

Methoprene



Molecular formula: $C_{19}H_{34}O_3$
Molecular weight: 310.48
CAS Registry No.: 40596-69-8
Merck Index: 6063

SAMPLE
Matrix: solutions
Sample preparation: 5 mL Water + 100 µL 22.7 µg/mL dipentyl phthalate in MeCN + 3 mL MTBE, vortex for 1 min, centrifuge at 2500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 100 µL mobile phase, vortex for 30 s, inject a 20 µL aliquot.

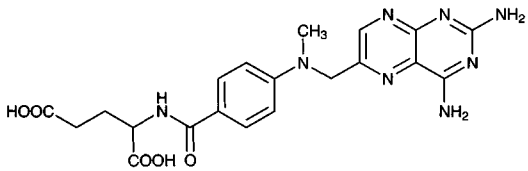
HPLC VARIABLES
Column: 70 × 4.6 3 µm Ultrasphere XL-ODS
Mobile phase: MeCN:water 90:10
Flow rate: 1
Injection volume: 20
Detector: UV 255

CHROMATOGRAM
Retention time: 6.5
Internal standard: dipentyl phthalate (4)
Limit of detection: 2.5 ng/mL
Limit of quantitation: 5 ng/mL

KEY WORDS
water; groundwater

REFERENCE
Allen,C.R.; Dickinson,C.M. Determination of methoprene in water samples by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1990**, *13*, 371–381.

Methotrexate



Molecular formula: $C_{20}H_{27}N_8O_5$
Molecular weight: 454.45
CAS Registry No.: 59-05-2
Merck Index: 6065

SAMPLE
Matrix: blood
Sample preparation: Mix 20 µL serum with 20 µL 2 M perchloric acid, vortex for a few seconds, centrifuge at 10000 g for 2 min. Inject a 20 µL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 µm Inertsil ODS-2 (GL Sciences, Japan)
Mobile phase: MeCN:25 mM pH 7.0 sodium phosphate buffer 8:92 containing 25 mM hydrogen peroxide
Flow rate: 1
Injection volume: 20
Detector: F ex 379 em 457 following post-column reaction. The column effluent flowed through a 5 m × 0.5 mm i.d. stainless-steel reaction coil at 160° and a 3 m × 0.5 mm i.d. stainless-steel coil at 15° to the detector.

CHROMATOGRAM
Retention time: 10
Limit of detection: 20 nM

OTHER SUBSTANCES
Extracted: metabolites, leucovorin

KEY WORDS
serum; post-column reaction; comparison with immunoassay

REFERENCE
Kubo,H.; Umiguchi,Y.; Fukumoto,M.; Kinoshita,T. Fluorometric determination of methotrexate in serum by high-performance liquid chromatography using in-line oxidation with hydrogen peroxide, *Anal.Sci.*, **1992**, *8*, 789–792.

SAMPLE
Matrix: blood
Sample preparation: Condition a C18 Sep-Pak Classic SPE cartridge with 20 mL MeOH and 3 mL 200 mM pH 6 phosphate buffer. Gently vortex a 200-500 μL aliquot of serum with 1 ml 4 μg/mL IS in 200 mM pH 6 phosphate buffer. Add to the SPE cartridge, wash with 1 mL water, dry under vacuum for 5 min, elute with 2 mL MeOH. Dry the eluate under nitrogen at 60°. Reconstitute the residue in 200-300 μL 5 mM HCl, inject a 100μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Beckmann Ultrasphere ODS
Mobile phase: MeCN:buffer 10:90 (Buffer was 10 mM pH 2.5 KH_2PO_4 containing 20 mM tetramethylammonium chloride.)
Flow rate: 1
Injection volume: 100
Detector: UV 313

CHROMATOGRAM
Retention time: 12.71
Internal standard: 8-chlorotheophylline (8.42)
Limit of detection: 20 nM
Limit of quantitation: 30 nM

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; SPE

REFERENCE
Aboleneen,H.; Simpson,J.; Backes,D. Determination of methotrexate in serum by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *681*, 317–322.

SAMPLE
Matrix: blood
Sample preparation: Inject 100 μL plasma onto column A and elute to waste with mobile phase A, after 4 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.

HPLC VARIABLES
Column: A 25 × 4 25 μm C8-alkyl-diol silica (ADS); B 125 × 4 5 μm LiChrospher RP-18
Mobile phase: A MeCN:pH 7.4 phosphate buffer 2:98 containing 2 mM tetrabutylammonium hydrogen sulfate; B MeCN:pH 7.4 phosphate buffer 18:82 containing 5 mM tetrabutylammonium hydrogen sulfate
Column temperature: 4
Flow rate: 1

Injection volume: 100
Detector: UV 307

CHROMATOGRAM
Retention time: 9.5-10
Limit of detection: 10 ng/mL

KEY WORDS
column-switching; plasma

REFERENCE
Yu,Z.; Westerlund,D. Ion-pair chromatography of methotrexate in a column-switching system using an alkyl-diol silica precolumn for direct injection of plasma, *J.Chromatogr.A*, **1996**, *742*, 113–120.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 500 μL 2.5 μg/mL sulfafurazole in 10% trichloroacetic acid in 100 mM HCl, vortex for 20 s, centrifuge at 8300 g for 5 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4 5 μm 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 313

CHROMATOGRAM
Retention time: 5
Internal standard: sulfafurazole (UV 254) (23)
Limit of detection: 40 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

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: Add 2 mg ascorbic acid to each 1 mL blood, centrifuge at 800 g in the cold. 1 mL Plasma + 1.5 mL MeOH, vortex, centrifuge at 800 g. Remove the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL water, inject the whole amount.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: Gradient. A was 250 mM pH 5.0 phosphate buffer. B was MeOH:250 mM pH 5.0 phosphate buffer 1:1. A:B 100:0 for 15 min, to 50:50 over 15 min, to 0:100 over 5 min, maintain at 0:100 for 5 min.
Flow rate: 2
Injection volume: 200
Detector: UV 310

CHROMATOGRAM
Retention time: 29

Internal standard: methotrexate

OTHER SUBSTANCES
Extracted: leucovorin

KEY WORDS
plasma; methotrexate is IS

REFERENCE
Wainer,I.W.; Stiffin,R.M. Direct resolution of the stereoisomers of leucovorin and 5-methyltetrahydrofolate using a bovine serum albumin high-performance liquid chromatographic chiral stationary phase coupled to an achiral phenyl column, *J.Chromatogr.*, **1988**, *424*, 158–162.

SAMPLE
Matrix: blood
Sample preparation: 1 Volume plasma + 2 volumes MeCN, mix thoroughly, centrifuge at 2000 g for 3 min. Remove the supernatant and add it to 4 volumes chloroform, vortex briefly, inject a 30 μL aliquot of the aqueous supernatant.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100RP8
Mobile phase: MeCN:25 mM pH 3.9 sodium acetate buffer 12:88
Flow rate: 1.5
Injection volume: 30
Detector: UV 307

CHROMATOGRAM
Retention time: 7
Limit of detection: 12 ng/mL
Limit of quantitation: 40 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Cociglio,M.; Hillaire-Buys,D.; Alric,C. Determination of methotrexate and 7-hydroxymethotrexate by liquid chromatography for routine monitoring of plasma levels, *J.Chromatogr.B*, **1995**, *674*, 101–110.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond-Elut SPE cartridge with 1 mL MeOH and 1.5 mL 50 mM pH 2.7 phosphate buffer. 1 mL Plasma + 1 mL 50 mM pH 6.5 phosphate buffer, mix, add to the SPE cartridge at 2 mL/min, wash with 2 mL 50 mM pH 2.7 phosphate buffer, wash with 1 mL 100 mM NaOH, wash with 1 mL 50 mM pH 2.7 phosphate buffer, elute with 1.5 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 3.2 30 μm Spherisorb C18
Column: 150 × 3.9 5 μm Novapak C18
Mobile phase: DMF:MeCN:3% hydrogen peroxide:14 mM pH 6.5 phosphate buffer 4:3.3:0.5:92.2
Flow rate: 1
Injection volume: 100
Detector: F ex 350 em 465, following post-column photolytic oxidation in a 3 m long × 0.38 mm i.d. length of polyethylene (PE-20) tubing with a Spectroline pencil UV lamp (UV 254 nm)

CHROMATOGRAM
Retention time: 4

Limit of detection: 0.05 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; SPE; human; dog; pharmacokinetics; post-column photochemical derivatization; post-column reaction

REFERENCE
Lu,G.; Jun,H.W. Determination of trace methotrexate and 7-OH-methotrexate in plasma by high-performance liquid chromatography with fluorimetric detection, *J.Liq.Chromatogr.*, **1995**, *18*, 155–171.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 301

CHROMATOGRAM
Retention time: 3.06
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen;

dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood

Sample preparation: Condition an Isolute HAX 200 mg SPE cartridge (International Sorbent Technology) with 3 mL MeOH and 3 mL 100 mM phosphoric acid. Dilute 0.01-1 mL plasma with 2 mL 100 mM phosphoric acid, add to the SPE cartridge at 2.5 mL/min, wash with 2 mL MeOH:100 mM phosphoric acid 5:95, wash with 3 mL pH 8.6 Na_2HPO_4, wash with MeOH: water 5:95, air dry for 2 min, elute with 2 mL 20 g/L trifluoroacetic acid in MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL 100 mM phosphoric acid, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 80 × 4.6 3 μm C18 (Perkin-Elmer)
Mobile phase: MeCN:100 mM pH 6.5 phosphate buffer:30% hydrogen peroxide 6.5:93.3:0.2
Column temperature: 22
Flow rate: 1
Injection volume: 20
Detector: F ex 350 em 435 following post-column reaction. The column effluent flowed through a 10 m × 0.3 mm coil irradiated with a 254 nm UV lamp at 37° and flowed to the detector.

CHROMATOGRAM

Limit of quantitation: 0.1 nM

KEY WORDS

post-column photochemical derivatization; SPE; plasma; comparison with immunoassays; post-column reaction

REFERENCE

Albertioni,F.; Rask,C.; Eksborg,E.; Poulsen,J.H.; Pettersson,B.; Beck,O.; Schroeder,H.; Peterson,C. Evaluation of clinical assays for measuring high-dose methotrexate in plasma, *Clin.Chem.*, **1996**, *42*, 39–44.

SAMPLE

Matrix: blood

Sample preparation: 500 μL Plasma + 500 μL acetone, vortex vigorously for 1 min, centrifuge at 3000 rpm for 4 min. Remove a 600 μL aliquot of the supernatant and add it to 600 μL butanol and 800 μL diethyl ether, mix vigorously, centrifuge at 2000 rpm for 2 min. Remove a 300 μL aliquot of the lower aqueous layer and evaporate it to dryness under reduced pressure, reconstitute the residue with 150 μL 0.9% NaCl in water, filter (0.45 μm), inject a 50 μL aliquot onto column A and column B in series and elute with mobile phase A, after 5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. (Methotrexate is oxidized on column A to the fluorescent compounds 2,4-diaminopteridine-6-carboxylic acid and 2,4-diaminopteridine-6-carboxaldehyde.)

HPLC VARIABLES

Column: A 70 × 4.5 cerium(IV) trihydroxyperoxide; B 250 × 4.6 5 μm ODS/TM silica (Preparation of cerium(IV) trihydroxyperoxide is as follows. Add 15 mL 35% ammonia solution with stirring to a cooled solution of 11.18 g cerium(III) chloride heptahydrate in 100 mL water, add 20 mL 30% hydrogen peroxide with stirring over 15 min, collect the orange-red precipitate by filtration, wash the solid several times with water, wash with acetone, dry under vacuum at

room temperature overnight to obtain cerium(IV) trihydroxyperoxide, discard after 6 months. Suspend 1 g cerium(IV) trihydroxyperoxide in chloroform, degas under vacuum with stirring for 10 min, add the suspension to a 100 × 7 reservoir on top of the column, pump the slurry into the column using acetone as a purge solvent at 5 mL/min for 20 min, wash the column with acetone:pH 3.5 phosphate buffer 50:50 at 1 mL/min for 20 min, equilibrate with mobile phase A at 0.5 mL/min for 1 h (Analyst 1996, 121, 183).)

Mobile phase: A 40 mM pH 3.5 phosphate buffer; B MeCN:50 mM pH 6.6 phosphate buffer 10:90

Flow rate: A 0.2; B 1

Injection volume: 50

Detector: F ex 367 em 463

CHROMATOGRAM

Retention time: 5 (2,4-diaminopteridine-6-carboxylic acid), 6 (2,4-diaminopteridine-6-carboxaldehyde)

Limit of detection: 2.78 ng/mL

KEY WORDS

derivatization; plasma; column-switching

REFERENCE

Emara,S.; Razee,S.; Khedr,A.; Masujima,T. On-line precolumn derivatization for HPLC determination of methotrexate using a column packed oxidant, *Biomed.Chromatogr.*, **1997**, *11*, 42–46.

SAMPLE

Matrix: blood, CSF

Sample preparation: 250 μL Plasma or CSF + 25 μL 10 mg/mL ascorbic acid + 250 μL ice-cold 1.5 M perchloric acid, vortex, let stand in ice-water for 5 min, centrifuge at 4° at 3000 g for 5 min. Remove 350 μL of the supernatant with 50 μL 8 M potassium acetate, keep in ice-water for 2 min, centrifuge at 4° at 3000 g for 2 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 200 × 3 5 μm Hypersil ODS glass column

Mobile phase: Gradient. A was 10 mM ammonium formate adjusted to pH 3.5 with HCl. B was MeCN:10 mM ammonium formate 25:75 adjusted to pH 3.5 with HCl. A:B from 85:15 to 5:95 over 21 min, maintain at 5:95 for 1 min, re-equilibrate at initial conditions for 11 min.

Flow rate: 0.4

Injection volume: 100

Detector: UV 305

CHROMATOGRAM

Retention time: 20

Limit of detection: 50 nM

OTHER SUBSTANCES

Extracted: leucovorin, N^5-methyltetrahydrofolate

KEY WORDS

plasma

REFERENCE

van Tellingen,O.; van der Woude,H.R.; Beijnen,J.H.; van Beers,C.J.T.; Nooyen,W.J. Stable and sensitive method for the simultaneous determination of N^5-methyltetrahydrofolate, leucovorin, methotrexate and 7-hydroxymethotrexate in biological fluids, *J.Chromatogr.*, **1989**, *488*, 379–388.

SAMPLE

Matrix: blood, saliva, urine

Sample preparation: Condition a 3 mL Isolute C8 SPE cartridge (International Sorbent Technology) with 3 mL MeOH and 3 mL 100 mM phosphoric acid at about 3 mL/min, do not allow to dry. 1 mL Plasma, 10 μL urine, or 100 μL saliva + 2 mL 100 mM phosphoric acid, wash with 2 mL MeOH:water 5:95, dry with air, elute with 2 mL MeOH:trifluoroacetic acid 98:2.

Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL water, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 80 × 4.6 3 μm C18 (Perkin-Elmer)
Mobile phase: MeCN:10 mM pH 6.5 phosphate buffer:30% hydrogen peroxide 6:93.8:0.2
Flow rate: 1
Injection volume: 10
Detector: F ex 350 em 435 following post-column photochemical reaction. The column effluent flowed through a 10 m × 0.3 mm i.d. reaction coil, illuminated with a UV 254 lamp at 37°, and then to the detector.

CHROMATOGRAM
Retention time: 10
Limit of detection: 10 nM (urine), 0.1 nM (plasma, saliva)

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: acetaminophen, allopurinol, amoxicillin, ara-C, aspirin, azathioprine, chloroquine, ciprofloxacin, F-ara-A, ibuprofen, 6-mercaptopurine, naproxen, sulfamethoxazole, trimethoprim

KEY WORDS
post-column photochemical derivatization; plasma; pharmacokinetics; SPE; post-column reaction

REFERENCE
Albertioni,F.; Pettersson,B.; Beck,O.; Rask,C.; Seideman,P.; Peterson,C. Simultaneous quantitation of methotrexate and its two main metabolites in biological fluids by a novel solid-phase extraction procedure using high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *665*, 163–170.

SAMPLE
Matrix: blood, urine
Sample preparation: Centrifuge heparinized blood at 16000 g for 15 min, inject a 10-80 μL aliquot. Inject a 10 μL aliquot of urine.

HPLC VARIABLES
Guard column: 40 μm LC-18 (Supelco)
Column: 250 × 4.6 10 μm LiChrosorb RP-18
Mobile phase: MeCN:DMF:50 mM pH 6.2 phosphate buffer:30% hydrogen peroxide 7:5.6:100:0.15 (Prepare and sonicate 1 day before use. Degas with helium during use.)
Column temperature: 45
Flow rate: 1
Injection volume: 10-80
Detector: F 370 em 417 (cut-off filter following post-column reaction. The column effluent flowed through a 1.59 m × 0.25 mm ID PTFE coil irradiated by a Sylvania G8T5 germicidal lamp at 254 nm to the detector.

CHROMATOGRAM
Retention time: 4
Limit of detection: 0.4 ng

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
post-column reaction; post-column photochemical derivatization; whole blood

REFERENCE
Salamoun,J.; Smrz,M.; Kiss,F.; Salamounova,A. Column liquid chromatography of methotrexate and its metabolites using a post column photochemical reactor and fluorescence detection, *J.Chromatogr.*, **1987**, *419*, 213–223.

SAMPLE
Matrix: blood, urine
Sample preparation: Mix plasma or urine with 2.5 volumes of MeCN, vortex, centrifuge, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 30 × 2.1 7 μm CX-300 (Applied Biosystems)
Column: 250 × 2.9 10 μm SCX (Whatman)
Mobile phase: MeCN:20 mM $(NH_4)H_2PO_4$ 30:70, adjust pH to 1.6 with phosphoric acid
Flow rate: 2
Injection volume: 50
Detector: UV 313

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Park,J.M.; Ahn,B.-N.; Yoon,E.J.; Lee,M.G.; Shim,C.-K.; Kim,C.-K. The pharmacokinetics of methotrexate after intravenous administration of methotrexate-loaded proliposomes to rats, *Biopharm.Drug Dispos.*, **1994**, *15*, 391–407.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4.6 5 μm C18
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 1.2
Injection volume: 20
Detector: UV 300

CHROMATOGRAM
Retention time: 2.73

OTHER SUBSTANCES
Simultaneous: granisetron

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10 μL aliquot of an aqueous solution.

HPLC VARIABLES
Guard column: 5 μm Lichrospher 60 RP-select B
Column: 125 × 3 3 μm Hypersil BDS
Mobile phase: Gradient. A was MeCN. B was 5 mM monobasic potassium phosphate adjusted to pH 2.3 with phosphoric acid. A:B 7:93 for 5 min, to 13:87 over 15 min, to 21:79 over 6 min, maintain at 21:79 for 1 min, back to 7:93 over 2 min, re-equilibrate at initial conditions for 5 min.
Flow rate: 0.5
Injection volume: 10
Detector: UV 310

CHROMATOGRAM
Retention time: 21

OTHER SUBSTANCES
Simultaneous: leucovorin

REFERENCE
Mandl,A.; Lindner,W. Improved detection of leucovorin in mixed folates and antifolates by reversed-phase liquid chromatography and on-line post-column UV irradiation, *Chromatographia*, **1996**, *43*, 327–330.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a saline solution.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:buffer 35:65, pH 3.0 (Buffer was 4.88 mM KH_2PO_4 containing 1.623 mM phosphoric acid.)
Flow rate: 1
Detector: UV 303

CHROMATOGRAM
Retention time: 5
Limit of detection: 5 pmole

KEY WORDS
saline

REFERENCE
Chatelut,E.; Suh,P.; Kim,S. Sustained-release methotrexate for intracavitary chemotherapy, *J.Pharm.Sci.*, **1994**, *83*, 429–432.

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: 4.72 (A), 2.93 (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, 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, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 5 μm Lichrospher 100 RP-18
Mobile phase: MeOH:100 mM pH 7.3 phosphate buffer 20:80 to 32:68
Flow rate: 0.8
Injection volume: 20
Detector: UV 300

REFERENCE
Smal,M.A.; Dong,Z.; Cheung,H.T.A.; Asano,Y.; Escoffier,L.; Costello,M.; Tattersall,M.H.N. Activation and cytotoxicity of 2-α-aminoacyl prodrugs of methotrexate, *Biochem.Pharmacol.*, **1995**, *49*, 567–574.

SAMPLE
Matrix: urine
Sample preparation: Add 250 μL 1 M pH 5.0 acetate buffer to a 1.0 mL urine, vortex and add a 250 μL aliquot of the mixture to a Sep-Pak silica cartridge and dry by aspiration of air. Wash the cartridge with 5 mL ethyl acetate and 5 mL EtOH:MeOH 60:40 and dry by aspiration of air. Elute with 3 mL of 1% ammonia in MeOH, dry eluate under a stream of nitrogen in a water bath at 37°. Reconstitute dried residue with 100 μL mobile phase and inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2 (GL Science, Tokyo, Japan)
Mobile phase: MeCN:50 mM pH 5.3 phosphate buffer 9.5:90.5
Flow rate: 1.1
Injection volume: 20
Detector: UV 303

CHROMATOGRAM
Retention time: 6.5
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

Noninterfering: diclofenac, felbinac, fenbufen, indomethacin, loxoprofen, naproxen, piroxicam, sulindac

KEY WORDS
SPE

REFERENCE
Hirai,T.; Matsumoto,S.; Kishi,I. Determination of methotrexate and its main metabolite 7-hydroxymethotrexate in human urine by high-performance liquid chromatography with normal solid-phase extraction, *J.Chromatogr.B*, **1997**, *690*, 267–273.

SAMPLE
Matrix: urine
Sample preparation: Condition a 100 mg Bond Elut phenyl SPE cartridge with 1 mL MeOH and 1 mL water. Centrifuge urine at 1000 g. Adjust pH of 1 mL supernatant to 5.0 ± 0.1 with 2 M trichloroacetic acid then with 0.6 M trichloroacetic acid, add to the SPE cartridge, wash with 2 mL water, wash with 1 mL ethyl acetate, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL water, inject a 10-50 μL aliquot onto column A and elute to waste with mobile phase A, after 9.5 min divert the effluent from column B onto column A, after another 5 min remove column A from the circuit and start the gradient, elute column B with mobile phase B and monitor the effluent from column B. Re-equilibrate both columns with mobile phase A for 15 min. Wash both columns separately with MeCN at the end of the day.

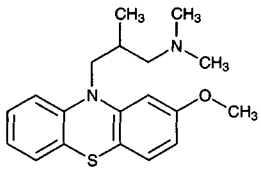
HPLC VARIABLES
Column: A 250 × 4 10 μm Nucleosil 100 SB strong anion-exchange (maintained at 25 ± 1°); B 250 × 4 10 μm LiChrospher 100 RP-18e
Mobile phase: A MeCN:buffer 1:99; B Gradient. MeCN:buffer from 1:99 to 25:75 over 24 min. (Buffer was 314 mM formic acid adjusted to pH 2.7 with 25% ammonia.)
Flow rate: 1
Injection volume: 10-50
Detector: UV 310

CHROMATOGRAM
Retention time: 34
Limit of quantitation: 4 ng/mL

KEY WORDS
column-switching; heart-cut; SPE

REFERENCE
Mader,R.M.; Rizovski,B.; Steger,G.G.; Rainer,H.; Proprentner,R.; Kotz,R. Determination of methotrexate in human urine at nanomolar levels by high-performance liquid chromatography with column switching, *J.Chromatogr.*, **1993**, *613*, 311–316.

Methotrimeprazine



Molecular formula: $C_{19}H_{24}N_2OS$
Molecular weight: 328.48
CAS Registry No.: 60-91-1
Merck Index: 6066

SAMPLE

Matrix: blood
Sample preparation: 2-5 mL Plasma + 1 mL 1 M NaOH + 8 mL diethyl ether:chloroform 4:1, shake for 10 min, centrifuge. Remove the upper organic layer and evaporate it to dryness at 50° under a stream of nitrogen. Dissolve the residue in 50 µL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 µm Spherisorb nitrile-bonded silica
Mobile phase: MeCN:MeOH:100 mM K_2HPO_4 adjusted to pH 6.5 with orthophosphoric acid 6:4:7
Detector: E, Model LCA 15-EDT Research, glassy carbon electrode +0.85 V

CHROMATOGRAM

Retention time: 4
Internal standard: methotrimeprazine
Limit of detection: 0.2 ng/mL

OTHER SUBSTANCES

Simultaneous: prochlorperazine
Noninterfering: chlorpromazine

KEY WORDS

plasma; methotrimeprazine is IS

REFERENCE

Sankey,M.G.; Holt,J.E.; Kaye,C.M. A simple and sensitive H.P.L.C. method for the assay of prochlorperazine in plasma, *Br.J.Clin.Pharmacol.*, **1982**, *13*, 578–580.

SAMPLE

Matrix: blood
Sample preparation: Work under yellow light. 1 mL Serum + 2 mL water + 2 mL 2 M NaOH, vortex for 10 s, add 5 mL water-saturated n-heptane:isoamyl alcohol 99:1, shake gently for 20 min, centrifuge at 4° at 2800 g, remove organic layer and repeat the extraction. Combine the organic layers and evaporate them to dryness under reduced pressure. Dissolve the residue in 500 µL MeCN, inject a 30 µL aliquot (store at 5°).

HPLC VARIABLES

Column: 250 × 4.6 5 µm Nucleosil 100 CN
Mobile phase: MeCN:pyridine:140 mM sodium acetate pH 3.1 698:2:300
Flow rate: 0.9
Injection volume: 100
Detector: E, Environmental Sciences Assoc. Coulochem II, Model 5011 detector cell, oxidative screen mode, screen electrode +0.35 V, sample electrode +0.65 V

CHROMATOGRAM

Retention time: 9
Limit of detection: 0.2 ng/mL

OTHER SUBSTANCES

Simultaneous: chlorprothixene
Interfering: promethazine

KEY WORDS
serum; recirculate mobile phase

REFERENCE
Bagli,M.; Rao,M.L.; Höflich,G. Quantification of chlorprothixene, levomepromazine and promethazine in human serum using high-performance liquid chromatography with coulometric electrochemical detection, *J.Chromatogr.B*, **1994**, *657*, 141–148.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 252

CHROMATOGRAM
Retention time: 8.59
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine;

amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, milk

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL water. 1 mL Serum or milk + 5 mL 0.5 (serum) or 1 (milk) M HCl, mix, add to the SPE cartridge, wash with 5 mL water, wash with 5 mL MeOH:water 20:80, elute with 5 mL MeOH:water 60:40, evaporate eluate to dryness under vacuum at 60°, dissolve residue in 100 μL mobile phase, inject whole amount.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Develosil C8-5 (Nomura)
Mobile phase: MeCN:0.5% KH_2PO_4 adjusted to pH 4.5 with 50% phosphoric acid 35:65
Flow rate: 1
Injection volume: 100
Detector: UV 254

CHROMATOGRAM

Retention time: 10
Internal standard: methotrimeprazine (levomepromazine)

OTHER SUBSTANCES

Extracted: chlorpromazine

KEY WORDS

serum; SPE; methotrimeprazine is IS

REFERENCE

Ohkubo,T.; Shimoyama,R.; Sugawara,K. Determination of chlorpromazine in human breast milk and serum by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *614*, 328–332.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 1 mL Plasma + 4 mL water + 1 mL 160 ng/mL thioridazine + 0.8 mL 1 M NaOH + 15 mL n-heptane:isoamyl alcohol 98.5:1.5, shake for 10 min, centrifuge at 700 g, remove the organic layer, repeat the extraction twice more. Combine the organic layers and evaporate them to dryness, reconstitute the residue in 10 mL 50 mM HCl, add 20 mL diethyl ether, shake for 3 min. Remove the aqueous layer and make it alkaline with 1 mL 5 M NaOH, add 10 mL n-heptane:isoamyl alcohol 98.5:1.5, shake for 10 min, centrifuge at 700 g. Remove the organic layer and evaporate it to dryness, dissolve the residue in 1 mL MeCN, inject a 50 μL aliquot. Urine. 20 mL Urine + 3 mL 1 M HCl + 1 mL 1 μg/mL thioridazine, wash with diethyl ether, make the aqueous layer alkaline with 5 M NaOH, add n-heptane: isoamyl alcohol 98.5:1.5, shake for 10 min, centrifuge at 700 g. Remove the organic layer and evaporate it to dryness, dissolve the residue in 1 mL MeCN, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 150 × 4 5 μm Nucleosil C18
Mobile phase: MeCN:pyridine:THF:100 mM pH 3.5 acetate buffer 68.9:0.1:1:30 containing 20 mM sodium perchlorate
Flow rate: 0.7
Injection volume: 10-50
Detector: E, Yanaco Model VMD-101, glassy carbon working electrode 0.95 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 7.0
Internal standard: thioridazine (9.5)
Limit of detection: 0.5 ng/mL (urine), 2 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: chlorpromazine

KEY WORDS
plasma

REFERENCE
Murakami,K.; Ueno,T.; Hijikata,J.; Shirasawa,K.; Muto,T. Simultaneous determination of chlorpromazine and levomepromazine in human plasma and urine by high-performance liquid chromatography using electrochemical detection, *J.Chromatogr.*, **1982**, *227*, 103–112.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 251.1

CHROMATOGRAM
Retention time: 15.842

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 3.7

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 60:1.5:0.5:38
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 2.41

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
Guard column: 20 × 4.6 5 μm Supelguard LC18-DB (Supelco)
Column: 250 × 4.6 5 μm Supelcosil C18-DB
Mobile phase: MeCN:THF:buffer 47.5:2.5:50 (Buffer was 500 mM pH 5.0 ammonium acetate containing 25 mM sodium dodecyl sulfate.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 24.53
Internal standard: methoxypromazine (20.41)

OTHER SUBSTANCES
Simultaneous: metabolites

REFERENCE
Loennechen,T.; Dahl,S.G. High-performance liquid chromatography of levomepromazine (methotrimeprazine) and its main metabolites, *J.Chromatogr.*, **1990**, *503*, 205–215.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 30 × 3.2 7 μm SI 100 ODS (not commercially available)
Column: 150 × 3.2 7 μm SI 100 ODS (not commercially available)
Mobile phase: MeCN:buffer 31.2:68.8 (Buffer was 6.66 g KH_2PO_4 and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)
Flow rate: 0.5-1
Detector: UV 202, 247

CHROMATOGRAM
Retention time: 4.5
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, nicotine, oxazepam, procaine, promazine, propafenone, propranolol, salicylamide, temazepam, tetracaine, thiopental, triamterene, verapamil, zolpidem, zopiclone

REFERENCE
Below,E.; Burrmann,M. Application of HPLC equipment with rapid scan detection to the identification of drugs in toxicological analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 4131–4144.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Lichrosphere cyanopropyl
Mobile phase: Carbon dioxide:MeOH:isopropylamine 94:6:0.03
Column temperature: 50

Flow rate: 3
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 3.4

OTHER SUBSTANCES
Simultaneous: triflupromazine, carphenazine, promazine, perphenazine, chlorprothixene, deserpidine, thiothixene, reserpine
Also analyzed: acetophenazine, ethopropazine, promethazine, propiomazine

KEY WORDS
SFC; pressure 200 bar

REFERENCE
Berger,T.A.; Wilson,W.H. Separation of drugs by packed column supercritical fluid chromatography. 1. Phenothiazine antipsychotics, *J.Pharm.Sci.*, **1994**, *83*, 281–286.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 15.23 (A), 7.19 (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, 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, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 25 ng/mL solution in pH 4.0 acetate/citrate buffer.

HPLC VARIABLES
Column: 150 × 0.32 3 μm Hypersil C18
Mobile phase: MeCN:pH 4.0 acetate/citrate buffer 45:55
Injection volume: 20
Detector: UV

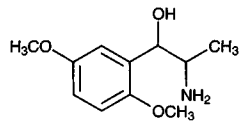
CHROMATOGRAM
Retention time: 11.5

OTHER SUBSTANCES
Simultaneous: chlorpromazine, thioridazine

KEY WORDS
microcolumn

REFERENCE
Streel,B.; Ceccato,A.; Chiap,P.; Hubert,P.; Crommen,J. Injection-generated solvent and pH gradients for sample enrichment on injection of large volumes in microcolumn liquid chromatography, *Biomed.Chromatogr.*, **1995**, *9*, 254–256.

Methoxamine



Molecular formula: $C_{11}H_{17}NO_3$
Molecular weight: 211.26
CAS Registry No.: 390-28-3, 61-16-5 (HCl)
Merck Index: 6067

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 50 μL water + 100 μL 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 μL saturated sodium carbonate and 200 μL 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 μL mobile phase, vortex for 5 s, centrifuge for 5 min, inject a 20-60 μL aliquot of the supernatant. Urine. Dilute urine 10 times with water. 100 μL Diluted urine + 50 μL water + 100 μL saturated sodium carbonate + 200 μL 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 μL mobile phase, vortex for 5 s, centrifuge for 5 min, inject a 20-60 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 50 mm long pellicular ODS (Whatman)

Column: 100 × 4.6 5 μm 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: 18 (-), 20 (+)
Internal standard: methoxamine

OTHER SUBSTANCES
Extracted: atenolol

KEY WORDS
plasma; derivatization; methoxamine is IS; chiral

REFERENCE
Mehvar,R. Liquid chromatographic analysis of atenolol enantiomers in human plasma and urine, *J.Pharm.Sci.*, **1989**, *78*, 1035–1039.

SAMPLE
Matrix: perfusate
Sample preparation: 30 μL Perfusate (artificial CSF) + 10 μL 200 mM perchloric acid. Mix a 25 μL aliquot with 12.5 μL reagent, let stand for 2 min, inject an aliquot. (Prepare a stock solution by dissolving 27 mg o-phthalaldehyde in 1 mL MeOH, add 5 μL β-mercaptoethanol, add 9 mL 100 mM pH 9.3 sodium tetraborate containing 10 μM EDTA. This solution is good for 5 days in a sealed amber bottle at room temperature. Prepare the working reagent by diluting 1 mL of the stock solution with 3 mL 100 mM pH 9.3 sodium tetraborate containing 10 μM EDTA, allow to stand for 24 h before use.)

HPLC VARIABLES
Column: two columns 150 × 4.6 5 μm M.S. Gel C18 (ESA)
Mobile phase: MeOH:buffer 8:92 adjusted to pH 3.0 with phosphoric acid (Buffer was 54 mM NaH_2PO_4 containing 1.24 mM sodium heptanesulfonate.)
Column temperature: 33
Flow rate: 1.2
Detector: E, ESA Coulochem Electrode Array System Model 5500, detector temp 33°, oxidation potential 900 mV

CHROMATOGRAM
Retention time: 5.00
Limit of quantitation: 0.5 ng/mL

OTHER SUBSTANCES
Extracted: apomorphine, dopamine, hydralazine, isoproterenol, morphine, norepinephrine, phenylephrine

KEY WORDS
rat

REFERENCE
Acworth,I.N.; Yu,J.; Ryan,E.; Gariepy,K.C.; Gamache,P.; Hull,K.; Maher,T. Simultaneous measurement of monoamine, amino acid, and drug levels, using high performance liquid chromatography and coulometric array technology: application to in vivo microdialysis perfusate analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 685–705.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.7

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions
Sample preparation: Evaporate an ethyl acetate solution 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: 14.0, 14.8 (enantiomers)

OTHER SUBSTANCES
Simultaneous: atenolol

KEY WORDS
derivatization; chiral

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: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenyl-

butazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Chirex 3020 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 60:35:5 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1
Injection volume: 20
Detector: UV 290

KEY WORDS

chiral; α = 1.11 for enantiomers

REFERENCE

Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 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: 6.41 (A), 3.62 (B)

OTHER SUBSTANCES

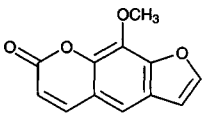
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, 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, haloper-

idol, 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, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
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.

Methoxsalen



Molecular formula: $C_{12}H_8O_4$
Molecular weight: 216.19
CAS Registry No.: 298-81-7
Merck Index: 6068
Lednicer No.: 1 333

SAMPLE
Matrix: blood
Sample preparation: 1 mL Whole blood + 2.5 mL 1 M pH 9 borate buffer, mix well, add 8 mL n-hexane:isopropanol 95:5, shake at 80-100 strokes/min for 10 min, centrifuge at 5° at 1500 g. Remove 7.0 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil silica
Mobile phase: Dichloromethane:MeCN 95:5
Flow rate: 2.2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 3.8
Limit of detection: 10-15 ng/mL

KEY WORDS
normal phase; whole blood; dog; human; pharmacokinetics

REFERENCE
Puglisi,C.V.; de Silva,J.A.F.; Meyer,J.C. Determination of 8-methoxypsoralen, a photoactive compound, in blood by high pressure liquid chromatography, *Anal.Lett.*, **1977**, *10*, 39–50.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL Bond Elut C18 SPE cartridge with 9 mL MeOH and 9 mL water. Add 500 μL plasma to the SPE cartridge, wash with 9 mL water, elute with 500 μL 500 ng/mL 5-methoxypsoralen in MeOH, vortex the eluate, inject a 20 μL aliquot of the eluate.

HPLC VARIABLES
Column: 100 mm long Spheri-5 RP-18
Mobile phase: MeCN:MeOH:water 10:25:65
Flow rate: 0.7
Injection volume: 20
Detector: UV 300

CHROMATOGRAM
Retention time: 5.0
Internal standard: 5-methoxypsoralen (bergapten) (8.5)
Limit of detection: 1.5 ng/mL

OTHER SUBSTANCES
Noninterfering: acetaminophen, alprazolam, amikacin, amobarbital, caffeine, carbamazepine, chloramphenicol, diazepam, disopyramide, gentamicin, lidocaine, lithium, lorazepam, meprobamate, mexiletine, phenobarbital, phenytoin, procainamide, propranolol, quinidine, salicylic acid, theophylline, tobramycin, valproic acid, vancomycin

KEY WORDS
SPE; plasma

REFERENCE
Ketchum,C.H.; Robinson,C.A.,Jr.; Huang,S.T. Analysis of 8-methoxypsoralen by high-performance liquid chromatography, *Clin.Chem.*, **1990**, *36*, 1956–1957.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL 60 μm Silicacart C18 SPE cartridge (Tessek) with 5 mL MeOH and 5 mL water. 2 mL Plasma + 2 mL MeCN + 1 mL 6.25 μg/mL griseofulvin in MeOH, shake by hand for 1-2 s, centrifuge at 500 g for 10 min, add 4 mL of the supernatant to the SPE cartridge, wash with 5 mL water, elute with 2 mL MeOH. Evaporate the eluate to dryness under vacuum at 38°, reconstitute in 1 mL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Spherisorb 5 ODS
Mobile phase: MeCN:10 mM phosphoric acid 34:66, pH 2.82
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: UV 248

CHROMATOGRAM
Retention time: 7.59
Internal standard: griseofulvin (12.13)
Limit of detection: 15 ng/mL

KEY WORDS
plasma; SPE

REFERENCE
Kucová,D.; Marysková,D.; Davídková,P.; Gasparic,J. High-performance liquid chromatographic determination of methoxsalen in plasma after liquid-solid extraction, *J.Chromatogr.*, **1993**, *614*, 340–344.

SAMPLE
Matrix: blood

Sample preparation: Inject 25 μL plasma onto column A with mobile phase A, elute column A with mobile phase A to waste, after 8 min backflush the contents of column A onto column B with mobile phase B, monitor the effluent from column B. (Re-equilibrate column A with mobile phase A for 2 min before next injection.)

HPLC VARIABLES

Column: A 250 × 4 25 μm C8-Alkyl-Diol Silica (some preparation details given); B 125 × 4 5 μm LiChrospher RP-18
Mobile phase: A water; B MeOH:water 60:40
Flow rate: A 1; B 0.8
Injection volume: 25
Detector: F ex 312 em 540

CHROMATOGRAM

Retention time: 4
Limit of detection: 0.39 ng/mL
Limit of quantitation: 0.79 ng/mL

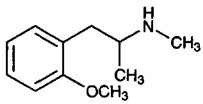
KEY WORDS

plasma; column-switching; protect from light

REFERENCE

Vielhauer,S.; Rudolphi,A.; Boos,K.-S.; Seidel,D. Evaluation and routine application of the novel restricted-access precolumn packing material Alkyl-Diol Silica: coupled-column high-performance liquid chromatographic analysis of the photoreactive drug 8-methoxypsoralen in plasma, *J.Chromatogr.B*, **1995**, *666*, 315–322.

Methoxyphenamine



Molecular formula: $C_{11}H_{17}NO$
Molecular weight: 179.26
CAS Registry No.: 93-30-1, 5588-10-3 (HCl)
Merck Index: 6077

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.3

OTHER SUBSTANCES

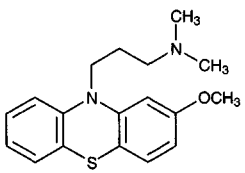
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene,

dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Methoxypromazine



Molecular formula: $C_{18}H_{22}N_2OS$
Molecular weight: 314.45
CAS Registry No.: 61-01-8, 3403-42-7 (maleate)
Merck Index: 6080
Lednicer No.: 1 387

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: 5.3

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquin-

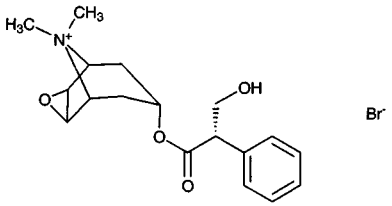
amide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Methscopolamine bromide

Molecular formula: $C_{18}H_{24}BrNO_4$
Molecular weight: 398.30
CAS Registry No.: 155-41-9
Merck Index: 6084



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 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Partisil-10-ODS
Mobile phase: MeCN:buffer 50:50 (Buffer was 2.85 mM ethylenediamine sulfate adjusted to pH 7.44 ± 0.02 with 1 M ammonium hydroxide.)

Flow rate: 3.8
Injection volume: 30
Detector: UV 216.5

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: aposcopolamine, pheniramine, phenylpropanolamine, 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
Sample preparation: Prepare a 1.5 mg/mL solution, inject a 10 μL aliquot.

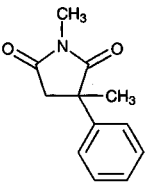
HPLC VARIABLES
Guard column: Supelguard LC-8-DB (Supelco)
Column: 50 × 4.6 Supelcosil LC-8-DB
Mobile phase: MeCN:buffer 10:90 containing 0.02% triethylamine (Buffer was KH_2PO_4 adjusted to pH 2.0 with phosphoric acid.)
Column temperature: 35
Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 2

OTHER SUBSTANCES
Simultaneous: chlorpheniramine, phenylpropanolamine, pseudoephedrine, triprolidine

REFERENCE
Supelco Catalog, **1992**, p. 179.

Methsuximide



Molecular formula: $C_{12}H_{13}NO_2$
Molecular weight: 203.24
CAS Registry No.: 77-41-8
Merck Index: 6085
Lednicer No.: 1 228

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 100 μL buffer + 1.5 mL IS in 5% isopropanol in chloroform, vortex for 30 s, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of air at room temperature, reconstitute the residue in 100 μL mobile phase, inject a 6-10 μL aliquot. (Buffer was 13.6 g KH_2PO_4 in 90 mL water, pH adjusted to 6.8 with about 3 mL 10 M NaOH, made up to 100 mL.)

HPLC VARIABLES

Guard column: 20 × 4.6 Supelguard LC-1 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil LC-1 (Supelco)
Mobile phase: MeOH:MeCN:buffer 17.5:17.5:65 (Buffer was 2.72 g KH_2PO_4 in 1.9 L water, pH adjusted to 6.3 with about 2 mL 1 M NaOH, made up to 2 L.)
Flow rate: 2
Injection volume: 6-10
Detector: UV 204

CHROMATOGRAM

Retention time: 5.30
Internal standard: 3-isobutyl-1-methylxanthine (3.15)

OTHER SUBSTANCES

Extracted: acetaminophen, amobarbital, barbital, caffeine, carbamazepine, chloramphenicol, ethosuximide, mephobarbital, pentobarbital, phenobarbital, phenytoin, primidone, secobarbital, theophylline, thiopental
Also analyzed: acetanilide, acetylcysteine, 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: solutions
Sample preparation: Prepare a solution in MeOH, inject a 3 μL aliquot.

HPLC VARIABLES

Guard column: 50 × 2.1 Whatman Co:Pell ODS
Column: 125 × 4.5 5 μm 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: 12.3

OTHER SUBSTANCES

Simultaneous: amobarbital, barbital, butabarbital, carbamazepine, ethosuximide, ethotoin, ethylphenacemide, glutethimide, heptabarbital, pheneturide, phenobarbital, phenylethylmalonamide, phenytoin, primidone, secobarbital, sulfamethoxazole, sulthiame
Interfering: cyclobarbital, pentobarbital

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: solutions

Sample preparation: Inject a 30-100 μL aliquot.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: Gradient. MeCN:7.5 g/L NaH_2PO_4 adjusted to pH 3.2 with phosphoric acid 5:95 to 22:78 over 24 min, to 45:55 over 10 min, maintain at 45:55 for 5 min. Re-equilibrate with 5:95 for 5 min.
Column temperature: 50
Flow rate: 3
Injection volume: 30-100
Detector: UV 210

CHROMATOGRAM
Retention time: 19.0

OTHER SUBSTANCES
Simultaneous: acetaminophen, amitriptyline, amobarbital, butabarbital, butalbital, caffeine, chlordiazepoxide, clomipramine, codeine, desipramine, diazepam, ethchlorvynol, ethotoin, flurazepam, glutethimide, hexobarbital, imipramine, lidocaine, mesantoin, methaqualone, methyprylon, nirvanol, nitrazepam, nortriptyline, oxazepam, pentobarbital, phenobarbital, phenylpropanolamine, phenytoin, primidone, procainamide, propranolol, quinidine, salicylic acid, secobarbital, theophylline

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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: 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: 6.6

OTHER SUBSTANCES
Simultaneous: barbital, carbamazepine, diazepam, ethotoin, mephenytoin, phenacemide, phenobarbital, phensuximide

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REFERENCE
Vydac HPLC Catalog, 1994-5, **1994**, p. 26.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 62 × 2 packed with chiral packing (Prepare packing by dissolving 4-chloro-3-methylphenylcarbamate cellulose in THF, coat on Nucleosil 1000-7, dry at 60° for 3 h under reduced pressure.)
Mobile phase: Hexane:isopropanol 90:10
Flow rate: 0.1
Injection volume: 20
Detector: UV 254

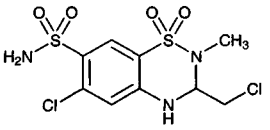
CHROMATOGRAM

Retention time: k' 4.10

KEY WORDS
narrow-bore; chiral; α 1.12

REFERENCE
Chankvetadze,B.; Chankvetadze,L.; Sidamonidze,S.; Yashima,E.; Okamoto,Y. Enantioseparation of some chiral pharmaceuticals using narrow-bore liquid chromatography, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 695–699.

Methyclothiazide



Molecular formula: $C_9H_{11}Cl_2N_3O_4S_2$
Molecular weight: 360.24
CAS Registry No.: 135-07-9
Merck Index: 6086
Lednicer No.: 1 360

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 2 mL 100 mM NaOH + 8 mL ethyl acetate, vortex vigorously for 30 s, centrifuge at 10° at 2000 g for 10 min. Remove the organic layer and pass it through a 35 × 6 column of anhydrous magnesium sulfate, rinse column with 2 mL ethyl acetate. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μL 8 μg/mL phenacetin in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 10 μm μBondapak C18
Mobile phase: MeOH:water 35:65
Column temperature: 35
Flow rate: 1.5
Injection volume: 20
Detector: UV 225

CHROMATOGRAM
Retention time: 7.5
Internal standard: phenacetin (11)
Limit of detection: 1.5 ng/mL

OTHER SUBSTANCES
Noninterfering: clonidine, methyldopa, prazosin, propranolol

KEY WORDS
plasma

REFERENCE
Hartman,C.A.; Kucharczyk,N.; Sofia,R.D.; Perhach,J.L.,Jr. Determination of methyclothiazide in human plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *226*, 510–513.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 226.3

CHROMATOGRAM
Retention time: 15.36

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 2 mL 1 M pH 4.1 NaH_2PO_4 + 4 mL ethyl acetate, vortex for 2 min, centrifuge at 1500 g for 5 min. Remove the organic phase and add it to 5 mL 100 mM pH 7.5 Na_2HPO_4, vortex for 2 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL MeCN:10 mM pH 3.0 phosphate buffer, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm LiCHrosorb RP-18
Mobile phase: Gradient. MeCN:10 mM pH 3.0 phosphate buffer 10:90 for 1.5 min then to 35:65 over 2 min
Column temperature: 50
Flow rate: 1.5
Injection volume: 5
Detector: UV 271

CHROMATOGRAM
Retention time: 4.7
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Extracted: chlorothiazide, hydrochlorothiazide, quinethazone, chlorthalidone, furosemide, metolazone, mefruside, bendroflumethiazide, cyclopenthiazide, bumetanide
Simultaneous: indapamide, clorexolone, ethacrynic acid
Noninterfering: aspirin, albuterol, allopurinol, alprenolol, atenolol, captopril, carbimazole, clonidine, coloxyl, danthron, diazepam, digoxin, doxepin, glibenclamide, hydralazine, indomethacin, labetalol, metformin, methyldopa, metoprolol, mianserin, minoxidil, nifedipine, nitrazepam, oxazepam, oxprenolol, pindolol, prazosin, propranolol, senokot, theophylline, trifluoperazine
Interfering: clopamide

REFERENCE
Fullinfaw,R.O.; Bury,R.W.; Moulds,R.F.W. Liquid chromatographic screening of diuretics in urine, *J.Chromatogr.*, **1987**, *415*, 347–356.

SAMPLE
Matrix: urine

Sample preparation: 2 mL Urine + 0.5 g solid buffer I (pH 5-5.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and vortex it with 2 mL 5% aqueous lead acetate for 10 s, centrifuge at 600 g for 5 min, remove and keep organic phase. 2 mL Urine + 0.5 g solid buffer II (pH 9-9.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and combine it with previous organic layer. Evaporate to dryness at 50° under a stream of nitrogen, reconstitute in 300 μL 50 μg/mL β-hydroxyethyltheophylline in MeOH, inject 5 μL aliquot. (Solid buffer I was KH_2PO_4:Na_2HPO_4 99:1, solid buffer II was $NaHCO_3$:K_2CO_3 3:2.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm HP Hypersil ODS (A) or HP LiChrosorb RP-18 (B)
Mobile phase: Gradient. MeCN:buffer from 15:85 at 2 min to 80:20 at 20 min (Buffer was 50 mM NaH_2PO_4 containing 16 mM propylamine hydrochloride, adjusted to pH 3 with concentrated phosphoric acid.)
Flow rate: 1
Injection volume: 5
Detector: UV 230, UV 275

CHROMATOGRAM

Retention time: 11.7 (A), 12.6 (B)
Internal standard: β-hydroxyethyltheophylline (3.7 (A), 4.4 (B))
Limit of detection: 500 ng/mL

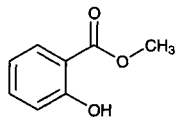
OTHER SUBSTANCES

Extracted: furosemide, metolazone, amiloride, acetazolamide, chlorothiazide, hydrochlorothiazide, quinethazone, triamterene, hydroflumethiazide, chlorthalidone, dichlorphenamide, trichloromethiazide, benzthiazide, cyclothiazide, polythiazide, bendroflumethiazide, ethacrynic acid, bumetanide, probenecid, spironolactone, canrenone, flumethiazide
Noninterfering: acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil

REFERENCE

Cooper,S.F.; Massé,R.; Dugal,R. Comprehensive screening procedure for diuretics in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 65–88.

Methyl salicylate



Molecular formula: $C_8H_8O_3$
Molecular weight: 152.15
CAS Registry No.: 119-36-8
Merck Index: 6200

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood + 100 μL 2 M NaOH + 10 mL dichloromethane, rotate for 10 min, centrifuge for 5 min. Remove 6 mL of the organic layer and evaporate it to dryness, reconstitute the residue in 1 mL 100 mM NaOH, heat at 60° for 2 h, cool, add 200 μL 1 M HCl, add 200 μL 1 mg/mL β-hydroxyethyltheophylline in MeOH, add 5 mL dichloromethane, rotate for 10 min, centrifuge for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 200 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm ODS (Altex)
Mobile phase: MeOH:water:glacial acetic acid 40:66:1
Flow rate: 1.5
Injection volume: 20
Detector: UV 250

CHROMATOGRAM
Retention time: 6.4 (salicylic acid), 13 (unhydrolyzed methyl salicylate)
Internal standard: β-hydroxyethyltheophylline (2)
Limit of quantitation: 10 μg/mL

OTHER SUBSTANCES
Simultaneous: theophylline
Noninterfering: acetaminophen, amobarbital, butalbital, caffeine, carbamazepine, glutethimide, ibuprofen, indomethacin, meprobamate, methaqualone, pentobarbital, phenobarbital, phenylbutazone, phenytoin, primidone, secobarbital

KEY WORDS
whole blood; methyl salicylate is hydrolyzed to salicylic acid

REFERENCE
Levine,B.; Caplan,Y.H. Liquid chromatographic determination of salicylate and methyl salicylate in blood and application to a postmortem case, *J.Anal.Toxicol.*, **1984**, *8*, 239–241.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 6-10 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 Supelguard LC-1 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil LC-1 (Supelco)
Mobile phase: MeOH:MeCN:buffer 17.5:17.5:65 (Buffer was 2.72 g KH_2PO_4 in 1.9 L water, pH adjusted to 6.3 with about 2 mL 1 M NaOH, made up to 2 L.)
Flow rate: 2
Injection volume: 6-10
Detector: UV 204

CHROMATOGRAM
Retention time: 7.17 (tails)
Internal standard: 5-ethyl-5-p-tolybarbituric acid (tolybarb) (4.80)

OTHER SUBSTANCES
Simultaneous: acetaminophen, acetanilide, N-acetylcysteine, N-acetylprocainamide, amobarbital, ampicillin, aspirin, barbital, butabarbital, butalbital, caffeine, carbamazepine, chloramphenicol, chlorpropamide, codeine, cyheptamide, diazoxide, diflunisal, diphylline, disopyramide, ethchlorvynol, gentisic acid, glutethimide, heptabarbital, hexobarbital, ibuprofen, indomethacin, ketoprofen, mefenamic acid, mephenytoin, mephobarbital, methaqualone, methsuximide, methyprylon, morphine, naproxen, nirvanol, oxphenylbutazone, pentobarbital, phenacetin, phenobarbital, phensuximide, phenytoin, procainamide, salicylamide, salicylic acid, secobarbital, sulfamethoxazole, sulindac, theophylline, thiopental, tolmetin, trimethoprim, vancomycin
Noninterfering: amikacin, gentamicin, meprobamate, netilmicin, quinidine, tetracycline, tobramycin, valproic acid
Interfering: ethosuximide, cimetidine, primidone, phenylbutazone

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

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil C8
Mobile phase: MeCN:water 55:45 containing 10 mM sodium dodecanesulfonate
Column temperature: 40
Flow rate: 1

Detector: UV 254

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: diphenhydramine

REFERENCE
Supelco Catalog, **1993,** p. 531.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

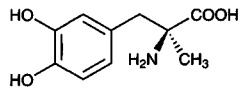
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole,

thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Methyldopa



Molecular formula: $C_{10}H_{13}NO_4$
Molecular weight: 211.22
CAS Registry No.: 555-30-6, 41372-08-1 (sesquihydrate)
Merck Index: 6132
Lednicer No.: 1 95

SAMPLE
Matrix: blood
Sample preparation: Add 50 µL 2 M HCl to 50 µL plasma, make up to 170 µL with water, centrifuge at 1400 rpm for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 8 4 µm NovaPak C18
Mobile phase: MeCN:MeOH:10 mM pH 3 sodium acetate buffer 47.25:15.75:37
Flow rate: 2
Detector: F ex 276 em 310

CHROMATOGRAM
Retention time: 5.15
Internal standard: methyldopa

OTHER SUBSTANCES
Extracted: propofol
Noninterfering: albuterol, ampicillin, amoxicillin, amphotericin B, bleomycin, ceftazidime, cefoxitin, cephalexin, ciprofloxacin, dobutamine, dopamine, epinephrine, erythromycin, esmolol, fluconazole, gentamicin, labetalol, metoclopramide, miconazole, nitroglycerin, nitroprusside, norepinephrine, paclitaxel, penicillin G benzathine, ranitidine, streptomycin, tetracycline

KEY WORDS
plasma; methyldopa is IS

REFERENCE

el-Yazigi,A.; Hussein,R.F. Microdetermination of propofol in plasma by a rapid and sensitive liquid chromatographic method, *J.Pharm.Biomed.Anal.*, **1996**, *15*, 99–104.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 50 µL 10 µg/mL dopa, vortex, add 50 µL perchloric acid, vortex for 5 s, let stand at -20° for 15 min, centrifuge at 4° at 4000 rpm for 15 min, inject a 17 µL aliquot of the supernatant.

HPLC VARIABLES
Column: 125 × 4 5 µm Hypersil ODS
Mobile phase: MeOH:buffer 16:130 (Buffer contained 23.8 g/L phosphoric acid, 15.4 g/L KH_2PO_4, and 3.4 g/L PIC B8.)

Flow rate: 1
Injection volume: 17
Detector: E, Metrohm 656, +0.75 V

CHROMATOGRAM
Internal standard: dopa
Limit of quantitation: 50 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Dilger,C.; Salama,Z.; Jaeger,H. Determination of methyldopa in plasma using high-performance liquid chromatography with electrochemical detection. Application to pharmacokinetic/bioavailability studies, *Arzneimittelforschung*, **1987**, *37*, 1399–1401.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 3 mL ice-cold MeOH:500 mM perchloric acid 98:2, centrifuge at 4° at 4000 g for 3 min. Remove 200 μL of the supernatant and add it to 100 μL 80 ng/mL N-methyldopamine, evaporate to dryness under vacuum, reconstitute in 200 μL mobile phase, inject a 5-20 μL aliquot. Urine. 1 mL Urine + 50 mL water, inject a 10 μL aliquot. (To deconjugate adjust pH to 1, flush with nitrogen, heat in a boiling water bath for 1 h, dilute with 50 mL water, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeOH:13 mM sodium acetate containing 0.5 mM sodium 1-octanesulfonate and 0.5 mM disodium EDTA 14:86, pH 3.10
Flow rate: 1
Injection volume: 5-20
Detector: E, ESA Model 5100 A Coulochem, Model 5011 A analytical cell, first electrode +0.40 V, second electrode -0.30 V

CHROMATOGRAM
Retention time: 6.5
Internal standard: N-methyldopamine (11)

OTHER SUBSTANCES
Extracted: norepinephrine, epinephrine, dopamine, dihydroxyphenylacetic acid, 3-O-methylmethyldopa, homovanilic acid

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Lucarelli,C.; Betto,P.; Ricciarello,G.; Grossi,G. High-performance liquid chromatographic determination of L-3-(3,4-dihydroxyphenyl)-2-methylalanine (α-methyldopa) in human urine and plasma, *J.Chromatogr.*, **1991**, *541*, 285–296.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 2.96

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Powder tablets or contents of capsules, weigh out an amount equivalent to about 100 mg levodopa, add 30 mL 0.1 M HCl, sonicate, make up to 50 mL with 0.1 M HCl, mix, filter (0.45 μm), discard first 5 mL filtrate. 5 mL Filtrate + 10 mL 2 mg/mL methyldopa in 0.1 M HCl, make up to 100 mL with mobile phase, mix, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: 3% aqueous acetic acid
Flow rate: 1.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 4.5
Internal standard: methyldopa

OTHER SUBSTANCES
Simultaneous: levodopa

KEY WORDS
capsules; tablets; methyldopa 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
Sample preparation: Grind beads from sustained-release microcapsules containing 500 mg methyldopa to a fine powder, add 50 mL 50 mM sulfuric acid, sonicate for 15 min, make up to 100 mL with 50 mM sulfuric acid. Filter, reject the first 10 mL of the filtrate, make up a 10 mL aliquot to 100 mL with 50 mL sulfuric acid, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm CN (Phenomenex)

Mobile phase: MeOH:water:acetic acid 20:80:2 containing 5 mM sodium 1-heptanesulfonate, pH adjusted to 2.60
Flow rate: 1.6
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 4.5

OTHER SUBSTANCES
Simultaneous: degradation products, 3-O-methylmethyldopa

KEY WORDS
microcapsules; stability-indicating

REFERENCE
Metwally,M.E.-S. Stability-indicating high-performance liquid chromatographic assay for α-methyldopa in sustained-release capsules, *J.Chromatogr.*, **1991**, *549*, 221–228.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve in mobile phase, filter, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm μBondapak C18
Mobile phase: MeOH:50 mM ammonium acetate adjusted to pH 4.1 with 0.6 M acetic acid 1:99
Flow rate: 0.9
Detector: E, Coulochem model 5100A, screen electrode +0.3 V, sample electrode +0.6 V and UV 280

CHROMATOGRAM
Retention time: 8.59
Limit of detection: 1400 ng/mL (UV), 20 ng/mL (E)

OTHER SUBSTANCES
Simultaneous: hydroxydopa, carbidopa, levodopa, methoxytyrosine, methylcarbidopa, impurities

KEY WORDS
stability-indicating; tablets

REFERENCE
Kafil,J.B.; Dhingra,B.S. Stability-indicating method for the determination of levodopa, levodopa-carbidopa and related impurities, *J.Chromatogr.A*, **1994**, *667*, 175–181.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Grind tablets, weigh out a portion, dissolve in 50 mL mobile phase, sonicate, filter (No. 4 sintered glass plate), dilute, inject an aliquot. Capsules. Dissolve 10 capsules (without opening) in 100 mL mobile phase, sonicate, inject an aliquot. Injections, ampules, sprays. Dilute, inject an aliquot.

HPLC VARIABLES
Column: 120 × 4.6 Spherisorb C18 ODS-2
Mobile phase: Isopropanol:buffer 5:95 (Buffer was 100 mM sodium dodecyl sulfate containing 25 mM Na_2HPO_4, pH adjusted to 3.0 with HCl.)
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 4.5
Limit of detection: 4 ng/mL

OTHER SUBSTANCES
Simultaneous: carbidopa, dopamine, epinephrine, hydrochlorothiazide, isoproterenol, levodopa, 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: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeOH:30 mM pH 2.8 sodium phosphate buffer 5:95
Flow rate: 1
Detector: UV 201

REFERENCE
Walter,E.; Janich,S.; Roessler,B.J.; Hilfinger,J.M.; Amidon,G.L. HT29-MTX/Caco-2 cocultures as an in vitro model for the intestinal epithelium: In vitro-in vivo correlation with permeability data from rats and humans, *J.Pharm.Sci.*, **1996**, *85*, 1070–1076.

SAMPLE
Matrix: solutions

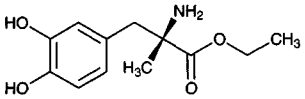
HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide,

hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Methyldopate



Molecular formula: $C_{12}H_{17}NO_4$
Molecular weight: 239.27
CAS Registry No.: 2544-09-4, 2508-79-4 (HCl)

SAMPLE
Matrix: solutions
Sample preparation: Dilute with 5% dextrose, inject a 15 μL aliquot.

HPLC VARIABLES
Column: Waters microparticulate C18
Mobile phase: MeOH:350 mM acetic acid and 5 mM sodium heptanesulfonate 35:65
Flow rate: 1.6-2.0
Injection volume: 15
Detector: F ex 285 em 315

CHROMATOGRAM
Retention time: 4.50

OTHER SUBSTANCES
Simultaneous: theophylline, terbutaline
Interfering: isoproterenol

REFERENCE
Williams,D.A.; Fung,E.Y.Y.; Newton,D.W. Ion-pair high-performance liquid chromatography of terbutaline and catecholamines with aminophylline in intravenous solutions, *J.Pharm.Sci.*, **1982**, *71*, 956–958.

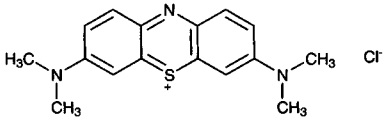
SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 20:1.5:0.5:78
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 1.98

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

Methylene blue



Molecular formula: $C_{16}H_{18}ClN_3S$
Molecular weight: 319.86
CAS Registry No.: 61-73-4, 7220-79-3 (trihydrate)
Merck Index: 6137

SAMPLE
Matrix: blood
Sample preparation: Condition a 40 μm Sepralyte non-bonded silica SPE cartridge (Analytichem) with 1 mL 1 M HCl, with 3 mL water, and with 500 μL 2 M $(NH_4)H_2PO_4$. Centrifuge 1 mL plasma at 4° at 12000 g for 1 min, add the supernatant to the SPE cartridge at 1 mL/min, vortex the precipitate in the centrifuge tube with 500 μL 0.3% ascorbic acid in 12 mM pH 3.0 citrate buffer, add the mixture to the SPE cartridge, wash with 500 μL water, wash with 500 μL MeCN saturated with tetramethylammonium chloride, apply suction for 5 min, elute with 300 μL elution solvent by centrifuging at 4° at 2000 g for 5 min, inject an aliquot of the eluate. (The elution solvent was MeOH:1 M tetramethylammonium chloride:1 M citric acid:water 32:10:20:38.)

HPLC VARIABLES
Column: 75 × 3.9 4 μm Nova-pak ODS
Mobile phase: MeOH:THF:triethylamine phosphate solution: 1 M tetramethylammonium chloride:water 30:1:10:2:57 (Triethylamine phosphate was 67.8 mL orthophosphoric acid in 800 mL water, adjust pH to 3.0 with triethylamine, make up to 1 L with water.) (Filter mobile phase but do not degas.)
Flow rate: 1
Injection volume: 50
Detector: UV 658

CHROMATOGRAM
Retention time: 7
Internal standard: methylene blue

OTHER SUBSTANCES
Extracted: mitoxantrone

KEY WORDS
plasma; SPE; use polypropylene containers; methylene blue is IS

REFERENCE

Lin,K.T.; Rivard,G.E.; Leclerc,J.-M. High-performance liquid chromatographic determination of mitoxantrone in plasma utilizing non-bonded silica gel for solid-phase isolation to reduce adsorptive losses on glass during sample preparation, *J.Chromatogr.*, **1989**, *465*, 75–86.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6, 5 μm TSKgel ODS 80 T_M (TOSOH, Japan)

Mobile phase: MeCN:25 mM pH 6.5 imidazole buffer 60:40 containing 10 mM sodium 1-propanesulfonate

Flow rate: 1.0

Injection volume: 50

Detector: Chemiluminescence following post-column reaction. The column effluent mixed with 0.25 mM bis(4-nitro-2-(3,6,9-trioxadecyloxycarbonyl)phenyl) oxalate (Wako) and 25 mM hydrogen peroxide in MeCN pumped at 1.3 mL/min and the mixture flowed to the detector (a red sensitive photomultiplier).

CHROMATOGRAM

Retention time: 3.5

Limit of detection: 120 fmol

OTHER SUBSTANCES

Simultaneous: pyridine 1, oxazine 1, 3,3′-diethylthiadicarbocyanine iodide (DTDCI)

KEY WORDS

peroxyoxalate chemiluminescence (PO-CL) detection

REFERENCE

Kimoto,K.; Gohda,R.; Murayama,K.; Santa,F.; Fukushima,T.; Homma,H.; Imai,K. Sensitive detection of near-infrared fluorescent dyes using high-performance liquid chromatography with peroxyoxalate chemiluminescence detection system, *Biomed.Chromatogr.*, **1996**, *10*, 189–190.

SAMPLE

Matrix: solutions

Sample preparation: Inject a 100 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 μBondapak C18

Mobile phase: MeCN:buffer 40:60 (Prepare buffer by mixing 400 mL MeCN, 300 mL water, 5 mL glacial acetic acid, and a vial of low-UV PIC B5 (pentanesulfonic acid, Waters), make up to 1 L with water.)

Flow rate: 1

Injection volume: 100

Detector: UV 664

CHROMATOGRAM

Retention time: 4.6

REFERENCE

Haddad,P.R.; Heckenberg,A.L. Trace determination of sulfide by reversed-phase ion-interaction chromatography using pre-column derivatization, *J.Chromatogr.*, **1988**, *447*, 415–420.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a solution in MeOH, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm CN (Alltech)

Mobile phase: MeOH:100 mM sodium acetate 60:40 containing 50 mg/L disodium EDTA, pH adjusted to 4.5 with aldehyde free glacial acetic acid
Flow rate: 0.8
Injection volume: 5-25
Detector: E, Bioanalytical Systems LC-4B, glassy carbon working electrode +1.000 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 11.2
Limit of quantitation: 5.7 ng

OTHER SUBSTANCES
Simultaneous: metabolites, leucogentian violet
Interfering: gentian violet

REFERENCE
Roybal,J.E.; Munns,R.K.; Hurlbut,J.A.; Shimoda,W. High-performance liquid chromatography of gentian violet, its demethylated metabolites, leucogentian violet and methylene blue with electrochemical detection, *J.Chromatogr.*, **1989**, *467*, 259–266.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 20 × 4 Keystone ODS/H
Mobile phase: MeCN:10 mM KH_2PO_4 40:60 containing 15 mM sodium dodecyl sulfate, pH 2.8
Flow rate: 2
Detector: UV 280

CHROMATOGRAM
Retention time: 1

OTHER SUBSTANCES
Simultaneous: thionin

REFERENCE
Keystone Scientific Catalog, 1993-4, p. 49.

SAMPLE
Matrix: tissue
Sample preparation: 100-300 mg minced tissue + 500 μL MeCN:MeOH:water, mix, filter (10000 molecular weight cut-off), inject a 20 μL aliquot of the ultrafiltrate.

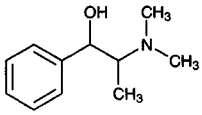
HPLC VARIABLES
Column: 60 × 4.6 3 μm Hypersil ODS
Mobile phase: MeOH:100 mM ammonium acetate 70:30
Flow rate: 0.4
Injection volume: 20
Detector: MS, Hewlett-Packard 5988A, particle beam, EI, 70 eV, source 300°, desolvation chamber 35° or Finnigan MAT 4500 quadrupole, thermospray, vaporizer 180-195°, source 250

CHROMATOGRAM
Retention time: 6
Limit of detection: 1.3 ppm (particle beam)

KEY WORDS
cow; muscle

REFERENCE
Voyksner,R.D.; Smith,C.S.; Knox,P.C. Optimization and application of particle beam high-performance liquid chromatography/mass spectrometry to compounds of pharmaceutical interest, *Biomed.Environ.Mass.Spectrom.*, **1990**, *19*, 523–534.

Methylephedrine



Molecular formula: $C_{11}H_{17}NO$
Molecular weight: 179.26
CAS Registry No.: 552-79-4
Merck Index: 6145

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

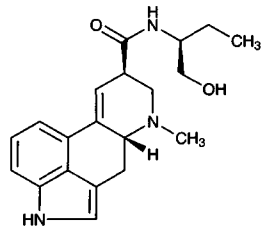
Retention time: 2.9

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Methylergonovine



Molecular formula: $C_{20}H_{25}N_3O_2$
Molecular weight: 339.44
CAS Registry No.: 113-42-8, 57432-61-8 (maleate)
Merck Index: 6147

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 1 mL 500 mM potassium carbonate, mix for 5 s, add 10 mL ethyl acetate, shake horizontally for 15 min, centrifuge at 700-1000 g for 5 min. Remove 8 mL of the upper organic phase and add it to 700 µL 50 mM phosphoric acid, shake horizontally for 15 min, centrifuge at 750 g for 2 min, discard the organic phase. Heat the aqueous phase at 50° in a vortex evaporator to remove residual organic solvent, inject a 400 µL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 µm Supelcosil LC-8
Mobile phase: MeCN:10 mM pH 7 potassium phosphate buffer 30:70
Column temperature: 60
Flow rate: 1
Injection volume: 400
Detector: F ex 315 em 440

CHROMATOGRAM
Retention time: 4.5
Limit of detection: 0.005 ng/mL
Limit of quantitation: 0.02 ng/mL

OTHER SUBSTANCES
Extracted: methysergide

KEY WORDS
plasma; protect from sunlight; pharmacokinetics

REFERENCE
Smith,H.T.; Molinaro,N.C. High-performance liquid chromatographic method for the determination of methysergide and methylergonovine in human plasma, *J.Chromatogr.*, **1988**, *424*, 416–423.

SAMPLE
Matrix: blood
Sample preparation: 0.1-0.7 mL Plasma or whole blood + 2.5 mL HCl/ammonia solution + 200 µL 100 nM ergometrine in water, mix, add to an Extrelut silica SPE cartridge, let stand for 20 min, elute with two 5 mL portions of ethyl acetate. Evaporate the eluate to dryness under a stream of nitrogen below 40°, reconstitute the residue in mobile phase, inject a 25-150 µL aliquot. (HCl/ammonia solution was 2 M HCl and 1 M ammonia mixed to a pH of 9.0.)

HPLC VARIABLES
Column: 250 mm long 5 µm Hypersil C18 ODS
Mobile phase: MeCN:10 mM ammonium carbonate 30:70
Flow rate: 2

Injection volume: 25-150
Detector: F ex 328 em 415

CHROMATOGRAM
Retention time: 3.0
Internal standard: ergonovine (ergometrine) (2.4)
Limit of detection: 0.6 nM

OTHER SUBSTANCES
Extracted: methysergide

KEY WORDS
rat; plasma; whole blood; SPE; pharmacokinetics

REFERENCE
Bredberg,U.; Paalzow,L. Pharmacokinetics of methysergide and its metabolite methylergometrine in the rat, *Drug Metab.Dispos.*, **1990**, *18*, 338–343.

SAMPLE
Matrix: formulations
Sample preparation: Injections. 1 mL Injection (200 μg/mL) + 300 mg NaCl + 200 μL 10% ammonia + 5 mL dichloromethane, shake vigorously for 10 min, let stand for a few min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 4 mL water, mix an aliquot with an equal volume of 30 μg/mL 17α-hydroxyprogesterone in MeOH, inject a 20 μL aliquot. Tablets. Weigh out amount of powdered tablets equivalent to about 200 μg compound, add 1 mL water, sonicate for 2 min, add 300 mg NaCl, add 200 μL 10% ammonia, add 5 mL dichloromethane, shake vigorously for 10 min, let stand for a few min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 4 mL water, mix an aliquot with an equal volume of 30 μg/mL 17α-hydroxyprogesterone in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 5 μm LiChrosorb RP-18
Mobile phase: MeCN:50 mM pH 3.5 acetate buffer 40:60 containing 1.5 mM triethylamine
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 9
Internal standard: 17α-hydroxyprogesterone (12)

OTHER SUBSTANCES
Simultaneous: benzyl alcohol, ergonovine
Noninterfering: ascorbic acid

KEY WORDS
injections; tablets

REFERENCE
Tokunaga,H.; Kimura,T.; Kawamura,J. Determination of ergometrine maleate and methylergometrine maleate in pharmaceutical preparations by high-performance liquid chromatography, *Chem.Pharm.Bull.(Tokyo)*, **1983**, *31*, 3988–3993.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.2

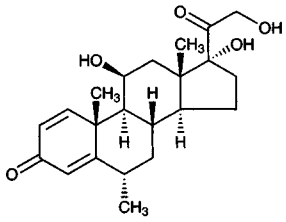
OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Methylprednisolone



Molecular formula: $C_{22}H_{30}O_5$
Molecular weight: 374.48
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 (suleptanate)
Merck Index: 6189
Lednicer No.: 1 193, 196

SAMPLE

Matrix: blood, urine
Sample preparation: Condition a 3 mL 500 mg Sep-Pak Vac C18 SPE cartridge with 3 mL MeOH and 3 mL water. 1 mL Serum or urine + 500 µL 200 mM pH 3.85 acetate buffer (serum only) + 400 µL 2.5 µM IS in mobile phase, mix, centrifuge. Add the supernatant to the SPE cartridge, wash with 3 mL acetone:water 20:80, 3 mL water, and 3 mL hexane. Elute with 3 mL diethyl ether into tubes containing 1 mL 200 mM NaOH, vortex, centrifuge. Dry the organic layer under a stream of nitrogen. Reconstitute the residue in 250 µL mobile phase, mix for 5 min. Inject a 60 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Spherex C18 (Phenomenex USA)
Mobile phase: MeOH:THF:water 3:25:72
Flow rate: 1.0
Injection volume: 60
Detector: UV 254

CHROMATOGRAM

Retention time: 21.0
Internal standard: fludrocortisone (15.9)
Limit of detection: 5 nM

OTHER SUBSTANCES

Extracted: 11-deoxycortisol, dexamethasone, hydrocortisone, prednisolone

KEY WORDS

serum; SPE

REFERENCE

McWhinney,B.C.; Ward,G.; Hickman,P.E. Improved HPLC method for simultaneous analysis of cortisol, 11-deoxycortisol, prednisolone, methylprednisolone, and dexamethasone in serum and urine, *Clin.Chem.*, **1996**, *42*, 979–981.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 245.2

CHROMATOGRAM
Retention time: 18.887

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil Ph (GL Science, Japan)
Mobile phase: MeCN:THF:buffer 21:4:75 (Buffer was 11.6 g ammonium acetate in 750 mL water, adjusted to pH 5.8 with acetic acid.)
Column temperature: 40
Flow rate: 1.6
Injection volume: 15
Detector: UV 254

OTHER SUBSTANCES
Simultaneous: methylprednisolone 17-suleptanate, PNU-675004

REFERENCE
Okamoto,H.; Mori,K.; Ohtsuka,K.; Ohuchi,H.; Ishii,H. Effect of ionic strength on solution of PNU-67590A, a micellar prodrug of methylprednisolone, *Pharm.Res.*, **1997**, *14*, 1181–1185.

SAMPLE
Matrix: urine
Sample preparation: Activate 3-mL 500 mg Bakerbond C18 cartridge with 2 mL MeOH and 2 mL water. Filter sample. Add 2 mL urine to the SPE cartridge, wash with two 2 mL portions of 25 mM borate buffer and with 200 mL/L acetone in water. Add 1 mL hexane and air-dry under reduced pressure for 4 min. Elute with two 1 mL portions of ethyl acetate. Dry the eluate under a stream of nitrogen and dissolve in 75 μL 400 mL/L MeOH, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm LiChrospher 100 C18
Mobile phase: MeCN:MeOH:water 3:43:54
Column temperature: 40
Flow rate: 1
Injection volume: 25
Detector: UV 242

CHROMATOGRAM
Retention time: 39.3-40.1
Internal standard: 6α-methylprednisolone

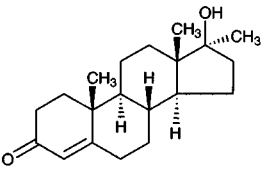
OTHER SUBSTANCES
Extracted: hydrocortisone

Simultaneous: metabolites, alprazolam, amlodipine, aspirin, carbamazepine, citalopram, cortisone, dexamethasone, digoxin, enalapril, ferrous sulfate, fluoxetine, furosemide, gabapentin, 5-hydroxyindoleacetic acid, lamotrigine, metyrapone, naproxen, oxazepam, oxcarbazepine, oxybutynin, phenobarbital, phenytoin, prednisone, prednisolone, spironolactone, valproic acid, vigabatrin, zopiclone
Noninterfering: octreotide
Interfering: corticosterone

KEY WORDS
SPE; methylprednisolone is IS

REFERENCE
Turpeinen,U.; Markkanen,H.; Välimäki,M.; Stenman,U.-H. Determination of urinary free cortisol by HPLC, *Clin.Chem.*, **1997**, *43*, 1386–1391.

Methyltestosterone



Molecular formula: $C_{25}H_{38}O_2$
Molecular weight: 302.46
CAS Registry No.: 58-18-4
Merck Index: 6206
Lednicer No.: 1 172, 4 11

SAMPLE
Matrix: formulations
Sample preparation: 1 Tablet + 4 mL 50 mM KH_2PO_4, rotate 15 min, add 2 mL 1 μg/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 × 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: 14.1

OTHER SUBSTANCES
Simultaneous: norgestrel, norethindrone, norethindrone acetate, 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: formulations
Sample preparation: Oils. 1 mL Sample + 25 mL MeOH:water 90:10, shake vigorously for 5 min, centrifuge, inject a 10 μL aliquot of the supernatant. Tablets. Grind a tablet to a fine powder, add 25 mL MeOH, sonicate for 5-10 min, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate. Suspensions (aqueous). Make up 5 mL to 50

mL with MeOH, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:water 75:25
Flow rate: 1.5
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: 8.0
Limit of detection: 5 μg/mL

OTHER SUBSTANCES
Simultaneous: calusterone, mesterolone (UV 210), norethandrolone, trenbolone acetate, benzyl benzoate, nandrolone acetate, testosterone acetate, stanozolol, oxymetholone, nandrolone propionate, methenolone acetate, testosterone propionate, aspirin, caffeine, formebolone, benzyl alcohol, testolactone, cortisone, fluoxymesterone, norethindrone, oxandrolone (UV 210), boldenone, ethisterone, methandrostenolone, nandrolone, norgestrel, testosterone, dehydroepiandrosterone (UV 210), mibolerone
Interfering: methandriol (UV 210), norethindrone acetate

KEY WORDS
oils; tablets; suspensions

REFERENCE
Walters,M.J.; Ayers,R.J.; Brown,D.J. Analysis of illegally distributed anabolic steroid products by liquid chromatography with identity confirmation by mass spectrometry or infrared spectrophotometry, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 904–926.

SAMPLE
Matrix: formulations
Sample preparation: Crush tablets, weigh out amount equivalent to 10 mg steroid, dissolve in 10 mL MeOH, sonicate for 15 min, filter. 1 mL Filtrate + 5 mL MeOH + 4 mL water, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: Gradient. MeOH:water from 70:30 to 100:0 over 15 min, maintain at 100:0 for 15 min.
Flow rate: 1
Injection volume: 25
Detector: UV 240

CHROMATOGRAM
Retention time: 10.8

OTHER SUBSTANCES
Simultaneous: boldenone, boldenone acetate, boldenone undecylenate, clostebol acetate, danazol (UV 280), fluoxymesterone, methandriol, methandriol-3-acetate, methandriol dipropionate, methandrostenolone, nandrolone, nandrolone decanoate, nandrolone phenylpropionate, nandrolone propionate, stanolone, stanozolol, testosterone, testosterone acetate, testosterone cypionate, testosterone enanthate, testosterone isobutyrate, testosterone propionate, testosterone undecanoate
Noninterfering: oxandrolone, oxymetholone, testosterone decanoate, testosterone isocaproate

KEY WORDS
tablets

REFERENCE

Lurie,I.S; Sperling,A.R.; Meyers,R.P. The determination of anabolic steroids by MECC, gradient HPLC, and capillary GC, *J.Forensic Sci.*, **1994**, *39*, 74–85.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 4 × 4 5 μm Spherisorb ODS-2
Column: 250 × 4 5 μm Spherisorb ODS-2
Mobile phase: MeOH:water 67:33
Flow rate: 1
Injection volume: 100
Detector: UV 238

CHROMATOGRAM

Retention time: 12.65

OTHER SUBSTANCES

Simultaneous: spironolactone

REFERENCE

Kaukonen,A.M.; Vuorela,P.; Vuorela,H.; Mannermaa,J.-P. High-performance liquid chromatography methods for the separation and quantitation of spironolactone and its degradation products in aqueous formulations and of its metabolites in rat serum, *J.Chromatogr.A*, **1998**, *797*, 271–281.

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: 18

OTHER SUBSTANCES

Simultaneous: prednisone, prednisolone, prednisolone succinate, hydrocortisone acetate, norethindrone, 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: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:water 60:40
Injection volume: 250
Detector: UV

CHROMATOGRAM

Retention time: 7

OTHER SUBSTANCES
Simultaneous: diethylstilbestrol, trenbolone, nandrolone, zeranol, dienestrol, medroxyprogesterone
Interfering: hexestrol

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: Inject an aliquot of a 100 μg/mL solution in MeOH.

HPLC VARIABLES
Guard column: 70 × 2.1 Whatman CO:Pell ODS
Column: 300 × 3.9 Bondex C18
Mobile phase: MeOH:water 70:30
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Simultaneous: boldenone, nandrolone, methandrostenolone, testosterone, danazol, fluoxymesterone

REFERENCE
Noggle,F.T.,Jr.; Clark,C.R.; DeRuiter,J. Liquid chromatographic and spectral analysis of the 17-hydroxy anabolic steroids, *J.Chromatogr.Sci.*, **1990**, *28*, 162–166.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 0.5 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN:water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 24.1

OTHER SUBSTANCES
Simultaneous: acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, diflunisal, doxapram, estrone, fluoxymesterone, mefenamic acid, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, testosterone, testosterone propionate, tranylcypromine, tripelennamine
Interfering: danthron

KEY WORDS
details for purification of triethylamine in paper

REFERENCE

Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 25 μg/mL solution in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Partisil 10 ODS-1
Mobile phase: MeOH:water 55:45
Column temperature: 40
Flow rate: 1.5
Detector: UV 240

CHROMATOGRAM
Retention time: k' 5.413

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), methylprednisolone acetate (UV 240), methylprednisolone (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: tissue
Sample preparation: 1 g Tissue + 10 mL Chloroform:MeOH 2:1, homogenize for 1 min (Polytron setting 5), filter, rinse tube with an additional 10 mL chloroform:MeOH, filter, combine filtrates, add 4 mL water, vortex for 1 min, centrifuge at 600 g for 10 min. Remove organic layer and dry it under air at 40°. Reconstitute with 200 μL MeOH, add to an activated Sep-Pak C18 cartridge, wash tube onto cartridge with 200 μL MeOH, elute with 5 mL each 0, 25, 50, 75, 100% MeOH, collect 5 mL fractions, inject a 100 μL aliquot of each fraction. (Elutes in 75% MeOH fraction.)

HPLC VARIABLES
Guard column: in line guard column
Column: 80 mm long 10 μm μBondapak C18 radially compressed
Mobile phase: MeCN:water 45:55
Flow rate: 3
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 6.15
Limit of quantitation: 1000 ng/g

OTHER SUBSTANCES
Simultaneous: testosterone

KEY WORDS
fish; muscle; tilapia aurea; SPE

REFERENCE
Goudie,C.A. Extraction of a synthetic androgen from fish muscle and quantitation by high performance liquid chromatography, *Steroids*, **1984**, *44*, 241–252.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize 1-5 g tissue in 10-50 mL chloroform:MeOH 2:1, add 0.2 volume water, centrifuge at 1800 g for 10 min, extract aqueous phase again with 2 volumes of chloroform, evaporate organic phases under vacuum, dissolve residues in MeCN, pass through a Lipidex 5000 column and a Sep Pak silica cartridge, inject an aliquot.

HPLC VARIABLES
Guard column: 25 × 3.8 5 μm ODS 2
Column: 150 × 4.6 5 μm Hypersil C8 plus 150 × 4.6 5 μm Nucleosil C18
Mobile phase: MeOH:water 75:25
Flow rate: 0.8
Injection volume: 50
Detector: F ex 340 em 470 following post-column reaction. The eluate from the column mixed with freshly prepared 0.6 mM nicotinamide-adenine dinucleotide (NAD) in 20 mM pH 9 pyrophosphate buffer pumped at 1.2 mL/min and flowed into a 950 μL static mixing chamber then through an immobilized enzyme reactor into the fluorescence detector. The Chrom Sep immobilized enzyme reactor was prepared by pumping 10 mM pH 7.8 KH_2PO_4 through it at 0.8 mL/min. Ten 100 μL samples of a solution of 25 U/mL 3α-hydroxysteroid dehydrogenase in 10 mM pH 7.8 KH_2PO_4 were injected onto the reactor which was then equilibrated with 10 mM pH 7.8 KH_2PO_4 for 20 min. The reactor was then attached to the chromatographic system.

CHROMATOGRAM
Retention time: 21.2 (5β-17-methyltestosterone), 22.6 (5α-17-methyltestosterone)
Limit of detection: 1 ng/g

KEY WORDS
immobilized enzyme reactor; fish; muscle; SPE

REFERENCE
Cravedi,J.P.; Delous,G. Use of an immobilized enzyme reactor for the analysis of residues of 17α-methyltestosterone in trout by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *564*, 461–467.

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: 9
Limit of detection: about 6 ng/mL

OTHER SUBSTANCES
Simultaneous: 17 β-trenbolone, zeranol, trans-diethylstilbestrol, medroxyprogesterone, nandrolone

KEY WORDS
cow

REFERENCE
Jansen,E.H.; Both-Miedema,R.; van Blitterswijk,H.; Stephany,R.W. Separation and purification of several anabolics present in bovine urine by isocratic high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *299*, 450–455.

SAMPLE
Matrix: urine
Sample preparation: Add 10 mL urine to a Supelclean LC-18 SPE tube at a flow rate of 2 mL/min, wash with 4 mL 25 mM sodium borate buffer, wash with 4 mL 40% MeOH, wash with 4 mL 20% acetone, elute with two 500 μL aliquots of 73% MeOH, evaporate under nitrogen at 40°, reconstitute with 1 mL mobile phase, inject a 200 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Microsorb silica
Mobile phase: Cyclohexane:ethyl acetate 40:60
Injection volume: 200
Detector: F ex 247 em 547, after post-column reaction with 30 mM $Tb(NO_3)_3$ in ethyl acetate using a 50 cm tightly coiled capillary tube to ensure mixing

CHROMATOGRAM
Retention time: 16
Limit of detection: 100 pg/mL

OTHER SUBSTANCES
Extracted: testosterone acetate, progesterone, bolasterone, testosterone

KEY WORDS
SPE; normal phase; post-column reaction

REFERENCE
Amin,M.; Harrington,K.; von Wandruszka,R. Determination of steroids in urine by micellar HPLC with detection by senstized terbium fluorescence, *Anal.Chem.*, **1993**, *65*, 2346–2351.

SAMPLE
Matrix: urine
Sample preparation: 5 mL Urine + 1 mL 200 mM pH 7.0 sodium phosphate buffer + 50 μL β-glucuronidase (E. coli K12, Boehringer Mannheim), heat at 55° for 1 h, cool to room temperature, add 1 g sodium bicarbonate:potassium carbonate 1:2, add 1 g sodium sulfate, add 5 mL n-pentane, shake for 20 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 50 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Hypersil BDS-C18
Mobile phase: Gradient. MeCN:1 mM phosphoric acid from 25:75 to 30:70 over 10 min, to 35:65 over 6.5 min, maintain at 35:65 for 11.5 min.
Column temperature: 40
Flow rate: 1.2
Injection volume: 20
Detector: UV 240

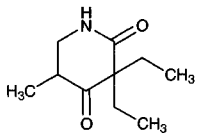
CHROMATOGRAM
Retention time: 25
Internal standard: methyltestosterone

OTHER SUBSTANCES
Extracted: epitestosterone, testosterone

KEY WORDS
methyltestosterone is IS

REFERENCE
Navajas,R.; Imaz,C.; Carreras,D.; García,M.; Pérez,M.; Rodríguez,C.; Rodríguez,A.F.; Cortés,R. Determination of epitestosterone and testosterone in urine by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *673*, 159–164.

Methyprylon



Molecular formula: $C_{10}H_{17}NO_2$
Molecular weight: 183.25
CAS Registry No.: 125-64-4
Merck Index: 6216

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

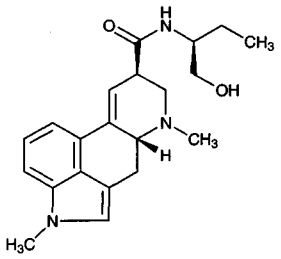
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tol-

metin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Methysergide



Molecular formula: $C_{21}H_{27}N_3O_2$
Molecular weight: 353.46
CAS Registry No.: 361-37-5, 129-49-7 (maleate)
Merck Index: 6217
Lednicer No.: 2 477

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 1 mL 500 mM potassium carbonate, mix for 5 s, add 10 mL ethyl acetate, shake horizontally for 15 min, centrifuge at 700-1000 g for 5 min. Remove 8 mL of the upper organic phase and add it to 700 μL 50 mM phosphoric acid, shake horizontally for 15 min, centrifuge at 750 g for 2 min, discard the organic phase. Heat the aqueous phase at 50° in a vortex evaporator to remove residual organic solvent, inject a 400 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-8
Mobile phase: MeCN:10 mM pH 7 potassium phosphate buffer 30:70
Column temperature: 60
Flow rate: 1
Injection volume: 400
Detector: F ex 315 em 440

CHROMATOGRAM
Retention time: 9
Limit of detection: 0.005 ng/mL
Limit of quantitation: 0.02 ng/mL

OTHER SUBSTANCES
Extracted: methylergonovine

KEY WORDS
plasma; protect from sunlight; pharmacokinetics

REFERENCE
Smith,H.T.; Molinaro,N.C. High-performance liquid chromatographic method for the determination of methysergide and methylergonovine in human plasma, *J.Chromatogr.*, **1988**, *424*, 416–423.

SAMPLE
Matrix: blood
Sample preparation: 0.1-0.7 mL Plasma or whole blood + 2.5 mL HCl/ammonia solution + 200 μL 100 nM ergometrine in water, mix, add to an Extrelut silica SPE cartridge, let stand for 20 min, elute with two 5 mL portions of ethyl acetate. Evaporate the eluate to dryness under a stream of nitrogen below 40°, reconstitute the residue in mobile phase, inject a 25-150 μL aliquot. (HCl/ammonia solution was 2 M HCl and 1 M ammonia mixed to a pH of 9.0.)

HPLC VARIABLES
Column: 250 mm long 5 μm Hypersil C18 ODS
Mobile phase: MeCN:10 mM ammonium carbonate 30:70
Flow rate: 2
Injection volume: 25-150
Detector: F ex 328 em 415

CHROMATOGRAM
Retention time: 7.0
Internal standard: ergonovine (ergometrine) (2.4)
Limit of detection: 0.6 nM

OTHER SUBSTANCES
Extracted: methylergonovine (methylergometrine)

KEY WORDS
rat; plasma; whole blood; SPE; pharmacokinetics

REFERENCE
Bredberg,U.; Paalzow,L. Pharmacokinetics of methysergide and its metabolite methylergometrine in the rat, *Drug Metab.Dispos.*, **1990**, *18*, 338–343.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.2

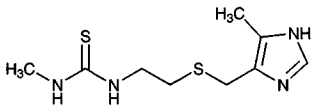
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide,

phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Metiamide



Molecular formula: $C_9H_{16}N_4S_2$
Molecular weight: 244.37
CAS Registry No.: 34839-70-8
Lednicer No.: 2 252

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' 0.50

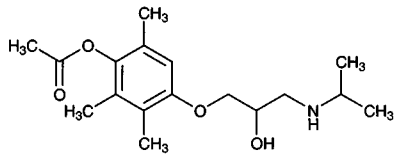
OTHER SUBSTANCES

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, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Metipranolol



Molecular formula: $C_{17}H_{27}NO_4$
Molecular weight: 309.41
CAS Registry No.: 22664-55-7, 36592-77-5 (HCl)
Merck Index: 6221
Lednicer No.: 5 17

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 281

CHROMATOGRAM

Retention time: 5.30
Limit of detection: <120 ng/mL

KEY WORDS

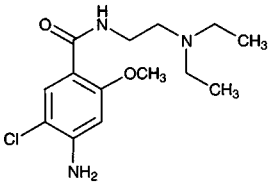
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide;

imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

Metoclopramide



Molecular formula: $C_{14}H_{22}ClN_3O_2$
Molecular weight: 299.80
CAS Registry No.: 364-62-5, 54143-47-6 (HCl monohydrate), 7232-21-5 (HCl)
Merck Index: 6226
Lednicer No.: 4 41

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 10 μg/mL quinidine sulfate in water + 100 μL 1 M NaOH + 5 mL chlorobutane:MeCN 90:10, shake thoroughly for 1 min, centrifuge at 2500 g for 5 min. Remove the organic phase and add it to 100 μL 100 mM HCl, mix for 1 min, centrifuge at 2500 g for 5 min, inject a 20 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: 250 × 4.55 5 μm Spherisorb CN
Mobile phase: MeCN:20 mM pH 3.0 KH_2PO_4 40:60
Flow rate: 2
Injection volume: 20
Detector: UV 275

CHROMATOGRAM
Retention time: 4.6
Internal standard: quinidine sulfate (5.8)
Limit of detection: 3 ng/mL

KEY WORDS
plasma

REFERENCE

Buss,D.C.; Hutchings,A.D.; Scott,S.; Routledge,P.A. A rapid liquid chromatographic method for the determination of metoclopramide in human plasma, *Ther.Drug Monit.*, **1990**, *12*, 293–296.

SAMPLE
Matrix: blood
Sample preparation: Add 10 μL 20 μg/mL oxaprotiline in MeOH to 990 μL plasma or serum. Inject 100 μL plasma or serum onto column A with mobile phase A and elute to waste, after 15 min elute column A onto column B with mobile phase B for 2 min. Remove column A from circuit and re-equilibrate it with mobile phase A for 5 min. Chromatograph on column B with mobile phase B.

HPLC VARIABLES
Column: A 20 × 4.6 10 μm Hypersil MOS C8; B 20 × 4.6 5 μm Hypersil CPS CN + 250 × 4.6 5 μm Nucleosil 100 CN

Mobile phase: A MeOH:water 5:95; B MeOH:MeCN:10 mM pH 6.8 potassium phosphate buffer 188:578:235
Flow rate: 1.5
Injection volume: 100
Detector: UV 214

CHROMATOGRAM
Retention time: 5.7
Internal standard: oxaprotiline (9.5)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Simultaneous: clozapine, fluvoxamine, doxepin, amitriptyline, clomipramine, fluoxetine, imipramine, norfluoxetine, nortriptyline, desipramine, maprotiline
Noninterfering: haloperidol, spiroperidol, pimozide, fluspirilene, trifluperidol, perazine, chlordiazepoxide, clobazam, diazepam, nordiazepam, flurazepam, lorazepam, nitrazepam, oxazepam, carbamazepine

KEY WORDS
plasma; serum; column-switching

REFERENCE
Härtter,S.; Wetzel,H.; Hiemke,C. Automated determination of fluvoxamine in plasma by column-switching high-performance liquid chromatography, *Clin.Chem.*, **1992**, *38*, 2082–2086.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 275

CHROMATOGRAM
Retention time: 3.92
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine;

lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, tissue

Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM

Retention time: 1.38
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES

Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, mianserin, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, trazodone, trihexyphenidyl, trimipramine, triprolidine

Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

Interfering: moclobemide, tranylcypromine, pentobarbital

KEY WORDS
serum; whole blood; liver

REFERENCE
McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Condition a 3 mL C8 Analytichem SPE cartridge with 1 volume (2.7 mL) MeOH and 1 volume buffer A, do not allow to dry. Mix 1 mL plasma + 100 μL 10 μg/mL amisulpride and 10 μg/mL alpiropride in 10 mM HCl + 1 mL buffer A, add to SPE cartridge, rinse the sample container with 1 mL buffer A and add the rinse to the SPE cartridge, wash with 1 volume water, wash with 2 mL buffer B, dry the column for 1 min, wash with 200 μL acetone, dry for 30 s, elute with 1 mL buffer C, add 50 μL buffer D, evaporate to dryness under a stream of air, reconstitute in 200 μL mobile phase, sonicate for 1 min, inject an aliquot. Urine. Connect a Baker 3 mL ion exchange quaternary aminesilicane-bonded silica gel SPE cartridge on top of a 3 mL Baker carboxylic acid-bonded silica gel SPE cartridge, condition with 1 volume (2.7 mL) buffer D, 1 volume of water, 1 volume of MeOH, and 1 volume of water. Mix 1 mL urine + 100 μL 10 μg/mL amisulpride and 10 μg/mL alpiropride in 10 mM HCl + 1 mL water, add to SPE cartridges, rinse the sample container with 2 mL water and add the rinse to the SPE cartridges, wash with 1 mL water, remove the top column, wash the bottom column with 1 volume of water and 2 volumes of MeOH, dry the column for 1 min, elute with 1 mL buffer D, evaporate the eluate to dryness under a stream of air at 45°, reconstitute in 200 μL mobile phase, sonicate for 1 min, inject an aliquot. (Buffer A was 10 mL triethylamine in 1 L water, pH adjusted to 7.00 with acetic acid. Buffer B was MeOH:water 20:80. Buffer C was 10 mL triethylamine + 7 mL acetic acid in 1 L MeOH. Buffer D was 2.10 mL concentrated HCl in 250 mL MeOH (100 mM).)

HPLC VARIABLES
Guard column: 10 cm long Chrompack reverse-phase pellicular material
Column: 250 × 4.6 10 μm LiChrosorb RP-8
Mobile phase: MeCN:MeOH:buffer 160:80:760 (Buffer was 10 mL triethylamine + 760 mL water adjusted to pH 6.8 with acetic acid (about 4.2 mL).)
Flow rate: 2
Injection volume: 175
Detector: UV 230

CHROMATOGRAM
Retention time: 7.1
Internal standard: amisulpride (4.9), alpiropride (6.0)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: alizapride, aspirin, theophylline, acetaminophen, caffeine, isosorbide-5-mononitrate, nitrofurantoin, codeine, acenocoumarol, carbamazepine, nitrazepam, clonazepam
Noninterfering: indomethacin, orphenadrine, furosemide, cisplatin, amitriptyline, isosorbide dinitrate, propranolol

KEY WORDS
plasma; SPE

REFERENCE
de Jong,A.P.; Wittebrood,A.J.; du Châtinier,W.M.; Bron,J. Liquid chromatographic analysis of alizapride and metoclopramide in human plasma and urine using solid-phase extraction, *J.Chromatogr.*, **1987**, *419*, 233–242.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 250 μL Plasma + 300 μL 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 μL mobile phase, inject a 50 μL aliquot. Urine. Dilute 100 μL urine + 75 μL 2 mg/mL metoclopramide to 10 mL with water, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 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: 8
Internal standard: metoclopramide

OTHER SUBSTANCES
Simultaneous: oxazepam, desmethyldiazepam, chlorodiazepam, diazepam, theophylline, theobromine, acetaminophen, chlorpromazine, ranitidine

KEY WORDS
plasma; metoclopramide is IS

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: Condition a 100 mg Isolute MF C18 (International Sorbent Technology) SPE cartridge with 2 mL MeCN, 2 mL water, and 500 μL buffer. Mix 1 mL urine and 25 mL water. Mix 1 mL diluted urine or plasma with 1 mL buffer, vortex, add to the SPE cartridge, wash with 1 mL buffer, wash with 1 mL MeCN:water 30:70, wash with 80 μL MeOH, air dry for 30 s, elute with 500 μL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 250 μL mobile phase, inject a 100 μL aliquot. (Prepare buffer by dissolving 6.18 g boric acid and 7.46 g KCl in 1 L water. Mix 500 mL of this solution with 185 mL 100 mM NaOH, pH 9.)

HPLC VARIABLES
Guard column: 20 × 4.6 40 μm Pelliguard Si
Column: 250 × 4.6 Chiralpak AS amylose carbamate (J.T. Baker)
Mobile phase: n-Heptane:EtOH:diethylamine 70:29.8:0.2
Column temperature: 28
Flow rate: 0.5
Injection volume: 100
Detector: F ex 280 em 370

CHROMATOGRAM
Retention time: 11.5
Internal standard: metoclopramide

OTHER SUBSTANCES
Extracted: amisulpride

KEY WORDS
mobile phase reservoir at 34°; plasma; SPE; metoclopramide is IS

REFERENCE
Ascalone,V.; Ripamonti,; Malavasi,B. Stereospecific determination of amisulpride, a new benzamide derivative, in human plasma and urine by automated solid-phase extraction and liquid chromatography on a chiral column. application to pharmacokinetics, *J.Chromatogr.B*, **1996**, *676*, 95–105.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 213.4

CHROMATOGRAM
Retention time: 9.915

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with water.

HPLC VARIABLES
Column: Waters C18 column (PN 86344)
Mobile phase: Sodium acetate 2.7 g in 500 mL water, 500 mL MeCN, 1 mL glacial acetic acid, and 2 mL tetramethylammonium hydroxide in methanol (1:5)
Flow rate: 1.5
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 5.5

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

HPLC VARIABLES
Column: 250 × 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: 7.9
Limit of detection: 49 ng/mL

OTHER SUBSTANCES
Simultaneous: ondansetron

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: Dilute 1 mL formulation to 10 mL with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 μBondapak phenyl
Mobile phase: MeCN:MeOH:0.01% acetic acid:0.005 N sulfonic acid 20:15:40:25
Flow rate: 1
Detector: UV 230

CHROMATOGRAM
Retention time: 10.0

OTHER SUBSTANCES
Simultaneous: fluorouracil

KEY WORDS
injections; 5% dextrose; stability-indicating

REFERENCE
Wang,D.-P.; Chang,L.-C.; Lee,D.K.T.; Wong,C.-Y. Stability of fluorouracil-metoclopramide hydrochloride admixture, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 98–99.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 25-75 μL aliquot of an aqueous solution.

HPLC VARIABLES
Guard column: 25 × 4 5 μm Bondapak C18
Column: 150 × 3.9 5 μm Bondapak C18
Mobile phase: MeCN:10 mM pH 5.5 sodium phosphate buffer containing 5 mM triethylamine 17:83
Flow rate: 1.2
Injection volume: 25-75
Detector: UV 230

CHROMATOGRAM
Retention time: 4.4

OTHER SUBSTANCES
Simultaneous: tramadol

REFERENCE
Zaghloul,I.Y.; Radwan,M.A. High performance liquid chromatographic determination of tramadol in pharmaceutical dosage forms, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 779–787.

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: 5.3

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Centrifuge.

HPLC VARIABLES

Column: 100 × 5 4 μm Novapack radial compression RCM 8x10
Mobile phase: MeCN:buffer 60:40 (Buffer was 10 mM KH_2PO_4, pH 4.5.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 274

OTHER SUBSTANCES

Also analyzed: alizapride, bromopride, clebopride, domperidone, metopimazine

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 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: 7.92 (A), 4.26 (B)

OTHER SUBSTANCES

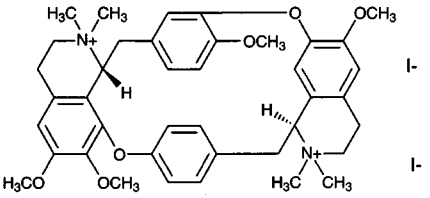
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, 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, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, 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, pro-

pantheline, 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, trifluopromazine, 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.

Metocurine iodide



Molecular formula: $C_{40}H_{48}I_2N_2O_6$
Molecular weight: 906.64
CAS Registry No.: 7601-55-0
Merck Index: 6227

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond Elut C18 SPE cartridge with 2 column volumes of THF, 2 volumes of MeOH, and 2 volumes of water. 1 mL Plasma + 100 μL 5 μg/mL D-tubocurarine chloride in 10 mM HCl, add to the SPE cartridge, wash with 2 volumes of water, elute with 250 μL mobile phase. Evaporate the eluate and reconstitute with 100 μL mobile phase, vortex, centrifuge at 12800 g for 5 min, inject an aliquot.

HPLC VARIABLES
Guard column: 10 μm CN Guard-Pak (Waters)
Column: 10 μm Radial-Pak CN (Waters)
Mobile phase: MeCN:MeOH:water:1 M pH 2.5 dibutylamine phosphate 40:10:10:1
Flow rate: 2.4
Detector: UV 204

CHROMATOGRAM
Retention time: 13.2
Internal standard: D-tubocurarine chloride (5.9)
Limit of quantitation: 25 ng/mL

KEY WORDS
SPE; plasma; pharmacokinetics

REFERENCE
Avram,M.J.; Shanks,C.A. Determination of D-tubocurarine chloride or metocurine iodide in human plasma by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1984**, *306*, 398–404.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Plasma + 250 μL picric acid (1:50 dilution of saturated picric acid solution) + 250 μL tubocurarine solution + 250 μL water + 2.5 mL dichloromethane:isopropanol 85:15, vortex for 15 s, centrifuge at 1500 g for 10 min. Remove the organic phase and evaporate it to dryness at 40° under a stream of nitrogen, reconstitute the residue in 150-250 μL MeCN: water 40:60, centrifuge at 1500 g for 4 min, inject a 20-100 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 μPorasil
Mobile phase: MeCN:2 mM sulfuric acid 50:50
Flow rate: 2
Injection volume: 20-100
Detector: UV 210

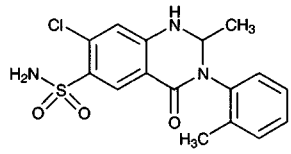
CHROMATOGRAM
Retention time: 5.3
Internal standard: tubocurarine (3.7)
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Also analyzed: atracurium, alcuronium

KEY WORDS
plasma

REFERENCE
Bjorksten,A.R.; Beemer,G.H.; Crankshaw,D.P. Simple high-performance liquid chromatographic method for the analysis of the non-depolarizing neuromuscular blocking drugs in clinical anaesthesia, *J.Chromatogr.*, **1990**, *533*, 241–247.

Metolazone



Molecular formula: $C_{16}H_{16}ClN_3O_3S$
Molecular weight: 365.84
CAS Registry No.: 17560-51-9
Merck Index: 6231
Lednicer No.: 2 384

SAMPLE
Matrix: blood
Sample preparation: Clean an Amalytichem C2 ethyl sorbent cartridge with 1 mL MeCN and 1 mL buffer. 25 μL Plasma + 1 mL buffer, add to cartridge, wash with 1 mL buffer, blow dry with nitrogen for 1 min, elute cartridge directly on to 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 × 4.6 30 μm C18 Alltech pellicular packing
Column: 150 × 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: 8.3
Internal standard: metolazone

OTHER SUBSTANCES
Noninterfering: bumetanide, chlorothiazide, hydrochlorothiazide, chlorthalidone, ibuprofen, acetaminophen, salicylic acid

KEY WORDS
plasma; SPE; metolazone is IS

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: Condition a C2 ethyl SPE cartridge (Analytichem) with 1.5 mL MeCN:MeOH 50:50 and 1.5 mL buffer. 500 μL Whole blood + 1.5 mL water, vortex for 15 s, centrifuge at 13000 g for 5 min. 250 μL Plasma or diluted whole blood + 25 μL 1 μg/mL IS in water + 250 μL buffer, add to the SPE cartridge, wash with 1 mL MeOH:buffer 10:90, wash with 1 mL hexane, purge with nitrogen for 1 min, elute the contents of the SPE cartridge onto the column with mobile phase. (Buffer was 25 mM (?) K_2HPO_4 adjusted to pH 7.0 with concentrated phosphoric acid.)

HPLC VARIABLES

Guard column: 30 mm long 40-50 μm pellicular C18
Column: 100 × 4.6 3 μm Spherisorb ODS C18
Mobile phase: MeCN:KH_2PO_4 30:70 adjusted to pH 3.0 with concentrated phosphoric acid
Flow rate: 1
Detector: F ex 235 em 410

CHROMATOGRAM

Retention time: 5.83
Internal standard: 7-chloro-1,2,3,4-tetrahydro-2-methyl-3-(3-methylphenyl)-4-oxo-6-quinazolinesulfonamide (7.40)
Limit of detection: 0.02 ng/mL
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES

Noninterfering: bumetanide, furosemide, hydrochlorothiazide, ibuprofen, indomethacin

KEY WORDS

plasma; whole blood; protect from light; SPE

REFERENCE

Farthing,D.; Sica,D.A.; Fakhry,I.; Gehr,T.W.B. Novel high-performance liquid chromatographic method using solid-phase on-line elution for determination of metolazone in plasma and whole blood, *J.Chromatogr.B*, **1994**, *653*, 171–176.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a solution in mobile phase, inject a 30 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spherisorb C6
Mobile phase: MeOH:water 40:60
Flow rate: 1.4
Injection volume: 30
Detector: UV 254

CHROMATOGRAM

Retention time: 7.8

OTHER SUBSTANCES

Simultaneous: acetaminophen, aldosterone, allopurinol, amitriptyline, caffeine, calcitriol, cephalothin, chlordiazepoxide, chlorothiazide, corticosterone, cortisone, dexamethasone, diazepam, ephedrine, ethinyl estradiol, furosemide, hydrocortisone, ibuprofen, imipramine, indomethacin, mechlorethamine, methylprednisone, nandrolone, naproxen, phenacetin, phenobarbital, phenytoin, prednisolone, prednisone, probenecid, progesterone, propranolol, sulfasalazine, testosterone, theophylline, vincristine

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: 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), 5.16 (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, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

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 + 2 mL 1 M pH 4.1 NaH_2PO_4 + 4 mL ethyl acetate, vortex for 2 min, centrifuge at 1500 g for 5 min. Remove the organic phase and add it to 5 mL 100

mM pH 7.5 Na_2HPO_4, vortex for 2 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL MeCN:10 mM pH 3.0 phosphate buffer, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm LiCHrosorb RP-18
Mobile phase: Gradient. MeCN:10 mM pH 3.0 phosphate buffer 10:90 for 1.5 min then to 35:65 over 2 min
Column temperature: 50
Flow rate: 1.5
Injection volume: 5
Detector: UV 271

CHROMATOGRAM
Retention time: 5.3
Limit of quantitation: 1000 ng/mL

OTHER SUBSTANCES
Extracted: chlorothiazide, hydrochlorothiazide, quinethazone, chlorthalidone, clopamide, methyclothiazide, furosemide, mefruside, bendroflumethiazide, cyclopenthiazide, bumetanide
Simultaneous: indapamide, clorexolone, ethacrynic acid
Noninterfering: aspirin, albuterol, allopurinol, alprenolol, atenolol, captopril, carbimazole, clonidine, coloxyl, danthron, diazepam, digoxin, doxepin, glibenclamide, hydralazine, indomethacin, labetalol, metformin, methyldopa, metoprolol, mianserin, minoxidil, nifedipine, nitrazepam, oxazepam, oxprenolol, pindolol, prazosin, propranolol, senokot, theophylline, trifluoperazine

REFERENCE
Fullinfaw,R.O.; Bury,R.W.; Moulds,R.F.W. Liquid chromatographic screening of diuretics in urine, *J.Chromatogr.*, **1987**, *415*, 347–356.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 0.5 g solid buffer I (pH 5-5.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and vortex it with 2 mL 5% aqueous lead acetate for 10 s, centrifuge at 600 g for 5 min, remove and keep organic phase. 2 mL Urine + 0.5 g solid buffer II (pH 9-9.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and combine it with previous organic layer. Evaporate to dryness at 50° under a stream of nitrogen, reconstitute in 300 μL 50 μg/mL β-hydroxyethyltheophylline in MeOH, inject 5 μL aliquot. (Solid buffer I was KH_2PO_4:Na_2HPO_4 99:1, solid buffer II was $NaHCO_3$:K_2CO_3 3:2.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm HP Hypersil ODS (A) or HP LiChrosorb RP-18 (B)
Mobile phase: Gradient. MeCN:buffer from 15:85 at 2 min to 80:20 at 20 min (Buffer was 50 mM NaH_2PO_4 containing 16 mM propylamine hydrochloride, adjusted to pH 3 with concentrated phosphoric acid.)
Flow rate: 1
Injection volume: 5
Detector: UV 230, UV 275

CHROMATOGRAM
Retention time: 12.16 (A), 12.3 (B)
Internal standard: β-hydroxyethyltheophylline (3.7 (A), 4.4 (B))
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Extracted: amiloride, acetazolamide, chlorothiazide, hydrochlorothiazide, quinethazone, triamterene, flumethiazide, hydroflumethiazide, chlorthalidone, dichlorphenamide, trichloromethiazide, methyclothiazide, benzthiazide, cyclothiazide, polythiazide, bendroflumethiazide, ethacrynic acid, bumetanide, probenecid, spironolactone, canrenone

Noninterfering: acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil
Interfering: furosemide

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: Mix urine by inversion, vortex for 15 s. 100 µL Urine + 300 µL water, mix, inject a 40 µL aliquot.

HPLC VARIABLES

Guard column: 30 mm long 40-50 µm pellicular C18
Column: 150 × 4.6 5 µm Nucleosil C18
Mobile phase: MeCN:KH_2PO_4 35:65 adjusted to pH 3.0 with concentrated phosphoric acid
Flow rate: 1
Injection volume: 40
Detector: F ex 240 em 450

CHROMATOGRAM

Retention time: 7.1
Limit of detection: 4.2 ng/mL

OTHER SUBSTANCES

Simultaneous: bumetanide, furosemide, salicylic acid
Noninterfering: acetaminophen, chlorothiazide, hydrochlorothiazide, ibuprofen

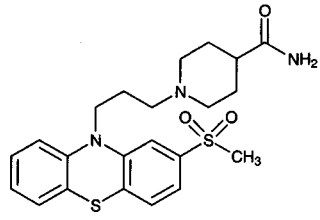
KEY WORDS

protect from light; pharmacokinetics

REFERENCE

Farthing,D.; Fakhry,I.; Gehr,T.W.; Sica,D.A. Quantitation of metolazone in urine by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1990**, *534*, 228–232.

Metopimazine



Molecular formula: $C_{22}H_{27}N_3O_3S_2$
Molecular weight: 445.61
CAS Registry No.: 14008-44-7
Merck Index: 6233
Lednicer No.: 1 153

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

Noninterfering: acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil
Interfering: furosemide

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: Mix urine by inversion, vortex for 15 s. 100 µL Urine + 300 µL water, mix, inject a 40 µL aliquot.

HPLC VARIABLES

Guard column: 30 mm long 40-50 µm pellicular C18
Column: 150 × 4.6 5 µm Nucleosil C18
Mobile phase: MeCN:KH_2PO_4 35:65 adjusted to pH 3.0 with concentrated phosphoric acid
Flow rate: 1
Injection volume: 40
Detector: F ex 240 em 450

CHROMATOGRAM

Retention time: 7.1
Limit of detection: 4.2 ng/mL

OTHER SUBSTANCES

Simultaneous: bumetanide, furosemide, salicylic acid
Noninterfering: acetaminophen, chlorothiazide, hydrochlorothiazide, ibuprofen

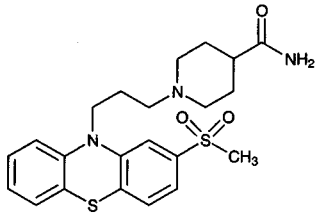
KEY WORDS

protect from light; pharmacokinetics

REFERENCE

Farthing,D.; Fakhry,I.; Gehr,T.W.; Sica,D.A. Quantitation of metolazone in urine by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1990**, *534*, 228–232.

Metopimazine



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SAMPLE

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Sample preparation: Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

HPLC VARIABLES

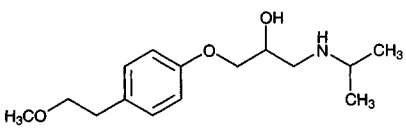
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.1

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Metoprolol



Molecular formula: $C_{15}H_{25}NO_3$
Molecular weight: 267.37
CAS Registry No.: 37350-58-6, 56392-17-7 (tartrate), 119637-66-0 (fumarate), 98418-47-4 (succinate), 37350-58-6 (tartrate)
Merck Index: 6235
Lednicer No.: 2 109

SAMPLE
Matrix: blood
Sample preparation: Add 1.5 mL MeCN to 500 μL serum, centrifuge, evaporate the supernatant to dryness, redissolve the residue in 200 μL water. Inject onto column A, wash with MeCN:

water 10:90 or MeOH:water 20:80 for 20 min, backflush the contents of column A onto column B with mobile phase, elute with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 25 × 4 25 μm pore diameter 6 nm LiChrospher RP-18 ADS (Merck); B 125 × 4 5 μm endcapped LiChroCART HPLC-cartridge RP-18 (Merck)
Mobile phase: MeCN:50 mM pH 4 K_3PO_4 buffer 27:73
Column temperature: 40
Flow rate: 1
Injection volume: 200
Detector: UV 242, UV 230

CHROMATOGRAM

Retention time: 3.0

OTHER SUBSTANCES

Extracted: celiprolol, tiracizine, talinolol, oxprenolol, metabolites

KEY WORDS

serum; column-switching

REFERENCE

Oertel,R.; Richter,K.; Gramatté,T.; Kirch,W. Determination of drugs in biological fluids by high-performance liquid chromatography with on-line sample processing, *J.Chromatogr.A*, **1998**, *797*, 203–209.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 50 μL 200 μg/mL (R)-(-)-flecainide in water + 1 mL pH 11 borate buffer, mix, add 5 mL diisopropyl ether (Caution! Diisopropyl ether readily forms explosive peroxides!), shake on a reciprocal shaker for 15 min, centrifuge at -10° at 4000 rpm, freeze in dry ice/acetone. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 100 μL 10 mM HCl, adjust pH to 11 with 200 μL borate buffer, add 50 μL 100 μg/mL (+)-4-(6-methoxy-2-naphthyl)-2-butyl chloroformate in MeCN, mix, let stand at room temperature for 1 h, add 3 mL dichloromethane, swirl mix for 30 s. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 100 μL n-hexane:MeOH 100:6, inject a 20 μL aliquot. (Synthesis of (+)-4-(6-methoxy-2-naphthyl)-2-butyl chloroformate is as follows. Slowly add 2 mmoles 4-(6-methoxy-2-naphthyl)-2-butanone (nabumetone) in 20 mL anhydrous diethyl ether to 0.55 mmoles lithium aluminum hydride in anhydrous diethyl ether, when the reaction is complete (monitor by TLC) cautiously add water, add 6 M HCl, remove the organic layer. Extract the aqueous layer 3 times with 10 mL portions of diethyl ether. Combine the organic layers and dry them over anhydrous sodium sulfate, evaporate to dryness to obtain racemic 4-(6-methoxy-2-naphthyl)-2-butanol. Dissolve 0.5 mmole 4-(6-methoxy-2-naphthyl)-2-butanol in 20 mL dry dichloromethane, add 0.55 mmole dry pyridine, stir, add 1 mmole (1S)-(-)-camphanoyl chloride ((1S)-(-)-camphanic chloride) in small portions, stir at room temperature overnight, wash 3 times with 1 M HCl, wash with water, wash with saturated sodium bicarbonate solution, wash with water until neutral. Dry the organic layer over anhydrous magnesium sulfate, evaporate to dryness to obtain the camphanoyl ester. Purify by preparative HPLC (250 × 50 (R)-DNBPG (J.T. Baker), n-hexane:isopropanol 70:30, 65 mL/min, UV 220) to obtain diastereomerically pure camphanoyl esters. Add 5 mmole diastereomerically pure camphanoyl ester dissolved in 30 mL dry diethyl ether dropwise under argon to 2.5 mmoles lithium aluminum hydride stirred in diethyl ether at 0°, stir at room temperature for 1 h, stir in an ice bath, cautiously add 10% aqueous ammonium chloride, remove the organic layer, extract the aqueous layer three times with diethyl ether. Combine the organic layers and dry them over anhydrous magnesium sulfate, evaporate to dryness. Dissolve 1 g in 10 mL dichloromethane, purify by flash chromatography on a 500 × 40 column filled with 200 g 40-63 μm silica gel 60 using dichloromethane:MeOH 99.5:0.5 at 45 mL/min to obtain enantiomerically pure 4-(6-methoxy-2-naphthyl)-2-butanol. Dissolve 0.5 mmole (+)-4-(6-methoxy-2-naphthyl)-2-butanol and 0.5 mmole triethylamine in 10 mL dry toluene, cool to 0°, stir, add 1 mL 20% phosgene in dry toluene, stir for 4 h, filter, evaporate under reduced pressure to obtain (+)-4-(6-methoxy-2-naphthyl)-2-butyl chloroformate as an oil.)

HPLC VARIABLES

Guard column: 20 × 4 6 μm Ultrasep ES 100 (Bischoff, Germany)

Column: 125 × 3 3 μm Nucleosil 120
Mobile phase: n-Hexane:MeOH 100:0.4
Flow rate: 1
Injection volume: 20
Detector: UV 230, F ex 270 em 350

CHROMATOGRAM
Retention time: 24.5 ((S)-(-)), 26.5 ((R)-(+))
Internal standard: (R)-(-)-flecainide (37.5)
Limit of detection: 0.9 ng/mL
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, flecainide, metoprolol, mexiletine, propafenone, propranolol, tocainide

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KEY WORDS
derivatization; plasma; chiral; pharmacokinetics; normal phase; protect from light

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REFERENCE
Büschges,R.; Devant,R.; Mutschler,E.; Spahn-Langguth,H. 4-(6-Methoxy-2-naphthyl)-2-butyl chloroformate enantiomers: New reagents for the enantiospecific analysis of amino compounds in biogenic matrices, *J.Pharm.Biomed.Anal.*, **1996**, *15*, 201–220.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 10.722

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk

Sample preparation: Dissolve 10 μmole compound (as free base or hydrochloride) in 500 μL MeCN, add 250 μL 5% sodium carbonate (for hydrochlorides only), add 500 μL 100 mM reagent in MeCN, vortex for 1 min, heat at 60° for 2 h, add 100 μmole L-proline, heat at 60° for 30 min. Remove a 100 μL aliquot and dilute it with mobile phase, neutralize with acetic acid, inject a 10 μL aliquot. Prepare the reagent ((R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate) as follows. Add 0.7 mL carbon disulfide to 6 mL (1R,2R)-(-)-1,2-diaminocyclohexane, 12 mL water, and 12 mL EtOH, heat the oil bath to 80°, add 2.8 mL carbon disulfide dropwise (making sure that the product does not start to precipitate), when addition is complete reflux for 1 h, acidify with 500 μL 5 M HCl, reflux for 12 h, cool, filter, wash the solid with a little cold EtOH to give trans-4,5-tetramethyleneimidazolidine-2-thione as a white fluffy solid (mp 148-150°) (Tetrahedron 1993, 49, 4419). Stir 7.97 g 3,5-dinitrobenzoyl chloride in 30 mL dichloroethane at 50°, add a solution of 6 g trans-4,5-tetramethyleneimidazolidine-2-thione in 120 mL dichloroethane containing a catalytic amount of 4-(dimethylamino)pyridine over 15 min, reflux for 2 h, remove the crystals of (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate by filtration, evaporate the filtrate to dryness and dissolve the residue in 60 mL dichloroethane, reflux for 16 h to obtain more (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate (mp >250°, $[\alpha]_{546}$ = -133° (c = 1) in MeCN).

HPLC VARIABLES

Column: 125 × 4 5 μm Lichrospher 60 RP Select B
Mobile phase: MeCN:20 mM ammonium acetate 55:45
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: k' 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: perfusate
Sample preparation: Mix 1 mL perfusate with 50 μL 6.46 μM nadolol in water and inject a 50 μL aliquot.

HPLC VARIABLES

Column: 100 × 8 4 μm NovaPak C18
Mobile phase: MeCN:triethylamine:water 9:0.3:91, adjusted to pH 3.0 with orthophosphoric acid
Flow rate: 3.0
Injection volume: 50
Detector: F ex 224 em no emission filter

CHROMATOGRAM

Retention time: 15.5
Internal standard: nadolol (5.55)

OTHER SUBSTANCES

Simultaneous: metabolites

KEY WORDS

liver; rat; pharmacokinetics

REFERENCE
Wang,B.; Semple,H.A. Inhibition of metoprolol metabolism by amino acids in perfused rat livers. Insights into the food effect?, *Drug Metab.Dispos.*, **1997**, *25*, 287–295.

SAMPLE
Matrix: perfusate
Sample preparation: Dilute perfusate 101 times with water and inject a 10 μL aliquot.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:water:acetic acid 19:80:1 containing 625 nM 1-heptane-sulfonic acid
Flow rate: 2
Injection volume: 10
Detector: F ex 225 em300

CHROMATOGRAM
Retention time: 8.1
Limit of quantitation: 300 ng/mL

OTHER SUBSTANCES
Extracted: atenolol

KEY WORDS
rat

REFERENCE
Lindahl,A.; Krondahl,E.; Grudén,A.-C.; Ungell,A.-L.; Lennernäs,H. Is the jejunal permeability in rats age-dependent?, *Pharm.Res.*, **1997**, *14*, 1278–1281.

SAMPLE
Matrix: solutions
Sample preparation: Inject 15 or 50 μL aliquot.

HPLC VARIABLES
Guard column: 5 μm LiChrospher 60 RP-Select B
Column: 125 × 4 5 μm LiChrospher 60 RP-Select B
Mobile phase: MeCN:MeOH:30 mM pH 4.5 phosphate buffer 10:20:70 (A) or 20:47:33 (B)
Flow rate: 1.5(A), 2 (B)
Injection volume: 50 (A), 15 (B)
Detector: UV 223

REFERENCE
Galia,E.; Nicolaides,E.; Hörter,D.; Löbenberg,R.; Reppas,C.; Dressman,J.B. Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs, *Pharm.Res.*, **1998**, *15*, 698–705.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 bidentate C18 silane column (Preparation is as follows. Reflux 60 g 7 μm Zorbax PSM300 silica in 600 mL 75 ppm HF in water for 72 h (Caution! HF is highly toxic!), wash with 1.5 L water, wash with 500 mL acetone, dry overnight under vacuum (30 in. Hg). Add to 570 mL water boil for 10 h, cool to room temperature, wash with 500 mL acetone, dry overnight at 110° under vacuum (30 in. Hg). Heat 6 g of this material at 110° under vacuum (30 in. Hg) and place it in a dry nitrogen atmosphere. Add 60 mL dry xylene, 240 μL pyridine, and 4.9 mL dichlorodimethyldioctadecyldisiloxane (?) (Petrarch Systems, Bristol, PA). Reflux under nitrogen for 80 h, cool, wash with 300 mL toluene, 300 mL dichloromethane, 300 mL MeOH, 300 mL MeOH:water 50:50, and 300 mL acetone. Dry at 110° under vacuum (30 in. Hg overnight) (cf. US Pat. 4 746 572).)
Mobile phase: MeCN:17 mM pH 11 K_3PO_4 buffer 50:50
Column temperature: 40

Flow rate: 1
Injection volume: 5
Detector: not given

CHROMATOGRAM
Retention time: 2.4

OTHER SUBSTANCES
Simultaneous: pindolol

REFERENCE
Kirkland,J.J.; van Straten,M.A.; Claessens,H.A. Reversed-phase high-performance liquid chromatography of basic compounds at pH 11 with silica-based column packings, *J.Chromatogr.A*, **1998**, *797*, 111–120.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 40:60 containing 100 mM KCl
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 275

OTHER SUBSTANCES
Also analyzed: disopyramide, lidocaine

REFERENCE
Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE
Matrix: solutions
Sample preparation: 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 1079, 28, 125) in 30 mL dry dioxane (Caution! Dioxane is a carcinogen!) dropwise to a mixture of 2.2 g 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride) in 20 mL dry dioxane stirred at 0°, add 3 g anhydrous sodium carbonate at room temperature, stir, filter, evaporate to give a colorless solid. Dissolve 8.3 g of this solid in 30 mL dry dioxane, add 2 g N-(2-aminoethyl)-3-aminopropyltrimethoxysilane, add 1.5 g anhydrous sodium carbonate, reflux with stirring for 40 h, filter, add 3 g dried 10 μm LiChrosorb Si 100, reflux with slow stirring for 10 h, cool, filter. Wash the solid with dioxane, MeOH, and diethyl ether, dry under reduced pressure (J.Chromatogr. 1984, 292, 427).)
Mobile phase: Hexane:1,2-dichloroethane: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 KH_2PO_4 adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 0.6-1
Injection volume: 10-100
Detector: UV 270

OTHER SUBSTANCES
Also analyzed: 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

Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

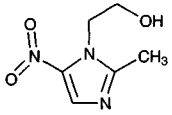
CHROMATOGRAM
Retention time: k' 0.74

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

Metronidazole



Molecular formula: $C_6H_9N_3O_3$
Molecular weight: 171.16
CAS Registry No.: 443-48-1, 69198-10-3 (HCl), 13182-89-3 (benzoate), 73334-05-1 (phosphate)
Merck Index: 6242
Lednicer No.: 1 240

SAMPLE
Matrix: blood
Sample preparation: Prepare a 250 × 5 column filled with 1.5 g Extrelut. 1 mL Serum + 100 μL 100 μg/mL ornidazole in MeOH:water 1:1 + 1 mL 1 M Na_2HPO_4, shake for 5 s, add to column, After 15 min elute with 10 mL dichloromethane, evaporate the eluate to dryness in a stream of nitrogen, redissolve residue in 100 μL MeOH:water 1:1, shake for 30 s, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 100 × 2.1 5 μm MOS-Hypersil RP-8
Mobile phase: MeOH:10 mM KH_2PO_4 30:70
Flow rate: 0.2
Injection volume: 5
Detector: UV 318

CHROMATOGRAM
Retention time: 2.45
Internal standard: ornidazole (3.36)

Limit of detection: 30 ng/mL

KEY WORDS
serum; SPE

REFERENCE
Róna,K.; Gachályi,B. Simple liquid chromatographic method for the determination of ornidazole and metronidazole in human serum, *J.Chromatogr.*, **1987**, *420*, 228–230.

SAMPLE
Matrix: blood, eggs, tissue
Sample preparation: Serum. 2 mL Serum + 1 mL 20% trichloroacetic acid in MeOH, vortex for 1 min, centrifuge at 3500 rpm. Filter (0.45 μm) the supernatant, inject a 20 μL aliquot of the filtrate. Eggs. Condition a 3 mL Bakerbond octadecyl SPE cartridge with 5 mL MeOH, 10 mL water, and 2 mL 1% trichloroacetic acid. Homogenize 5 g blended whole egg and 5 mL pH 4.5 phosphate buffer, add 25 mL MeCN, homogenize, centrifuge at 3500 rpm for 10 min. Remove the supernatant and evaporate it to 2 mL under reduced pressure at 40°, add 10 mL 1% trichloroacetic acid to the residue, add the mixture to the SPE cartridge, wash with 10 mL water, dry under vacuum, elute with 2 mL 0.1% triethylamine in MeCN. Evaporate the eluate to dryness, reconstitute with 2 mL mobile phase, inject a 20 μL aliquot. Tissue. Condition a 3 mL Bakerbond octadecyl SPE cartridge with 5 mL MeOH, 10 mL water, and 2 mL 1% trichloroacetic acid. Homogenize 5 g tissue and 25 mL MeCN, centrifuge at 3500 rpm for 10 min, remove the supernatant, extract the residue with 15 mL MeCN. Combine the supernatants, add 20 mL n-hexane, shake. Remove the lower layer and evaporate it to 2 mL, add 10 mL 1% trichloroacetic acid to the residue, add the mixture to the SPE cartridge, wash with 10 mL water, dry under vacuum, elute with 2 mL 0.1% triethylamine in MeCN. Evaporate the eluate to dryness, reconstitute with 2 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 5 μm Bakerbond BDS-C18
Mobile phase: MeCN:50 mM pH 4.3 ammonium acetate buffer 30:70
Flow rate: 1
Injection volume: 20
Detector: UV 350

CHROMATOGRAM
Retention time: 2.61
Limit of quantitation: 5 ng/mL (serum), 2 ng/g (tissue, eggs)

OTHER SUBSTANCES
Extracted: dimetronidazole
Noninterfering: chloramphenicol, nitrofurans, sulfonamides

KEY WORDS
hen; serum; liver; muscle; SPE

REFERENCE
Semeniuk,S.; Posyniak,A.; Niedzielska,J.; Zmudski,J. Determination of nitroimidazole residues in poultry tissues, serum and eggs by high-performance liquid chromatography, *Biomed.Chromatogr.*, **1995**, *9*, 238–242.

SAMPLE
Matrix: blood, gastric juice, saliva
Sample preparation: Plasma, saliva. Mix 500 μL plasma or saliva with 50 μL 50% perchloric acid (w/v), vortex briefly, add 1.5 g solid anhydrous potassium carbonate to neutral pH. Add 300 μL MeCN, mix, centrifuge at 11600 g for 6 min., remove a 180 μL aliquot of the supernatant, evaporate under a stream of nitrogen at 50°, reconstitute the residue with 500 μL mobile phase, inject a 100 μL aliquot. Gastric juice. Mix 500 μL gastric juice with 20 μL 10 μg/mL IS in water and 50 μL 50% perchloric acid, vortex briefly, add 1.5 g solid anhydrous potassium carbonate to neutral pH. Add 300 μL MeCN, mix, centrifuge at 11600 g for 6 min, remove a 180 μL aliquot of the supernatant, evaporate under a stream of nitrogen at 50°, reconstitute the residue with 500 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2 5 μm Hypersil ODS
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:buffer 10:90 (Buffer was 50 mM KH_2PO_4 containing 0.1% triethylamine, adjusted to pH 7.0 with orthophosphoric acid.)
Flow rate: 1
Injection volume: 100
Detector: UV 317

CHROMATOGRAM
Retention time: 6.7
Internal standard: tinidazole (13.9)
Limit of quantitation: 250 ng/mL (plasma, gastric juice), 100 ng/mL (saliva)

OTHER SUBSTANCES
Extracted: active metabolite

KEY WORDS
plasma

REFERENCE
Jessa,M.J.; Barrett,D.A.; Shaw,P.N.; Spiller,R.C. Rapid and selective high-performance liquid chromatographic method for the determination of metronidazole and its active metabolite in human plasma, saliva and gastric juice, *J.Chromatogr.B*, **1996**, *677*, 374–379.

SAMPLE
Matrix: blood, urine
Sample preparation: 100 μL Urine or 1 mL plasma + 100 ng IS + 1 mL MeCN, centrifuge. Remove supernatant and add it to 1 mL buffer, add 6 mL dichloromethane:petroleum ether: isopropanol 45:45:10, rotate for 10 min. Remove the upper organic layer and evaporate it under nitrogen at 60°. Dissolve residue in 100 μL mobile phase, inject. (Buffer contained 80 g $NaHCO_3$ and 30 g K_2CO_3 per liter, pH 9.5.)

HPLC VARIABLES
Column: 466 × 5 5 μm Spherisorb ODS
Mobile phase: MeCN:MeOH:45 mM pH 4.5 KH_2PO_4 40:3:57, containing 40 g/L $NaClO_4$ and 40 g/L trimethylammonium chloride
Flow rate: 1
Injection volume: 100
Detector: UV 338

CHROMATOGRAM
Retention time: 3.8
Internal standard: 4-(3-dimethylaminopropyl)-4-chloroquinoline (5)
Limit of quantitation: < 10 ng/mL

OTHER SUBSTANCES
Simultaneous: chloroquine

KEY WORDS
plasma

REFERENCE
Okonkwo,P.O.; Eta,E.I. Simultaneous determination of chloroquine and metronidazole in human biological fluid by high-pressure liquid chromatography, *Life Sci.*, **1988**, *42*, 539–545.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the

residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 320

CHROMATOGRAM
Retention time: 6.778

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablets to a fine powder 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 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak phenyl
Mobile phase: 20 mM KH_2PO_4, pH 4.2
Flow rate: 2.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Internal standard: phenylpropanolamine hydrochloride (3.5)

OTHER SUBSTANCES
Noninterfering: mannitol, excipients

KEY WORDS
tablets; stability-indicating

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: Dissolve 5 g suspension (containing about 200 mg metronidazole benzoate) in 100 mL MeOH. Dilute 2.5 mL of this solution and 2 mL 200 μg/mL ethyl paraben in MeOH to 10 mL with MeOH, filter, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 Varian MicroPak MCH-10 reverse phase
Mobile phase: 20 mM ammonium acetate in MeCN:water 47:53, adjusted to pH 4.5 using glacial acetic acid
Flow rate: 2
Injection volume: 10
Detector: UV 270

CHROMATOGRAM
Retention time: 5.56 (metronidazole benzoate), 2.9 (metronidazole with 12% MeCN in mobile phase)
Internal standard: ethyl paraben (2.78)
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Simultaneous: 2-methyl-5-nitroimidazole, misonidazole, tinidazole, benzoic acid, methyl paraben, propyl paraben

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KEY WORDS
suspensions; stability-indicating

REFERENCE
Sa'sa',S.I.; Khalil,H.S.; Jalal,I.M. Determination of metronidazole benzoate in liquid preparations by reversed phase HPLC, *J.Liq.Chromatogr.*, **1986**, *9*, 3617–3631.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out 2 g metronidazole benzoate suspension containing 3.5% metronidazole benzoate, add 80 mL MeOH:water 80:20, sonicate for a few minutes, make up to 100 mL with MeOH water 80:20, centrifuge at 900 g for 10 min, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 2 30-38 μm Whatman Co:Pell
Column: 250 × 4.6 10 μm Perkin-Elmer C18
Mobile phase: MeOH:water 60:40 containing 5 mM acetate buffer and 50 mM KNO_3, pc_H* 5.2
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 5 (metronidazole), 12 (metronidazole benzoate)

OTHER SUBSTANCES
Simultaneous: benzoic acid, methylparaben, propylparaben

KEY WORDS
suspensions

REFERENCE
Pashankov,P.P.; Kostova,L.L. Reversed-phase high-performance liquid chromatography of metronidazole benzoate in suspension dosage form, *J.Chromatogr.*, **1987**, *394*, 382–387.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 1:5 with water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm μBondapak 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: 5.76

OTHER SUBSTANCES
Simultaneous: desacetylcefotaxime, cefotaxime

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: formulations
Sample preparation: Mix an aliquot with an equal volume of 5 mg/mL cefoxitin, dilute with water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Resolv (Waters)
Mobile phase: MeCN:buffer 18:86 (Buffer was 2.46 g anhydrous sodium acetate, 8 mL glacial acetic acid, and 200 mg tetrabutylammonium hydrogen sulfate in 1 L water, pH 3.0.)
Flow rate: 1.2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 1.8
Internal standard: cefoxitin (3.0)

OTHER SUBSTANCES
Simultaneous: cefotaxime

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 50-fold with water, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeCN:20 mM KH_2PO_4 7:93 containing 10 mM triethylamine, adjusted to pH 4.8 with HCl
Flow rate: 1.5
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 3.1

OTHER SUBSTANCES
Simultaneous: ceftazidime, ceftizoxime, ceftriaxone
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: 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:45 mM pH 4.5 KH_2PO_4 15:85
Flow rate: 1
Injection volume: 20
Detector: UV 325

CHROMATOGRAM
Retention time: 7.1
Limit of detection: 50 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
Sample preparation: Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb ODS C18
Mobile phase: MeCN:buffer 15:85 (Buffer was 10 mM NaH_2PO_4 adjusted to pH 8 with trimethylamine.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 250

CHROMATOGRAM
Retention time: 2

OTHER SUBSTANCES
Simultaneous: miconazole

REFERENCE
Faouzi,M.E.A.; Dine,T.; Luyckx,M.; Brunet,C.; Mallevais,M.-L.; Goudaliez,F.; Gressier,B.; Cazin,M.; Kablan,J.; Cazin,J.C. Stability, compatibility and plasticizer extraction of miconazole injection added to infusion solutions and stored in PVC containers, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1363–1372.

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: 4.83 (A), 3.86 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE

Matrix: tissue
Sample preparation: Condition a 1 mL Accubond C18 SPE cartridge (J&W Scientific) with three 1 mL portions of MeOH and three 1 mL portions of water. Homogenize (Tekmar tissuemiser) 250 ng vaginal tissue, 1 mL water, and 1 mL 400 ng/mL tinidazole in water in ice for 30 s, rinse homogenizer. Combine homogenate and rinse and add 100 μL 5% trichloroacetic acid, mix, centrifuge at 1500 g for 30 min, remove the supernatant, wash the precipitate with 1 mL water. Add the supernatant and the wash to the SPE cartridge, wash with two 1 mL portions of water, dry under vacuum for 10 min, elute with four 250 μL aliquots of MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Zorbax SB-phenyl
Mobile phase: MeOH:buffer 15:85 (Buffer was 10 mM KH_2PO_4 adjusted to pH 4.0 with 10% phosphoric acid
Flow rate: 1
Injection volume: 100
Detector: UV 313

CHROMATOGRAM

Retention time: 9.2
Internal standard: tinidazole (23.2)
Limit of detection: 100 ng/g

OTHER SUBSTANCES

Simultaneous: cefazolin, cefmetazole, cephalexin

KEY WORDS

SPE; human; dog; vaginal tissue

REFERENCE

Venkateshwaran,T.G.; Stewart,J.T. Determination of metronidazole in vaginal tissue by high-performance liquid chromatography using solid-phase extraction, *J.Chromatogr.B*, **1995**, *672*, 300–304.

SAMPLE

Matrix: urine
Sample preparation: Dilute urine 1:2 with MeOH, let stand for 1 h, centrifuge at 5000 g for 1 h. Dilute the supernatant 1:1 with water, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 120 × 4.6 5 μm LiChrosorb Si 60
Mobile phase: MeOH:200 mM pH 7.0 potassium phosphate:water 25:30:45 containing 2.5 mM N-cetyl-N,N,N-trimethylammonium bromide (Use a 150 × 4.6 column of 15-25 μm LiChroprep Si 60 between pump and injector.)
Column temperature: 35
Flow rate: 0.5 for 9 min, then 1
Injection volume: 20
Detector: UV 312

CHROMATOGRAM

Retention time: 5
Limit of detection: 2 ng/mL
Limit of quantitation: 5 ng/mL

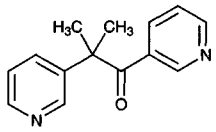
OTHER SUBSTANCES

Extracted: metabolites

REFERENCE

Thomsen,U.G.; Cornett,C.; Tjornelund,J.; Hansen,S.H. Separation of metronidazole, its major metabolites and their conjugates using dynamically modified silica, *J.Chromatogr.A*, **1995**, *697*, 175–184.

Metyrapone



Molecular formula: $C_{14}H_{14}N_2O$
Molecular weight: 226.28
CAS Registry No.: 54-36-4, 908-35-0 (ditartrate)
Merck Index: 6246

SAMPLE

Matrix: blood
Sample preparation: 250 μL Whole blood + 500 μL 1 M NaOH + 100 μL 500 μM 2,3'-dipyridyl in water + 1 g NaCl, extract twice with 1 mL portions of dichloromethane. Combine the organic layers and evaporate them to dryness at 40°, reconstitute the residue in 25 μL MeOH, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Spherisorb ODS-1
Mobile phase: MeCN:67 mM pH 7.4 phosphate buffer 25:75 (Place a 100 × 4.6 column of 40-60 μm silica between pump and injector.)
Injection volume: 20
Detector: UV 261

CHROMATOGRAM

Retention time: 10.5
Internal standard: 2,3'-dipyridyl (7)
Limit of detection: 4.4 μM

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

rat; whole blood

REFERENCE

Usansky,J.I.; Damani,L.A. Assay of metyrapone, metyrapol and the isomeric mono-N-oxides of metyrapone in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *563*, 283–298.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 100 μL 1 M NaOH + 250 mg NaCl + 100 μL 249 μg/mL oxprenolol in MeOH, vortex for 30 s, add 5 mL diethyl ether:dichloromethane 80:20, vortex for 2 min, centrifuge at 1500 g for 20 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 500 μL mobile phase, inject a 100 μL aliquot. Urine. 1 mL Urine + 100 μL 1 M NaOH + 250 mg NaCl + 100 μL 249 μg/mL oxprenolol in MeOH, vortex for 30 s, add 5 mL diethyl ether:dichloromethane 80:20, vortex for 2 min, centrifuge at 1500 g for 20 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 1 mL water, add 100 μL 1 M HCl, vortex for 30 s, add 5 mL hexane:diethyl ether 50:50, vortex for 1 min, centrifuge at 1500 g for 10 min, freeze in dry ice-acetone. Discard the organic phase, thaw the aqueous phase and add it to 200 μL 1 M NaOH and 250 mg NaCl, vortex for 30 s, add 5 mL diethyl ether:dichloromethane 80:20, vortex for 2 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 500 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 75 × 4.6 Ultremex 3 silica
Column: 250 × 4.6 10 μm Chiralcel OJ-CSP cellulose tris(4-methylbenzoate)
Mobile phase: Hexane:EtOH 92:8 containing 0.1% diethylamine (plasma) or hexane:EtOH 94:6 containing 0.2% diethylamine (urine)

Flow rate: 1
Injection volume: 100
Detector: UV 261

CHROMATOGRAM
Retention time: 17 (plasma), 20 (urine)
Internal standard: oxprenolol (10)
Limit of detection: 30 ng/mL
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; chiral (for metabolites)

REFERENCE
Chiarotto,J.A.; Wainer,I.W. Determination of metyrapone and the enantiomers of its chiral metabolite metrapol in human plasma using coupled achiral-chiral liquid chromatography, *J.Chromatogr.B*, **1995**, *665*, 147–154.

SAMPLE
Matrix: microsomal incubation
Sample preparation: 2 mL Microsomal incubation + 500 μL 1 M NaOH + 500 μL 500 μM 2,3'-dipyridyl in water + 1 g NaCl, extract twice with 1 mL portions of dichloromethane. Combine the organic layers and evaporate them to dryness at 40°, reconstitute the residue in 25 μL MeOH, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil ODS-1
Mobile phase: MeCN:67 mM pH 7.4 phosphate buffer 20:80 (Place a 100 × 4.6 column of 40-60 μm silica between pump and injector.)
Flow rate: 2
Injection volume: 20
Detector: UV 261

CHROMATOGRAM
Retention time: 16
Internal standard: 2,3'-dipyridyl (10)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; liver

REFERENCE
Usansky,J.I.; Damani,L.A. Assay of metyrapone, metyrapol and the isomeric mono-N-oxides of metyrapone in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *563*, 283–298.

SAMPLE
Matrix: urine
Sample preparation: Buffer urine to 4.9 by mixing with an equal volume of pH 4.9 200 mM sodium phosphate buffer. Inject a 40 μL aliquot onto column A with mobile phase A, after 3 min backflush the contents of column A onto column B with mobile phase B and start the gradient. At the end of the run re-equilibrate for 10 min.

HPLC VARIABLES
Column: A 20 × 4 5 μm Hypersil octadecylsilica ODS; B 200 × 4.6 5 μm Shiseido SG-120 polymer-based C18

Mobile phase: A water; B Gradient. MeCN:buffer from 7:93 to 15:85 over 3.5 min, to 50:50 over 8.5 min, maintain at 50:50 for 11 min (Buffer was 6.9 g $NaH_2PO_4.H_2O$ in 1 L water, pH adjusted to 3.1 with phosphoric acid.)
Flow rate: 1
Injection volume: 40
Detector: UV 270

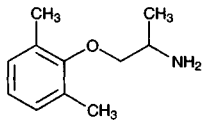
CHROMATOGRAM
Retention time: 12.2
Limit of detection: 2000 ng/mL

OTHER SUBSTANCES
Extracted: acetazolamide, amiloride, bendroflumethiazide, benzthiazide, bumetanide, caffeine, carbamazepine, chlorothiazide, chlorthalidone, clopamide, dichlorfenamide, ethacrynic acid, furosemide, hydrochlorothiazide, 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.

Mexiletine



Molecular formula: $C_{11}H_{17}NO$
Molecular weight: 179.26
CAS Registry No.: 31828-71-4, 5370-01-4 (HCl)
Merck Index: 6257

SAMPLE
Matrix: blood
Sample preparation: Mix 400 µL serum with 20 µL 10 µg/mL IS in MeOH and 50 µL 10% sodium carbonate. Add 4 mL diisopropyl ether, shake vigorously for 4 min, centrifuge, freeze at -20°. Mix the organic layer with 100 µL 10 mM HCl, vortex carefully for 45 s using a microshaker, centrifuge, evaporate the aqueous phase to dryness under a stream of argon in a 56° water bath. Reconstitute the residue in 100 µL mobile phase, inject a 50 µL aliquot. (Caution! Diisopropyl ether readily forms explosive peroxides!)

HPLC VARIABLES
Guard column: 20 × 4.6 5 µm Supelguard LC-CN
Column: 150 × 4.6 5 µm Supelcosil LC-CN
Mobile phase: MeCN:water:500 mM KH_2PO_4 36:62:2
Flow rate: 1.8
Injection volume: 50
Detector: UV 210

CHROMATOGRAM
Retention time: 3.5
Internal standard: LU41616 (2'-[2-hydroxy-3-(3"-hydroxy-3"-methylbutylamino)propoxy]-3-phenylpropiophenone hydrochloride) (7.7)
Limit of detection: 5 ng/mL
Limit of quantitation: 40 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, diltiazem, propafenone
Simultaneous: acebutolol, amiodarone, aprobarbital, atenolol, bupranolol, celiprolol, clobazam, debrisoquine, diazepam, flecainide, gallopamil, hexobarbiltal, lidocaine, mephenytion, meto-

polol, nadolol, pentobarbital, phenacetin, prazosin, procainamide, progesterone, propranolol, quinidine, sotalol, theophylline, verapamil

KEY WORDS
serum

REFERENCE
Kunicki,P.K.; Sitkiewicz,D. High performance liquid chromatographic analysis of some antiarrhythmic drugs in human serum using cyanopropyl derivatized silica phase, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1169–1181.

SAMPLE
Matrix: blood
Sample preparation: 1 mL serum + 250 μL triethylamine + 100 μL 1 M NaOH + 100 μL 2 μg/mL IS in 10 mM HCl + 1.75 mL dichloromethane, rotate slowly for 20 min, centrifuge at 3600 g for 10 min. Remove 1 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 500 μL 10 mM HCl, inject a 200 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil CPS (CN)
Mobile phase: MeCN:buffer 5:95 (Buffer was 973.5 mL water, 25 mL PIC B-8 low-UV reagent + 1 mL butylamine + 0.5 mL PIC D-4 reagent.)
Flow rate: 2
Injection volume: 200
Detector: UV 215

CHROMATOGRAM
Retention time: 21
Internal standard: 1-(2,4-dimethylphenoxy)-2-aminopropane hydrochloride (KOE 768-CL) (29)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: acebutolol, acenocoumarol, amiodarone, aspirin, atenolol, betaxolol, digoxin, dipyridamole, flecainide, furosemide, hydrochlorothiazide, metoprolol, nifedipine, phenprocoumon, pindolol, propafenone, quinidine, sotalol, spironolactone, sulfinpyrazone, triamterene, verapamil

KEY WORDS
serum

REFERENCE
Krämer,B.K.; Ress,K.M.; Mayer,F.; Kühlkamp,V.; Liebich,H.M.; Risler,T.; Seipel,L. Rapid high-performance liquid chromatographic method for the quantification of mexiletine and its metabolites in serum, *J.Chromatogr.*, **1989**, *493*, 414–420.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum or plasma + 100 μL 500 mM sodium carbonate + 100 μL 5 μg/mL N-propionylprocainamide in water, mix gently, add 5 mL dichloromethane, shake gently for 5 min, centrifuge. Remove the lower organic layer and add it to 200 μL 10 mM HCl, vortex for 15 s, centrifuge, inject a 20 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 100 × 5 NovaPak cyano HP radial compression
Mobile phase: MeCN:buffer 10:90, final pH adjusted to 6.0 (Buffer was 5 mM acetate buffer containing 0.05% triethylamine.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 6.8
Internal standard: N-propionylprocainamide (5.9)
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Simultaneous: N-acetylprocainamide, disopyramide, lidocaine, procainamide, quinidine, tocainide
Noninterfering: carbamazepine, desmethyldoxepin, digoxin, doxepin, ethosuximide, lithium, phenobarbital, phenytoin, primidone, propranolol, theophylline, valproic acid

KEY WORDS
serum; plasma

REFERENCE
vasBinder,E.; Annesley,T. Liquid chromatographic analysis of mexiletine in serum, with alternate application to tocainide, procainamide, and N-acetylprocainamide, *Biomed.Chromatogr.*, **1991**, *5*, 19–22.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 0.4 μg/mL methylmexiletine hydrochloride in water + 1 mL 150 mM barium hydroxide + 1 mL 170 mM zinc sulfate, vortex, add 500 μL 2 M NaOH, extract twice with 5 mL portions of diethyl ether. Combine the organic layers and evaporate them to dryness at 45°, reconstitute the residue in 20 μL 30 mM HCl, add 60 μL 100 mM sodium borate, add 60-80 μL reagent, keep at 4°, inject an aliquot. (Reagent was 40 mg o-phthalaldehyde and 50 mg N-acetyl-L-cysteine in 5 mL MeOH, prepare daily.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Apex C18 (Jones Chromatography)
Mobile phase: MeOH:50 mM sodium acetate 65:35, pH 7.3
Flow rate: 1
Injection volume: 100
Detector: F ex 350 em 445

CHROMATOGRAM
Retention time: 14 (S-(+)), 15 (R-(-))
Internal standard: methylmexiletine (23, 24 (enantiomers))
Limit of detection: 1.5 ng/mL

OTHER SUBSTANCES
Noninterfering: metabolites

KEY WORDS
chiral; plasma; pharmacokinetics; derivatization

REFERENCE
Abolfathi,Z.; Bélanger,P.-M.; Gilbert,M.; Rouleau,J.R.; Turgeon,J. Improved high-performance liquid chromatographic assay for the stereoselective determination of mexiletine in plasma, *J.Chromatogr.*, **1992**, *579*, 366–370.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 30 μL 1 μg/mL IS in water + 200 μL 300 mM barium hydroxide, vortex, add 200 μL 300 mM zinc sulfate, add 200 μL 2 M NaOH, add 5 mL diethyl ether, vortex, centrifuge at 2000 g for 5 min, repeat extraction. Combine the organic layers and evaporate them to 1 mL under a stream of nitrogen at 37°, add 300 μL 100 mM HCl, vortex, discard the ether layer. Wash the aqueous layer with 2 mL diethyl ether, add 300 μL 2 M NaOH to the aqueous layer, add 10 μL 1 mg/mL 2-anthroyl chloride in dry dichloromethane, vortex for 3 min, extract with 1 mL dichloromethane. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject an aliquot. (Prepare 2-anthroyl chloride as follows. Reflux 500 mg anthra-

quinone-2-carboxylic acid with 30 mL 14% aqueous ammonia, add 2 g zinc dust in small portions over 30 min, reflux with stirring for 1 h, filter, acidify the filtrate to pH 3 with dilute HCl, filter, recrystallize from hot EtOH to give 2-anthracenecarboxylic acid (mp 278-279°, yield 230%). Add 100 mg 2-anthracenecarboxylic acid in 100 mL dry benzene (Caution! Benzene is a carcinogen!) to 500 μL oxalyl chloride with stirring, heat slowly on a water bath for 30 min, add 500 μL oxalyl chloride, heat for 30 min, cool, filter. Evaporate the filtrate to obtain 40 mg pale yellow 2-anthroyl chloride. Purify by dissolving in dichloromethane and precipitating with hexane. Purify further by HPLC by injecting 100 μL aliquots of a 50 mg/mL solution in MeCN onto a 250 × 9.4 10 μm ODS-2 Magnum-9 (Whatman) column and eluting with MeCN at 0.7 mL/min. Using a UV 230 detector collect the appropriate fraction (about 11 min) and evaporate to dryness to obtain pure compound.)

HPLC VARIABLES

Guard column: 150 × 4.6 5 μm silica (Alltech)
Column: 250 × 4.6 5 μm Pirkle 1-A phenylglycine (Regis)
Mobile phase: Hexane:isopropanol:chloroform 78:7:15
Flow rate: 0.8
Injection volume: 20
Detector: F ex 270 em 420

CHROMATOGRAM

Retention time: 11.5 (R-(-)), 12.5 (S-(+))
Internal standard: 1-(2',6'-dimethylphenoxy)-2-ethanamine hydrochloride (Boehringer Ingelheim KPE-2963) (14.5)
Limit of detection: 0.5 ng/mL
Limit of quantitation: 2.5 ng/mL

KEY WORDS

derivatization; serum; chiral; pharmacokinetics

REFERENCE

Kwok,D.K.W.; Igwemezie,L.; Kerr,C.R.; McErlane,K.M. High-performance liquid chromatographic analysis using a highly sensitive fluorogenic reagent, 2-anthroyl chloride, and stereoselective determination of the enantiomers of mexiletine in human serum, *J.Chromatogr.B*, **1994**, *661*, 271–280.

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: 1.4
Internal standard: protriptyline (4)

OTHER SUBSTANCES

Extracted: acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, chlorpromazine, desipramine, dextromethorphan, diazepam, diphenhydramine, doxepin, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, ibuprofen, imipramine, maprotiline, methadone, methaqualone, midazolam, norchlorimipramine, nordiazepam, nordoxepin, norfluoxetine, nortriptyline, norverapamil, pentazocine, promazine, propafenone, propoxyphene, propranolol, protriptyline, temazepam, trazodone, trimipramine, verapamil

Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin

Interfering: encainide, lidocaine, quinidine

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: 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 KH_2PO_4 36:62:2
Flow rate: 1.8
Detector: UV 210

CHROMATOGRAM

Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: diltiazem, 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*, 394–394.

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)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 262

CHROMATOGRAM

Retention time: 4.95
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood
Sample preparation: 500 μL Plasma + 50 μL 300 mM NaOH, mix, add 4 mL diisopropyl ether (Caution! Diisopropyl ether readily forms explosive peroxides!), vortex for 2 min, centrifuge at 1800 g for 10 min, repeat the extraction. Combine the organic layers and add them to 50 μL 100 mM HCl in MeOH, evaporate to dryness under a stream of nitrogen, reconstitute with 100 μL aqueous 100 mM HCl, add 100 μL 2 M NaOH, add 200 μL water, vortex for 15 s, let stand for 15 min, add 100 μL 4 mg/mL 2-naphthoyl chloride in dichloromethane, vortex for 2 min,

add 2 mL hexane:isopropanol 90:10, vortex for 2 min, centrifuge at 1800 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL hexane:isopropanol 90:10, inject an aliquot. (The 300 mM NaOH, 2 M NaOH, and aqueous 100 mM HCl solutions should be washed with diisopropyl ether before use.)

HPLC VARIABLES

Guard column: 50 mm long Chiralcel OJ
Column: 250 × 4.6 10 μm Chiralcel OJ
Mobile phase: Hexane:EtOH 71:29
Flow rate: 1
Injection volume: 10
Detector: F ex 230 em 340

CHROMATOGRAM

Retention time: 7.2 (R-(-)), 8.9 (S-(+))
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Noninterfering: amiodarone, captopril, clobazam, clonazepam, disopyramide, ergotamine, nitrazepam, nordisopyramide, procainamide, propafenone, sotalol

KEY WORDS

plasma; derivatization; chiral

REFERENCE

Lanchote,V.L.; Bonato,P.S.; Dreossi,S.A.C.; Gonçalves,P.V.B.; Cesarino,E.J.; Bertucci,C. High-performance liquid chromatographic determination of mexiletine enantiomers in plasma using direct and indirect enantioselective separations, *J.Chromatogr.B*, **1996**, *685*, 281–289.

SAMPLE

Matrix: blood, saliva, urine
Sample preparation: Serum, saliva. 1 mL Serum or saliva + 30 ng IS + 200 μL 150 mM barium hydroxide solution, vortex, add 200 μL 150 mM zinc sulfate solution, mix, add 200 μL 2 M NaOH, extract twice with 5 mL portions of diethyl ether. Combine the organic layers and evaporate them to 1 mL under a stream of nitrogen at 37°, add 300 μL 100 mM HCl, extract. Remove the aqueous layer and wash it twice with 2 mL portions of diethyl ether, add 300 μL 2 M NaOH to the aqueous layer, add 10 μL 1 mg/mL 2-anthroyl chloride, vortex for 3 min, extract with 1 mL dichloromethane. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject an aliquot. Urine. Make urine alkaline with 2 M NaOH, extract twice with 5 mL portions of diethyl ether. Combine the organic layers and evaporate them to 1 mL under a stream of nitrogen at 37°, add 300 μL 100 mM HCl, extract. Remove the aqueous layer and wash it twice with 2 mL portions of diethyl ether, add 300 μL 2 M NaOH to the aqueous layer, add 10 μL 1 mg/mL 2-anthroyl chloride, vortex for 3 min, extract with 1 mL dichloromethane. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Pirkle 1-A phenylglycine (Regis) + 150 × 4.6 5 μm silica (Alltech) in series
Mobile phase: Hexane:isopropanol:chloroform 39:3.5:75.5
Flow rate: 0.8
Injection volume: 20
Detector: F ex 270 em 420

CHROMATOGRAM

Internal standard: 1-(2',6'-dimethylphenoxy)-2-ethanamine (KOE 2963)

KEY WORDS

chiral; serum; derivatization; pharmacokinetics

REFERENCE

Kwok,D.W.; Kerr,C.R.; McErlane,K.M. Pharmacokinetics of mexiletine enantiomers in healthy human subjects. A study of the in vivo serum protein binding, salivary excretion and red blood cell distribution of the enantiomers, *Xenobiotica*, **1995**, *25*, 1127–1142.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 1 mL Plasma + 50 μL 20 μg/mL 4-methylmexiletine in MeOH + 1 mL 200 mM pH 9 borate buffer + 7 mL diethyl ether, shake on a reciprocating shaker for 10 min, centrifuge at 3000 g for 5 min, freeze in MeOH/dry ice. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 250 μL MeOH:10 mM phosphoric acid 48:52 containing 300 mg/L sodium 1-octanesulfonate, inject a 100 μL aliquot. Urine. 100 μL Urine + 300 μL 1 M pH 5.5 sodium acetate buffer + 2500 U β-glucuronidase (Helix pomatia Type H-5, Sigma), vortex, heat at 37° for 4 h, add 100 μL 20 μg/mL 4-methylmexiletine in MeOH, add 1 mL 200 mM pH 10 borate buffer, add 7 mL diethyl ether, shake on a reciprocating shaker for 10 min, centrifuge at 3000 g for 5 min, freeze in MeOH/dry ice. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 250 μL MeOH:10 mM phosphoric acid 48:52 containing 300 mg/L sodium 1-octanesulfonate, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 4.6 Wakosil ODS (Wako)

Column: 250 × 4.6 Wakosil ODS (Wako)

Mobile phase: Gradient. A was MeOH:10 mM phosphoric acid 16:84 containing 300 mg/L sodium 1-octanesulfonate. B was MeOH:10 mM phosphoric acid 80:20 containing 300 mg/L sodium 1-octanesulfonate. A:B from 65:35 to 20:80 over 10 min, maintain at 20:80 for 4 min, return to initial conditions over 1 min, re-equilibrate for 10 min.

Flow rate: 1

Injection volume: 100

Detector: F ex 345 em 445 following post-column derivatization. The column effluent was mixed with reagent pumped at 0.6 mL/min and the mixture flowed through a 5 m × 0.5 mm i.d. tefzel tube to the detector. (Reagent was 300 mg o-phthalaldehyde in 100 mL EtOH, add 900 mL 200 mM pH 10 borate buffer, add 500 μL 2-mercaptoethanol.)

CHROMATOGRAM

Retention time: 12.5

Internal standard: 4-methylmexiletine (14)

Limit of detection: 2 ng/mL (plasma)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; post-column reaction; pharmacokinetics

REFERENCE

Tateishi,T.; Harada,K.; Ebihara,A. Fluorescence detection of mexiletine and its p-hydroxylated and hydroxymethylated metabolites in human plasma and urine by high-performance liquid chromatography using post-column derivatization with o-phthalaldehyde, *J.Liq.Chromatogr.*, **1994**, *17*, 659–671.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 11.468

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Reflux a 10% excess of reagent in toluene for 10 min, add the drug, let stand at room temperature for 10 min, cool, dilute, inject an aliquot. (The reagent was N-(p-toluenesulfonyl)prolyl azide and was prepared as follows. Mix 40-45 mmoles L-(-)-proline, 40 mL THF, and 200 mL 10% potassium carbonate, add 37-43 mmoles p-toluenesulfonyl chloride in 40 mL THF dropwise, heat at 50° and maintain at pH 8 or above for 3 h, cool, acidify to pH 2, extract with chloroform. Extract the organic layers with potassium carbonate in water. Acidify the aqueous layer and extract it with chloroform. Dry the chloroform layer and evaporate it to dryness, recrystallize the resulting 1-[(p-toluene)sulfonyl]proline from petroleum ether and benzene (Caution! Benzene is a carcinogen!) (Anal.Chem. 1984, 56, 958). Suspend 86 mmoles 1-[(p-toluene)sulfonyl]proline in 15 mL water and add sufficient acetone to give a clear solution, cool to 0°, add 10.2 g triethylamine in 175 mL acetone, slowly add 12.5 g ethyl chloroformate in 45 mL acetone while maintaining the temperature at 0°, stir at 0° for 30 min, add dropwise 8.6 g sodium azide in 30 mL water, stir at 0° for 1 h, pour into ice water, extract with ether, dry over anhydrous magnesium sulfate, evaporate under reduced pressure at room temperature to give N-(p-toluenesulfonyl)prolyl azide (cf J.Org.Chem. 1961, 26, 3511).)

HPLC VARIABLES
Column: 300 × 4 7-9 μm silica gel
Mobile phase: Petroleum ether:isopropanol 97:3
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Retention time: 29.8, 44.3 (enantiomers)

KEY WORDS
derivatization; chiral; normal phase

REFERENCE
Zhou,Y.; Sun,Z.P.; Lin,D.K. Liquid chromatographic evaluation of a new chiral derivatizing agent for enantiomeric resolution of amine and alcohol drugs, *J.Liq.Chromatogr.*, **1990**, *13*, 875–885.

SAMPLE
Matrix: microbial broth
Sample preparation: Dilute microbial broth 1:20. 250 μL Diluted microbial broth + 5 μg IS + 100 μL 200 mM sodium carbonate, add 2 mL diethyl ether, vortex for 15 s, centrifuge at 1800

g for 4 min, remove 1.5 mL of the ether layer, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 300 μL chloroform, add 75 μL 0.1% S-(+)-1-(1-naphthyl)ethyl isocyanate in chloroform, vortex for 10 s, evaporate to dryness under a stream of nitrogen. Reconstitute the residue in 220 μL chloroform, add 30 μL 0.33% n-butylamine in chloroform, inject a 10-65 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 Partisil 5
Mobile phase: Hexane:chloroform:MeOH 65:34:1
Flow rate: 0.8 for 12 min then 2.5 for 23 min
Injection volume: 10-65
Detector: F ex 280 em 340

CHROMATOGRAM
Retention time: 7.0 (S-(+)), 7.6 (R-(-))
Internal standard: prenalterol ((±)-1-(4-hydroxyphenoxy)-3-isopropylaminopropan-2-ol) (27.7, 32.4 (enantiomers))
Limit of quantitation: 400 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
chiral; derivatization; normal phase

REFERENCE
Freitag,D.G.; Foster,R.T.; Coutts,R.T.; Pasutto,F.M. High-performance liquid chromatographic method for resolving the enantiomers of mexiletine and two major metabolites isolated from microbial fermentation media, *J.Chromatogr.*, **1993**, *616*, 253–259.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 50 μL aliquot of a solution in MeOH:triethylamine 99:1 with 20 μL 0.1% FLOPIC in dry toluene, vortex briefly, let stand at room temperature in the dark for 30 min, add 50 μL 1% ethanolamine in MeOH, let stand at room temperature for 15 min, evaporate to dryness under reduced pressure, reconstitute with 100 μL mobile phase, sonicate for 30 s, inject a 20 μL aliquot. (FLOPIC is (-)-(S)-flunoxaprofen isocyanate; synthesis is as follows. Dissolve 1 g (+)-(S)-flunoxaprofen in 30 mL acetone, cool to 0°, add a solution of 500 μL triethylamine in 2 mL acetone dropwise, add a solution of 370 μL ethyl chloroformate in 2 mL acetone dropwise, stir at 0° for 15 min, add a solution of 250 mg sodium azide in 1 mL water dropwise (Caution! Sodium azide is highly toxic!), stir for 1 h, pour into 60 mL ice water, stir for 10 min, filter, wash the solid with two 50 mL aliquots of ice-water, dry under reduced pressure to obtain flunoxaprofen azide. Dissolve 100 mg flunoxaprofen azide in 3 mL dry toluene, reflux for 10-15 min, cool to room temperature, filter. Evaporate the filtrate to dryness under reduced pressure and dry under reduced pressure to obtain FLOPIC as a crystalline solid (mp 93-94°), store in a desiccator under reduced pressure.)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova Pak C18
Mobile phase: MeOH:water:THF 62:35:3
Flow rate: 1
Injection volume: 20
Detector: F ex 296 em 356

CHROMATOGRAM
Retention time: 20.9 (S), 22.7 (R)

OTHER SUBSTANCES
Simultaneous: flecainide

KEY WORDS
derivatization; chiral

REFERENCE
Martin,E.; Quinke,K.; Spahn,H.; Mutschler,E. (-)-(S)-Flunoxaprofen and (-)-(S)-naproxen isocyanate: two new fluorescent chiral derivatizing agents for an enantiospecific determination of primary and secondary amines, *Chirality*, **1989**, *1*, 223–234.

SAMPLE
Matrix: solutions
Sample preparation: 50 μL 5 mg/mL Mexiletine in 100 mM HCl + 50 μL buffer + 100 μL reagent, swirl for 1 min, place on ice for 5 min, add 2 mL mobile phase, inject a 5 μL aliquot. (Buffer was 100 mM sodium borate adjusted to pH 9.50 with 2 M NaOH. Reagent was 13.40 g o-phthaldialdehyde and 16.3 mg N-acetyl-L-cysteine in 1 mL MeOH, protect from light, keep on ice.)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeOH:MeCN:buffer 60:1.5:40 (Buffer was 3 mL/L glacial acetic acid in water, pH adjusted to 7.20 with 2 M NaOH.)
Flow rate: 1
Injection volume: 5
Detector: F ex 338 em 425 or UV 254

CHROMATOGRAM
Retention time: 12.38 (S-(+)) (α = 1.11)
Limit of detection: 75 pg (F)

KEY WORDS
derivatization; protect from light; chiral; $\alpha \approx 1.11$

REFERENCE
Desai,D.M.; Gal,J. Enantiospecific drug analysis via the *ortho*-phthalaldehyde/homochiral thiol derivatization method, *J.Chromatogr.*, **1993**, *629*, 215–228.

SAMPLE
Matrix: solutions
Sample preparation: 10 μL 100 μM Mexiletine hydrochloride in 200 mM pH 8.0 borate buffer containing 4 mM disodium EDTA + 30 μL 50 mM 4-fluoro-7-nitro-2,1,3-benzoxadiazole in MeCN, mix, heat at 60° for 5 min, add 960 μL MeOH:acetic acid 99:1, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 60 (sic) 5 μm Ultron ES-phCD phenylcarbamylated β-cyclodextrin (Sinwa Kakou)
Mobile phase: MeCN:MeOH:water 10:40:50
Flow rate: 0.8
Injection volume: 10
Detector: F ex 470 em 530

CHROMATOGRAM
Retention time: 60 (R-(-)), 65 (S-(+))

KEY WORDS
chiral; derivatization

REFERENCE
Fukushima,T.; Kato,M.; Santa,T.; Imai,K. Enantiomeric separation and spectrofluorometric detection of the racemic drugs, (±)-1-(2,5-dimethylphenoxy)-2-propamine (mexiletine) and (3RS)-4-amino-3-hydroxybutanoic acid (GABOB), derivatized with 4-fluoro-7-nitro-2,1,3-benzoxadiazole on a phenylcarbamylated cyclodextrin bonded stationary phase, *Analyst*, **1995**, *120*, 381–383.

SAMPLE
Matrix: solutions

Sample preparation: Mix 20 µL of a 1 mM solution in MeOH or water with 50 µL pH 8 borate buffer and 50 µL 18 mM 2-(6-methoxy-2-naphthyl)-1-propyl chloroformate in acetone, vortex, let stand at room temperature for 30 min, add 100 µL 10 mM trans-4-hydroxy-L-proline in water, mix, let stand for 2 min, add 2 mL dichloromethane, vortex for 30 s. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 100 µL mobile phase, inject an aliquot. Prepare 2-(6-methoxy-2-naphthyl)-1-propyl chloroformate as follows. Stir 1.5 mmoles lithium aluminum hydride in THF, slowly add 2 mmoles (S)-naproxen in 20 mL anhydrous THF, reflux for 1 h, evaporate most of the solvent, cautiously add water with stirring, acidify with 6 N HCl, extract three times with diethyl ether. Combine the organic layers and dry them over anhydrous sodium sulfate, evaporate to dryness, chromatograph on silica gel with dichloromethane:MeOH 100:2 (flash chromatography), evaporate eluate to dryness, dry under vacuum over KOH to give 2-(6-methoxy-2-naphthyl)propanol as a white solid (mp 92-3°). Stir 0.5 mmoles 2-(6-methoxy-2-naphthyl)propanol and 0.5 mmoles triethylamine in 10 mL dry toluene at 0°, add 1 mL 20% phosgene in toluene (Caution! Phosgene is highly toxic, perform reaction in a chemical fume hood!) (Fluka), stir for 4 h, filter, evaporate to dryness under reduced pressure, dry under vacuum to give 2-(6-methoxy-2-naphthyl)-1-propyl chloroformate (mp 60°). Store under vacuum over phosphorus pentoxide at room temperature.)

HPLC VARIABLES

Column: 250 × 4 5 µm Zorbax-SIL
Mobile phase: n-Hexane:isopropanol 100:0.25
Flow rate: 1.5
Injection volume: 100
Detector: UV 230, F ex 270 em 365

CHROMATOGRAM

Retention time: k' 22.5 (S-(+)), k' 23.3 (R-(-))

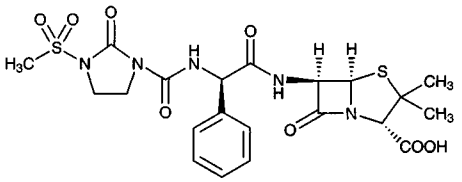
KEY WORDS

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.

Mezlocillin



Molecular formula: $C_{21}H_{25}N_5O_8S_2$
Molecular weight: 539.59
CAS Registry No.: 51481-65-3, 59798-30-0 (Na salt)
Merck Index: 6259
Lednicer No.: 3 206

SAMPLE

Matrix: bile, blood, urine
Sample preparation: Serum. 0.5 mL serum + 0.5 mL MeCN mix in 7 mL tube on vortex mixer; shake by rotation (20 rpm) 10 min; centrifuge 10 min 1000 g; transfer supernatant to another tube, add 7 aliquots dichloromethane; equilibrate 10 min; shake by rotation (20 rpm) 10 min; centrifuge 10 min 1000 g; inject aliquot of upper aqueous layer. Urine. Centrifuge urine and dilute 1:20. Bile. Centrifuge bile and dilute 1:10

HPLC VARIABLES

Column: 150 × 4.6 5 µm Ultrasphere ODS
Mobile phase: 24:76 MeCN:20 mM ammonium acetate adjusted to pH 5 with glacial acetic acid
Flow rate: 1
Injection volume: 20
Detector: UV 214

CHROMATOGRAM
Retention time: 5.2
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Also analyzed: ampicillin, azlocillin, aztreonam, cefmenoxime, cefoperazone, cefsulodin, cefotaxime, ceftazidime, ceftriaxone, cloxacillin, desacetylcefotaxime, 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 μL Serum + methicillin + 2 mL MeCN, vortex for 1 min, centrifuge at 3000 g for 10 min. Remove the supernatant and add it to 5 mL dichloromethane, vortex, centrifuge at 3000 g for 10 min, inject a 15 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: 300 mm long μBondapak C18
Mobile phase: MeCN:100 mM pH 6.1 sodium phosphate buffer 25:75
Flow rate: 2.5
Injection volume: 15
Detector: UV 229

CHROMATOGRAM
Internal standard: methicillin
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Extracted: piperacillin

KEY WORDS
serum; pharmacokinetics

REFERENCE
Martens,M.G.; Faro,S.; Feldman,S.; Cotton,D.B.; Dorman,K.; Riddle,G.D. Pharmacokinetics of the acylureidopenicillins piperacillin and mezlocillin in the postpartum patient, *Antimicrob.Agents Chemother.*, **1987**, *31*, 2015–2017.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 200 μL 400 mM HCl + 3.5 mL dichloromethane, extract. Extract the organic phase with 200 μL 20 mM pH 6.2 phosphate buffer, inject a 30-60 μL aliquot.

HPLC VARIABLES
Column: C18
Mobile phase: MeCN:20 mM pH 3.0 sodium phosphate buffer 24:76
Flow rate: 1
Injection volume: 30-60
Detector: UV 254

CHROMATOGRAM
Internal standard: mezlocillin

OTHER SUBSTANCES
Extracted: piperacillin

KEY WORDS
serum; mezlocillin is IS

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, tissue, urine
Sample preparation: Serum, plasma. Dilute serum or plasma 1:2 to 1:10 with buffer, centrifuge, inject a 20 μL aliquot of supernatant. Urine. Dilute urine 1:10 to 1:100 with buffer, centrifuge, inject a 20 μL aliquot of supernatant. Tissue (lung, gut). Cut tissue with a scalpel, homogenize with 1-3 mL buffer, centrifuge at 9600 g for 5 min three times, inject a 20 μL aliquot. Tissue (chondral). Cut tissue with a scalpel, homogenize with 3-6 mL buffer in an ice bath for 2-3 min, centrifuge at 9600 g for 5 min four or five times, inject a 100 μL aliquot. Dilute human pleural samples with buffer, centrifuge, inject a 20 μL aliquot. (Buffer was 66.6 mM K_2HPO_4 adjusted to pH 7.40 with KH_2PO_4.)

HPLC VARIABLES
Column: 200 × 4 5 μm Nucleosil C18
Mobile phase: MeCN:buffer 24:76, adjusted to pH 4.0 with phosphoric acid (Buffer was 57.4 mM K_2HPO_4 adjusted to pH 4.0 with phosphoric acid.)
Flow rate: 1
Injection volume: 20-100
Detector: UV 220

CHROMATOGRAM
Retention time: 16
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: Condition a Bondelut SPE cartridge (cat. no. 607101) with two 1 mL portions of MeCN and 1 mL buffer. Dilute urine 1:10 with buffer. 100 μL Serum or diluted urine + 100 μL 190 μg/mL nafcillin in buffer, vortex for 10 s, add to the SPE cartridge, wash with 1 mL buffer, wash with 500 μL MeCN:buffer 15:85, elute with 500 μL MeCN:buffer 70:30, inject a 10 μL portion of the eluate. (Buffer was 70 mM KH_2PO_4 adjusted to pH 5.0 with 5 M NaOH.)

HPLC VARIABLES
Guard column: 45 × 4 10 μm Spherisorb C-18
Column: 300 × 4 μBondapak C-18
Mobile phase: MeCN:50 mM pH 7.0 KH_2PO_4 27:73
Flow rate: 1.1
Injection volume: 10
Detector: UV 220

CHROMATOGRAM
Retention time: 4.2
Internal standard: nafcillin (7.5)
Limit of detection: 1.6 μg/mL

OTHER SUBSTANCES
Noninterfering: acetaminophen, dexamethasone, diazepam, furosemide, morphine

KEY WORDS
serum; SPE

REFERENCE
Fiore,D.; Auger,F.A.; Drusano,G.L.; Dandu,V.R.; Lesko,L.J. Improved micromethod for mezlocillin quantitation in serum and urine by high-pressure liquid chromatography, *Antimicrob.Agents Chemother.*, **1984**, *26*, 775–777.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm cyano
Mobile phase: MeCN:20 mM NaH_2PO_4 10:90 adjusted to pH 4.2 with phosphoric acid
Flow rate: 2.5
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 4.80

OTHER SUBSTANCES
Simultaneous: granisetron (UV 300)

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10-20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeOH:67 mM pH 3.0 KH_2PO_4 45:55
Flow rate: 1
Injection volume: 10-20
Detector: UV 231.1

CHROMATOGRAM
Retention time: 12.9
Internal standard: mezlocillin

OTHER SUBSTANCES
Simultaneous: azlocillin

KEY WORDS
mezlocillin is IS

REFERENCE
Barriere,S.L.; Catlin,D.H.; Orlando,P.L.; Noe,A.; Frost,R.W. Alteration in the pharmacokinetic disposition of ciprofloxacin by simultaneous administration of azlocillin, *Antimicrob.Agents Chemother.*, **1990**, *34*, 823–826.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 40 μL aliquot of an aqueous solution.

HPLC VARIABLES
Column: 250 × 4.6 5 μm LiChrospher C18
Mobile phase: MeCN:KH_2PO_4 20:80, pH 5.5
Column temperature: 37.5
Flow rate: 1.7
Injection volume: 40
Detector: UV 220

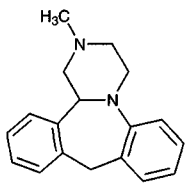
CHROMATOGRAM
Retention time: 7.7
Internal standard: mezlocillin

OTHER SUBSTANCES
Simultaneous: piperacillin

KEY WORDS
mezlocillin is IS

REFERENCE
Kinzig,M.; Sörgel,F.; Brismar,B.; Nord,C.E. Pharmacokinetics and tissue penetration of tazobactam and piperacillin in patients undergoing colorectal surgery, *Antimicrob.Agents Chemother.*, **1992**, *36*, 1997–2004.

Mianserin



Molecular formula: $C_{18}H_{20}N_2$
Molecular weight: 264.37
CAS Registry No.: 24219-97-4, 21535-47-7 (HCl)
Merck Index: 6260
Lednicer No.: 2 451

SAMPLE
Matrix: Blood, tissue, urine
Sample preparation: Serum, urine. 500 μL Serum or urine + 100 μL 2 μg/mL diazepam + 200 μL 20% sodium carbonate + 500 μL water + 3 mL n-hexane:isoamyl alcohol 98.5:1.5, mix for 2 min, centrifuge at 1200 g for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, inject a 10 μL aliquot. Tissue. Homogenize 1 g sample with 9 mL 100 mM HCl and 100 μL 20 μg/mL diazepam, centrifuge at 15000 g for 10 min. Add 500 μL 20% sodium carbonate and 4 mL n-hexane:isoamyl alcohol 98.5:1.5 to 1 mL of the supernatant, mix for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, filter by microconcentrator (Microcon-30, Grace). Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 2 μm TSK gel Super-Octyl (A) or 100 × 4.6 5 μm Hypersil MOS-C8 (B), (Yokogawa, Japan)
Mobile phase: MeOH:20 mM pH 7 KH_2PO_4 60:40
Flow rate: 0.6
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 9.3 (A), 13.5 (B)
Internal standard: diazepam (4.4, A)

SAMPLE
Matrix: solutions
Sample preparation: Inject a 40 μL aliquot of an aqueous solution.

HPLC VARIABLES
Column: 250 × 4.6 5 μm LiChrospher C18
Mobile phase: MeCN:KH_2PO_4 20:80, pH 5.5
Column temperature: 37.5
Flow rate: 1.7
Injection volume: 40
Detector: UV 220

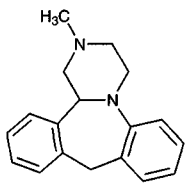
CHROMATOGRAM
Retention time: 7.7
Internal standard: mezlocillin

OTHER SUBSTANCES
Simultaneous: piperacillin

KEY WORDS
mezlocillin is IS

REFERENCE
Kinzig,M.; Sörgel,F.; Brismar,B.; Nord,C.E. Pharmacokinetics and tissue penetration of tazobactam and piperacillin in patients undergoing colorectal surgery, *Antimicrob.Agents Chemother.*, **1992**, *36*, 1997–2004.

Mianserin



Molecular formula: $C_{18}H_{20}N_2$
Molecular weight: 264.37
CAS Registry No.: 24219-97-4, 21535-47-7 (HCl)
Merck Index: 6260
Lednicer No.: 2 451

SAMPLE
Matrix: Blood, tissue, urine
Sample preparation: Serum, urine. 500 μL Serum or urine + 100 μL 2 μg/mL diazepam + 200 μL 20% sodium carbonate + 500 μL water + 3 mL n-hexane:isoamyl alcohol 98.5:1.5, mix for 2 min, centrifuge at 1200 g for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, inject a 10 μL aliquot. Tissue. Homogenize 1 g sample with 9 mL 100 mM HCl and 100 μL 20 μg/mL diazepam, centrifuge at 15000 g for 10 min. Add 500 μL 20% sodium carbonate and 4 mL n-hexane:isoamyl alcohol 98.5:1.5 to 1 mL of the supernatant, mix for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, filter by microconcentrator (Microcon-30, Grace). Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 2 μm TSK gel Super-Octyl (A) or 100 × 4.6 5 μm Hypersil MOS-C8 (B), (Yokogawa, Japan)
Mobile phase: MeOH:20 mM pH 7 KH_2PO_4 60:40
Flow rate: 0.6
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 9.3 (A), 13.5 (B)
Internal standard: diazepam (4.4, A)

Limit of quantitation: 50 ng/mL (serum, urine), 500 ng/mL (tissue)

OTHER SUBSTANCES

Extracted: amitriptyline, amoxapine, clomipramine, desipramine, dothiepin, doxepin, imipramine, maprotiline, melitracen, nortriptyline

Noninterfering: barbital, carbamazepine, ethosuximide, hexobarbital, lofepramine, pentobarbital, phenobarbital, phenytoin, primidone, sulpiride, trimethadione, trimipramine

KEY WORDS

serum; brain; liver

REFERENCE

Tanaka,E.; Terada,M.; Nakamura,T.; Misawa,S.; Wakasugi,C. Forensic analysis of eleven cyclic antidepressants in human biological samples using a new reversed-phase chromatographic column of 2 μm porous microspherical silica gel, *J.Chromatogr.B*, **1997**, *692*, 405–412.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 279

CHROMATOGRAM

Retention time: 6.02

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclam-

ide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 13.787

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: bulk

Sample preparation: Dissolve 2 mg mianserin in 1 mL EtOH, dilute with 4 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Chiralcel OD or 250 × 4.6 Chiralpak AD

Mobile phase: EtOH:n-hexane 95:5 or n-hexane:2-propanol 90:10

Flow rate: 0.5 or 1

Injection volume: 20

Detector: UV 250

KEY WORDS
chiral

REFERENCE
Selditz,U.; Liao,Y.; Franke,J.P.; de Zeeuw,R.A.; Wikström,H. Direct enantiomeric separation of mianserin and 6-azamianserin derivatives using chiral stationary phases, *J.Chromatogr.A*, **1998**, *803*, 169–177.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

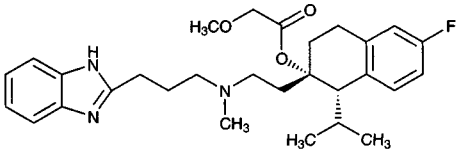
HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.4

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Mibefradil



Molecular formula: $C_{29}H_{38}FN_3O_3$
Molecular weight: 495.64
CAS Registry No.: 116644-53-2, 116666-63-8 (2.HCl)
Merck Index: 6261

SAMPLE
Matrix: blood
Sample preparation: Add 100-30 ng/mL IS, extract either using dichloromethane or using a Bond-Elut C2 SPE cartridge.

HPLC VARIABLES
Column: 3μm C6 (Gromsil or Spherisorb)
Mobile phase: MeCN:9.5 mM pH 3.9 phosphate buffer 55:45 to 70:30
Flow rate: 1
Detector: F ex 270 em 300

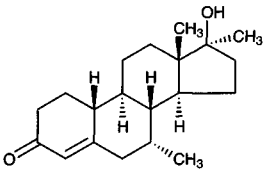
CHROMATOGRAM
Internal standard: Ro 40-6792

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; marmoset; cynomolgus monkey; human; plasma; pharmacokinetics; SPE

REFERENCE
Wiltshire,H.R.; Sutton,B.M.; Heeps,G.; Betty,A.M.; Angus,D.W.; Harris,S.R.; Worth,E.; Welker,H.A. Metabolism of the calcium antagonist, mibefradil (POSICOR, Ro 40-5967). Part III. Comparative pharmacokinetics of mibefradil and its major metabolites in rat, marmoset, cynomolgus monkey and man, *Xenobiotica*, **1997**, *27*, 557–571.

Mibolerone



Molecular formula: $C_{20}H_{30}O_2$
Molecular weight: 302.46
CAS Registry No.: 3704-09-4
Merck Index: 6262
Lednicer No.: 2 144

SAMPLE
Matrix: formulations
Sample preparation: Oils. 1 mL Sample + 25 mL MeOH:water 90:10, shake vigorously for 5 min, centrifuge, inject a 10 μL aliquot of the supernatant. Tablets. Grind a tablet to a fine powder, add 25 mL MeOH, sonicate for 5-10 min, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate. Suspensions (aqueous). Make up 5 mL to 50

mL with MeOH, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:water 75:25
Flow rate: 1.5
Injection volume: 10
Detector: UV 240

CHROMATOGRAM

Retention time: 7.3
Limit of detection: 5 μg/mL

OTHER SUBSTANCES

Simultaneous: methyltestosterone, methandriol (UV 210), norethindrone acetate, calusterone, mesterolone (UV 210), norethandrolone, trenbolone acetate, benzyl benzoate, nandrolone acetate, testosterone acetate, stanozolol, oxymetholone, nandrolone propionate, methenolone acetate, testosterone propionate, aspirin, caffeine, formebolone, benzyl alcohol, testolactone, cortisone, fluoxymesterone, norethindrone, oxandrolone (UV 210), boldenone, ethisterone, methandrostenolone, nandrolone, norgestrel, testosterone
Interfering: dehydroepiandrosterone (UV 210)

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: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

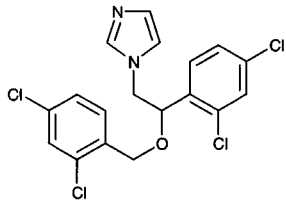
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide,

hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Miconazole



Molecular formula: $C_{18}H_{14}Cl_4N_2O$
Molecular weight: 416.13
CAS Registry No.: 22916-47-8, 22832-87-7 (nitrate)
Merck Index: 6266
Lednicer No.: 2 249

SAMPLE

Matrix: blood
Sample preparation: 400 μL Plasma + 400 μL water + 50 μL MeOH + 100 μL 1 M KOH + 6 mL hexane:dichloromethane 50:50, shake for 3 min, centrifuge at 4000 rpm for 6 min. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH, inject a 25 μL aliquot.

HPLC VARIABLES

Guard column: 80 × 4 CoPell ODS
Column: 300 × 4 μBondapak C18
Mobile phase: MeCN:10 mM pH 8.0 NaH_2PO_4 buffer 66:34
Flow rate: 2
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 10
Internal standard: miconazole

OTHER SUBSTANCES

Extracted: sulconazole

KEY WORDS
plasma; dog; miconazole is IS

REFERENCE
Fass,M.; Zaro,B.; Chaplin,M.; Matin,S. Reversed-phase high-pressure liquid chromatographic analysis of sulconazole in plasma, *J.Pharm.Sci.*, **1981**, *70*, 1338–1340.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 202.8

CHROMATOGRAM
Retention time: 21.53

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Powders. Extract a sample equivalent to about 10 mg miconazole twice with 20 mL portions of MeOH with magnetic stirring, filter extracts, combine, dilute to 50 mL with MeOH. Remove a 2.5 mL aliquot and add it to 2 mL 200 μg/mL econazole in MeOH, dilute to 10 mL with MeOH, inject a 10 μL aliquot. Creams. Condition a Baker diol SPE cartridge with 6 mL dichloromethane. Add a sample equivalent to 10 mg miconazole to 30 mL dichloromethane, sonicate for 2 min, make up to 50 mL with dichloromethane, filter, add 2 mL of the filtrate to the SPE cartridge. Wash with two 3 mL portions of n-hexane:dichloromethane 4:1, aspirate to dryness, elute with three 1 mL portions of MeOH:100 mM triethylamine adjusted to pH 7.0 with acetic acid 4:1. Combine the eluates and dilute them to 5 mL with MeOH. Remove a 2.5 mL aliquot and add it to 1 mL 200 μg/mL econazole in MeOH, dilute to 5 mL with MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Nova-Pak RP-18
Mobile phase: MeOH:THF:100 mM triethylamine adjusted to pH 7.0 with acetic acid 70:12:18
Flow rate: 0.8
Injection volume: 10
Detector: UV 230

CHROMATOGRAM
Retention time: 3.5
Internal standard: econazole (2.5)

KEY WORDS
creams; powders; SPE

REFERENCE
Cavrini,V.; Di Pietra,A.M.; Gatti,R. Analysis of miconazole and econazole in pharmaceutical formulations by derivative UV spectroscopy and liquid chromatography (HPLC), *J.Pharm.Biomed.Anal.*, **1989**, *7*, 1535–1543.

SAMPLE
Matrix: formulations
Sample preparation: Dilute peritoneal dialysis fluid with an equal volume of 4 μg/mL p-dichlorobenzene in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 37-50 μm Bondapak C18/Corasil
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:50 mM pH 4.6 $(NH_4)H_2PO_4$ 85:15
Flow rate: 1
Injection volume: 20
Detector: UV 229

CHROMATOGRAM
Retention time: 8.5
Internal standard: p-dichlorobenzene (4.5)

KEY WORDS
stability-indicating; peritoneal dialysis fluid

REFERENCE
Holmes,S.E.; Aldous,S. Stability of miconazole in peritoneal dialysis fluid, *Am.J.Hosp.Pharm.*, **1991**, *48*, 286–290.

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

CHROMATOGRAM
Retention time: 18
Internal standard: ketoconazole (7)

OTHER SUBSTANCES
Simultaneous: clotrimazole, ketoconazole, bifonazole, tioconazole, isoconazole, econazole, fenticonazole

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: Dilute with mobile phase, add metronidazole, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb ODS C18
Mobile phase: MeCN:buffer 15:85 (Buffer was 10 mM NaH_2PO_4 adjusted to pH 8 with trimethylamine.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 250

CHROMATOGRAM
Retention time: 3.93
Internal standard: metronidazole (2)

KEY WORDS
injections; stability-indicating; 5% dextrose; saline

REFERENCE
Faouzi,M.E.A.; Dine,T.; Luyckx,M.; Brunet,C.; Mallevais,M.-L.; Goudaliez,F.; Gressier,B.; Cazin,M.; Kablan,J.; Cazin,J.C. Stability, compatibility and plasticizer extraction of miconazole injection added to infusion solutions and stored in PVC containers, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1363–1372.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: RCSS Guard-Pak (Waters)
Column: 100 × 8 C18 Radial Pak (Waters)
Mobile phase: MeOH:0.75% acetic acid 30:70, pH adjusted to 5.5 with triethylamine
Flow rate: 3
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 14.1

OTHER SUBSTANCES
Simultaneous: acetaminophen, N-acetylprocainamide, cefaclor, cefamandole, cefazolin, cefotaxime, cefoxitin, cephalexin, cephalothin, cephapirin, chloramphenicol, cimetidine, moxalactam, procainamide, sulfamethoxazole, theophylline, tobramycin, vancomycin

REFERENCE
Danzer,L.A. Liquid-chromatographic determination of cephalosporins and chloramphenicol in serum, *Clin.Chem.*, **1983**, *29*, 856–858.

SAMPLE
Matrix: solutions
Sample preparation: Centrifuge at 10000 rpm, dilute the supernatant with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:50 mM $(NH_4)H_2PO_4$ 85:15
Detector: UV 230

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: tissue
Sample preparation: Skin sample extracted with 500 μL mobile phase, vortex 1 min, centrifuge at 8000 rpm for 10 min, inject 40 μL aliquot.

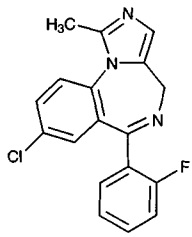
HPLC VARIABLES
Column: 125 × 4.5 Whatman 5 μm reverse-phase C18
Mobile phase: MeCN:10 mM KH_2PO_4 80:20
Flow rate: 0.7
Injection volume: 40
Detector: UV 214

CHROMATOGRAM
Retention time: 5.5
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.

Midazolam



Molecular formula: $C_{18}H_{13}ClFN_3$
Molecular weight: 325.77
CAS Registry No.: 59467-70-8, 59467-96-8 (HCl), 59467-94-6 (maleate)
Merck Index: 6270
Lednicer No.: 3 197

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL MeOH + 500 μL 1 M NaOH + 3 mL hexane, shake for 5 min, centrifuge at 3000 rpm for 5 min. Evaporate 2 mL of the organic phase to dryness under a stream of nitrogen. Dissolve residue in 200 μL mobile phase, inject a 75 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm YMC-Guardpak ODS-AM (GL Sciences)
Column: 250 × 4.6 5 μm Inertsil ODS (GL Sciences)

Mobile phase: MeCN:10 mM pH 6.5 phosphate buffer 80:20
Flow rate: 1
Injection volume: 75
Detector: UV 245

CHROMATOGRAM
Limit of detection: 50 ng/mL (sic)

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Takedomi,S.; Matsuo,H.; Yamano,K.; Yamamoto,K.; Iga,T.; Sawada,Y. Quantitative prediction of the interaction of midazolam and histamine H_2 receptor antagonists in rats, *Drug Metab.Dispos.*, **1998**, *26*, 318–323.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL MeOH + 500 μL 1 M NaOH + 3 mL hexane, shake for 5 min, centrifuge at 3000 rpm for 5 min. Evaporate 2 mL of the organic phase to dryness under a stream of nitrogen. Dissolve residue in 200 μL mobile phase, inject a 75 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm YMC-Guardpack ODS-AM (GL Sciences, Japan)
Column: 250 × 4.6 5 μm Inertsil ODS
Mobile phase: MeCN:10 mM pH 6.5 phosphate buffer 8:2
Flow rate: 1
Injection volume: 75
Detector: UV 245

CHROMATOGRAM
Limit of detection: 50 ng/mL

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Takedomi,S.; Matsuo,H.; Yamano,K.; Yamamoto,K.; Iga,T.; Sawada,Y. Quantitative prediction of the interaction of midazolam and histamine H_2 receptor antagonists in rats, *Drug Metab.Dispos.*, **1998**, *26*, 318–323.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL 1 M NaOH + 2 mL 10% (v/v) isopropanol in dichloromethane containing 25 ng/mL IS, vortex for 60 s, centrifuge at 2000 g for 45 s. Evaporate organic phase under a gentle stream of air at 60° for 10 min. Dissolve residue in 1 mL hexane:MTBE 2:1, add 200 μL 20 mM orthophosphoric acid, vortex for 30 s, centrifuge at 3200 g for 45 s. Carefully aspirate upper organic layer, add 7 μL 1 M NaOH to acidic aqueous phase to adjust pH to 6.6-6.9, inject an 80 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm C8 Symmetry (Waters)
Mobile phase: MeCN:THF:10 mM pH 6.7 phosphate buffer 35:5:60
Flow rate: 1
Injection volume: 80
Detector: UV 220

CHROMATOGRAM
Retention time: 7.4
Internal standard: climazolam (8.4)
Limit of detection: 10 ng/mL
Limit of quantitation: 12.5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: amoxicillin, caffeine, carbamazepine, cisapride, dexamethasone, dopamine, furosemide, gentamicin, indomethacin, morphine, phenobarbital, ranitidine, theophylline

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Lee,T.C.; Charles,B. Measurement by HPLC of midazolam and its major metabolite, 1-hydroxymidazolam in plasma of very premature neonates, *Biomed.Chromatogr.*, **1996**, *10*, 65–68.

SAMPLE
Matrix: blood
Sample preparation: Add 25 μL 40 μg/mL diazepam in MeOH, 40 μL 2% NaOH, and 3.5 mL cyclohexane:diethyl ether 31:69 to 1 mL plasma. Extract on a rotary mixer at 4° for 10 min, centrifuge at 4° at 2000 g for 10 min. Remove a 3.3 mL aliquot of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°. Dissolve the residue in 300 μL MeCN: water 5:95, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 5 × 0.8 μ-Precolumn cartridge C18 (LC Packings)
Column: 150 × 0.8 3 μm Hypersil C18 BDS
Mobile phase: Gradient. MeCN:10 mM pH 7.0 sodium phosphate buffer 35:65 for 16 min, to 60: 40 over 1 min, maintain at 60:40.
Flow rate: 0.016
Injection volume: 20
Detector: UV 240 for 17.6 min then UV 300

CHROMATOGRAM
Retention time: 15.4
Internal standard: diazepam (19.5)
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, 1'-hydroxymidazolam

KEY WORDS
pharmacokinetics; capillary HPLC; plasma

REFERENCE
Eeckhoudt,S.L.; Desager,J.-P.; Horsmans,Y.; De Winne,A.J.; Verbeeck,R.K. Sensitive assay for midazolam and its metabolite 1'-hydroxymidazolam in human plasma by capillary high-performance liquid chromatography, *J.Chromatogr.B*, **1998**, *710*, 165–171.

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL 1 M pH 9.0 borate buffer to 50 μL serum, mix well, add 1 mL chloroform:diethyl ether 95:5 (Caution! Chloroform is a carcinogen!), vortex for 1 min, centrifuge at 1100 for 5 min, evaporate the organic layer to dryness under nitrogen at 40°, resuspend the residue in 50 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 2.1 C18 symmetry column (Waters)
Mobile phase: MeCN:MeOH:14.9 mM pH 3 sodium acetate 23:10:67
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 4.7
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
pharmacokinetics; rat; serum

REFERENCE
Ma,F.; Lau,C.E. Determination of midazolam and its metabolites in serum microsamples by high-performance liquid chromatography and its application to pharmacokinetics in rats, *J.Chromatogr.B*, **1996**, *682*, 109–113.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 14.873

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Cool microsomal incubation on ice, add 100 μL MeCN and IS, centrifuge, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:MeOH:10 mM phosphate buffer 22.5:37.5:40
Flow rate: 0.8
Detector: UV 220

CHROMATOGRAM
Retention time: 14.1
Internal standard: phenacetin (5.4)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
liver

REFERENCE
von Moltke,L.L.; Greenblatt,D.J.; Schmider,J.; Duan,S.X.; Wright,C.E.; Harmatz,J.S.; Shader,R.I. Midazolam hydroxylation by human liver microsomes in vitro: Inhibition by fluoxetine, norfluoxetine, and by azole antifungal agents, *J.Clin.Pharmacol.*, **1996**, *36*, 783–791.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Cool incubation mixture on ice, add 100 μL MeCN and IS, centrifuge, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:MeOH:10 mM phosphate buffer 22.5:37.5:40
Flow rate: 0.8
Detector: UV 220

CHROMATOGRAM
Retention time: 14.2
Internal standard: phenacetin (5.4)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
liver

REFERENCE
von Moltke,L.L.; Greenblatt,D.J.; Schmider,J.; Duan,S.X.; Wright,C.E.; Harmatz,J.S.; Shader,R.I. Midazolam hydroxylation by human liver microsomes in vitro: Inhibition by fluoxetine, norfluoxetine, and by azole antifungal agents, *J.Clin.Pharmacol.*, **1996**, *36*, 783–791.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 2 mg/mL solution in 0.9% sodium chloride, dilute 1:1000 with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: Bakerbond phenyl column
Mobile phase: MeCN:20 mM sodium dihydrogen phosphate 80:20
Flow rate: 2
Detector: UV 240

CHROMATOGRAM
Retention time: 9.2

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
stability-indicating

REFERENCE
Stiles,M.L.; Allen,L.V.,Jr.; Prince,S.J. Stability of deferoxamine mesylate, floxuridine, fluorouracil, hydromorphone hydrochloride, lorazepam, and midazolam hydrochloride in polypropylene infusion-pump syringes, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 1583–1588.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize brain in 100 mM NaOH (5 mL/g) by sonication. Add 60 μL 1 μg/mL IS in MeOH to a tube, dry in a water bath under a stream of nitrogen at 50°. Add 250 μL homogenate (ca. 50 mg tissue equivalent) and 250 μL 100 mM pH 13 NaOH, vortex thoroughly. Add 2 mL toluene, mix (50 inversions), centrifuge at 15 000 rpm at 4° for 20 min. Dry the organic phase under a stream of nitrogen. Add 2 mL toluene to the aqueous phase and repeat the extraction. Combine the organic layers and dry under a stream of nitrogen. Reconstitute the residue in 100 μL mobile phase, inject a 40 μL aliquot.

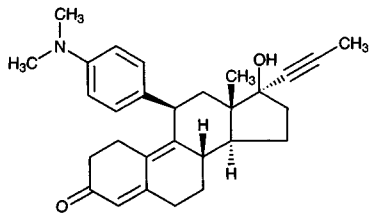
HPLC VARIABLES
Column: 100 × 4.6 3 μm Rainin C8 Microsorb
Mobile phase: MeCN:MeOH:25 mM potassium phosphate buffer 18.5:16.5:65
Flow rate: 0.9
Injection volume: 40
Detector: UV 240

CHROMATOGRAM
Retention time: 4.2
Internal standard: halazepam (6.2)
Limit of quantitation: 500 ng/g

KEY WORDS
brain; rat

REFERENCE
Jiang,Q.; Walton,N.Y.; Gunawan,S.; Treiman,D.M. High-performance liquid chromatographic determination of midazolam in rat brain, *J.Chromatogr.B*, **1996**, *683*, 276–280.

Mifepristone



Molecular formula: $C_{29}H_{35}NO_2$
Molecular weight: 429.60
CAS Registry No.: 84371-65-3
Merck Index: 6273
Lednicer No.: 5 53

SAMPLE
Matrix: blood
Sample preparation: Add 200-400 μL plasma or serum to a Pasteur pipette packed with 3 mL 60-80 mesh Chromosorb W-NAW:ethylene glycol 80:20 (w/w), let stand for 30 min, elute with 5 mL n-hexane:ethyl acetate 95:5. Evaporate the eluate and reconstitute the residue in mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: Lichrosorb RP-18
Mobile phase: MeOH:water:triethanolamine 90:10:0.05
Flow rate: 1.5
Injection volume: 100
Detector: UV 304

CHROMATOGRAM
Retention time: 4.33
Limit of detection: 4 ng

KEY WORDS
plasma; serum; SPE; pharmacokinetics

REFERENCE
Heikinheimo,O.; Tevilin,M.; Shoupe,D.; Croxatto,H.; Lahteenmaki,P. Quantitation of RU 486 in human plasma by HPLC and RIA after column chromatography, *Contraception*, **1986**, *34*, 613–624.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 50 μL 12.6 μg/mL IS in MeOH + 650 μL water, mix, inject a 400 μL aliquot into column A and elute to waste with mobile phase A for 7 min, elute 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, monitor the effluent from column B. Wash column A with MeOH:water 70:30 for 5 min then re-equilibrate with water.

HPLC VARIABLES
Column: A 30 × 4 Serumout (Sekisui); B 300 × 3.9 μBondapak C18
Mobile phase: A water; B MeCN:MeOH:50 mM pH 3.1 phosphate buffer 27:18:60
Column temperature: 25
Flow rate: A 0.6; B 1
Injection volume: 400
Detector: UV 305 or E, Sekisui ECD-120, +1.0 V, Ag/AgCl reference electrode

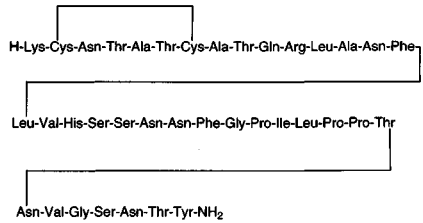
CHROMATOGRAM
Retention time: 30.1
Internal standard: 13-ethyl-17-hydroxy-18,19-dinorpregna-4,9,11-trien-20-yn-3-one (R2323) (35.3)
Limit of detection: 0.9 ng (E), 6.6 ng (UV)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; pharmacokinetics; column-switching

REFERENCE
Nagoshi,K.; Hayashi,N.; Sekiba,K. Automated direct assay system for RU38486, an antiprogesterone-antiglucocorticoid agent, and its metabolites using high performance liquid chromatography, *Acta Med.Okayama.*, **1991**, *45*, 81–87.

Miloxacin



Molecular formula: $C_{12}H_9NO_6$
Molecular weight: 263.21
CAS Registry No.: 37065-29-5
Merck Index: 6283

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood. Filter serum through a 0.45 μm syringe filter with a cellulose acetate membrane, inject a 50 μL aliquot of the filtrate. Tissue. Add 1 mL MeCN:THF 95:5 to 1 g muscle, homogenize with a Pencil Mixer (Iuchi, Japan) for 2 min, centrifuge at 1500 g for 5 min, filter the supernatant through a syringe filter unit, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μL Hisep shielded hydrophobic phase precolumn (Supelco)
Column: 150 × 4.6 5 μL Hisep shielded hydrophobic phase (Supelco)
Mobile phase: MeCN:buffer 15:85 (Buffer was 50 mM citric acid:200 mM pH 2.5 Na_2HPO_4 buffer containing 10 mM tetra-*n*-butyl ammonium bromide 85:15.)
Flow rate: 1

Injection volume: 20-50
Detector: UV 265

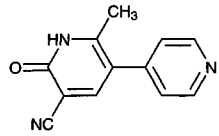
CHROMATOGRAM
Retention time: 11.5
Limit of detection: 50 ng/mL (serum), 100 ng/mL (muscle)

OTHER SUBSTANCES
Extracted: sulfamonomethoxine, oxolinic acid

KEY WORDS
fish; muscle; serum

REFERENCE
Ueno,R.; Aoki,T. High-performance liquid chromatographic method for the rapid and simultaneous determination of sulfamonomethoxine, miloxacin and oxolinic acid in serum and muscle of cultured fish, *J.Chromatogr.B*, **1996**, *682*, 179–181.

Milrinone



Molecular formula: $C_{12}H_9N_3O$
Molecular weight: 211.22
CAS Registry No.: 78415-72-2
Merck Index: 6284

SAMPLE
Matrix: blood, tissue
Sample preparation: Homogenize (Ystral D7801) tissue with 2 volumes of 10 mM sodium bicarbonate containing 2.5% acetic acid (final pH 3), centrifuge at 20000 g for 30 min. 100 μL Plasma or tissue homogenate + 375 μL water, mix, add 25 μL 50% acetic acid, vortex, centrifuge at 7000 g for 5 min. Filter (Amicon Centricon-30 microconcentrator) while centrifuging in a refrigerated centrifuge at 5500 g for 35 min, inject an aliquot of the filtrate.

HPLC VARIABLES
Guard column: 10 × 3 RP-2 (Chrompack)
Column: 250 × 4 RP Select B RT (Merck)
Mobile phase: MeOH:25 mM pH 3.75 ammonium acetate containing 0.01% tetramethylammonium hydroxide
Flow rate: 0.75
Injection volume: 100
Detector: UV 331

CHROMATOGRAM
Retention time: 5
Limit of detection: 5 ng/mL

KEY WORDS
rat; liver; heart; brain; lung; plasma

REFERENCE
Verrijk,R.; van Rooij,H.H.; Wemer,J.; Porsius,A.J. High-performance liquid chromatographic determination of milrinone in biological tissues and fluids, *J.Chromatogr.*, **1989**, *491*, 265–268.

SAMPLE
Matrix: blood, urine
Sample preparation: Urine. Acidify urine to pH 3.75 with 50% acetic acid, filter (Amicon Centricon-30 microconcentrator), inject an aliquot. Plasma. 100 μL Plasma + 375 μL water, mix,

add 25 μL 50% acetic acid, vortex, centrifuge at 7000 g. Filter (Amicon Centricon-30 microconcentrator) while centrifuging in a refrigerated centrifuge at 5500 g for 35 min, inject an aliquot of the filtrate.

HPLC VARIABLES
Guard column: 10 × 3 RP-2 (Chrompack)
Column: 250 × 4 RP Select B RT (Merck)
Mobile phase: MeOH:25 mM pH 3.75 ammonium acetate containing 0.01% tetramethylammonium hydroxide
Flow rate: 0.75
Injection volume: 100
Detector: UV 331

CHROMATOGRAM
Limit of detection: 5 ng/mL

KEY WORDS
rat; plasma; pharmacokinetics

REFERENCE
Verrijk,R.; Vleeming,W.; van Rooij,H.H.; Wemer,J.; Porsius,A.J. Plasma elimination of milrinone in rats in relation to its hemodynamic effects, *J.Pharm.Sci.*, **1990**, *79*, 236–239.

SAMPLE
Matrix: formulations
Sample preparation: 1 mL Sample + 99 mL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil ODS III
Mobile phase: MeOH:500 mM pH 7 borate buffer:water 40:2:58
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 2.5

OTHER SUBSTANCES
Simultaneous: degradation products
Noninterfering: digoxin

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: 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:water 22:10:68 adjusted to pH 7.1 with 10 M NaOH
Flow rate: 2
Injection volume: 20
Detector: UV 268

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Simultaneous: procainamide, degradation products

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: 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 is 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

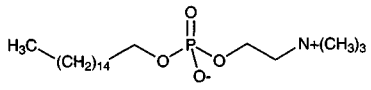
CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: atropine

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.

Miltefosine



Molecular formula: $C_{21}H_{46}NO_4P$
Molecular weight: 407.57
CAS Registry No.: 58066-85-6
Merck Index: 6285

SAMPLE
Matrix: blood
Sample preparation: Lyophilize 1 mL serum, extract residue 3 times with 1 mL portions of MeOH. Combine the extracts, add 500 μL of a saturated solution of potassium tert-butoxide in MeOH, heat at 50° for 15 min, add 1 mL 3 M HCl, heat at 50° for 15 min, neutralize with 250 μL 2 M NaOH, wash twice with 2 mL portions of n-hexane. Extract the MeOH/water phase with chloroform. Evaporate the chloroform layer to dryness under a stream of nitrogen, reconstitute the residue in 500 μL MeCN:MeOH:water 20:57:23, inject an aliquot.

HPLC VARIABLES
Guard column: 70 × 3.2 30-40 μm SC-201 RP (Vydac)

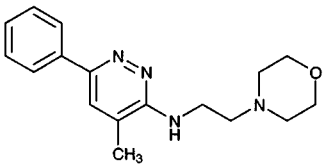
Column: 250 × 4.6 5 μm Nucleosil 120-5 C18
Mobile phase: MeCN:MeOH:water 20:57:23 containing 20 mM choline chloride
Flow rate: 2
Detector: UV 203 or radioactivity

CHROMATOGRAM
Retention time: 22

KEY WORDS
rat; serum; pharmacokinetics; tritium-labeled

REFERENCE
Unger,C.; Fleer,E.; Damenz,W.; Hilgard,P.; Nagel,G.; Eibl,H. Hexadecylphosphocholine: determination of serum concentrations in rats, *J.Lipid Mediat.*, **1991**, *3*, 71–78.

Minaprine



Molecular formula: $C_{17}H_{22}N_4O$
Molecular weight: 298.39
CAS Registry No.: 25905-77-5, 25953-17-7 (2.HCl)
Merck Index: 6287
Lednicer No.: 4 120

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

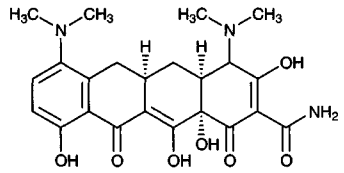
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 204

CHROMATOGRAM
Retention time: 11.225

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Minocycline



Molecular formula: $C_{23}H_{27}N_3O_7$
Molecular weight: 457.48
CAS Registry No.: 10118-90-8, 13614-98-7 (HCl)
Merck Index: 6289
Lednicer No.: 1 214

SAMPLE

Matrix: blood
Sample preparation: Mix 1 mL plasma with 400 μL 500 mM pH 5 KH_2PO_4 and 5 mL ethyl acetate, shake for 1 min, centrifuge at ≥2000 g for 2 min, mix 4 mL of the upper organic phase with 500 μL 20 mM HCl, shake for 1 min, centrifuge at ≥2000 g for 2 min, inject a 20 μL aliquot of the lower aqueous phase.

HPLC VARIABLES

Column: 125 × 4.0 Nucleosil 5-CN
Mobile phase: MeOH:20 mM perchloric acid containing 4 mM triethylamine 20:80
Flow rate: 1
Detector: UV 350

CHROMATOGRAM

Retention time: 2.8
Limit of quantitation: 30 ng/mL

KEY WORDS

plasma

REFERENCE

Mascher,H.J. Determination of minocycline in human plasma by high-performance liquid chromatography with UV detection after liquid-liquid extraction, *J.Chromatogr.A*, **1998**, *812*, 339–342.

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: 150 × 3.9 10 μm Waters RP phenyl
Mobile phase: Gradient. A 0.1 M diammonium EDTA:1 M diethanolamine (to pH 7.3 with 85% phosphoric acid):isopropanol:water 10:50:185:755. B 0.1 M diammonium EDTA:1 M diethanolamine (to pH 7.3 with 85% phosphoric acid):isopropanol:water 10:50:400:540. From 100% A to 100% B over 6 min
Flow rate: 2
Injection volume: 80
Detector: UV 340

CHROMATOGRAM

Retention time: 4.1
Limit of detection: 35 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
Sample preparation: 200 μL Serum + 50 μL 20 μg/mL oxytetracycline in water + 250 μL 500 mM trichloroacetic acid, vortex for 30 s, centrifuge at 10000 g for 10 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: Nova-Pak phenyl guard
Column: 4 μm Nova-Pak phenyl radial compression
Mobile phase: MeCN:MeOH:100 mM oxalic acid 11:9:80 adjusted to pH 2.7 with 1 M HCl
Flow rate: 2
Injection volume: 50
Detector: UV 350

CHROMATOGRAM
Retention time: 8-9
Internal standard: oxytetracycline (13.5-14)
Limit of quantitation: 620 ng/mL

KEY WORDS
serum; pharmacokinetics

REFERENCE
Birmingham,K.; Vaughan,L.M.; Strange,C. Rapid serum minocycline assay for pleurodesis monitoring using high-performance liquid chromatography with radial compression, *Ther.Drug Monit.*, **1995**, *17*, 268–272.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 22.637

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk, formulations

Sample preparation: Bulk. Dissolve 25 mg minocycline hydrochloride in solvent, make up to 25 mL with solvent, inject an aliquot. Capsules, tablets. Add an amount equivalent to 25 mg minocycline hydrochloride to 25 mL solvent, sonicate for 5 min, centrifuge at 2500 g for 5 min, filter (1.5 μm), inject an aliquot of the filtrate. (Solvent was 10 mM NaOH containing 0.1% sodium sulfite.)

HPLC VARIABLES
Column: 250 × 4.6 8 μm 100 Å PLRP-S poly(styrene-divinylbenzene) (Polymer Labs)
Mobile phase: t-Butanol:200 mM pH 10.5 phosphate buffer:TBAS solution:EDTA solution:water 9:10:10:10:61 (TBAS solution was 20 mM tetrabutylammonium sulfate adjusted to pH 10.5 with NaOH solution. EDTA solution was 10 mM EDTA adjusted to pH 10.5 with NaOH solution.)
Column temperature: 60
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 20

OTHER SUBSTANCES
Simultaneous: impurities

KEY WORDS
capsules; tablets

REFERENCE
Weng,N.; Thuranira,J.; Vermeulen,K.; Roets,E.; Hoogmartens,J. Quantitative analysis of minocycline by liquid chromatography on poly(styrene-divinylbenzene), *J.Liq.Chromatogr.*, **1992**, *15*, 2529–2549.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Bulk. Prepare a 10-100 μg/mL solution in buffer, inject an aliquot. Capsules, tablets. Prepare a 1 mg/mL solution of capsule contents or crushed tablets in buffer, sonicate for 10 min, filter (0.45 μm), dilute with buffer, inject an aliquot. (Buffer was 20 mM sodium perchlorate adjusted to pH 2.0 with perchloric acid.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm 100 Å PLRP-S polystyrene-divinylbenzene (Polymer Laboratories)
Mobile phase: MeCN:buffer 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: 15

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: cells
Sample preparation: 100 μL Cell suspension + 100 μL cefoperazone solution + 100 μL Hanks balanced salt solution, sonicate 30 min, add 800 μL MeCN, centrifuge at 13000 g for 5 min,

remove supernatant. Dry supernatant under air, dissolve in 100 μL mobile phase, inject 75 μL.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:50 mM pH 3.1 KH_2PO_4 45:55
Flow rate: 1
Injection volume: 75
Detector: UV 353

CHROMATOGRAM
Retention time: 3
Internal standard: tetracycline
Limit of detection: 100-1000 ng/mL

REFERENCE
Darouiche,R.O.; Hamill,R.J. Antibiotic penetration of and bactericidal activity within endothelial cells, *Antimicrob.Agents Chemother.*, **1994**, *38*, 1059–1064.

SAMPLE
Matrix: formulations
Sample preparation: Inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Vydac 208TP54 C8
Mobile phase: MeCN:buffer 22:78 (Buffer was 50 mM K_2HPO_4 adjusted to pH 6.5 with phosphoric acid.)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 7.2

OTHER SUBSTANCES
Simultaneous: degradation products
Noninterfering: rifampin

KEY WORDS
injections; saline; 5% dextrose; stability-indicating

REFERENCE
Pearson,S.D.; Trissel,L.A. Stability and compatibility of minocycline hydrochloride and rifampin in intravenous solutions at various temperatures, *Am.J.Hosp.Pharm.*, **1993**, *50*, 698–702.

SAMPLE
Matrix: honey
Sample preparation: Condition a 500 mg Baker-10 C18 SPE cartridge with 10 mL MeOH, 10 mL water, and 10 mL saturated aqueous disodium EDTA. Condition a 500 mg Baker-10 COOH cartridge with MeOH:ethyl acetate 10:90. Dissolve 25 g honey in 50 mL 100 mM pH 4.0 disodium EDTA-McIlvaine buffer, filter. Add the filtrate to the C18 SPE cartridge, wash with 20 mL water, wash with 400 μL ethyl acetate, air dry under vacuum for 5 min, elute with 50 mL MeOH:ethyl acetate 10:90. Add a 5 mL aliquot to the COOH SPE cartridge, wash with 5 mL MeOH (?), elute with 10 mL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 75 × 4.6 3 μm Chemcosorb 3C8 (Chemco)
Mobile phase: MeCN:MeOH:10 mM aqueous oxalic acid 3:2:16, pH 3.0
Flow rate: 1
Injection volume: 100
Detector: UV 350

CHROMATOGRAM
Retention time: 2
Limit of detection: 0.1 ppm

OTHER SUBSTANCES
Extracted: chlortetracycline, demeclocycline (demethylchlortetracycline), doxycycline, methacycline, oxytetracycline, tetracycline

KEY WORDS
SPE

REFERENCE
Oka,H.; Ikai,Y.; Kawamura,N.; Uno,K.; Yamada,M.; Harada,K.; Suzuki,M. Improvement of chemical analysis of antibiotics. XII. Simultaneous analysis of seven tetracyclines in honey, *J.Chromatogr.*, **1987**, *400*, 253–261.

SAMPLE
Matrix: milk
Sample preparation: Fill a disposable polypropylene column (Bio-Rad Econo-Pac column) with Chelating Sepharose Fast Flow (Pharmacia) and condition it with 10 mL water, 1.5 mL 100 mM copper sulfate, and 100 mL water. Condition a 6 mL SupelClean ENVI-Chrom P SPE cartridge with 2 mL MeOH and 5 mL water. Homogenize 10 g tissue with 20-30 mL 100 mM pH 4 succinic acid buffer. Centrifuge the homogenate at 2000 g at 10° for 15-20 min. Add the supernatant to the metal chelate affinity column, wash sequentially with 5 mL 500 mM NaCl, 10 mL water, 10 mL MeOH, 10 mL water, and 3 mL McIlvaine buffer, discard the clear effluent. Elute with 8 mL McIlvaine-EDTA-NaCl buffer. Add the eluate to the SPE cartridge under gravity, rinse the column with 2.5 mL water, add the rinse to the SPE cartridge. Wash the SPE cartridge with 2.5 mL water. Dry the SPE cartridge by drawing air through it for 2-3 min. Elute with 5 mL MeOH. Evaporate the eluate to dryness under nitrogen at 40-50°, dissolve the residue in 1 mL water. Inject a 100 μL aliquot. (McIlvaine buffer was 500 mM NaCl and 100 mM EDTA (Carson, M.C. J. AOAC Int. 1993, 76, 329).)

HPLC VARIABLES
Column: 150 × 3.9 5 μm PLRP-S (Polymer Labs, USA)
Mobile phase: MeOH:5 mM oxalic acid 58:42
Flow rate: 0.5
Injection volume: 100
Detector: MS, HP 5989, NICI, high energy dynode, HP 59980B particle beam interface 60°, helium sheath 40-45 p.s.i., source 250°, quadrupole 100°, source pressure 1 Torr with methane reagent gas, m/z 378-483

CHROMATOGRAM
Retention time: 6.75

OTHER SUBSTANCES
Extracted: chlortetracycline, demeclocycline, doxycycline, oxytetracycline, tetracycline

KEY WORDS
metal chelate affinity chromatography; cow; SPE

REFERENCE
Carson,M.C.; Ngoh,M.A.; Hadley,S.W. Confirmation of multiple tetracycline residues in milk and oxytetracycline in shrimp by liquid chromatography-particle beam mass spectrometry, *J.Chromatogr.B*, **1998**, *712*, 113–128.

SAMPLE
Matrix: milk
Sample preparation: Prepare a column as follows. Swirl Chelating Sepharose Fast Flow resin (Pharmacia) in its bottle, add it to a polypropylene column to give a bed volume of 1.0-1.2 mL, wash 3 times with 2 mL portions of water, wash with 2 mL 10 mM copper sulfate, wash with two 2 mL portions of water. Centrifuge 5 mL milk at 10° at 1500 g for 15 min, remove the

lower layer and add it to 10 mL succinate buffer, mix, centrifuge at 1500 g for 30 min, add the supernatant to the column. Wash with 2 mL succinate buffer, wash with 2 mL water, wash with 2 mL MeOH, wash with 2 mL water, wash with 700 μL citrate/phosphate buffer (be careful not to disturb bed), elute with 2.5 mL citrate/phosphate buffer (column is white and eluate is blue). Filter (Amicon Centricon 30, MW 30000 cut-off; pre-washed by centrifuging with 2 mL water) while centrifuging at 5000 g for 30-90 min, inject a 600 μL aliquot of the ultrafiltrate. (Prepare succinate buffer by dissolving 11.8 g succinic acid in 980 mL water, adjust pH to 4.0 with 10 M NaOH, make up to 1 L. Prepare the citrate/phosphate buffer by dissolving 12.9 g citric acid monohydrate, 10.9 g Na_2HPO_4, 37.2 g disodium EDTA dihydrate, and 29.2 g NaCl in 1 L water.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm PLRP-S (Polymer Labs)
Mobile phase: Gradient. MeCN:MeOH:10 mM oxalic acid 0:0:100 for 1 min, to 22:8:70 over 5 min, maintain at 22:8:70 for 11 min, return to initial conditions.
Flow rate: 1
Injection volume: 600
Detector: UV 355

CHROMATOGRAM

Retention time: 11.7
Limit of detection: 0.50 ng/mL
Limit of quantitation: 1.03 ng/mL

OTHER SUBSTANCES

Extracted: chlortetracycline, demeclocycline, doxycycline, methacycline, oxytetracycline, tetracycline
Noninterfering: chloramphenicol, gentian violet, hydromycin B, ivermectin, spectinomycin, sulfa drugs

KEY WORDS

cow; SPE; ultrafiltrate

REFERENCE

Carson,M.C. Simultaneous determination of multiple tetracycline residues in milk using metal chelate affinity chromatography, *J.AOAC Int.*, **1993**, *76*, 329–334.

SAMPLE

Matrix: urine
Sample preparation: Adjust the pH of 80 mL urine to 6.5 by adding 5.6 g NaH_2PO_4 and 10 g sodium sulfite, extract with 100 mL ethyl acetate. Remove the organic layer and evaporate it to dryness under reduced pressure at room temperature, reconstitute the residue in 1 mL mobile phase, inject a 20-100 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 5 μm Nucleosil SA (strong cation-exchange)
Mobile phase: EtOH:buffer 48:52 (Buffer was 100 mM pH 4.6 citrate containing 0.05% disodium EDTA.)
Flow rate: 1
Injection volume: 20-100
Detector: UV 350

CHROMATOGRAM

Retention time: 5

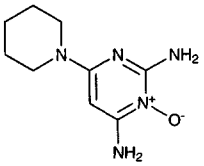
OTHER SUBSTANCES

Extracted: metabolites

REFERENCE

Nelis,H.J.C.F.; De Leenheer,A.P. Metabolism of minocycline in humans, *Drug Metab.Dispos.*, **1982**, *10*, 142–146.

Minoxidil



Molecular formula: $C_9H_{15}N_5O$
Molecular weight: 209.25
CAS Registry No.: 38304-91-5
Merck Index: 6290
Lednicer No.: 1 262

SAMPLE

Matrix: blood
Sample preparation: Condition a Fisher Prepsep C18 SPE column with 2 volumes MeOH then 2 volumes water. 1 mL Plasma + 50 μL 0.056 μg/mL IS in water, vortex for 15 s, add to SPE column, wash with 2 volumes water, wash with 6 mL acetone, elute with ten 200 μL fractions of MeOH, dry at 50° under a stream of nitrogen, reconstitute in 100 μL mobile phase, vortex for 10 s, inject a 10-60 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water 11:98, adjusted to pH 3.0 with phosphoric acid
Flow rate: 1.5
Injection volume: 10-60
Detector: E, Environmental Sciences Assoc. Model 5100 A, guard cell ESA Model 5020 +0.90 V, detector ESA model 5011 with detector 1 +0.30 V and detector 2 +0.80 V (UV 280 J.Pharm.Sci.1990, 79, 483)

CHROMATOGRAM

Retention time: 12 (minoxidil), 18 (minoxidil sulfate)
Internal standard: 2,4-diamino-6-diethylaminopyrimidine 3-oxide (10)
Limit of detection: 500 pg/mL

KEY WORDS

plasma; SPE

REFERENCE

Carrum,G.; Abernethy,D.R.; Sadhukhan,M.; Wright,C.E. Minoxidil analysis in human plasma using high-performance liquid chromatography with electrochemical detection. Application to pharmacokinetic studies, *J.Chromatogr.*, **1986**, *381*, 127–135.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 231

CHROMATOGRAM

Retention time: 4.01

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30
Detector: UV 231.1

CHROMATOGRAM
Retention time: 9.76

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve a sample in MeOH to a concentration of about 1 mg/mL, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Spherisorb SCX
Mobile phase: MeOH:water 80:20 containing 20 mM ammonium formate and 2.3 mL/L trifluoroacetic acid
Flow rate: 1
Injection volume: 1-10
Detector: UV 270

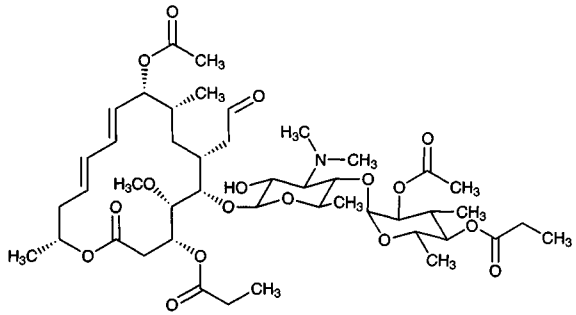
CHROMATOGRAM
Retention time: 4.7

OTHER SUBSTANCES
Simultaneous: cimetidine, clomipramine, halofantrine, haloperidol, reserpine, verapamil

REFERENCE
Law,N.; Appleby,J.R.G. Re-evaluation of strong cation-exchange high-performance liquid chromatography for the analysis of basic drugs, *J.Chromatogr.A*, **1996**, *725*, 335–341.

Miokamycin

Molecular formula: $C_{45}H_{71}NO_{17}$
Molecular weight: 898.06
CAS Registry No.: 55881-07-7
Merck Index: 6291



SAMPLE
Matrix: bulk
Sample preparation: Dissolve 60 mg of bulk compound in 50 mL MeCN, add 5 mL 1.2 mg/mL josamycin in MeCN, mix, inject an aliquot.

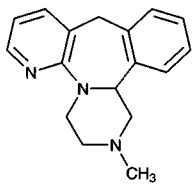
HPLC VARIABLES
Column: 150 × 2.6 5 μm Spheri ODS-2

Mobile phase: MeCN:buffer 75:25 (Buffer was 10 mM ammonium acetate containing 1 mM K_2HPO_4 adjusted to pH 6.5 with 10 mM phosphoric acid.)
Column temperature: 25
Flow rate: 1.5
Detector: UV 232

CHROMATOGRAM
Retention time: 7.0
Internal standard: josamycin (3.2)

REFERENCE
Marini,D.; Balestrieri,F.; Pollino,G. Dosaggio mediante HPLC della miocamicina e delle sue principali impurezze [HPLC analysis of miokamycin and its principle impurities], *Boll.Chim.Farm.*, **1986**, *125*, 193–196.

Mirtazepine



Molecular formula: $C_{17}H_{19}N_3$
Molecular weight: 265.36
CAS Registry No.: 61337-67-5
Merck Index: 6295
Lednicer No.: 5 177

SAMPLE
Matrix: formulations
Sample preparation: Extract 300 mg powdered tablet with 100 mL MeCN:water 50:50, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:MeOH:THF:buffer 14.875:12.67:7.455:65 (Buffer was 18 g/L tetramethylammonium hydroxide pentahydrate in water adjusted to pH 7.4 with 85% phosphoric acid.)
Column temperature: 40
Flow rate: 1.5
Injection volume: 10
Detector: UV 210

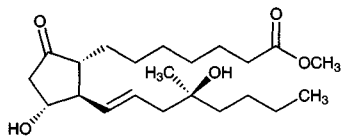
CHROMATOGRAM
Limit of detection: 0.01-0.04%
Limit of quantitation: 0.02-0.12%

OTHER SUBSTANCES
Simultaneous: impurities

KEY WORDS
tablets; comparison with capillary electrophoresis

REFERENCE
Wynia,G.S.; Windhorst,G.; Post,P.C.; Maris,F.A. Development and validation of a capillary electrophoresis method within a pharmaceutical quality control environment and comparison with high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *773*, 339–350.

Misoprostol



Molecular formula: $C_{22}H_{38}O_5$
Molecular weight: 382.54
CAS Registry No.: 59122-46-2
Merck Index: 6297

SAMPLE

Matrix: formulations
Sample preparation: Extract polymeric controlled-release formulations under supercritical fluid conditions using carbon dioxide:formic acid 95:5 at 330 atmospheres at 75° in a 500 μL cell, restrictor temperature 100°, collection solvent 15 mL hexane:EtOH 2:1, collection solvent temperature 0°, extraction time 1 h. Evaporate the collection solvent to dryness, reconstitute with 1 mL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Supelco ODS
Mobile phase: MeCN:MeOH:water 45:20:35
Flow rate: 1.5
Injection volume: 10
Detector: UV 280 following post-column reaction. The column effluent mixed with 4 M KOH pumped at 0.5 mL/min and the mixture flowed at 80° to the detector.

CHROMATOGRAM

Retention time: 7.5
Limit of detection: <0.1 ppt

OTHER SUBSTANCES

Simultaneous: degradation products

KEY WORDS

polymeric controlled-release formulations; SFE; post-column reaction

REFERENCE

Roston,D.A.; Sun,J.J.; Collins,P.W.; Perkins,W.E.; Tremont,S.J. Supercritical fluid extraction-liquid chromatography method development for a polymeric controlled-release drug formulation, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1513–1520.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in MeOH:water 60:40, inject a 20 μL aliquot onto column A with mobile phase A. When the 11R,16S and 11S,16R enantiomers elute (as one peak) switch effluent into the 250 μL sample loop of an injection valve. When the whole peak has been collected inject the contents of the sample loop onto column B with mobile phase B.

HPLC VARIABLES

Column: A 150 × 4.6 3 μm Supelcosil ODS; B 100 × 4.6 LKB EnantioPac
Mobile phase: A MeOH:water 60:40; B Isopropanol:20 mM pH 5.7 phosphate buffer 4:96
Column temperature: 28
Flow rate: A 0.7; B 0.4
Injection volume: 20
Detector: A UV 205; B UV 205

CHROMATOGRAM

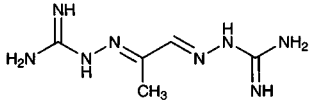
Retention time: 43 (11R,16S and 11S,16R from column A), 37 (11S,16R from column B), 55 (11R,16S from column B)

KEY WORDS

chiral; column-switching

REFERENCE
Roston,D.A.; Wijayaratne,R. Two-dimensional liquid chromatographic method for resolution of prostaglandin enantiomers, *Anal.Chem.*, **1988**, *60*, 948–950.

Mitoguazone



Molecular formula: $C_5H_{12}N_8$
Molecular weight: 184.20
CAS Registry No.: 459-86-9
Merck Index: 6299

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 10 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil BDS C8
Mobile phase: MeCN:buffer 11:89 (Buffer was 50 mM KH_2PO_4 containing 1 g/L sodium heptanesulfonate, adjusted to pH 3.0 with concentrated phosphoric acid.)
Column temperature: 40
Flow rate: 2
Injection volume: 20
Detector: UV 283

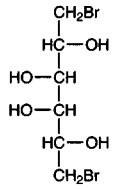
CHROMATOGRAM
Retention time: 18

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
comparison with capillary electrophoresis and TLC

REFERENCE
Thomson,C.E.; Gray,M.R.; Baxter,M.P. The use of capillary electrophoresis as part of a specificity testing strategy for mitoguazone dihydrochloride HPLC methods, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1103–1101.

Mitolactol



Molecular formula: $C_6H_{12}Br_2O_4$
Molecular weight: 307.97
CAS Registry No.: 10318-26-0
Merck Index: 6300

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 1 mL ice-cold MeOH, let stand at -20° for 20 min, centrifuge at 1500 g for 5 min. Remove a 1 mL aliquot of the supernatant and add it to 1 mL 5% diethyldithiocarbamate and 2 mL 50 mM pH 7.4 potassium phosphate buffer, heat at 50° for 1 h, extract with 5 mL chloroform. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute, inject an aliquot.

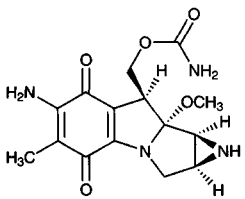
HPLC VARIABLES
Column: 250 × 4.6 5 μm Alltech CN
Mobile phase: Heptane:isopropanol:acetic acid 74.4:21.6:4
Flow rate: 1.3
Detector: UV 254

CHROMATOGRAM
Retention time: 9.9
Limit of detection: 500 nM

KEY WORDS
derivatization; plasma; human; mouse; pharmacokinetics

REFERENCE
Henner,W.D.; Furlong,E.A.; Kelley,S.L.; Rosowsky,A. Assay for mitolactol and its bifunctional alkylating metabolites in plasma, *J.Pharm.Sci.*, **1985**, *74*, 983–986.

Mitomycin



Molecular formula: $C_{15}H_{18}N_4O_5$
Molecular weight: 334.33
CAS Registry No.: 50-07-7
Merck Index: 6301

SAMPLE
Matrix: aqueous humor
Sample preparation: 100 μL Aqueous humor + 25 μL 5 μg/mL 4-aminoacetophenone in water, vortex, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: Microsorb C18
Column: 50 × 4.6 3 μm Microsorb C18 (Short-One)
Mobile phase: MeOH:water 28:72 containing $(NH_4)H_4PO_4$ adjusted to pH 7.0 with dilute ammonium hydroxide
Flow rate: 1.5
Injection volume: 100
Detector: UV 365

CHROMATOGRAM
Retention time: 2.3
Internal standard: 4-aminoacetophenone (2.7)
Limit of detection: 6.25 ng/mL

REFERENCE
Li,W.Y.; Seah,S.K.L.; Koda,R.T. Determination of mitomycin C in human aqueous humor and serum by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *619*, 148–153.

SAMPLE
Matrix: aqueous humor, tissue
Sample preparation: Tissue. Add 5 mL cold MeCN to 200 mg conjunctiva or sclera, homogenize, sonicate for 30 min, centrifuge at 4° at 3000 rpm for 10 min, evaporate the organic layer to dryness under reduced pressure at 40°, reconstitute with 400 μL mobile phase, sonicate, filter, inject an aliquot. Aqueous humor. Add 4 mL ethyl acetate to 200 μL aqueous humor, stir. Sonicate for 30 min, centrifuge at 4° at 3000 rpm for 10 min, evaporate the organic layer to dryness under reduced pressure at 40°, reconstitute with 400 μL mobile phase, sonicate, filter, inject an aliquot.

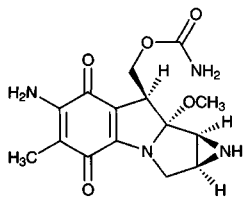
HPLC VARIABLES
Column: 250 × 4.6 5 μm Alltech CN
Mobile phase: Heptane:isopropanol:acetic acid 74.4:21.6:4
Flow rate: 1.3
Detector: UV 254

CHROMATOGRAM
Retention time: 9.9
Limit of detection: 500 nM

KEY WORDS
derivatization; plasma; human; mouse; pharmacokinetics

REFERENCE
Henner,W.D.; Furlong,E.A.; Kelley,S.L.; Rosowsky,A. Assay for mitolactol and its bifunctional alkylating metabolites in plasma, *J.Pharm.Sci.*, **1985**, *74*, 983–986.

Mitomycin



Molecular formula: $C_{15}H_{18}N_4O_5$
Molecular weight: 334.33
CAS Registry No.: 50-07-7
Merck Index: 6301

SAMPLE
Matrix: aqueous humor
Sample preparation: 100 μL Aqueous humor + 25 μL 5 μg/mL 4-aminoacetophenone in water, vortex, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: Microsorb C18
Column: 50 × 4.6 3 μm Microsorb C18 (Short-One)
Mobile phase: MeOH:water 28:72 containing $(NH_4)H_4PO_4$ adjusted to pH 7.0 with dilute ammonium hydroxide
Flow rate: 1.5
Injection volume: 100
Detector: UV 365

CHROMATOGRAM
Retention time: 2.3
Internal standard: 4-aminoacetophenone (2.7)
Limit of detection: 6.25 ng/mL

REFERENCE
Li,W.Y.; Seah,S.K.L.; Koda,R.T. Determination of mitomycin C in human aqueous humor and serum by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *619*, 148–153.

SAMPLE
Matrix: aqueous humor, tissue
Sample preparation: Tissue. Add 5 mL cold MeCN to 200 mg conjunctiva or sclera, homogenize, sonicate for 30 min, centrifuge at 4° at 3000 rpm for 10 min, evaporate the organic layer to dryness under reduced pressure at 40°, reconstitute with 400 μL mobile phase, sonicate, filter, inject an aliquot. Aqueous humor. Add 4 mL ethyl acetate to 200 μL aqueous humor, stir. Sonicate for 30 min, centrifuge at 4° at 3000 rpm for 10 min, evaporate the organic layer to dryness under reduced pressure at 40°, reconstitute with 400 μL mobile phase, sonicate, filter, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Zorbax ODS
Mobile phase: MeOH:20 mM pH 7.0 sodium phosphate buffer 30:70
Column temperature: 40
Flow rate: 1
Detector: UV 360

CHROMATOGRAM
Retention time: 5.6
Limit of detection: 20 ng/g (conjunctiva and sclera), 20 ng/mL (aqueous humor)

KEY WORDS
rabbit; ocular tissue; conjunctiva; sclera

REFERENCE
Chen,B.-M.; Xia,L.-W.; Xia,X.-B. Determination of mitomycin C in rabbit ocular tissue after topical administration by high performance liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2659–2667.

SAMPLE
Matrix: blood
Sample preparation: Add 50 μL plasma to 50 μL 2 μg/mL porfiromycin in MeCN. Vortex the mixture for 0.5 min and centrifuge at 2200 g at 4° for 10 min. Mix 50 μL supernatant with 50 μL water. Inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 AM-312 ODS (YMC, Japan)
Mobile phase: Gradient. A was MeCN:50 mM pH 7.0 phosphate buffer 10:90 containing 5 mM sodium 1-octanesulphonate. B was MeCN:50 mM pH 7.0 phosphate buffer 50:50 containing 5 mM sodium 1-octanesulphonate. A:B from 100:0 to 70:30 over 20 min, to 0:100 over 1 min, maintain at 0:100 for 5 min, to 100:0 over 1 min, maintain at 100:0 for 10 min.
Flow rate: 1
Injection volume: 50
Detector: UV 360

CHROMATOGRAM
Internal standard: porfiromycin
Limit of detection: 10 ng/mL

KEY WORDS
rat; plasma

REFERENCE
Fuse,E.; Takai,K.; Okuno,K.; Kobayashi,S. Hepatic extraction ratio of 5-fluorouracil in rats. Dose dependence and effect of uracil and interleukin-2, *Biochem.Pharmacol.*, **1996**, *52*, 561–568.

SAMPLE
Matrix: blood
Sample preparation: Centrifuge plasma at 15800 g at 4° for 5 min. 500 μL Plasma + 50 μL 4 μg/mL IS in MeOH:water 50:50 + 1 mL MeCN, stir for 1 min, centrifuge at 15800 g at 4° for 10 min. Lyophilize the supernatant in a vacuum centrifuge (Hetovac VR-1, Allerod, Denmark). Reconstitute the residue in 150 μL MeOH:10 mM pH 6.5 NaH_2PO_4 30:70. Inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Hypersil ODS
Mobile phase: MeOH:10 mM pH 6.5 NaH_2PO_4 30:70
Column temperature: 30
Flow rate: 1.3
Injection volume: 50
Detector: UV 365

CHROMATOGRAM
Retention time: 4.92
Internal standard: porfiromycin (6.64)
Limit of detection: 1 ng/mL
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Simultaneous: dexamethasone, lorazepam, mezlocillin, ondansetron, meperidine

KEY WORDS
plasma

REFERENCE
Joseph,G.; Biederbick,W.; Woschée,U.; Theisohn,M.; Klaus,W. Sensitive and convenient high-performance liquid chromatographic method for the determination of mitomycin C in human plasma, *J.Chromatogr.B*, **1997**, *698*, 261–267.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 10 mL chloroform:isopropanol 50:50, shake for 1 min, centrifuge at 2500 g for 5 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 30-40°, reconstitute the residue in 100 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:10 mM pH 6.0 phosphate buffer 30:70
Flow rate: 1
Injection volume: 10
Detector: UV 365

CHROMATOGRAM
Retention time: 7.5
Limit of detection: 1 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Den Hartigh,J.; van Oort,W.J.; Bocken,M.C.Y.M.; Pinedo,H.M. High-performance liquid chromatographic determination of the antitumor agent mitomycin C in human blood plasma, *Anal.Chim.Acta*, **1981**, *127*, 47–53.

SAMPLE
Matrix: blood
Sample preparation: Condition a 500 mg Baxter C18 SPE cartridge with 1 volume of MeOH and 2 volumes of water. 500 μL Serum + 100 μL 5 μg/mL 4-aminoacetophenone in water, mix, add to the SPE cartridge, wash with two 500 μL aliquots of water, elute with three 500 μL aliquots of MeOH. Combine the eluates and evaporate them to dryness under a stream of air at 40-50°, reconstitute the residue in 150 μL water, centrifuge at 13700 g for 5 min, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: Microsorb C18
Column: 150 × 4.6 5 μm C18
Mobile phase: MeOH:water 28:72 containing $(NH_4)H_4PO_4$ adjusted to pH 7.0 with dilute ammonium hydroxide
Flow rate: 1.5
Injection volume: 50
Detector: UV 365

CHROMATOGRAM
Retention time: 7.5
Internal standard: 4-aminoacetophenone (9.0)
Limit of detection: 10 ng/mL

KEY WORDS
serum; SPE

REFERENCE
Li,W.Y.; Seah,S.K.L.; Koda,R.T. Determination of mitomycin C in human aqueous humor and serum by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *619*, 148–153.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 400 μL MeCN, vortex, centrifuge at 12000 g for 2 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 15 × 3.2 NewGuard RP18
Column: 220 × 4.6 5 μm Spheri-5 RP18
Mobile phase: MeCN:10 mM phosphoric acid 80:20
Flow rate: 0.8
Injection volume: 50
Detector: UV 340

CHROMATOGRAM
Retention time: 6.66
Internal standard: mitomycin C

OTHER SUBSTANCES
Extracted: novobiocin

KEY WORDS
serum; mitomycin C is IS

REFERENCE
Zuhowski,E.G.; Gutheil,J.C.; Egorin,M.J. Rapid and sensitive high-performance liquid chromatographic assay for novobiocin in human serum, *J.Chromatogr.B*, **1994**, *655*, 147–152.

SAMPLE
Matrix: blood
Sample preparation: Centrifuge plasma at 2000 g for 15 min, inject a 25 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm CAPCELL PAK MF Ph-1 internal surface reversed-phase (Shiseido, Tokyo)
Mobile phase: Water
Flow rate: 1
Injection volume: 25
Detector: UV 365

CHROMATOGRAM
Retention time: 5.0
Limit of detection: 5 ng/mL
Limit of quantitation: 20 ng/mL

KEY WORDS
direct injection; plasma

REFERENCE
Song,D.; Au,J.L.-S. Direct injection isocratic high-performance liquid chromatographic analysis of mitomycin C in plasma, *J.Chromatogr.B*, **1996**, *676*, 165–168.

SAMPLE
Matrix: blood, urine
Sample preparation: Prepare an SPE column of 100 mg 100-200 μm Amberlite XAD-2 in a Pasteur pipette, wash with three 3 mL portions of MeOH, wash with 10 mL water. 2 mL plasma, serum, or urine + 100 μL 1 μg/mL porfiromycin in water, add to the SPE column, wash with three 2 mL portions of water, elute with three 2 mL aliquots of MeOH. Evaporate the eluate to dryness under reduced pressure at 57°, reconstitute the residue in 200 μL mobile phase, mix on a whirlmixer for 2 min, inject a 10-100 μL aliquot.

HPLC VARIABLES
Column: 100 × 3 5 μm Hypersil-MOS
Mobile phase: MeCN:50 mM pH 7 phosphate buffer 10:90
Flow rate: 2
Injection volume: 10-100
Detector: UV 360 or E, E.G. & G. Par 310, hanging mercury drop electrode -600 mV, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 3
Internal standard: porfiromycin (5)
Limit of detection: 250 pg (E), 150 pg (UV)

KEY WORDS
SPE; serum; plasma; pharmacokinetics; human; rat

REFERENCE
Tjaden,U.R.; Langenberg,J.P.; Ensing,K.; Van Bennekom,W.P.; de Bruijn,E.A.; van Oosterom,A.T. Determination of mitomycin C in plasma, serum and urine by high-performance liquid chromatography with ultraviolet and electrochemical detection, *J.Chromatogr.*, **1982**, *232*, 355–367.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Condition a 40 × 8 polypropylene SPE column containing 1 mL Porapak Q (Waters) with 20 volumes MeOH and 40 volumes water. 0.2-2.5 mL Plasma or thawed red blood cells + 100-200 ng porfiromycin + 5 volumes water, centrifuge red blood cell ghosts at 8000 g for 90 min, add to the SPE column, wash with 20 mL water, elute with 6 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL water, inject a 100 μL aliquot. Urine. Dilute urine 100 (rabbit) or 10 (human) fold, add porfiromycin, inject an aliquot.

HPLC VARIABLES
Column: 10 μm C8 (Hewlett-Packard)
Mobile phase: Gradient. MeCN:water from 5:95 to 41:59 over 8 min. Wash with MeCN for 5 min, re-equilibrate at initial conditions for 5 min.
Flow rate: 2
Injection volume: 100
Detector: UV 360

CHROMATOGRAM
Retention time: 6.2
Internal standard: porfiromycin (6.7)
Limit of quantitation: 5 ng

KEY WORDS
pharmacokinetics; plasma; rabbit; human; red blood cells; SPE

REFERENCE
van Hazel,G.A.; Kovach,J.S. Pharmacokinetics of mitomycin C in rabbit and human, *Cancer Chemother.Pharmacol.*, **1982**, *8*, 189–192.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 2 mL MeOH and 5 mL water. 1 mL Plasma or urine + 100 μL 100 μg/mL desipramine chloride, add to the SPE cartridge, wash with 3 mL water, elute with 4 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, vortex, centrifuge, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 70 mm long 5 μm LiChrosorb RP-8
Column: 150 × 4 5 μm LiChrosorb RP-8
Mobile phase: MeOH:buffer:water 25:10:65 (plasma) or 20:10:70 (urine) (Buffer was pH 7.0 phosphate buffer, μ = 0.1.)
Flow rate: 0.7
Injection volume: 100
Detector: UV 365

CHROMATOGRAM
Retention time: 7
Limit of quantitation: 500 ng/mL (urine), 2 ng/mL (plasma)

KEY WORDS
plasma; SPE; desipramine prevents adsorption on glass; pharmacokinetics

REFERENCE
Eksborg,S.; Ehrsson,H.; Lindfors,A. Liquid chromatographic determination of mitomycin C in human plasma and urine, *J.Chromatogr.*, **1983**, *274*, 263–270.

SAMPLE
Matrix: formulations
Sample preparation: Emulsion. 500 μL Emulsion + 10 mL 400 μg/mL hydroquinone in MeOH + 40 mL 0.1% 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: 10
Internal standard: hydroquinone (4.2)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: carboplatin, epirubicin, 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: reaction mixtures

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm NewGuard RP-18
Column: 250 × 4 5 μm Nucleosil C18
Mobile phase: MeOH:10 mM pH 6.0 phosphate buffer 7:13
Flow rate: 0.6
Detector: UV 364

REFERENCE
Ishiki,N.; Onishi,H.; Machida,Y. Conversion characteristics of the conjugates of mitomycin C with estradiol benzoate in various pH media, *Chem.Pharm.Bull.*, **1997**, *45*, 1345–1349.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Silverson) tumor tissue with 2 volumes 154 mM KCl. 1 mL Homogenate + 10 μL 0.5-5 μg/mL porfiromycin in MeOH + 5 mL chloroform:isopropanol:ethyl acetate 40:40:20, vortex vigorously for 15 min, centrifuge at 4° at 1000 g for 15 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 300 μL mobile phase, vortex for 2 min, centrifuge at 15000 g, filter, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: MeOH:18 mM pH 5.8 sodium phosphate buffer 26:74
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: UV 310, UV 360

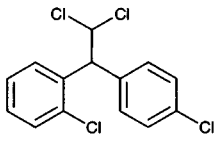
CHROMATOGRAM
Retention time: 9.27
Internal standard: porfiromycin (13.11)
Limit of detection: 20 ng/g

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
tumor; rat

REFERENCE
Cummings,J.; Chirrey,L.; Willmott,N.; Halbert,G.W.; Smyth,J.F. Determination of mitomycin C, 2,7-diaminomitosene, 1,2-*cis*- and 1,2-*trans*-1-hydroxy-2,7-diaminomitosene in tumour tissue by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 105–113.

Mitotane



Molecular formula: $C_{14}H_{10}Cl_4$
Molecular weight: 320.04
CAS Registry No.: 53-19-0
Merck Index: 6302

SAMPLE
Matrix: blood

Sample preparation: 200 μL Plasma + 300 μL acetone, vortex, centrifuge at 26 000 g for 5 min, inject a 50 μL aliquot of the supernatant

HPLC VARIABLES
Guard column: 20 mm long Supelguard (Supelco)
Column: 150 × 4.6 3 μm Supelcosil LC-18
Mobile phase: MeOH:buffer 80:20 (Buffer was 50 mM KH_2PO_4 adjusted to pH 7.0 with KOH.)
Column temperature: 50
Flow rate: 1.25
Injection volume: 50
Detector: UV 230

CHROMATOGRAM
Retention time: 6.2
Limit of detection: 250 nM

OTHER SUBSTANCES
Extracted: metabolites, o,p'-(dichlorodiphenyl)-2,2-dichloroethene

KEY WORDS
plasma

REFERENCE
Andersen,A.; Warren,D.J.; Nome,O.; Vesterhus,L.; Slordal,L. A high-pressure liquid chromatographic method for measuring mitotane [1,1-(*o,p'*-dichlorodiphenyl)-2,2-dichloroethane] and its metabolite 1,1-(*o,p'*-dichlorodiphenyl)-2,2-dichloroethene in plasma, *Ther.Drug Monit.*, **1995**, *17*, 526[94]531.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Powder tablet, add 10 mg DDE, extract four times with 10 mL isooctane. Combine extracts, filter, make up to 50 mL with isooctane. Dilute an aliquot 1:10, inject an aliquot. Emulsions. 5 mL Emulsion + 10 mg IS, extract with 40 mL isooctane:EtOH 98:2 for 15 min. Make up isooctane layer to 50 mL with isooctane. Remove a 1 mL aliquot and make up to 10 mL with isooctane, inject an aliquot.

HPLC VARIABLES
Guard column: 100 × 4.6 30-38 μm HC Pellosil (Whatman)
Column: 250 × 4.6 Partisil PxS 5/25
Mobile phase: Isooctane:cyclohexane 80:20 (Flush column with isooctane:EtOH 98:2 at 2 mL/min for 15 min to remove corn oil from emulsions, re-equilibrate with 120 mL mobile phase.)
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 9
Internal standard: 1,1-bis-(p-chlorophenyl)-2,2-dichloroethylene (p,p'-DDE) (4.5)

KEY WORDS
tablets; emulsions; normal phase

REFERENCE
Musial,S.P.; Freeman,C.J.; Sinsheimer,J.E. Mitotane (*o,p'*-DDD) emulsion and tablet analysis by high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *319*, 467–470.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 30 × 4.6 10 μm spherical C-18 MCP (Brownlee)

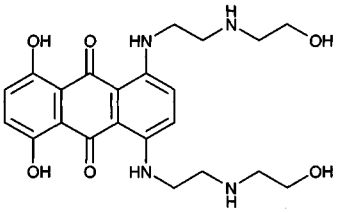
Column: 100 × 5 Nova-Pak C-18
Mobile phase: Gradient. MeOH:water:acetic acid 50:50:0.2 for 40 min then to MeCN:water:acetic acid 55:45:0.2 over 10 min

CHROMATOGRAM
Retention time: 40-50

OTHER SUBSTANCES
Simultaneous: metabolites

REFERENCE
Cai,W.; Counsell,R.E.; Djanegara,T.; Schteingart,D.E.; Sinsheimer,J.E.; Wotring,L.L. Metabolic activation and binding of mitotane in adrenal cortex homogenates, *J.Pharm.Sci.*, **1995**, *84*, 134–138.

Mitoxantrone



Molecular formula: $C_{22}H_{28}N_4O_6$
Molecular weight: 444.49
CAS Registry No.: 65271-80-9, 70476-82-3 (di HCl)
Merck Index: 6303
Lednicer No.: 3 75

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond-Elut C18 SPE cartridge with 10 mL MeOH and 5 mL water. Add 1-2 mL plasma to the SPE cartridge, wash with 5 mL water, elute with 300 µL 500 mM methanolic HCl, inject an aliquot.

HPLC VARIABLES
Guard column: 70 × 2.1 Co:Pell ODS (Whatman)
Column: 300 × 3.9 10 µm µBondapak C18
Mobile phase: MeCN:200 mM pH 4.0 ammonium acetate 25:75
Flow rate: 1.5
Detector: UV 658

CHROMATOGRAM
Retention time: 4
Limit of detection: 1 ng/mL

KEY WORDS
plasma; SPE

REFERENCE
Peng,Y.-M.; Ormberg,D.; Alberts,D.S.; Davis,T.P. Improved high-performance liquid chromatography of the new antineoplastic agents bisantrene and mitoxantrone, *J.Chromatogr.*, **1982**, *233*, 235–247.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 50 µL 100 µg/mL bisantrene, adjust to pH 11 with 50 µL 1 M NaOH, add 5 mL dichloromethane, extract. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 250 µL mobile phase, centrifuge at 15600 g for 1 min, inject a 150 µL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 37-50 µm C18/Corasil
Column: 300 × 3.9 10 µm µBondapak C18

Mobile phase: MeCN:80 mM pH 3.0 sodium formate 28:72
Flow rate: 1
Injection volume: 150
Detector: E, BAS LC-4B detector, TL-5 glassy carbon electrode at +0.75 V, Ag/AgCl reference electrode or UV 660

CHROMATOGRAM
Retention time: 5.5
Internal standard: bisantrene (8)
Limit of detection: 0.1 ng/mL (electrochemical)

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Choi,K.E.; Sinkule,J.A.; Han,D.S.; McGrath,S.C.; Daly,K.M.; Larson,R.A. High-performance liquid chromatographic assay for mitoxantrone in plasma using electrochemical detection, *J.Chromatogr.*, **1987**, *420*, 81–88.

SAMPLE
Matrix: blood
Sample preparation: Condition a 40 μm Sepralyte non-bonded silica SPE cartridge (Analytichem) with 1 mL 1 M HCl, with 3 mL water, and with 500 μL 2 M $(NH_4)H_2PO_4$. 1 mL Plasma + 5 μL 5 μg/mL methylene blue, centrifuge at 4° at 12000 g for 1 min, add the supernatant to the SPE cartridge at 1 mL/min, vortex the precipitate in the centrifuge tube with 500 μL 0.3% ascorbic acid in 12 mM pH 3.0 citrate buffer, add the mixture to the SPE cartridge, wash with 500 μL water, wash with 500 μL MeCN saturated with tetramethylammonium chloride, apply suction for 5 min, elute with 300 μL elution solvent by centrifuging at 4° at 2000 g for 5 min, inject an aliquot of the eluate. (The elution solvent was MeOH:1 M tetramethylammonium chloride:1 M citric acid:water 32:10:20:38.)

HPLC VARIABLES
Column: 75 × 3.9 4 μm Nova-pak ODS
Mobile phase: MeOH:THF:triethylamine phosphate solution: 1 M tetramethylammonium chloride:water 30:1:10:2:57 (Triethylamine phosphate was 67.8 mL orthophosphoric acid in 800 mL water, adjust pH to 3.0 with triethylamine, make up to 1 L with water.) (Filter mobile phase but do not degas.)
Flow rate: 1
Injection volume: 50
Detector: UV 658

CHROMATOGRAM
Retention time: 3.5
Internal standard: methylene blue (7)
Limit of quantitation: 1 ng/mL

KEY WORDS
plasma; SPE; use polypropylene containers

REFERENCE
Lin,K.T.; Rivard,G.E.; Leclerc,J.-M. High-performance liquid chromatographic determination of mitoxantrone in plasma utilizing non-bonded silica gel for solid-phase isolation to reduce adsorptive losses on glass during sample preparation, *J.Chromatogr.*, **1989**, *465*, 75–86.

SAMPLE
Matrix: blood, tissue
Sample preparation: Add 1 mL solution containing 10 μg/mL hexanesulfonic acid, 500 μg/mL ascorbic acid and 8 μg/mL ametantrone to 1 mL whole blood or tissue homogenate, vortex for 30 s. Add 1 mL 100 mM pH 9.5 borate buffer, 300 μL 1 M NaOH, vortex for 30 s. Extract using a horizontal linear shaker (Infors HT, Infors, Switzerland) with 5 mL dichloromethane at 150 rpm for 60 min. Centrifuge at 2800 g for 15 min, evaporate organic layer to dryness under

reduced pressure, dissolve the residue in 150 μL mobile phase. Inject a 92 μL aliquot. (Silanize all glassware.)

HPLC VARIABLES
Guard column: 11 × 4 5 μm Nucleosil C18
Column: 250 × 4 5 μm Nucleosil C18
Mobile phase: MeCN:160 mM pH 2.7 ammonium formate buffer 33:67 containing 250 mM (sic) hexanesulfonic acid
Flow rate: 1
Injection volume: 92
Detector: UV 658

CHROMATOGRAM
Retention time: 5.4-6.7
Internal standard: ametantrone (6.9-8.3)
Limit of detection: 2 ng/mL
Limit of quantitation: 5 ng/mL

KEY WORDS
mouse; liver; heart; spleen; kidney; whole blood

REFERENCE
Rentsch,K.M.; Schwender,R.A.; Hänseler,E. Determination of mitoxantrone in mouse whole blood and different tissues by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *679*, 185–192.

SAMPLE
Matrix: cells
Sample preparation: 1 mL Bone marrow suspension (20000 cells/μL) + 50 ng ametantrone + 1 mL 100 mM pH 10.0 borate buffer + 5 mL dichloromethane, extract, centrifuge at 1000 g for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 1 mL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:160 mM pH 2.7 ammonium formate buffer 30:70 containing 25 mM hexanesulfonic acid, 2 mM NaCl, and 1.34 mM EDTA
Flow rate: 1.6
Injection volume: 100
Detector: E, AMOR/Spark, glassy carbon electrode +0.75 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 3.64
Internal standard: ametantrone (4.99)
Limit of detection: 1 ng/mL

KEY WORDS
rat; bone marrow; pharmacokinetics

REFERENCE
de Vries,A.J.; Nooter,K. Quantification of mitoxantrone in bone marrow by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1991**, *563*, 435–442.

SAMPLE
Matrix: formulations
Sample preparation: Condition a 3 mL Supelclean LC-18 SPE cartridge (Supelco) with 1 mL MeOH and 1 mL water. Dissolve one volume liposome preparation in 3 volumes EtOH, add 500 μL to the SPE cartridge, wash with 1 mL water, elute with 1 mL 500 mM HCl in MeOH, inject an aliquot of the eluate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm LiChrospher RP-18

Mobile phase: MeCN:10 mM KH_2PO_4 40:60, pH adjusted to 3.0 with orthophosphoric acid
Flow rate: 1
Detector: UV 242

CHROMATOGRAM
Retention time: 4.2
Internal standard: propyl paraben (11.4)
Limit of quantitation: 12.5 ng/mL

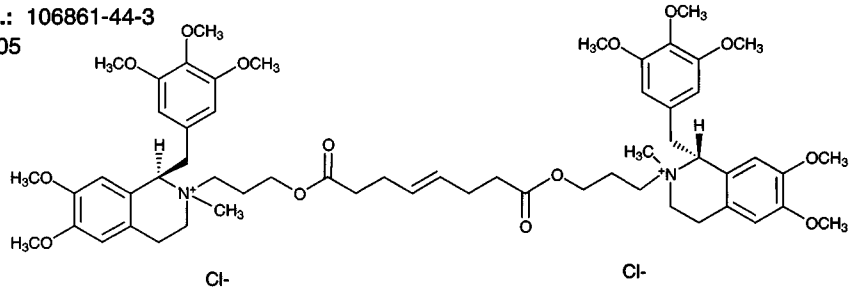
OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
liposomes; SPE; stability-indicating

REFERENCE
Law,S.-L.; Jang,T.-F. High-performance liquid chromatographic determination of mitoxantrone in liposome preparations using solid-phase extraction and its application in stability studies, *J.Chromatogr.A*, **1994**, *670*, 234–238.

Mivacurium chloride

Molecular formula: $C_{58}H_{80}Cl_2N_2O_{14}$
Molecular weight: 1100.18
CAS Registry No.: 106861-44-3
Merck Index: 6305



SAMPLE
Matrix: bile, blood, urine
Sample preparation: Condition a Bond Elut C1 SPE cartridge with 3 mL MeOH and 3 mL water, do not allow to dry. Dilute bile 1:3 with water. Add 50 μL 70 mg/mL phenylmethanesulfonyl fluoride (an enzyme inhibitor) in DMF to 5 mL blood before centrifuging at 3000 g for 10 min to prepare plasma. 1 mL Plasma, urine, or diluted bile, add to the SPE cartridge, wash with 3 mL water, wash with 3 mL MeCN, wash with 3 mL MeOH, wash with 3 mL water, elute with 1 mL eluant, mix eluate thoroughly, inject a 20-100 μL aliquot. (Eluant was MeOH: 50 mM pH 3 KH_2PO_4 80:20.)

HPLC VARIABLES
Guard column: 5 μm Spherisorb S5C1 methylsilyl
Column: 150 × 4.6 5 μm Spherisorb S5C1 methylsilyl
Mobile phase: MeCN:MeOH:50 mM pH 3 KH_2PO_4 70:0.35:30
Flow rate: 1
Injection volume: 20-100
Detector: UV 210

CHROMATOGRAM
Retention time: 5.7
Internal standard: mivacurium chloride
Limit of quantitation: 30 ng/mL (bile), 10 ng/mL (plasma, urine)

OTHER SUBSTANCES
Extracted: doxacurium
Simultaneous: pancuronium, atracurium, tubocurarine succinylcholine, metocurine

KEY WORDS
plasma; dog; human; SPE; mivacurium is IS

REFERENCE
DeAngelis,R.; Loebs,P.; Maehr,R.; Savarese,J.; Welch,R. High-performance liquid chromatographic analysis of doxacurium, a new long-acting neuromuscular blocker, *J.Chromatogr.*, **1990**, *525*, 389–400.

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 8 mL water. Condition a QMA anion-exchange SPE cartridge with 5 mL MeOH and 8 mL water. 1 mL Plasma containing 330 μg phospholine iodide (esterase inhibitor) + 1 mL 100 ng/mL IS in saline, add to the C18 SPE cartridge, wash with 4 mL water, wash with 4 mL MeOH, wash with 4 mL MeCN, elute with 2.8 mL MeCN:6 M HCl 99.67:0.33. Add the eluate to the anion-exchange SPE cartridge, elute with 900 μL MeCN:6 M HCl 99.67:0.33. Evaporate the eluate to dryness under vacuum at 55°, reconstitute the residue in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 5 μm LiChrosphere 60 RP Select B
Column: 125 × 4.6 5 μm LiChrosphere 60 RP Select B
Mobile phase: MeCN:water 40 60 containing 5 mM octanesulfonic acid (PIC B-8, low UV)
Column temperature: 35
Flow rate: 1
Injection volume: 50
Detector: F ex 202 em 320

CHROMATOGRAM
Retention time: 8.4 (trans-trans), 9.3 (cis-trans), 10.2 (cis-cis)
Internal standard: bis-[3-[trans-1,2,3,4-tetrahydro-6,7-dimethoxy-N-methyl-1-(3,4,5-trimethoxybenzyl)isoquinolinium]propyl]-1,3-phenylenedipropionate dihydrochloride (BW 785U77) (13.1)
Limit of quantitation: 5 ng/mL

KEY WORDS
plasma; SPE

REFERENCE
Brown,A.R.; James,C.D.; Welch,R.M.; Harrelson,J.C. Stereoselective high-performance liquid chromatographic assay with fluorometric detection for the isomers of mivacurium in human plasma, *J.Chromatogr.*, **1992**, *578*, 302–308.

SAMPLE
Matrix: blood
Sample preparation: Condition a 300 mg PrepSep C18 SPE cartridge with 3 mL MeOH and 3 mL water. 1 mL Plasma + 100 μL 4 μg/mL IS in physiological saline (adjusted to pH 3 with 100 mM HCl) + 1 mL water, mix, add to SPE cartridge, wash with 3 mL water, wash with 3 mL MeCN, wash with 3 mL water, elute with two 750 μL portions of MeOH:50 mM pH 3 ammonium diphosphate 80:20. Evaporate the eluates to dryness under vacuum, reconstitute the residue in 300 μL mobile phase, inject a 150 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4.6 5 μm LiChrosphere 60 RP select B
Column: 125 × 4.6 5 μm LiChrosphere 60 RP select B
Mobile phase: MeCN:water 37.5:62.5 containing 5.2 mM octanesulfonic acid (PIC B-8)
Column temperature: 35
Flow rate: 2
Injection volume: 150

Detector: F ex 202 em 290

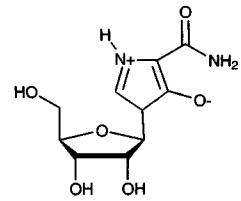
CHROMATOGRAM
Retention time: 7.1 (trans-trans), 7.9 (cis-trans), 8.9 (cis-cis)
Internal standard: bis-3-[trans-1,2,3,4-tetrahydro-6,7-dimethoxy-N-methyl-1-(3,4,5-trimethoxybenzyl)isoquinolinium] propyl-1,3-phenylenedipropionate dichloride (BW785U77) (12)
Limit of quantitation: 3.9 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Lacroix,M.; Tu,T.M.; Donati,F.; Varin,F. High-performance liquid chromatographic assays with fluorometric detection for mivacurium isomers and their metabolites in human plasma, *J.Chromatogr.B*, **1995**, *663*, 297–307.

Mizoribine



Molecular formula: $C_9H_{13}N_3O_6$
Molecular weight: 259.22
CAS Registry No.: 50924-49-7
Merck Index: 6306

SAMPLE
Matrix: blood
Sample preparation: 500 µL Plasma + 50 µL 65 µg/mL 3-methylxanthine in water + 50 µL 70% perchloric acid, mix, add 50µL saturated potassium chloride, mix well, centrifuge. Remove a 350 µL aliquot of the supernatant and add it to 35 µL 10 M NaOH, wash with 1.5 mL dichloromethane. Remove a 250 µL aliquot of the aqueous phase and neutralize it with 20 µL 2 M HCl, inject an aliquot.

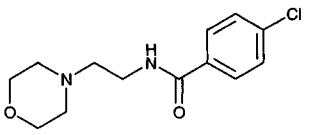
HPLC VARIABLES
Column: 200 × 4.6 5 µm Hypersil ODS
Mobile phase: Gradient. MeOH:20 mM Na_2HPO_4 2:98, pH 3, containing 0.04% octanesulfonic acid, after 1.1 min MeOH:20 mM Na_2HPO_4 6:94, pH 3, containing 0.04% octanesulfonic acid (step gradient).
Column temperature: 40
Flow rate: 1.3
Injection volume: 2
Detector: UV 275

CHROMATOGRAM
Retention time: 2.5
Internal standard: 3-methylxanthine (5)
Limit of detection: 100 ng/mL
Limit of quantitation: 250 ng/mL

KEY WORDS
dog; plasma; human

REFERENCE
Erdmann,G.R.; Gruber,S.A.; McGuiggan,M.M.; Cipolle,R.J.; Canafax,D.M. Determination of mizoribine in plasma using ion-pair high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *494*, 354–360.

Moclobemide



Molecular formula: $C_{13}H_{17}ClN_2O_2$
Molecular weight: 268.74
CAS Registry No.: 71320-77-9
Merck Index: 6309
Lednicer No.: 4 39

SAMPLE

Matrix: bile, blood, tissue, vitreous humor
Sample preparation: Bile, blood, vitreous humor. Mix 1 mL sample with 2 μg IS, 1 mL deionized water, and 1 mL 200 mM sodium carbonate. Add 6 mL hexane:butanol 95:5, extract for 30 min, centrifuge. Remove the organic layer and add it to 100 μL 0.2% orthophosphoric acid, extract for 30 min, centrifuge. Inject a 30 μL aliquot of the aqueous layer. Tissue. Mix 500 μL liver homogenate with 2 μg IS, 1 mL deionized water, and 1 mL 200 mM sodium carbonate. Add 6 mL hexane:butanol 95:5, extract for 30 min, centrifuge. Remove the organic layer and add it to 400 μL 0.2% orthophosphoric acid, extract again for 30 min, centrifuge. Inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Column: 100 × 4.6 Spheri-5RP-18 (Applied Biosystems)
Mobile phase: MeCN:100 mM $NaH_2PO_4.2H_2O$ containing 1% diethylamine 15:85, adjusted to pH 5.8
Flow rate: 0.9
Injection volume: 30
Detector: UV 220, UV 254

CHROMATOGRAM

Internal standard: R011-9900 (Roche Australia)

KEY WORDS

liver; use silanized glassware

REFERENCE

McIntyre,I.M.; King,C.V.; Staikos,V.; Gall,J.; Drummer,O.H. A fatality involving moclobemide, sertraline, and pimozide, *J.Forensic Sci.*, **1997**, *42*, 951–953.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 200 μL 5 μg /mL IS in MeOH:water 30:70 + 1 mL 500 mM Na_2HPO_4 + 10 mL distilled n-butyl chloride, extract for 10 min, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 400 μL mobile phase, centrifuge at 1000 g for 5 min, inject a 40 μL aliquot.

HPLC VARIABLES

Column: 125 × 3.2 7 μm Lichrosorb RP-18
Mobile phase: MeCN:water:25% ammonia 25:75:0.15 (Wash column with MeCN:water 50:50 at the end of the day.)
Flow rate: 1.5
Injection volume: 40
Detector: UV 237

CHROMATOGRAM

Retention time: 4.3
Internal standard: p-methyl-N-(2-morpholinoethyl)benzamide (2.9)
Limit of detection: 20 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Raaflaub,J.; Haefelfinger,P.; Trautmann,K.H. Single-dose pharmacokinetics of the MAO-inhibitor moclobemide in man, *Arzneimittelforschung*, **1984**, *34*, 80–82.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 239

CHROMATOGRAM

Retention time: 4.71

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE
Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM
Retention time: 1.30
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES
Simultaneous: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, mianserin, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, trazodone, trihexyphenidyl, trimipramine, triprolidine
Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine
Interfering: metoclopramide, tranylcypromine, pentobarbital

KEY WORDS
serum; whole blood; liver

REFERENCE
McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 500 μL Plasma + 400 μL saturated sodium phosphate solution (pH 11) + 100 μL 8 μg/mL IS in water, vortex for a few s, add to a glass Extrelut 1 SPE cartridge, let stand for 10 min, elute with two 6 mL portions of freshly distilled dichloromethane. Evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 250 μL mobile phase, vortex for 30 s, inject a 75 μL aliquot. Urine. 200 μL Urine + 700 μL saturated sodium phosphate solution (pH 11) + 100 μL 20 μg/mL IS in water, vortex for a few s, add to a glass Extrelut 1 SPE cartridge, let stand for 10 min, elute with two 6 mL portions of freshly distilled dichloromethane. Evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 750 μL mobile phase, vortex for 30 s, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 Spherisorb S5 C6
Mobile phase: MeCN:67 mM KH_2PO_4 30:320 adjusted to pH 3.9 with dilute HCl
Flow rate: 1.3
Injection volume: 50-75
Detector: UV 240

CHROMATOGRAM
Retention time: 7.2
Internal standard: p-iodo-N-(2-morpholinoethyl)benzamide (Ro 11-9900) (12.7)
Limit of quantitation: 75 ng/mL (urine), 20 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Geschke,R.; Körner,J.; Eggers,H. Determination of the new monoamine oxidase inhibitor moclobemide and three of its metabolites in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *420*, 111–120.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 10.218

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: hepatocytes
Sample preparation: Add an equal volume of MeOH to the hepatocytes, centrifuge at 5000 g for 15 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Guard column: 20 mm long 5 μm Nucleosil C18
Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: Gradient. MeOH:buffer 12:88 for 45 min, then 80:20 for 15 min (step gradient). (Buffer was 25 mM pH 3.9 sodium phosphate buffer containing 0.2% N-hexylamine.)
Flow rate: 1
Detector: UV 250

CHROMATOGRAM
Retention time: 34

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; human

REFERENCE
Vallès,B.; Coassolo,P.; De Sousa,G.; Aubert,C.; Rahmani,R. In vitro hepatic biotransformation of moclobemide (Ro 11-1163) in man and rat, *Xenobiotica*, **1993**, *23*, 1101–1111.

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: 6.86 (A), 3.85 (B)

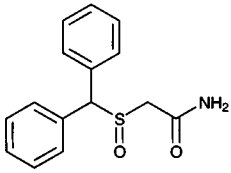
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, 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
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.

Modafinil



Molecular formula: $C_{15}H_{15}NO_2S$
Molecular weight: 273.36
CAS Registry No.: 68693-11-8
Merck Index: 6311

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 100 mM HCl + 100 µL MeOH + 100 µL 40 µg/mL IS in MeOH + 10 mL diethyl ether, shake for 10 min, centrifuge at 0-4° at 4000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 200 µL mobile phase, vortex for 30 s, inject a 25 µL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 µm µBondapak C18
Mobile phase: MeCN:water:acetic acid 15:42:1.2
Flow rate: 1.4
Injection volume: 25
Detector: UV 236

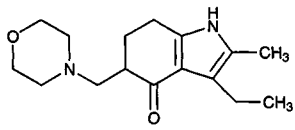
CHROMATOGRAM
Retention time: 11.0
Internal standard: [bis-(4-fluorophenyl)methylsulfinyl]acetic acid (24.0)
Limit of detection: 40 ng/mL
Limit of quantitation: 130 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Moachon,G.; Matinier,D. Simultaneous determination of modafinil and its acid metabolite by high-performance liquid chromatography in human plasma, *J.Chromatogr.B*, **1994**, *654*, 91–96.

Molindone



Molecular formula: $C_{16}H_{24}N_2O_2$
Molecular weight: 276.38
CAS Registry No.: 7416-34-4, 15622-65-8 (HCl)
Merck Index: 6315
Lednicer No.: 2 455

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elut CN SPE cartridge with MeOH and then with water. Add 1 mL plasma to the SPE cartridge, wash with 200 μL water, elute with five 200 μL portions of MeOH. Evaporate the eluate to dryness under a stream of air at 35°, reconstitute the residue in 200 μL 750 ng/mL p-aminobenzonitrile in mobile phase, inject an aliquot.

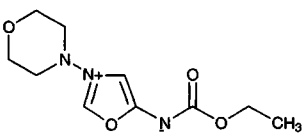
HPLC VARIABLES
Column: 250 × 4.6 Zorbax CN
Mobile phase: Cyclopentane:MeOH:THF:morpholine 90:7:2.9:0.1
Column temperature: 35
Detector: UV 254

CHROMATOGRAM
Internal standard: p-aminobenzonitrile
Limit of detection: 3 ng/mL

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Zetin,M.; Cramer,M.; Garber,D.; Plon,L.; Paulshock,M.; Hoffman,H.E.; Schary,W.L. Bioavailability of oral and intramuscular molindone hydrochloride in schizophrenic patients, *Clin.Ther.*, **1985**, *7*, 169–175.

Molsidomine



Molecular formula: $C_9H_{14}N_4O_4$
Molecular weight: 242.23
CAS Registry No.: 25717-80-0
Merck Index: 6316
Lednicer No.: 3 140

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 312.8

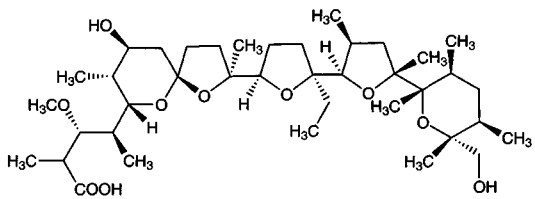
CHROMATOGRAM
Retention time: 10.007

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Monensin

Molecular formula: $C_{36}H_{62}O_{11}$
Molecular weight: 670.88
CAS Registry No.: 17090-79-8
Merck Index: 6329



SAMPLE
Matrix: eggs, tissue
Sample preparation: 5 g Pulverized frozen tissue or 5 g homogenized whole eggs + 2 mL water + 13 mL MeOH, homogenize for 30 s. Sonicate for 10 min and centrifuge at 2000 g for 10 min. Add 4 mL 100 mM NaOH to a 2 mL aliquot of the supernatant, extract with 2 mL and 1 mL hexane:toluene 50:50 (v/v) for 30 s by inversion, centrifuge at 1500 g for 10 min. Evaporate the combined extracts to dryness under a stream of nitrogen at 60°. Dissolve the residue in 200 µL MeCN:water 75:25. Inject a 20 µL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 µm Inertsil ODS-2 (GL Sciences, Japan)
Mobile phase: MeCN:MeOH:THF:water:trifluoroacetic acid 67:10:10:13:0.1
Flow rate: 1
Injection volume: 20
Detector: MS, VG Platform, Megaflow electrospray probe, positive ion mode, source at 125°, cone voltage 25 V, m/z 693

CHROMATOGRAM
Retention time: 7
Limit of detection: 0.5-1 ng/g
Limit of quantitation: 2 ng/g

OTHER SUBSTANCES
Extracted: narasin, salinomycin

KEY WORDS
domestic fowl; muscle; liver

REFERENCE
Blanchflower,W.J.; Kennedy,D.G. Determination of monensin, salinomycin, and narasin in muscle, liver and eggs from domestic fowl using liquid chromatography-electrospray mass spectrometry, *J.Chromatogr.B*, **1996**, *675*, 225–233.

SAMPLE
Matrix: feed
Sample preparation: 20 g Ground feed + 50 mL MeOH, shake on a reciprocating shaker for 1 h, allow to settle, centrifuge an aliquot of the supernatant at 2500 rpm for 5 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 Partisil 5ODS-3
Mobile phase: MeOH:water:glacial acetic acid 94:5.9:0.1
Flow rate: 0.7
Injection volume: 50
Detector: UV 520 following post-column reaction with the reagent pumped at 0.7 mL/min. The mixture passed through a 7.6 m × 0.05 mm i.d. coil of stainless steel tubing maintained at 70° to the detector. (Prepare reagent by stirring 900 mL MeOH and slowly and cautiously adding 20 mL concentrated sulfuric acid (Caution! Corrosive! Exothermic reaction!), cool in an ice bath, add 100 g vanillin, mix to dissolve, make up to 1 L with MeOH. Degas under vacuum with sonication for 2 min.)

CHROMATOGRAM
Retention time: 11.5
Limit of detection: 250 ng/g

OTHER SUBSTANCES
Simultaneous: narasin, salinomycin

KEY WORDS
post-column reaction

REFERENCE
Blanchflower,W.J.; Rice,D.A.; Hamilton,J.T.G. Simultaneous high-performance liquid chromatographic determination of monensin, narasin and salinomycin in feeds using post-column derivatisation, *Analyst*, **1985**, *110*, 1283–1287.

SAMPLE
Matrix: feed
Sample preparation: 20 g Ground Feed + 200 mL hexane:ethyl acetate 90:10, stir at high speed for 2 h, let stand. Remove an aliquot equivalent to 1 g feed and evaporate it to dryness under reduced pressure at 40°, reconstitute with 2 mL MeOH, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 60 × 4.6 3 μm C18 (Hewlett-Packard)
Mobile phase: MeOH:5% acetic acid 90:10
Flow rate: 0.5
Injection volume: 20-25
Detector: UV 520 following post-column reaction. The column effluent mixed with the reagent pumped at 1 mL/min and the mixture flowed through a 1.5 mL reaction coil (Kratos Model 510) at 95° to the detector. (Reagent was 40 g/L vanillin in MeOH:sulfuric acid 100:2. Keep in an ice bath and prepare fresh daily.)

CHROMATOGRAM
Retention time: 5.5
Limit of detection: 0.5 ppm

OTHER SUBSTANCES
Extracted: narasin, salinomycin

KEY WORDS
post-column reaction

REFERENCE

Lapointe,M.R.; Cohen,H. High-speed liquid chromatographic determination of monensin, narasin, and salinomycin in feeds, using post-column derivatization, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 480–484.

SAMPLE

Matrix: feed, premix

Sample preparation: Feed. Shake 5 g feed with 15 mL MeOH for 2 h, filter, evaporate the filtrate to 3 mL and make up to 10 mL with MeOH, inject a 3 μL aliquot. Premix. Shake 0.5 g premix with 15 mL MeOH for 2 h, filter, make up the filtrate to 50 mL with MeOH, inject a 3 μL aliquot.

HPLC VARIABLES

Column: 150 × 1 5 μm Separon SGX C18 glass column (Tessek Prague)

Mobile phase: MeOH:water:glacial acetic acid 94:5.9:0.1

Flow rate: 0.02

Injection volume: 3

Detector: UV 592 following post-column derivatization. The column effluent mixed with the reagent pumped at 0.015 mL/min and the mixture flowed through a 150 × 1 reactor containing 40-70 μm acid-washed glass beads at 75° to the detector. (The reagent was 500 mM 4-dimethylaminobenzaldehyde in 1.2 M sulfuric acid in MeOH.)

CHROMATOGRAM

Retention time: 11 (monensin B), 14 (monensin A)

Limit of detection: 1.7 μg/mL (monensin A)

OTHER SUBSTANCES

Extracted: narasin, salinomycin

KEY WORDS

microbore; post-column reaction

REFERENCE

Fejglova,Z.; Dolezal,J.; Hrdlicka,A.; Frgalova,K. Microbore HPLC determination of polyether antibiotics using postcolumn derivatization with benzaldehyde reagents, *J.Liq.Chromatogr.*, **1994**, *17*, 359–372.

SAMPLE

Matrix: fermentation solutions

Sample preparation: Homogenize (mechanical grinder) 5 mL fermentation broth with 45 mL MeOH, allow to settle at 5° for 1 h, filter (0.45 μm) the supernatant, dilute the filtrate with MeOH (if necessary), inject a 10 μL aliquot.

HPLC VARIABLES

Column: Little Champ C18 (Regis)

Mobile phase: MeOH:buffer 85:15 (Buffer was 1 g/L (?) $(NH_4)H_2PO_4$ adjusted to pH 3.0 with phosphoric acid.)

Flow rate: 1.5

Injection volume: 10

Detector: UV 520 following post-column reaction. The column effluent mixed with the reagent pumped at 1.5 mL/min and the mixture flowed through a 3 m × 0.25 mm ID stainless steel coil at 120° to the detector. (Prepare reagent by making a 3% solution of vanillin in MeOH, chill solution, slowly add concentrated sulfuric acid to a final concentration of 3%. Store at 0°, discard after 2 weeks.)

CHROMATOGRAM

Retention time: 2 (monensin B), 2.7 (monensin A)

KEY WORDS

post-column reaction

REFERENCE

Neely,F.L. Determination of monensin in fermentation broth by HPLC with post-column derivatization, *J.Liq.Chromatogr.*, **1992**, *15*, 1513–1522.

SAMPLE
Matrix: formulations
Sample preparation: 100 μL Liposome + 50 μL Tween 20, vortex for 5 min, add 600 μL MeOH, centrifuge at 10000 rpm for 30 min, dilute the supernatant 10-fold with MeOH, inject an aliquot.

HPLC VARIABLES
Column: 70 × 4.6 3 μm Ultrasphere XL-ODS (Beckman)
Mobile phase: MeCN:MeOH:dichloromethane:water:acetic acid 20:45:25:9.5:0.5
Flow rate: 0.33
Detector: UV 520 following post-column reaction. The column effluent mixed with the reagent pumped at 0.67 mL/min and the mixture flowed through a Beckman 231 reactor at 70° to the detector. (Prepare the reagent by dissolving 8 g vanillin in 100 mL cooled MeOH, add 4 g concentrated sulfuric acid, mix, keep in an ice bath.)

CHROMATOGRAM
Retention time: 6.05
Limit of quantitation: 100 ng/mL

KEY WORDS
post-column reaction; liposomes

REFERENCE
Ferdous,A.J.; Bennefield,S.D.; Singh,M. A modified HPLC method for monensin analysis in liposomes and nanocapsules and its comparison with spectrophotometric and radioactive methods, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1775–1780.

SAMPLE
Matrix: milk, tissue
Sample preparation: Tissue. Condition a Sep-Pak silica gel SPE cartridge (No. 51900) with 3 mL dichloromethane, do not allow it to go dry. Sonicate or blend 10 g ground or minced tissue and 75 mL MeOH:water 85:15, centrifuge at 2000 rpm for 10 min. Remove the supernatant and add it to 50 (muscle, liver), 40 (kidney) or 60 (fat) mL 100 mg/mL NaCl, extract twice with 35 mL portions of dichloromethane. Combine the organic layers and evaporate them to dryness under vacuum at 45°, reconstitute the residue in 7 mL dichloromethane, add it to the SPE cartridge at no more than 5 mL/min, rinse out the flask with 3 mL dichloromethane, add the rinse to the SPE cartridge, wash with 10 mL dichloromethane, elute with 5 mL dichloromethane:MeOH 95:5. Evaporate the eluate to dryness under a stream of nitrogen or air at 45°, reconstitute the residue in 1 mL MeOH:water 90:10, filter (Gelman Acrodisc CR PTFE 0.45 μm), inject a 100 μL aliquot. Milk. Condition a Sep-Pak silica gel SPE cartridge (No. 51900) with 3 mL dichloromethane, do not allow it to go dry. Sonicate (ultrasonic cell disrupter) or blend 40 mL milk and 160 mL MeOH for 30 s, let stand for 10-15 min, centrifuge at 2000 rpm for 10 min. Decant the supernatant and add it to 60 mL 100 mg/mL NaCl, extract twice with 70 mL portions of dichloromethane. Combine the organic layers and evaporate them to dryness under vacuum at 45°, reconstitute the residue in 7 mL dichloromethane, add it to the SPE cartridge at no more than 5 mL/min, rinse out the flask with 3 mL dichloromethane, add the rinse to the SPE cartridge, wash with 10 mL dichloromethane, elute with 5 mL dichloromethane:MeOH 95:5. Evaporate the eluate to dryness under a stream of nitrogen or air at 45°, reconstitute the residue in 1 mL MeOH:water 90:10, filter (Gelman Acrodisc CR PTFE 0.45 μm), inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Partisil 5 ODS-3 25 LC
Mobile phase: MeOH:water:acetic acid 94:6:0.1
Flow rate: 0.7
Injection volume: 100
Detector: UV 520 following post-column reaction. The column effluent mixed with the reagent pumped at 0.7 mL/min and flowed through a 0.5 mm i.d. × 6 m stainless steel tube held at 98° to the detector. (The reagent was prepared by slowly adding 20 mL concentrated sulfuric acid to 950 mL MeOH (Caution! Exothermic!), allow to cool to room temperature, add 30 g vanillin while stirring.)

CHROMATOGRAM
Retention time: 8
Limit of quantitation: 25 ppb (tissue), 5 ppb (milk)

OTHER SUBSTANCES
Simultaneous: narasin, salinomycin
Noninterfering: bacitracin, bambermycin, lasalocid, lincomycin, nicarbazin, tylosin

KEY WORDS
cow; muscle; liver; fat; kidney; post-column reaction; SPE

REFERENCE
Moran,J.W.; Turner,J.M.; Coleman,M.R. Determination of monensin in edible bovine tissues and milk by liquid chromatography, *J.AOAC Int.*, **1995**, *78*, 668–673.

SAMPLE
Matrix: premix
Sample preparation: 5 g Feed premix + 75 mL MeOH:water 90:10, let stand overnight with periodic swirling, make up to 100 mL with MeOH:water 90:10, dilute an aliquot to a monensin concentration of 2 mg/mL, inject a 40 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 Zorbax C8
Mobile phase: MeOH:water 90:10
Injection volume: 40
Detector: RI

CHROMATOGRAM
Retention time: retention volume 12-14 mL

OTHER SUBSTANCES
Noninterfering: arprinocid, lasalocid, narasin, salinomycin

KEY WORDS
premix

REFERENCE
Macy,T.D.; Loh,A. High pressure liquid chromatographic determination of monensin in feed premixes, *J.Assoc.Off.Anal.Chem.*, **1983**, *66*, 284–286.

SAMPLE
Matrix: solutions
Sample preparation: Condition a Mega Bond Elut silica gel SPE cartridge with benzene (Caution! Benzene is a carcinogen!). Evaporate a solution in MeOH to dryness, add 5 mL 5.28 mg/mL 1-bromoacetylpyrene in MeCN, add 5 mL 1.28 mg/mL Kryptofix 222 in MeCN, heat at 50° for 1.5 h, cool. Either inject this solution directly or evaporate it to dryness, dissolve the residue in 5 mL benzene:chloroform 50:50, rinse out the flask with two 5 mL portions of benzene:chloroform 50:50, filter, add the filtrate to the SPE cartridge, elute with two 5 mL portions of benzene:acetone 70:30. Evaporate the eluate to dryness, reconstitute the residue in 10 mL MeCN, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Develosil 5C18
Mobile phase: MeOH:water 97:3
Flow rate: 1
Detector: F ex 360 em 420

CHROMATOGRAM
Retention time: 13.5
Internal standard: 18,19-dihydrosalinomycin (25), 18,19-dihydro-20-ketosalinomycin (16.5)

Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES

Simultaneous: lasalocid, narasin, salinomycin

KEY WORDS

derivatization; SPE

REFERENCE

Asukabe,H.; Murata,H.; Harada,K.-I.; Suzuki,M.; Oka,H.; Ikai,Y. Improvement of chemical analysis of antibiotics. XX. Basic study on high-performance liquid chromatographic determination of four polyether antibiotics pre-derivatized with 1-bromoacetylpyrene, *J.Chromatogr.A*, **1993**, *657*, 349–356.

SAMPLE

Matrix: tissue

Sample preparation: Homogenize (Tissuemizer) 10 g tissue and 25 mL solvent for 1 min, wash blades with 3-4 mL solvent, combine with homogenate, shake for 30 min, centrifuge at 800 g for 15 min, decant. Add 25 mL solvent to residue, mix thoroughly, shake vigorously for 1 min, centrifuge at 800 g for 15 min, add supernatants to a 75 × 20 column of 80-200 mesh alumina (Fisher), rinse container onto column with 25 mL solvent, add 100 mL solvent to the column. Combine all the eluates and add 100 mL 5% NaCl, shake vigorously, let stand 2-3 min, add 30 mL dichloromethane, shake vigorously for 30 s, repeat extraction twice. Combine the dichloromethane layers and evaporate them to dryness under reduced pressure at 48-50°, reconstitute the residue in 1 mL solvent, add to a 75 × 20 column of 25-100 μm Sephadex LH-20, rinse flask with two 3.5 mL portions of solvent and add the rinses to the column, add 10 mL solvent to the column, discard the first 18 mL of eluate, add 10 mL solvent to the column, collect this fraction, evaporate to dryness under a stream of nitrogen at 48-50°, reconstitute the residue in 1 mL dichloromethane, evaporate to dryness under a stream of nitrogen at 48-50°, repeat twice, reconstitute in MeOH, evaporate to dryness under a stream of nitrogen at 48-50°, reconstitute in 100 μL pyridine and 100 μL acetic anhydride, let stand overnight at room temperature, add to 35 mL water, rinse vial with 5 mL water. Combine the aqueous layers and add 40 mL petroleum ether, shake vigorously for 1 min, separate and preserve the organic layer. Add 40 mL anhydrous ethyl ether to the aqueous phase, shake for 1 min. Combine the organic phases and wash with 20 mL 10% HCl, twice with 20 mL water, and with 40 mL saturated NaCl (shake for 1 min each time). Filter the organic layer through a glass fiber filter containing 20-30 g anhydrous sodium sulfate, wash the sodium sulfate with 10-15 mL petroleum ether, evaporate the filtrate to dryness under a stream of nitrogen at 48-50°. Take up the residue in 1 mL solvent and put it in another tube, wash flask into tube with three 2 mL portions of solvent, evaporate almost to dryness under a stream of nitrogen at 48-50°, make up to 1 mL with solvent, mix, add 500 μL 9-anthryldiazomethane solution, let stand in the dark for 30 min, evaporate to dryness under a stream of nitrogen at 48-50°, reconstitute in 1 mL hexane. Condition a Baker-10 silica SPE cartridge with 5-10 mL hexane, do not allow to dry. Add the hexane solution to the SPE cartridge, rinse tube with 9 mL hexane, add rinse to the SPE cartridge. Wash SPE cartridge with 10 mL hexane:dichloromethane 50:50, with 10 mL hexane:dichloromethane 20:80, with 10 mL dichloromethane, and with 1 mL MeOH. Elute with 1 mL MeOH, inject a 20 μL aliquot of the eluate. (Solvent was MeOH:water 80:20. Prepare 9-anthryldiazomethane solution as follows. Add 1100 g manganous sulfate tetrahydrate in 1.5 L water and 1170 mL 40% NaOH over 1 h to a hot stirred solution of 960 g potassium permanganate in 6 L water, stir for 1 h, centrifuge, wash solid with water until washings are colorless, dry solid at 100-120°, grind the activated manganese dioxide to a fine powder. Add 8.5 g 85% hydrazine hydrate (Caution! Hydrazine hydrate is a carcinogen!) to 8.8 g 9-anthraldehyde dissolved in 150 mL EtOH, stir at room temperature for 3 h, filter off solid, dry under vacuum, recrystallize from EtOH to give 9-anthraldehyde hydrazone as red-yellow crystals, mp 124-6°. Dissolve 220 mg 9-anthraldehyde hydrazone in 100 mL anhydrous ethyl ether, add 800 mg activated manganese dioxide, add 600 μL EtOH saturated with KOH, stir vigorously for 30 min, filter (glass fiber), wash solid with 20 mL anhydrous ethyl ether, evaporate to reduce volume, make up to 100 mL with anhydrous ethyl ether, store in a dark flask in the dark in a refrigerator. Discard after 30 days (J.Assoc.Off.Anal.Chem. 1985, 68, 1149).)

HPLC VARIABLES

Guard column: pellicular C18 (Alltech)

Column: 200 × 4.6 5 μm RP-C8 (Hewlett-Packard)

Mobile phase: Gradient. A was MeCN. B was MeCN:water 10:90. A:B 20:80 for 9 min, 10:90 for 7 min, 20:80 for 1 min.

Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: F ex 365 em 418 (filter)

CHROMATOGRAM
Retention time: 11.5
Limit of detection: 0.15 ppm

OTHER SUBSTANCES
Extracted: salinomycin, narasin

KEY WORDS
cow; liver; SPE; derivatization

REFERENCE
Martinez,E.E.; Shimoda,W. Liquid chromatographic determination of multiresidue fluorescent derivatives of ionophore compounds, monensin, salinomycin, narasin, and lasalocid, in beef liver tissue, *J.Assoc.Off.Anal.Chem.*, **1986**, *69*, 637–641.

SAMPLE
Matrix: tissue
Sample preparation: Blend 20 g ground or minced tissue with 150 mL MeOH:water 85:15 for 30 s or until homogeneous, centrifuge at 2500 rpm for 15 min. Remove the supernatant and mix it with 40 mL 10% NaCl in water, extract three times with 25 mL portions of carbon tetrachloride (Caution! Carbon tetrachloride is a carcinogen!), evaporate the extracts to dryness under reduced pressure at ≤40°, reconstitute with 5 mL chloroform, add to a SepPak silica SPE cartridge at 2 mL/min, rinse flask with 3 mL chloroform, add the rinse to the SPE cartridge, wash with 10 mL hexane:chloroform 10:90, elute with 10 mL chloroform:MeOH 95:5, evaporate the eluate to dryness under reduced pressure or a stream of air at ≤40°, reconstitute with 2 mL MeOH:water 90:10, filter (0.45 μm), inject a 200 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Partisil 5 ODS-3 25 LC
Mobile phase: MeOH:water:acetic acid 94:6:0.1
Flow rate: 0.7
Injection volume: 200
Detector: UV 520 following post-column reaction. The column effluent mixed with the reagent pumped at 0.7 mL/min and the mixture flowed through a 6.1 m × 0.5 mm ID stainless steel coil at 98° to the detector. (Prepare reagent by cautiously adding 20 mL concentrated sulfuric acid to 950 mL MeOH, allow to cool to room temperature, allow 30 g vanillin while stirring.)

CHROMATOGRAM
Retention time: 11
Limit of detection: 5 ng/g
Limit of quantitation: 25 ng/g

OTHER SUBSTANCES
Simultaneous: narasin, salinomycin
Noninterfering: bacitracin, bambermycin, lasalocid, lincomycin, nicarbazin, tylosin

KEY WORDS
post-column reaction; SPE; chicken; muscle; liver; skin

REFERENCE
Moran,J.W.; Rodewald,J.M.; Donoho,A.L.; Coleman,M.R. Determination of monensin in chicken tissues by liquid chromatography with postcolumn derivatization, *J.AOAC Int.*, **1994**, *77*, 885–890.

SAMPLE
Matrix: tissue

Sample preparation: Condition a 500 mg Bond Elut silica SPE cartridge with 5 mL isooctane:ethyl acetate 90:10. Homogenize (Polytron) 5 g tissue with 10 mL iso-octane:ethyl acetate 90:10 for 30 s, shake mechanically for 5 min, centrifuge at 1500 g for 5 min, remove the supernatant, extract twice more with 10 mL portions of isooctane:ethyl acetate 90:10. Combine the supernatants and dry them over 1 g anhydrous sodium sulfate, add to the SPE cartridge at 3 mL/min, wash with 10 mL dichloromethane, dry under vacuum for 30 s, elute with two 3 mL portions of dichloromethane:MeOH 90:10. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 500 μL MeOH:water 90:10, vortex for 30 s, sonicate for 5 min, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 × 3.2 5 μm Inertsil ODS-2
Mobile phase: MeOH:10 mM pH 4.0 ammonium acetate 6:94
Flow rate: 0.5
Injection volume: 100
Detector: UV 520 following post-column reaction. The column effluent mixed with the reagent pumped at 0.3 mL/min and the mixture flowed through a 150 × 4.6 column packed with 75 μm glass beads at 100° to the detector. (Prepare reagent by adding 2.5 mL sulfuric acid to 125 mL cold MeOH, add 9.5 g vanillin, prepare fresh each day.)

CHROMATOGRAM

Retention time: 10.0
Limit of detection: 2 ppb
Limit of quantitation: 3 ppb

OTHER SUBSTANCES

Extracted: narasin, salinomycin
Noninterfering: bacitracin, chlortetracycline, furazolidone, lasalocid, lincomycin, melengestrol acetate, oxytetracycline, penicillin G, roxarsone, tetracycline, tilmicosin, tylosin, virginiamycin

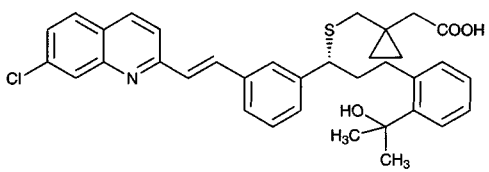
KEY WORDS

post-column reaction; cow; chicken; muscle; fat; kidney; liver; SPE

REFERENCE

Gerhardt,G.C.; Salisbury,C.D.C.; Campbell,H.M. Determination of ionophores in the tissues of food animals by liquid chromatography, *Food Addit.Contam.*, **1995**, *12*, 731–737.

Montelukast



Molecular formula: $C_{35}H_{36}ClNO_3S$, $C_{35}H_{35}ClNNaO_3S$ (monosodium salt)
Molecular weight: 586.19
CAS Registry No.: 158966-92-8, 151767-02-1 (monosodium salt)
Merck Index: 6340

SAMPLE

Matrix: blood
Sample preparation: Mix 200 μL plasma with 40 μL 5 μg/mL IS and 400 μL MeCN, vortex, centrifuge. Inject an aliquot of the supernatant.

HPLC VARIABLES

Column: 50 × 4.6 Apex C18
Mobile phase: MeCN:5 mM pH 3.5 ammonium phosphate buffer 62:38
Flow rate: 1.5
Detector: F ex 350 em 400

CHROMATOGRAM

Internal standard: montelukast analog containing a dimethylmethylene group instead cyclopropyl group

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Balani,..; Xu,X.; Pratha,V.; Koss,M.A.; Amin,R.D.; Dufresne,C.; Miller,R.R.; Arison,B.H.; Doss,G.A.; Chiba,M.; Freeman,A.; Holland,S.D.; Schwartz,J.I.; Lasseter,K.C.; Gertz,B.J.; Isenberg,J.I.; Rogers,J.D.; Lin,J.H.; Baillie,T.A. Metabolic profiles of montelukast sodium (Singulair), a potent cysteinyl leukotriene1 receptor antagonist, in human plasma and bile, *Drug Metab.Dispos.*, **1997**, *25*, 1282–1287.

SAMPLE
Matrix: blood
Sample preparation: Mix 300 μL plasma with 20 μL 3 μg/mL IS in MeOH:water 70:30 and 400 μL MeCN. Vortex, centrifuge at 10000 rpm for 10 min. Inject 60 μL of the supernatant onto column A, elute to waste with mobile phase A for 5 min, elute to waste for 5 min with MeCN:mobile phase A 12:88. Backflush the contents of column A onto column B with mobile phase B, after 3.5 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:mobile phase A 80:20 for 5 min then re-equilibrate with mobile phase A.)

HPLC VARIABLES
Column: A 10 × 3 5 μm Chromspher 5 Biomatrix + 50 × 4.6 5 μm Chromspher 5 Biomatrix; B 10 × 3 5 μm Chiral AGP + 100 × 4 5 μm Chiral AGP
Mobile phase: A MeOH:10 mM pH 3.6 ammonium acetate 10:100; B MeCN:10 mM pH 5.8 ammonium acetate 32.5:100.
Flow rate: 1
Injection volume: 60
Detector: F ex 350 em 400

CHROMATOGRAM
Retention time: 20 (S), 23 (R)
Internal standard: 1-(((1(R)-(3-(2-(7-chloro-2-quinolinyl)-(E)-ethenyl)-phenyl) (3-(2-(1-hydroxy-1-methylethyl)phenyl)propyl)thio)-methyl)-isopropane)acetate (30)
Limit of detection: 9.6 ng/mL

KEY WORDS
plasma; column-switching; chiral

REFERENCE
Liu,L.; Cheng,H.; Zhao,J.J.; Rogers,J.D. Determination of montelukast (MK-0476) and its S-enantiomer in human plasma by stereoselective high-performance liquid chromatography with column-switching, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 631–638.

SAMPLE
Matrix: blood, bile
Sample preparation: Plasma. Deproteinize plasma with MeCN and evaporate under a stream of nitrogen at 37°. Reconstitute with MeCN:1 mM pH 3.5 ammonium acetate 90:10. Inject an aliquot. Bile. Centrifuge bile and inject an aliquot directly.

HPLC VARIABLES
Column: 250 × 4.6 Beckman C18
Mobile phase: Gradient. A was MeCN. B was 1 mM pH 3.5 ammonium acetate buffer. A:B from 35:65 to 45:55 over 5 min, to 55:45 over 35 min, to 87:13 over 20 min, to 95:5 over 0.3 min
Flow rate: 1.1
Detector: MS, PE-SCIEX III tandem mass, turbo-ion spray interface, positive ion mode, m/z 586. The column effluent mixed with MeCN:1% trifluoroacetic acid in water 90:10 pumped at 50-100 μL/min and the mixture flowed to the detector.

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics; radiolabeled

REFERENCE
Balani,S.K.; Xu,X.; Pratha,V.; Koss,M.A.; Amin,R.D.; Dufresne,C.; Miller,R.R.; Arison,B.H.; Doss,G.A.; Chiba,M.; Freeman,A.; Holland,S.D.; Schwartz,J.I.; Lasseter,K.C.; Gertz,B.J.; Isenberg,J.I.; Rogers,J.D.; Lin,J.H.; Baillie,T.A. Metabolic profiles of montelukast sodium (Singulair), a potent cysteinyl leukotriene1 receptor antagonist, in human plasma and bile, *Drug Metab.Dispos.*, **1997**, *25*, 1282–1287.

SAMPLE
Matrix: blood, bile
Sample preparation: Plasma. Deproteinize plasma with MeCN. Evaporate under a stream of nitrogen at 37°. Reconstitute the residue with MeCN:1 mM pH 3.5 ammonium acetate 90:10. Inject an aliquot. Bile. Centrifuge bile and inject an aliquot directly.

HPLC VARIABLES
Column: 250 × 4.6 Beckman C18
Mobile phase: Gradient. A was MeCN. B was 1 mM pH 3.5 ammonium acetate buffer. A:B from 35:65 to 45:55 over 5 min, to 55:45 over 35 min, to 87:13 over 20 min, to 95:5 over 0.3 min
Flow rate: 1.1
Detector: Radioactivity, Ramona 5 (Raytest) following post-column reaction. The column effluent mixed with Packard Flow -Scint III cocktail pumped at 2.2 mL/min and this mixture flowed to the detector.

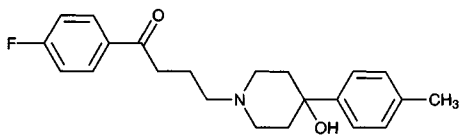
CHROMATOGRAM
Internal standard: montelukast analog containing dimethylmethylene group instead cyclopropyl group

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics; radiolabeled

REFERENCE
Balani,S.K.; Xu,X.; Pratha,V.; Koss,M.A.; Amin,R.D.; Dufresne,C.; Miller,R.R.; Arison,B.H.; Doss,G.A.; Chiba,M.; Freeman,A.; Holland,S.D.; Schwartz,J.I.; Lasseter,K.C.; Gertz,B.J.; Isenberg,J.I.; Rogers,J.D.; Lin,J.H.; Baillie,T.A. Metabolic profiles of montelukast sodium (Singulair), a potent cysteinyl leukotriene1 receptor antagonist, in human plasma and bile, *Drug Metab.Dispos.*, **1997**, *25*, 1282–1287.

Moperone



Molecular formula: $C_{22}H_{26}FNO_2$
Molecular weight: 355.45
CAS Registry No.: 1050-79-9, 3871-82-7 (HCl)
Merck Index: 6343

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 247

CHROMATOGRAM

Retention time: 5.62
Limit of detection: <120 ng/mL

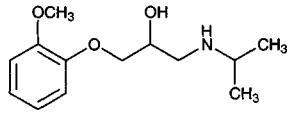
KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

Moprolol



Molecular formula: $C_{13}H_{21}NO_3$
Molecular weight: 239.31
CAS Registry No.: 5741-22-0, 27058-84-0 (HCl), 77164-20-6 (l-form), 113482-87-4 (l-form HCl)
Merck Index: 6345
Lednicer No.: 2 109

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL Bond Elut CN SPE cartridge with 1 volume dichloromethane, 1 volume MeOH, and 1 volume pH 10 carbonate buffer, do not allow to go dry. 1 mL Plasma + 10 μL 100 ng/mL IS in water + 500 μL pH 10 carbonate buffer, mix, add to the SPE cartridge, wash with two volumes of pH 10 carbonate buffer, centrifuge at 4000 rpm for 15 s, elute with two volumes of dichloromethane while centrifuging at 1000 rpm for 3 min. Evaporate the eluate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 1 mL diethyl ether, add 250 μL trifluoroacetic anhydride, let stand at room temperature for 45 min, evaporate to dryness under a stream of nitrogen, reconstitute with 500 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 5 μm LiChrosorb CN
Mobile phase: n-Pentane:diethyl ether 55:45
Flow rate: 1
Injection volume: 100
Detector: UV 223

CHROMATOGRAM

Retention time: 4.7
Internal standard: 1-(3-chloroisoxazol-5-yl)-2-(tert-butylamino)ethanol (Zambon Group, Milan) (5)

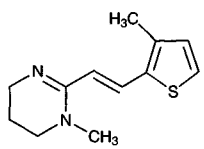
KEY WORDS

derivatization; SPE; plasma; an aliquot of the effluent can be further analyzed by GC using an electron capture detector

REFERENCE

Gianesello,V.; Brenn,E.; Figini,G.; Gazzaniga,A. Determination by coupled high-performance liquid chromatography-gas chromatography of the β-blocker levomoprolol in plasma following ophthalmic administration, *J.Chromatogr.*, **1989**, *473*, 343–352.

Morantel



Molecular formula: $C_{12}H_{16}N_2S$
Molecular weight: 220.34
CAS Registry No.: 20574-50-9, 26155-31-7 (tartrate)
Merck Index: 6348
Lednicer No.: 1 266

SAMPLE

Matrix: feed
Sample preparation: 5 g Ground feed + 100 mL methanolic HCl, shake on a reciprocating shaker for 30 min. Remove a 40 mL aliquot and centrifuge at 2000 rpm for 10 min. Remove 5 mL of the supernatant and make up to 100 mL with MeCN, mix, centrifuge at 2000 rpm for

10 min, inject an aliquot of the supernatant. (Prepare methanolic HCl by slowly adding 8.5 mL concentrated HCl to 992 mL MeOH:water 50:50.)

HPLC VARIABLES
Guard column: 400 mg Corasil II
Column: 250 × 4.6 Zorbax Sil
Mobile phase: MeCN:acetic acid:water:diethylamine 95:2:2:1
Flow rate: 1.6
Injection volume: 25
Detector: UV 313

CHROMATOGRAM
Retention time: 7.3

OTHER SUBSTANCES
Simultaneous: cis-isomer, degradation products, pyrantel

KEY WORDS
protect from light

REFERENCE
Goras,J.T.; Gauthier,A.R. Liquid chromatographic determination of morantel tartrate in cattle feed, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 598–601.

SAMPLE
Matrix: milk
Sample preparation: 20 mL Milk + 1.5 mL HCl + 1 mL 37.4 ng/mL pyrantel in water, heat at 95° with stirring for 2 h, cool in an ice bath, basify with 2.5 mL 12 M KOH, add 25 mL toluene, shake, centrifuge, repeat extraction. Combine the toluene layers and extract them vigorously twice with 4 mL portions of 100 mM HCl. Combine the aqueous layers and basify them with 2.5 mL 12 M KOH, heat at 95° for 5 h, acidify with 3 mL HCl, cool in an ice bath, extract twice with 5 mL portions of chloroform. Combine the organic layers, add 500 μL 100 mM NaOH, mix, centrifuge. Remove the aqueous layer and place it on a vortex evaporator for 5 min to remove residual chloroform, inject an aliquot. (Prepare 12 M KOH by cautiously dissolving 790 g KOH pellets in enough water to make 1 L in a fume hood, use an ice bath.)

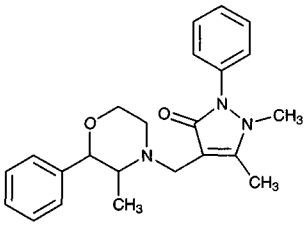
HPLC VARIABLES
Guard column: 30 mm long μC18 Corasil Bondapak
Column: 100 × 5 Radial-Pak A (Waters)
Mobile phase: MeOH:MeCN:water:acetic acid 40:10:49:1
Flow rate: 1
Injection volume: 100
Detector: UV 313

CHROMATOGRAM
Retention time: 6.6 (as 3-(3-methyl-2-thienyl)-acrylic acid, the hydrolysis product)
Internal standard: pyrantel (as 3-(2-thienyl)acrylic acid, the hydrolysis product) (4.2)
Limit of quantitation: 1 ppb

KEY WORDS
protect from light

REFERENCE
Lynch,M.J.; Mosher,F.R.; Brunner,L.A.; Bartolucci,S.R. Liquid chromatographic determination and identification of morantel-related residues as precursors of 3-(3-methyl-2-thienyl) acrylic acid (CP-20,107) in bovine milk, *J.Assoc.Off.Anal.Chem.*, **1986**, *69*, 931–935.

Morazone



Molecular formula: $C_{23}H_{27}N_3O_2$
Molecular weight: 377.49
CAS Registry No.: 6536-18-1, 50321-35-2 (HCl)
Merck Index: 6349
Lednicer No.: 2 261

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

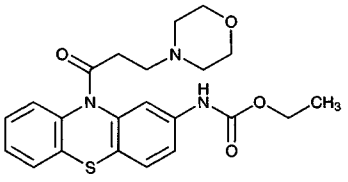
Retention time: 1.5

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Moricizine



Molecular formula: $C_{22}H_{25}N_3O_4S$
Molecular weight: 427.52
CAS Registry No.: 31883-05-3, 29560-58-8 (HCl)
Merck Index: 6351
Lednicer No.: 4 200

SAMPLE
Matrix: bile, blood, urine
Sample preparation: 1 mL Bile, plasma, or urine + 20 μL 40 μg/mL clozapine in EtOH + 200 μL 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 μl mobile phase, vortex for 30 s, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 25 × 4.6 30 μm C18 (Merck)
Column: 100 × 8 10 μm μBondapak 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: 6.2
Internal standard: clozapine (9.7)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; rat

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: 1 mL Serum + 100 μL 200 mM NaOH + 5 mL 100 mM pH 9.0 boric acid/KCl/NaOH buffer + 10 mL dichloromethane, shake for 30 min, centrifuge. Remove the organic layer and wash it with 5 mL water, evaporate the organic layer to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 10 μm μPorasil
Mobile phase: Hexane:THF:MeOH:water 60:27:6.3:0.7
Flow rate: 2
Detector: UV 268

CHROMATOGRAM
Retention time: 4.6
Limit of detection: 2 ng/mL

KEY WORDS
serum; normal phase; pharmacokinetics

REFERENCE
Rice,P.J.; LeClair,I.O.; Stone,W.L.; Mehta,A.V. Pharmacokinetics of moricizine in young patients, *J.Clin.Pharmacol.*, **1995**, *35*, 1016–1019.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax C8
Mobile phase: MeCN:buffer 42:58 (Buffer was 5 μM sodium octanesulfonate, 0.2% glacial acetic acid, and 0.1% triethylamine.)
Column temperature: 35
Flow rate: 2.5
Detector: UV 254

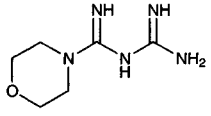
CHROMATOGRAM
Retention time: 5
Internal standard: N-butyl-p-aminobenzoate (7)

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
buffer; protect from light

REFERENCE
King,S.-Y.P.; Sigvardson,K.W.; Dudzinski,J.; Torosian,G. Degradation kinetics and mechanisms of moricizine hydrochloride in acidic medium, *J.Pharm.Sci.*, **1992**, *81*, 586–591.

Moroxydine



Molecular formula: $C_6H_{13}N_5O$
Molecular weight: 171.20
CAS Registry No.: 3731-59-7, 3160-91-6 (HCl)
Merck Index: 6354

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18

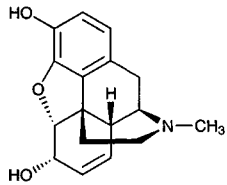
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 236.9

CHROMATOGRAM
Retention time: 2.873

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Morphine



Molecular formula: $C_{17}H_{19}NO_3$
Molecular weight: 285.34
CAS Registry No.: 57-27-2, 52-26-6 (HCl), 630-81-9 (HBr), 64-13-3 (sulfate)
Merck Index: 6359
Lednicer No.: 1 286

SAMPLE
Matrix: bile, blood
Sample preparation: 0.5 mL Blood or bile + 10 (blood) or 15 (bile) μL 100 μg/mL nalorphine in MeOH + 300 μL 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) μL 0.2% phosphoric acid, gently rotate for 30 min, discard organic layer, inject 50 μL 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 × 3.9 5 μm Nova-Pak phenyl
Mobile phase: MeCN:10 mM pH 6.6 NaH_2PO_4 10:90
Flow rate: 1.2
Injection volume: 50
Detector: UV 210 and F ex 220 em 370 (cut-off)

CHROMATOGRAM
Retention time: 5.4
Internal standard: nalorphine (23.5)
Limit of detection: 200 ng/mL (bile), 100 ng/mL (blood)

OTHER SUBSTANCES
Simultaneous: hydrocodone, dihydrocodeine, codeine, oxycodone, 6-monoacetylmorphine

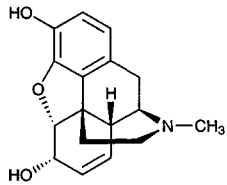
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 236.9

CHROMATOGRAM
Retention time: 2.873

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Morphine



Molecular formula: $C_{17}H_{19}NO_3$
Molecular weight: 285.34
CAS Registry No.: 57-27-2, 52-26-6 (HCl), 630-81-9 (HBr), 64-13-3 (sulfate)
Merck Index: 6359
Lednicer No.: 1 286

SAMPLE
Matrix: bile, blood
Sample preparation: 0.5 mL Blood or bile + 10 (blood) or 15 (bile) μL 100 μg/mL nalorphine in MeOH + 300 μL 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) μL 0.2% phosphoric acid, gently rotate for 30 min, discard organic layer, inject 50 μL 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 × 3.9 5 μm Nova-Pak phenyl
Mobile phase: MeCN:10 mM pH 6.6 NaH_2PO_4 10:90
Flow rate: 1.2
Injection volume: 50
Detector: UV 210 and F ex 220 em 370 (cut-off)

CHROMATOGRAM
Retention time: 5.4
Internal standard: nalorphine (23.5)
Limit of detection: 200 ng/mL (bile), 100 ng/mL (blood)

OTHER SUBSTANCES
Simultaneous: hydrocodone, dihydrocodeine, codeine, oxycodone, 6-monoacetylmorphine

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 μL Bile, 3 mL blood, or 5 mL tissue homogenate + 1 mL 200 μg/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 carefully adding powdered ammonium carbonate until the solution was 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 μL MeOH, vortex for 30 s, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm RP-18 Spheri-5
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:50 mM pH 7 phosphate buffer 40:60 Place a 70 × 2 30-38 μm 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: 4.2
Internal standard: nalorphine (14.72)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: codeine, hydromorphone, 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.; Sutheimer,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: bile, perfusate
Sample preparation: Dilute bile 100-fold with water. Dilute venous perfusate 1:4 with water. Inject an aliquot.

HPLC VARIABLES
Column: 300 mm long 10 μm Econosil C18
Mobile phase: MeCN:0.1% trifluoroacetic acid 4:96, adjusted to pH 2.4 with 1 M NaOH
Flow rate: 0.8

Detector: F ex 280 em 335

CHROMATOGRAM
Retention time: 13
Limit of quantitation: 270 nM

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
liver; pig; pharmacokinetics

REFERENCE
Milne,R.W.; Jensen,R.H.; Larsen,C.; Evans,A.M.; Nation,R.L. Comparison of the disposition of hepatically-generated morphine-3-glucuronide and morphine-6-glucuronide in isolated perfused liver from the guinea pig, *Pharm.Res.*, **1997**, *14*, 1014–1018.

SAMPLE
Matrix: blood
Sample preparation: Condition a 500 mg Bond Elut LRC C2 SPE cartridge with 2 mL MeOH, 2 mL MeCN:water:trifluoroacetic acid 80:20:0.1, and 5 mL Tris buffer. Mix 100 μL 100 ng/mL IS with 2 mL 50 mM Tris buffer adjusted to pH 7.5 with concentrated HCl and 500 μL plasma. Add sample to the SPE cartridge and draw through by vacuum at 2 mL/min. Wash with 20 mL Tris buffer, dry with vacuum for 1 min. Elute with 2 mL MeCN:water:trifluoroacetic acid 80:20:0.1, evaporate the eluate to dryness under a stream of nitrogen at 40°. Reconstitute the residue in 1 mL MeOH, vortex for 40 s, centrifuge at 3000 rpm for 15 min, decant the supernatant, evaporate to dryness. Reconstitute the residue in 200 μL mobile phase, vortex for 20 s. Inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 4 μm Nova-Pak phenyl Guard-Pak
Column: two 75 × 3.9 4 μm Nova-Pak phenyl columns in series
Mobile phase: MeOH:10 mM potassium phosphate monobasic 10:90, adjusted to pH 4.0 with 85% phosphoric acid
Flow rate: 1
Injection volume: 50
Detector: F ex 210 em 335 or E, ESA Coulochem II, Model 5200, Model 5011 high-sensitivity analytical cell, cell 1 -300 mV, cell 2 -450 mV, Model 5020 guard cell -750 mV

CHROMATOGRAM
Retention time: 14.5
Internal standard: noroxymorphone (12)
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Rotshteyn,Y.; Weingarten,B. A highly sensitive assay for the simultaneous determination of morphine, morphine-3-glucuronide, and morphine-6-glucuronide in human plasma by high-performance liquid chromatography with electrochemical and fluorescence detection, *Ther.Drug Monit.*, **1996**, *18*, 179–188.

SAMPLE
Matrix: blood
Sample preparation: Activate a Sep-Pak SPE cartridge by rinsing successively with 10 mL MeOH, 5 mL MeCN:10 mM pH 2.1 NaH_2PO_4 buffer 25:75, and 5 mL water. 100 μL Plasma + 3 mL 500 mM pH 3 sodium bicarbonate/sodium carbonate buffer, mix, add to the SPE cartridge. Wash with 20 mL 5 mM pH 9.3 sodium bicarbonate/sodium carbonate buffer, 0.5 mL water,

and 0.35 mL MeCN:10mM pH 2.1 NaH_2PO_4 buffer 25:75, elute with an additional 0.8 mL of MeCN:10 mM pH 2.1 NaH_2PO_4 buffer 25:75, inject an aliquot of the eluate.

HPLC VARIABLES
Guard column: 15 × 3.2 C18
Column: 150 × 4.6 C18
Mobile phase: MeCN:0.8 mM 1-dodecylsulfate sodium in 10 mM pH 2.1 NaH_2PO_4 buffer 26.5:73.5
Flow rate: 0.8
Injection volume: 250
Detector: UV 210

CHROMATOGRAM
Retention time: 9.03

OTHER SUBSTANCES
Extracted: metabolites, morphine-3-β-D-glucuronide, morphine-6-β-D-glucuronide

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Wu,D.; Kang,Y.-S.; Bickel,U.; Pardridge,.M. Blood-brain barrier permeability to morphine-6-glucuronide is markedly reduced compared with morphine, *Drug Metab.Dispos.*, **1997**, *25*, 768–771.

SAMPLE
Matrix: blood
Sample preparation: Activate a Sep-Pak Light C18 SPE cartridge with 3 mL MeOH and 3 mL water. Mix 1 mL serum with 1 mL 500 mM pH 9.3 potassium carbonate. Add to the SPE cartridge, wash with 5 mL 5 mM pH 9.3 potassium carbonate and 250 μL water. Dry the SPE cartridge with air for 30 s, wash with 200 μL MeCN:30 mM pH 2 potassium phosphate buffer 16:84. Elute with 600 μL MeCN:30 mM pH 2 potassium phosphate buffer 16:84. Dilute the eluate with an equal volume of water, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 YMC ODS-AL
Mobile phase: Gradient. A. 3 mM formic acid in MeCN. B. 3 mM formic acid in water. A:B from 96:4 to 30:70 over 3.5 min (sic).
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: MS, Fisons VG Platform, electrospray, spray voltage 2.4 kV, sampling cone voltage 40 V, SIM m/z 286.2

CHROMATOGRAM
Retention time: 3.8
Limit of detection: 20 pg
Limit of quantitation: 840 pg/mL

OTHER SUBSTANCES
Extracted: metabolites (m/z 462.2)

KEY WORDS
serum; SPE

REFERENCE
Tyrefors,N.; Hyllbrant,B.; Ekman,L.; Johansson,M.; Långström,B. Determination of morphine, morphine-3-glucuronide and morphine-6-glucuronide in human serum by solid-phase extraction and liquid chromatography-mass spectrometry with electrospray ionisation, *J.Chromatogr.A*, **1996**, *729*, 279–285.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL 100 mg ethyl SPE cartridge (J.T.Baker) with 2 mL MeOH, 1 mL water, and 2 mL 1 mM pH 9.3 ammonium hydrogen carbonate buffer. Mix 1 mL serum with 200 μL 1 μg/mL IS in water. Add to the SPE cartridge, wash with 1 mL 1 mM pH 9.3 ammonium hydrogen carbonate buffer, elute with 1 mL MeOH. Evaporate the eluate to dryness, reconstitute the residue in 100 μL mobile phase, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 2.1 5 μm Supelcosil LC-Si (Supelco)
Mobile phase: MeCN:MeOH:water:formic acid 5.2:59.8:34.65:0.35
Flow rate: 0.23
Injection volume: 5
Detector: MS, API I MS single quadrupole, ionspray, capillary tip 5000 V, interface plate 650 V, source 60°, positive ion mode, SIM, m/z 286

CHROMATOGRAM
Retention time: 18.79
Internal standard: nalorphine (15.4)
Limit of quantitation: 4 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, codeine, diamorphine

KEY WORDS
serum; pharmacokinetics; SPE; mouse

REFERENCE
Zuccaro,P.; Ricciarello,R.; Pichini,S.; Pacifici,R.; Altieri,I.; Pellegrini,M.; D'Ascenzo,G. Simultaneous determination of heroin, 6-monoacetylmorphine, morphine, and its glucuronides by liquid chromatography-atmospheric pressure ionspray-mass spectrometry, *J.Anal.Toxicol.*, **1997**, *21*, 268–277.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Whole blood + 1 mL 1 M pH 9 borate buffer + 100 μL 1 μg/mL nalorphine + 5 mL ethyl acetate, vortex thoroughly, centrifuge, repeat the extraction. Combine the extracts and add them to 4 mL 50 mM sulfuric acid, extract. Discard the organic layer and saturate the aqueous layer with freshly ground ammonium carbonate, extract with 8 mL ethyl acetate, filter (phase-separating paper). Evaporate the organic layer and to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 ODS Hypersil
Mobile phase: MeCN:10 mM KH_2PO_4 20:80 containing 20 mM octanesulfonic acid, adjusted to pH 2.5 with orthophosphoric acid
Injection volume: 20
Detector: E, BAS LC-4B, TL-5A thin-layer flow cell +0.870 V

CHROMATOGRAM
Retention time: 9
Internal standard: nalorphine (19)
Limit of quantitation: 5 ng/mL

KEY WORDS
whole blood

REFERENCE
Logan,B.K.; Oliver,J.S.; Smith,H. The measurement and interpretation of morphine in blood, *Forensic Sci.Int.*, **1987**, *35*, 189–195.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elut SPE cartridge with 1 mL MeOH, 1 mL water, and 1 mL 100 mM pH 9.0 sodium bicarbonate buffer, do not allow to go dry. 100 μL Plasma + 20 μL 1 μg/mL nalorphine in MeOH:water 30:70 + 750 μL 100 mM pH 9.0 sodium bicarbonate buffer, vortex for 10 s, add to the SPE cartridge, wash with 1 mL 100 mM pH 9.0 sodium bicarbonate buffer, wash with 1 mL water, wash with 100 μL MeOH:water 50:50, dry under vacuum for 10 min, elute with three 250 μL aliquots of MeOH. Evaporate the eluate to dryness at 45° in a vacuum centrifuge for 1 h, reconstitute with 25 μL 100 mM pH 11.4 sodium carbonate or 25 μL pH 9.5 sodium bicarbonate, add 25 μL 1 mg/mL dansyl chloride in acetone, vortex, let stand in the dark at 45° for 20 min, add 250 μL toluene, vortex for 2 min, centrifuge at 12500 g for 1 min, inject a 100-200 μL aliquot of the upper organic layer.

HPLC VARIABLES
Guard column: 10 × 4.6 3 μm silica
Column: 150 × 4.6 3 μm Spherisorb 3CN
Mobile phase: n-Hexane:isopropanol:ammonia 95:5:0.25 (Place a silica column between the pump and the injector.)
Flow rate: 1.5
Injection volume: 100-200
Detector: F ex 340 em 500

CHROMATOGRAM
Retention time: 5.7
Internal standard: nalorphine (2.8)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: 6-acetylmorphine

KEY WORDS
plasma; derivatization; pharmacokinetics; SPE; normal phase

REFERENCE
Barrett,D.A.; Shaw,P.N.; Davis,S.S. Determination of morphine and 6-acetylmorphine in plasma by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1991**, *566*, 135–145.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL 500 mg Bond Elut C8 SPE cartridge with 5 mL MeOH, 3 mL MeCN:10 mM NaH_2PO_4 10:90, and 5 mL water. 1 mL Plasma + 0.5 μg nalorphine in water + 1 mL 500 mM ammonium sulfate, add to the SPE cartridge, wash with 6 mL 5 mM ammonium sulfate, elute with 1 mL MeCN:10 mM NaH_2PO_4 10:90, inject a 100 μL aliquot of the eluate.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:buffer 26:74 (Buffer was 10 mM NaH_2PO_4 containing 1 mM sodium dodecyl sulfate, pH adjusted to 2.1 with orthophosphoric acid.)
Flow rate: 1
Injection volume: 100
Detector: F ex 210 em 340

CHROMATOGRAM
Retention time: 13.5
Internal standard: nalorphine (22)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, glucuronides, normorphine

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Glare,P.A.; Walsh,T.D.; Pippenger,C.E. A simple, rapid method for the simultaneous determination of morphine and its principal metabolites in plasma using high-performance liquid chromatography and fluorometric detection, *Ther.Drug Monit.*, **1991**, *13*, 226–232.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Baxter C18 SPE cartridge with 3 mL MeOH and 3 mL water. 1 mL Plasma + 2 mL 500 mM pH 9.3 ammonium sulfate + 30 μL 1 μg/mL naltrexone, add to the SPE cartridge, wash with 3 mL 5 mM pH 9.3 ammonium sulfate, wash with 3 mL water, dry under vacuum, elute with 1 mL MeOH:triethylamine 99.5:0.5. Evaporate the eluate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: C18 (Upchurch)
Column: 100 × 3.2 5 μm Spherisorb C8
Mobile phase: MeOH:50 mM Na_2HPO_4 15:85 containing 3 mM 1-heptanesulfonic acid, pH adjusted to 3.5 with orthophosphoric acid
Flow rate: 0.8
Injection volume: 20
Detector: E, ESA Coulochem, guard cell + 650 mV, analytical cell +250 mV and +600 mV (monitored)

CHROMATOGRAM
Retention time: 5.2
Internal standard: naltrexone (16.4)
Limit of quantitation: 1.2 ng/mL

OTHER SUBSTANCES
Extracted: hydromorphone

KEY WORDS
plasma; SPE

REFERENCE
Bouquillon,A.I.; Freeman,D.; Moulin,D.E. Simultaneous solid-phase extraction and chromatographic analysis of morphine and hydromorphone in plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1992**, *577*, 354–357.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL 40 μm 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 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 200 × 4.5 5 μm LiChrosphere diol
Mobile phase: MeCN:50 mM NaH_2PO_4 80:20 pH adjusted to 3 with orthophosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 7

Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, glucuronides, normorphine, codeine

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: Rock 5 mL whole blood + 10 mL water + 8.5 mL Na_2WO_4 in a 50 mL stoppered tube for 1 min, add 6 mL $NiCl_2$, rock for 5 min, add 15 mL dichloromethane:isobutyl alcohol:THF 30:45:25, centrifuge at 2500 g for 15 min. Remove organic phase and repeat the process. Filter all organic phases through a 40-90 μm filter and evaporate to dryness in a 100 mL porcelain dish at a moderate temperature in a sand bath. Take up residue in 500 μL MeCN: water 80:20, inject a 20 μL aliquot. (Na_2WO_4 prepared by mixing 10 g $Na_2WO_4.2H_2O$ in 38 mL of 2 M NaOH and 2.5 g of $NaHCO_3$ and making up to 100 mL. $NiCl_2$ was 17% w/v $NiCl_2$ in water.)

HPLC VARIABLES
Column: 200 × 4.6 5 μm Hypersil C8
Mobile phase: A = MeCN; B = 20 mM n-propylamine adjusted to pH 5 with 85% phosphoric acid. A:B from 15:85 to 20:80 over 5 min to 45:55 over another 15 min to 65:35 over another 5 min
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 8
Limit of detection: 0.20 ppm

OTHER SUBSTANCES
Extracted: buprenorphine, caffeine, cocaine, codeine, diamorphine, ethylmorphine, lidocaine, methaqualone, 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: 100 μL Plasma + 200 μL ethyl p-hydroxybenzoate in MeOH, stir thoroughly, centrifuge at 15000 rpm for 10 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100RP-8(e)
Mobile phase: MeCN:buffer 23:77 (Buffer was 50 mM NaH_2PO_4 containing 5 mM sodium dodecylsulfonate, pH adjusted to 2.1 with phosphoric acid.)
Column temperature: 33
Flow rate: 1
Injection volume: 20

Detector: E, Shimadzu L-ECD-6A, +0.80 V

CHROMATOGRAM
Internal standard: ethyl p-hydroxybenzoate (F ex 280 em 330)
Limit of detection: 30 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, glucuronides (F ex 280 em 330)

KEY WORDS
pharmacokinetics; plasma

REFERENCE
Matsuzawa,T.; Wada,Y.; Shimoyama,M.; Nakajima,K.; Seki,T.; Sugibayashi,K.; Morimoto,Y. The effect of different routes of administration on the metabolism of morphine: The disposition of morphine and its metabolites after topical application, *Biopharm.Drug Dispos.*, **1994**, *15*, 665–678.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL 50 mg Bond Elut C8 SPE cartridge with 1 mL MeOH and 1 mL water. Add 200 μL buffer to the SPE cartridge, add 200 μL serum, add 200 μL buffer, wash with 1 mL buffer, elute with two 150 μL portions of MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 50 μL mobile phase or water, inject a 20 μL aliquot. (Buffer was 1 mM pH 9 ammonium bicarbonate.)

HPLC VARIABLES
Column: 250 × 8.4 Nucleosil 5C18
Mobile phase: Gradient. MeCN:25 mM tetraethylammonium phosphate buffer 1:99 for 8 min, 3:97 for 15 min (step gradient).
Flow rate: 1
Injection volume: 20
Detector: F ex 245 em 345

CHROMATOGRAM
Retention time: 6.97
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, glucuronides

KEY WORDS
serum; SPE

REFERENCE
Aderjan,R.; Hofmann,S.; Schmitt,G.; Skopp,G. Morphine and morphine glucuronides in serum of heroin consumers and in heroin-related deaths determined by HPLC with native fluorescence detection, *J.Anal.Toxicol.*, **1995**, *19*, 163–168.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Baker CN SPE cartridge with 2 column volumes of MeOH, 2 column volumes of water, and 1 mL 500 mM pH 9.3 diammonium sulfate. 600 μL Serum + 500 μL 500 mM pH 9.3 diammonium sulfate buffer, mix, add to the SPE cartridge, wash with 2 mL 50 mM pH 9.3 diammonium sulfate buffer, elute with 2 mL chloroform:isopropanol 90:10. Evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: Cp-Sper C8 (Chrompack)
Mobile phase: MeCN:10 mM pH 2.1 KH_2PO_4 11:89 containing 0.4 g/L heptanesulfonic acid

Flow rate: 2
Injection volume: 20
Detector: E, ESA, model 5010 analytical cell, detector 1 0.3 V, detector 2 0.4 V (monitored)

CHROMATOGRAM
Retention time: 3.5
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, glucuronides

KEY WORDS
serum; SPE; pharmacokinetics

REFERENCE
Koopman-Kimenai,P.M.; Vree,T.B.; Booij,L.H.D.J.; Hasenbros,M.A.W.M. Pharmacokinetics of epidurally administered nicomorphine with its metabolites and glucuronide conjugates in patients undergoing pulmonary surgery during combined epidural local anaesthetic block and general anaesthesia, *Biopharm.Drug Dispos.*, **1995**, *16*, 507–520.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 50 μL 50 μg/mL nalorphine hydrochloride, add to a Sep-Pak tC18 SPE cartridge, wash with 2 mL water, wash with 3 mL acetone:water 5:95, elute with 4 mL MeOH:water 70:30. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 TSK-gel ODS-120T (Tosoh)
Mobile phase: MeCN:buffer 25:75 (Buffer was 10 mM NaH_2PO_4 containing 1 mM sodium dodecyl sulfate, adjusted to pH 2.1 with phosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: E, Shimadzu L-ECD-6A, 0.8 V

CHROMATOGRAM
Internal standard: nalorphine
Limit of detection: 0.5 ng/mL (1 mL plasma)

KEY WORDS
plasma; rabbit; SPE; pharmacokinetics

REFERENCE
Matsumoto,Y.; Yamamoto,I.; Watanabe,Y.; Matsumoto,M. Enhancing effect of viscous sodium hyaluronate solution on the rectal absorption of morphine, *Biol.Pharm.Bull.*, **1995**, *18*, 1744–1749.

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 μL 1 μg/mL IS in 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 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil ABZ
Mobile phase: Gradient. MeOH:water from 15:85 to 60:40 over 10 min. (Convex gradient where MeOH% = -0.46exp(-x/1.18) + 0.6 where × = 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 286

CHROMATOGRAM
Retention time: 6.17
Internal standard: codeine (7.03 min, m/z 300), naltrexone (7.27 min, m/z 342)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; human; mouse; SPE; pharmacokinetics; LC-MS

REFERENCE
Pacifici,R.; Pichini,S.; Altieri,I.; Caronna,A.; Passa,A.R.; Zuccaro,P. High-performance liquid chromatographic-electrospray mass spectrometric determination of morphine and its 3- and 6-glucuronides: application to pharmacokinetic studies, *J.Chromatogr.B*, **1995**, *664*, 329–334.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 287

CHROMATOGRAM
Retention time: 3.32
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; bupren-

orphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, CSF

Sample preparation: Prepare 500 mg 3 mL Bond Elut C2 cartridges by rinsing with 2 mL MeOH then 2 mL 50 mM pH 7.5 Tris-HCl buffer. Apply 1 mL serum or CSF + 1 mL 50 mM pH 7.5 Tris-HCl buffer to the column and wash column with 10 mL 50 mM pH 7.5 Tris-HCl buffer. Elute with 2 mL 50% MeCN containing 0.1% trifluoroacetic acid. Freeze dry eluent or dry an aliquot at 40° under a stream of nitrogen, dissolve residue in 2560 μL mobile phase, inject 20-200 μL aliquot.

HPLC VARIABLES

Guard column: Hexyl

Column: 150 × 4.6 Spherisorb S5 C6

Mobile phase: Gradient. A 0.1% trifluoroacetic acid in water; B 0.1% trifluoroacetic acid in 40% MeCN. 16% B for 2 min then to 50% B over 10 min then to 100% B over 2 min, after 7 min return to original conditions over 2 min.

Flow rate: 1

Injection volume: 20-200

Detector: F ex 280 em 335

CHROMATOGRAM

Retention time: 8

Limit of detection: 0.87 ng/mL

OTHER SUBSTANCES

Extracted: codeine, normorphine, metabolites

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, CSF

Sample preparation: Condition 4 × 4 10 μm LiChrosphere 60 RP-select B SPE cartridge with buffer at 1 mL/min for 3 min and 0.5 mL/min for 4.5 min. Inject a 100-400 μL aliquot on to column A and elute to waste with buffer for 10 min, elute the contents of column A on to column B with the mobile phase and start the gradient. (Buffer was 100 mM ammonium sulfate adjusted to pH 9.3 with 25% ammonia in water.)

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrosphere 60 RP-select B
Column: 250 × 4 5 μm LiChrosphere 60 RP-select B
Mobile phase: Gradient. A was 200 mM pH 3.0 potassium phosphate buffer. B was MeCN:200 mM pH 3.0 potassium phosphate buffer 20:80. A:B from 80:20 to 40:60 over 12.5 min. After each run wash with MeCN:MeOH:water 40:40:20 for 6 min at 1.2 mL/min, re-equilibrate at initial conditions at 1.2 mL/min for 6 min.
Flow rate: 0.8
Injection volume: 100-400
Detector: F ex 210 em 350

CHROMATOGRAM
Retention time: 9.5
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, glucuronides

KEY WORDS
plasma; SPE

REFERENCE
Huwyler,J.; Rufer,S.; Kusters,E.; Drewe,J. Rapid and highly automated determination of morphine and morphine glucuronides in plasma by on-line solid-phase extraction and column liquid chromatography, *J.Chromatogr.B*, **1995**, *674*, 57–63.

SAMPLE
Matrix: blood, CSF, urine, vitreous humor
Sample preparation: Condition a 200 mg Bond Elut SPE cartridge with 1 mL MeOH, 1 mL water, and 2 mL buffer. Centrifuge 1.5 mL serum, CSF, urine, or vitreous humor at 14000 g for 5 min, vortex 1 mL supernatant with 2 mL buffer and 100 ng internal standard. Centrifuge at 5000 g for 10 min, slowly add 2 mL supernatant to the SPE cartidge, wash with 2 mL buffer, dry under vacuum for 5 min. Elute with 500 μL MeOH:500 mM acetic acid 90:10 under gravity. Dry the eluate under a stream of nitrogen, reconstitute in 100 μL mobile phase, centrifuge at 14000 g for 4 min, inject a 10-20 μL aliquot. (Prepare buffer by adjusting pH of 900 mL 960 mg/L ammonium carbonate to 9.3 with concentrated ammonium hydroxide and 1 M ammonium hydroxide, make up to 1 L.)

HPLC VARIABLES
Column: 125 × 3 4 μm Superspher RP 18
Mobile phase: MeCN:50 mM pH 3 ammonium formate buffer 5:95
Flow rate: 0.6 for 4 min, to 1.1 over 3 min, maintain at 1.1 for 10 min
Injection volume: 10-20
Detector: MS, Finnigan MAT SSQ 7000 single quadrupole, 100-500u, 10 V, positive ion, sheath gas nitrogen pressure 70 p.s.i., auxiliary gas nitrogen 20 mL/min; heated vaporizer temperature 400°, heated capillary temperature 170°, corona current, 5 μ A, m/z 286

CHROMATOGRAM
Retention time: 4.1
Internal standard: morphine-d3
Limit of detection: 500 pg/mL
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, codeine, 6-monoacetylmorphine

KEY WORDS
serum; SPE

REFERENCE
Bogusz,M.J.; Maier,R.-D.; Erkens,M.; Driessen,S. Determination of morphine and its 3- and 6-glucuronides, codeine, codeine glucuronide and 6-monoacetylmorphine in body fluids by liquid chromatography atmospheric pressure chemical ionization mass spectrometry, *J.Chromatogr.B*, **1997**, *703*, 115–127.

SAMPLE
Matrix: blood, tissue
Sample preparation: 50-100 mg Brain tissue + 2.6 ng/mL nalbuphine hydrochloride in MeOH, homogenize (PTFE pestle), sonicate (Soniprep 150) at 23 μm amplitude for 1 min, centrifuge at 4° at 15000 g for 30 min. Evaporate the supernatant in a centrifugal evaporator, reconstitute with 3 mL 10 mM di-(2-ethylhexyl)phosphoric acid in ethyl acetate, add 1 mL 50 mM pH 7.0 sodium phosphate buffer, wash, centrifuge at 4° at 3000 g for 10 min. Remove 2.8 mL of the organic phase and add it to 500 μL 170 mM orthophosphoric acid, vortex for 30 s, centrifuge at 4° at 3000 g for 10 min. Remove the aqueous phase and adjust the pH to 2.5-3.0 with 24 μL 11.2 M ammonia solution, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: SCX Newguard
Column: 250 × 4.6 10 μm Partisil 10SCX
Mobile phase: MeCN:buffer 15:85 (Buffer was 76 mM orthophosphoric acid adjusted to pH 3.0 with ammonia.)
Column temperature: 28
Flow rate: 3
Injection volume: 100
Detector: E, Bioanalytical Systems, TL-5A glassy carbon working electrode +0.95 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 4.5
Internal standard: nalbuphine (6)
Limit of detection: 1.3 ng

KEY WORDS
rat; plasma; brain; pharmacokinetics

REFERENCE
Borg,P.J.; Sitaram,B.R.; Taylor,D.A. Ion-pair extraction and liquid chromatographic analysis of morphine in rat brain and plasma, *J.Chromatogr.*, **1993**, *621*, 165–172.

SAMPLE
Matrix: blood, urine
Sample preparation: 10 mL Serum, plasma, whole blood, or urine + 10 mL nalorphine in water + 25 mL saturated ammonium sulfate solution + 500 μL concentrated HCl, heat at 120° for 30 min, filter (Whatman No. 1 paper), adjust the pH of the filtrate to 9.0 with 25% NaOH, extract with 125 mL chloroform:isopropanol 80:20, repeat extraction with 50 mL chloroform:isopropanol 80:20. Combine the organic phases and wash them twice with 15 mL portions of 50 mM sodium borate solution, extract the organic phase twice with 10 mL portions of 1 M sulfuric acid. Combine the aqueous extracts and add 4 mL saturated ammonium sulfate, adjust pH to 9.0 with 25% NaOH, extract twice with 5 mL portions of chloroform:isopropanol 80:20. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 50 μL water, add 100 μL 0.1% dansyl chloride in acetone, add 50 μL 200 mM sodium carbonate, let stand at room temperature in the dark for 3 h, add 1 mL toluene, vortex for 2 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.5 3 μm Spherisorb S3W silica
Mobile phase: n-Hexane:isopropanol:ammonia 97:2.7:0.3
Flow rate: 1.5
Injection volume: 20
Detector: F ex 330-380 (filters) em 410-500 (filters)

CHROMATOGRAM
Retention time: 9.5
Internal standard: nalorphine (5)
Limit of detection: 0.2 pmole

OTHER SUBSTANCES
Noninterfering: acetaminophen, amitriptyline, amphetamine, atropine, benzoylecgonine, caffeine, carbamazepine, carisoprodol, chlorpromazine, chlorprothixene, cimetidine, cocaine, codeine, cyclizine, dextromethorphan, diazepam, dihydrocodeine, diphenoxilate, disopyramide, doxepin, doxylamine, emetine, erythromycin, ethylmorphine, flurazepam, glutethimide, hydrocodone, hydrocortisone, hydromorphone, hydroxyzine, imipramine, lidocaine, loxapine, meperidine, metapyrilene, methadone, methamphetamine, methocarbamol, methylphenidate, naloxone, nicotine, nordiazepam, nortriptyline, orphenadrine, oxycodone, papaverine, pentazocine, phenacetin, phencyclidine, phenmetrazine, phenolphthalein, phentermine, phenytoin, prazepam, procainamide, propoxyphene, propranolol, protriptyline, pyrilamine, quinine, spironolactone, strychnine, terpin hydrate, thioridazine, thiothixene, triamterene, trifluoperazine, triflupromazine, trihexyphenidyl, trimethoprim, trimetobenzamide, tripelennamine

KEY WORDS
derivatization; serum; plasma; whole blood; normal phase

REFERENCE
Tagliaro,F.; Frigerio,A.; Dorizzi,R.; Lubli,G.; Marigo,M. Liquid chromatography with pre-column dansyl derivatisation and fluorimetric detection applied to the assay of morphine in biological samples, *J.Chromatogr.*, **1985**, *330*, 323–331.

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 μL MeCN:30 mM pH 2.1 potassium phosphate buffer 15:85 at 0.75 mL/min, elute with 600 μL 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 μL MeCN:30 mM pH 2.1 potassium phosphate buffer 15:85 at 0.75 mL/min, elute with 600 μL MeCN:30 mM pH 2.1 potassium phosphate buffer 15:85 at 0.75 mL/min, inject a 400 μL aliquot of the eluate.

HPLC VARIABLES
Column: 100 × 4 3 μm 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: E, ESA Model 5100 A Coulochem, Model 5010 detector cell, first electrode 0.25 V, second electrode 0.35 V

CHROMATOGRAM
Retention time: 8
Limit of detection: 0.5 nM

OTHER SUBSTANCES
Extracted: metabolites, codeine (UV 214), norcodeine (UV 214), normorphine

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 μL Plasma or serum + 100 μL MeOH + 200 μL MeCN + 100 μL buffer, vortex for 1 min, centrifuge at 4000 rpm for 15 min, add the supernatant to the SPE cartridge, wash with two 3 mL portions of water, dry under vacuum for 10 min, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 100 μL 2.5 μg/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 μL Urine + 100 μL MeOH + 200 μL MeCN + 500 μL buffer, vortex for 1 min, centrifuge at 2000 rpm for 5 min, add the supernatant to the SPE cartridge, wash with two 3 mL portions of water, dry under vacuum for 10 min, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 100 μL 2.5 μg/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 × 4.6 5 μm Adsorbosphere HS C18
Mobile phase: MeCN:MeOH:1.2% ammonium acetate 15:40:45
Flow rate: 0.8
Detector: UV 239

CHROMATOGRAM

Retention time: 5.6
Internal standard: flufenamic acid (24.39)
Limit of quantitation: 300 ng/mL (urine), 100 ng/mL (plasma, serum)

OTHER SUBSTANCES

Extracted: codeine, monoacetylmorphine, 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: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 211.1

CHROMATOGRAM
Retention time: 3.315

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Dilute with mobile phase, filter (0.45 μm)

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak 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: 4

OTHER SUBSTANCES
Simultaneous: codeine, meconic acid, O^6-codeine methyl ether, α-codeimethine

REFERENCE
Ayyangar,N.R.; Bhide,S.R.; Kalkote,U.R. Assay of semi-synthetic codeine base with simultaneous determination of α-codeimethine and O6-codeine methyl ether as by-product impurities by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *519*, 250–255.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 750 μg/mL solution in 10 mM pH 2.5 orthophosphoric acid, sonicate for 10 min, filter (0.2 μm), inject a 15 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100
Column: 125 × 4 3 μm Spherisorb ODS-1
Mobile phase: Gradient. A was water containing 5 mL/L 85% orthophosphoric acid and 0.56 mL/L hexylamine. B was MeCN:water 90:10 containing 5 mL/L 85% orthophosphoric acid and 0.56 mL/L hexylamine. A:B from 91:9 to 86:14 over 4 min, maintain at 86:14 for 13 min, to 55:45 over 11 min, maintain at 55:45 for 8 min, re-equilibrate at initial conditions for 20 min.
Flow rate: 0.7
Injection volume: 15
Detector: UV 210

CHROMATOGRAM
Retention time: 2.9

OTHER SUBSTANCES
Simultaneous: acetaminophen, acetylcodeine, benzocaine, caffeine, cocaine, codeine, diamorphine, lidocaine, 6-monoacetylmorphine, 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: Bulk. Weigh out 100 mg, dissolve in 25 mL MeOH:water:acetic acid 24:72:1, dilute with MeOH:water:acetic acid 24:72:1 to a final concentration of 240 μg/mL, filter (0.45 μm), inject a 20 μL aliquot. Injections. Dilute with MeOH:water:acetic acid 24:72:1 to a final concentration of 240 μg/mL, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:5 mM sodium 1-heptanesulfonate:acetic acid 24:72:1
Flow rate: 1.5
Injection volume: 20
Detector: UV 284

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: phenol, 2-mercaptobenzothiazole (UV 230), pseudomorphine (UV 230)

KEY WORDS
injections

REFERENCE
Bello,A.C.; Jhangiani,R.K. Liquid chromatographic determination of morphine sulfate and some contaminants in injections and bulk drug material: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 1046–1048.

SAMPLE
Matrix: formulations
Sample preparation: Dilute a 5% bupivacaine hydrochloride injection and 25 mg/mL morphine sulfate injection with 0.9% NaCl injections to a bupivacaine concentration of 625 μg/mL and a morphine concentration of 100 μg/mL, inject a 15 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeCN:50 mM pH 7.5 phosphate buffer 25:75
Flow rate: 1
Injection volume: 15
Detector: UV 280

CHROMATOGRAM
Retention time: 2.2

OTHER SUBSTANCES
Simultaneous: degradation products
Noninterfering: bupivacaine

KEY WORDS
injections; stability-indicating

REFERENCE
Johnson,C.E.; Christen,C.; Perez,M.M.; Ma,M. Compatibility of bupivacaine hydrochloride and morphine sulfate, *Am.J.Health-Syst.Pharm.*, **1997**, *54*, 61–64.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with MeOH:water 500 mM pH 7 sodium borate 35:65:2, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Whatman PXS ODS-3 C18
Mobile phase: MeCN:MeOH:water:85% phosphoric acid: sodium octanesulfonate 7.5:12.5:80:0.5:0.108 (v/v/v/v/w)
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

OTHER SUBSTANCES
Simultaneous: milrinone, phenol

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: Filter (0.22 μm), dilute to 25 μg/mL with mobile phase, inject a 15 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:50 mM pH 7.5 phosphate buffer 12:88
Flow rate: 1
Injection volume: 15
Detector: UV 280

CHROMATOGRAM
Retention time: 3.2

OTHER SUBSTANCES
Noninterfering: midazolam

KEY WORDS
stability-indicating; 5% dextrose; injections

REFERENCE
Johnson,C.E.; Bhatt-Mehta,V.; Mancari,S.C.; McKown,J.A. Stability of midazolam hydrochloride and morphine sulfate during simulated intravenous coadministration, *Am.J.Hosp.Pharm.*, **1994**, *51*, 2812–2815.

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 KH_2PO_4 50:50, pH adjusted to 5.40 with 1 M NaOH
Flow rate: 1.5
Injection volume: 10
Detector: UV 216

CHROMATOGRAM
Retention time: 6.7

OTHER SUBSTANCES
Simultaneous: hydromorphone, ondansetron

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 βC, *Am.J.Hosp.Pharm.*, **1994**, *51*, 2138–2142.

SAMPLE
Matrix: formulations
Sample preparation: Dilute formulation 1:100 with water, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 5 × 4 35-60 μm Perisorb RP18
Column: 250 × 4 10 μm LiChrosorb RP18
Mobile phase: MeOH:MeCN:2.72 g/L KH_2PO_4 12:2:86
Injection volume: 50
Detector: UV 220

CHROMATOGRAM
Retention time: 6.52

KEY WORDS
injections; water

REFERENCE
Sadjak,A.; Wintersteiger,R. Compatibility of morphine, baclofen, floxuridine and fluorouracil in an implantable medication pump, *Arzneimittelforschung*, **1995**, *45*, 93–98.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: MeCN:20 mM KH_2PO_4 adjusted to pH 6.0 with 1 M KOH 50:50
Flow rate: 1
Injection volume: 20
Detector: UV 235

CHROMATOGRAM
Retention time: 6.6
Limit of detection: 258 ng/mL

OTHER SUBSTANCES
Simultaneous: hydromorphone, bupivacaine

KEY WORDS
saline; injections

REFERENCE
Venkateshwaran,T.G.; Stewart,J.T. HPLC determination of morphine-hydromorphone-bupivacaine and morphine-hydromorphone-tetracaine mixtures in 0.9% sodium chloride injection, *J.Liq.Chromatogr.*, **1995**, *18*, 565–578.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm cyano
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 1
Injection volume: 20
Detector: UV 300

CHROMATOGRAM
Retention time: 3.70

OTHER SUBSTANCES
Simultaneous: granisetron, mechlorethamine (UV 200)

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: hair
Sample preparation: Incubate 20-200 mg hair in 2 mL 250 mM HCL at 45° overnight. Extract and neutralize with a commercial liquid-liquid method (Toxi-Tubes A, Analytical systems, Laguna Hills). Reconstitute the residue with mobile phase A, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 3 μm C18 (NBS, ESA)
Mobile phase: Gradient. A:B 93:7 for 11.5 min, to 50:50 over 8 min, maintain at 50:50 for 5 min, to 10:90 over 10 min, maintain at 10:90 for 15.5 min, re-equilibrate at initial conditions for 7.5 min. A was MeCN:20 mM pH 7.0 monobasic sodium phosphate 10:90. B was MeCN:20 mM pH 7.0 monobasic sodium phosphate 50:50.
Column temperature: 37
Flow rate: 0.8
Injection volume: 10
Detector: E, Two ESA CoulArray modules, each containing four electrochemical cells, 450 mV at electrode 1, 550 mV at electrode 2, 650 mV at electrode 3, 750 mV at electrode 4, 850 mV at electrode 5, 900 mV at electrode 6, 950 mV at electrode 7, 1000 mV at electrode 8, solid-state palladium reference electrode built in the coulometric cells. Increase all cell potentials to 1200 for 60 s at the end of each analysis. Allow the electrodes to stabilize for 7.5 min before the next injection.

CHROMATOGRAM
Retention time: 4.66
Limit of detection: 17 pg

OTHER SUBSTANCES
Simultaneous: 6-acetylmorphine, 3,4-methylenedioxymetamphenamine, buprenorphine, cocaine, codeine, diamorphine, dihydrocodeine, ethylmorphine, hydrocodone, lidocaine, methadone, naloxone, procaine, thebaine

KEY WORDS
hair

REFERENCE
Achilli,G.; Cellerino,G.P.; Melzi d'Eril,G.V.; Tagliaro,F. Determination of illicit drugs and related substances by high-performance liquid chromatography with an electrochemical coulometric-array detector, *J.Chromatogr.A*, **1996**, *729*, 273–277.

SAMPLE
Matrix: hair
Sample preparation: Wash 100-200 mg hair with 10 mL ethyl ether and 12 mL 10 mM HCl, add 3 mL 100 mM HCl, heat at 45° for 12 h, neutralize with 100 μL 3 M NaOH, add to an extraction tube containing sodium carbonate, sodium bicarbonate, and organic solvents (Toxi-Tubes A, Analytical Systems), add 2 mL water, vortex for 2 min, centrifuge at 759 g for 10 min, remove the organic layer, add dichloromethane:dichloroethane:heptane 18:18:64 to the aqueous layer, extract. Combine the organic layers and evaporate them to dryness, reconstitute with 50 μL water, add 50 μL 1 mg/mL dansyl chloride in acetone, add 50 μL 100 mM sodium carbonate, let stand in the dark at room temperature for at least 1.5 h, add 1 mL toluene, vortex for 2 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb silica
Mobile phase: Hexane:isopropanol:ammonia 95:4.5:0.5
Flow rate: 2
Injection volume: 20
Detector: F ex 330-380 (bandpass filter) em 410-500 (bandpass filter)

CHROMATOGRAM
Retention time: 13
Limit of detection: 60 pg

OTHER SUBSTANCES
Noninterfering: codeine

KEY WORDS
derivatization; derivatization at C3 hydroxy group; normal phase

REFERENCE
Marigo,M.; Tagliaro,F.; Poiesi,C.; Lafisca,S.; Neri,C. Determination of morphine in the hair of heroin addicts by high performance liquid chromatography with fluorimetric detection, *J.Anal.Toxicol.*, **1986**, *10*, 158–161.

SAMPLE
Matrix: meconium
Sample preparation: 500 mg Meconium + 5 mL water + 1 drop 500 mM HCl, vortex for 1 min, sonicate for 5 min, vortex for 1 min, centrifuge at 2683 g for 10 min. Remove the supernatant and add it to 5 mL water and 1 drop 500 mM HCl, vortex for 1 min, sonicate for 5 min, vortex for 1 min, centrifuge at 2683 g for 10 min. Make up the supernatant to 20 mL with pH 9.0 borax buffer, add it to an Extrelut SPE cartridge, after 10 min elute with 60 mL dichloromethane:isopropanol 80:20. Add 1 drop 2% tartaric acid in water and evaporate the eluate under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-18 DB
Mobile phase: MeOH:MeCN:THF:triethylamine:water 100:25:7:1.5:600 containing 77 mM KH_2PO_4
Flow rate: 1
Injection volume: 20
Detector: UV 204

CHROMATOGRAM
Retention time: k' 1.15
Limit of detection: 500 ng/g

OTHER SUBSTANCES
Extracted: amphetamine
Noninterfering: caffeine, benzoylecgonine, cocaine, codeine

KEY WORDS
SPE

REFERENCE
Franssen,R.M.E.; Stolk,L.M.L.; van den Brand,W.; Smit,B.J. Analysis of morphine and amphetamine in meconium with immunoassay and HPLC-diode-array detection, *J.Anal.Toxicol.*, **1994**, *18*, 294–295.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Prepare a 1 mL 100 mg C18 Bond Elut by washing with 1 mL MeOH, 1 mL water, 1 mL 5 mM pH 9.0 carbonate buffer. Mix 100 μL microsomal incubation, 20 μL 25 μg/mL 10,11-dihydrocarbamazepine in MeCN:water 25:75, 600 μL 200 mM pH 10.2 carbonate buffer, 80 μL 20 mM tetrabutylammonium hydrogen sulfate in water with vortex mixing after each addition. Add to 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 × 2 Phase Separations pellicular ODS
Column: 250 × 4.6 5μm 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: 11
Internal standard: 10,11-dihydrocarbamazepine
Limit of detection: 250 pg/mL

OTHER SUBSTANCES
Simultaneous: codeine, norcodeine, metabolites

KEY WORDS
SPE

REFERENCE
Pawula,M.; Shaw,P.N.; Barrett,D.A. Determination of codeine and its metabolites in mirosomal incubates by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *653*, 106–111.

SAMPLE
Matrix: perfusate
Sample preparation: 30 μL Perfusate (artificial CSF) + 10 μL 200 mM perchloric acid. Mix a 25 μL aliquot with 12.5 μL reagent, let stand for 2 min, inject an aliquot. (Prepare a stock solution by dissolving 27 mg o-phthalaldehyde in 1 mL MeOH, add 5 μL β-mercaptoethanol, add 9 mL 100 mM pH 9.3 sodium tetraborate containing 10 μM EDTA. This solution is good for 5 days in a sealed amber bottle at room temperature. Prepare the working reagent by diluting 1 mL of the stock solution with 3 mL 100 mM pH 9.3 sodium tetraborate containing 10 μM EDTA, allow to stand for 24 h before use.)

HPLC VARIABLES
Column: two columns 150 × 4.6 5 μm M.S. Gel C18 (ESA)
Mobile phase: MeOH:buffer 8:92 adjusted to pH 3.0 with phosphoric acid (Buffer was 54 mM NaH_2PO_4 containing 1.24 mM sodium heptanesulfonate.)
Column temperature: 33
Flow rate: 1.2
Detector: E, ESA Coulochem Electrode Array System Model 5500, detector temp 33°, oxidation potential 280 mV

CHROMATOGRAM
Retention time: 11.10

Limit of quantitation: 0.5 ng/mL

OTHER SUBSTANCES
Extracted: apomorphine, dopamine, hydralazine, isoproterenol, methoxamine, norepinephrine, phenylephrine

KEY WORDS
rat; derivatization

REFERENCE
Acworth,I.N.; Yu,J.; Ryan,E.; Gariepy,K.C.; Gamache,P.; Hull,K.; Maher,T. Simultaneous measurement of monoamine, amino acid, and drug levels, using high performance liquid chromatography and coulometric array technology: application to in vivo microdialysis perfusate analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 685–705.

SAMPLE
Matrix: perfusate, urine
Sample preparation: Perfusate. Add 200 μL 1 μg/mL IS and 500μL 200 mM pH 9.0 potassium buffer to 500 μL perfusate, add 6 mL chloroform:n-butyl alcohol 80:20, rotate at 33 rpm for 10 min, centrifuge at 1800 g for 10 min. Add 200 μL 0.05% sulfuric acid to 5 mL organic phase. Mix for 10 min and centrifuge at 1800 g for 10 min. Inject a 100 μL aliquot of the acidic phase. (Caution! Chloroform is a carcinogen!) Urine. Directly inject a 100 μL aliquot of diluted urine.

HPLC VARIABLES
Guard column: Nova-Pak C18 Guard-Pak
Column: 4 μm Nova-Pak C18 Radial-Pak cartridge
Mobile phase: MeCN:MeOH:70 mM pH 3.0 potassium dihydrogen orthophosphate buffer 1.5:1:97.5
Flow rate: 1
Injection volume: 100
Detector: UV 210

CHROMATOGRAM
Retention time: 6.2
Internal standard: normorphine (4.9)
Limit of quantitation: 13 pM (perfusate), 26 pM (urine)

KEY WORDS
pharmacokinetics; rat; kidney

REFERENCE
Disposition of morphine in the rat isolated perfused kidney: Concentration ranging studies, *J.Pharmacol.Exp.Ther.*, **1997**, *282*, 1518–1525.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Adsorbosphere C18 (Alltech)
Mobile phase: MeCN:water:20 mM pH 3.0 KH_2PO_4 25:55:20, containing 10 μL triethylamine per 100 mL
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 6.4

OTHER SUBSTANCES
Also analyzed: codeine, diamorphine, fentanyl, meperidine

REFERENCE

Lichtman,A.H.; Meng,Y.; Martin,B.R. Inhalation exposure to volatilized opioids produces antinociception in mice, *J.Pharmacol.Exp.Ther.*, **1996**, *279*, 69–76.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 50 μL aliquot.

HPLC VARIABLES

Column: Et 280/4 Nucleosil 100 C18
Mobile phase: 1 M triethylammonium phosphate buffer (Fluka):water 1:40
Injection volume: 50
Detector: F ex 220 em 340

OTHER SUBSTANCES

Simultaneous: morphine glucuronides

REFERENCE

Skopp,G.; Lutz,R.; Pötsch,L.; Ganβmann,B.; Klinder,K.; Schmidt,A.; Aderjan,R.; Mattern,R. An in vitro experiment for postmortem vascular permeation. The passage of morphine and morphine glucuronides across a vascular wall, *J.Forensic Sci.*, **1997**, *42*, 486–491.

SAMPLE

Matrix: solutions
Sample preparation: Weigh 35.5 mg morphine sulfate and 15.0 mg ondansetron hydrochloride in a 10 mL volumetric flask, add 0.9% sodium chloride, shake vigorously for 2 min, add 0.9% sodium chloride to volume. Dilute 1:5, 1:7.5, and 1:20, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 220 × 4.6 5 μm underivatized silica (Brownlee Silica Applied Biosystems, Inc.,San Jose)
Mobile phase: MeOH:10 mM pH 4.0 aqueous monobasic potassium phosphate (adjusted with 10% phosphoric acid) 40:60
Flow rate: 1
Injection volume: 20
Detector: UV 233

CHROMATOGRAM

Retention time: 6.7
Limit of detection: 210 ng/mL

OTHER SUBSTANCES

Simultaneous: ondansetron

REFERENCE

Venkateshwaran,T.G.; Stewart,J.T.; King,D.T. HPLC determination of morphine-ondansetron and meperidine-ondansetron mixtures in 0.9% sodium chloride injection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1329–1338.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm IB-SIL C8 (Phenomenex)
Mobile phase: MeCN:20 mM sodium dihydrogen phosphate 40:60
Flow rate: 1
Injection volume: 20
Detector: UV 285

CHROMATOGRAM

Retention time: 4.8

OTHER SUBSTANCES
Simultaneous: doxorubicin (7.3)

REFERENCE
Zhang,H.; Ye,L.; Stewart,J.T. HPLC determinations of doxorubicin with selected medications in 0.9% sodium chloride injection USP, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 2375–2385.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 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 1.5 μA. The mixture flowed through a 20 s reaction coil (3.9 m (?) × 0.33 mm ID) to the detector.

CHROMATOGRAM
Retention time: 4
Limit of detection: 0.4 ng

OTHER SUBSTANCES
Simultaneous: codeine, 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 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: 3.03

OTHER SUBSTANCES
Simultaneous: ethoheptazine, morphine-3-glucuronide, pholcodeine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine, pemoline, benzphetamine, diethylpropion, mazindol, tranylcypromine, caffeine, fenethyline, phendimetrazine, methylphenidate, phenelzine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine, norpipanone, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine

Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: prolintane 2-phenethylamine, morphine-N-oxide, codeine, codeine-N-oxide

REFERENCE

Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 500 μg/mL solution in MeOH:water 50:50, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Zorbax C8
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L MeCN:water 20:80. A:B from 100:0 to 0:100 over 30 min. (Purify triethylamine as follows. Wash neutral alumina (Merck) 3 times with 2 bed volumes of pentane, 3 times with 2 bed volumes of dichloromethane, and 3 times with 2 bed volumes of MeOH, allow solvent to evaporate in a fume hood overnight, heat alumina at 130° for 2 h. Prepare a 14 cm column of the washed alumina in a 290 × 22 tube, pass through a head volume of MeOH, pass through triethylamine. When triethylamine starts to elute discard the first 20 mL, use the next 20 mL, discard the column.)
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM

Retention time: 6.5

OTHER SUBSTANCES

Simultaneous: acetophenone, amphetamine, desipramine, ethylmorphine, imipramine, mefenamic acid, methamphetamine, phenylbutazone, salicylic acid

KEY WORDS

also details of isocratic elution

REFERENCE

Hill,D.W. Evaluation of alkyl bonded silica and solvent phase modifiers for the efficient elution of basic drugs on HPLC, *J.Liq.Chromatogr.*, **1990**, *13*, 3147–3175.

SAMPLE

Matrix: solutions
Sample preparation: 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: 16.5
Limit of detection: 3.8 ng/mL

OTHER SUBSTANCES

Simultaneous: buprenorphine, butorphanol, ethylmorphine, codeine, nalbuphine, nalorphine, meperidine, tramadol, fentanyl
Noninterfering: thiopentone, succinylcholine, pancuronium, diazepam, atropine, neostigmine

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: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 5.60 (A), 3.20 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, 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

details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE

Matrix: urine
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH, 3 mL MeCN: 10 mM ammonium acetate 40:60 adjusted to pH 3 with acetic acid, and 5 mL water. 5 mL

Urine + 5 mL 500 mM ammonium acetate, adjusted to pH 9.5 with ammonia, mix, add to the SPE cartridge, wash with 20 mL 5 mM pH 9.5 ammonium acetate, wash with 0.5 mL water. Elute with 2 mL MeCN:10 mM ammonium acetate 40:60 adjusted to pH 3 with acetic acid, inject a 50 μL aliquot of the eluate.

HPLC VARIABLES

Column: 150 × 4.6 L-column ODS (Chemical Inspection & Testing Institute, Tokyo)
Mobile phase: Gradient. MeCN:100 mM ammonium acetate 0:100 for 1 min, to 40:60 over 20 min.
Flow rate: 1
Injection volume: 50
Detector: UV 210; MS Shimadzu model QP-1100EX thermospray, vaporizer temperature from 170 to 150° over 20 min. SIM, m/z 286

CHROMATOGRAM

Retention time: 13
Limit of detection: 2–40 ng/mL

OTHER SUBSTANCES

Extracted: 6-acetylmorphine, amphetamine, benzoylecgonine, cocaine, ephedrine, methamphetamine, methylephedrine, morphine-3-glucuronide, morphine-6-glucuronide

KEY WORDS

SPE

REFERENCE

Tatsuno,M.; Nishikawa,M.; Katagi,M.; Tsuchihashi,H. Simultaneous determination of illicit drugs in human urine by liquid chromatography-mass spectrometry, *J.Anal.Toxicol.*, **1996**, *20*, 281–286.

SAMPLE

Matrix: urine
Sample preparation: Pack a 110 × 10 mm polypropylene disposable column having support frits with 830 mg aldehyde-activated silica (Clifmar Associates, UK), wash the packed column with 50 mL phosphate buffered saline. Add 5 mL phosphate buffered saline to the column, add 500 μL unpurified antisera (J.Pharm.Biomed.Anal. 1994, 12, 353), close the column, place on a rotamixer for 2 h. Wash the immunocolumn with 10 mL phosphate buffered saline, carefully add 5 mL 1 M pH 6 glycine buffer, rotate the column overnight, wash with 10 mL 0.3% HCl and 20 mL phosphate buffered saline. Dilute 100 μL urine with 900 μL phosphate buffered saline, add 1 mL of the diluted urine to the immunocolumn, wash with 15-20 mL phosphate buffered saline, elute with two 1 mL portions of EtOH:phosphate buffered saline 40:60 (pH 4), collect the second eluted fraction, inject an aliquot of the fraction. (Phosphate buffered saline (pH 7.2-7.4) was 8 g NaCl, 200 mg KH_2PO_4, 200 mg KCl, and 2.9 g K_2HPO_4 dissolved in 1 L water.)

HPLC VARIABLES

Column: 250 × 5 5 μm Hypersil CPS (Jones Chromatography, UK)
Mobile phase: MeCN:65 mM pH 2.5 phosphate buffer containing 1.5 mM sodium lauryl sulfate 13:87
Flow rate: 1
Injection volume: 100
Detector: E, ESA Coulochem model 5100A, +450 mV

CHROMATOGRAM

Retention time: 8

KEY WORDS

immunoaffinity; SPE

REFERENCE

Rashid,B.A.; Aherne,G.W.; Katmeh,M.F.; Kwasowski,P.; Stevenson,D. Determination of morphine in urine by solid-phase immunoextraction and high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.A*, **1998**, *797*, 245–250.

SAMPLE

Matrix: urine

Sample preparation: 500 μL Urine + N-ethylnordiazepam + chlorpheniramine + 100 μL buffer, centrifuge at 11000 g for 30 s, inject a 500 μL aliquot onto column A with mobile phase A, after 0.6 min backflush column A with mobile phase A to waste for 1.6 min, elute column A with 250 μL mobile phase B, with 200 μL mobile phase C, and with 1.15 mL mobile phase D. Elute column A to waste until drugs start to emerge then elute onto column B. Elute column B to waste until drugs started to emerge, then elute onto column C. When all the drugs have emerged from column B remove it from the circuit, elute column C with mobile phase D, monitor the effluent from column C. Flush column A with 7 mL mobile phase E, with mobile phase D, and mobile phase A. Flush column B with 5 mL mobile phase E then with mobile phase D. (Buffer was 6 M ammonium acetate adjusted to pH 8.0 with 2 M KOH.)

HPLC VARIABLES

Column: A 10 × 2.1 12-20 μm PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 × 3.2 11 μm Aminex A-28 (Bio-Rad); C 25 × 3.2 5 μm C8 (Phenomenex) + 150 × 4.6 5 μm silica (Macherey-Nagel)

Mobile phase: A 0.1% pH 8.0 potassium borate buffer; B 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid; C MeCN:buffer 40:60 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); D MeCN:buffer 33:67 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); E MeCN:buffer 70:30 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)

Column temperature: ambient (column A), 40 (columns B and C)

Flow rate: A 5; B-E 1

Injection volume: 500

Detector: UV 210, UV 235

CHROMATOGRAM

Retention time: k' 5.0

Internal standard: N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)

Limit of detection: 300 ng/mL

OTHER SUBSTANCES

Extracted: caffeine, cotinine, benzoylecgonine, secobarbital, oxazepam, phenobarbital, nordiazepam, diazepam, phenylpropanolamine, phentermine, amphetamine, phenmetrazine, lidocaine, ephedrine, pentazocine, methamphetamine, desipramine, nortriptyline, diphenhydramine, methadone, imipramine, flurazepam, amitriptyline, codeine, hydromorphone, hydrocodone

KEY WORDS

column-switching

REFERENCE

Binder,S.R.; Regalia,M.; Biaggi-McEachern,M.; Mazhar,M. Automated liquid chromatographic analysis of drugs in urine by on-line sample cleanup and isocratic multi-column separation, *J.Chromatogr.*, **1989**, *473*, 325–341.

SAMPLE

Matrix: urine

Sample preparation: 10 mL Urine + 500 μL 100 μg/mL nalorphine hydrobromide in MeOH + 1 mL concentrated HCl, heat at 100° for 1 h, cool, add 500 μL 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 μL 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 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm Lichrosorb
Column: 250 × 4 5 μm 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: 14
Internal standard: nalorphine (6)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: codeine
Noninterfering: acetaminophen, aspirin, amitriptyline, buprenorphine, caffeine, carbamazepine, chlorpromazine, desipramine, dextromethorphan, doxepin, ephedrine, fenfluramine, imipramine, lidocaine, loxapine, meperidine, methadone, methaqualone, naloxone, naltrexone, nicotine, orphenadrine, oxycodone, papaverine, pentazocine, phendimetrazine, phenmetrazine, phentermine, phenylpropanolamine, phenytoin, primidone, procaine, promethazine, propoxyphene, propyphenazone, theobromine, theophylline, trazodone, trifluprom azine, trimethoprim, trimipramine

KEY WORDS
SPE; normal phase

REFERENCE
Ferrara,S.D.; Tedeschi,L.; Frison,G.; Castagna,F. Solid-phase extraction and HPLC-UV confirmation of drugs of abuse in urine, *J.Anal.Toxicol.*, **1992**, *16*, 217–222.

SAMPLE
Matrix: urine
Sample preparation: 1 mL Urine + 10 μL nalorphine solution + 3000-3500 U glucuronidase (Patella vulgata, Sigma) + 300 μL 1.1 M pH 5 sodium acetate buffer, heat overnight at 55°, add 500 μL 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 μL 0.2% phosphoric acid, rotate gently for 30 min, inject a 50 μL 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 × 3.9 5 μm 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 or UV 210

CHROMATOGRAM
Retention time: 5.9
Internal standard: nalorphine (25.2)
Limit of detection: 40 ng/mL

OTHER SUBSTANCES
Extracted: codeine, 6-monoacetylmorphine
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: 1 mL Urine + 0.5 mL 1% trichloroacetic acid, centrifuge at 5200 g for 10 min, filter (0.2 μm), inject 20 μL aliquot

HPLC VARIABLES
Column: 250 × 4 Lichrospher 5μm 60 RP-select B
Mobile phase: Gradient. MeCN:50 mM pH 3.2 potassium phosphate buffer from 10:90 to 75:25 over 7 min, hold at 75:25 for 3 min, return to 10:90 over 5 min, equilibrate at 10:90 for 5 min
Flow rate: 1.5
Injection volume: 20
Detector: UV 190-370

CHROMATOGRAM
Retention time: 2.5

OTHER SUBSTANCES
Extracted: amitriptyline, codeine, amphetamine, benzoylecgonine, meperidine, norpropoxyphene, nordiazepam
Also analyzed: phenylpropanolamine, lidocaine, diphenhydramine, nortriptyline, ephedrine, cocaine (different gradient).

REFERENCE
Li,S.; Gemperline,P.J.; Briley,K.; Kazmierczak,S. Identification and quantitation of drugs of abuse in urine using the generalized rank annihilation method of curve resolution, *J.Chromatogr.B*, **1994**, *655*, 213–223.

SAMPLE
Matrix: urine
Sample preparation: Condition a 300 mg Bond Elut Certify SPE cartridge with 2 mL MeOH and 2 mL water. 5 mL Urine + 1 mL concentrated HCl, vortex, heat at 120° for 30 min, cool, adjust pH to between 7.0 and 8.0 with 10 M KOH. 5 mL Urine or hydrolysed urine + nalorphine, add to the SPE cartridge, wash with 2 mL water, wash with 1 mL pH 4 acetate buffer, wash with 2 mL MeOH, elute with 2 mL dichloromethane:isopropanol 80:20 containing 2% ammonia. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 0.5-1 mL pentane:dichloromethane 90:10. (Use unhydrolysed urine to determine diamorphine and unconjugated compounds.)

HPLC VARIABLES
Column: 200 × 2 3 μm Hypersil
Mobile phase: Pentane:dichloromethane:MeOH containing 0.5% diethylamine 65:29.8:5.2
Flow rate: 0.4
Injection volume: 50
Detector: UV 280

CHROMATOGRAM
Retention time: 15
Internal standard: nalorphine (5)
Limit of detection: 7 ng/mL

OTHER SUBSTANCES
Extracted: diamorphine, 6-monoacetylmorphine, pholcodine, dihydrocodeine, codeine
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 + 10 mg β-glucuronidase/arylsulfatase (Helix pomatia, Sigma), heat at 37° overnight, add an equal volume of buffer, centrifuge at 2000 *g* for 5 min, inject an aliquot of the supernatant onto column A with mobile phase A and elute to waste. After 2.5 min backflush the contents of column A onto column B with mobile phase B, monitor the effluent from column B. Re-equilibrate both columns for 12.5 min before the next injection. (Buffer was 200 mM boric acid adjusted to pH 9.5 with 5 M NaOH.)

HPLC VARIABLES
Column: A 10 × 4.6 5 μm Spherisorb cyanopropyl; B 250 × 4.6 Capcell Pak C18 UG-120 (Shiseido)
Mobile phase: A water; B Gradient. MeCN:buffer from 3:97 to 30:70 over 30 min, to 40:60 over 8 min (Buffer was 3.4 mL/L phosphoric acid adjusted to pH 3.0 with 5 M NaOH.)
Flow rate: A 1.25; B 1
Injection volume: 100
Detector: UV 220

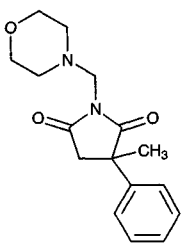
CHROMATOGRAM
Retention time: 6.3
Limit of detection: 250 ng/mL

OTHER SUBSTANCES
Extracted: acebutolol, alprenolol, amphetamine, atenolol, bopindolol, codeine, ephedrine, labetalol, metoprolol, 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.

Morsuximide



Molecular formula: $C_{16}H_{20}N_2O_3$
Molecular weight: 288.35
CAS Registry No.: 3780-72-1

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb RP18
Mobile phase: EtOH:water 10:90 containing 10 mM β-cyclodextrin and 0.5 mM tri-O-methyl-β-cyclodextrin
Column temperature: 25
Flow rate: 0.95

Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 3.4, k' 3.9 (enantiomers)

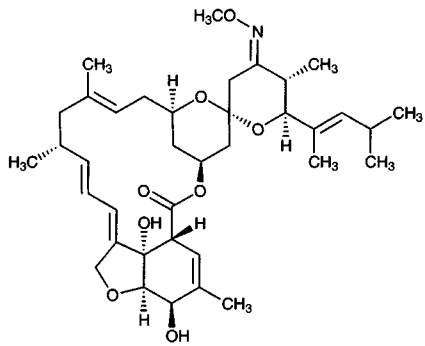
OTHER SUBSTANCES
Extracted: mephenytoin

KEY WORDS
chiral

REFERENCE
Nowakowski,R.; Bielejewska,A.; Duszczyk,K.; Sybilska,D. Chiral discrimination by high-performance liquid chromatography with joint use of two cyclodextrin additives, *J.Chromatogr.A*, **1997**, *782*, 1–11.

Moxidectin

Molecular formula: $C_{37}H_{53}NO_8$
Molecular weight: 639.83
CAS Registry No.: 113507-06-5
Merck Index: 6373



SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL 100 mg Supelclean LC18 SPE cartridge with 5 mL MeOH and 5 mL water at 6 mL/min. 1 mL Plasma + 1 mL MeCN + 250 µL water, mix for 20 min, centrifuge at 2000 g for 2 min, add 2.2 mL supernatant to the SPE cartridge at 3 mL/min, wash with 2 mL water, wash with 1 mL MeOH:water 25:75 at 3 mL/min, dry under nitrogen at 6 mL/min for 10 s, elute with 1.2 mL MeOH at 3 mL/min. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute with 100 µL N-methylimidazole:MeCN 1:1, add 150 µL trifluoroacetic anhydride:MeCN 1:2, let stand for <30 s, inject a 100 µL aliquot.

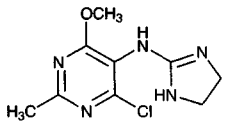
HPLC VARIABLES
Column: Supelcosil C18
Mobile phase: MeCN:MeOH:0.2% acetic acid 62:30:8
Flow rate: 1.5
Injection volume: 100
Detector: F ex 383 em 447

CHROMATOGRAM
Retention time: 9.2
Limit of quantitation: 0.1 ng/mL

KEY WORDS
plasma; SPE; cow; derivatization; pharmacokinetics

REFERENCE
Alvinerie,M.; Sutra,J.F.; Badri,M.; Galtier,P. Determination of moxidectin in plasma by high-performance liquid chromatography with automated solid-phase extraction and fluorescence detection, *J.Chromatogr.B*, **1995**, *674*, 119–124.

Moxonidine



Molecular formula: $C_9H_{12}ClN_5O$
Molecular weight: 241.68
CAS Registry No.: 75438-57-2
Merck Index: 6375

SAMPLE
Matrix: solutions

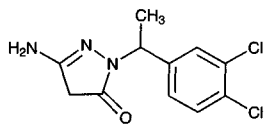
HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 0.86

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, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE
Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Muzolimine



Molecular formula: $C_{11}H_{11}Cl_2N_3O$
Molecular weight: 272.13
CAS Registry No.: 55294-15-0
Merck Index: 6397
Lednicer No.: 3 137

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 2 mg cysteine + 100 μL 200 μg/mL IS in MeOH + 5 mL hexane:dichloromethane 60:40, vortex for 30 s, centrifuge at 550 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:50 mM pH 4.2 phosphate buffer 45:55
Flow rate: 1.5
Injection volume: 100
Detector: UV 272

CHROMATOGRAM
Retention time: 4.0
Internal standard: 3-amino-1-(1-(3,4-dichlorophenyl)ethyl)pyrazol-5-one (7.2)
Limit of quantitation: 50 ng/mL

KEY WORDS
plasma; dog; human; pharmacokinetics

REFERENCE
Osman,M.A.; Dunning,L.K.; Bhavnagri,V.P.; Cheng,L.K. Determination of muzolimine in plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *496*, 478–484.

SAMPLE
Matrix: urine
Sample preparation: 1 mL Urine + 4 mg cysteine + 100 µL 200 µg/mL IS in MeOH + 5 mL hexane:dichloromethane 60:40, vortex for 30 s, centrifuge at 550 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 µL mobile phase, inject a 100 µL aliquot.

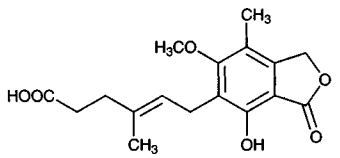
HPLC VARIABLES
Column: 150 × 3.9 5 µm Nova Pak phenyl
Mobile phase: MeCN:50 mM pH 4.2 phosphate buffer 28>72
Flow rate: 1.5
Injection volume: 100
Detector: UV 272

CHROMATOGRAM
Retention time: 4.4
Internal standard: N-ethyl-3-(3-methylphenoxy)azetidine-1-carboxamide (5.6)
Limit of quantitation: 250 ng/mL

KEY WORDS
dog; human

REFERENCE
Osman,M.A.; Dunning,L.K.; Bhavnagri,V.P.; Cheng,L.K. Determination of muzolimine in plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *496*, 478–484.

Mycophenolic acid



Molecular formula: $C_{17}H_{20}O_6$
Molecular weight: 320.34
CAS Registry No.: 24280-93-1
Merck Index: 6408

SAMPLE
Matrix: blood
Sample preparation: Mix 50 µL plasma with 100 µL MeCN containing 100 mM phosphoric acid. Inject a 5 µL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 4.6 3.5 µm Zorbax SB-C18
Mobile phase: MeCN:40 mM pH 2.1 phosphoric acid 30:70
Flow rate: 1.5
Injection volume: 5
Detector: UV 215

CHROMATOGRAM
Retention time: 9.7
Limit of quantitation: 500 nM

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE
Svensson,J.O.; Brattström,C.; Säwe,J. A simple HPLC method for simultaneous determination of mycophenolic acid and mycophenolic acid glucuronide in plasma (Abstract 75), *Ther.Drug Monit.*, **1997**, *19*, 566.

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL MeCN containing 15 μg/mL IS, 20 μL 150 g/L perchloric acid, and 20 μL 250 g/L sodium tungstate to 200 μL plasma. Mix and centrifuge at 10 000 g for 5 min. Inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Symmetry C18 (Waters)
Mobile phase: Gradient. A was MeCN:20 mM pH 3.0 phosphate buffer 25:75. B was MeCN:20 mM pH 6.5 phosphate buffer 70:30. A:B 94:6 for 4.5 min, to 62:38 over 7 min, to 0:100 over 2 min.
Flow rate: 1.2
Injection volume: 50
Detector: UV 254, UV 215

CHROMATOGRAM
Internal standard: carboxybutoxy ether of mycophenolic acid
Limit of detection: 10 ng/mL (UV 215); 30 ng/mL (UV 254)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE
Andreeva,M.; Niedmann,D.; Schütz,E.; Armstrong,V.W.; Oellerich,M. An HPLC procedure for simultaneous determination of mycophenolic acid and its glucuronide in human plasma (Abstract 73), *Ther.Drug Monit.*, **1997**, *19*, 565.

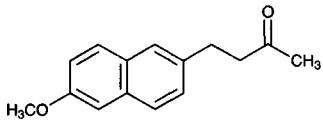
SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 2.1 Hypersil C18
Mobile phase: MeCN:0.1% acetic acid 43:57
Flow rate: 0.15
Injection volume: 20
Detector: MS, Hewlett Packard 5989, electrospray, selected ion monitoring m/z=319

CHROMATOGRAM
Retention time: 8
Internal standard: (E)-6-[1,3-dihydro-4-(4-carboxybutoxy)-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl-4-methyl-4-hexenoic acid (m/z=419)
Limit of detection: 15.4 ng/mL

REFERENCE
Korecka,M.; Van Breemen,R.B.; Nikolic,D.; Nowak,I.; Shaw,L.M. Mycophenolic acid measurement in plasma: Validation of an HPLC method by comparison with an HPLC-mass spectrometry procedure (Abstract 60), *Ther.Drug Monit.*, **1997**, *19*, 562.

Nabumetone



Molecular formula: $C_{15}H_{16}O_2$
Molecular weight: 228.29
CAS Registry No.: 42924-53-8
Merck Index: 6428

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

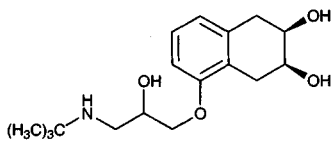
CHROMATOGRAM
Retention time: k' 7.59

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

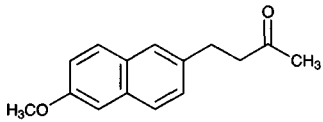
Nadolol



Molecular formula: $C_{17}H_{27}NO_4$
Molecular weight: 309.41
CAS Registry No.: 4200-33-9
Merck Index: 6431
Lednicer No.: 2 110

REFERENCE
Korecka,M.; Van Breemen,R.B.; Nikolic,D.; Nowak,I.; Shaw,L.M. Mycophenolic acid measurement in plasma: Validation of an HPLC method by comparison with an HPLC-mass spectrometry procedure (Abstract 60), *Ther.Drug Monit.*, **1997**, *19*, 562.

Nabumetone



Molecular formula: $C_{15}H_{16}O_2$
Molecular weight: 228.29
CAS Registry No.: 42924-53-8
Merck Index: 6428

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

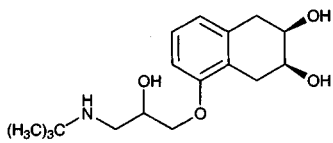
CHROMATOGRAM
Retention time: k' 7.59

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

Nadolol



Molecular formula: $C_{17}H_{27}NO_4$
Molecular weight: 309.41
CAS Registry No.: 4200-33-9
Merck Index: 6431
Lednicer No.: 2 110

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 80 μL 250 ng/mL atenolol in EtOH + 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: 6.5
Internal standard: atenolol (4)
Limit of detection: 1 ng/mL

KEY WORDS
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: 500 μL Plasma + 100 μL 1 M NaOH, vortex gently for 1 min, add to an Extrelut-1 SPE cartridge, rinse out the tube with 300 μL water, add the rinse to the SPE cartridge, let stand for 15 min, elute with 10 mL diethyl ether. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL water and 400 μL MeOH, evaporate to dryness under a stream of nitrogen at 50°, reconstitute with 50 μL MeOH, add 10 μL 5 mg/mL (R)-(-)-1-(1-naphthyl)ethylisocyanate in MeCN, vortex for 30 s, heat at 45° for 5 min, evaporate to dryness under a stream of nitrogen at room temperature, reconstitute with 100 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm YMC-AM-303 ODS (YMC)
Mobile phase: MeCN:water 40:60
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: F ex 285 em 340

CHROMATOGRAM
Retention time: 31 (SR), 33 (RS), 35 (RR), 37 (SS)
Limit of quantitation: 2.5 ng/mL

KEY WORDS
derivatization; pharmacokinetics; dog; plasma; chiral; SPE

REFERENCE
Hoshino,M.; Yajima,K.; Suzuki,Y.; Okahira,A. Determination of nadolol diastereomers in dog plasma using chiral derivatization and reversed-phase high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.B*, **1994**, *661*, 281–289.

SAMPLE
Matrix: blood

Sample preparation: Condition a 500 mg non-end-capped cyanopropyl SPE cartridge (Varian) with 1 mL MeOH and 1 mL water. Condition a 100 mg phenylboronic acid SPE cartridge (Varian) with 1 mL MeOH, 2 mL 100 mM HCl, 4 mL 0.3% ammonium hydroxide, and 2 mL 100 mM pH 8.5 ammonium sulphate. 1 mL Plasma + 80 ng IS, add to the cyanopropyl SPE cartridge, wash with 3 mL water, wash with 3 mL MeCN, elute with 2.5 mL alkaline MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 1 mL MeOH, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 50 μL distilled 1,2-dimethoxyethane, add 20 μL reagent, let stand at room temperature for 1 h, add 300 μL water, extract with 1 mL dichloromethane. Wash the organic layer with 300 μL water, evaporate the organic layer to dryness under a stream of nitrogen, reconstitute the residue in 1 mL MeOH:water 40:60. Add this solution to the phenylboronic SPE column, wash with 2 mL water, wash with 100 μL MeOH, wash with 1 mL hexane:ethyl acetate 80:20, wash with 1 mL hexane, elute with 5 mL MeOH:triethylamine 95:5. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH:2% tetramethylethylenediamine 50:50, inject an 80 μL aliquot. (Reagent was 0.1% R(-)-1-(naphthyl)ethylisocyanate in 1,2-dimethoxyethane.)

HPLC VARIABLES

Column: 250 × 4.6 5 μm C18 (Beckman)
Mobile phase: MeOH:THF:buffer 52:7:41 (Buffer was 0.4% tetramethylethylenediamine adjusted to pH 3.0 with trifluoroacetic acid.)
Column temperature: 28
Flow rate: 1
Injection volume: 80
Detector: F ex 230 em 330

CHROMATOGRAM

Retention time: 20.6 (SRS), 21.8 (RSR), 26.2 (SSR), 28.2 (RRS)
Internal standard: 5-(3-[methylethylamino]-2-hydroxypropoxy)-1,2,3,4-tetrahydro-2,3-naphthalenediol (SQ 11559, Bristol-Myers Squibb) (Racemate A was separated by HPLC using the above system with MeOH:100 mM ammonium acetate 18:82 mobile phase and UV 230.) (13.5, 15.5 (enantiomers))
Limit of detection: 1 ng/mL
Limit of quantitation: 2.5 ng/mL

KEY WORDS

chiral; plasma; SPE; pharmacokinetics; derivatization

REFERENCE

Belas,F.J.; Phillips,M.A.; Srinivas,N.R.; Barbhaiya,R.H.; Blair,I.A. Simultaneous determination of nadolol enantiomers in human plasma by high-performance liquid chromatography using fluorescence detection, *Biomed.Chromatogr.*, **1995**, *9*, 140–145.

SAMPLE

Matrix: blood
Sample preparation: 200 μL Plasma + 50 μL 4 μg/mL IS + 250 μL 10 M NaOH, vortex for 10 s, add 5 mL MTBE, rotate for 15 min, centrifuge at 2600 rpm 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, inject a 100 μL aliquot (maintain samples at 5° until injection).

HPLC VARIABLES

Column: 250 × 4.6 C18 octadecylsilane (Beckmann)
Mobile phase: MeCN:water 16:84 containing 5.75 g/L $(NH_4)H_2PO_4$, pH 4.2
Flow rate: 1.4
Injection volume: 100
Detector: F ex 230 em 330

CHROMATOGRAM

Retention time: 3.3
Internal standard: desmethyl nadolol (4.3)
Limit of quantitation: 5 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Srinivas,N.R.; Shyu,W.C.; Shah,V.R.; Campbell,D.A.; Barbhaiya,R.H. High-performance liquid chromatographic assay for the quantitation of nadolol in human plasma using fluorescence detection, *Biomed.Chromatogr.*, **1995**, *9*, 75–79.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 50 μL 2 μg/mL desmethylnadolol + 750 μL 1 M NaOH, vortex for 10 s, add 5 mL dichloromethane, rotate for 15 min, centrifuge at 2600 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL 0.1% R-(-)-naphthylethylisocyanate in dichloromethane, let stand for 1 h, reconstitute with 150 μL mobile phase, inject a 125 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm C18 ODS (Beckman)
Mobile phase: MeOH:THF:water:phosphoric acid 52:7:41:0.001 adjusted to pH 3 with tetramethylene diamine
Flow rate: 1.4
Injection volume: 125
Detector: F ex 230 em 330

CHROMATOGRAM
Retention time: 21 (SRS), 23 (RSR), 28 (SSR), 30 (RRS)
Internal standard: desmethylnadolol (13, 15 (enantiomers))
Limit of quantitation: 2 ng/mL

KEY WORDS
plasma; chiral; derivatization

REFERENCE
Srinivas,N.R.; Shyu,W.C.; Dhah,V.R.; Campbell,D.A.; Barbhaiya,R.H. Stereoselective analysis of nadolol in human plasma, *Biomed.Chromatogr.*, **1995**, *9*, 226–228.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 270

CHROMATOGRAM
Retention time: 3.69
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM
Retention time: 6.77

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk, formulations

HPLC VARIABLES
Column: 250 × 4.6 10 μm Chiralcel OD
Mobile phase: EtOH:hexane:diethylamine 15:85:0.4 (A) or EtOH:hexane:diethylamine 20:80:0.4 (B)
Column temperature: 23
Flow rate: 1
Detector: UV 245

CHROMATOGRAM
Retention time: 6.94 (RSR , RRS (A)), 9.30 (SRS (A)), 10.96 (SSR (A)) or 6.51 (RRS (B)), 6.98 (RSR (B)), 9.19 (SRS (B)), 10.83 (SSR (B))

KEY WORDS
chiral

REFERENCE
Aboul-Enein,H.Y.; Abou-Basha,L.I. HPLC separation of nadolol and enantiomers on Chiralcel OD column, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 383–392.

SAMPLE
Matrix: formulations
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: 6
Internal standard: atenolol (4.5)

OTHER SUBSTANCES
Simultaneous: impurities, acebutolol, alprenolol, metoprolol, oxprenolol, pindolol, practolol, propranolol, sotalol, timolol

KEY WORDS
tablets; stability-indicating

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: 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 (S)-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 50:50:1
Flow rate: 1
Injection volume: 40
Detector: F ex 225 em 290 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

HPLC VARIABLES
Column: 100 × 8 4 μm NovaPak C18
Mobile phase: MeCN:triethylamine:water 9:0.3:91, adjusted to pH 3.0 with orthophosphoric acid
Flow rate: 3.0
Injection volume: 50
Detector: F ex 224 em no emission filter

CHROMATOGRAM
Retention time: 5.55

OTHER SUBSTANCES
Simultaneous: metoprolol

REFERENCE
Wang,B.; Semple,H.A. Inhibition of metoprolol metabolism by amino acids in perfused rat livers. Insights into the food effect?, *Drug Metab.Dispos.*, **1997**, *25*, 287–295.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.9

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 0.5-2 mg/mL solution in mobile phase, add a 5-fold molar excess of 1-naphthylisocyanate, mix, let stand for 15 min, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm CHI-1-LEU (R)-N-(3,5-dinitrobenzoyl)-L-leucine covalently bound to aminopropyl silica (Hichrom)
Mobile phase: n-Hexane:EtOH:MeCN 90:10:2
Flow rate: 2
Injection volume: 20
Detector: UV 239

CHROMATOGRAM
Retention time: 26 (SS), 28 (RS), 32 (SR), 36 (RR)

KEY WORDS
chiral; derivatization

REFERENCE
Dyas,A.M.; Robinson,M.L.; Fell,A.F. Direct separation of nadolol enantiomers on a Pirkle-type chiral stationary phase, *J.Chromatogr.*, **1991**, *586*, 351–355.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in mobile phase, add 1 μL 1-naphthylisocyanate for each mL of solution, let stand for 20 min, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 3 μm aminopropylsilica (HPLC Technology) (Prepare column by passing through a solution of 5 g (R)-N-(3,5)-dinitrobenzoyl-L-leucine and 5 g 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline in THF, wash with THF, wash with dichloromethane, wash with 100 mL dichloromethane containing 10 g trifluoroacetic anhydride, wash with dichloromethane, equilibrate with mobile phase. [J.Chromatogr.,1991,586,351])
Mobile phase: n-Hexane:isopropanol 80:20
Flow rate: 2
Injection volume: 20
Detector: UV 239

CHROMATOGRAM
Retention time: 26, 28, 31, 36 (different enantiomers)

KEY WORDS
chiral

REFERENCE
Dyas,A.M.; Robinson,M.L.; Fell,A.F. Influence of the structure of the alcoholic modifier on the enantioselective separation of nadolol, *J.Chromatogr.A*, **1994**, *660*, 249–253.

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, fen-

camfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm YMC-Pack ODS-AM-303 end-capped (YMC)
Mobile phase: MeOH:50 mM pH 7.2 phosphate buffer 30:70
Column temperature: 40
Flow rate: 1
Injection volume: 5
Detector: UV 220 or 230

CHROMATOGRAM

Retention time: 14 (racemate A), 15.5 (racemate B)

KEY WORDS

diastereomers

REFERENCE

Hoshino,M.; Matsui,E.; Yajima,K.; Okahira,A. Direct high-performance liquid chromatographic separation of the racemates and diastereomers of nadolol, *J.Chromatogr.A*, **1994**, *664*, 104–110.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)

Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 1.86

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, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE
Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 5.64 (A), 3.39 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, 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
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' 3.43

KEY WORDS
chiral; α 1.36

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: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 0.51

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE

Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

SAMPLE

Matrix: urine

Sample preparation: 3 mL Urine + 500 μL 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 μL aliquot.

HPLC VARIABLES

Guard column: μBondapak C18

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeCN:water 40:60 containing 5 mM KH_2PO_4/K_2HPO_4 adjusted to pH 6.5 with phosphoric acid or 1 M KOH

Column temperature: 30 ± 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: 3.08

Limit of quantitation: 100 ppb

OTHER SUBSTANCES

Extracted: alprenolol, metoprolol, 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. Re-equilibrate both columns for 12.5 min before the next injection. (Buffer was 200 mM boric acid adjusted to pH 9.5 with 5 M NaOH.)

HPLC VARIABLES

Column: A 10 × 4.6 5 μm Spherisorb cyanopropyl; B 250 × 4.6 Capcell Pak C18 UG-120 (Shiseido)

Mobile phase: A water; B Gradient. MeCN:buffer from 3:97 to 30:70 over 30 min, to 40:60 over 8 min (Buffer was 3.4 mL/L phosphoric acid adjusted to pH 3.0 with 5 M NaOH.)

Flow rate: A 1.25; B 1

Injection volume: 100

Detector: UV 220

CHROMATOGRAM

Retention time: 9.5

Limit of detection: 250 ng/mL

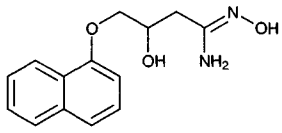
OTHER SUBSTANCES

Extracted: acebutolol, alprenolol, amphetamine, atenolol, bopindolol, codeine, ephedrine, labetalol, metoprolol, morphine, 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.

Nadoxolol



Molecular formula: $C_{14}H_{16}N_2O_3$
Molecular weight: 260.29
CAS Registry No.: 54063-51-3, 35991-93-6 (HCl)
Merck Index: 6432

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 211.1

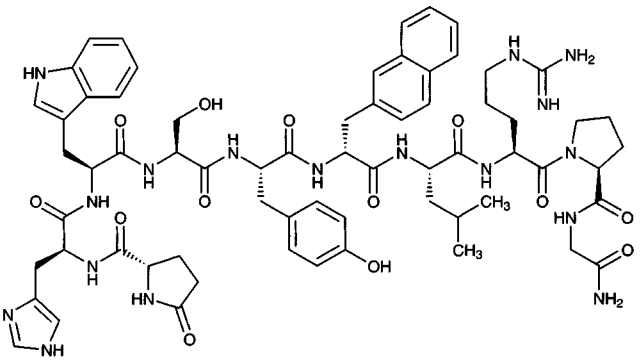
CHROMATOGRAM
Retention time: 12.445

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Nafarelin

Molecular formula: $C_{66}H_{83}N_{17}O_{13}$
Molecular weight: 1322.49
CAS Registry No.: 76932-56-4, 86220-42-0 (acetate)
Merck Index: 6437



SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 200 × 3 Spherisorb S5ODS-2
Mobile phase: Gradient. A was 0.05% phosphoric acid containing 0.5% $(NH_4)_2SO_4$. B was MeCN. A:B from 82:18 to 64:36 over 25 min, maintain at 64:36 for 2.5 min, return to initial conditions over 1 min, re-equilibrate for 6.5 min. or Isocratic MeCN:0.05% phosphoric acid containing 0.5% $(NH_4)_2SO_4$ 24:76
Flow rate: 0.5
Detector: UV 210

CHROMATOGRAM
Retention time: 29 (gradient), 64 (isocratic)

OTHER SUBSTANCES
Simultaneous: buserelin, deslorelin, gonadorelin, goserelin, leuprolide

KEY WORDS
comparison with capillary electrophoresis

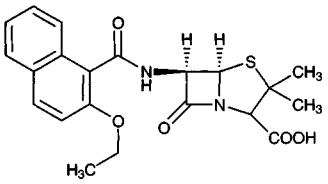
REFERENCE
Corran,P.H.; Sutcliffe,N. Identification of gonadorelin (LHRH) derivatives: comparison of reversed-phase high-performance liquid chromatography and micellar electrokinetic chromatography, *J.Chromatogr.*, **1993**, *636*, 87–94.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:170 mM KH_2PO_4 30:70
Detector: UV 225

REFERENCE
Nerenberg,C.; Foreman,J.; Chu,N.; Chaplin,M.D.; Kushinsky,S. Radioimmunoassay of nafarelin ([6-(3-(2-naphthyl)-D-alanine)]-luteinizing hormone-releasing hormone) in plasma or serum, *Anal.Biochem.*, **1984**, *141*, 10–16.

Nafcillin



Molecular formula: $C_{21}H_{22}N_2O_5S$
Molecular weight: 414.48
CAS Registry No.: 147-52-4, 985-16-0 (Na salt), 7177-50-6 (Na salt monohydrate)
Merck Index: 6438
Lednicer No.: 1 412

SAMPLE

Matrix: blood
Sample preparation: 400 μL Serum + 400 μL MeCN, vortex for 10 s, shake slowly for 15 min, centrifuge at 3000 g for 10 min. Remove the supernatant and add it to 4 mL dichloromethane, vortex for 10 s, shake for 15 min, centrifuge at 3000 g for 10 min, inject a 50 μL aliquot of the upper aqueous layer.

HPLC VARIABLES

Column: μBondapak C18
Mobile phase: MeCN:water:200 mM ammonium acetate 28:62:10, pH 5.6
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 9.0
Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Extracted: cloxacillin, dicloxacillin, methicillin, oxacillin
Noninterfering: amdinocillin (mecillinam), amikacin, amoxicillin, ampicillin, carbenicillin, cefamandole, cefazolin, ceforanide, cefatoxamine, cefoxitin, cephalexin, cephaloridine, cephalothin, cephradine, cepharin, chloramphenicol, clindamycin, co-trimoxazole, fluorocytosine, gentamicin, metronidazole, moxalactam, penicillin, piperacillin, sulfamethoxazole, theophylline, ticarcillin, tobramycin, trimethoprim, vancomycin

KEY WORDS

serum

REFERENCE

Rudrik,J.T.; Bawdon,R.E. Determination of penicillinase-resistant penicillins in serum using high-pressure liquid chromatography, *J.Liq.Chromatogr.*, **1981**, *4*, 1525–1545.

SAMPLE

Matrix: blood
Sample preparation: 500 μL Serum + 50 μL 1 M sulfuric acid, mix for 30 s, add 2 mL dichloromethane, mix for 2 min, centrifuge at 1130 g for 2 min. Remove 1.8 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 10-20 μL aliquot.

HPLC VARIABLES

Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate 20:80
Flow rate: 2
Injection volume: 10-20
Detector: UV 254

CHROMATOGRAM

Retention time: 4
Internal standard: nafcillin

OTHER SUBSTANCES
Extracted: cloxacillin

KEY WORDS
serum; nafcillin is IS

REFERENCE
Jamaluddin,A.B.M.; Sarwar,G.; Rahim,M.A.; Rahman,M.K. Assay for cloxacillin in human serum utilising high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1989**, *490*, 243–246.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Serum or urine + 50 μL 1 M sulfuric acid, mix for 5 s, add 2 mL dichloromethane, shake for 2 min, centrifuge at 2000 rpm for 2 min. Remove the organic layer and add it to 1 mL 40 mM pH 6.8 NaH_2PO_4, shake for 2 min, centrifuge at 2000 rpm for 2 min. Place the aqueous layer in a separate tube, agitate to remove traces of dichloromethane, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: Co:Pell ODS
Column: 100 × 4.6 10 μm MPLC Concept RP-8 (Brownlee)
Mobile phase: MeCN:40 mM NaH_2PO_4 6.2:20, pH 4.5
Flow rate: 1.6
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 6.60
Internal standard: nafcillin

OTHER SUBSTANCES
Extracted: cloxacillin

KEY WORDS
serum; nafcillin is IS

REFERENCE
Teare,F.W.; Kwan,R.H.; Spino,M.; MacLeod,S.M. High-pressure liquid chromatographic assay of cloxacillin in serum and urine, *J.Pharm.Sci.*, **1982**, *71*, 938–941.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Bondelut SPE cartridge (cat. no. 607101) with two 1 mL portions of MeCN and 1 mL buffer. Dilute urine 1:10 with buffer. 100 μL Serum or diluted urine + 100 μL buffer, vortex for 10 s, add to the SPE cartridge, wash with 1 mL buffer, wash with 500 μL MeCN:buffer 15:85, elute with 500 μL MeCN:buffer 70:30, inject a 10 μL portion of the eluate. (Buffer was 70 mM KH_2PO_4 adjusted to pH 5.0 with 5 M NaOH.)

HPLC VARIABLES
Guard column: 45 × 4 10 μm Spherisorb C-18
Column: 300 × 4 μBondapak C-18
Mobile phase: MeCN:50 mM pH 7.0 KH_2PO_4 27:73
Flow rate: 1.1
Injection volume: 10
Detector: UV 220

CHROMATOGRAM
Retention time: 7.5
Internal standard: nafcillin

OTHER SUBSTANCES
Extracted: mezlocillin
Noninterfering: acetaminophen, dexamethasone, diazepam, furosemide, morphine

KEY WORDS
serum; SPE; nafcillin is IS

REFERENCE
Fiore,D.; Auger,F.A.; Drusano,G.L.; Dandu,V.R.; Lesko,L.J. Improved micromethod for mezlocillin quantitation in serum and urine by high-pressure liquid chromatography, *Antimicrob.Agents Chemother.*, **1984**, *26*, 775–777.

SAMPLE
Matrix: cells
Sample preparation: 100 μL Cell suspension + 100 μL cefoperazone solution + 100 μL Hanks balanced salt solution, sonicate 30 min, add 800 μL MeCN, centrifuge at 13000 g for 5 min, remove supernatant. Dry supernatant under air, dissolve in 100 μL mobile phase, inject 75 μL.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:20 mM pH 5.0 sodium acetate 30:70
Flow rate: 1.5
Injection volume: 75
Detector: UV 224

CHROMATOGRAM
Retention time: 8.0
Internal standard: 5-(p-Hydroxyphenyl)-5-phenylhydantoin
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 with water (if necessary), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak phenyl
Mobile phase: MeOH:water 50:50 containing 10 mM ammonium acetate
Flow rate: 2
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 4.5

KEY WORDS
saline; 5% dextrose; stability-indicating

REFERENCE
Das Gupta,V.; Stewart,K.R. Quantitation of carbenicillin disodium, cefazolin sodium, cephalothin sodium, nafcillin sodium, and ticarcillin disodium by high-pressure liquid chromatography, *J.Pharm.Sci.*, **1980**, *69*, 1264–1267.

SAMPLE
Matrix: formulations

Sample preparation: Blend tablets and capsules with water in a high-speed blender for 5 min, filter, dilute with mobile phase, inject a 20 μL aliquot. Dilute oral suspensions and injections with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 70 mm long Co:Pell ODS
Column: 300 × 4.6 10 μm Chromegabond C18 (E.S. Industries)
Mobile phase: MeCN:MeOH:10 mM KH_2PO_4 19:11:70
Flow rate: 1
Injection volume: 20
Detector: UV 225

CHROMATOGRAM
Retention time: 21.0
Limit of detection: 659 ng/mL

OTHER SUBSTANCES
Simultaneous: amoxicillin, ampicillin, cloxacillin, dicloxacillin, methicillin, oxacillin, penicillin G, penicillin V

KEY WORDS
tablets; capsules; oral suspensions; injections

REFERENCE
Briguglio,G.T.; Lau-Cam,C.A. Separation and identification of nine penicillins by reverse phase liquid chromatography, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 228–231.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 1:100 with water, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 100 × 2.1 3 μm Hypersil C18
Mobile phase: MeCN:20 mM pH 6.0 sodium acetate 22:78
Column temperature: 40
Flow rate: 0.5
Injection volume: 10
Detector: UV 230, 254

CHROMATOGRAM
Retention time: 2.45

OTHER SUBSTANCES
Simultaneous: lidocaine (broad tailing peak)

KEY WORDS
injections; saline; 5% dextrose; stability-indicating

REFERENCE
Hagan,R.L.; Carr-Lopez,S.M.; Strickland,J.S. Stability of nafcillin sodium in the presence of lidocaine hydrochloride, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 521–523.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 250 × 4.6 5 μm Bakerbond C18
Mobile phase: MeCN:50 mM sodium acetate 30:70
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 6.17

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: milk
Sample preparation: Mix 10 mL milk with 2 mL 100 mM tetraethylammonium chloride, add 40 mL MeCN slowly with continual stirring, let stand for 10 min, decant the supernatant through a plug of glass wool. Collect 40 mL filtrate, add 2 mL buffer, evaporate to 1-2 mL under reduced pressure at 40-50°, dilute to 4 mL with water, filter (0.45 μm PVDF). Inject a 2 mL aliquot onto a 150 × 4.6 5 μm Supelcosil LC-18 column, elute with MeCN:10 mM KH_2PO_4 0:100 for 3 min, to 60:40 over 37 min at 1 mL/min, collect a 1.5-2 mL aliquot containing the compound (ca. 30.3 min), evaporate to <1 mL under reduced pressure, make up to 1 mL with water, inject an aliquot. (Prepare the buffer by mixing 10 mM KH_2PO_4 and 10 mM Na_2HPO_4 in a 5:1 ratio, pH 6.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-18-DB
Mobile phase: MeCN:buffer 38:62 (Buffer was 2 mM phosphoric acid containing 8 mM potassium dihydrogen phosphate.)
Flow rate: 1
Injection volume: 200
Detector: UV 215

REFERENCE
Moats,W.A.; Romanowski,R.D. Multiresidue determination of β-lactam antibiotics in milk and tissues with the aid of high-performance liquid chromatographic fractionation for clean up, *J.Chromatogr.A*, **1998**, *812*, 237–247.

SAMPLE
Matrix: milk
Sample preparation: Condition a 3 mL 500 mg Baker-10 C18 SPE cartridge (J.T. Baker) with 3 mL MeOH and 3 mL distilled water. Add 20 mL MeCN to 10 mL milk, vortex for 1 min, centrifuge at 1500 g for 10 min, concentrate the supernatant to 2-3 mL on a rotary evaporator at 40°, add to the SPE cartridge, dry the cartridge under reduced pressure for 3 min, elute with 1 mL MeOH, filter (0.45 μm) the eluate, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Kaseisorb LC ODS-300-5 (Tokyo Kasei)
Mobile phase: MeCN:MeOH:50 mM KH_2PO_4 buffer 20:10:80 containing 5 mM sodium 1-decanesulfonate, adjusted to pH 3.5 with concentrated phosphoric acid
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 24
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: ampicillin, cloxacillin, dicloxacillin, penicillin G

KEY WORDS
SPE

REFERENCE
Takeba,K.; Fujinuma,K.; Miyazaki,T.; Nakazawa,H. Simultaneous determination of β-lactam antibiotics in milk by ion-pair liquid chromatography, *J.Chromatogr.A*, **1998**, *812*, 205–211.

SAMPLE
Matrix: milk
Sample preparation: 50 g Milk + 2 drops penicillinase (Difco Laboratories), let stand 1 h at 37°, add 50 MeCN, shake vigorously for 1 min, centrifuge at 9000 g for 10 min, decant, add 5 g NaCl, swirl to dissolve, add 100 mL dichloromethane, shake for 1 min, centrifuge at 1000 g for 10 min. Remove top aqueous layer and extract organic layer with 25 mL 10% NaCl by shaking and centrifuging as before. Combine aqueous layers, add 1 mL 0.3% mercuric chloride in water, let stand 30 min, add 1 mL 2 M HCl, extract with three 50 mL portions of dichloromethane by shaking each portion for 1 min and centrifuging at 1000 g for 10 min, filter dichloromethane extracts through 30 g anhydrous sodium sulfate, evaporate to dryness under reduced pressure at 35°, if water remains add 5-10 mL MeOH to flask and complete evaporation. Dissolve residue in 1 mL 10% acetic acid, add 0.5 mL 0.08% dansyl hydrazine in 10% acetic acid, let stand 90 min to overnight in the dark, transfer reaction mixture to a separatory funnel with three 25 mL portions of dichloromethane, add 5 mL 2 M HCl, shake for 1 min, wash organic layer with 5 mL 5% $NaHCO_3$ solution, filter through 10-20 g anhydrous sodium sulfate. Extract acid aqueous layer again with 25 mL dichloromethane. Combine dichloromethane layers and evaporate to dryness at 35° under reduced pressure. Dissolve residue in 2 mL IS solution, inject a 20 μL aliquot. (Prepare IS solution by dissolving 10 μL benzaldehyde in 100 mL dichloromethane, evaporate 1 mL to dryness under reduced pressure, dissolve residue in 1 mL 10% acetic acid, add 0.5 mL 0.08% dansyl hydrazine in 10% acetic acid, let stand 90 min to overnight in the dark, transfer reaction mixture to a separatory funnel with three 25 mL portions of dichloromethane, add 5 mL 2 M HCl, shake for 1 min, wash organic layer with 5 mL 5% $NaHCO_3$ solution, filter through 10-20 g anhydrous sodium sulfate. Extract acid aqueous layer again with 25 mL dichloromethane. Combine dichloromethane layers and evaporate to dryness at 35° under reduced pressure. Dissolve residue in 100 mL MeCN then dilute an aliquot 1:4 with MeCN.)

HPLC VARIABLES
Column: 250 × 4 10 μm Lichrosorb RP-18
Mobile phase: MeCN:water 58:42
Flow rate: 1
Injection volume: 20
Detector: F ex 254 em 500 filter

CHROMATOGRAM
Retention time: 9.0
Internal standard: benzaldehyde (derivatized) (12.18)
Limit of detection: 5 ng/g

OTHER SUBSTANCES
Extracted: penicillin V, phenethicillin, penicillin G, oxacillin, cloxacillin, dicloxacillin, methicillin

KEY WORDS
derivatization

REFERENCE
Munns,R.K.; Shimoda,W.; Roybal,J.E.; Vieira,C. Multiresidue method for determination of eight neutral β-lactam penicillins in milk by fluorescence-liquid chromatography, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 968–971.

SAMPLE
Matrix: milk
Sample preparation: Add 2 volumes MeCN to milk, stand 5 min, decant aqueous portion, suction filter, extract with an equal volume of 1:1 methylene chloride:hexane, centrifuge aqueous phase at 3000 rpm for 10 min. Dilute 3:1 with 20 mM sodium acetate buffer and filter (0.2 μm nylon). Inject 50 μL onto column with mobile phase A, run mobile phase A for 30 min and elute to waste. After 30 min switch to mobile phase B and elute through detector.

HPLC VARIABLES
Column: 100 × 8 Radial-Pak 10 μm μBondapak C18
Mobile phase: A 20 mM sodium acetate buffer; B Gradient. MeCN:MeOH:20 mM sodium acetate buffer from 15:10:75 to 30:0:70 over 15 min and hold at 30:0:70
Flow rate: A 3; B 2
Injection volume: 50
Detector: E, Waters 464 pulsed electrochemical detector using a thin layer cell with a Ag/AgCl reference electrode. E1 = 1300 mV for 0.166 s, E2 = 1500 mV for 0.166 s, E3 = -200 mV for 0.333 s.

CHROMATOGRAM
Retention time: 18
Limit of detection: 0.2 ppm

OTHER SUBSTANCES
Simultaneous: penicillin V, ampicillin, methicillin, penicillin G, oxacillin, dicloxacillin, cloxacillin

KEY WORDS
column-switching

REFERENCE
Kirchmann,E.; Earley,R.L.; Welch,L.E. The electrochemical detection of penicillins in milk, *J.Liq.Chromatogr.*, **1994**, *17*, 1755–1772.

SAMPLE
Matrix: milk
Sample preparation: Condition a Bond Elut C8 SPE cartridge with 5 mL MeOH and 5 mL water. 20 mL Milk + 20 mL buffer, heat at 60° for 20 min or until milk curdles, centrifuge for 10 min, add the supernatant to the SPE cartridge, wash with two 2.5 mL portions of water, elute with 2.5 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, extract the residue with three 100 μL portions of 50 mM pH 6.0 potassium phosphate buffer, filter (0.2 μm), inject an aliquot of the filtrate. (Buffer was 545 mL 100 mM citric acid, 455 mL 200 mM Na_2HPO_4, and 74.4 g EDTA, adjust to pH 4.5 with ammonium hydroxide, make up to 2 L with water.)

HPLC VARIABLES
Column: 250 × 4.6 10 μm Lichrosorb RP-8
Mobile phase: MeOH:50 mM pH 6.0 potassium phosphate buffer 35:65
Flow rate: 1
Injection volume: 200
Detector: UV 210 or Charm II assay

CHROMATOGRAM
Retention time: 67.00

OTHER SUBSTANCES
Extracted: ampicillin, ceftiofur, cephapirin, cloxacillin, dicloxacillin, oxacillin, penicillin G
Simultaneous: amoxicillin

KEY WORDS
SPE

REFERENCE
Zomer,E.; Quintana,J.; Saul,S.; Charm,S.E. LC-Receptograms: A method for identification and quantitation of β-lactams in milk by liquid chromatography with microbial receptor assay, *J.AOAC Int.*, **1995**, *78*, 1165–1172.

SAMPLE
Matrix: 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: 19

OTHER SUBSTANCES
Simultaneous: dicloxacillin, methicillin, penicillin G, penicillin V, cloxacillin, carbenicillin

REFERENCE
Moats,W.A. Effect of the silica support of bonded reversed-phase columns on chromatography of some antibiotic compounds, *J.Chromatogr.*, **1986**, *366*, 69–78.

SAMPLE
Matrix: solutions
Sample preparation: React the antibiotic, triethylamine, and 1-(2,5-dihydroxyphenyl)-2-bromoethanone in a 1:2:4 molar ratio in DMF at 45° for 2 h (use dibenzo-18-crown-6 to make the sodium salt soluble), inject a 10 μL aliquot. (Preparation of 1-(2,5-dihydroxyphenyl)-2-bromoethanone is as follows. Stir 27.6 g 1,4-dimethoxybenzene and 28 mL bromoacetyl bromide at 0°, add 53.4 g aluminum bromide over 10 min (an exothermic reactions ensues), let stand at room temperature for 12 h, add 100 mL 48% HBr, add 100 g ice, stir for 1 h, extract twice with 200 mL portions of diethyl ether. Combine the extracts and wash them 3 times with 200 mL portions of water, dry over 40 g anhydrous magnesium sulfate, evaporate to dryness, recrystallize the product 3 times from EtOH to yield 1-(2,5-dihydroxyphenyl)-2- bromoethanone monobromoacetate (mp 105-107°). Dissolve 11 g 1-(2,5-dihydroxyphenyl)-2-bromoethanone monobromoacetate in 200 mL warm dry MeOH saturated with HBr, stir for 18 h, add 200 mL water, cool to -10°. Collect the yellow solid and dry it under vacuum at 50° for 48 h, recrystallize from toluene:heptane 50:50 then toluene to obtain 1-(2,5-dihydroxyphenyl)-2-bromoethanone as yellow needles (mp 117-119°).)

HPLC VARIABLES
Column: 250 × 4 7 μm RP-18 LiChrocart (Merck)
Mobile phase: MeOH:100 mM pH 6.5 sodium acetate 58:42
Flow rate: 1
Injection volume: 10
Detector: E, Bioanalytical Systems Model LC4B, glassy carbon electrode 0.8 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 30.7

OTHER SUBSTANCES
Extracted: carbenicillin, cephapirin, cloxacillin, dicloxacillin, hetacillin, methicillin, oxacillin, penicillin G

KEY WORDS
derivatization

REFERENCE
Munns,R.K.; Roybal,J.E.; Shimoda,W.; Hurlbut,J.A. 1-(4-Hydroxyphenyl)-, 1-(2,4-dihydroxyphenyl)- and 1-(2,5-dihydroxyphenyl)-2-bromoethanones: new labels for determination of carboxylic acids by high-performance liquid chromatography with electrochemical and ultraviolet detection, *J.Chromatogr.*, **1988**, *442*, 209–218.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 2.5-5 μg/mL solution, inject a 20 μL aliquot.

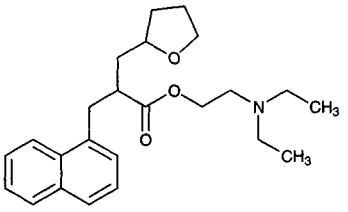
HPLC VARIABLES
Column: 80 × 4.6 3.65 μm Zorbax Rx-SIL (similar to Zorbax SB-C8 (Mac-Mod Analytical))

Mobile phase: MeCN:0.1% trifluoroacetic acid 44:56
Flow rate: 1
Injection volume: 20
Detector: UV 235

CHROMATOGRAM
Retention time: k' 2.7

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.

Nafronyl



Molecular formula: $C_{24}H_{33}NO_3$
Molecular weight: 383.53
CAS Registry No.: 31329-57-4, 3200-06-4 (acid oxalate)
Merck Index: 6440
Lednicer No.: 2 213

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 225.2

CHROMATOGRAM
Retention time: 15.832

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

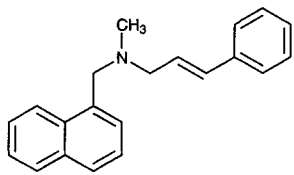
SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher100RP-18
Column: 250 × 4 5 μm Spherisorb ODS 2
Mobile phase: MeCN:buffer 60:40. (Buffer was 20 mM sodium acetate containing 0.28% triethylamine, adjusted to pH 4.5 with acetic acid.)
Flow rate: 1.5
Detector: UV 280

CHROMATOGRAM
Retention time: k' 5.25

REFERENCE
Yang,H.; Thyrion,F.C. Determination of six pharmaceuticals and their degradation products in reversed-phase high performance liquid chromatography by using amine additives, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1347–1357.

Naftifine



Molecular formula: $C_{21}H_{21}N$
Molecular weight: 287.40
CAS Registry No.: 65472-88-0, 65473-14-5 (HCl)
Merck Index: 6442
Lednicer No.: 4 55

SAMPLE
Matrix: blood, lymph, tissue
Sample preparation: Grind skin in a mortar under liquid nitrogen. Homogenize blood, lymph, or skin with 10 volumes of EtOH, centrifuge at 6000 g for 10 min, wash the sediment twice with EtOH. Evaporate, reconstitute, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Lichrosrb RP8
Mobile phase: Gradient. MeOH:10 mM pH 7.2 Sorensen buffer from 50:50 to 100:0 over 30 min
Detector: UV 260

CHROMATOGRAM
Retention time: 30

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
rat; skin

REFERENCE
Grimus,R.C.; Schuster,I. The role of the lymphatic transport in the enteral absorption of naftifine by the rat, *Xenobiotica*, **1984**, *14*, 287–294.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 100 μg/mL solution in MeOH, inject an aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 60 RP-Select-B
Mobile phase: MeCN: pH 2 trifluoroacetic acid buffer 35:65.
Flow rate: 1

Detector: UV 254

CHROMATOGRAM
Retention time: 7.33

OTHER SUBSTANCES
Also analyzed: cloxyquin, chlorphenesin, sulbentine, tolnaftate

KEY WORDS
photodegradation; kinetics

REFERENCE
Thoma,K.; Kübler,N.; Reimann,E. Untersuchung der Photostabilität von Antimykotica. 3. Mitteilung: Photostabilität lokal wirksamer Antimykotica [Photodegradation of antimycotic drugs. 3. Communication: Photodegradation of topical antimycotics], *Pharmazie*, **1997**, *52*, 362–373.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 400 μg/mL solution in MeOH, inject an aliquot.

HPLC VARIABLES
Column: 125 × 2 4 μm Superspher 60 RP-18
Mobile phase: Gradient. A was MeOH:water 90:10. B was MeOH:pH 2 trifluoroacetic acid buffer 15:85. A:B 0:100 for 10 min, 20:80 for 15 min, then A:B 80:20 (step gradient).
Flow rate: 0.5
Detector: UV 225

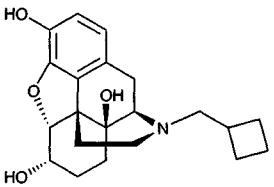
CHROMATOGRAM
Retention time: 11.3

OTHER SUBSTANCES
Also analyzed: cloxyquin, chlorphenesin, sulbentine, tolnaftate

KEY WORDS
photodegradation; kinetics

REFERENCE
Thoma,K.; Kübler,N.; Reimann,E. Untersuchung der Photostabilität von Antimykotica. 3. Mitteilung: Photostabilität lokal wirksamer Antimykotica [Photodegradation of antimycotic drugs. 3. Communication: Photodegradation of topical antimycotics], *Pharmazie*, **1997**, *52*, 362–373.

Nalbuphine



Molecular formula: $C_{21}H_{27}NO_4$
Molecular weight: 357.45
CAS Registry No.: 20594-83-6, 23277-43-2 (HCl)
Merck Index: 6444
Lednicer No.: 2 319

SAMPLE
Matrix: blood
Sample preparation: Add 1 mL of pH 9 borate buffer to 500 μL plasma, extract with 10 mL chloroform:isopropanol 98:2, shake mechanically for 15 min, centrifuge at 2000 rpm for 10 min. Remove the organic layer using phase-separating filter paper and add it to 200 μL 17 mM phosphoric acid, shake mechanically for 2 min, centrifuge at 2000 rpm for 5 min. Inject a 50 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Hypersil ODS
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:pH 3.4 phosphate buffer 20:80
Column temperature: 50
Flow rate: 1.0
Injection volume: 50
Detector: E, ESA Coulochem II Model 5200, Model 5020 guard cell 550 mV, Model 5021 analytical cell in oxidation screening mode E1=60 mV, E2=450 mV

CHROMATOGRAM
Retention time: 13.4
Limit of detection: 0.1 ng/mL
Limit of quantitation: 0.3 ng/mL

OTHER SUBSTANCES
Noninterfering: metabolites, caffeine, codeine, naloxone, theobromine, theophylline

KEY WORDS
plasma; recycle mobile phase; pharmacokinetics

REFERENCE
de Cazanove,F.; Kinowski,J.-M.; Audran,M.; Rochette,A.; Bressolle,F. Determination of nalbuphine in human plasma by high-performance liquid chromatography with electrochemical detection. Application to a pharmacokinetic study, *J.Chromatogr.B*, **1997**, *690*, 203–210.

SAMPLE
Matrix: blood
Sample preparation: Condition a 10 mL 130 mg Bond Elut Certify SPE cartridge with 2 mL MeOH and 2 mL 200 mM pH 8 Tris buffer without column drying. Dilute 500 μL plasma with 2 mL 200 mM pH 8 Tris buffer, add 50 ng IS, pass slowly through the column. Wash with 2 mL water, 2 mL 10 mM pH 3.3 acetic acid and dry completely. Wash with 3 mL MeOH and elute with two 2 mL portions of dichlormethane:2-propanol:ammonia 79:20:1. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase containing 2% of isopropanol, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 I.D. 5 μm LiChrospher C18
Mobile phase: MeOH:water 20:80 containing 24 mM KH_2PO_4 and 60 mM EDTA, pH 3.4
Flow rate: 0.8
Injection volume: 50
Detector: E, ESA Coulochem, Model 5020 guard cell +0.5 V, Model 5011 dual electrode analytical cell operating in the oxidation screening mode, E1 +0.06 V, E2 +0.4 V

CHROMATOGRAM
Retention time: 13.6
Internal standard: 6-monoacetylmorphine (8.4)
Limit of detection: 20 pg/mL
Limit of quantitation: 100 pg/mL

OTHER SUBSTANCES
Noninterfering: morphine, nalorphine, naloxone, naltrexone, norbuprenorphine

KEY WORDS
SPE; plasma; pharmacokinetics

REFERENCE
Nicolle,E.; Veitl,S.; Guimier,C.; Bessard,G. Modified method of nalbuphine determination in plasma: validation and application to pharmacokinetics of the rectal route, *J.Chromatogr.B*, **1997**, *690*, 89–97.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 500 mM pH 9.25 sodium carbonate buffer + 3 mL hexane:isoamyl alcohol 9:1, mix on a rotary shaker for 30 min, centrifuge at 1880 g for 20 min, freeze at -20° for 1 h (for rabbit plasma perform on half-scale). Remove the organic layer and evaporate it to dryness under a stream of air, reconstitute in 250 μL mobile phase, inject a 200 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Applied Biosystems pre-column
Column: 100 × 2 10 μm μPorasil
Mobile phase: MeCN:5 mM pH 3.75 sodium acetate 80:20
Flow rate: 1
Injection volume: 200
Detector: F ex 210 em 345

CHROMATOGRAM
Retention time: 11.7
Internal standard: nalbuphine

OTHER SUBSTANCES
Simultaneous: butorphanol, morphine, ethylmorphine, codeine, fentanyl, meperidine, tramadol, buprenorphine
Noninterfering: thiopentone, succinylcholine, pancuronium, diazepam, atropine, neostigmine
Interfering: nalorphine

KEY WORDS
plasma; human; pig; dog; rabbit; nalbuphine is IS

REFERENCE
Ho,S.-T.; Wang,J.-J.; Ho,W.; Hu,O.Y.-P. Determination of buprenorphine by high-performance liquid chromatography with fluorescence detection: application to human and rabbit pharmacokinetic studies, *J.Chromatogr.*, **1991**, *570*, 339–350.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 100 μL 200 ng/mL 6-monoacetylmorphine in water + 2 mL buffer + 10 mL hexane:dichloromethane:isopropanol 69:30:1, shake mechanically at 60 rpm for 15 min, centrifuge at 2000 g at 4° for 5 min. Remove the upper organic layer and add it to 200 μL 17 mM phosphoric acid, shake for 2 min, centrifuge at 2000 g at 4° for 5 min, inject a 50 μL aliquot of the aqueous layer. (Buffer was 100 mM boric acid and 100 mM KCl adjusted to pH 9 with 100 mM NaOH.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Lichrospher C18
Mobile phase: MeOH:water 20:80 containing 24 mM KH_2PO_4 and 0.06 mM disodium EDTA adjusted to pH 3.4 with orthophosphoric acid
Flow rate: 0.8
Injection volume: 50
Detector: E, ESA Model 5100A Coulochem, Model 5020 guard cell 0.50 V, Model 5011 analytical cell in oxidation screening mode, first electrode 0.06 V, second electrode 0.40 V

CHROMATOGRAM
Retention time: 8.5
Internal standard: 6-monoacetylmorphine (5.7)
Limit of detection: 0.1 ng/mL

OTHER SUBSTANCES
Extracted: naloxone
Noninterfering: buprenorphine, codeine, dextromoramide, dextropropoxyphene, ethylmorphine, fentanyl, methadone, morphine, nalorphine, norcodeine, pholcodine

KEY WORDS
plasma

REFERENCE
Nicolle,E.; Michaut,S.; Serre-Debeauvais,F.; Bessard,G. Rapid and sensitive high-performance liquid chromatographic assay for nalbuphine in plasma, *J.Chromatogr.B*, **1995**, *663*, 111–117.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 259

CHROMATOGRAM
Retention time: 3.67
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil;

lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 5 μm Biophase ODS
Mobile phase: MeCN:buffer:water 20:10:70 containing 3 mM sodium 1-heptanesulfonate, pH 3.7 (Buffer was 2.1 M acetic acid containing 400 mM ammonium acetate.)
Flow rate: 1
Injection volume: 50
Detector: E, Bioanalytical Systems Model 4B, glassy carbon electrode 0.95 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 13.58

OTHER SUBSTANCES

Simultaneous: ritodrine

REFERENCE

Kuhnert,P.; Erhard,P.; Dixon,A.; Kuhnert,B.; Gross,T. Determination of ritodrine in plasma using HPLC, *J.Liq.Chromatogr.*, **1983**, *6*, 2775–2783.

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: 6.56 (A), 3.61 (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, mazin-

dol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, 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
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.

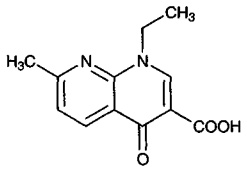
SAMPLE
Matrix: urine
Sample preparation: 1.25 mL (?) Urine + 250 µL 20000 U/mL β-glucuronidase + 500 µL 1 M pH 5.2 sodium acetate buffer, heat at 56° for 12 h, add 1 mL 40% pH 9.2 phosphate buffer, add 50 µL 10 µg/mL levallorphan, add 10 mL chloroform:isopropanol:n-heptane 50:17:33, agitate, centrifuge at 2000 g for 10 min. Remove the aqueous phase and add it to 5 mL 200 mM HCl, extract. Remove the aqueous phase and add it to 1 mL 40% pH 9.2 phosphate buffer, add 500 µL concentrated ammonia solution, extract with 5 mL chloroform. Remove the organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, inject a 70 µL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPak C18
Mobile phase: MeOH:THF:10 mM pH 2.6 KH_2PO_4 65:5:30
Column temperature: 30
Flow rate: 0.8
Injection volume: 70
Detector: UV 215-238 (scan mode)

CHROMATOGRAM
Retention time: 3.83
Internal standard: levallorphan (4.38)
Limit of detection: 25 ng/mL

REFERENCE
Kintz,P.; Tracqui,A.; Mangin,P. Determination of nalbuphine using high-performance liquid chromatography coupled to photodiode-array detection and gas chromatography coupled to mass spectrometry, *J.Chromatogr.*, **1992**, *579*, 172–176.

Nalidixic acid



Molecular formula: $C_{12}H_{17}N_2O_3$
Molecular weight: 232.24
CAS Registry No.: 389-08-2, 3374-05-8 (Na salt), 15769-77-4 (Na salt monohydrate)
Merck Index: 6446
Lednicer No.: 1 429

SAMPLE

Matrix: blood
Sample preparation: Condition a 100 mg Bond-Elut C18 SPE cartridge with two 3 mL portions of MeOH and 3 mL of 0.05% orthophosphoric acid. Centrifuge plasma at 500 g for 5 min. Mix 200 μL centrifuged plasma with 3.2 mL buffer, vortex for 1 min. Add the mixture to the SPE cartridge under low vacuum, wash with 1 mL water and 500 μL 0.05% orthophosphoric acid solution, dry under vacuum. Elute with six 250 μL portions of MeCN. Evaporate the combined eluates to dryness under a stream of nitrogen at 40°. Reconstitute the residue with 200 μL buffer, vortex, sonicate, let stand for 30 min, vortex again. Inject a 50 μL aliquot. (Buffer was 1/15 M pH 8.0 phosphate buffer.)

HPLC VARIABLES

Guard column: 4 × 4 5 μm LiChroSpher 100 RP-18 end-capped
Column: 125 × 4 5 μm LiChroSpher 100 RP-18 end-capped
Mobile phase: MeCN:20 mM pH 2.3 orthophosphoric acid:dimethylformamide 10:60:30
Flow rate: 0.8
Injection volume: 50
Detector: UV 340

CHROMATOGRAM

Retention time: 9.66
Internal standard: nalidixic acid

OTHER SUBSTANCES

Extracted: oxolinic acid
Noninterfering: 2-phenoxyethanol

KEY WORDS

plasma; sea bass; SPE; nalidixic acid is IS; fish

REFERENCE

Loussouarn,S.; Pouliquen,H.; Armand,F. High-performance liquid chromatographic determination of oxolinic acid in the plasma of seabass (Dicentrarchus labrax) anaesthetized with 2-phenoxyethanol, *J.Chromatogr.B*, **1997**, *698*, 251–259.

SAMPLE

Matrix: blood
Sample preparation: Filter 1 mL plasma using a micropartition system (MPS-1, Amicon, MA) while centrifuging at 2000 g for 20 min at 10°, inject an aliquot of the ultrafiltrate.

HPLC VARIABLES

Column: 250 × 4.6 Spherisorb ODS-2 endcapped
Mobile phase: MeCN:MeOH:buffer 16:34:50 containing 5 mM tetrabutylammonium sulfate, adjusted to pH 2.5 with 1 NaOH (Buffer was 100 mM citric acid containing 200 mM ammonium perchlorate.)
Column temperature: 37
Flow rate: 0.8
Detector: UV 258

CHROMATOGRAM
Retention time: 8.96
Internal standard: cinoxacin (6.26)

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Zlotos,G.; Bücker,A.; Kinzig-Schippers,M.; Sorgel,F.; Holzgrabe,U. Plasma protein binding of gyrase inhibitors, *J.Pharm.Sci.*, **1998**, *87*, 215–220.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 258.2

CHROMATOGRAM
Retention time: 16.008

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Measure out powdered tablets or suspension containing 150 mg nalidixic acid, add 400 mL MeOH, sonicate for 30 min, shake for 30 min, sonicate for 30 min. Make up to 500 mL with MeOH, mix , filter. Dilute 3.0 mL filtrate and 1.0 mL 800 μg/mL sulfanilic acid in mobile phase to 25 mL with MeOH. Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:buffer 67.5:32.5 (Prepare mobile phase by dissolving 784 mg K_2HPO_4 in 325 mL water. Dissolve 2.62 g hexadecyltrimethylammonium bromide in 350 mL MeOH. Combine solutions, add 325 mL MeOH, mix.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 4.9
Internal standard: sulfanilic acid (3.5)

KEY WORDS
tablets; suspensions

REFERENCE
Walker,S.T. Liquid chromatographic determination of nalidixic acid in pharmaceutical preparations, *J.AOAC Int.*, **1996**, *79*, 431–433.

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: 10
Internal standard: trimethoprim (3)
Limit of quantitation: 100 μg/mL

OTHER SUBSTANCES
Simultaneous: phenazopyridine

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: Pulverize tablets, add 10 mL water, swirl for 2-3 min, make up to 250 mL with MeOH, shake mechanically for 5 min. Remove a 5 mL aliquot and centrifuge it at 3000 rpm for 5 min. Remove 100 μL of the supernatant and add it to 200 μL 1 mg/mL methyl p-hydroxybenzoate, make up to 10 mL with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:50 mM ammonium acetate 30:5:65, pH 5
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7.27
Internal standard: methyl p-hydroxybenzoate (4.0)

KEY WORDS
tablets

REFERENCE
Foda,N.H. High-performance liquid chromatographic determination of nalidixic acid in tablets, *J.Liq.Chromatogr.*, **1995**, *18*, 4135–4147.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm 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: 5
Internal standard: nalidixic acid

OTHER SUBSTANCES
Simultaneous: ciprofloxacin, fenbufen, felbinac

KEY WORDS
nalidixic acid is IS

REFERENCE
Naora,K.; Katagiri,Y.; Ichikawa,N.; Hayashibara,M.; Iwamoto,K. Simultaneous high-performance liquid chromatographic determination of ciprofloxacin, fenbufe and felbinac in rat plasma, *J.Chromatogr.*, **1990**, *530*, 186–191.

SAMPLE
Matrix: tissue
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL MeOH and 20 mL water. Homogenize 5 g tissue with 100 mL MeOH:0.1% metaphosphoric acid 40:60 at high speed for 2 min, filter homogenate through a 2 mm layer of Hyflo Super-Cel. Evaporate the filtrate to about 20 mL under reduced pressure at 40°, add the residue to the SPE cartridge at 5 mL/min, wash with 20 mL water, wash with 10 mL MeOH:water 5:95, elute with 10 mL MeOH. Evaporate the eluate to dryness under reduced pressure, reconstitute the residue in 1 mL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Kaseisorb LC ODS 300-5 (Tokyo Kasei Kogyo)
Mobile phase: MeCN:5 mM NaH_2PO_4 40:60
Flow rate: 0.5
Injection volume: 10
Detector: F ex 325 em 365

CHROMATOGRAM
Retention time: 11
Limit of detection: 10 ng/g

OTHER SUBSTANCES
Extracted: oxolinic acid, piromidic acid (UV 280)
Noninterfering: ampicillin, chlortetracycline, colistin, doxycycline, erythromycin, oxytetracycline, sodium nifulstyrate, spiramycin, sulfamdimethoxine, sulfamonomethoxine, sulfisozole, tetracycline, thiamphenicol

KEY WORDS
eel; fish; trout; carp; muscle; SPE

REFERENCE

Horie,M.; Saito,K.; Hoshino,Y.; Nose,N.; Mochizuki,E.; Nakazawa,H. Simultaneous determination of nalidixic acid, oxolinic acid and piromidic acid in fish by high-performance liquid chromatography with fluorescence and UV detection, *J.Chromatogr.*, **1987**, *402*, 301–308.

SAMPLE

Matrix: tissue

Sample preparation: Condition a 500 mg Bond Elut C18 SPE cartridge with 5 mL MeOH and 10 mL water. Homogenize 5 g tissue with 100 mL MeOH:0.2% metaphosphoric acid 40:60 for 2 min, filter through a 1 mm layer of Hyflo Super-Cel. Evaporate the filtrate under reduced pressure at 40° to 10 mL, add the residue to the SPE cartridge, wash with 10 mL water, elute with 10 mL MeOH. Evaporate the eluate to dryness under reduced pressure, take up the residue in 1 mL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 Newguard RP-8
Column: 150 × 4.6 5 μm Inertsil ODS
Mobile phase: MeCN:5 mM oxalic acid 45:55
Flow rate: 0.5
Injection volume: 10
Detector: UV 265

CHROMATOGRAM

Retention time: 11
Limit of detection: 50 ng/g

OTHER SUBSTANCES

Extracted: sulfadimethoxine, sulfamonomethoxine, sulfisozole, oxolinic acid, piromidic acid, sodium nifurstyrenate, furazolidone

KEY WORDS

fish; SPE

REFERENCE

Horie,M.; Saito,K.; Hoshino,Y.; Nose,N.; Nakazawa,H.; Yamane,Y. Simultaneous determination of residual synthetic antibacterials in fish by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *538*, 484–491.

SAMPLE

Matrix: tissue

Sample preparation: Condition a 200 mg Bond Elut C18 SPE cartridge with 5 mL MeOH and 10 mL water. Homogenize 5 g tissue with 100 mL MeOH:0.2% metaphosphoric acid 1:2 at high speed for 2 min, filter homogenate through a 2 mm layer of Hyflo Super-Cel. Evaporate the filtrate to about 30 mL under reduced pressure at 50°, add the residue to the SPE cartridge, wash with 20 mL water, elute with 10 mL MeOH. Evaporate the eluate to dryness under reduced pressure, reconstitute the residue in 1 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:100 mM pH 4.5 ammonium acetate buffer 40:60
Column temperature: 35
Flow rate: 0.8
Injection volume: 20
Detector: MS, Shimadzu LCMS-QP 1000 quadrupole, Vestec thermospray interface, filament-on mode (ionization by electron beam), vaporizer 165°, ion source block 260°, repeller potential 0 V, electron multiplier 3000 V, ionization potential 1000 eV, positive ion mode, scan m/z 150-400; SIM m/z 233

CHROMATOGRAM

Retention time: 4
Limit of detection: 10 ng/g

OTHER SUBSTANCES
Extracted: oxolinic acid, piromidic acid

KEY WORDS
fish; muscle; thermospray; SPE

REFERENCE
Horie,M.; Saito,K.; Nose,N.; Tera,M.; Nakazawa,H. Confirmation of residual oxolinic acid, nalidixic acid and piromidic acid in fish by thermospray liquid chromatography-mass spectrometry, *J.Liq.Chromatogr.*, **1993**, *16*, 1463–1472.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Oster model 54841) 10 g catfish tissue with 50 mL acetone for 10-20 s, centrifuge at 3000 rpm for 2 min, filter (paper) supernatant. Repeat extraction twice more, combine extracts, add 15 mL n-propanol, evaporate to 15 mL under reduced pressure at 40°. Transfer residue to another container using 20 mL acetone, 30 mL hexane, and 60 mL 3% NaCl. Shake vigorously for 10 s, centrifuge at 3000 rpm for 1 min, discard hexane layer, wash with another 30 mL hexane. Extract the remaining mixture twice with 25 mL portions of chloroform by shaking vigorously for 1 min. Combine the chloroform extracts and add them to 25 mL 100 mM NaOH, shake vigorously for 1 min. Discard the chloroform layer and wash the aqueous layer with 25 mL chloroform with gentle rocking (10 rocks). Add the aqueous layer to 25 mL 75 mM phosphoric acid (pH 6 ± 0.5), extract twice with 25 mL portions of chloroform (vigorous shaking for 1 min). Filter (paper) extracts and evaporate them to dryness under reduced pressure at 40°, add 2 mL MeCN, evaporate to dryness, reconstitute in 1 mL mobile phase, sonicate briefly, filter (0.45 μm), inject a 100 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm PLRP-S polymer (Polymer Laboratories)
Mobile phase: MeCN:THF:20 mM phosphoric acid 16:12:72
Column temperature: 46
Injection volume: 100
Detector: F ex 325 em 365

CHROMATOGRAM
Retention time: 9.9
Limit of quantitation: 10 ng/g

OTHER SUBSTANCES
Extracted: flumequine, oxolinic acid, piromidic acid (UV 280)

KEY WORDS
catfish; fish

REFERENCE
Munns,R.K.; Turnipseed,S.B.; Pfenning,A.P.; Roybal,J.E.; Holland,D.C.; Long,A.R.; Plakas,S.M. Determination of residues of flumequine and nalidixic, oxolinic, and piromidic acids in catfish by liquid chromatography with fluorescence and UV detection, *J.AOAC Int.*, **1995**, *78*, 343–352.

SAMPLE
Matrix: urine
Sample preparation: Make up 1 mL urine to 25 mL with mobile phase, filter (0.45 μm). Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeCN:400 μM oxalic acid in water 28:72
Flow rate: 2.0
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 4.24
Limit of detection: 3.09 μg/mL

OTHER SUBSTANCES
Simultaneous: metabolites, cinoxacin, oxolinic acid, pipemidic acid, piromidic acid

KEY WORDS
antibiotics

REFERENCE
Durán Merá,I.; Galeano Díaz,T.; Rodríguez Cáceres,M.I.; Salinas López,F. Determination of the chemotherapeutic quinolonic and cinolonic derivatives in urine by high-performance liquid chromatography with ultraviolet and fluorescence detection in series, *J.Chromatogr.A*, **1997**, *787*, 119–127.

SAMPLE
Matrix: urine
Sample preparation: Make up 1 mL urine to 25 mL with deionized water. Adjust to pH 2.5-3 with HCl, extract with 25 mL chloroform. Separate the organic layer, dry the organic phase with sodium sulfate, evaporate it to dryness. Dissolve the residue in 3 mL MeCN, dilute to 10mL with water, filter (0.45 μm). Inject a 20 μL aliquot.

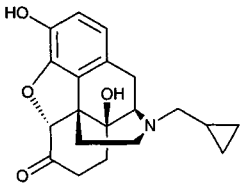
HPLC VARIABLES
Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeCN:400 μM oxalic acid in water 28:72
Flow rate: 2.0
Injection volume: 20
Detector: F ex 260 em 360

CHROMATOGRAM
Retention time: 4.24
Limit of detection: 21.6 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, oxolinic acid

REFERENCE
Durán Merá,I.; Galeano Díaz,T.; Rodríguez Cáceres,M.I.; Salinas López,F. Determination of the chemotherapeutic quinolonic and cinolonic derivatives in urine by high-performance liquid chromatography with ultraviolet and fluorescence detection in series, *J.Chromatogr.A*, **1997**, *787*, 119–127.

Nalmefene



Molecular formula: $C_{21}H_{25}NO_3$
Molecular weight: 339.43
CAS Registry No.: 55096-26-9
Merck Index: 6447

SAMPLE
Matrix: blood
Sample preparation: Make plasma alkaline and extract with MTBE. Evaporate the organic layer to dryness under nitrogen, reconstitute the residue in 100 μL MeOH:water 90:10, inject an aliquot.

HPLC VARIABLES
Column: Zorbax SB-C18

CHROMATOGRAM
Retention time: 4.24
Limit of detection: 3.09 μg/mL

OTHER SUBSTANCES
Simultaneous: metabolites, cinoxacin, oxolinic acid, pipemidic acid, piromidic acid

KEY WORDS
antibiotics

REFERENCE
Durán Merá,I.; Galeano Díaz,T.; Rodríguez Cáceres,M.I.; Salinas López,F. Determination of the chemotherapeutic quinolonic and cinolonic derivatives in urine by high-performance liquid chromatography with ultraviolet and fluorescence detection in series, *J.Chromatogr.A*, **1997**, *787*, 119–127.

SAMPLE
Matrix: urine
Sample preparation: Make up 1 mL urine to 25 mL with deionized water. Adjust to pH 2.5-3 with HCl, extract with 25 mL chloroform. Separate the organic layer, dry the organic phase with sodium sulfate, evaporate it to dryness. Dissolve the residue in 3 mL MeCN, dilute to 10mL with water, filter (0.45 μm). Inject a 20 μL aliquot.

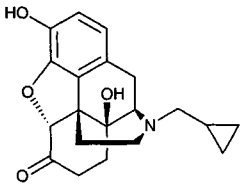
HPLC VARIABLES
Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeCN:400 μM oxalic acid in water 28:72
Flow rate: 2.0
Injection volume: 20
Detector: F ex 260 em 360

CHROMATOGRAM
Retention time: 4.24
Limit of detection: 21.6 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, oxolinic acid

REFERENCE
Durán Merá,I.; Galeano Díaz,T.; Rodríguez Cáceres,M.I.; Salinas López,F. Determination of the chemotherapeutic quinolonic and cinolonic derivatives in urine by high-performance liquid chromatography with ultraviolet and fluorescence detection in series, *J.Chromatogr.A*, **1997**, *787*, 119–127.

Nalmefene



Molecular formula: $C_{21}H_{25}NO_3$
Molecular weight: 339.43
CAS Registry No.: 55096-26-9
Merck Index: 6447

SAMPLE
Matrix: blood
Sample preparation: Make plasma alkaline and extract with MTBE. Evaporate the organic layer to dryness under nitrogen, reconstitute the residue in 100 μL MeOH:water 90:10, inject an aliquot.

HPLC VARIABLES
Column: Zorbax SB-C18

Mobile phase: MeOH:10mM pH 4.0 ammonium formate 40:60
Flow rate: 1
Detector: MS, Sciex API IIIplus tandem mass, positive ion mode, 316.0/298.0 parent/product ions

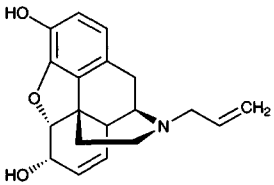
CHROMATOGRAM
Retention time: 6.9
Internal standard: nalmefene

OTHER SUBSTANCES
Extracted: oxycodone

KEY WORDS
plasma; nalmefene is IS

REFERENCE
Kaisershot,C.; Pierce,A.; Talaat,R. Quantitative analysis of oxycodone and its metabolites noroxycodone and oxymorphone in human plasma by high-performance liquid chromatography with ionspray tandem mass spectrometry (Abstract 2129), *Pharm.Res.*, **1997**, *14*, S262.

Nalorphine



Molecular formula: $C_{19}H_{21}NO_3$
Molecular weight: 311.38
CAS Registry No.: 62-67-9, 57-29-4 (HCl)
Merck Index: 6448

SAMPLE
Matrix: bile, blood
Sample preparation: 0.5 mL Blood or bile 300 μL 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) μL 0.2% phosphoric acid, gently rotate for 30 min, discard organic layer, inject 50 μL 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 × 3.9 5 μm Nova-Pak phenyl
Mobile phase: MeCN:10 mM pH 6.6 NaH_2PO_4 10:90
Flow rate: 1.2
Injection volume: 50
Detector: UV 210 and F ex 220 em 370 (cut-off)

CHROMATOGRAM
Retention time: 23.5
Internal standard: nalorphine

OTHER SUBSTANCES
Simultaneous: hydrocodone, oxycodone, dihydrocodeine, morphine, codeine, 6-monoacetylmorphine
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, nalorphine is IS

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 μL Bile, 3 mL blood, or 5 mL tissue homogenate + 1 mL 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 carefully adding powdered ammonium carbonate until the solution was 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 μL MeOH, vortex for 30 s, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm RP-18 Spheri-5
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:50 mM pH 7 phosphate buffer 40:60 (Place a 70 × 2 30-38 μm CoPell 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: 14.72
Internal standard: nalorphine

OTHER SUBSTANCES
Extracted: codeine, 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

KEY WORDS
nalorphine is IS

REFERENCE
Hepler,B.R.; Sutheimer,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: 200 μL Plasma + 200 μL 3.5% sodium carbonate, extract with 4 mL dry ether. Extract the organic layer with 200 μL 20 mM phosphoric acid. Inject an aliquot of the phosphoric acid solution.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax C8
Mobile phase: MeCN:MeOH:EDTA:70 mM KH_2PO_4 6:10:0.02:83.98
Flow rate: 1
Detector: E, LCB4, oxidation potential 900 mV

CHROMATOGRAM
Retention time: 12.5
Internal standard: nalorphine

OTHER SUBSTANCES
Extracted: oxymorphone

KEY WORDS
plasma; rat; nalorphine is IS

REFERENCE
Hussain,M.A.; Aungst,B.J. Intranasal absorption of oxymorphone, *J.Pharm.Sci.*, **1997**, *86*, 975–976.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL 100 mg ethyl SPE cartridge (J.T.Baker) with 2 mL MeOH, 1 mL water, and 2 mL 1 mM pH 9.3 ammonium hydrogen carbonate buffer. Add 1 mL serum to the SPE cartridge, wash with 1 mL 1 mM pH 9.3 ammonium hydrogen carbonate buffer, elute with 1 mL MeOH. Evaporate the eluate to dryness, reconstitute the residue in 100 μL mobile phase, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 2.1 5 μm Supelcosil LC-Si (Supelco)
Mobile phase: MeCN:MeOH:water:formic acid 5.2:59.8:34.65:0.35
Flow rate: 0.23
Injection volume: 5
Detector: MS, API I MS single quadrupole, ionspray, capillary tip 5000 V, interface plate 650 V, source 60°, positive ion mode, orifice 70 V, SIM, m/z 312

CHROMATOGRAM
Retention time: 15.4
Internal standard: nalorphine

OTHER SUBSTANCES
Extracted: codeine, diamorphine, morphine

KEY WORDS
serum; SPE; nalorphine is IS; mouse

REFERENCE
Zuccaro,P.; Ricciarello,R.; Pichini,S.; Pacifici,R.; Altieri,I.; Pellegrini,M.; D'Ascenzo,G. Simultaneous determination of heroin, 6-monoacetylmorphine, morphine, and its glucuronides by liquid chromatography-atmospheric pressure ionspray-mass spectrometry, *J.Anal.Toxicol.*, **1997**, *21*, 268–277.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Whole blood + 1 mL 1 M pH 9 borate buffer + 5 mL ethyl acetate, vortex thoroughly, centrifuge, repeat the extraction. Combine the extracts and add them to 4 mL 50 mM sulfuric acid, extract. Discard the organic layer and saturate the aqueous layer with freshly ground ammonium carbonate, extract with 8 mL ethyl acetate, filter (phase-separating paper). Evaporate the organic layer and to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 ODS Hypersil
Mobile phase: MeCN:10 mM KH_2PO_4 20:80 containing 20 mM octanesulfonic acid, adjusted to pH 2.5 with orthophosphoric acid
Injection volume: 20
Detector: E, BAS LC-4B, TL-5A thin-layer flow cell +0.870 V

CHROMATOGRAM
Retention time: 19
Internal standard: nalorphine

OTHER SUBSTANCES
Extracted: morphine

KEY WORDS
whole blood; nalorphine is IS

REFERENCE
Logan,B.K.; Oliver,J.S.; Smith,H. The measurement and interpretation of morphine in blood, *Forensic Sci.Int.*, **1987**, *35*, 189–195.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elut SPE cartridge with 1 mL MeOH, 1 mL water, and 1 mL 100 mM pH 9.0 sodium bicarbonate buffer, do not allow to go dry. 100 μL Plasma + 750 μL 100 mM pH 9.0 sodium bicarbonate buffer, vortex for 10 s, add to the SPE cartridge, wash with 1 mL 100 mM pH 9.0 sodium bicarbonate buffer, wash with 1 mL water, wash with 100 μL MeOH:water 50:50, dry under vacuum for 10 min, elute with three 250 μL aliquots of MeOH. Evaporate the eluate to dryness at 45° in a vacuum centrifuge for 1 h, reconstitute with 25 μL 100 mM pH 11.4 sodium carbonate or 25 μL pH 9.5 sodium bicarbonate, add 25 μL 1 mg/mL dansyl chloride in acetone, vortex, let stand in the dark at 45° for 20 min, add 250 μL toluene, vortex for 2 min, centrifuge at 12500 g for 1 min, inject a 100-200 μL aliquot of the upper organic layer.

HPLC VARIABLES
Guard column: 10 × 4.6 3 μm silica
Column: 150 × 4.6 3 μm Spherisorb 3CN
Mobile phase: n-Hexane:isopropanol:ammonia 95:5:0.25 (Place a silica column between the pump and the injector.)
Flow rate: 1.5
Injection volume: 100-200
Detector: F ex 340 em 500

CHROMATOGRAM
Retention time: 2.8
Internal standard: nalorphine

OTHER SUBSTANCES
Extracted: morphine, 6-acetylmorphine

KEY WORDS
plasma; derivatization; SPE; nalorphine is IS

REFERENCE
Barrett,D.A.; Shaw,P.N.; Davis,S.S. Determination of morphine and 6-acetylmorphine in plasma by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1991**, *566*, 135–145.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL 500 mg Bond Elut C8 SPE cartridge with 5 mL MeOH, 3 mL MeCN:10 mM NaH_2PO_4 10:90, and 5 mL water. 1 mL Plasma + 1 mL 500 mM ammonium sulfate, add to the SPE cartridge, wash with 6 mL 5 mM ammonium sulfate, elute with 1 mL MeCN:10 mM NaH_2PO_4 10:90, inject a 100 μL aliquot of the eluate.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:buffer 26:74 (Buffer was 10 mM NaH_2PO_4 containing 1 mM sodium dodecyl sulfate, pH adjusted to 2.1 with orthophosphoric acid.)

Flow rate: 1
Injection volume: 100
Detector: F ex 210 em 340

CHROMATOGRAM
Retention time: 22
Internal standard: nalorphine

OTHER SUBSTANCES
Extracted: morphine, normorphine

KEY WORDS
plasma; SPE; nalorphine is IS

REFERENCE
Glare,P.A.; Walsh,T.D.; Pippenger,C.E. A simple, rapid method for the simultaneous determination of morphine and its principal metabolites in plasma using high-performance liquid chromatography and fluorometric detection, *Ther.Drug Monit.*, **1991**, *13*, 226–232.

SAMPLE
Matrix: blood
Sample preparation: Add 500 μL plasma to a Sep-Pak tC18 SPE cartridge, wash with 2 mL water, wash with 3 mL acetone:water 5:95, elute with 4 mL MeOH:water 70:30. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 TSK-gel ODS-120T (Tosoh)
Mobile phase: MeCN:buffer 25:75 (Buffer was 10 mM NaH_2PO_4 containing 1 mM sodium dodecyl sulfate, adjusted to pH 2.1 with phosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: E, Shimadzu L-ECD-6A, 0.8 V

CHROMATOGRAM
Internal standard: nalorphine

OTHER SUBSTANCES
Extracted: morphine

KEY WORDS
plasma; rabbit; SPE; nalorphine is IS

REFERENCE
Matsumoto,Y.; Yamamoto,I.; Watanabe,Y.; Matsumoto,M. Enhancing effect of viscous sodium hyaluronate solution on the rectal absorption of morphine, *Biol.Pharm.Bull.*, **1995**, *18*, 1744–1749.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 287

CHROMATOGRAM

Retention time: 3.52
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood
Sample preparation: Condition a 3 mL Bond Elut Certify SPE cartridge with 2 mL MeOH and 2 mL water. 1 mL Plasma + 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 μL mobile phase, inject a 60 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 3 μm Basic C8 (YMC)
Mobile phase: MeCN:5 mM $(NH_4)_2HPO_4$ 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: 18
Internal standard: nalorphine

OTHER SUBSTANCES
Extracted: codeine
Simultaneous: morphine, norcodeine

KEY WORDS
plasma; SPE; nalorphine is IS

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, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 211.1

CHROMATOGRAM
Retention time: 4.76

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 µL aliquot.

HPLC VARIABLES
Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20

Detector: UV 254

CHROMATOGRAM
Retention time: 1.70

OTHER SUBSTANCES
Simultaneous: levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: pemoline, benzphetamine, diethylpropion, mazindol, tranylcypromine, caffeine, fenethyline, phendimetrazine, methylphenidate, phenelzine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, phenazocine, norpipanone

REFERENCE
Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.8

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, naloxone, naphazoline, nicotine, nifedipine, nomifensine,

nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in 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: 11.6
Limit of detection: 3.1 ng/mL

OTHER SUBSTANCES

Simultaneous: buprenorphine, butorphanol, ethylmorphine, fentanyl, morphine, codeine, meperidine, tramadol
Noninterfering: thiopentone, succinylcholine, pancuronium, diazepam, atropine, neostigmine
Interfering: nalbuphine

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: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: urine

Sample preparation: 10 mL Urine + 1 mL concentrated HCl, heat at 100° for 1 h, cool, add 500 μL 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 μL 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 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 5 μm Lichrosorb

Column: 250 × 4 5 μm 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: 6
Internal standard: nalorphine

OTHER SUBSTANCES
Extracted: morphine, codeine
Noninterfering: acetaminophen, aspirin, amitriptyline, buprenorphine, caffeine, carbamazepine, chlorpromazine, desipramine, dextromethorphan, doxepin, ephedrine, fenfluramine, imipramine, lidocaine, loxapine, meperidine, methadone, methaqualone, naloxone, naltrexone, nicotine, orphenadrine, oxycodone, papaverine, pentazocine, phendimetrazine, phenmetrazine, phentermine, phenylpropanolamine, phenytoin, primidone, procaine, promethazine, propoxyphene, propyphenazone, theobromine, theophylline, trazodone, trifluprom azine, trimethoprim, trimipramine

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KEY WORDS
SPE; normal phase; nalorphine is IS

REFERENCE
Ferrara,S.D.; Tedeschi,L.; Frison,G.; Castagna,F. Solid-phase extraction and HPLC-UV confirmation of drugs of abuse in urine, *J.Anal.Toxicol.*, **1992**, *16*, 217–222.

SAMPLE
Matrix: urine
Sample preparation: 1 mL Urine + 3000-3500 U glucuronidase (Patella vulgata, Sigma) + 300 μL 1.1 M pH 5 sodium acetate buffer, heat overnight at 55°, add 500 μL 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 μL 0.2% phosphoric acid, rotate gently for 30 min, inject a 50 μL 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 × 3.9 5 μm 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 or UV 210

CHROMATOGRAM
Retention time: 25.2
Internal standard: nalorphine
Limit of detection: 40 ng/mL

OTHER SUBSTANCES
Extracted: codeine, morphine, 6-monoacetylmorphine
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

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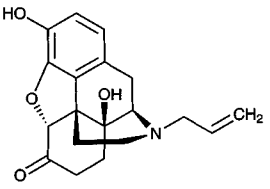
KEY WORDS
nalorphine is IS

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.

Naloxone



Molecular formula: $C_{19}H_{21}NO_4$
Molecular weight: 327.38
CAS Registry No.: 465-65-6, 357-08-4 (HCl), 51481-60-8 (HCl dihydrate)
Merck Index: 6449
Lednicer No.: 1 289

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 50 μL 2 M NaOH + 4.5 mL chloroform:isopropanol 90:10 (Caution! Chloroform is a carcinogen!), vortex for 1 min. Centrifuge at 2000 g for 20 min. Evaporate the organic phase to dryness under a stream of nitrogen at 40°. Reconstitute the residue with 70 μL mobile phase. Inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 30-40 μm Perisorb RP-18 (Upchurch Scientific, USA)
Column: 250 × 4.6 5 μm KR100-5-C18 (Biosains, Malaysia)
Mobile phase: MeCN:buffer 85.5;14.5 (Buffer was 100 mM disodium hydrogen orthophosphate adjusted to pH 3.5 with 85% phosphoric acid.)
Flow rate: 0.8
Injection volume: 50
Detector: E, DECADE (Antec Leyden), glassy carbon working electrode, oxidative mode +0.95 V, Ag/AgCl reference electrode saturated with LiCl

CHROMATOGRAM
Retention time: 6.98

OTHER SUBSTANCES
Extracted: naltrexone

KEY WORDS
naloxone is IS; plasma

REFERENCE
Peh,K.K.; Billa,N.; Yuen,K.H. Simple liquid chromatographic method for the determination of naltrexone in human plasma using amperometric detection, *J.Chromatogr.B*, **1997**, *701*, 140–145.

SAMPLE
Matrix: blood
Sample preparation: Buffer plasma to pH 8.6, extract with MTBE, evaporate under a stream of nitrogen, reconstitute the residue with 50 μL MeOH:water 50:50, inject an aliquot.

HPLC VARIABLES
Column: 5 μm Hypersil ODS-5
Mobile phase: MeOH:20mM pH 4.0 ammonium acetate 90:10
Detector: MS, Sciex API IIIplus tandem mass, positive ion mode, scan 328.2/310.0 parent product ion

CHROMATOGRAM
Retention time: 3.2

Internal standard: naloxone

KEY WORDS
plasma; naloxone is IS

REFERENCE
Selenka,J.M.; Talaat,R.E. Quantitative analysis of naltrexone in human plasma by high-performance liquid chromatography with ionspray tandem mass spectrometry (Abstract APQ 1239), *Pharm.Res.*, **1996**, *13*, S60.

SAMPLE
Matrix: blood
Sample preparation: Condition a 50 mg Isolute CBA SPE cartridge (Hengoed, UK) with full column volumes of MeOH and water. 1 mL plasma + 15 ng IS, add to the SPE cartridge, wash with two column volumes of water. Dry SPE cartridge under vacuum. Elute with one column volume of 1% ammonia in MeOH. Evaporate eluate to dryness under vacuum at 50°, reconstitute the residue in 150 μL mobile phase, vortex, inject an aliquot.

HPLC VARIABLES
Guard column: Brownlee Newguard C2 (Anachem, UK)
Column: 100 × 4 5 μm cyano-propyl column (Capitol HPLC, UK)
Mobile phase: MeOH:40 mM pH 5.3 potassium phosphate buffer 60:40
Flow rate: 1
Injection volume: 50
Detector: E, ESA Coulochem 2, Model 5020 guard cell, Model 5011 analytical cell, detector 1 + 450 mV, detector 2 + 850 mV, guard cell + 900 mV

CHROMATOGRAM
Retention time: 5.3
Internal standard: sumatriptan succinate (6.1)
Limit of detection: 250 pg

KEY WORDS
plasma; SPE

REFERENCE
Franklin,M.; Odontiadis,J. Determination of naloxone in human plasma by high-performance liquid chromatography with coulometric determination, *J.Chromatogr.B*, **1996**, *679*, 199–203.

SAMPLE
Matrix: blood
Sample preparation: Condition a C18 SPE cartridge (J.T. Baker) with 2 mL MeOH and 2 mL 10 mM pH 7 potassium phosphate buffer. Add 1 mL plasma to the SPE cartridge, wash with 2 mL water, dry under a stream of nitrogen, elute with 3 mL benzene:butanol 85:15 (Caution! Benzene is a carcinogen!). Evaporate the eluate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-18 DB
Mobile phase: MeCN:10 mM pH 3.00 potassium phosphate buffer 15:85
Flow rate: 0.8
Injection volume: 20
Detector: E, ESA Coulochem 5100A, 5011A analytical cell, first electrode +0.20 V, second electrode +0.70 V (monitored), palladium reference electrode

CHROMATOGRAM
Retention time: 5.85
Internal standard: naloxone

OTHER SUBSTANCES
Extracted: naltrexone

KEY WORDS
plasma; SPE; naloxone is IS

REFERENCE
Zuccaro,P.; Altieri,I.; Betto,P.; Pacifici,R.; Ricciarello,G.; Pini,L.A.; Sternieri,E.; Pichini,S. Determination of naltrexone and 6β-naltrexol in plasma by high-performance liquid chromatography with coulometric detection, *J.Chromatogr.*, **1991**, *567*, 485–490.

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 2 mL MeOH and with 2 mL 109 mM sodium octyl sulfate. 250 μL Plasma + 80 ng naltrexone + 750 μL water + 1 mL buffer + 200 μL 0.3 mM sodium octyl sulfate, add to the SPE cartridge, wash four times with 2.5 mL water, wash twice with 2.5 mL portions of MeOH:water 50:50, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 65°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot. (Buffer was 83.5 mL 100 mM HCl and 17.5 mL boric acid solution, pH 9.0. Boric acid solution was 1.237 g boric acid + 90 mL water + 10 mL 1 M HCl.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Econosphere C18
Mobile phase: MeCN:buffer 15:85 (Buffer was 100 mM monochloroacetic acid containing 2.59 mM sodium octyl sulfate and 2.39 mM EDTA, pH adjusted to 4.5 with NaOH.)
Flow rate: 1
Injection volume: 20
Detector: E, Bioanalytical Systems LC-4A, glassy carbon electrode +0.89 V

CHROMATOGRAM
Retention time: 7.0
Internal standard: naltrexone (10.4)
Limit of detection: 1 ng/mL

KEY WORDS
plasma; wash polypropylene tubes with 6.3 M nitric acid; SPE; pharmacokinetics

REFERENCE
Reid,R.W.; Deakin,A.; Leehey,D.J. Measurement of naloxone in plasma using high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1993**, *614*, 117–122.

SAMPLE
Matrix: blood
Sample preparation: Rock 5 mL whole blood + 10 mL water + 8.5 mL Na_2WO_4 in a 50 mL stoppered tube for 1 min, add 6 mL $NiCl_2$, rock for 5 min, add 15 mL dichloromethane:isobutyl alcohol:THF 30:45:25, centrifuge at 2500 g for 15 min. Remove organic phase and repeat the process. Filter all organic phases through a 40-90 μm filter and evaporate to dryness in a 100 mL porcelain dish at a moderate temperature in a sand bath. Take up residue in 500 μL MeCN: water 80:20, inject a 20 μL aliquot. (Na_2WO_4 prepared by mixing 10 g $Na_2WO_4.2H_2O$ in 38 mL of 2 M NaOH and 2.5 g of $NaHCO_3$ and making up to 100 mL. $NiCl_2$ was 17% w/v $NiCl_2$ in water.)

HPLC VARIABLES
Column: 200 × 4.6 5 μm Hypersil C8
Mobile phase: A = MeCN; B = 20 mM n-propylamine adjusted to pH 5 with 85% phosphoric acid. A:B from 15:85 to 20:80 over 5 min to 45:55 over another 15 min to 65:35 over another 5 min
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 9
Limit of detection: 0.30 ppm

OTHER SUBSTANCES
Extracted: buprenorphine, caffeine, cocaine, codeine, diamorphine, ethylmorphine, lidocaine, methaqualone, morphine, 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: 500 μL Plasma + 100 μL 200 ng/mL 6-monoacetylmorphine in water + 2 mL buffer + 10 mL hexane:dichloromethane:isopropanol 69:30:1, shake mechanically at 60 rpm for 15 min, centrifuge at 2000 g at 4° for 5 min. Remove the upper organic layer and add it to 200 μL 17 mM phosphoric acid, shake for 2 min, centrifuge at 2000 g at 4° for 5 min, inject a 50 μL aliquot of the aqueous layer. (Buffer was 100 mM boric acid and 100 mM KCl adjusted to pH 9 with 100 mM NaOH.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Lichrospher C18
Mobile phase: MeOH:water 20:80 containing 24 mM KH_2PO_4 and 0.06 mM disodium EDTA adjusted to pH 3.4 with orthophosphoric acid
Flow rate: 0.8
Injection volume: 50
Detector: E, ESA Model 5100A Coulochem, Model 5020 guard cell 0.50 V, Model 5011 analytical cell in oxidation screening mode, first electrode 0.06 V, second electrode 0.40 V

CHROMATOGRAM
Retention time: 3.6
Internal standard: 6-monoacetylmorphine (5.7)

OTHER SUBSTANCES
Extracted: nalbuphine
Noninterfering: buprenorphine, codeine, dextromoramide, dextropropoxyphene, ethylmorphine, fentanyl, methadone, morphine, nalorphine, norcodeine, pholcodine
Interfering: naltrexone

KEY WORDS
plasma

REFERENCE
Nicolle,E.; Michaut,S.; Serre-Debeauvais,F.; Bessard,G. Rapid and sensitive high-performance liquid chromatographic assay for nalbuphine in plasma, *J.Chromatogr.B*, **1995**, *663*, 111–117.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 283

CHROMATOGRAM

Retention time: 3.56
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromet azine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 14.028

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm RP-18 Spheri-5
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:50 mM pH 7 phosphate buffer 40:60 (Place a 70 × 2 30-38 μm CoPell 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: 20.81

OTHER SUBSTANCES
Extracted: acetaminophen, atropine, codeine, epinephrine, ethylmorphine, hydrocodone, hydromorphone, hydroxyzine, morphine, nalorphine, norcodeine, normorphine, oxycodone, pentazocine, phenylpropanolamine, pseudomorphine, scopolamine, secobarbital
Noninterfering: brompheniramine, chloroprocaine, dextromethorphan, diazepam, diphenhydramine, fentanyl, flurazepam, meperidine, methadone, neostigmine, propoxyphene

REFERENCE
Hepler,B.R.; Sutheimer,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: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2

Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 1.55

OTHER SUBSTANCES
Simultaneous: methylphenidate, phenelzine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, norpipanone, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine
Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: pemoline, benzphetamine, diethylpropion, mazindol, tranylcypromine, caffeine, fenethyline, phendimetrazine, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, dextropropoxyphene, nalorphine, phenazocine

REFERENCE
Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.2

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metopro-

lol, mianserin, morazone, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxy-

phenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 6.16 (A), 3.51 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, 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, trifluopromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine
Sample preparation: Buffer the urine sample at pH 9.7. Extract with ethyl acetate:heptane (4:1). Dry the organic phase and reconstitute with mobile phase.

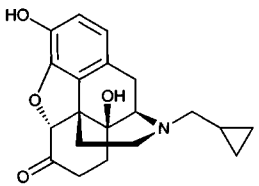
HPLC VARIABLES
Column: Hypersil silica
Mobile phase: MeCN:0.06% trifluoroacetic acid 90:10
Detector: MS, Sciex API III, heated nebulizer, positive ion mode

CHROMATOGRAM
Limit of quantitation: 0.2 ng/mL

OTHER SUBSTANCES
Extracted: buprenorphine, norbuprenorphine

REFERENCE
Johnson,R.A.; Haan,D.E.; James,C.A.; Hopkins,N.K. Determination of linezolid, PNU-100766, in human plasma and urine using high-performance liquid chromatography with ultraviolet detection (Abstract 2487), *Pharm.Res.*, **1997**, *14*, S374.

Naltrexone



Molecular formula: $C_{20}H_{23}NO_4$
Molecular weight: 341.41
CAS Registry No.: 16590-41-3, 16676-29-2 (HCl)
Merck Index: 6450
Lednicer No.: 2 319

SAMPLE
Matrix: blood
Sample preparation: Buffer plasma to pH 8.6, add IS, extract with MTBE, evaporate under a stream of nitrogen, reconstitute the residue with 50 μL MeOH:water 50:50, inject an aliquot.

HPLC VARIABLES
Column: 5 μm Hypersil ODS-5
Mobile phase: MeOH:20mM pH 4.0 ammonium acetate 90:10
Detector: MS, Sciex API IIIplus tandem mass, positive ion mode, scan 342.0/324.0 parent product ion

CHROMATOGRAM
Retention time: 3.7
Internal standard: naloxone (3.2)
Limit of quantitation: 100 pg/mL

KEY WORDS
plasma

REFERENCE
Selenka,J.M.; Talaat,R.E. Quantitative analysis of naltrexone in human plasma by high-performance liquid chromatography with ionspray tandem mass spectrometry (Abstract APQ 1239), *Pharm.Res.*, **1996**, *13*, S60.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 50 μL 250 ng/mL IS + 50 μL 2 M NaOH + 4.5 mL chloroform:isopropanol 90:10 (Caution! Chloroform is a carcinogen!), vortex for 1 min. Centrifuge at 2000 g for 20 min. Evaporate the organic phase to dryness under a stream of nitrogen at 40°. Reconstitute the residue in 70 μL mobile phase. Inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 30-40 μm Perisorb RP-18 (Upchurch Scientific, USA)
Column: 250 × 4.6 5 μm KR100-5-C18 (Biosains, Malaysia)
Mobile phase: MeCN:buffer 85.5:14.5 (Buffer was 100 mM disodium hydrogen orthophosphate adjusted to pH 3.5 with 85% phosphoric acid.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: E, DECADE (Antec Leyden), glassy carbon working electrode, oxidative mode +0.95 V, Ag/AgCl reference electrode saturated with LiCl

CHROMATOGRAM
Retention time: 9.42
Internal standard: naloxone (6.98)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Peh,K.K.; Billa,N.; Yuen,K.H. Simple liquid chromatographic method for the determination of naltrexone in human plasma using amperometric detection, *J.Chromatogr.B*, **1997**, *701*, 140–145.

SAMPLE
Matrix: blood
Sample preparation: Condition a C18 SPE cartridge (J.T. Baker) with 2 mL MeOH and 2 mL 10 mM pH 7 potassium phosphate buffer. 1 mL Plasma + 100 μL 100 ng/mL naloxone, add to the SPE cartridge, wash with 2 mL water, dry under a stream of nitrogen, elute with 3 mL benzene:butanol 85:15 (Caution! Benzene is a carcinogen!). Evaporate the eluate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-18 DB
Mobile phase: MeCN:10 mM pH 3.00 potassium phosphate buffer 15:85
Flow rate: 0.8
Injection volume: 20
Detector: E, ESA Coulochem 5100A, 5011A analytical cell, first electrode +0.20 V, second electrode +0.70 V (monitored), palladium reference electrode

CHROMATOGRAM
Retention time: 7.40
Internal standard: naloxone (5.85)

Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Zuccaro,P.; Altieri,I.; Betto,P.; Pacifici,R.; Ricciarello,G.; Pini,L.A.; Sternieri,E.; Pichini,S. Determination of naltrexone and 6β-naltrexol in plasma by high-performance liquid chromatography with coulometric detection, *J.Chromatogr.*, **1991**, *567*, 485–490.

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 2 mL MeOH and with 2 mL 109 mM sodium octyl sulfate. 250 μL Plasma + 80 ng naltrexone + 750 μL water + 1 mL buffer + 200 μL 0.3 mM sodium octyl sulfate, add to the SPE cartridge, wash four times with 2.5 mL water, wash twice with 2.5 mL portions of MeOH:water 50:50, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 65°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot. (Buffer was 83.5 mL 100 mM HCl and 17.5 mL boric acid solution, pH 9.0. Boric acid solution was 1.237 g boric acid + 90 mL water + 10 mL 1 M HCl.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Econosphere C18
Mobile phase: MeCN:buffer 15:85 (Buffer was 100 mM monochloroacetic acid containing 2.59 mM sodium octyl sulfate and 2.39 mM EDTA, pH adjusted to 4.5 with NaOH.)
Flow rate: 1
Injection volume: 20
Detector: E, Bioanalytical Systems LC-4A, glassy carbon electrode +0.89 V

CHROMATOGRAM
Retention time: 10.4
Internal standard: naltrexone

OTHER SUBSTANCES
Extracted: naloxone

KEY WORDS
plasma; wash polypropylene tubes with 6.3 M nitric acid; SPE; naltrexone is IS

REFERENCE
Reid,R.W.; Deakin,A.; Leehey,D.J. Measurement of naloxone in plasma using high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1993**, *614*, 117–122.

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 μL 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 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil ABZ
Mobile phase: Gradient. MeOH:water from 15:85 to 60:40 over 10 min. (Convex gradient where MeOH% = -0.46exp(-x/1.18) + 0.6 where x = 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 342

CHROMATOGRAM
Retention time: 7.27
Internal standard: naltrexone
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: morphine (m/z 286)

KEY WORDS
serum; human; mouse; SPE; LC-MS; naltrexone is IS

REFERENCE
Pacifici,R.; Pichini,S.; Altieri,I.; Caronna,A.; Passa,A.R.; Zuccaro,P. High-performance liquid chromatographic-electrospray mass spectrometric determination of morphine and its 3- and 6-glucuronides: application to pharmacokinetic studies, *J.Chromatogr.B*, **1995**, *664*, 329–334.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 225

CHROMATOGRAM
Retention time: 3.49
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; bupren-

orphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 205.2

CHROMATOGRAM

Retention time: 6.087

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: C18 (Upchurch)

Column: 100 × 3.2 5 μm Spherisorb C8

Mobile phase: MeOH:50 mM Na_2HPO_4 15:85 containing 3 mM 1-heptanesulfonic acid, pH adjusted to 3.5 with orthophosphoric acid
Flow rate: 0.8
Injection volume: 20
Detector: E, ESA Coulochem, guard cell + 650 mV, analytical cell +250 mV and +600 mV (monitored)

CHROMATOGRAM
Retention time: 16.4
Internal standard: naltrexone

OTHER SUBSTANCES
Simultaneous: hydromorphone, morphine

KEY WORDS
naltrexone is IS

REFERENCE
Bouquillon,A.I.; Freeman,D.; Moulin,D.E. Simultaneous solid-phase extraction and chromatographic analysis of morphine and hydromorphone in plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1992**, *577*, 354–357.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

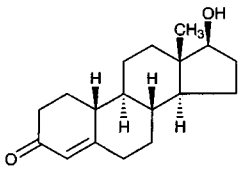
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam,

norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Nandrolone



Molecular formula: $C_{18}H_{26}O_2$
Molecular weight: 274.40
CAS Registry No.: 434-22-0, 360-70-3 (decanoate), 52279-57-9 (p-hydroxyphenylpropionate), 62- 90-8 (phenpropionate), 7207-92-3 (propionate), 22263-51-0 (cyclotate)
Merck Index: 6452
Lednicer No.: 1 164

SAMPLE

Matrix: bile, tissue, urine

Sample preparation: Urine. 50 mL Urine + 6 g Amberlite XAD-2, mix for 15 min, transfer settled Amberlite XAD-2 to a 100 × 9 glass column, wash with 10 mL water, dry under a stream of nitrogen, elute with 25 mL MeOH:ethyl acetate 50:50. Evaporate eluate to dryness at 50° and take up residue in 2 mL 250 mM pH 4.8 acetate buffer. Add 50 μL Helix pomatia juice containing a minimum 40 U/mL β-glucuronidase and 20 U/mL arylsulfatase and incubate at 37° for 2 h. Add 4 drops 6 M HCl and 20 mL ethyl acetate, mix for 15 min, remove water layer, incubate ethyl acetate layer at 37° for 1 h, wash with two 3 mL portions of 10% $NaHCO_3$, wash with 3 mL water. Evaporate to dryness, dissolve in 70 mL MeCN:water 5:95, analyze a 53 mL aliquot. Bile. 3 mL Bile + 2 mL 250 mM pH 4.8 acetate buffer + 50 μL Helix pomatia juice containing a minimum 40 U/mL β-glucuronidase and 20 U/mL arylsulfatase, incubate at 37° for 2 h. Add 4 drops 6 M HCl and 20 mL ethyl acetate, mix for 15 min, remove water layer, incubate ethyl acetate layer at 37° for 1 h, wash with two 3 mL portions of 10% $NaHCO_3$, wash with 3 mL water. Evaporate to dryness, dissolve in 70 mL MeCN:water 5:95, analyze a 53 mL aliquot. Liver, kidney. Add 80 mL 100 mM pH 9.5 Tris buffer containing 20 mg subtilopeptidase A (11.6 U/mg) to 20 g minced sample, incubate at 60° for 3.5 h, filter over glass wool, add 6 g Amberlite XAD-2 to the filtrate, mix for 15 min, transfer settled Amberlite XAD-2 to a 100 × 9 glass column, wash with 10 mL water, dry under a stream of nitrogen, elute with five 10 mL portions of MeOH. Evaporate eluate to dryness and take up residue in 2 mL 250 mM pH 4.8 acetate buffer. Add 50 μL Helix pomatia juice containing a minimum 40 U/mL β-glucuronidase and 20 U/mL arylsulfatase and incubate at 37° for 2 h. Add 4 drops 6 M HCl and 20 mL ethyl acetate, mix for 15 min, remove water layer, incubate ethyl acetate layer at 37° for 1 h, wash with two 3 mL portions of 10% $NaHCO_3$, wash with 3 mL water. Evaporate to dryness, dissolve in 70 mL MeCN:water 5:95, analyze a 53 mL aliquot. Meat. Add 80 mL 100 mM pH 9.5 Tris buffer containing 20 mg subtilopeptidase A (11.6 U/mg) to 20 g minced sample, incubate at 60° for 3.5 h, filter over glass wool, add 6 g Amberlite XAD-2 to the filtrate, mix for 15 min, transfer settled Amberlite XAD-2 to a 100 × 9 glass column, wash with 10 mL water, dry under a stream of nitrogen, elute with five 10 mL portions of MeOH. Evaporate eluate to dryness and

take up residue in 70 mL MeCN:water 5:95, analyze a 53 mL aliquot. Condition column A with 20 mL water then add 53 mL sample, flush column A with 10 mL water. Condition column B with 10 mL water. Elute column A onto column B with 20 mL water containing 250 μg/mL norgestrel and 5% MeCN. Switch column B into circuit with column C and elute with mobile phase. Recondition column A with MeOH:water 70:30.

HPLC VARIABLES

Column: A 10 × 10 immuno precolumn (with immunoglobulin G immobilized on cyanogen bromide-activated Sepharose 4B, prepared as J.Chromatogr. 1988, 452, 419-433); B 10 × 2 Chrompack reverse phase column; C Chromsep reverse phase guard column (Chrompack) + 100 × 3 5 μm Chromspher glass column
Mobile phase: MeCN:water 35:65
Flow rate: 0.4
Injection volume: 53000
Detector: UV 247

CHROMATOGRAM

Retention time: 9 (α), 6 (β)
Limit of detection: 50 ng/L

OTHER SUBSTANCES

Simultaneous: trenbolone (at UV 340)

KEY WORDS

meat; liver; kidney; SPE; column-switching

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: formulations
Sample preparation: Oils. 1 mL Sample + 25 mL MeOH:water 90:10, shake vigorously for 5 min, centrifuge, inject a 10 μL aliquot of the supernatant. Tablets. Grind a tablet to a fine powder, add 25 mL MeOH, sonicate for 5-10 min, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate. Suspensions (aqueous). Make up 5 mL to 50 mL with MeOH, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:water 75:25
Flow rate: 1.5
Injection volume: 10
Detector: UV 240

CHROMATOGRAM

Retention time: 5.8 (nandrolone), 13.5 (nandrolone acetate)
Limit of detection: 5 μg/mL

OTHER SUBSTANCES

Simultaneous: dehydroepiandrosterone (UV 210), mibolerone, methyltestosterone, methandriol (UV 210), norethindrone acetate, calusterone, mesterolone (UV 210), norethandrolone, trenbolone acetate, benzyl benzoate, testosterone acetate, stanozolol, oxymetholone, nandrolone propionate, methenolone acetate, testosterone propionate, aspirin, caffeine, formebolone, benzyl alcohol, testolactone, cortisone, fluoxymesterone, norethindrone, oxandrolone (UV 210), boldenone
Interfering: ethisterone, methandrostenolone, norgestrel, 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: formulations
Sample preparation: Crush tablets, weigh out amount equivalent to 10 mg steroid, dissolve in 10 mL MeOH, sonicate for 15 min, filter. 1 mL Filtrate + 5 mL MeOH + 4 mL water, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: Gradient. MeOH:water from 70:30 to 100:0 over 15 min, maintain at 100:0 for 15 min.
Flow rate: 1
Injection volume: 25
Detector: UV 240

CHROMATOGRAM
Retention time: 7.7 (nandrolone), 26.4 (nandrolone decanoate), 20.7 (nandrolone phenylpropionate), 17.3 (nandrolone propionate)

OTHER SUBSTANCES
Simultaneous: boldenone, boldenone acetate, boldenone undecylenate, clostebol acetate, danazol (UV 280), fluoxymesterone, methandriol, methandriol-3-acetate, methandriol dipropionate, methandrostenolone, methyltestosterone, stanolone, stanozolol, testosterone, testosterone acetate, testosterone cypionate, testosterone enanthate, testosterone isobutyrate, testosterone propionate, testosterone undecanoate
Noninterfering: oxandrolone, oxymetholone, testosterone decanoate, testosterone isocaproate

KEY WORDS
tablets

REFERENCE
Lurie,I.S.; Sperling,A.R.; Meyers,R.P. The determination of anabolic steroids by MECC, gradient HPLC, and capillary GC, *J.Forensic Sci.*, **1994**, *39*, 74–85.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:water 60:40
Injection volume: 250
Detector: UV

CHROMATOGRAM
Retention time: 4.5

OTHER SUBSTANCES
Simultaneous: diethylstilbestrol, trenbolone, zeranol, dienestrol, hexestrol, 17α-methyltestosterone, medroxyprogesterone

REFERENCE
Jansen,E.H.J.M.; Both-Miedema,R.; van den Berg,R.H. Application of optimization procedures for the separation of anabolic compounds by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 57–64.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 100 μg/mL, inject a 5 μL aliquot.

HPLC VARIABLES
Guard column: 70 × 2.1 CO:Pell ODS
Column: 300 × 3.9 Bondex C18 (Phenomenex)
Mobile phase: MeOH:water 75:25
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 4 (nandrolone), 10 (nandrolone propionate), 22 (nandrolone phenylpropionate)

OTHER SUBSTANCES
Also analyzed: boldenone, boldenone acetate, boldenone benzoate

REFERENCE
Noggle,F.T.,Jr.; Clark,C.R.; DeRuiter,J. Liquid chromatographic and mass spectral analysis of the anabolic 17-hydroxy steroid esters, *J.Chromatogr.Sci.*, **1990**, *28*, 263–268.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 100 μg/mL solution in MeOH.

HPLC VARIABLES
Guard column: 70 × 2.1 Whatman CO:Pell ODS
Column: 300 × 3.9 Bondex C18
Mobile phase: MeOH:water 70:30
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: methyltestosterone, danazol, boldenone, testosterone
Interfering: methandrostenolone, fluoxymesterone

REFERENCE
Noggle,F.T.,Jr.; Clark,C.R.; DeRuiter,J. Liquid chromatographic and spectral analysis of the 17-hydroxy anabolic steroids, *J.Chromatogr.Sci.*, **1990**, *28*, 162–166.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 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' 3.573

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), methylprednisolone acetate (UV 240), methylprednisolone (UV 240), methyltestosterone (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: tissue

Sample preparation: Condition a 500 mg Bond Elut silica SPE cartridge with 5 mL EtOH and 5 mL cyclohexane. Homogenize 5 g tissue with 11 mL 40 mM pH 4.1 sodium acetate buffer. Add 500 μL 17 mg/mL β-glucuronidase (type H-5) in acetate buffer, mix. Incubate at 37° overnight. Mix with 5 mL 2 M aqueous tris(hydroxymethyl)aminomethane. Fill an Extrelut cartridge with 25% of an Extrelut 20 sachet, mix the remainder of the sachet with the sample and add it to the cartridge. Rinse the sample tube with 15 mL butan-2-ol:hexane 5:95 and add the rinse to the cartridge. Elute with 100 mL butan-2-ol:hexane 5:95. Extract the eluate with two 10 mL portions of MeCN. Evaporate the combined MeCN extracts to dryness after adding two 2 mL portions of heptane. Reconstitute the residue with 2 mL cyclohexane. Add to the SPE cartridge Wash the tube with two 2 mL and two 1 mL portions of cyclohexane. Add to the SPE cartridge. Wash with 2 mL cyclohexane. Elute with 5 mL acetone:cyclohexane 25:75. Evaporate the eluate and redissolve the residue in 400 μL MeOH and 3.6 mL 85° water. Cool to room temperature and add to an immunoaffinity column (Randox Laboratories, UK). Complete the transfer with three 1 mL portions of hot water, cooled before adding to the column. Wash with 2 mL 1 mM pH 10 carbonate buffer, elute with 4 mL MeOH:water 70:30. Dilute the eluate with 12 mL water. Inject a 4 mL aliquot onto column A and then backflush the contents of column A onto column B with mobile phase. Monitor the effluent from column B.

HPLC VARIABLES

Column: A Chromspher (type R2) (Chrompack, UK); B 200 × 3 5 μm Chromspher C18 (Chrompack, UK)
Mobile phase: MeCN:water 35:65
Flow rate: 0.5
Injection volume: 4000
Detector: UV 247

CHROMATOGRAM

Retention time: 12 (β epimer), 9 (α epimer)
Limit of detection: 500 pg/g

OTHER SUBSTANCES

Extracted: trenbolone

KEY WORDS

pig; cow; liver; corned beef; SPE; column-switching

REFERENCE

Stubbings,G.W.; Cooper,A.D.; Shepherd,M.J.; Croucher,M.; Airs,D.; Farrington,W.H.H.H.; Shearer,G. Determination of 19-nortestosterone and trenbolone in animal tissues by high-performance liquid chromatography with immunoaffinity clean-up, *Food Addit.Contam.*, **1998**, *15*, 293–301.

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

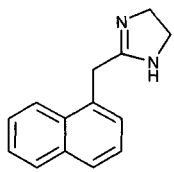
CHROMATOGRAM
Retention time: 5.5
Limit of detection: about 6 ng/mL

OTHER SUBSTANCES
Simultaneous: 17α-methyltestosterone, 17β-trenbolone, zeranol, trans-diethylstilbestrol, medroxyprogesterone

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.

Naphazoline



Molecular formula: $C_{14}H_{14}N_2$
Molecular weight: 210.28
CAS Registry No.: 835-31-4, 550-29-2 (HCl)
Merck Index: 6455
Lednicer No.: 1 241

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 1 mL 600 mM perchloric acid, vortex, let stand for 10 min, centrifuge at 2000 g for 15 min. Remove 800 μL of the supernatant and add it to 500 μL 1.2 M potassium hydrogen carbonate in a 10 mL tube, mix, let stand for a few min, add 500 μL 2 M NaOH, add 4 mL dichloromethane, shake gently for 15 min, centrifuge at 500 g for 10 min. Remove 3.5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL 25 mM pH 5 sodium phosphate buffer, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil phenyl
Mobile phase: MeOH:buffer 44:56 (Buffer was 25 mM pH 5 sodium phosphate buffer containing 5 mM sodium 1-heptanesulfonate.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 214

CHROMATOGRAM
Retention time: 11
Limit of detection: 1 ng/mL

KEY WORDS
rat; plasma

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

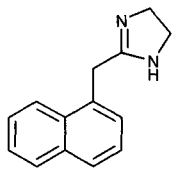
CHROMATOGRAM
Retention time: 5.5
Limit of detection: about 6 ng/mL

OTHER SUBSTANCES
Simultaneous: 17α-methyltestosterone, 17β-trenbolone, zeranol, trans-diethylstilbestrol, medroxyprogesterone

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.

Naphazoline



Molecular formula: $C_{14}H_{14}N_2$
Molecular weight: 210.28
CAS Registry No.: 835-31-4, 550-29-2 (HCl)
Merck Index: 6455
Lednicer No.: 1 241

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 1 mL 600 mM perchloric acid, vortex, let stand for 10 min, centrifuge at 2000 g for 15 min. Remove 800 μL of the supernatant and add it to 500 μL 1.2 M potassium hydrogen carbonate in a 10 mL tube, mix, let stand for a few min, add 500 μL 2 M NaOH, add 4 mL dichloromethane, shake gently for 15 min, centrifuge at 500 g for 10 min. Remove 3.5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL 25 mM pH 5 sodium phosphate buffer, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil phenyl
Mobile phase: MeOH:buffer 44:56 (Buffer was 25 mM pH 5 sodium phosphate buffer containing 5 mM sodium 1-heptanesulfonate.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 214

CHROMATOGRAM
Retention time: 11
Limit of detection: 1 ng/mL

KEY WORDS
rat; plasma

REFERENCE
Chabenat,C.; Boucly,P. Determination of naphazoline in rat plasma using column liquid chromatography with ultraviolet detection, *Biomed.Chromatogr.*, **1992**, *6*, 241–243.

SAMPLE
Matrix: blood, urine
Sample preparation: 50-100 μL Serum or urine + 250 μL buffer + 5 mL dichloromethane, shake mechanically for 20 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in 100 (serum) or 500 (urine) μL 20 mM pH 3.75 KH_2PO_4, vortex for 15 s, inject a 10 (urine) or 90 (serum) μL aliquot. (Buffer was 100 mM potassium hydrogen carbonate and 100 mM potassium carbonate, pH 10.0.)

HPLC VARIABLES
Guard column: 50 × 2 Co:Pell ODS
Column: Resolve C18 (Waters)
Mobile phase: MeCN:buffer 40:60 (Buffer was 20 mM KH_2PO_4 adjusted to pH 3.75 with 85% phosphoric acid.)
Flow rate: 1.2
Injection volume: 10-90
Detector: UV 210

CHROMATOGRAM
Retention time: 10
Internal standard: naphazoline

OTHER SUBSTANCES
Extracted: tolazoline
Noninterfering: acetaminophen, N-acetylprocainamide, carbamazepine, chloramphenicol, desipramine, digoxin, disopyramide, dopamine, ethosuximide, gentamicin, imipramine, lidocaine, methotrexate, phenobarbital, phenytoin, primidone, procainamide, propranolol, quinidine, salicylic acid, theophylline, valproic acid

KEY WORDS
naphazoline is IS; serum

REFERENCE
Cwik,M.J.; Chiu,G.P.; Fischer,J.H.; Chow-Tung,E.; Currie,B.L. Quantitative determination of tolazoline in serum and urine, *J.Chromatogr.*, **1985**, *338*, 123–130.

SAMPLE
Matrix: formulations
Sample preparation: 5 mL Ophthalmic solution + 5 mL 1.5 mg/mL tetrahydrozoline hydrochloride in water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeOH:buffer 30:70 (Buffer was 6 g sodium citrate dihydrate and 4 g anhydrous citric acid in 700 mL water, add 7 mL perchloric acid, adjust pH to 2.2 ± 0.2 with perchloric acid.)
Flow rate: 2
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 4.37
Internal standard: tetrahydrozoline (5.31)

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
ophthalmic solutions; stability-indicating

REFERENCE
Bauer,J.; Krogh,S. High-performance liquid chromatographic stability-indicating assay for naphazoline and tetrahydrozoline in ophthalmic preparations, *J.Pharm.Sci.*, **1983**, *72*, 1347–1349.

SAMPLE
Matrix: formulations
Sample preparation: 2 mL Sample + 1 mL 200 μg/mL emetine hydrochloride in water, make up to 10 mL with mobile phase, filter (0.45 μm), inject a 50-100 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Technosphere RP C-8 (HPLC Technology)
Mobile phase: MeCN:40 mM tetramethylammonium bromide:1 M acetic acid 80:15:5 (apparent pH 4.5)
Flow rate: 1.5
Injection volume: 50-100
Detector: UV 260

CHROMATOGRAM
Retention time: 1.40
Internal standard: emetine (1.75)
Limit of quantitation: 50 μg/mL

OTHER SUBSTANCES
Simultaneous: benzalkonium (C12, C14, C16)
Interfering: tetrahydrozoline

KEY WORDS
nasal; ophthalmic

REFERENCE
Santoni,G.; Medica,A.; Gratteri,P.; Furlanetto,S.; Pinzauti,S. High-performance liquid chromatographic determination of benzalkonium and naphazoline or tetrahydrozoline in nasal and ophthalmic solutions, *Farmaco*, **1994**, *49*, 751–754.

SAMPLE
Matrix: formulations
Sample preparation: Dilute nasal solution 10-fold with water, filter (0.45 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 125 × 4 5 μm Aluspher RP-select B (Merck)
Mobile phase: Gradient. MeCN:1 mM NaOH from 10:90 to 80:20 over 25 min.
Column temperature: 25
Flow rate: 1.2
Injection volume: 10
Detector: UV 224 or UV 283

CHROMATOGRAM
Retention time: 9.5
Limit of detection: 0.5 ng (UV 224)

OTHER SUBSTANCES
Simultaneous: ephedrine, oxymetazoline, xylometazoline

KEY WORDS
nasal solution

REFERENCE
De Orsi,D.; Gagliardi,L.; Cavazzutti,G.; Mediati,M.G.; Tonelli,D. Simultaneous determination of ephedrine and 2-imidazolines in pharmaceutical formulations by reversed-phase HPLC, *J.Liq.Chromatogr.*, **1995**, *18*, 3233–3242.

SAMPLE
Matrix: formulations
Sample preparation: Dilute ophthalmic solution 1:10 with water,inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeOH:water 57:43 containing 22 mM heptanesulfonic acid and 1% acetic acid
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 8.5

OTHER SUBSTANCES
Simultaneous: antazoline, degradation products

KEY WORDS
ophthalmic solutions; stability-indicating

REFERENCE
Ruckmick,S.C.; Marsh,D.F.; Duong,S.T. Synthesis and identification of the primary degradation product in a commercial ophthalmic formulation using NMR, MS, and a stability-indicating HPLC method for antazoline and naphazoline, *J.Pharm.Sci.*, **1995**, *84*, 502–507.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of an aqueous solution.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax C8
Mobile phase: MeCN:buffer 75:25 (Buffer was 12 g KH_2PO_4 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: 5.5
Internal standard: naphazoline hydrochloride

OTHER SUBSTANCES
Simultaneous: cyclobenzaprine, amitriptyline
Interfering: desipramine

KEY WORDS
naphazoline is IS

REFERENCE
Heinitz,M.L. Determination of cyclobenzaprine in tablets by high-performance liquid chromatography, *J.Pharm.Sci.*, **1982**, *71*, 656–658.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 3.1

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 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: 8

OTHER SUBSTANCES
Simultaneous: atropine, codeine, dansylamide, dansylcadaverine, doxorubicin, methylatropine, 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 30:1.5:0.5:68
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 2.52

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 MeOH:water 40:60, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.1 RSIL C18 (RSL, Eke, Belgium)
Mobile phase: MeOH:water 40:60 containing 20 mM sodium 1-octanesulfonate and 10 mM N,N-dimethyloctylamine, pH adjusted to 3.0 with orthophosphoric acid
Column temperature: 25
Flow rate: 1
Injection volume: 10
Detector: UV 220

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: degradation products, antazoline, coumazoline, lidocaine, oxymetazoline, prednisolone, sulfadimidine, sulfanilamide, sulfathiazole, tenaphtoxaline, tetrahydrozoline, tolazoline, tramazoline, xylometazoline

REFERENCE
De Schutter,J.A.; Van den Bossche,W.; De Moerloose,P. Stability-indicating analysis of tetryzoline hydrochloride in pharmaceutical formulations by reversed-phase ion-pair liquid chromatography, *J.Chromatogr.*, **1987**, *391*, 303–308.

SAMPLE
Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 7.85

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, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 9.13 (A), 4.77 (B)

OTHER SUBSTANCES

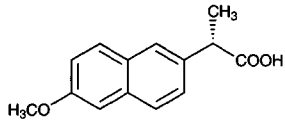
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, 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, pro-

pantheline, 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
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.

Naproxen



Molecular formula: $C_{14}H_{14}O_3$
Molecular weight: 230.26
CAS Registry No.: 22204-53-1, 26159-34-2 (sodium salt)
Merck Index: 6504
Lednicer No.: 1 86

SAMPLE
Matrix: blood
Sample preparation: Precipitate plasma with phosphoric acid, extract with dichloromethane containing IS. Centrifuge at 3000 rpm for 10 min. Remove a 4 mL aliquot of the upper layer and evaporate it to dryness under a stream of nitrogen. Reconstitute the residue with 500 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water:glacial acetic acid 40:60:0.1
Flow rate: 2.5
Injection volume: 20
Detector: UV 232

CHROMATOGRAM
Retention time: 6.5
Internal standard: ibuprofen (15.5)

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Niazi,S.K.; Alam,S.M.; Ahmad,S.I. Partial-area method in bioequivalence assessment: Naproxen, *Biopharm.Drug Dispos.*, **1997**, *18*, 103–116.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Whole blood + 20 μL 500 mM pH 2.5 NaH_2PO_4 + 1 mL ethyl acetate, vortex for 1 min. Centrifuge at 10000 rpm for 10 min, evaporate the organic layer to dryness under a stream of nitrogen at 50°. Dissolve the residue in 200 μL 75 mM pH 7 Na_2HPO_4 buffer:MeOH 50:50. Inject a 100 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm MicroPak C18

Mobile phase: MeCN:buffer 45:55 (Buffer was 75 mM sodium acetate adjusted to pH 3.3 with acetic acid.)
Flow rate: 2
Injection volume: 100
Detector: E, BAS LC-4B, glassy carbon working electrode 1.1 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 3.5
Internal standard: naproxen

OTHER SUBSTANCES
Extracted: diclofenac

KEY WORDS
rat; whole blood; pharmacokinetics; naproxen is IS

REFERENCE
Torres-López,J.E.; López-Muñoz,J.; Castañeda-Hernández,G.; Flores-Murrieta,F.J.; Granados-Soto,V. Pharmacokinetic-pharmacodynamic modeling of the antinociceptive effect of diclofenac in the rat, *J.Pharmacol.Exp.Ther.*, **1997**, *282*, 685–690.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 10 μL 2.5 μg/mL 6-methoxy.2-acetonaphthone in MeCN + 500 μL pH 7.4 phosphate buffer + 100 μL 37% HCl + 5 mL ethyl acetate, stir for 5 min, centrifuge at 1500 g for 10 min. Remove the supernatant into another test tube and extract the residue again. Evaporate the combined organic phases to dryness under a gentle nitrogen flow at 40°. Reconstitute the residue in 500 μL MeCN, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 2.5 μm Viosfer ODS
Mobile phase: MeCN:1% acetic acid 50:50
Flow rate: 1.8
Injection volume: 10
Detector: UV 238

CHROMATOGRAM
Retention time: 2.58
Internal standard: 6-methoxy-2-acetonaphthone (3.75)
Limit of detection: 1 μg/mL

KEY WORDS
pharmacokinetics; plasma

REFERENCE
Marzo,A.; Monti,N.; Ripamonti,M.; Marzo,P.; Wool,C.; Cerutti,R.; Maggi,G.C. Comparative bioavailability study on naproxen betainate sodium salt monohydrate and naproxen sodium salt in healthy volunteers, *Arzneimittelforschung*, **1997**, *47*, 385–389.

SAMPLE
Matrix: blood
Sample preparation: Acidify 100 μL whole blood with 20 μL 500 mM pH 2.5 NaH_2PO_4 buffer, add 1 mL ethyl acetate, vortex at maximal speed for 1 min, centrifuge at 10000 rpm for 10 min, evaporate the organic layer to dryness under nitrogen stream at 50°, reconstitute the residue with 200 μL MeOH:75 mM pH 7 Na_2HPO_4 buffer 1:1, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 40 × 4 37-50 μm Corasil C18 (Waters)
Column: 300 × 4 10 μm Micro Pak C18 (Varian)
Mobile phase: MeCN:75 mM sodium acetate adjusted to pH 3.3 with glacial acetic acid 45:55
Flow rate: 2

Injection volume: 100
Detector: E, LC-4B, glassy carbon electrode +1100 mV, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 3.5
Internal standard: naproxen

OTHER SUBSTANCES
Extracted: diclofenac

KEY WORDS
rat; whole blood; naproxen is IS

REFERENCE
Torres-López,J.E.; Robles,M.B.; Pérez-Urizar,J.; Flores-Murrieta,F.J.; Granados-Soto,V. Determination of diclofenac in micro-whole blood samples by high-performance liquid chromatography with electrochemical detection. Application in a pharmacokinetic study, *Arzneimittelforschung*, **1997**, *47*, 1040–1043.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 500 μL 500 mM HCl, vortex for 1 min, add 5 mL ethyl acetate, extract for 20 min, centrifuge at 2500 rpm for 10 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 200 μL MeCN, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 5 μm C18 (Machery & Nagel)
Mobile phase: MeCN:water:acetic acid 50:50:0.1
Flow rate: 1
Injection volume: 100
Detector: UV 280

CHROMATOGRAM
Internal standard: naproxen

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, tissue
Sample preparation: 500 μL Plasma or tissue (homogenized in phosphate buffer in a ratio 1:10), 10 μL 5 μg/μL 6-methoxy-2-acetonaphthone in MeCN, 500 μL pH 7.4 phosphate buffer, 100 μL 37% HCl, and 5 mL ethyl acetate stir for 5 min, centrifuge at 1500 g for 10 min. Pipette the supernatant into another test tube and extract the residue again. Evaporate combined organic layer to dryness under a gentle nitrogen flow at 40°, reconstitute the residue in 500 μL MeCN and inject an aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrosorb RP18
Mobile phase: MeCN:1% acetic acid 40:60
Flow rate: 1.5
Injection volume: 10
Detector: UV 238

CHROMATOGRAM
Retention time: 4.51
Internal standard: 6-methoxy-2-acetonaphthone (7.22)
Limit of detection: 10 ng
Limit of quantitation: 1 μg/mL (plasma), 10 μg/g (tissue)

KEY WORDS
gastric wall; heart; kidney; liver; lung; pharmacokinetics; plasma; rat

REFERENCE
Marzo,A.; Ripamonti,M.; Benatti,P.; Marzo,P.; Wool,C.; Cerutti,R.; Reiner,V. Absorption and distribution of naproxen in rats orally treated with naproxen betainate sodium salt monohydrate. Comparison with naproxen, *Arzneimittelforschung*, **1997**, *47*, 381–384.

SAMPLE
Matrix: dialysate
Sample preparation: Inject a 10 μL aliquot of dialysate (pH 7.4 isotonic phosphate buffer).

HPLC VARIABLES
Guard column: 37-50 μm Corasil C18
Column: 100 × 4 5 μm 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

REFERENCE
Evrard,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: perfusate
Sample preparation: Mix 1 mL perfusate with 100 μL 1 M HCl and 8 mL diethyl ether. Vortex for 1 min, centrifuge at 3000 rpm for 5 min. Remove the organic layer and evaporate it to dryness under nitrogen. Reconstitute the residue with 2 mL mobile phase. Inject a 30 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Econosphere C18
Mobile phase: MeOH:40 mM pH 8.0 phosphate buffer 40:60
Injection volume: 30
Detector: UV 330

CHROMATOGRAM
Retention time: 7.5
Internal standard: naproxen (5.2)

OTHER SUBSTANCES
Extracted: piroxicam

KEY WORDS
naproxen is IS

REFERENCE
Takamatsu,N.; Welage,L.S.; Idkaidek,N.M.; Liu,D.Y.; Lee,P.I.-D.; Hayashi,Y.; Rhie,J.K.; Lennernäs,H.; Barnett,J.L.; Shah,V.P.; Lesko,L.; Amidron,G.L. Human intestinal permeability of piroxicam, propranolol, phenylalanine, and PEG 400 determined by jejunal perfusion, *Pharm.Res.*, **1997**, *14*, 1127–1132.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeCN:water:1% glacial acetic acid 50:49:1
Flow rate: 1
Detector: UV 243

REFERENCE
Walter,E.; Janich,S.; Roessler,B.J.; Hilfinger,J.M.; Amidon,G.L. HT29-MTX/Caco-2 cocultures as an in vitro model for the intestinal epithelium: In vitro-in vivo correlation with permeability data from rats and humans, *J.Pharm.Sci.*, **1996**, *85*, 1070–1076.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 40:60adjusted to pH 5.5 with NaOH
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 230

OTHER SUBSTANCES
Also analyzed: carbamazepine, fenbufen, indomethacin, ketoprofen, α.-naphthoquinone, tolmetin

REFERENCE
Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE
Matrix: solutions
Sample preparation: 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 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: urine
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL ethyl acetate and dry by aspiration of air. Evaporate an aliquot of 100 μL 20 μg/mL IS in MeOH to dryness at 37°.

Add 1 mL urine, vortex, add 250 μL 1 M pH 5.0 acetate buffer, vortex. Add 250 μL of the mixture to the SPE cartridge, dry by aspiration of air, elute with 3 mL ethyl acetate, evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject a 10-30 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:50 mM pH 5.0 phosphate buffer 42:58
Flow rate: 0.9
Injection volume: 10-30
Detector: UV 230

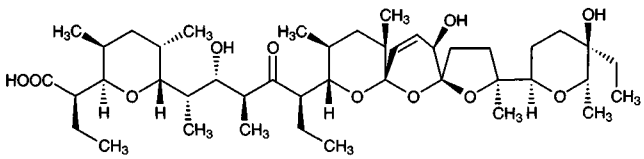
CHROMATOGRAM
Retention time: 7
Internal standard: indomethacin (18.5)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: diclofenac, ibuprofen, felbinac, fenbufen, flurbiprofen, ketoprofen, loxoprofen, mefenamic acid, piroxicam, sulindac

KEY WORDS
SPE

REFERENCE
Hirai,T.; Matsumoto,S.; Kishi,I. Simultaneous analysis of several non-steroidal anti-inflammatory drugs in human urine by high-performance liquid chromatography with normal solid-phase extraction, *J.Chromatogr.B*, **1997**, *692*, 375–388.

Narasin



Molecular formula: $C_{43}H_{72}O_{11}$
Molecular weight: 765.04
CAS Registry No.: 55134-13-9
Merck Index: 6506

SAMPLE
Matrix: bulk, feed, premix
Sample preparation: 100 mg Bulk or 5 g premix or feed + 200 mL MeOH:water 90:10, shake on a gyratory shaker at 200 rpm for 1 h, allow to settle. Dilute an aliquot to 20 μg/mL with MeOH:water 90:10, filter (0.45 μm), inject a 200 μL aliquot.

HPLC VARIABLES
Guard column: C18
Column: 250 × 4.6 Partisil 5 ODS-3 25 LC
Mobile phase: MeOH:water:acetic acid 94:6:0.1
Flow rate: 0.7
Injection volume: 200
Detector: UV 520 following post-column reaction. The column effluent mixed with the reagent pumped at 0.7 mL/min and the mixture flowed through a 6.1 m × 0.5 mm ID stainless steel coil at 98° to the detector. (Prepare reagent by cautiously adding 20 mL concentrated sulfuric acid to 950 mL MeOH, allow to cool to room temperature, add 30 g vanillin while stirring.)

CHROMATOGRAM
Retention time: 14
Limit of quantitation: 2.5 ppm

OTHER SUBSTANCES
Extracted: monensin

Noninterfering: bacitracin, bambermycin, lincomycin, nicarbazin, tylosin

KEY WORDS
post-column reaction

REFERENCE
Rodewald,J.M.; Moran,J.W.; Donoho,A.L.; Coleman,M.R. Determination of narasin in raw material, premix, and animal feeds by liquid chromatography and correlation to microbiological assay, *J.AOAC Int.*, **1994**, *77*, 821–828.

SAMPLE
Matrix: eggs, tissue
Sample preparation: 5 g Pulverized frozen tissue or 5 g homogenized whole eggs + 2 mL water + 13 mL MeOH, homogenize for 30 s. Sonicate for 10 min and centrifuge at 2000 g for 10 min. Add 4 mL 100 mM NaOH to a 2 mL aliquot of the supernatant, extract with 2 mL and 1 mL hexane:toluene 50:50 (v/v) for 30 s by inversion, centrifuge at 1500 g for 10 min. Evaporate the combined extracts to dryness under a stream of nitrogen at 60°. Dissolve the residue in 200 μL MeCN:water 75:25. Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2 (GL Sciences, Japan)
Mobile phase: MeCN:MeOH:THF:water:trifluoroacetic acid 67:10:10:13:0.1
Flow rate: 1
Injection volume: 20
Detector: MS, VG Platform, Megaflow electrospray probe, positive ion mode, source at 125°, cone voltage 25 V, m/z 787

CHROMATOGRAM
Retention time: 8.3
Limit of detection: 0.5-1 ng/g
Limit of quantitation: 2 ng/g

OTHER SUBSTANCES
Extracted: monensin, salinomycin

KEY WORDS
domestic fowl; muscle; liver

REFERENCE
Blanchflower,W.J.; Kennedy,D.G. Determination of monensin, salinomycin, and narasin in muscle, liver and eggs from domestic fowl using liquid chromatography-electrospray mass spectrometry, *J.Chromatogr.B*, **1996**, *675*, 225–233.

SAMPLE
Matrix: feed
Sample preparation: 20 g Ground Feed + 200 mL hexane:ethyl acetate 90:10, stir at high speed for 2 h, let stand. Remove an aliquot equivalent to 1 g feed and evaporate it to dryness under reduced pressure at 40°, reconstitute with 2 mL MeOH, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 60 × 4.6 3 μm C18 (Hewlett-Packard)
Mobile phase: MeOH:5% acetic acid 90:10
Flow rate: 0.5
Injection volume: 20-25
Detector: UV 520 following post-column reaction. The column effluent mixed with the reagent pumped at 1 mL/min and the mixture flowed through a 1.5 mL reaction coil (Kratos Model 510) at 95° to the detector. (Reagent was 40 g/L vanillin in MeOH:sulfuric acid 100:2. Keep in an ice bath and prepare fresh daily.)

CHROMATOGRAM
Retention time: 8.2

Limit of detection: 1 ppm

OTHER SUBSTANCES
Extracted: monensin, salinomycin

KEY WORDS
post-column reaction

REFERENCE
Lapointe,M.R.; Cohen,H. High-speed liquid chromatographic determination of monensin, narasin, and salinomycin in feeds, using post-column derivatization, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 480–484.

SAMPLE
Matrix: feed, premix
Sample preparation: Feed. Shake 5 g feed with 15 mL MeOH for 2 h, filter, evaporate the filtrate to 3 mL and make up to 10 mL with MeOH, inject a 3 μL aliquot. Premix. Shake 0.5 g premix with 15 mL MeOH for 2 h, filter, make up the filtrate to 50 mL with MeOH, inject a 3 μL aliquot.

HPLC VARIABLES
Column: 150 × 1 5 μm Separon SGX C18 glass column (Tessek Prague)
Mobile phase: MeOH:water:glacial acetic acid 94:5.9:0.1
Flow rate: 0.02
Injection volume: 3
Detector: UV 592 following post-column derivatization. The column effluent mixed with the reagent pumped at 0.015 mL/min and the mixture flowed through a 150 × 1 reactor containing 40-70 μm acid-washed glass beads at 75° to the detector. (The reagent was 500 mM 4-dimethylaminobenzaldehyde in 1.2 M sulfuric acid in MeOH.)

CHROMATOGRAM
Retention time: 18
Limit of detection: 2.3 μg/mL

OTHER SUBSTANCES
Extracted: monensin, salinomycin

KEY WORDS
microbore; post-column reaction

REFERENCE
Fejglova,Z.; Dolezal,J.; Hrdlicka,A.; Frgalova,K. Microbore HPLC determination of polyether antibiotics using postcolumn derivatization with benzaldehyde reagents, *J.Liq.Chromatogr.*, **1994**, *17*, 359–372.

SAMPLE
Matrix: fermentation solutions
Sample preparation: Homogenize 5 mL fermentation broth with 25 mL MeOH, filter (0.45 μm) the supernatant, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 50 × 4.6 3 μm Little Champ ODS (Regis)
Mobile phase: MeOH:100 mM pH 4 KH_2PO_4 buffer 93:7
Flow rate: 2
Injection volume: 20
Detector: UV 520 following post-column reaction. The column effluent mixed with 10% sulfuric acid in MeOH pumped at 1 mL/min and with 6% vanillin in MeOH pumped at 1 mL/min and the mixture flowed through a 1.5 m × 0.25 mm ID stainless steel coil at 120° to the detector.

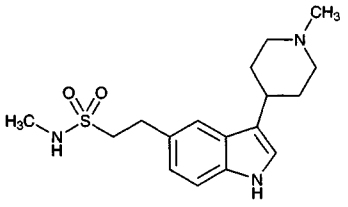
CHROMATOGRAM
Retention time: 2

KEY WORDS
post-column reaction

REFERENCE
Neely,F.L. A rapid HPLC determination of narasin in fermentation broth, *Chromatographia*, **1991**, *31*, 277–280.

Naratriptan

Molecular formula: $C_{17}H_{25}N_3O_2S$
Molecular weight: 335.47
CAS Registry No.: 121679-13-8



SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL Lichrolut C18-Select B SPE cartridge with 3 mL MeOH and 3 mL water. Mix 100 or 300 μL plasma with 500 μL 40 ng/mL IS in MeOH:water 50:50, add 800 μL MeOH, vortex, centrifuge at 15000 rpm for 10 min. Evaporate the supernatant to dryness under a stream of nitrogen at 40°, reconstitute the sample in 1 mL MeOH:water 10:90. Add 1 mL of the sample to the SPE cartridge, wash with 5 mL water, wash with 3 mL MeOH:water 20:80, elute with 2 mL MeCN containing 10% 1 M HCl, evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 300 μL mobile phase, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 150 × 2 4 μm Nova-Pak C8
Mobile phase: Gradient. A was MeCN:20 mM ammonium acetate 10:90. B was MeCN:20 mM ammonium acetate 80:20. A:B from 80:20 to 20:80 over 20 min, re-equilibrate at initial conditions for 10 min
Column temperature: 35
Flow rate: 0.5
Injection volume: 30
Detector: MS, Finnigan MAT SSQ-700 or TSQ-7000, API, ESI, 4.8 kV needle, +5.8 V to the capillary, +44.6 V to the tube lens, source 230°, m/z 339

CHROMATOGRAM
Internal standard: MDL 74,967

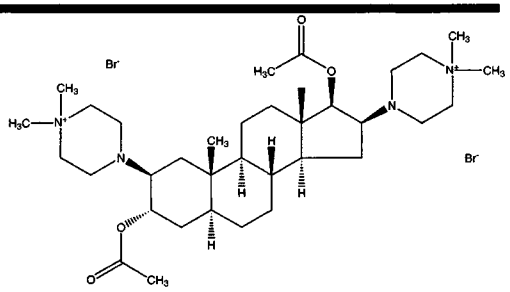
OTHER SUBSTANCES
Noninterfering: MDL 74,721, sumatriptan

KEY WORDS
plasma; rabbit; SPE; pharmacokinetics

REFERENCE
Duléry,B.D.; Petty,M.A.; Schoun,J.; David,M.; Huebert,N.D. A method using a liquid chromatographic-electrospray-mass spectrometric assay for the determination of antimigraine compounds: preliminary pharmacokinetics of MDL 74,721, sumatriptan and naratriptan, in rabbit, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1009–1020.

Natamycin

Molecular formula: $C_{33}H_{47}NO_{13}$
Molecular weight: 665.8
CAS Registry No.: 7681-93-8
Merck Index: 6513



SAMPLE

Matrix: blood, tissue

Sample preparation: Serum. 50-150 μL Serum + 2 mL MeOH:acetic acid 90:10, vortex for 30 s, leave in the dark for 1 h, centrifuge at 1000 g for 10 min, decant, filter (0.45 μm), inject a 100 μL aliquot. Tissue. 100-200 mg Tissue + 500 μL 1 mM pH 7.4 phosphate buffer, vortex, homogenize using a manual glass homogenizer, add 2 mL MeOH:acetic acid 90:10, vortex for 30 s, leave in the dark for 1 h, centrifuge at 1000 g for 10 min, decant, filter (0.45 μm), inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 2 Alltech C18
Column: 300 × 3.9 10 μm μBondapak RP-C18
Mobile phase: MeCN:10 mM pH 4.0 acetate buffer 37:63
Flow rate: 1 for 6 min, then 2
Injection volume: 100
Detector: UV 303

CHROMATOGRAM

Retention time: 6
Internal standard: natamycin

OTHER SUBSTANCES

Extracted: amphotericin (UV 383)

KEY WORDS

serum; lung; liver; natamycin is IS

REFERENCE

Polikandritou Lambros,M.; Abbas,S.A.; Bourne,D.W.A. New high-performance liquid chromatographic method for amphotericin B analysis using an internal method, *J.Chromatogr.B*, **1996**, *685*, 135–140.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 303.3

CHROMATOGRAM
Retention time: 13.637

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: cheese
Sample preparation: Cut cheese into small pieces, weigh out 5 g, add 50 mL MeOH (100 mL for cheese rind), stir or shake for 1.5 h, add 25 mL water (50 mL for cheese rind), let stand at -15 to -20° for 1 h, filter (paper) while cold, discard first 5 mL filtrate, filter (0.45 μm), filter (0.20 μm) again, dilute with two volumes of water, add 75-150 mL to an activated Sep-Pak C18 SPE cartridge at 25 mL/min, wash with 10 mL water, elute with 3 mL MeOH, make up eluate to 5 mL with MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 100 × 2.1 30-40 μm Perisorb RP-8
Column: 150 × 4.6 5 μm Lichrosorb RP-8
Mobile phase: MeOH:water:acetic acid 60:40:5
Flow rate: 1
Injection volume: 20
Detector: UV 303

CHROMATOGRAM
Limit of detection: 500 ng/g

KEY WORDS
SPE

REFERENCE
de Ruig,W.G.; van Oostrom,J.J.; Leenheer,K. Spectrometric and liquid chromatographic determination of natamycin in cheese and cheese rind, *J.Assoc.Off.Anal.Chem.*, **1987**, *70*, 944–948.

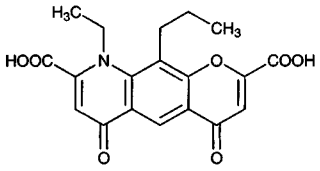
SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 140 mM, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 50 × 4.6 3 μm Econosphere C18
Mobile phase: MeOH:buffer 50:50 (Buffer was 6.3 g NaH_2PO_4 in 1 L water, adjust pH to 2.6 with phosphoric acid.)
Column temperature: 50
Flow rate: 1
Injection volume: 10
Detector: UV 318

CHROMATOGRAM
Retention time: 10

REFERENCE
Backes,B.J.; Rychnovsky,S.D. A reverse-phase HPLC assay for measuring the interaction of polyene macrolide antifungal agents with sterols, *Anal.Biochem.*, **1992**, *205*, 96–99.

Nedocromil



Molecular formula: $C_{19}H_{17}NO_7$
Molecular weight: 371.35
CAS Registry No.: 69049-73-6, 69049-74-7 (Na salt), 101626-68-0 (Ca salt)
Merck Index: 6524
Lednicer No.: 4 209

SAMPLE
Matrix: urine
Sample preparation: Condition a 100 mg Isolute phenyl SPE cartridge with 2 mL MeOH and 2 mL 100 mM HCl. Mix 5 mL urine with 1 mL water. Adjust pH to 1 with 4 mL 250 mM hydrochloric acid, vortex for 30 s. Remove 2 mL aliquot, add it to the SPE cartridge, allow to elute through the bed over 2-3 min, dry under full vacuum for 3 min. Wash with 2 mL 100 mM HCl, 2 mL MeOH:100 mM HCl 30:70 and dry under full vacuum for 3 min. Elute with 2 mL MeOH, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase, vortex for 30 s and inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 I.D. 5 μm Spherisorb S5C8
Column: 250 × 4.6 I.D. 5 μm Spherisorb S5C8
Mobile phase: MeOH:45 mM phosphate buffer:500 mM dodecyl triethyl ammonium phosphate 55:44.76:0.24 adjusted to pH 2.3 with orthophosphoric acid.
Flow rate: 0.85
Injection volume: 20
Detector: UV 238

CHROMATOGRAM
Retention time: 23.5
Internal standard: nedocromil

OTHER SUBSTANCES
Extracted: cromolyn disodium

KEY WORDS
SPE; nedocromil is IS

REFERENCE
Aswania,O.A.; Corlett,S.A.; Chrystyn,H. Development and validation of an ion-pair liquid chromatographic method for the quantitation of sodium cromoglycate in urine following inhalation, *J.Chromatogr.B*, **1997**, *690*, 373–378.

SAMPLE
Matrix: urine
Sample preparation: Inject 200 μL urine through an in-line filter on to 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, after 4 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 30 × 4 Hypersil 5-ODS; B 250 × 4.6 Zorbax SAX

Mobile phase: A MeOH:9.2 mM sulfuric acid (Pass mobile phase through a 250 × 4.6 25-40 μm silica (HPLC Technology) column to saturate it with silica.); B MeOH:buffer 50:50 (Buffer was 180 mM Na_2HPO_4 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: A 5 (0.2 when no sample is being chromatographed); B 1
Injection volume: 200
Detector: UV 253

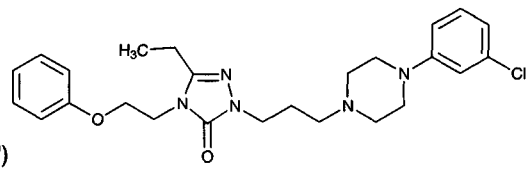
CHROMATOGRAM
Retention time: 8
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Simultaneous: acetaminophen, albuterol, aspartame, aspirin, beclomethasone dipropionate, caffeine, cromolyn, isoproterenol, menthol, minocromil, quinoline yellow, reproterol, riboflavin, saccharin, salicylic acid, sorbitan trioleate, terbutaline, theophylline

KEY WORDS
column-switching

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.

Nefazodone



Molecular formula: $C_{25}H_{32}ClN_5O_2$
Molecular weight: 470.01
CAS Registry No.: 83366-66-9, 82752-99-6 (HCl)
Merck Index: 6527
Lednicer No.: 4 98

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 500 μL saturated sodium bicarbonate solution + 50 μL 4 μg/mL aprindine in EtOH, vortex for 20 s, add 5 mL butyl chloride, tumble mix for 17 min, centrifuge at 600 g for 2 min. Remove 4.5 mL of the upper organic layer and evaporate it to dryness at 42° over 17 min, cool for 30 s, reconstitute the residue in 200 μL 10 mM $(NH_4)_2HPO_4$, vortex for 20 s, inject an aliquot

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax phenyl
Mobile phase: MeCN:MeOH:buffer 45:10:45 (Buffer was 20 mL 1 M $(NH_4)_2HPO_4$ adjusted to pH 3.0 with phosphoric acid, 10 mL 1 M tetramethylammonium hydroxide adjusted to pH 3.0 with phosphoric acid, and 860 mL water.)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 12.7
Internal standard: aprindine (15.3)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: diazepam, haloperidol

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Franc,J.E.; Duncan,G.F.; Farmen,R.H.; Pittman,K.A. High-performance liquid chromatographic method for the determination of nefazodone and its metabolites in human plasma using laboratory robotics, *J.Chromatogr.*, **1991**, *570*, 129–138.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond Elut cyanopropyl SPE cartridge with one volume MeOH and one volume water, do not allow to dry. 1 mL Plasma + 30 ng gepirone, add to the SPE cartridge, wash with 2 column volumes of water, dry under vacuum, elute with one column volume of MeOH:ammonia 90:10. Evaporate the eluate to dryness under vacuum, reconstitute the residue in 150 μL mobile phase, vortex, inject an aliquot.

HPLC VARIABLES
Guard column: 5 μm C2 (Capital HPLC, Edinburgh)
Column: 150 × 4.6 5 μm cyanopropyl (Capital HPLC, Edinburgh)
Mobile phase: MeCN:MeOH:40 mM pH 6 potassium phosphate buffer 22.5:17.5:60
Flow rate: 1.4
Injection volume: 50
Detector: E, ESA coulometric Model 5100A, Model 5020 guard cell 0.80 V, detector 1 0.75 V, detector 2 0.5 V

CHROMATOGRAM
Retention time: 3.2
Internal standard: gepirone (5.6)
Limit of quantitation: 0.9 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Franklin,M. Determination of nefazodone and its metabolites in plasma by high-performance liquid chromatography with coulometric detection, *J.Pharm.Biomed.Anal.*, **1993**, *11*, 1109–1113.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Add 5 mL plasma to a conditioned Sep-Pak C8 SPE cartridge, wash with 2 mL 10 mM pH 5.0 ammonium acetate buffer, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 800 μL 10 mM pH 5.0 ammonium acetate buffer, add 400 μL MeOH, filter (0.5 μm), inject a 200 μL aliquot. Urine. Hydrolyze urine with β-glucuronidase/arylsulfatase (Helix pomatia, Sigma) at 37° overnight, add a 5 mL aliquot to a conditioned Sep-Pak C8 SPE cartridge, wash with 2 mL 10 mM pH 5.0 ammonium acetate buffer, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 800 μL 10 mM pH 5.0 ammonium acetate buffer, add 400 μL MeOH, filter (0.5 μm), inject a 200 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax Rx C8
Mobile phase: Gradient. MeOH:10 mM pH 5.0 ammonium acetate buffer from 60:40 to 90:10 over 20 min, to 100:0 (step-gradient).
Flow rate: 1
Injection volume: 200
Detector: UV 254 or radioactivity

CHROMATOGRAM
Retention time: 22

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; dog; plasma; SPE

REFERENCE
Mayol,R.F.; Cole,C.A.; Luke,G.M.; Colson,K.L.; Kerns,E.H. Characterization of the metabolites of the antidepressant drug nefazodone in human urine and plasma, *Drug Metab.Dispos.*, **1994**, *22*, 304–311.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + 100 µL 1 µg/mL aprindine + 100 µL 1 M sodium carbonate + 100 µL saturated ammonium sulfate + 5 mL n-butyl chloride, extract, centrifuge, freeze the aqueous phase in isopropanol/dry ice. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 30 µL EtOH, add 90 µL mobile phase, inject a 70 µL aliquot.

HPLC VARIABLES
Guard column: 37-50 µm Corasil Type II
Column: 300 × 3.9 7-8 µm Zorbax silica
Mobile phase: MeCN:10 mM ammonium acetate 58:42, pH 5.0
Flow rate: 1
Injection volume: 70
Detector: UV 254

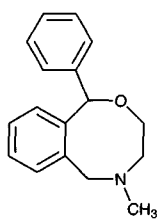
CHROMATOGRAM
Retention time: 8.4
Internal standard: aprindine (11.2)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Barbhaiya,R.H.; Marathe,P.H.; Greene,D.S.; Mayol,R.F.; Shukla,U.A.; Gammans,R.R.; Pittman,K.A.; Robinson,D. Safety, tolerance, and preliminary pharmacokinetics of nefazodone after administration of single and multiple oral doses to healthy adult male volunteers: A double blind, Phase I study, *J.Clin.Pharmacol.*, **1995**, *35*, 974–984.

Nefopam



Molecular formula: $C_{17}H_{19}NO$
Molecular weight: 253.34
CAS Registry No.: 13669-70-0, 23327-57-3 (HCl)
Merck Index: 6529
Lednicer No.: 2 447

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the

residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 12.653

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, loraze-

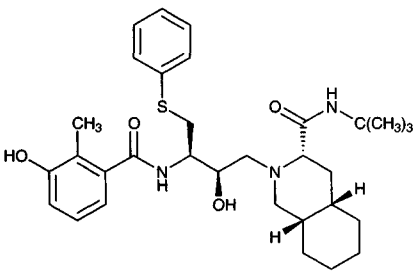
pam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Nelfinavir

Molecular formula: $C_{32}H_{45}N_3O_4S$
Molecular weight: 567.79
CAS Registry No.: 159989-64-7, 159989-65-8 (mesylate)



SAMPLE

Matrix: formulations
Sample preparation: Dissolve oral powder granules in MeCN:MeOH:water 70:20:10. Heat at 40° with shaking, inject a 10 µL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Capcell Pak UG120A C18
Mobile phase: Gradient. MeCN:5 mM pH 6.0 tetrabutylammonium sulfate from 50:50 to 65:35 over 7.0 min, maintain at 65:35 for 2 min, to 90:10 over 9 min.
Flow rate: 1.1
Injection volume: 10
Detector: UV 210

OTHER SUBSTANCES

Simultaneous: coupling reagents, degradation products, excipients, synthetic intermediates

KEY WORDS

oral powder; stability-indicating

REFERENCE

Tsai,W.-C.; Liu,J.; Tyle,P.; Wilke,T.L. Development of a stability-indicating HPLC method for nelfinavir mesylate (ag1343) oral powder formulation (Abstract 3347), *Pharm.Res.*, **1997**, *14*, S580.

SAMPLE
Matrix: solutions

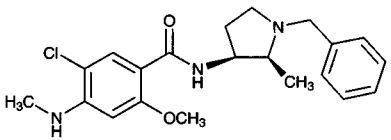
HPLC VARIABLES
Column: 150 × 3.9 5 μm Delta-pak C4 (Waters)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mM ammonium dihydrogen phosphate and 1 mM 1-heptanesulfonic acid sodium salt, pH adjusted to 4.8 with ammonium hydroxide.)
Flow rate: 0.6
Injection volume: 35
Detector: UV 210

CHROMATOGRAM
Retention time: 22-27

OTHER SUBSTANCES
Simultaneous: indinavir, ritonavir, saquinavir
Noninterfering: didanosine, lamivudine, stavudine, zalcitabine, zidovudine

REFERENCE
Iayewardene,A.L.; Zhu,F.; Aweeka,F.T.; Gambertoglio,J.G. Simple high-performance liquid chromatographic determination of the protease inhibitor indinavir in human plasma, *J.Chromatogr.B*, **1998**, *707*, 203–211.

Nemonapride



Molecular formula: $C_{21}H_{36}ClN_3O_2$
Molecular weight: 387.91
CAS Registry No.: 93664-94-9
Merck Index: 6533

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

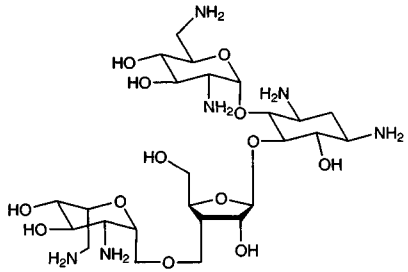
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 212.2

CHROMATOGRAM
Retention time: 14.985

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Neomycin



Molecular formula: $C_{23}H_{46}N_6O_{13}$ (neomycin B)
Molecular weight: 614.65 (neomycin B)
CAS Registry No.: 1404-04-2, 1406-04-8 (undecylenate), 1405-10-3 (sulfate), 1405-12-5 (palmitate)
Merck Index: 6542

SAMPLE
Matrix: blood, broth
Sample preparation: Condition a 3 mL 100 mg Isolute carboxypropyl CBA-bonded silica SPE cartridge (Jones Chromatography) with 1 mL MeOH and 1 mL pH 7.4 phosphate buffer. Add 1 mL plasma or Iso-sensitest broth to the SPE cartridge, wash with 2 mL pH 7.4 phosphate buffer and 4 mL 200 mM pH 9 borate buffer. Dry the cartridge with approximately 30 mL of air, elute with 1 mL MeCN:200 mM pH 10.5 borate buffer 50:50. Adjust the pH value of a 1 mL portion of the eluate to 8.9 by the addition of 200 μL 800 mM boric acid, add 200 μL 7 mM fluorenylmethyl chloroformate in MeCN, let stand at ambient temperature for 15 min, add 50 μL 100 mM glycine, inject a 20 μL aliquot of the mixture. (Prepare pH 7.4 phosphate buffer by mixing appropriate volumes of 20 mM NaH_2PO_4 and Na_2HPO_4 solutions. Prepare borate buffer by adjusting the pH of boric acid solution with 45% KOH.)

HPLC VARIABLES
Column: 200 × 4.6 3 μm ODS Hypersil
Mobile phase: MeCN:water 90:10
Flow rate: 1
Injection volume: 20
Detector: F ex 260 em 315

CHROMATOGRAM
Retention time: 10
Internal standard: sisomycin (14)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: netilmicin

KEY WORDS
derivatization; plasma; SPE

REFERENCE
Stead,D.A.; Richards,R.M.E. Sensitive high-performance liquid chromatographic assay for aminoglycosides in biological matrices enables the direct estimation of bacterial drug uptake, *J.Chromatogr.B*, **1997**, *693*, 415–421.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of an aqueous solution.

HPLC VARIABLES
Column: 250 × 4.6 8 μm PLRP-S 1000 Å poly(styrene-divinylbenzene) (Polymer Laboratories)

Mobile phase: Water containing 70 g/L sodium sulfate, 1.4 g/L sodium 1-octanesulfonate, and 50 mL/L 200 mM pH 3.0 phosphate buffer
Column temperature: 35
Flow rate: 1
Injection volume: 20
Detector: E, Dionex PED-1 pulsed electrochemical detector at 35°, 3 mm dia. gold working electrode, E_1 +0.05 V, E_2 +0.75 V, E_3 -0.15 V, t_1 0-0.40 s, t_2 0.41-0.60 s, t_3 0.61-1.00 s, measure signal between 0.2 and 0.4 s, stainless steel counter electrode, Ag/AgCl reference electrode, following post-column reaction. The column effluent mixed with 500 mM NaOH pumped at 0.3 mL/min and the mixture flowed through a 1.2 m long 500 µL coil to the detector. (Prepare 500 mM NaOH solution by diluting 50% NaOH with helium-degassed water. Clean gold electrode after each 60 analyses.)

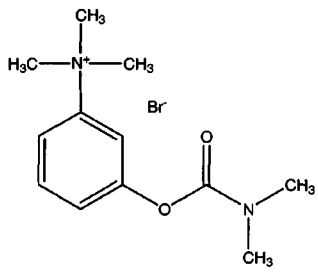
CHROMATOGRAM
Retention time: 23 (neomycin B)
Limit of detection: 5 ng
Limit of quantitation: 15 ng

OTHER SUBSTANCES
Simultaneous: neamine, neomycin C, neomycin LP-A, neomycin LP-B, paromamine, paromomycin I, paromomycin II

KEY WORDS
post-column reaction

REFERENCE
Adams,E.; Schepers,R.; Roets,E.; Hoogmartens,J. Determination of neomycin sulfate by liquid chromatography with pulsed electrochemical detection, *J.Chromatogr.A*, **1996**, *741*, 233–240.

Neostigmine bromide



Molecular formula: $C_{12}H_{19}BrN_2O_2$
Molecular weight: 303.20
CAS Registry No.: 114-80-7, 59-99-4 (neostigmine), 51-60-5 (neostigmine methylsulfate)
Merck Index: 6553

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 100 µL 500 ng/mL edrophonium in water + 500 µL 100 mM picric acid in 100 mM NaOH (pH adjusted to 7) + 400 µL 100 mM NaH_2PO_4 + 12 mL water saturated dichloromethane, shake vigorously for 5 min, centrifuge at 2000 g for 10 min. Remove 10 mL of the organic phase and add it to 200 µL 1 mM tetrabutylammonium hydrogen sulfate, shake vigorously for 30 s, centrifuge at 2000 g for 2 min, discard most of the organic layer, centrifuge at 2000 g for 1 min, inject a 50 µL aliquot of the aqueous layer. (Store glassware in 100 mM tetramethylammonium chloride solution and wash 5 times with water before use.)

HPLC VARIABLES
Guard column: 50 × 3.2 30-40 µm Perisorb RP-2 (Merck)
Column: 150 × 4.6 5 µm Ultrasphere octyl
Mobile phase: MeCN:water 20:80 containing 10 mM sodium heptanesulfonate, 10 mM NaH_2PO_4, and 2.5 mM tetramethylammonium chloride, pH adjusted to 3 with concentrated sulfuric acid
Flow rate: 2
Injection volume: 50
Detector: UV 214

CHROMATOGRAM
Retention time: 5
Internal standard: edrophonium (3.5)
Limit of quantitation: 5 ng/mL

KEY WORDS
serum; pharmacokinetics

REFERENCE
De Ruyter,M.G.M.; Cronnelly,R.; Castagnoli,N.,Jr. Reversed-phase, ion-pair liquid chromatography of quaternary ammonium compounds: determination of pyridostigmine, neostigmine and edrophionium in biological fluids, *J.Chromatogr.*, **1980**, *183*, 193–201.

SAMPLE
Matrix: blood
Sample preparation: Extract serum.

HPLC VARIABLES
Column: 150 × 3.3 7 μm Separon SGX
Mobile phase: 80 mM ammonium perchlorate in MeOH
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 2.34

OTHER SUBSTANCES
Simultaneous: nicotine, strychnine, amrinone

KEY WORDS
serum

REFERENCE
Eigendorf,H.G.; Nagel,S. Zur Analytik von Amrinone (Cordemcura). 2. Mitteillung: Hochdruckflüssigchromatographie [The analysis of amrinone (Cordemcura). 2. High pressure liquid chromatography], *Pharmazie*, **1987**, *42*, 631.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL 200 mg octadecyl SPE cartridge with 2 column volumes of MeOH and 2 column volumes of 100 mM pH 4 phosphate buffer. 1 mL Serum + 5 μL 100 mM pH 4 NaH_2PO_4, vortex for 15 s, add 1 mL 1 mM reagent in 100 mM pH 4 phosphate buffer, mix for 30 s, add to the SPE cartridge, wash with 4 mL water, elute with 200 μL MeOH:water 95:5. Evaporate the eluate to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot. (Synthesize reagent, sodium α-(3,4-dimethoxyphenyl) cinnamonitrile-2'-sulfonate, as follows. Add 5 mL 10% KOH in water to a stirred solution of 20 mmoles 3,4-(dimethoxyphenyl)acetonitrile and 20 mmoles 2-formylbenzenesulfonic acid, sodium salt hydrate (sodium benzaldehyde-2-sulfonate) in 50 mL EtOH at 50°, stir at 50° for 5 min, cool (evaporate to near dryness, if necessary), filter to obtain sodium α-(3,4-dimethoxyphenyl) cinnamonitrile-2'-sulfonate (mp of p-toluidine salt is 218-223°) (J. Chem. Eng. Data 1975, 20, 215).)

HPLC VARIABLES
Column: 250 × 4.6 5 μm diol (ES Industries, Marlton NJ)
Mobile phase: MeOH:50 mM pH 4 NaH_2PO_4 20:80 containing 500 μM sodium α-(3,4-dimethoxyphenyl) cinnamonitrile-2'-sulfonate
Flow rate: 1
Injection volume: 50
Detector: F ex 243 em 418 (cutoff filter) following post-column extraction. The column effluent mixed with dichloromethane pumped at 1 mL/min and the mixture flowed through a 90 cm × 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.

CHROMATOGRAM
Retention time: 11.16
Internal standard: neostigmine

OTHER SUBSTANCES
Extracted: physostigmine, eseroline
Also analyzed: amantadine, amphetamine, atropine, chlorpheniramine, clidinium bromide, N,N-dimethyl-N-benzyltetradecylammonium chloride, guanethidine, hydralazine, imipramine, malachite green, promazine, propantheline bromide
Noninterfering: chlordiazepoxide

KEY WORDS
post-column extraction; SPE; neostigmine is IS; serum; silanize glassware; post-column reaction

REFERENCE
Quinn,K.D.; Stewart,J.T. A high performance liquid chromatographic post-column fluorescent ion pair extraction system: application to physostigmine and its metabolite eseroline in human serum, *Biomed. Chromatogr.*, **1991**, *5*, 8–13.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 4.782

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: perfusate
Sample preparation: Direct injection of a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 octyl Spherisorb S5-ODS

Mobile phase: MeCN:buffer 30:70 (Buffer was 10 mM sodium octanesulfonate, 10 mM NaH_2PO_4, 2.5 mM tetramethylammonium chloride, adjust to pH 3 with sulfuric acid.)
Column temperature: 30
Flow rate: 1.1
Injection volume: 100
Detector: UV 214

KEY WORDS
calibration range 2-10 μM

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: solutions

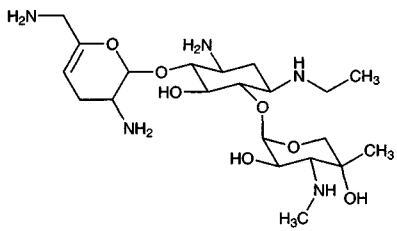
HPLC VARIABLES
Column: 50 × 4.6 5 μm Suplex pKb-100 (Supelco)
Mobile phase: MeCN:buffer 30:70 (Buffer was 10 mM pH 7.0 sodium phosphate containing 5 mM sodium dodecyl sulfate.)
Column temperature: 35
Flow rate: 2
Detector: UV 210

CHROMATOGRAM
Retention time: 2.2

OTHER SUBSTANCES
Simultaneous: 4'-methylphenazone, edrophonium chloride

REFERENCE
Supelco Catalog, **1994**, p. 771.

Netilmicin



Molecular formula: $C_{21}H_{41}N_5O_7$
Molecular weight: 475.59
CAS Registry No.: 56391-56-1, 56391-57-2 (sulfate)
Merck Index: 6563

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 20 μL 10 μg/mL gentamicin C1a in water + 50 μL buffer, vortex for 15 s, add 200 μL MeCN, vortex for 15 s, centrifuge at 2000 g for 5 min. Filter (0.45 μm, Millex-HV4) the supernatant and add 300 μL of the filtrate to 20 μL 250 mg/mL 1-fluoro-2,4-dinitrobenzene in MeCN. Heat at 80° for 2 h, cool rapidly to room temperature, filter (0.45 μm, Millex-HV4), inject a 50 μL aliquot of the filtrate. (Buffer was prepared by dissolving 3.81 g disodium tetraborate decahydrate in water, adjusting pH to 10 with NaOH, and making up to 100 mL with water.)

HPLC VARIABLES
Guard column: 33 × 4.6 5 μm C18 (Perkin-Elmer)
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water:acetic acid 70:30:0.1

Flow rate: 2.2
Injection volume: 50
Detector: UV 365

CHROMATOGRAM

Retention time: 13.5
Internal standard: gentamicin C1a (11.0)
Limit of quantitation: 500 ng/mL

KEY WORDS

serum; guinea pig; human; derivatization

REFERENCE

Dionisotti,S.; Bamonte,F.; Gamba,M.; Ongini,E. High-performance liquid chromatographic determination of netilmicin in guinea-pig and human serum by fluorodinitrobenzene derivatization with spectrophotometric detection, *J.Chromatogr.*, **1988**, *434*, 169–176.

SAMPLE

Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL trichloroacetic acid + 100 μL 25 μg/mL gentamicin, shake for 30 s, centrifuge at 3200 rpm for 5 min, add 100 μL 1 M NaOH to the supernatant, shake (?) for 30 s, add 1 mL pH 11 KH_2PO_4, add 2 mL dichloromethane, shake for 10 s, centrifuge at 3200 rpm for 5 min. Remove the aqueous layer and add it to 1 mL reagent, shake for 30 s, add 500 mg anhydrous sodium carbonate, shake for 30 s, add 500 μL isopropanol, shake, centrifuge for 5 min, inject an aliquot of the supernatant. (Reagent was 5 mg o-phthaldialdehyde, 500 μL MeOH, 300 μL 2-mercaptoethanol, and 5 mL 100 mM pH 10.4 borate buffer. Prepare fresh each day.)

HPLC VARIABLES

Column: 300 × 4 10 μm RP-18
Mobile phase: Gradient. A was water:acetic acid:100 mM heptanesulfonic acid 80:10:10. B was MeCN. A:B from 60:40 to 50:50 over 10 min, re-equilibrate at initial conditions for 10 min.
Flow rate: 2
Detector: F ex 337 em 437

CHROMATOGRAM

Retention time: 6.5
Internal standard: gentamicin (11)
Limit of detection: 100 ng/mL

KEY WORDS

plasma; derivatization

REFERENCE

Santos,M.; Garcia,E.; López,F.G.; Lanao,J.M.; Dominguez-Gil,A. Determination of netilmicin in plasma by HPLC, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1059–1062.

SAMPLE

Matrix: blood, broth
Sample preparation: Condition a 3 mL 100 mg Isolute carboxypropyl CBA-bonded silica SPE cartridge (Jones Chromatography) with 1 mL MeOH and 1 mL pH 7.4 phosphate buffer. Add 1 mL plasma or Iso-sensitest broth to the SPE cartridge, wash with 2 mL pH 7.4 phosphate buffer and 4 mL 200 mM pH 9 borate buffer. Dry the cartridge with approximately 30 mL of air, elute with 1 mL MeCN:200 mM pH 10.5 borate buffer 50:50. Adjust the pH value of a 1 mL portion of the eluate to 8.9 by the addition of 200 μL 800 mM boric acid, add 200 μL 7 mM fluorenylmethyl chloroformate in MeCN, let stand at ambient temperature for 15 min, add 50 μL 100 mM glycine, inject a 20 μL aliquot of the mixture. (Prepare pH 7.4 phosphate buffer by mixing appropriate volumes of 20 mM NaH_2PO_4 and Na_2HPO_4 solutions. Prepare borate buffer by adjusting the pH of boric acid solution with 45% KOH.)

HPLC VARIABLES

Column: 200 × 4.6 3 μm ODS Hypersil

Mobile phase: MeCN:water 90:10
Flow rate: 1
Injection volume: 20
Detector: F ex 260 em 315

CHROMATOGRAM
Retention time: 18
Internal standard: sisomycin (14)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: neomycin

KEY WORDS
derivatization; plasma; SPE

REFERENCE
Stead,D.A.; Richards,R.M.E. Sensitive high-performance liquid chromatographic assay for aminoglycosides in biological matrices enables the direct estimation of bacterial drug uptake, *J.Chromatogr.B*, **1997**, *693*, 415–421.

SAMPLE
Matrix: blood, dialysate, urine
Sample preparation: Plasma. Condition a 3 mL Baker cyanopropylsilane CN SPE cartridge with 2 mL MeOH, 2 mL water, and 2 mL buffer. 1 mL Plasma + 100 μL 100 μg/mL dibekacin in water, vortex for 15 s, add 1 mL buffer, vortex for 15 s, centrifuge at 3100 g at 4° for 7 min, add to SPE cartridge, wash with 500 μL water, wash with 250 μL mobile phase, elute to dryness. Elute with 250 μL mobile phase, inject an aliquot of the eluate. Urine, dialysate. Dilute 1:100 with water, add 100 μL 100 μg/mL dibekacin per 1 mL of sample, mix well, inject a 100 μL aliquot. (Buffer was 0.94 g sodium hexanesulfonate in 300 mL water, add 500 μL glacial acetic acid, dilute to 500 mL with water.)

HPLC VARIABLES
Guard column: 10 × 4.6 5 μm Hypersil C18
Column: 150 × 4.6 5 μm Hypersil C18
Mobile phase: MeOH:buffer 15:85 (Buffer was 3.48 g sodium hexanesulfonate + 28.4 g sodium sulfate in 2 L water, acidify to pH 3.4 with 2 mL glacial acetic acid.)
Column temperature: 25
Flow rate: 1.1
Injection volume: 100
Detector: F ex 338 em 418 (bandpass filter) following post-column reaction. The column effluent mixed with the reagent pumped at 0.4 mL/min and the mixture flowed through a 3 m × 0.05 mm i.d. knitted PTFE reaction coil at 25° to the detector (Derivatizing reagent was 0.4 g o-phthalaldehyde in 3 mL MeOH added to 390 mL buffer, add 2 mL β-mercaptoethanol, make up to 500 mL with water, store at 4°. Buffer was 1 M pH 10.4 borate from equal volumes of 1 M KOH and boric acid.)

CHROMATOGRAM
Retention time: 16
Internal standard: dibekacin

OTHER SUBSTANCES
Simultaneous: kanamycin, isepamicin, tobramycin, gentamicin

KEY WORDS
post-column reaction; SPE; plasma

REFERENCE
Maloney,J.A.; Awni,W.M. High-performance liquid chromatographic determination of isepamicin in plasma, urine and dialysate, *J.Chromatogr.*, **1990**, *526*, 487–496.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 206.4

CHROMATOGRAM
Retention time: 12.082

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve sample in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 8 μm 1000 Å poly(styrene-divinylbenzene) PLRP-S column (Polymer Labs., UK)
Mobile phase: THF:buffer 1:99 (Mobile phase was 35 g sodium sulfate, 500 mg sodium 1-octanesulfonate, 10 mL THF, and 50 mL 200 mM pH 3.0 phosphate buffer diluted to 1 L with water.)
Column temperature: 50
Flow rate: 1
Injection volume: 20
Detector: E, Dionex PED-1, pulsed electrochemical detection, gold working electrode, E1 +50 mV, E2 +750 mV, E3 -150 mV, t1 0-400 ms, t2 410-600 ms, t3 610-1000 ms, signal acquired 200-400 ms, Ag/AgCl reference electrode, stainless-steel counter electrode following post-column reaction. The column effluent mixed with 500 mM NaOH pumped at 0.3 mL/min and flowed through a 1.2 m (500 μL) reaction coil to the detector.

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
post-column reaction

REFERENCE
Adams,E.; Puelings,D.; Rafiee,M.; Roets,E.; Hoogmartens,J. Determination of netilmicin sulfate by liquid chromatography with pulsed electrochemical detection, *J.Chromatogr.A*, **1998**, *812*, 151–157.

SAMPLE
Matrix: reaction mixtures
Sample preparation: 50 µL Buffered reaction mixture + 50 µL isopropanol + 50 µL reagent, heat at 60° for 10 min, centrifuge at 1000 g for 2 min, immediately inject a 50 µL aliquot of the supernatant. (Reagent was 80 mM o-phthalaldehyde and 250 mM thioglycolic acid in 1 M boric acid, pH adjusted to 10.4 with 40% KOH.)

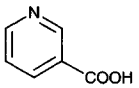
HPLC VARIABLES
Column: 100 × 5 Hypersil ODS
Mobile phase: A was MeOH:water:acetic acid 50:45:5 containing 5 g/L heptanesulfonic acid. B was MeOH:water:acetic acid 75:20:5 containing 5 g/L heptanesulfonic acid. A:B 10:90.
Flow rate: 2
Injection volume: 50
Detector: UV 330

CHROMATOGRAM
Retention time: 19

KEY WORDS
derivatization

REFERENCE
Lovering,A.M.; White,L.O.; Reeves,D.S. Identification of aminoglycoside-acetylating enzymes by high-pressure liquid chromatographic determination of their reaction products, *Antimicrob.Agents Chemother.*, **1984**, *26*, 10–12.

Niacin



Molecular formula: $C_6H_5NO_2$
Molecular weight: 123.11
CAS Registry No.: 59-67-6
Merck Index: 6612

SAMPLE
Matrix: blood
Sample preparation: 0.5 mL Plasma + 50 µL 100 µg/mL anthranilic acid, mix, add 3 mL acetone:water 2:1, centrifuge. Remove the supernatant and evaporate it to dryness, reconstitute the residue in 500 µL 100 mM HCl, add 1 mL, evaporate to dryness, reconstitute in 350 µL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 µm ODS-2 (Knauer)
Mobile phase: MeCN:10 mM pH 5.4 MOPS (3-(N-morpholino)propanesulfonic acid) buffer 25:75 containing 1 mM n-dodecylamine
Column temperature: 60
Flow rate: 1
Injection volume: 20
Detector: UV 262

CHROMATOGRAM
Retention time: 7.49
Internal standard: anthranilic acid (13)
Limit of detection: 100 ng/mL

REFERENCE
Adams,E.; Puelings,D.; Rafiee,M.; Roets,E.; Hoogmartens,J. Determination of netilmicin sulfate by liquid chromatography with pulsed electrochemical detection, *J.Chromatogr.A*, **1998**, *812*, 151–157.

SAMPLE
Matrix: reaction mixtures
Sample preparation: 50 µL Buffered reaction mixture + 50 µL isopropanol + 50 µL reagent, heat at 60° for 10 min, centrifuge at 1000 g for 2 min, immediately inject a 50 µL aliquot of the supernatant. (Reagent was 80 mM o-phthalaldehyde and 250 mM thioglycolic acid in 1 M boric acid, pH adjusted to 10.4 with 40% KOH.)

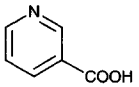
HPLC VARIABLES
Column: 100 × 5 Hypersil ODS
Mobile phase: A was MeOH:water:acetic acid 50:45:5 containing 5 g/L heptanesulfonic acid. B was MeOH:water:acetic acid 75:20:5 containing 5 g/L heptanesulfonic acid. A:B 10:90.
Flow rate: 2
Injection volume: 50
Detector: UV 330

CHROMATOGRAM
Retention time: 19

KEY WORDS
derivatization

REFERENCE
Lovering,A.M.; White,L.O.; Reeves,D.S. Identification of aminoglycoside-acetylating enzymes by high-pressure liquid chromatographic determination of their reaction products, *Antimicrob.Agents Chemother.*, **1984**, *26*, 10–12.

Niacin



Molecular formula: $C_6H_5NO_2$
Molecular weight: 123.11
CAS Registry No.: 59-67-6
Merck Index: 6612

SAMPLE
Matrix: blood
Sample preparation: 0.5 mL Plasma + 50 µL 100 µg/mL anthranilic acid, mix, add 3 mL acetone:water 2:1, centrifuge. Remove the supernatant and evaporate it to dryness, reconstitute the residue in 500 µL 100 mM HCl, add 1 mL, evaporate to dryness, reconstitute in 350 µL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 µm ODS-2 (Knauer)
Mobile phase: MeCN:10 mM pH 5.4 MOPS (3-(N-morpholino)propanesulfonic acid) buffer 25:75 containing 1 mM n-dodecylamine
Column temperature: 60
Flow rate: 1
Injection volume: 20
Detector: UV 262

CHROMATOGRAM
Retention time: 7.49
Internal standard: anthranilic acid (13)
Limit of detection: 100 ng/mL

KEY WORDS
plasma

REFERENCE
Zarzycki,P.K.; Kowalski,P.; Nowakowska,J.; Lamparczyk,H. High-performance liquid chromatographic and capillary electrophoretic determination of free nicotinic acid in human plasma and separation of its metabolites by capillary electrophoresis, *J.Chromatogr.A*, **1995**, *709*, 203–208.

SAMPLE
Matrix: blood, formulations, urine
Sample preparation: Tablets. Powder tablets, dissolve in water, inject a 10 μL aliquot. Injections. Dilute with water, inject a 10 μL aliquot. Plasma, urine. Condition a Lichrolut RP-18 (Merck) SPE cartridge with 3 mL MeOH and 3 mL water. Mix 40 μL plasma or 100 μL urine with twice the volume of MeCN for 2 min, add 100 μL water, centrifuge at 3500 rpm for 15 min, evaporate the supernatant under nitrogen at 45° to remove the organic solvents, add slowly to the SPE cartridge, collect the eluate. Evaporate to dryness under a stream of nitrogen at 45°. Reconstitute the residue with 500 μL MeOH containing 4.2 μg/mL IS. Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Lichrosorb RP-18
Mobile phase: Gradient. A was MeOH. B was 50 mM ammonium acetate. A:B from 5:95 to 15:85 over 6 min, to 30:70 over 7 min, maintain at 30:70 over 7 min
Flow rate: 1
Injection volume: 10
Detector: UV 270

CHROMATOGRAM
Retention time: 3.24
Internal standard: xanthine (4.56)
Limit of detection: 5 ng

OTHER SUBSTANCES
Extracted: ascorbic acid, folic acid, niacinamide, riboflavin, vitamin B12

KEY WORDS
plasma; SPE; tablets; injections

REFERENCE
Papadoyannis,I.N.; Tsioni,G.K.; Samanidou,V.F. Simultaneous determination of nine water and fat soluble vitamins after SPE separation and RP-HPLC analysis in pharmaceutical preparations and biological fluids, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 3203–3231.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 209.9

CHROMATOGRAM
Retention time: 3.163

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Dilute injections with water, inject a 50 μL aliquot. Dissolve tablets or capsule contents in water (warm if necessary), filter (0.5 μm PTFE), inject a 50 μL aliquot of the filtrate. (Dissolve tablets or other formulations containing proteinaceous material in water at 60°, add 5% trichloroacetic acid (to pH 4.4), filter, inject a 50 μL aliquot.)

HPLC VARIABLES
Guard column: pellicular Corasil
Column: 10 μm μBondapak C18
Mobile phase: Gradient. A was prepared by dissolving 1 g sodium dioctylsulfosuccinate in 170 mL MeOH, add 10 mL concentrated formic acid, make up to 800 mL with water, adjust pH to 2.5 with 1 M KOH, make up to 1 L. B was prepared by dissolving 1 g sodium dioctylsulfosuccinate in 450 mL MeOH, add 10 mL concentrated formic acid, make up to 800 mL with water, adjust pH to 4.6, make up to 1 L. A:B 100:0 for 19 min then 0:100 (step gradient) or A:B from 100:0 to 0:100 over 25 min (concave curve 9), maintain at 0:100 for 3 min, return to initial conditions over 2 min.
Flow rate: 1.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 5 (step gradient), 11 (curve gradient)

OTHER SUBSTANCES
Simultaneous: folic acid (UV 280), niacinamide, pyridoxamine (UV 280), thiamine, riboflavin, pyridoxine (UV 280), ascorbic acid (UV 280)

KEY WORDS
injections; capsules; tablets

REFERENCE
Woollard,D.C. New ion-pair reagent for the high-performance liquid chromatographic separation of B-group vitamins in pharmaceuticals, *J.Chromatogr.*, **1984**, *301*, 470–476.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 100 × 4 3 μm Hypersil BDS-C18
Mobile phase: Gradient. MeCN:water adjusted to pH 2.1 from 0.3:99.7 to 25:75 over 11 min
Flow rate: 0.5
Detector: UV 220

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: biotin, caffeine, citric acid, folic acid, niacinamide, pantothenic acid, riboflavin, saccharin, thiamine, pyridoxine, vitamin B12, ascorbic acid

KEY WORDS
tablets

REFERENCE
Hewlett Packard Leaflet 12-5091-7351 EUS, **1993**.

SAMPLE
Matrix: formulations
Sample preparation: Dilute liquid multivitamin formulations, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Lichrosorb RP-8
Mobile phase: Gradient. A was 10 mM KH_2PO_4 containing 5 mM sodium hexanesulfonate adjusted to pH 2.8 with phosphoric acid. B was MeOH. A:B from 90:10 to 71.8:28.2 over 4 min, maintain at 71.8:28.2 for 1.5 min, to 50:50 over 6.5 min, maintain at 50:50 for 5 min, return to initial conditions over 5 min
Flow rate: 1
Injection volume: 5
Detector: UV 272

CHROMATOGRAM
Retention time: 4.73
Internal standard: theobromine (8)
Limit of detection: 0.125 ng

OTHER SUBSTANCES
Simultaneous: folic acid, niacinamide, thiamine, riboflavin, pyridoxine (UV 290)

KEY WORDS
liquid multivitamins; degas solutions with helium; protect from light

REFERENCE
Blanco,D.; Sánchez,L.A.; Gutiérrez,M.D. Determination of water soluble vitamins by liquid chromatography with ordinary and narrow-bore columns, *J.Liq.Chromatogr.*, **1994**, *17*, 1525–1539.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 100 × 4.6 Spheri-5 RP-8
Mobile phase: Gradient. A was 100 mM pH 4.7 acetate buffer. B was MeCN:100 mM pH 4.7 acetate buffer 25:75.
Column temperature: 26
Flow rate: 4
Detector: UV 254

CHROMATOGRAM
Retention time: 1

OTHER SUBSTANCES
Simultaneous: pyridoxine, riboflavin, thiamine, niacinamide, ascorbic acid

REFERENCE
Rainin Catalog, C1-94, **1994**, p. 7.21.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 33 × 4.6 3 μm Supelcosil LC-8-DB
Mobile phase: MeOH:buffer 15:85 (Buffer was 4.3 mM sodium hexanesulfonate containing 0.1% triethylamine, adjusted to pH 2.8 with phosphoric acid.)
Column temperature: 35
Flow rate: 1
Detector: UV 200

CHROMATOGRAM
Retention time: 0.6

OTHER SUBSTANCES
Simultaneous: pantothenic acid, pyridoxine, riboflavin, thiamine, niacinamide, ascorbic acid

REFERENCE
Rainin Catalog, C1-94, **1994**, p. 780.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, meth-

yltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:50 mM KH_2PO_4 90:10
Flow rate: 1
Detector: UV 210

CHROMATOGRAM

Retention time: 1.5

OTHER SUBSTANCES

Simultaneous: biotin, folic acid, pantothenic acid, riboflavin, niacinamide

REFERENCE

MetaChem Catalog, **1995**, p. 21.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.16 μm PolyEncap ODS (n-octadecylacrylate copolymerized with vinyl silica in heptane, carrier Ultrasep ES 100; preparation described in paper)
Mobile phase: MeCN:pH 2.2 phosphate buffer 32:68
Flow rate: 1
Detector: UV 220

CHROMATOGRAM

Retention time: 2

OTHER SUBSTANCES

Simultaneous: aspirin, diazepam, diphenhydramine, o-hydroxyhippuric acid, MPPH, toluene

REFERENCE

Engelhardt,H.; Cuñat-Walter,M.A. Polymer encapsulated stationary phases with improved efficiency, *Chromatographia*, **1995**, *40*, 657–661.

SAMPLE
Matrix: tissue, food
Sample preparation: Condition a Sep-Pak Plus C18 SPE cartridge with 10 mL EtOH and 10 mL water. Weigh out 0.5-15 g cheese, semolina, or beef, make up to 200 mL with water, add 10 g calcium hydroxide, blend at high speed for 30 s, autoclave at 121° for 15 min, cool in an ice bath for at least 30 min, filter (Whatman 2V fluted paper). Add 100 mL filtrate to 300-320 mg oxalic acid, mix well, adjust pH to 6.5-7.0 with oxalic acid, filter (1-2 pieces Whatman No 42 paper) slowly, add 10 mL filtrate to the SPE cartridge, discard the first 6.5 mL effluent, collect the next 6.5 mL and add one drop 85% phosphoric acid, mix well, inject a 200 μL aliquot.

HPLC VARIABLES
Guard column: C18 Guard-Pak
Column: 150 × 4.6 5 μm C18 LC-18-DB (Supelco)
Mobile phase: MeCN:water:24 mM phosphoric acid 23:17:60 containing 1 g/L sodium dodecyl sulfate
Flow rate: 1.5
Injection volume: 200
Detector: UV 254

CHROMATOGRAM
Retention time: 9
Limit of detection: 500 ng/g

KEY WORDS
cow; muscle; semolina; cheese; SPE

REFERENCE
Tyler,T.A.; Genzale,J.A. Liquid chromatographic determination of total niacin in beef, semolina, and cottage cheese, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 467–469.

SAMPLE
Matrix: water
Sample preparation: Adjust 50 mL wastewater to pH 6.6 with acetic acid, add 50 mL ethyl acetate, shake vigorously for 5 min, let stand for 1 min, transfer the ethyl acetate layer to a flask, extract the residual aqueous layer with two 20 mL portions of ethyl acetate. Combine the organic layers and evaporate them at 90° to about 500 μL, dissolve the residue in 5 mL 10 mM HCl, make up to 50 mL with water, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2 (Vercopak)
Mobile phase: MeOH:buffer 20:80 (Buffer was 100 mM sodium acetate adjusted to pH 6.6 with 10 mM acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 260

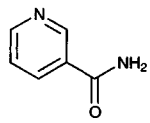
CHROMATOGRAM
Retention time: 3.3
Internal standard: niacin

OTHER SUBSTANCES
Extracted: sulfathiazole, sulfamethazine, sulfacetamide, sulfadiazine, sulfamerazine, sulfamethoxazole, sulfamonomethoxine

KEY WORDS
wastewater; niacin is IS

REFERENCE
Jen,J.-F.; Lee,H.-L.; Lee,B.-N. Simultaneous determination of seven sulfonamide residues in swine wastewater by high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *793*, 378–382.

Niacinamide



Molecular formula: $C_6H_6N_2O$
Molecular weight: 122.13
CAS Registry No.: 98-92-0
Merck Index: 6574

SAMPLE

Matrix: blood, formulations, urine

Sample preparation: Tablets. Powder tablets, dissolve in water, inject a 10 μL aliquot. Injections. Dilute with water, inject a 10 μL aliquot. Plasma, urine. Condition a Lichrolut RP-18 (Merck) SPE cartridge with 3 mL MeOH and 3 mL water. Mix 40 μL plasma or 100 μL urine with twice the volume of MeCN for 2 min, add 100 μL water, centrifuge at 3500 rpm for 15 min, evaporate the supernatant under nitrogen at 45° to remove the organic solvents, add slowly to the SPE cartridge, collect the eluate. Evaporate to dryness under a stream of nitrogen at 45°. Reconstitute the residue with 500 μL MeOH containing 4.2 μg/mL IS. Inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 5 μm Lichrosorb RP-18

Mobile phase: Gradient. A was MeOH. B was 50 mM ammonium acetate. A:B from 5:95 to 15:85 over 6 min, to 30:70 over 7 min, maintain at 30:70 over 7 min

Flow rate: 1

Injection volume: 10

Detector: UV 270

CHROMATOGRAM

Retention time: 8.13

Internal standard: xanthine (4.65)

Limit of detection: 5 ng

OTHER SUBSTANCES

Extracted: ascorbic acid, folic acid, niacin, riboflavin, vitamin B12

KEY WORDS

plasma; SPE; tablets; injections

REFERENCE

Papadoyannis,I.N.; Tsioni,G.K.; Samanidou,V.F. Simultaneous determination of nine water and fat soluble vitamins after SPE separation and RP-HPLC analysis in pharmaceutical preparations and biological fluids, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 3203–3231.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 214.6

CHROMATOGRAM
Retention time: 3.61

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formula, milk
Sample preparation: Mix 8.0 g powdered infant milk with 10 mL water to it. Mix the diluted powder or 10.5 g liquid infant milk with 1 g solid trichloroacetic acid, shake thoroughly with magnetic stirring for 10 min, centrifuge at 1250 g for 10 min, add 3 mL 4% trichloroacetic acid to the solid residue, mix thoroughly for 10 min, centrifuge, discard the solid phase. Combine the two acid extracts and make up to 10 mL with 4% trichloroacetic acid, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Guard column: 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Column: 250 × 4.6 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Mobile phase: MeOH:buffer 15:85 (Buffer was 5 mM octanesulfonic acid and 0.5% triethylamine, pH 3.6.)
Flow rate: 1
Injection volume: 20
Detector: UV 261 for 6 min, UV 287 for 2 min, UV 290 for 5 min, UV 282 for 3 min, UV 268 for 3.5 min, UV 361 for 20.5 min, UV 246 for 20 min

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CHROMATOGRAM
Retention time: 5
Limit of quantitation: ≤50 ng/mL

OTHER SUBSTANCES
Extracted: thiamine, riboflavin, pyridoxine, vitamin B12, folic acid, pyridoxal, pyridoxamine

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REFERENCE
Albalá-Hurtado,S.; Veciana-Nogués,M.; Izquierdo-Pulido,M.; Mariné-Font,A. Determination of water-soluble vitamins in infant milk by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *778*, 247–253.

SAMPLE
Matrix: formulations
Sample preparation: Pulverize tablets if necessary. Add tablets to 100 mL 5 mM pH 4.5 potassium phosphate buffer, sonicate at 75 W for 2 min, cool to room temperature, make up to 200 mL with buffer, filter (0.45 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb NH2 aminopropyl
Mobile phase: MeCN:5 mM KH_2PO_4 87:13 (Wash column with MeCN:water 10:90 at the end of the day.)
Column temperature: 25
Flow rate: 2
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 2

OTHER SUBSTANCES
Simultaneous: pantothenic acid, thiamine, riboflavin, pyridoxine

KEY WORDS
tablets

REFERENCE
Hudson,T.J.; Allen,R.J. Determination of pantothenic acid in multivitamin pharmaceutical preparations by reverse-phase high-performance liquid chromatography, *J.Pharm.Sci.*, **1984**, *73*, 113–115.

SAMPLE
Matrix: formulations
Sample preparation: Tablets without iron. Grind 5 tablets to a fine powder, add 10 mL monothioglycerol and 800 mL buffer, sonicate for 30 min, add 150 mL MeOH, make up to 1 L with buffer, filter (GF/C paper), discard first few mL, remove a 10 mL aliquot, make up to 25 mL with mobile phase, inject an aliquot. Tablets with dioctyl sodium sulfosuccinate. Grind 5 tablets to a fine powder, add 10 mL 2-monothioglycerol and 1 g barium chloride, make up to 1 L with buffer, stir vigorously for 30 min, filter (GF/C paper), discard first few mL, inject an aliquot. Capsules with iron. Contents of one capsule + 5 mL 2-monothioglycerol + 2 mL glacial acetic acid + 75 mL buffer, sonicate for 5 min, make up to 100 mL with buffer, stir vigorously for 30 min, filter (GF/C paper), add 300 mg cupferron, stir for 10 min, let stand for 1 h at room temperature, filter (GF/C paper), let stand for 30 min, filter again (if necessary), discard first few mL, inject an aliquot. (Buffer was 48 mL glacial acetic acid and 10 mL triethylamine in 1 L water, adjust pH to 3.6 ± 0.05 with acetic acid or triethylamine, make up to 1.7 L with water.)

HPLC VARIABLES
Column: 100 × 8 Radial Pak A C18 (Waters)
Mobile phase: MeOH:buffer 15:85 (Buffer was 2.20 g sodium heptanesulfonate, 100 mg EDTA, 48 mL glacial acetic acid, and 10 mL triethylamine made up to 1.7 L with water, adjust pH to 3.6 ± 0.05 with acetic acid or triethylamine.)
Flow rate: 2
Injection volume: 10
Detector: UV 280

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: thiamine, riboflavin, pyridoxine, ascorbic acid (UV 254)

KEY WORDS
multi-vitamin; protect from light; tablets; capsules

REFERENCE
Lam,F.-L.; Holcomb,I.J.; Fusari,S.A. Liquid chromatographic assay of ascorbic acid, niacinamide, pyridoxine, thiamine, and riboflavin in multivitamin-mineral preparations, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 1007–1011.

SAMPLE
Matrix: formulations
Sample preparation: Dilute injections with water, inject a 50 μL aliquot. Dissolve tablets or capsule contents in water (warm if necessary), filter (0.5 μm PTFE), inject a 50 μL aliquot of the filtrate. (Dissolve tablets or other formulations containing proteinaceous material in water at 60°, add 5% trichloroacetic acid (to pH 4.4), filter, inject a 50 μL aliquot.)

HPLC VARIABLES
Guard column: pellicular Corasil

Column: 10 μm μBondapak C18
Mobile phase: Gradient. A was prepared by dissolving 1 g sodium dioctylsulfosuccinate in 170 mL MeOH, add 10 mL concentrated formic acid, make up to 800 mL with water, adjust pH to 2.5 with 1 M KOH, make up to 1 L. B was prepared by dissolving 1 g sodium dioctylsulfosuccinate in 450 mL MeOH, add 10 mL concentrated formic acid, make up to 800 mL with water, adjust pH to 4.6, make up to 1 L. A:B 100:0 for 19 min then 0:100 (step gradient) or A:B from 100:0 to 0:100 over 25 min (concave curve 9), maintain at 0:100 for 3 min, return to initial conditions over 2 min.
Flow rate: 1.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 10 (step gradient), 11 (curve gradient)

OTHER SUBSTANCES
Simultaneous: folic acid (UV 280), niacin, pyridoxamine (UV 280), thiamine, riboflavin, pyridoxine (UV 280), ascorbic acid (UV 280)

KEY WORDS
injections; capsules; tablets

REFERENCE
Woollard,D.C. New ion-pair reagent for the high-performance liquid chromatographic separation of B-group vitamins in pharmaceuticals, *J.Chromatogr.*, **1984**, *301*, 470–476.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out 500 mg ground tablets, extract with water, make up to 50 or 100 mL with water, filter, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 10 C18
Mobile phase: MeOH:1% acetic acid 25:75
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Simultaneous: menadione hydrogen sulfite, pyridoxine, riboflavin, thiamine, ascorbic acid

KEY WORDS
tablets; multi-vitamin

REFERENCE
Sadlej-Sosnowska,N.; Blitek,D.; Wilczynska-Wojtulewicz,I. Determination of menadione sodium hydrogen sulphite and nicotinamide in multivitamin formulations by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *357*, 227–232.

SAMPLE
Matrix: formulations
Sample preparation: Dilute liquid multivitamin formulations, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Lichrosorb RP-8
Mobile phase: Gradient. A was 10 mM KH_2PO_4 containing 5 mM sodium hexanesulfonate adjusted to pH 2.8 with phosphoric acid. B was MeOH. A:B from 90:10 to 71.8:28.2 over 4 min,

maintain at 71.8:28.2 for 1.5 min, to 50:50 over 6.5 min, maintain at 50:50 for 5 min, return to initial conditions over 5 min
Flow rate: 1
Injection volume: 5
Detector: UV 272

CHROMATOGRAM
Retention time: 6.05
Internal standard: theobromine (8)
Limit of detection: 0.185 ng

OTHER SUBSTANCES
Simultaneous: folic acid, niacin, thiamine, riboflavin, pyridoxine (UV 290)

KEY WORDS
liquid multivitamins; degas solutions with helium; protect from light

REFERENCE
Blanco,D.; Sánchez,L.A.; Gutiérrez,M.D. Determination of water soluble vitamins by liquid chromatography with ordinary and narrow-bore columns, *J.Liq.Chromatogr.*, **1994**, *17*, 1525–1539.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Accubond Amino (J & W)
Mobile phase: MeCN:buffer 10 :90 (Buffer was 20 mM phosphoric acid adjusted to pH 3.0 with 20 mM NaOH.)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 1.8

OTHER SUBSTANCES
Simultaneous: p-aminobenzoic acid, pyridoxal, pyridoxamine, thiamine, riboflavin, pyridoxine, vitamin B12

REFERENCE
J & W Catalog, 1992-3, p. 277.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 100 × 4.6 Spheri-5 RP-8
Mobile phase: Gradient. A was 100 mM pH 4.7 acetate buffer. B was MeCN:100 mM pH 4.7 acetate buffer 25:75.
Column temperature: 26
Flow rate: 4
Detector: UV 254

CHROMATOGRAM
Retention time: 1.8

OTHER SUBSTANCES
Simultaneous: niacin, pyridoxine, riboflavin, thiamine, ascorbic acid

REFERENCE
Rainin Catalog, C1-94, **1994**, p. 7.21.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 33 × 4.6 3 μm Supelcosil LC-8-DB
Mobile phase: MeOH:buffer 15:85 (Buffer was 4.3 mM sodium hexanesulfonate containing 0.1% triethylamine, adjusted to pH 2.8 with phosphoric acid.)
Column temperature: 35
Flow rate: 1
Detector: UV 200

CHROMATOGRAM
Retention time: 0.75

OTHER SUBSTANCES
Simultaneous: niacin, pantothenic acid, pyridoxine, riboflavin, thiamine, ascorbic acid

REFERENCE
Rainin Catalog, C1-94, **1994**, p. 780.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphen-

butazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:50 mM KH_2PO_4 90:10
Flow rate: 1
Detector: UV 210

CHROMATOGRAM

Retention time: 2

OTHER SUBSTANCES

Simultaneous: biotin, folic acid, niacin, pantothenic acid, riboflavin

REFERENCE

MetaChem Catalog, **1995**, p. 21.

SAMPLE

Matrix: tissue
Sample preparation: Homogenize rat intestinal tissue in physiological saline. Removal a 3 mL aliquot and add it to 18 mL acetone, centrifuge at 10000 g for 10 min. Add the supernatant to 14 mL chloroform, mix intensively, centrifuge at 1000 g for 5 min. Filter (0.45 μm) a 500 μL aliquot of the upper aqueous phase, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 10 μm ODS Hypersil in an Upchurch C-135 B pre-column kit
Column: 250 × 4.6 5 μm ODS Hypersil
Mobile phase: Gradient. MeOH:5 mM pH 7.0 tetrabutylammonium phosphate from 10:90 to 70:30 over 10 min.
Flow rate: 1.2
Injection volume: 100
Detector: UV 410 following post-column derivatization. The column effluent mixed with 2% chloramine-T pumped at 0.5 mL/min and flowed through a 2 m × 0.5 mm i.d. coil of PTFE tubing kept at 60°. This mixture was mixed with 0.25% KCN containing 25 mM Tris and 40 mM HCl pumped at 0.5 mL/min. The mixture flowed through an 8 m × 0.5 mm i.d. coil of PTFE tubing kept at 60° to the detector.

CHROMATOGRAM

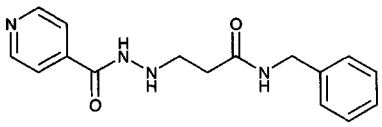
Retention time: 4.95
Limit of detection: 21 pmole

OTHER SUBSTANCES
Extracted: niacin

KEY WORDS
post-column reaction; rat; intestine

REFERENCE
Stein,J.; Hahn,A.; Rehner,G. High-performance liquid chromatographic determination of nicotinic acid and nicotinamide in biological samples applying post-column derivatization resulting in bathmochrome absorption shifts, *J.Chromatogr.B*, **1995**, *665*, 71–78.

Nialamide



Molecular formula: $C_{16}H_{18}N_4O_2$
Molecular weight: 298.34
CAS Registry No.: 51-12-7
Merck Index: 6575
Lednicer No.: 1 254

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 264

CHROMATOGRAM
Retention time: 3.07
Limit of detection: <120 ng/mL

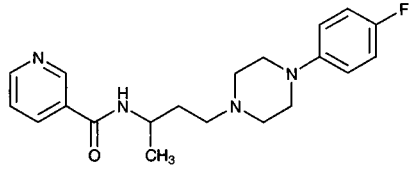
KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; almino-

profen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

Niaprazine



Molecular formula: $C_{20}H_{25}FN_4O$
Molecular weight: 356.44
CAS Registry No.: 27367-90-4
Merck Index: 6576

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

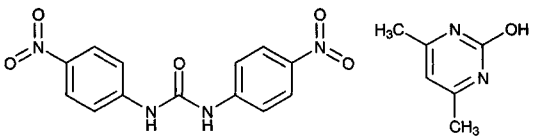
Retention time: 11.092

KEY WORDS

whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Nicarbazin



Molecular formula: $C_{19}H_{18}N_6O_6$
Molecular weight: 426.39
CAS Registry No.: 330-95-0
Merck Index: 6578

SAMPLE
Matrix: eggs
Sample preparation: Condition a 100 mg Bond Elut SPE cartridge (no. 601101) with two 1 mL portions of chloroform:MeCN 80:20 and with 1 mL chloroform, air dry. Stir 1 g homogenized egg and 50 μL 10% acetic acid and slowly add 2.5 mL MeCN, 4 min after the MeCN addition is complete add 0.25 g anhydrous sodium sulfate, stir for 2 min, centrifuge at 1400 g for 2 min, filter the supernatant through glass wool. Re-extract the sediment with 2.5 mL by stirring for 3 min, centrifuge at 1400 g for 2 min, filter the supernatant through glass wool, rinse the glass wool with 1 mL MeCN. Combine the filtrates and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 1 mL hexane:chloroform 50:50, add to the SPE cartridge, rinse the tube with 1 mL hexane:chloroform 50:50, add the rinse to the SPE cartridge, dry the SPE cartridge under vacuum for 1 min, elute with chloroform:MeCN 80:20. Collect the first 2 mL of eluate and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute with 1 mL mobile phase, vortex for 4 min, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 2.1 30-40 μm pellicular RP (Chrompack)
Column: two 100 × 3 5 μm ChromSpher C18 columns in series (Chrompack)
Mobile phase: MeCN:20 mM pH 4.8 acetate buffer:water 54:10:36
Flow rate: 0.6
Injection volume: 50
Detector: UV 360

CHROMATOGRAM
Retention time: 4.5 (dinitrocarbanilide)
Limit of detection: 2.5 ng/g

KEY WORDS
SPE

REFERENCE
Vertommen,M.H.; van der Laan,A.; Veenendaal-Hesselman,H.M. High-performance liquid chromatographic screening method for low levels of nicarbazin in eggs with off-line cartridge sample clean-up, *J.Chromatogr.*, **1989**, *481*, 452–457.

SAMPLE
Matrix: eggs
Sample preparation: 1 g Homogenized egg + 2.5 mL MeCN, vortex, stir for 4 min, add 300 mg anhydrous sodium sulfate, stir for 2 min, centrifuge at 1860 g for 2 min, decant the supernatant. Add 2.5 mL MeCN to the solid residue, vortex, stir for 3 min, centrifuge at 1860 g for 2 min, decant the supernatant. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 50-60°, reconstitute the residue in 1 mL mobile phase A, vortex for 15 s, sonicate for 3 min, filter (0.45 μm), inject a 250 μL aliquot onto column A with mobile phase A and elute to waste, after 5 min elute 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 and monitor the effluent from column B. (Clean column A with mobile phase A for 1 min at 1

mL/min and with MeCN at 2 mL/min for 13 min. Re-equilibrate with mobile phase A at 2 mL/min for 5 min.)

HPLC VARIABLES
Column: A 10 × 2.1 reverse-phase (Chrompack); B 10 × 2.1 pellicular reverse-phase (Chrompack) + 100 × 3 5 μm Chromspher C18 (Chrompack)
Mobile phase: A MeCN:water 20:80; B MeCN:10 mM pH 4.0 KH_2PO_4 50:50
Flow rate: 0.4
Injection volume: 250
Detector: UV 343

CHROMATOGRAM
Retention time: 6 (dinitrocarbanilide)
Limit of quantitation: 5 ng/g

KEY WORDS
column-switching

REFERENCE
Tarbin,J.A.; Shearer,G. High-performance liquid chromatographic method for the determination of the dinitrocarbanilide component of nicarbazin in eggs with on-line clean-up, *J.Chromatogr.*, **1993**, *613*, 354–358.

SAMPLE
Matrix: eggs, feed, litter, tissue
Sample preparation: Tissue, eggs, litter. 25 g Homogenized sample + 50 mL MeCN, blend (Büler H04) at low speed for 2-3 min, centrifuge at 1000 g for 10 min, remove the supernatant, re-extract the sediment with 30 mL MeCN, centrifuge at 1000 g for 10 min, combine the supernatants, shake well, discard the lower layer, add 80 mL dichloromethane, add 3 g NaCl, discard the lower layer, add 3 g anhydrous sodium sulfate, filter (paper). Evaporate the filtrate to dryness under reduced pressure at 45-50°, transfer to a smaller tube with 2 mL MeOH:MeCN:water 50:30:20 and 1 mL n-hexane, rinse in with four 1 mL portions of MeCN, evaporate to dryness under a stream of nitrogen at 50°. Reconstitute the residue in 2 mL MeOH:MeCN:water 50:30:20 and 0.5 mL n-hexane, centrifuge at 4000 rpm for 10 min, inject an aliquot of the MeOH layer (usually, but not always, the lower layer). Feed. 5 g Ground sample + 25 mL MeCN, blend (Büler H04) at low speed for 2-3 min, centrifuge at 1000 g for 10 min, remove the supernatant, re-extract the sediment with 25 mL MeCN, centrifuge at 1000 g for 10 min, combine the supernatants, evaporate to dryness under reduced pressure at 45-50°, transfer to a smaller tube with 2 mL MeOH:MeCN:water 50:30:20 and 1 mL n-hexane, rinse in with four 1 mL portions of MeCN, evaporate to dryness under a stream of nitrogen at 50°. Reconstitute the residue in 2 mL MeOH:MeCN:water 50:30:20 and 0.5 mL n-hexane, centrifuge at 4000 rpm for 10 min, inject an aliquot of the MeOH layer (usually, but not always, the lower layer).

HPLC VARIABLES
Column: 300 × 1 5 μm Supelcosil LC-18
Mobile phase: MeCN:100 mM pH 4.8 acetate buffer 60:30
Flow rate: 0.03
Injection volume: 0.5
Detector: UV 340

CHROMATOGRAM
Retention time: 9.5 (4,4'-dinitrocarbanilide)
Limit of detection: 25 pg

KEY WORDS
chicken; narrow bore; muscle; liver; nicarbazin is an equimolar mixture of 4,4'-dinitrocarbanilide and 4,6-dimethylpyrimidine

REFERENCE
Draisci,R.; Lucentini,L.; Boria,P.; Lucarelli,C. Micro high-performance liquid chromatography for the determination of nicarbazin in chicken tissues, eggs, poultry feed and litter, *J.Chromatogr.A*, **1995**, *697*, 407–414.

SAMPLE
Matrix: tissue
Sample preparation: Prepare a 12.5-15 mm bed of alumina (80-200 mesh, Brockman Activity I, Fisher) in a 5 mL pipette tip, condition with two 2 mL portions of chloroform:ethyl acetate 50:50. Blend (Polytron) 2.5 g ground tissue with 20 mL chloroform:ethyl acetate:DMSO 50:50:0.8 for 45 s, centrifuge at 3500 rpm for 5 min, discard the aqueous layer. Filter the organic layer through glass wool, add 2 mL filtrate to the alumina column, wash with 3.25 mL chloroform, dry with air pressure (column is dry when moisture no longer condenses on the outside, continue for another 3 min), elute with MeOH:pH 6.0 phosphate buffer 50:50, collect first 2 mL of eluate, purge eluate with helium for 2 min, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeOH:buffer 65:35 (Buffer was 50 mM KH_2PO_4 containing 1 mM EDTA, adjusted to pH 6.0 with NaOH.)
Flow rate: 1
Injection volume: 50
Detector: E, Bioanalytical Systems LC-45, glassy carbon electrode -0.8 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 8.5 (dinitrocarbanilide)
Limit of detection: 0.1-0.2 ppm
Limit of quantitation: 0.25 ppm

KEY WORDS
chicken; muscle; liver; SPE

REFERENCE
Parks,O.W. Rapid procedure for determination of nicarbazin residues in chicken tissues, *J.Assoc.Off. Anal.Chem.*, **1988**, *71*, 778–780.

SAMPLE
Matrix: tissue
Sample preparation: Connect two Sep-Pak alumina B SPE cartridges in series and condition with 4 mL DMF. Homogenize (Sorvall Omni-Mixer, do not substitute) 10 g ground liver or muscle with 25 mL ethyl acetate at half speed, decant supernatant, repeat homogenization twice more. Filter extracts, evaporate to an oily residue under vacuum, take up the residue in 10 mL MeCN, extract with 50 mL hexane. Extract the hexane layer twice with 10 mL portions of MeCN. Combine all the MeCN layers and wash with 50 mL hexane. Evaporate the MeCN layer to dryness under vacuum, reconstitute in 2 mL DMF, add to the SPE cartridges, wash with 14 mL DMF, eliminate as much DMF as possible, elute with 10 mL MeOH, evaporate the eluate to 2-3 mL under vacuum, make up to 10 mL with MeOH:water 75:25, inject a 50 μL aliquot.

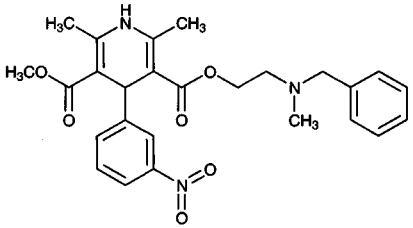
HPLC VARIABLES
Column: 250 × 4.6 3 μm IB-SIL C18 (Phenomenex)
Mobile phase: MeOH:water 75:25 containing 3.85 g/L ammonium acetate
Flow rate: 0.8-1
Injection volume: 50
Detector: UV 340 or MS, Finnigan 4600, negative ion thermospray, SIM m/z 164, 272, 302, filament on

CHROMATOGRAM
Retention time: 5 (4,4'-dinitrocarbanilide)
Limit of quantitation: 4 ppm

KEY WORDS
chicken; liver; muscle; SPE

REFERENCE
Leadbetter,M.G.; Matusik,J.E. Liquid chromatographic determination and liquid chromatographic-thermospray mass spectrometric confirmation of nicarbazin in chicken tissues: interlaboratory study, *J.AOAC Int.*, **1993**, *76*, 420–423.

Nicardipine



Molecular formula: $C_{26}H_{29}N_3O_6$
Molecular weight: 479.53
CAS Registry No.: 55985-32-5, 54527-84-3 (HCl)
Merck Index: 6579
Lednicer No.: 3 150

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL water. Mix 1 mL plasma with 10 µL 10 µg/mL IS in MeOH, dilute with 5 mL 1 M NaCl, mix briefly. Add the mixture to the SPE cartridge, wash with 10 mL water and 10 ml MeOH:water 40:60, elute with 5 mL MeOH:water 80:20. Dry the eluate under vacuum at 60° and dissolve the residue in 200 µL chloroform (Caution! Chloroform is a carcinogen!). Inject a 50 µL aliquot onto column A, wash with (?) for 30 s, elute the contents of column A onto column B with mobile phase, elute with mobile phase, monitor the effluent from column B.

HPLC VARIABLES
Column: A 10 × 4.6 5 µm TMS; B 250 × 4.6 5 µm Sumichiral OA-4500 (Sumika Chemical Analysis Service, Japan)
Mobile phase: MeOH:n-hexane:1,2-dichloroethane:trifluoroacetic acid 10:250:40:1
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 33.7 (+), 35.8 (-)
Internal standard: (+)-barnidipine (18)
Limit of detection: 2.5 ng/mL

KEY WORDS
plasma; SPE; chiral; column-switching

REFERENCE
Uno,T.; Ohkubo,T.; Sugawara,K. Enantioselective high-performance liquid chromatographic determination of nicardipine in human plasma, *J.Chromatogr.B*, **1997**, *698*, 181–186.

SAMPLE
Matrix: blood
Sample preparation: 200 µL Plasma or serum + 50 µL buffer + 50 µL 5 µg/mL butriptyline in water + 20 µL MTBE, vortex for 30 s, centrifuge at 9950 g for 2 min, inject an aliquot of the organic layer. (Buffer was 2 M Tris adjusted to pH 11.0 with 1 M NaOH.)

HPLC VARIABLES
Column: 125 × 5 5 µm Spherisorb S5SCX
Mobile phase: MeOH containing 20 mM ammonium perchlorate
Flow rate: 2
Injection volume: 100
Detector: E, Thames Chromatography TC100, V25-grade glassy-carbon electrode in a wall-jet assembly 1.1 V, Ag/AgCl reference electrode, range 100 nA

CHROMATOGRAM
Retention time: 4.5
Internal standard: butriptyline (7.5)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites, acebutolol, acetylprocainamide, ajamaline, amiodarone, amitriptyline, bupivacaine, chlorpromazine, desipramine, dextropropoxyphene, disopyramide, dothiepin, doxepin, fenethazine, fluazepam, hydroxypropafenone, imipramine, lignocaine, maprotiline, mepivacaine, metoprolol, mianserin, nortriptyline, norverapamil, phenazone, pindolol, prilocaine, propafenone, propranolol, protriptyline, quinidine, quinine, timolol, tocainide, trimipramine, verapamil, metabolites
Noninterfering: diazepam, mexiletine, nitrazepam
Interfering: oxprenolol, nadolol

KEY WORDS
plasma; serum

REFERENCE
Eastwood,R.J.; Galustian,C.; Bhamra,R.K.; Holt,D.W. High-performance liquid chromatographic method for the measurement of nicardipine in plasma or serum, *J.Chromatogr.*, **1990**, *530*, 463–468.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 238

CHROMATOGRAM
Retention time: 5.83
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acen-

ocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 µm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 205.2

CHROMATOGRAM

Retention time: 15.528

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: formulations

Sample preparation: Dilute with mobile phase, (if necessary), inject a 20 µL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Partisil ODS-3
Mobile phase: MeCN:MeOH:buffer 50:10:40 (Buffer was 20 mM KH_2PO_4 adjusted to pH 4.4-5.0 with phosphoric acid.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 237

CHROMATOGRAM
Retention time: 7.4

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
injections; stability-indicating

REFERENCE
Baaske,D.M.; DeMay,J.F.; Latona,C.A.; Mirmira,S.; Sigvardson,K.W. Stability of nicardipine hydrochloride in intravenous solutions, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 1701–1705.

SAMPLE
Matrix: formulations
Sample preparation: Filter (0.45 μm), dilute, and inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Partisil ODS-3
Mobile phase: MeCN:MeOH:20 mM KH_2PO_4 50:10:40, adjusted to pH 4.7
Flow rate: 1
Detector: UV 237

KEY WORDS
suspensions

REFERENCE
Maurin,M.B.; Rowe,S.M.; Koval,C.A.; Hussain,M.A. Solubilization of nicardipine hydrochloride via complexation and salt formation, *J.Pharm.Sci.*, **1994**, *83*, 1418–1420.

SAMPLE
Matrix: perfusate

HPLC VARIABLES
Column: 100 × 8 5 μm Novapak C18 radial compression
Mobile phase: MeCN:10 mM pH 4.5 phosphate buffer 70:30
Flow rate: 2
Detector: UV 237

OTHER SUBSTANCES
Also analyzed: felodipine, nifedipine, nimodipine, nitrendipine

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: perfusate
Sample preparation: Add perfusate to an equal volume of nitrendipine in MeOH, vortex, centrifuge, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 5C18
Mobile phase: MeCN:20 mM KH_2PO_4 60:40
Detector: UV 240

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

HPLC VARIABLES
Column: 250 × 4.6 cellulose tris(4-tert-butylphenylcarbamate)
Mobile phase: Hexane:isopropanol 90:10
Flow rate: 0.5
Detector: UV

CHROMATOGRAM
Retention time: 54 (-), 63 (+)

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: Centrifuge at 2000 g at 37° for 15 min.

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: nifedipine, nimodipine, nitrendipine, felodipine

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: Direct injection of a MeOH solution containing 200-1000 ng.

HPLC VARIABLES
Column: 250 × 4.6 Sumchiral OA-4500 (Sumika Chemical Analysis Service)
Mobile phase: n-Hexane:1,2-dichloroethane:MeOH:trifluoroacetic acid 250:140:10:1
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 25 (+), 26 (-) (α = 1.05)

KEY WORDS
chiral

REFERENCE
Ohkubo,T.; Uno,T.; Sugawara,K. Enantiomer separation of dihydropyridine derivative calcium antagonists by high-performance liquid chromatography with chiral stationary phases, *J.Chromatogr.A*, **1994**, *659*, 467–471.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 100 μM solution in buffer, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 column containing riboflavin binding proteins (Prepare as follows. Add riboflavin to saturate protein of egg yolk, homogenize with 3 volumes buffer, centrifuge, add the supernatant to a 500 × 30 column of DEAE-cellulose (Whatman) equilibrated with buffer, wash extensively with buffer to remove bound protein, elute riboflavin binding proteins (RFBP) with buffer containing 200 mM NaCl (RFBP has intense yellow color, absorption at 455 nm). Purify RFBP on a Sephadex G-100 column with 50 mM pH 7.5 Tris-HCl buffer as eluent, remove the bound riboflavin by extensive dialysis at pH 3.0. Add 4.5 g N,N-disuccinylimidyl carbonate to 3 g Nucleosil 5NH2 slurried in MeCN, filter, wash with MeCN, wash with 50 mM pH 7.5 phosphate buffer. Suspend 300 mg RFBP in 50 mM phosphate buffer, add the activated silica, mix gently for 2 h using a rotary evaporator, filter, wash with sterile water, wash with isopropanol:water 1:2, pack in a 100 × 4.6 column.) (Buffer was 100 mM pH 5.3 sodium acetate.)
Mobile phase: EtOH:50 mM pH 5.5 KH_2PO_4 5:95
Flow rate: 0.8
Injection volume: 20
Detector: UV

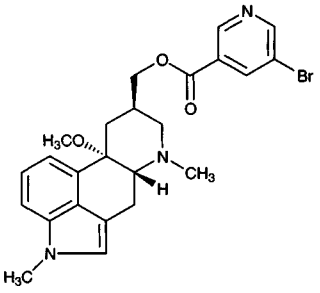
CHROMATOGRAM
Retention time: k' 10.61

OTHER SUBSTANCES
Simultaneous: bepridil, lorazepam, manidipine, oxazepam

KEY WORDS
chiral; α = 1.32

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.

Nicergoline



Molecular formula: $C_{24}H_{26}BrN_3O_3$
Molecular weight: 484.39
CAS Registry No.: 27848-84-6
Merck Index: 6580
Lednicer No.: 2 478

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 202.8

CHROMATOGRAM

Retention time: 15.452

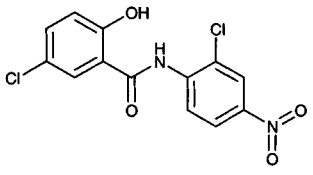
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Niclosamide



Molecular formula: $C_{13}H_8Cl_2N_2O_4$
Molecular weight: 327.12
CAS Registry No.: 50-65-7
Merck Index: 6602
Lednicer No.: 2 94

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 205.2

CHROMATOGRAM

Retention time: 23.92

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: formulations
Sample preparation: Dissolve sample in MeOH containing 10% formic acid, dilute with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil C18
Mobile phase: MeOH:buffer 19:81, pH 3.9 (Buffer was prepared by dissolving 6.6 g dibasic ammonium phosphate in 1 L water and adjusting to pH 3.9 with phosphoric acid.)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: 18.1

OTHER SUBSTANCES

Simultaneous: albendazole, fenbendazole, oxyclozanide

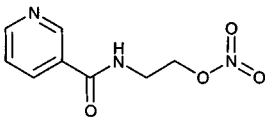
KEY WORDS

tablets; powder; liquid formulations

REFERENCE

van Tonder,E.C.; de Villiers,M.M.; Handford,J.S.; Malan,C.E.P.; Du Preez,J.L. Simple, robust and accurate high-performance liquid chromatography method for the analysis of several anthelmintics in veterinary formulations, *J.Chromatogr.A*, **1996**, *729*, 267–272.

Nicorandil



Molecular formula: $C_8H_9N_3O_4$
Molecular weight: 211.18
CAS Registry No.: 65141-46-0
Merck Index: 6608
Lednicer No.: 3 148

SAMPLE

Matrix: blood
Sample preparation: Condition two Bond Elut C18 SPE cartridges with 2 volumes of MeOH and 2 volumes of water. 1 mL Plasma + 50 μL 2 μg/mL IS in water, add to the SPE cartridge, wash with two 1 mL portions of water, elute with MeCN. Evaporate the eluate to less than 200 μL under a stream of nitrogen, add 1 mL water, add the mixture to another SPE cartridge, wash with two 1 mL portions of water, dry under vacuum for 10-15 min, wash with 2 mL MeCN, elute with 1 mL MeOH:water 95:5. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 160 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 phenyl (Brownlee)
Column: 150 × 4.6 5 μm Zorbax phenyl
Mobile phase: MeCN:isopropanol:water 12:2:86
Flow rate: 1
Injection volume: 160
Detector: UV 254 or photoconductivity (Tracor 965)

CHROMATOGRAM

Retention time: 15
Internal standard: N-[2-(nitrooxy)propyl]-3-pyridine carboxamide (24)
Limit of detection: 10 ng/mL, 2 ng/mL (photoconductivity)

KEY WORDS

plasma; dog; pharmacokinetics; SPE

REFERENCE

Schwende,F.J.; Lewis,R.C. Determination of nicorandil in plasma using high-performance liquid chromatography with photoconductivity and ultraviolet detection. Application to pre-clinical pharmacokinetics in beagle dogs, *J.Chromatogr.*, **1990**, *525*, 151–160.

SAMPLE

Matrix: blood
Sample preparation: Condition a Bond-Elut PH SPE cartridge with two 1 mL portions of MeOH and two 1 mL portions of water. 20 μL Plasma + 50 μL 10 μg/mL IS, add to the SPE cartridge, elute with 250 μL MeOH, inject a 40 μL aliquot of the eluate.

HPLC VARIABLES

Column: 10 μm μBondapak C18
Mobile phase: MeCN:50 mM pH 8.4 ammonium phosphate buffer 35:65
Flow rate: 1.2
Injection volume: 40
Detector: UV 254

CHROMATOGRAM

Retention time: 4.5
Internal standard: N-(2-hydroxypropyl)nicotinamide nitrate (5.5)
Limit of detection: 100 ng/mL

KEY WORDS

rat; plasma; pharmacokinetics; SPE

REFERENCE
Gomita,Y.; Eto,K.; Furuno,K.; Mimaki,Y.; Araki,Y. Influences of exposure to cigarette smoke on concentration of nicorandil in plasma of rats, *J.Pharm.Sci.*, **1992**, *81*, 228–231.

SAMPLE
Matrix: blood
Sample preparation: 180 μL Plasma + 10 μL water + 10 μL 210 μg/mL IS + 1 drop 100 mM NaOH + 1 mL ethyl acetate, vortex for 30 s, centrifuge at 2000 g for 10 min, repeat the extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, immediately reconstitute the residue in 200 μL water, vortex for 10 s, inject a 30-50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:water 45:55
Flow rate: 1
Injection volume: 30-50
Detector: UV 254

CHROMATOGRAM
Retention time: 5.5
Internal standard: N(3-hydroxypropyl) nicotinamide nitrate ester (6.7)
Limit of detection: 300 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, metabolites

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Bachert,E.L.; Fung,H.L. High performance liquid chromatographic method for stability and pharmacokinetic studies of nicorandil, *J.Chromatogr.*, **1993**, *619*, 336–341.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elut PH SPE cartridge with 2 mL MeOH and 2 mL water. 500 μL Plasma + 50 μL 2.5 μg/mL, mix, add to the SPE cartridge, wash with 2 mL water, wash with 1 mL MeOH:water 8:92, elute with 500 μL MeOH:water 50:50. Evaporate the eluate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 1 mL water, add 100 μL 2 M HCl, add 4 mL ethyl acetate, shake for 10 min, centrifuge at 2800 g for 10 min, discard the organic layer. Adjust the pH of the aqueous layer to 9-10 with 300 μL 1 M sodium carbonate, add 4 mL dichloromethane, shake for 10 min, centrifuge at 2800 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 100 μL water, inject a 45 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water:100 mM pH 8 borate buffer 15:70:15
Flow rate: 1
Injection volume: 45
Detector: UV 220

CHROMATOGRAM
Retention time: 8
Internal standard: N-[2-(nitroxy)propyl]-3-pyridinecarboxamide (SG-89) (11)
Limit of detection: 3 ng/mL

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Tanikawa,M.; Uzu,M.; Ohsawa,Y.; Fukushima,M. Sensitive method for determination of nicorandil in human plasma by reversed-phase high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1993**, *617*, 163–167.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 100 μL 1.5 M perchloric acid, vortex, centrifuge at 9600 g for 1 min, add to 100 μL 3 M potassium carbonate, vortex, centrifuge for 1 min, inject a 100-200 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Capcell Pak C8 AG-120 endcapped (Shiseido)
Mobile phase: MeOH:buffer 20:80 (Prepare by mixing 60 mL 500 mM acetic acid and 940 mL 500 mM pH 6.0 sodium acetate containing 20% MeOH and 20 mM hydrogen peroxide.)
Flow rate: 0.8
Injection volume: 100-200
Detector: F ex 323 em 386 following post-column reaction. The column effluent flowed through a 3 m × 0.25 mm ID Tefzel coil irradiated by two 4 W germicidal lamps (Nippon Denki, Tokyo) at about 40°, a 0.4 m × 0.1 mm ID stainless steel coil, and a 2 m × 0.25 mm ID PTFE coil to the detector.

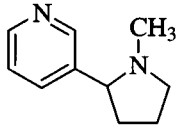
CHROMATOGRAM
Retention time: 10
Limit of detection: 7 ng/mL

OTHER SUBSTANCES
Noninterfering: histidine, kynurenic acid, niacin, phenylalanine, pyridoxine, thiamine, tyrosine

KEY WORDS
post-column reaction; post-column photochemical derivatization; plasma; pharmacokinetics

REFERENCE
Mawatari,K.-i.; Nakamura,Y.; Shimizu,R.; Sate,S.; Iinuma,F.; Watanabe,M. Fluorimetric determination of nicorandil in human plasma by a high-performance liquid chromatographic-postcolumn ultraviolet detection system equipped with on-line back-pressure tubing, *J.Chromatogr.B*, **1996**, *679*, 155–159.

Nicotine



Molecular formula: $C_{10}H_{14}N_2$
Molecular weight: 162.23
CAS Registry No.: 54-11-5, 96055-45-7 (nicotine polacrilex)
Merck Index: 6611

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL 100 ng/mL nicotine-methyl-d_3 to 1 mL heparinized plasma, vortex briefly, add 100 μL 5 M ammonium hydroxide and 8 mL dichloromethane, shake for 5 min on a horizontal shaker, centrifuge at 2500 rpm for 5 min, evaporate the organic phase to dryness under nitrogen at 37°, reconstitute the residue in 100 μL mobile phase, inject 10 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 2.0 BDS C18
Column: 100 × 3.0 3 μm BDS Hypersil C18
Mobile phase: MeCN:MeOH:10 mM ammonium acetate 53:32:15
Flow rate: 1.4

REFERENCE
Tanikawa,M.; Uzu,M.; Ohsawa,Y.; Fukushima,M. Sensitive method for determination of nicorandil in human plasma by reversed-phase high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1993**, *617*, 163–167.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 100 μL 1.5 M perchloric acid, vortex, centrifuge at 9600 g for 1 min, add to 100 μL 3 M potassium carbonate, vortex, centrifuge for 1 min, inject a 100-200 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Capcell Pak C8 AG-120 endcapped (Shiseido)
Mobile phase: MeOH:buffer 20:80 (Prepare by mixing 60 mL 500 mM acetic acid and 940 mL 500 mM pH 6.0 sodium acetate containing 20% MeOH and 20 mM hydrogen peroxide.)
Flow rate: 0.8
Injection volume: 100-200
Detector: F ex 323 em 386 following post-column reaction. The column effluent flowed through a 3 m × 0.25 mm ID Tefzel coil irradiated by two 4 W germicidal lamps (Nippon Denki, Tokyo) at about 40°, a 0.4 m × 0.1 mm ID stainless steel coil, and a 2 m × 0.25 mm ID PTFE coil to the detector.

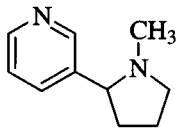
CHROMATOGRAM
Retention time: 10
Limit of detection: 7 ng/mL

OTHER SUBSTANCES
Noninterfering: histidine, kynurenic acid, niacin, phenylalanine, pyridoxine, thiamine, tyrosine

KEY WORDS
post-column reaction; post-column photochemical derivatization; plasma; pharmacokinetics

REFERENCE
Mawatari,K.-i.; Nakamura,Y.; Shimizu,R.; Sate,S.; Iinuma,F.; Watanabe,M. Fluorimetric determination of nicorandil in human plasma by a high-performance liquid chromatographic-postcolumn ultraviolet detection system equipped with on-line back-pressure tubing, *J.Chromatogr.B*, **1996**, *679*, 155–159.

Nicotine



Molecular formula: $C_{10}H_{14}N_2$
Molecular weight: 162.23
CAS Registry No.: 54-11-5, 96055-45-7 (nicotine polacrilex)
Merck Index: 6611

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL 100 ng/mL nicotine-methyl-d_3 to 1 mL heparinized plasma, vortex briefly, add 100 μL 5 M ammonium hydroxide and 8 mL dichloromethane, shake for 5 min on a horizontal shaker, centrifuge at 2500 rpm for 5 min, evaporate the organic phase to dryness under nitrogen at 37°, reconstitute the residue in 100 μL mobile phase, inject 10 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 2.0 BDS C18
Column: 100 × 3.0 3 μm BDS Hypersil C18
Mobile phase: MeCN:MeOH:10 mM ammonium acetate 53:32:15
Flow rate: 1.4

Injection volume: 10
Detector: MS, Perkin-Elmer Sciex API-III, APCI interface, nebulizer 480°, pressure 551 kPa, nitrogen 1.2 L/min, interface heater 55°, electron multiplier 4000 V, m/z 163.2 to 84.0

CHROMATOGRAM
Retention time: 0.57
Internal standard: nicotine-methyl-d_3 (0.57)
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES
Extracted: cotinine

KEY WORDS
pharmacokinetics; plasma

REFERENCE
Xu,A.S.; Peng,L.L.; Havel,J.A.; Petersen,M.E.; Fiene,J.A.; Hulse,J.D. Determination of nicotine and cotinine in human plasma by liquid chromatography-tandem mass spectrometry with atmospheric-pressure chemical ionization interface, *J.Chromatogr.B*, **1996**, *682*, 249–257.

SAMPLE
Matrix: blood
Sample preparation: Condition a Merck Extrelut-3 glass extraction column with 12 mL dichloromethane the day before. 1.5 mL Serum + 100 μL 3 μg/mL N-ethylnorcotinine in water + 1.4 mL 0.5 M NaOH. Add to column, after 15 min elute under gravity with dichloromethane: isopropanol 9:1. Add 300 μL 25 mM HCl in MeOH to the organic phase and evaporate it to dryness under nitrogen. Redissolve in 100 μL water and inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC8DB
Mobile phase: Gradient. A was MeCN:water 3.6:96.4 containing 2 mL/L triethylamine, 12 mM sodium heptanesulfonate, 12 mM K_2HPO_4, 12 mM citric acid adjusted to pH 4.7 with citric acid. B was MeCN:water 19.7:80.3 containing 2 mL/L triethylamine, 12 mM sodium heptanesulfonate, 12 mM K_2HPO_4, 12 mM citric acid adjusted to pH 5.2 with citric acid. A:B 100:0 for 15 min then to 50:50 over 20 min using a concave gradient. Re-equilibrate for 15 min before next injection.
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 14
Internal standard: N-ethylnorcotinine (33)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites, cotinine-N-oxide, trans-3'-hydroxycotinine, norcotinine, cotinine, caffeine

KEY WORDS
serum; SPE

REFERENCE
Zuccaro,P.; Altieri,I.; Rosa,M.; Passa,A.R.; Pichini,S.; Ricciarello,G.; Pacifici,R. Determination of nicotine and four metabolites in the serum of smokers by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1993**, *621*, 257–261.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 100 μL 70% perchloric acid, centrifuge at 2000 g for 10 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC8DB
Mobile phase: MeCN:MeOH:THF:buffer 4.3:2.3:0.3:93.1 (Buffer was 2 mL/L triethylamine containing 12 mM sodium heptanesulfonate, 12 mM K_2HPO_4, and 12 mM citric acid, adjusted to pH 4.7 with citric acid.)
Flow rate: 1.4
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 15
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, cotinine, caffeine

KEY WORDS
serum

REFERENCE
Pichini,S.; Altieri,I.; Passa,A.R.; Rosa,M.; Zuccaro,P.; Pacifici,R. Use of solvent optimization software for rapid selection of conditions for reversed-phase high-performance liquid chromatography of nicotine and its metabolites, *J.Chromatogr.A*, **1995**, *697*, 383–388.

SAMPLE
Matrix: blood, tissue
Sample preparation: Wash a C18 Sep Pak cartridge with 2 mL MeOH then 2 mL water. Blood. 1 mL Blood + 2 mL water, add 2 mL MeCN, cool to 5°, centrifuge. Tissue. 1 g Tissue + 4 mL water, homogenize, add 4 mL MeOH, centrifuge, extract pellet again with 2 mL MeOH. Combine supernatants, evaporate, take up in 50 μL 5 M NaOH, exctract with ethyl acetate, discard organic phase, add 100 μL 6 M HCl, extract with ethyl acetate, discard organic phase, add 8 mg of dodecyl sodium sulfate (at pH 2.0-2.1), add to cartridge, wash with MeOH/water, elute with MeOH, concentrate, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Alltech NH2
Mobile phase: Isopropanol:water 75:25
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Extracted: cotinine, cotinine N-oxide, nicotine N-oxide

KEY WORDS
SPE; liver

REFERENCE
Thompson,J.A.; Norris,K.J.; Petersen,D.R. Isolation and analysis of N-oxide metabolites of tertiary amines: quantitation of nicotine-1'-N-oxide formation in mice, *J.Chromatogr.*, **1985**, *341*, 349–359.

SAMPLE
Matrix: dialysate, saliva
Sample preparation: Saliva. Incubate 500 mg smokeless tobacco and 10 mL artificial saliva at 37° for 0.5-60 min, filter (0.45 HVLP), inject an aliquot. Dialysate (Method 1). Place 1.5 g tobacco (without sachet) in dialysis tube and tie ends. Press dialysis bag with a glass rod for 30 s , immerse it in 50 mL isotonic pH 7.4 phosphate buffer, incubate at 37°, inject an aliquot. (Method 2). Place 500 mg tobacco (with or without sachet) in dialysate tube, tie the ends,

immerse the dialysis bag in 25 mL artificial saliva and incubate at 37°. Remove an aliquot from the saliva surrounding the bag. Inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm Bondapak C18
Mobile phase: MeOH:buffer 40:60 (Buffer was 0.2% phosphoric acid adjusted to pH 7.25 with triethylamine.)
Flow rate: 1.5
Detector: UV 245

KEY WORDS
artificial saliva; tobacco

REFERENCE
Nasr,M.M.; Reepmeyer,J.C.; Tang,Y. In vitro study of nicotine release from smokeless tobacco, *J.AOAC Int.*, **1998**, *81*, 540–543.

SAMPLE
Matrix: meconium
Sample preparation: Condition a 3 mL C8 SPE cartridge (Backer-Bond, Gross Gerau, Germany) with 5 mL MeOH and 5 mL water. Condition a 3 mL silica SPE cartridge (Backer-Bond) with 5 mL chloroform. Place the C8 SPE cartridge on top of the silica SPE cartridge. Thaw and mix meconium, weigh a 2 g aliquot, add IS, and emulsify in 20 mL 100 mM pH 8.0 phosphate buffer. Vortex, centrifuge, filter the supernatant. Extract the supernatant 3 times with 2 mL portions of chloroform. Evaporate chloroform extracts to dryness and dissolve in 10 mL pH 9.0 boric buffer. Add to the SPE cartridges, remove the C8 column and elute the silica column with 3 mL MeOH:dichloromethane:ammonium hydroxide 30:70:1. Evaporate the eluate to dryness under nitrogen and redissolved in 100 μL water. Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm LiChrosorb C18
Mobile phase: MeCN:MeOH:water:pH 4.66 acetate buffer: acetic acid 2:29:50:20:1, pH adjusted to 4.3 with diethylamine
Flow rate: 1.0
Injection volume: 20
Detector: UV

CHROMATOGRAM
Retention time: 3.75
Internal standard: ephedrine
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: caffeine, cotinine

KEY WORDS
SPE

REFERENCE
Baranowski,J.; Pochopien,G.; Baranowska,I. Determination of nicotine, cotinine and caffeine in meconium using high-performance liquid chromatography, *J.Chromatogr.B*, **1998**, *707*, 317–321.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Extract microsomal incubation with isopropanol/dichloromethane, evaporate the extracts to a minimum volume, reconstitute with MeOH, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Microsorb C18
Mobile phase: MeCN:MeOH:60% perchloric acid 50:50:0.03
Detector: UV 255

CHROMATOGRAM
Retention time: 16.4 mL (retention volume)

OTHER SUBSTANCES
Extracted: metabolites, cotinine

KEY WORDS
pig; human; liver

REFERENCE
Berkman,C.E.; Park,S.B.; Wrighton,S.A.; Cashman,J.R. *In vitro-in vivo* correlations of human (*S*)-nicotine metabolism, *Biochem.Pharmacol.*, **1995**, *50*, 565–570.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 35 × 4 Dionex Ion Pac NGI
Mobile phase: MeCN:water 7:93 containing 500 μM hexanesulfonic acid
Flow rate: 1.0
Injection volume: 100
Detector: UV 262

CHROMATOGRAM
Retention time: 2.4
Limit of detection: 4 ng/mL

OTHER SUBSTANCES
Simultaneous: cotinine, 4-ethenylpyridine

REFERENCE
Bertoni,G.; Di Palo,V.; Tappa,R.; Possanzini,M. Fast determination of nicotine and 3-ethenylpyridine in indoor environments, *Chromatographia*, **1996**, *43*, 296–300.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.9

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, 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, dipipa-

none, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 3.3 7 μm Separon SGX
Mobile phase: 80 mM ammonium perchlorate in MeOH
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 2.26

OTHER SUBSTANCES

Simultaneous: amrinone, strychnine, nicotine, neostigmine

REFERENCE

Eigendorf,H.G.; Nagel,S. Zur Analytik von Amrinone (Cordemcura). 2. Mitteillung: Hochdruckflüssigchromatographie [The analysis of amrinone (Cordemcura). 2. High pressure liquid chromatography], *Pharmazie*, **1987**, *42*, 631.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 0.5 mg/mL solution in water, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN: water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30

Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 1.3

OTHER SUBSTANCES
Simultaneous: acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, diflunisal, doxapram, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, testosterone, testosterone propionate, tranylcypromine, tripelennamine

KEY WORDS
details for purification of triethylamine in paper

REFERENCE
Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 30 × 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 254

CHROMATOGRAM
Retention time: 0.7
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, 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, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Chirex 3014 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 50:35:15 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1
Injection volume: 20
Detector: UV 260

KEY WORDS

chiral; α = 1.10 for enantiomers

REFERENCE
Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

SAMPLE
Matrix: urine
Sample preparation: 500 μL Urine + 200 μL 4 M pH 4.7 acetate buffer + 100 μL 1.5 M KCN in water + 100 μL 400 mM chloramine T in water + 500 μL 50 mM 1,3-diethyl-2-thiobarbituric acid in acetone:water 50:50, mix, let stand at room temperature for 15 min, inject a 5 μL aliquot immediately.

HPLC VARIABLES
Guard column: 10 × 4.6 10 μm reversed-phase (Whatman)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:water 2:1 containing 20 mM pentanesulfonic acid
Flow rate: 2
Injection volume: 5
Detector: UV 546

CHROMATOGRAM
Retention time: 6
Limit of detection: 10 ng/mL (20 μL injection)

OTHER SUBSTANCES
Extracted: metabolites, cotinine

KEY WORDS
derivatization

REFERENCE
Barlow,R.D.; Thompson,P.A.; Stone,R.B. Simultaneous determination of nicotine, cotinine and five additional nicotine metabolites in the urine of smokers using pre-column derivatization and high performance liquid chromatography, *J.Chromatogr.*, **1987**, *419*, 375–380.

SAMPLE
Matrix: urine
Sample preparation: Centrifuge urine at 10000 g for 5 min, filter (Millipore Ultrafree-MC, 30 000 NMWL). Dilute a 10-150 μL aliquot to 150 μL with water, add 60 μL 5 μg/mL IS in buffer, add 30 μL 1.5 M KCN, add 15 μL 1 M chloramine T, add 75 μL 75 mM in 1,3-diethyl-2-thiobarbituric acid in water:acetone 50:50, mix vigorously for 30 s, centrifuge for 2 min, let stand for 11-12 min, inject a 50-250 μL aliquot. (Buffer was 6 M sodium acetate adjusted to pH 4.7 with 6 M trichloroacetic acid. The chloramine T and 1,3-diethyl-2-thiobarbituric acid solutions should be stored at 40°.)

HPLC VARIABLES
Guard column: 25 × 4 5 μm Nucleosil 100 C18
Column: 150 × 3.9 4 μm Nova Pak RP18
Mobile phase: Gradient. A was 60 mM sodium 1-pentanesulfonate adjusted to pH 3.8 with 1% orthophosphoric acid. B was MeOH:THF 95:5. C was MeCN:THF 97:3. A:B:C from 80:10:10 to 74:15:11 over 5 min, to 65:20:15 over 5 min, to 65:15:20 over 5 min, to 65:10:25 over 5 min, to 60:0:40 over 10 min, to 35:0:65 over 3 min.
Flow rate: From 1.55 to 1.45 over 15 min, maintain at 1.45.
Injection volume: 50-250
Detector: UV 529

CHROMATOGRAM
Retention time: 26
Internal standard: N'-ethylnorcotinine (21)
Limit of quantitation: 1.5 μM

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
derivatization; human; hamster; rat

REFERENCE
Rustemeier,K.; Demetriou,D.; Schepers,G.; Voncken,P. High-performance liquid chromatographic determination of nicotine and its urinary metabolites via their 1,3-diethyl-2-thiobarbituric acid derivatives, *J.Chromatogr.*, **1993**, *613*, 95–103.

SAMPLE
Matrix: urine
Sample preparation: 200 μL Urine + 100 μL 4 M pH 4.7 sodium acetate buffer + 40 μL 1.5 M KCN in water + 40 μL 400 mM chloramine T in water + 200 μL 78 mM barbituric acid in acetone:water 50:50, mix for 10 s, let stand at room temperature for 15 min, add 40 μL 1 M sodium metabisulfite in water (Clin. Chim. Acta 1987, 165, 45), inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 10 μm reversed-phase (Whatman)
Column: 250 × 4.6 5 μm Spherisorb S 5ODS2
Mobile phase: MeCN:50 mM pH 5.2 sodium acetate buffer 22:78
Flow rate: 1.5
Injection volume: 50
Detector: UV 490

CHROMATOGRAM
Retention time: 4.1
Limit of detection: 10 nM

OTHER SUBSTANCES
Extracted: cotinine

KEY WORDS
derivatization

REFERENCE
Ubbink,J.B.; Lagendijk,J.; Vermaak,W.H.H. Simple high-performance liquid chromatographic method to verify the direct barbituric acid assay for urinary cotinine, *J.Chromatogr.*, **1993**, *620*, 254–259.

SAMPLE
Matrix: urine
Sample preparation: Acidify urine to pH 2.00 with phosphoric acid, filter (Acrodisc 0.20 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Econosphere CN
Mobile phase: Isopropanol:20 mM sodium dodecyl sulfate 3:97, pH 4.60
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 260

CHROMATOGRAM
Retention time: 10.85
Limit of detection: 0.18 ppm

OTHER SUBSTANCES
Extracted: cotinine

REFERENCE
Reynolds,J.; Albazi,S.J. Simultaneous determination of nicotine and cotinine in untreated human urine by micellar liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 537–552.

SAMPLE
Matrix: urine
Sample preparation: Condition a 700 mg Extrelut-1 diatomaceous earth glass SPE cartridge with 6 mL dichloromethane, let stand for 1 day. 200 μL Urine + 200 μL 10 μg/mL N-ethylnornicotine in water + 600 μL 500 mM NaOH, add to the SPE cartridge, let stand for 10 min, elute with 5 mL dichloromethane:isopropanol 90:10. Add the eluate to 100 μL 25 mM HCl in MeOH and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm LC8DB (Supelchem)
Mobile phase: MeCN:water 9:80 containing 5 mL/L triethylamine, 670 mg/L sodium heptanesulfonate, 34 mM K_2HPO_4, and 34 mM citric acid, pH 4.4.
Flow rate: 1.6
Detector: UV 254

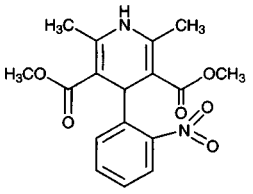
CHROMATOGRAM
Retention time: 4
Internal standard: N-ethylnornicotine (8.5)

OTHER SUBSTANCES
Extracted: metabolites, caffeine, cotinine

KEY WORDS
SPE

REFERENCE
Zuccaro,P.; Altieri,I.; Rosa,M.; Passa,A.R.; Pichini,S.; Pacifici,R. Solid-phase extraction of nicotine and its metabolites for high-performance liquid chromatographic determination in urine, *J.Chromatogr.B*, **1995**, *668*, 187–188.

Nifedipine



Molecular formula: $C_{17}H_{18}N_2O_6$
Molecular weight: 346.34
CAS Registry No.: 21829-25-4
Merck Index: 6617
Lednicer No.: 2 283; 4 106

SAMPLE
Matrix: blood
Sample preparation: Inject a 5 μL aliquot of serum directly.

HPLC VARIABLES
Column: 100 × 4.6 5-10 μm Silicalite (by sieving Silicalite, 3M Co.(?))
Mobile phase: MeCN:20 mM pH 6.9 phosphate buffer 7:93
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 1.35

KEY WORDS
serum

REFERENCE
Ambrose,D.L.; Fntz,J.S. High-performance liquid chromatographic determination of drugs and metabolites in human serum and urine using direct injection and a unique molecular sieve, *J.Chromatogr.B*, **1998**, *709*, 89–96.

SAMPLE
Matrix: blood
Sample preparation: Add 200 μL 1 M NaOH and 50 μL 2 μg/mL IS to 1 mL plasma, vortex for 10 s, add 3 mL hexane:dichloromethane 70:30, vortex for 5 min, centrifuge at 1400 g for 15 min, evaporate the organic layer under nitrogen at 50°, dissolve the dried sample in 250 μL mobile phase, inject a 50 μL aliquot onto column A in series with column B and elute with mobile phase. After 1 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. While column A is removed from the circuit flush it separately with mobile phase to remove strongly retained plasma components.

HPLC VARIABLES
Column: A 20 × 4.0 5 μm Hypersil BST ODS; B 200 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:10 mM pH 4 acetate buffer 75:25
Flow rate: 0.8
Injection volume: 50
Detector: E, Bioanalytical Systems Model BAS LC-3C equipped with BAS LC-44 thin-layer cell, glassy carbon electrode 1 V, Ag/AgCl/3 M KCl reference electrode

CHROMATOGRAM
Retention time: 4.7
Internal standard: dimethyl-1,4-(3-nitrophenyl)-3,5-pyridine-dicarboxylate (5.3)
Limit of detection: 450 pg/mL
Limit of quantitation: 1 ng/mL

KEY WORDS
dog; pharmacokinetics; plasma; column-switching

REFERENCE
Horváth,V.; Hrabéczy-Páll,A.; Niegreisz,Z.; Kocsi,E.; Horvai,G.; Gödörházy,L.; Tolokán,A.; Klebovich,I.; Balogh-Nemes,K. Sensitive high-performance liquid chromatographic determination of nifedipine in dog plasma using an automated sample preparation system with laboratory robot, *J.Chromatogr.B*, **1996**, *686*, 211–219.

SAMPLE
Matrix: blood
Sample preparation: Add 200 μL 1 M NaOH solution and 50 μL 2 μg/mL IS to 1 mL plasma, mix for 10 s, add 3 mL dichloromethane:n-hexane 30:70, vortex for 5 min, centrifuge. Evaporate a 2 mL aliquot of the organic layer to dryness in a stream of nitrogen at 50°, dissolve the residue in 250 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4 5 μm Hypersil BST ODS (Bio Separation Technologies, Hungary)
Column: 200 × 4.6 5 μm Hypersil ODS (Hewlett-Packard, USA)
Mobile phase: MeOH:10 mM pH 4 acetate buffer 75:25
Flow rate: 0.8
Injection volume: 50
Detector: E, Bioanalytical Systems BAS LC-3C equipped with BAS LC-44 thin-layer cell, glassy carbon electrode 1 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 4.9
Internal standard: dimethyl-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (5.5)
Limit of detection: 450 pg/mL
Limit of quantitation: 1 ng/mL

KEY WORDS
plasma

REFERENCE
Tolokán,A.; Gödörházy,L.; Horváth,V.; Hrabéczy-Páll,A.; Niegreisz,Z.; Kocsi,E.; Horvai,G.; Klebovich,I.; Balogh-Nemes,K. Economic approach to robotic sample pretreatment in high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *771*, 35–43.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 236.9

CHROMATOGRAM
Retention time: 19.485

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Weigh 10 tablets, powder finely. Weigh accurately powder containing 10 mg nifedipine, dissolve in MeOH and make up to 50 mL with MeOH. Add 1.6 mg IS, filter through a 45 μm membrane filter. Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Lichrosorb RP C18
Mobile phase: MeOH:water 55:45 pH 4.5
Flow rate: 1.0 for 4 min, then 2.0
Injection volume: 10
Detector: UV 260

CHROMATOGRAM
Retention time: 9.20
Internal standard: oxprenolol (4.35)
Limit of detection: 160 ng/mL

OTHER SUBSTANCES
Simultaneous: acebutolol, nifedipine oxidation products

KEY WORDS
comparison with GC and first-derivative spectroscopy; tablets

REFERENCE
el Walily,A.F.M. Analysis of nifedipine--acebutolol hydrochloride binary combination in tablets using UV-derivative spectroscopy, capillary gas chromatography and high performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 21–30.

SAMPLE
Matrix: formulations
Sample preparation: Crush five 10 mg tablets and mix with lactose to a nifedipine concentration 1 mg/500 mg of powder. Sonicate 500 mg aliquot of the powder in 10 mL MeOH at 20° for 10 min, dilute 1 mL of this solution to 10 mL with MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeOH:100 mM pH 5.8 ammonium acetate:triethylamine 70:30:0.1, pH adjusted to 5.8 with acetic acid
Flow rate: 1
Injection volume: 20
Detector: UV 238

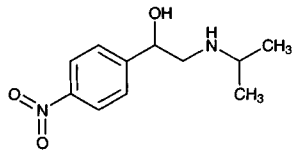
CHROMATOGRAM
Retention time: 5.0

OTHER SUBSTANCES
Simultaneous: photodegradation products

KEY WORDS
powder; stability-indicating

REFERENCE
Helin,M.M.; Kontra,K.M.; Naaranlahti,T.J.; Wallenius,K.J. Content uniformity and stability of nifedipine in extemporaneously compounded oral powders, *Am.J.Health-Syst.Pharm.*, **1998**, *55*, 1299–1301.

Nifenalol



Molecular formula: $C_{11}H_{16}N_2O_3$
Molecular weight: 224.26
CAS Registry No.: 7413-36-7, 5704-60-9 (HCl)
Merck Index: 6618

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 1.86

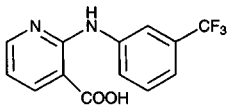
OTHER SUBSTANCES

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, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Niflumic acid



Molecular formula: $C_{13}H_9F_3N_2O_2$
Molecular weight: 282.22
CAS Registry No.: 4394-00-7, 65847-85-0 (β-morpholinoethyl ester)
Merck Index: 6620
Lednicer No.: 1 256

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 288

CHROMATOGRAM

Retention time: 8.63
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine;

lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 21.968

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

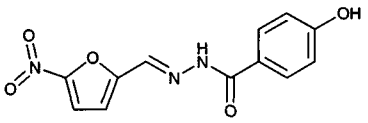
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Nifuroxazide



Molecular formula: $C_{12}H_9N_3O_5$
Molecular weight: 275.22
CAS Registry No.: 965-52-6
Merck Index: 6626

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

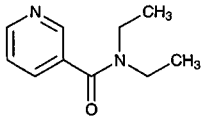
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 13.43

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Nikethamide



Molecular formula: $C_{10}H_{14}N_2O$
Molecular weight: 178.23
CAS Registry No.: 59-26-7
Merck Index: 6635
Lednicer No.: 1 253

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject

a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

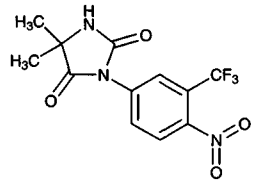
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 10.623

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Nilutamide



Molecular formula: $C_{12}H_{10}F_3N_3O_4$
Molecular weight: 317.22
CAS Registry No.: 63612-50-0
Merck Index: 6636

SAMPLE
Matrix: perfusate, tissue
Sample preparation: Homogenize (Polytron) rat lung with 8 mL saline using an ice bath. 250 μL Perfusate or lung homogenate + 40 ng norandrostenedione + 500 μL pH 7.0 phosphate buffer, vortex, add 4 mL dichloromethane, shake at 250 cycles/min for 15 min, centrifuge at 4° at 1700 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 MeOH:water 50:50, inject a 10-50 μL aliquot.

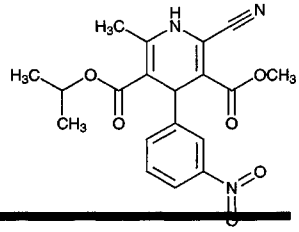
HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: Gradient. A was MeCN:MeOH:water 18.2:18.2:63.6. B was MeCN:MeOH:water 31:13:53. C was MeCN. A:B:C 100:0:0 for 3 min, to 0:85:15 over 5 min, maintain at 0:85:15 for 12 min, to 100:0:0 over 1 min, maintain at 100:0:0 for 14 min.
Injection volume: 10-50
Detector: UV 260

CHROMATOGRAM
Retention time: 18.5
Internal standard: norandrostenedione (22.4)
Limit of detection: 4 ng

KEY WORDS
lung; rat

REFERENCE
Camus,P.; Coudert,B.; d'Athis,P.; Foucher,P.; Delchambre,J.; Dupront,A.; Jeannin,L. Pharmacokinetics and metabolism of nilutamide in the isolated rat lung, *J.Pharmacol.Exp.Ther.*, **1991**, *259*, 1247–1255.

Nilvadipine



Molecular formula: $C_{19}H_{19}N_3O_6$
Molecular weight: 385.38
CAS Registry No.: 75530-68-6
Merck Index: 6637

SAMPLE
Matrix: perfusate
Sample preparation: 2 mL Perfusate + 100 µL 200 µg/mL nitrendipine in MeOH + 2 mL pH 9.0 phosphate buffer + 2 mL benzene:hexane 50:50 (Caution! Benzene is a carcinogen!), shake for 15 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 5C18
Mobile phase: MeCN:water 60:40 containing 5.25 mL/L 1% phosphoric acid, 2.5 g/L ammonium phosphate, and 10 mL/L 10% tetra-n-butylammonium hydroxide
Detector: UV 254

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: Direct injection of a MeOH solution containing 200-1000 ng.

HPLC VARIABLES
Column: 250 × 4.6 Sumchiral OA-4500 (Sumika Chemical Analysis Service)
Mobile phase: n-Hexane:1,2-dichloroethane:MeOH:trifluoroacetic acid 250:140:2:1
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 14 (-), 18.5 (+) (α = 1.39)

KEY WORDS
chiral

REFERENCE
Ohkubo,T.; Uno,T.; Sugawara,K. Enantiomer separation of dihydropyridine derivative calcium antagonists by high-performance liquid chromatography with chiral stationary phases, *J.Chromatogr.A*, **1994**, *659*, 467–471.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution of nilvadipine in a solution of human serum albumin in sodium phosphate buffer (I = 0.17), inject a 667-1330 μL aliquot onto column A and elute with mobile phase A, monitor the effluent from column A and divert the fraction containing the nilvadipine onto column B, elute the contents of column B onto column C with mobile phase B. (Wash column B with water for 4 min.)

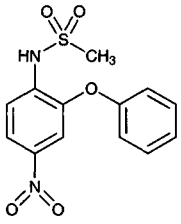
HPLC VARIABLES
Column: A 300 × 8 Develosil 100Diol5 (Nomura Chemical); B 10 × 4 Wakosil 5C4; C 150 × 6 Ultron ES-OVM
Mobile phase: A pH 7.4 sodium phosphate buffer (I = 0.17); B Isopropanol:pH 6.5 sodium phosphate buffer (I = 0.04)
Column temperature: 37
Flow rate: 1
Injection volume: 667-1330
Detector: UV 244

CHROMATOGRAM
Retention time: 8 (S), 10 (R)

KEY WORDS
column-switching; heart-cut; chiral; human serum albumin

REFERENCE
Shibukawa,A.; Nakao,C.; Sawada,T.; Terakita,A.; Morokoshi,N.; Nakagawa,T. Determination of the unbound concentration of hydrophobic drugs in albumin solutions by high-performance frontal analysis using a diol-silica column, *J.Pharm.Sci.*, **1994**, *83*, 868–873.

Nimesulide



Molecular formula: $C_{13}H_{12}N_2O_5S$
Molecular weight: 308.31
CAS Registry No.: 51803-78-2
Merck Index: 6640

SAMPLE
Matrix: blood
Sample preparation: 1 mL PLasma or urine + 10 μL 250 μg/mL tolbutamide in MeOH + 10 μL concentrated HCl + 8 mL toluene, extract for 15 min, centrifuge at 1500 g for 10 min, repeat extraction. 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 5 μL aliquot. (To hydrolyze conjugates adjust pH of 2 mL urine to 5.5 with 50 mM sodium acetate buffer, add 200 μL 200 U/mL β-glucuronidase, heat at 37° for 3 h, add 10 μL concentrated HCl, extract as above using twice the volume of IS solution.)

HPLC VARIABLES
Column: 300 × 4.6 10 μm C18 (Merck)
Mobile phase: MeOH:50 mM pH 5.0 phosphate buffer 50:50
Flow rate: 1
Injection volume: 5
Detector: UV 230

CHROMATOGRAM
Retention time: 9
Internal standard: tolbutamide (6)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: acetaminophen, aspirin, bezafibrate, doxepin, glibenclamide, salicylic acid, theophylline
Noninterfering: digoxin, flurazepam, tiadenol

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Castoldi,D.; Monzani,V.; Tofanetti,O. Simultaneous determination of nimesulide and hydroxynimesulide in human plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1988**, *425*, 413–418.

SAMPLE
Matrix: blood, tissue
Sample preparation: Tissue. Homogenize 2 g tissue in 10 mL MeCN, wash twice with 20 mL n-hexane. Evaporate the MeCN layer to dryness under reduced pressure at 50°, reconstitute in 100 μL 150 μg/mL 2-tert-butyl-4-methoxyphenol, inject a 20 μL aliquot. Serum. 1 mL Serum + 1 mL MeOH + 600 μL 1 M HCl, extract twice with 5 mL portions of benzene (Caution! Benzene is a carcinogen!). Combine the organic layers and extract twice with 2 mL portions of 200 mM NaOH. Combine the aqueous layers and acidify them with 2 M HCl, extract twice with 3 mL portions of benzene. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 100 μL 150 μg/mL 2-tert-butyl-4-methoxyphenol, inject a 20 μL aliquot.

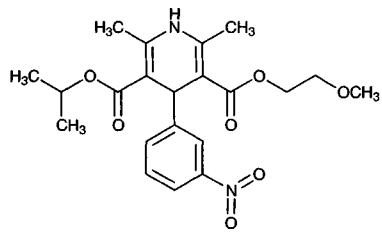
HPLC VARIABLES
Column: 300 × 4 5 μm μBondapak C18
Mobile phase: MeCN:water 65:35
Flow rate: 1.5
Injection volume: 20
Detector: UV 313

CHROMATOGRAM
Internal standard: 2-tert-butyl-4-methoxyphenol
Limit of detection: 50 ng/mL

KEY WORDS
muscle; serum; ovary; uterus; cervix

REFERENCE
Pulkkinen,M.O.; Vuento,M.; Macciocchi,A.; Monti,T. Distribution of oral nimesulide in female genital tissues, *Biopharm.Drug Dispos.*, **1991**, *12*, 113–117.

Nimodipine



Molecular formula: $C_{21}H_{26}N_2O_7$
Molecular weight: 418.45
CAS Registry No.: 66085-59-4
Merck Index: 6643
Lednicer No.: 3 149

SAMPLE
Matrix: blood
Sample preparation: 2 mL Serum + 2 mL water, mix, add 1 mL 1 M NaOH, add 12 mL hexane: ethyl ether 50:50, extract, centrifuge at 300 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 250 μL mobile phase, add 250 μL water, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 3 μm Spherisorb ODS
Mobile phase: MeOH:THF:water 47:15:38
Flow rate: 0.5
Injection volume: 50
Detector: UV 238

CHROMATOGRAM
Retention time: 13.08
Internal standard: nimodipine

OTHER SUBSTANCES
Extracted: nitrendipine

KEY WORDS
serum; nimodipine is IS

REFERENCE
Janis,R.A.; Krol,G.J.; Noe,A.J.; Pan,M. Radioreceptor and high-performance liquid chromatographic assays for the calcium channel antagonist nitrendipine in serum, *J.Clin.Pharmacol.*, **1983**, *23*, 266–273.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 10 μL 10 μg/mL nitrendipine + 1 mL ethyl acetate, shake horizontally for 3 min, centrifuge at 10000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40-50°, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2 30-40 μm Perisorb RP-18 (Upchurch)
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:water 65:35
Flow rate: 1
Injection volume: 100
Detector: UV 238

CHROMATOGRAM
Retention time: 9
Internal standard: nitrendipine (7.5)
Limit of quantitation: 10 ng/mL

KEY WORDS
monkey; plasma; pharmacokinetics; protect from light

REFERENCE
Qian,M.; Gallo,J.M. High-performance liquid chromatographic determination of the calcium channel blocker nimodipine in monkey plasma, *J.Chromatogr.*, **1992**, *578*, 316–320.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 200 μL 1 M NaOH + n-hexane:ethyl acetate 50:50, rotate for 10 min, centrifuge at 2000 g for 10 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 150 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 10 μm Daicel OJ
Column: 250 × 4.6 10 μm Daicel OJ
Mobile phase: n-Hexane:EtOH 85:15
Column temperature: 37
Flow rate: 0.3

Injection volume: 100
Detector: UV 238

CHROMATOGRAM
Retention time: 28.13 (S), 33.11 (R)

KEY WORDS
protect from light; serum; chiral; confirmation by GC/MS

REFERENCE
Fischer,C.; Schonberger,F.; Mück,W.; Heuck,K.; Eichelbaum,M. Simultaneous assessment of the intravenous and oral disposition of the enantiomers of racemic nimodipine by chiral stationary-phase high-performance liquid chromatography and gas chromatography/mass spectroscopy combined with a stable isotope technique, *J.Pharm.Sci.*, **1993**, *82*, 244–250.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 30 μL 800 ng/mL IS in MeCN + 300 μL 1 M NaOH + 5 mL diethyl ether:n-heptane 50:50, shake at 400 rpm for 5 min, centrifuge at 4° at 3000 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 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 2 8 μm Chira OJ MOD (Grom)
Column: 250 × 2 8 μm Chira OJ MOD (Grom)
Mobile phase: EtOH:n-heptane 20:80 containing 2 mM ammonium acetate
Column temperature: 35
Flow rate: 0.3
Injection volume: 50
Detector: MS, Sciex API III+, electrospray +4.2 kV, flow rate into MS was reduced to 0.05 mL/min, nebulizer gas nitrogen, m/z 436 (nimodipine), m/z 443 (IS) $(M+NH_4)^+$

CHROMATOGRAM
Retention time: 14.5, 15.5 (enantiomers)
Internal standard: heptadeuteronimodipine (deuterium in isopropyl group)
Limit of detection: 0.25 ng/mL
Limit of quantitation: 0.5 ng/mL

KEY WORDS
protect from light; plasma; pharmacokinetics; chiral

REFERENCE
Mück,W.M. Enantiospecific determination of nimodipine in human plasma by liquid chromatography-tandem mass spectrometry, *J.Chromatogr.A*, **1995**, *712*, 45–53.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8

Injection volume: 50
Detector: UV 238

CHROMATOGRAM
Retention time: 6.36
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE
Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 500 μL Microsomal incubation + 500 μL ice-cold MeOH, vortex for 15 s, let stand in ice, centrifuge at 0° at 10000 g for 5 min, inject a 40 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 4 Spherisorb S3ODS II
Mobile phase: MeOH:50 mM pH 6.6 ammonium acetate 60:40
Column temperature: 40
Flow rate: 0.5
Injection volume: 40
Detector: UV 218, UV 238

CHROMATOGRAM
Retention time: 11.8

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: hydroxymidazolam, midazolam, quinidine, quinine, verapamil
Noninterfering: cyclosporin A, erythromycin, naphthoflavone, naringenin, troleandomycin

KEY WORDS
rat; liver

REFERENCE
Leuenberger,P.M.; Ha,H.R.; Pletscher,W.; Meier,P.J.; Sticher,O. Measurement of nimodipine metabolism in rat liver microsomes by high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 2243–2255.

SAMPLE
Matrix: perfusate

HPLC VARIABLES
Column: 100 × 8 5 μm Novapak C18 radial compression
Mobile phase: MeCN:10 mM pH 4.5 phosphate buffer 70:30
Flow rate: 2
Detector: UV 237

OTHER SUBSTANCES
Also analyzed: felodipine, nicardipine, nifedipine, nitrendipine

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: Centrifuge at 2000 g at 37° for 15 min.

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: nicardipine, nifedipine, nitrendipine, felodipine

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: Prepare a 100 μM solution in buffer, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 column containing riboflavin binding proteins (Prepare as follows. Add riboflavin to saturate protein of egg yolk, homogenize with 3 volumes buffer, centrifuge, add the supernatant to a 500 × 30 column of DEAE-cellulose (Whatman) equilibrated with buffer, wash extensively with buffer to remove bound protein, elute riboflavin binding proteins (RFBP) with buffer containing 200 mM NaCl (RFBP has intense yellow color, absorption at 455 nm). Purify RFBP on a Sephadex G-100 column with 50 mM pH 7.5 Tris-HCl buffer as eluent, remove the bound riboflavin by extensive dialysis at pH 3.0. Add 4.5 g N,N-disuccinylimidyl carbonate to 3 g Nucleosil 5NH2 slurried in MeCN, filter, wash with MeCN, wash with 50 mM pH 7.5 phosphate buffer. Suspend 300 mg RFBP in 50 mM phosphate buffer, add the activated silica, mix gently for 2 h using a rotary evaporator, filter, wash with sterile water, wash with isopropanol:water 1:2, pack in a 100 × 4.6 column.) (Buffer was 100 mM pH 5.3 sodium acetate.)
Mobile phase: 50 mM pH 5.5 KH_2PO_4
Flow rate: 0.8
Injection volume: 20
Detector: UV

CHROMATOGRAM

Retention time: k' 0.72

OTHER SUBSTANCES

Simultaneous: flurbiprofen, isradipine, ketoprofen, suprofen

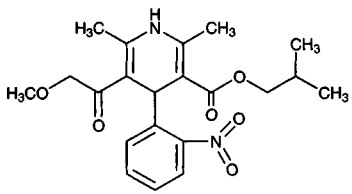
KEY WORDS

chiral; α = 1.11

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.

Nisoldipine



Molecular formula: $C_{20}H_{24}N_2O_6$
Molecular weight: 388.42
CAS Registry No.: 63675-72-9
Merck Index: 6658

SAMPLE

Matrix: blood
Sample preparation: Wrap tubes in Al foil. 1 mL Serum + 50 µL 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 µL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 250 × 4 Brownlee 10 µm RP8
Mobile phase: MeOH:50 mM buffer 30:70 (Buffer was 12 g NaH_2PO_4 in 2 L water adjusted to pH 4 ± 0.5 with 80% phosphoric acid.)
Flow rate: 0.75
Injection volume: 20
Detector: UV 350

CHROMATOGRAM

Retention time: 13
Internal standard: nisoldipine

OTHER SUBSTANCES
Extracted: nifedipine
Simultaneous: photodegradation products of nifedipine
Noninterfering: aspirin, acetaminophen, caffeine, chlorthalidone, diazepam, methyldopa, oxprenolol, propranolol

KEY WORDS
serum; furosemide; hydrochlorothiazide; timolol; triamterene; nisoldipine is IS

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: blood
Sample preparation: 1 mL Plasma + 50 μL MeOH + 100 μL 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 × 13 mm glass tube, evaporate to dryness without heating using a Speed Vac concentrator evaporator. Reconstitute in 200 μL mobile phase, vortex for 15 s, inject a 150 μL aliquot.

HPLC VARIABLES
Column: 100 × 8 4μm 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: 16
Internal standard: nisoldipine

OTHER SUBSTANCES
Extracted: nifedipine

KEY WORDS
plasma; analysis conducted under sodium lamps; nisoldipine is IS

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 Plasma + 100 μL saturated NaCl + 100 μL 1 M NaOH + 1.5 mL toluene, shake at 300 rpm for 15 min, centrifuge at 10° at 3000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under reduced pressure for 25 min, reconstitute the residue in 130 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 2 10 μm aminopropylsilica coated with tris(4-methylbenzoate)-modified cellulose (Daicel)
Column: 250 × 2 10 μm aminopropylsilica coated with tris(4-methylbenzoate)-modified cellulose (Daicel)
Mobile phase: n-Heptane:isopropanol containing 0.2% trifluoroacetic acid 88:12
Column temperature: 40
Flow rate: 0.2
Injection volume: 100
Detector: UV 230

CHROMATOGRAM
Retention time: 10.5 (+), 14.6 (-)

KEY WORDS
dog; rat; mouse; plasma; chiral; microbore; confirmation by GC/MS

REFERENCE
Zimmer,D.; Muschalek,V. Enantioselective assay for the determination of nisoldipine in dog, rat and mouse plasma by chiral microbore high-performance liquid chromatography combined with gas chromatography-mass spectrometry, *J.Chromatogr.A*, **1994**, *666*, 241–248.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Sumchiral OA-4600
Mobile phase: n-Hexane:1,2-dichloroethane:MeOH:trifluoroacetic acid 250:140:0.5:1
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 13, 15 (enantiomers)

KEY WORDS
chiral; α = 1.12

REFERENCE
Ohkubo,T.; Uno,T.; Sugawara,K. Enantiomer separation of dihydropyridine derivative calcium antagonists by high-performance liquid chromatography with chiral stationary phases, *J.Chromatogr.A*, **1994**, *659*, 467–471.

SAMPLE
Matrix: solutions

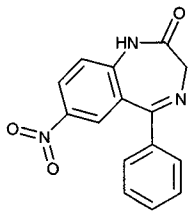
HPLC VARIABLES
Column: 62 × 2 packed with chiral packing (Prepare packing by dissolving 4-chloro-3-methylphenylcarbamate cellulose in THF, coat on Nucleosil 1000-7, dry at 60° for 3 h under reduced pressure.)
Mobile phase: Hexane:isopropanol 90:10
Flow rate: 0.1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 4.3

KEY WORDS
narrow-bore; chiral; α 1.18

REFERENCE
Chankvetadze,B.; Chankvetadze,L.; Sidamonidze,S.; Yashima,E.; Okamoto,Y. Enantioseparation of some chiral pharmaceuticals using narrow-bore liquid chromatography, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 695–699.

Nitrazepam



Molecular formula: $C_{15}H_{11}N_3O_3$
Molecular weight: 281.27
CAS Registry No.: 146-22-5
Merck Index: 6667
Lednicer No.: 1 366

SAMPLE

Matrix: bile, blood, gastric contents, tissue, urine
Sample preparation: Chop 5-g tissue and homogenize (Ultra Turrax T25) at 8500, 9500, 13500, 20500, and 24000 rpm for 1 min each. Add homogenate to 20 mL water. Dilute blood, urine, gastric contents, and bile four times with water. Mix 4 mL sample with 10 μL 1 mg/mL prazepam and 1 mL pH 7.4 phosphate buffer, vortex briefly, add 4 mL diethyl ether and mix for 15 min (Spiramix 10, Denley, UK). Separate the organic layer, add 4 mL diethyl ether to extraction sample, mix. Evaporate combined organic layers to dryness under a stream of dry air at 50°. Purify extracts by partition between 1 mL MeCN and 2 mL heptane, separate MeCN layer, evaporate it to dryness, reconstitute the residue in 100 μL MeOH and inject a 20 μL aliquot of the solution.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Apex II ODS
Column: 150 × 4.6 5 μm Apex II ODS
Mobile phase: MeCN:MeOH:10 mM phosphoric acid:10 mM Na_2HPO_4 40:20:36:4
Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM

Retention time: 3.5
Internal standard: prazepam (14.5)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES

Extracted: diazepam, oxazepam, temazepam

KEY WORDS

liver; lung; muscle; urine; pericardial fluid

REFERENCE

Pounder,D.J.; Adams,E.; Fuke,C.; Langford,A.M. Site to site variability of postmortem drug concentrations in liver and lung, *J.Forensic Sci.*, **1996**, *41*, 927–932.

SAMPLE

Matrix: blood
Sample preparation: Condition a 100 mg Bond-Elut C18 SPE cartridge with 2 mL MeOH and 2 mL water. Mix 1 mL plasma or serum with 200 μL 512 nM IS in MeOH:water 5:95, add to the SPE cartridge, wash with 2 mL water, wash with 50 μL MeOH. Elute with 200 μL and 100 μL MeOH, evaporate the eluate to dryness under a stream of air at 37°, reconstitute the residue with 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 4 μm Novapak C18
Mobile phase: MeCN:MeOH:10 mM pH 3.7 K_2HPO_4 30:2:10
Flow rate: 1.5
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 6.2
Internal standard: flunitrazepam (9.8)
Limit of detection: 5 nM

OTHER SUBSTANCES
Extracted: alprazolam, clonazepam
Simultaneous: amobarbital, carbamazepine, citalopram, clobazam, clozapine, diazepam, doxepin, ethosuximide, norclobazam, oxazepam, oxcarbamazepine, pentobarbital, phenobarbital, phenytoin, primidone, valproic acid, zopiclone
Interfering: medazepam, midazolam, nordiazepam, temazepam

KEY WORDS
SPE; plasma; serum

REFERENCE
Åkerman,K.K.; Jolkkonen,J.; Parviainen,M.; Penttilä,I. Analysis of low-dose benzodiazepines by HPLC with automated solid-phase extraction, *Clin.Chem.*, **1996**, *42*, 1412–1416.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 20 μL 20 μg/mL IS + 200 μL 1 M potassium carbonate + 3 mL chloroform, mix for 2 min, centrifuge at 1200 g for 5 min, aspirate aqueous phase. Evaporate the organic phase under a stream of nitrogen at 40°. Dissolve the residue in 100 μL mobile phase, inject a 20 μL aliquot. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES
Column: 100 × 4.6 2 μm TSK gel Super-ODS (A) or 100 × 4.6 5 μm Hypersil ODS-C18 (B)
Mobile phase: MeCN:5 mM pH 6 NaH_2PO_4 45:55
Flow rate: 0.65
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 9.1 (A), 34.2 (B)
Internal standard: diazepam (29.8 (A), 77.5 (B))
Limit of quantitation: 1 ng/mL (A)

OTHER SUBSTANCES
Extracted: bromazepam, chlordiazepoxide, clonazepam, estazolam, etizolam, flutazolam, haloxazolam, lorazepam, oxazolam, triazolam
Simultaneous: alprazolam
Noninterfering: barbital, carbamazepine, cloxazolam, ethosuximide, hexobarbital, mexazolam, oxazepam, pentobarbital, phenobarbital, phenytoin, primidone, trimethadione

KEY WORDS
serum

REFERENCE
Tanaka,E.; Terada,M.; Misawa,.; Wakasugi,C. Simultaneous determination of twelve benzodiazepines in human serum using a new reversed-phase chromatographic column on a 2-μm porous microspherical silica gel, *J.Chromatogr.B*, **1996**, *682*, 173–178.

SAMPLE
Matrix: blood
Sample preparation: Add 500 μL 200 mM pH 9.0 glycine buffer to 1 mL plasma, add 5 mL ethyl acetate, extract. Centrifuge at 700 g for 10 min, evaporate 4 mL of the organic phase to dryness under nitrogen at 60°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:buffer 69:31 (Mobile phase was 690 mL MeCN, 310 mL water, and 9 mL glacial acetic acid, adjusted to pH 5.0 with 5 M NaOH.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 10.5
Internal standard: nitrazepam

OTHER SUBSTANCES
Extracted: furosemide

KEY WORDS
plasma; nitrazepam is IS

REFERENCE
Jankowski,A.; Skorek-Jankowska,A.; Lamparczyk,H. Determination and pharmacokinetics of a furosemide-amiloride drug combination, *J.Chromatogr.B*, **1997**, *693*, 383–391.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 260

CHROMATOGRAM
Retention time: 4.03
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acen-

ocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 µm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 16.927

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions
Sample preparation: Inject an aliquot of a solution in mobile phase.

HPLC VARIABLES

Column: Nova-Pak C18

Mobile phase: MeOH:buffer 85:15 (Buffer was 90.7 mL 66.7 mM Na_2HPO_4 and 9.3 mL 66.7 mM KH_2PO_4 made up to 1 L with water, pH 7.6.)
Flow rate: 5 (sic)
Injection volume: 20
Detector: UV (wavelength not given)

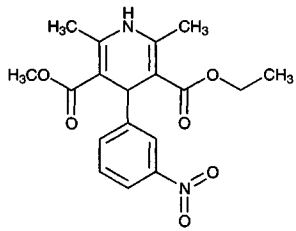
CHROMATOGRAM
Retention time: 4.32
Limit of detection: 100 nM

OTHER SUBSTANCES
Simultaneous: chlordiazepoxide, diazepam, flurazepam

KEY WORDS
comparison with capillary electrophoresis; capillary GC; and polarography

REFERENCE
McGrath,G.; McClean,S.; O'Kane,E.; Smyth,W.F.; Tagliaro,F. Study of the capillary zone electrophoretic behaviour of selected drugs, and its comparison with other analytical techniques for their formulation assay, *J.Chromatogr.A*, **1996**, *735*, 237–247.

Nitrendipine



Molecular formula: $C_{18}H_{20}N_2O_6$
Molecular weight: 360.37
CAS Registry No.: 39562-70-4
Merck Index: 6669

SAMPLE
Matrix: blood
Sample preparation: 2 mL Serum + 2 mL 20 ng/mL nimodipine in water, mix, add 1 mL 1 M NaOH, add 12 mL hexane:ethyl ether 50:50, extract, centrifuge at 300 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 250 μL mobile phase, add 250 μL water, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 3 μm Spherisorb ODS
Mobile phase: MeOH:THF:water 47:15:38
Flow rate: 0.5
Injection volume: 50
Detector: UV 238

CHROMATOGRAM
Retention time: 11.87
Internal standard: nimodipine (13.08)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum

REFERENCE
Janis,R.A.; Krol,G.J.; Noe,A.J.; Pan,M. Radioreceptor and high-performance liquid chromatographic assays for the calcium channel antagonist nitrendipine in serum, *J.Clin.Pharmacol.*, **1983**, *23*, 266–273.

Mobile phase: MeOH:buffer 85:15 (Buffer was 90.7 mL 66.7 mM Na_2HPO_4 and 9.3 mL 66.7 mM KH_2PO_4 made up to 1 L with water, pH 7.6.)
Flow rate: 5 (sic)
Injection volume: 20
Detector: UV (wavelength not given)

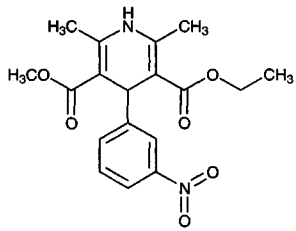
CHROMATOGRAM
Retention time: 4.32
Limit of detection: 100 nM

OTHER SUBSTANCES
Simultaneous: chlordiazepoxide, diazepam, flurazepam

KEY WORDS
comparison with capillary electrophoresis; capillary GC; and polarography

REFERENCE
McGrath,G.; McClean,S.; O'Kane,E.; Smyth,W.F.; Tagliaro,F. Study of the capillary zone electrophoretic behaviour of selected drugs, and its comparison with other analytical techniques for their formulation assay, *J.Chromatogr.A*, **1996**, *735*, 237–247.

Nitrendipine



Molecular formula: $C_{18}H_{20}N_2O_6$
Molecular weight: 360.37
CAS Registry No.: 39562-70-4
Merck Index: 6669

SAMPLE
Matrix: blood
Sample preparation: 2 mL Serum + 2 mL 20 ng/mL nimodipine in water, mix, add 1 mL 1 M NaOH, add 12 mL hexane:ethyl ether 50:50, extract, centrifuge at 300 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 250 μL mobile phase, add 250 μL water, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 3 μm Spherisorb ODS
Mobile phase: MeOH:THF:water 47:15:38
Flow rate: 0.5
Injection volume: 50
Detector: UV 238

CHROMATOGRAM
Retention time: 11.87
Internal standard: nimodipine (13.08)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum

REFERENCE
Janis,R.A.; Krol,G.J.; Noe,A.J.; Pan,M. Radioreceptor and high-performance liquid chromatographic assays for the calcium channel antagonist nitrendipine in serum, *J.Clin.Pharmacol.*, **1983**, *23*, 266–273.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 1 mL ethyl acetate, shake horizontally for 3 min, centrifuge at 10000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40-50°, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 2 30-40 μm Perisorb RP-18 (Upchurch)
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:water 65:35
Flow rate: 1
Injection volume: 100
Detector: UV 238

CHROMATOGRAM
Retention time: 7.5
Internal standard: nitrendipine

OTHER SUBSTANCES
Extracted: nimodipine

KEY WORDS
monkey; plasma; protect from light; nitrendipine is IS

REFERENCE
Qian,M.; Gallo,J.M. High-performance liquid chromatographic determination of the calcium channel blocker nimodipine in monkey plasma, *J.Chromatogr.*, **1992**, *578*, 316–320.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 236

CHROMATOGRAM
Retention time: 6.00
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazo-

lam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, gastric contents, tissue, urine

Sample preparation: 1 mL Blood, urine, or gastric contents or 1 g tissue homogenate + 500 μL buffer + 8 mL n-hexane:ethyl acetate 70:30, mix on a rotary mixer for 10 min, centrifuge at 3000 g for 8 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL 12.5 mM NaOH in MeOH:water 50:50, inject a 50 μL aliquot. (Buffer was 13.8 g potassium carbonate in 100 mL water, pH adjusted to 9.5 with concentrated HCl.)

HPLC VARIABLES

Guard column: 4 × 4 30 μm LiChrocart Aluspher RP-select B (Merck)

Column: 125 × 4 5 μm Aluspher RP-select B (Merck)

Mobile phase: Gradient. A was 12.5 mM NaOH in MeOH. B was 12.5 mM NaOH in water. A:B 10:90 for 5 min, to 90:10 over 15 min, maintain at 90:10 for 5 min, return to initial conditions over 1 min, re-equilibrate for 5 min.

Flow rate: 1

Injection volume: 50

Detector: UV 230, 254

CHROMATOGRAM

Retention time: 17

OTHER SUBSTANCES

Extracted: alprenolol, amitriptyline, bromazepam, carbamazepine, chlordiazepoxide, chlorpromazine, clonazepam, desipramine, diazepam, flunitrazepam, haloperidol, nordiazepam, nortriptyline, pindolol, zolpidem

Also analyzed: acebutolol, acetaminophen, alprazolam, amphetamine, atenolol, betaxolol, brotizolam, caffeine, camazepam, captopril, chloroquine, clobazam, clomipramine, clothiapine, clotiazepam, cloxazolam, cocaine, codeine, diclofenac, dihydralazine, dihydrocodeine, dihydroergotamine, diphenhydramine, domperidone, doxepin, droperidol, ergotamine, ethyl loflazepate, fenethylline, fluoxetine, flupentixol, flurazepam, furosemide, gliclazide, hydrochlorothiazide,

hydroxyzine, ibuprofen, imipramine, ketazolam, loprazolam, lorazepam, lormetazepam, maprotiline, medazepam, mepyramine, methadone, methaqualone, methyldopa, methylphenidate, metoclopramide, metoprolol, mexiletine, mianserin, midazolam, minoxidil, morphine, nadolol, nitrazepam, oxprenolol, papaverine, pentazocine, phenprocoumon, phenylbutazone, pipamperone, piritramide, practolol, prazepam, prazosin, promazine, promethazine, propoxyphene, propranolol, prothipendyl, quinine, sotalol, sulpride, thioridazine, trazodone, triazolam, trimipramine, tripelennamine, tyramine, verapamil, yohimbine

REFERENCE

Lambert,W.E.; Meyer,E.; De Leenheer,A.P. Systematic toxicological analysis of basic drugs by gradient elution of an alumina-based HPLC packing material under alkaline conditions, *J.Anal.Toxicol.*, **1995**, *19*, 73–78.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 236.9

CHROMATOGRAM

Retention time: 22.087

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: cytosol incubations

Sample preparation: 2 mL Incubation + 5 mL chlorobutane:1,2-dichloroethane 80:20, shake for 15 min, centrifuge at 4000 g for 10 min. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 400 μL mobile phase, inject an 80 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 μBondapak C18

Mobile phase: MeOH:10 mM ammonium phosphate buffer 66:34, pH 5.8

Flow rate: 1.5

Injection volume: 80

Detector: UV 230

OTHER SUBSTANCES

Extracted: diphenyl sulfoxide

KEY WORDS
rat; rabbit; nitrendipine is IS

REFERENCE
Lee,S.C.; Renwick,A.G. Sulphoxide reduction by rat and rabbit tissues *in vitro*, *Biochem.Pharmacol.*, **1995**, *49*, 1557–1565.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Condition a 100 mg Bond Elut C18 SPE cartridge with 2 mL MeOH and 1 mL 1% aqueous formic acid. Add the microsomal incubation to the SPE cartridge, wash with 1 mL 1% aqueous formic acid, elute with 1 mL MeCN. Evaporate the eluate to dryness under a stream of nitrogen in the dark at room temperature, reconstitute the residue in 1 mL MeCN: 0.5% phosphoric acid 10:90, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 10 μm Nucleosil RP C8
Mobile phase: Gradient. MeCN:0.5% phosphoric acid from 40:60 to 60:40 over 12 min.
Flow rate: 1.4
Injection volume: 50
Detector: UV 234, UV 345

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; liver; SPE

REFERENCE
Böcker,R.H.; Preuss,E.; Peter,R. High-performance liquid chromatography of the metabolites of nitrendipine and investigation into the metabolic pathways of this dihydropyridine, *J.Chromatogr.*, **1990**, *530*, 206–211.

SAMPLE
Matrix: microsomal incubations, perfusate
Sample preparation: Extract using a 100 mg 1 mL Bond Elut C2 SPE cartridge, elute with MeOH, evaporate eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 5 Hichrom HiRPB deactivated reverse-phase
Mobile phase: MeOH:25 mM pH 5.0 N,N,N',N'-tetramethylethylenediamine phosphate buffer 75:25
Flow rate: 1
Detector: UV 245

CHROMATOGRAM
Internal standard: felodipine
Limit of detection: 5 ng/mL

KEY WORDS
rat; liver; SPE

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: perfusate

HPLC VARIABLES
Column: 100 × 8 5 μm Novapak C18 radial compression
Mobile phase: MeCN:10 mM pH 4.5 phosphate buffer 70:30
Flow rate: 2
Detector: UV 237

OTHER SUBSTANCES
Also analyzed: felodipine, nicardipine, nifedipine, nimodipine

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: Prepare a solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: present but not specified
Column: 75 × 4.6 3 μm XL octyl (Beckman)
Mobile phase: MeCN:water 44:56 containing 5 mM heptanesulfonic acid
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6.2

REFERENCE
Greiner,P.O.; Angignard,D.; Cahn,J. High performance liquid chromatography of a new 1,4-dihydropyridine: applications to pharmacokinetic study in dogs, *J.Pharm.Sci.*, **1988**, *77*, 387–389.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10 μL aliquot of a solution in MeCN.

HPLC VARIABLES
Column: 100 × 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, 272, 276, 280, 314

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: nifedipine

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: Centrifuge at 2000 g at 37° for 15 min.

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: nicardipine, nifedipine, nimodipine, felodipine

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: Prepare a solution in mobile phase, inject an aliquot.

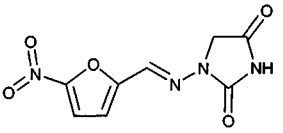
HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 5C18
Mobile phase: MeCN:water 60:40 containing 5.25 mL/L 1% phosphoric acid, 2.5 g/L ammonium phosphate, and 10 mL/L 10% tetra-n-butylammonium hydroxide
Detector: UV 254

OTHER SUBSTANCES
Simultaneous: nilvadipine

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.

Nitrofurantoin



Molecular formula: $C_8H_6N_4O_6$
Molecular weight: 238.16
CAS Registry No.: 67-20-9
Merck Index: 6696
Lednicer No.: 1 230

SAMPLE
Matrix: cell cultures
Sample preparation: Centrifuge cell culture at 4000 g for 30 min, filter (0.2 μm Acrodisk), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Prodigy ODS-3
Mobile phase: Gradient. A was MeCN containing 2 g/L ammonium acetate. B was water. A:B 5:95 for 2 min, to 80:20 over 30 min, 80:20 for 5 min

Flow rate: 1
Injection volume: 20
Detector: UV 376

CHROMATOGRAM
Retention time: 24.5

REFERENCE
Rafii,F.; Hansen,E.B.,Jr. Isolation of nitrofurantoin-resistant mutants of nitroreductase-producing Clostridium sp. Strains from the human intestinal tract, *Antimicrob.Agents Chemother.*, **1998**, *42*, 1121–1126.

SAMPLE
Matrix: milk
Sample preparation: Mix 50 mL cow milk with 25 mL 20% trichloroacetic acid, let stand for 15 min. Filter the samples and wash with water. Adjust the pH to 4.5-5 with NaOH, make up to 100 mL with water. Take a 25 mL aliquot and add it to a Sep-Pak Plus C18 SPE cartridge, elute with 2.5 mL mobile phase, pass nitrogen through eluate for at least 2 min (to remove oxygen), inject an aliquot.

HPLC VARIABLES
Guard column: Symmetry C18
Column: 150 × 3.9 4 μm Nova Pak C18
Mobile phase: MeCN:100mM aqueous sodium perchlorate:glacial acetic acid 28:72:0.5
Flow rate: 1
Injection volume: 20
Detector: E, ESA Coulochem II, Model 5011 analytical cell, porous carbon electrode -600 V, Model 5021 conditioning cell

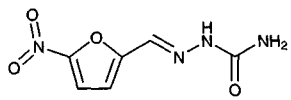
CHROMATOGRAM
Retention time: 2.2
Limit of detection: 4 ppb

OTHER SUBSTANCES
Extracted: furaltadone, furazolidone

KEY WORDS
cow; SPE

REFERENCE
Galeano Diaz,T.; Guiberteau Cabanillas,A.; Acedo Valenzuela,M.I.; Correa,C.A.; Salinas,F. Determination of nitrofurantoin, furazolidone and furaltadone in milk by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.A*, **1997**, *764*, 243–248.

Nitrofurazone



Molecular formula: $C_6H_6N_4O_4$
Molecular weight: 198.14
CAS Registry No.: 59-87-0
Merck Index: 6697
Lednicer No.: 1 229

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject

a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 260.6

CHROMATOGRAM
Retention time: 10.323

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: eggs, tissue
Sample preparation: Add 30 mL MeCN to 10 g homogenized shelled eggs, liver, or muscle, blend at low speed for 2 min, centrifuge at 1000 g for 5 min, add 10 mL 10% NaCl solution and 50 mL dichloromethane to the supernatant, shake for a few min. Filter the lower organic layer through 5 g anhydrous sodium sulfate, evaporate the filtrate to dryness using a rotary vacuum evaporator at 45°, redissolve the residue in 1 mL MeCN:MeOH:20 mM pH 4.6 sodium acetate 10:50:40, inject an aliquot. (Protect from light. Wash the 1 mL of MeCN:MeOH:20 mM pH 4.6 sodium acetate 10:50:40 three times with 1 mL n-hexane before use.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil L C18-DB
Mobile phase: MeCN:water 50:50 containing 1 mM ammonium acetate and 0.025% acetic acid
Flow rate: 0.6
Injection volume: 20
Detector: MS, PESCIEX API I, ionspray interface 5500 V, OR 60 V, m/z 199, split the column effluent so that 0.03 mL/min enters the MS

CHROMATOGRAM
Retention time: 5.2
Limit of detection: 3.2 ng/g

KEY WORDS
chicken; liver; muscle

REFERENCE
Draisci,R.; Giannetti,L.; Lucentini,L.; Palleschi,L.; Brambilla,G.; Serpe,L.; Gallo,P. Determination of nitrofuran residues in avian eggs by liquid chromatography-UV photodiode array detection and confirmation by liquid chromatography-ionspray mass spectrometry, *J.Chromatogr.A*, **1997**, *777*, 201–211.

SAMPLE
Matrix: eggs, tissue

Sample preparation: Add 30 mL MeCN to 10 g homogenized shelled eggs, liver, or muscle, blend at low speed for 2 min, centrifuge at 1000 g for 5 min, add 10 mL 10% NaCl solution and 50 mL dichloromethane to the supernatant, shake for a few min. Filter the lower organic layer through 5 g anhydrous sodium sulfate, evaporate the filtrate to dryness using a rotary vacuum evaporator at 45°, redissolve the residue in 1 mL MeCN:MeOH:20 mM pH 4.6 sodium acetate 10:50:40, inject an aliquot. (Protect from light. Wash the 1 mL of MeCN:MeOH:20 mM pH 4.6 sodium acetate 10:50:40 three times with 1 mL n-hexane before use.)

HPLC VARIABLES

Guard column: 10 × 4.6 μBondapak C18
Column: 150 × 4.6 5 μm Spherisorb ODS2 S5
Mobile phase: MeCN:20 mM pH 4.6 sodium acetate 21:79
Flow rate: 1
Injection volume: 50
Detector: UV 362

CHROMATOGRAM

Retention time: 5.3
Limit of detection: 2.5 ng/g

OTHER SUBSTANCES

Extracted: furazolidone, furaltadone

KEY WORDS

chicken; liver; muscle

REFERENCE

Draisci,R.; Giannetti,L.; Lucentini,L.; Palleschi,L.; Brambilla,G.; Serpe,L.; Gallo,P. Determination of nitrofuran residues in avian eggs by liquid chromatography-UV photodiode array detection and confirmation by liquid chromatography-ionspray mass spectrometry, *J.Chromatogr.A*, **1997**, *777*, 201–211.

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 Cuprophan 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: 6
Limit of detection: 2 ng/g (tissue), 1 ng/g (eggs)

OTHER SUBSTANCES

Extracted: furaltadone, furazolidone, nitrofurantoin

KEY WORDS

protect from light; cow; muscle; dialysis; column-switching

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 nitrofuran residues in edible products, *J.Chromatogr.*, **1990**, *500*, 453–468.

SAMPLE
Matrix: feed
Sample preparation: Grind feed to pass 20 mesh. 10 g Feed + 5 mL water, swirl, let stand for 5 min, add 50 mL DMF:water 95:5, shake vigorously for 15 s, let stand in the dark at room temperature overnight, filter (paper). Add 15 mL of the filtrate to 5 g alumina (Alcoa F-20, 80-200 mesh) in a 300 × 10 glass column, discard first several mL of eluate, collect remaining eluate, inject an aliquot

HPLC VARIABLES
Guard column: 100 × 2 μBondapak C18/Corasil
Column: 300 × 4 μBondapak C18
Mobile phase: MeCN:1% acetic acid 20:80
Detector: UV 280, UV 365

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Extracted: furazolidone, carbadox

KEY WORDS
protect from light

REFERENCE
Thorpe,V.A. Sample preparation of carbadox, furazolidone, nitrofurazone, and ethopabate in medicated feeds for high pressure liquid chromatography, *J.Assoc.Off.Anal.Chem.*, **1980**, *63*, 981–984.

SAMPLE
Matrix: food
Sample preparation: Condition a 6 mL Bond Elut C18 SPE cartridge with 5 mL MeOH and 5 mL water. Homogenize (Tissuemizer) 5 g finely-chopped (by hand) shrimp and 20 mL MeCN at medium speed for 45 s, centrifuge at 3000 rpm for 5 min, decant the supernatant. Add 30 mL hexane saturated with MeCN to the supernatant, shake for 30 s, discard the hexane layer. Add 10 mL EtOH to the MeCN layer, evaporate under reduced pressure at 45° to 2-5 mL (until liquid looks milky), add 2 mL EtOH, continue evaporation until there is 2 mL of a thick liquid, add 2 mL EtOH, evaporate to dryness. Add 2 mL water to the residue, sonicate for 5 min, add to the SPE cartridge, wash with 4 mL water, elute at ≤3 mL/min with 5 mL MeCN. Evaporate the eluate to dryness under a stream of nitrogen at ≤45° (remove promptly when dry), reconstitute the residue in 1 mL mobile phase, filter (0.45 μm), inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4 5 μm ODS Hypersil C18
Column: 200 × 4.6 5 μm ODS Hypersil C18
Mobile phase: MeCN:1% aqueous acetic acid 25:75
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: UV 375

CHROMATOGRAM
Retention time: 3.7
Limit of quantitation: 4 ng/g

OTHER SUBSTANCES
Extracted: furazolidone

KEY WORDS
SPE; shrimp

REFERENCE
Rupp,H.S.; Munns,R.K.; Long,A.R. Simultaneous determination of nitrofurazone and furazolidone in shrimp (*Penaeus vannamei*) muscle tissue by liquid chromatography with UV detection, *J.AOAC Int.*, **1993**, *76*, 1235–1239.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH:water 30:70, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Resolve spherical C18 (Waters)
Mobile phase: MeOH:water 35:65
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: UV 305

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: carbadox, furazolidone
Noninterfering: pyrantel

KEY WORDS
protect from light

REFERENCE
Roybal,J.E.; Munns,R.K.; Shimoda,W. Liquid chromatographic determination of carbadox residues in animal feed, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 653–657.

SAMPLE
Matrix: 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: 7.5
Limit of detection: 15 ng/mL

OTHER SUBSTANCES
Simultaneous: degradation products, carbadox, nitrofurantoin, furazolidone, furaltadone

REFERENCE
Kaniou,I.; Zachariadis,G.; Kalligas,G.; Tsoukali,H.; Stratis,J. Separation and determination of carbadox, nitrofurazone, nitrofurantoin, furazolidone, and furaltadone in their mixtures by thin layer and high performance liquid chromatography, *J.Liq.Chromatogr.*, **1994**, *17*, 1385–1398.

SAMPLE
Matrix: tissue

Sample preparation: Homogenize (Waring blender) 10 g muscle, liver, or kidney in 100 mL EtOH for 5 min, let stand for 5 min, filter through 10 g Celite 545 on top of a sintered glass filter, rinse blender with 100 mL EtOH and filter rinse. Add 25 mL 3.6% aqueous metaphosphoric acid to the combined filtrates, evaporate to 25 mL under reduced pressure at 45°. Remove residue, rinse out flask with 5 mL hexane and 3 mL water, combine, centrifuge at 0° at 27000 g for 30 min, discard hexane, rinse surface with 5 mL hexane, discard hexane. Remove aqueous layer, rinse out tube twice with 3 mL portions of water, combine, add 10 mL 1 M KH_2PO_4, make up to 100 mL with water, extract three times for 5 min with 50 mL ethyl acetate. Combine the extracts and dry them over 15 g anhydrous sodium sulfate, filter through glass wool, evaporate to dryness under reduced pressure at 45°. Take up residue in 3 mL ethyl acetate and add to alumina column, rinse flask with 2 mL ethyl acetate and add rinse to column. Elute with 20 mL EtOH:MeOH:ethyl acetate 10:10:80 and combine all the eluate. Evaporate to dryness under reduced pressure at 45°, reconstitute in 500 μL mobile phase, inject a 100 μL aliquot. (Prepare alumina column by slurrying 1 g aluminum oxide (Baker) in 20 mL ethyl acetate and adding to a 200 × 6 glass chromatographic column.)

HPLC VARIABLES

Guard column: Brownlee 10 μm RP-GU MPLC C-8
Column: 250 × 4.6 Brownlee RP-10A C-8
Mobile phase: MeCN:EtOH:10 mM ammonium acetate 25:5:70, pH 6.8
Flow rate: 1
Injection volume: 100
Detector: UV 350

CHROMATOGRAM

Retention time: 6.6
Limit of detection: 2 ng
Limit of quantitation: 10 ng

OTHER SUBSTANCES

Extracted: quinoxaline-2-carboxylic acid, furazolidone, carbadox, desoxycarbadox

KEY WORDS

protect from light; pig; muscle; liver; kidney

REFERENCE

MacIntosh,A.I.; Neville,G.A. Liquid chromatographic determination of carbadox, desoxycarbadox, and nitrofurazones in pork tissues, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 958–962.

SAMPLE

Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax TP 18/2) 3 g ground muscle + 6.8 mL MeCN for 6 s, centrifuge at 5000 rpm for 5 min. Remove 6.5 mL of the supernatant and add it to 2 mL 5 M NaCl, shake vigorously for 10 s, centrifuge at 3000 rpm for 2 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 43°, reconstitute the residue in 250 μL MeCN:buffer 20:80, add 1 mL hexane, mix (Whirlimixer), centrifuge for 4 min, discard the hexane layer, filter (Costar Spin-X 0.22 μm cellulose acetate) while centrifuging at 5600 g for 4 min, inject a 20 μL aliquot of the filtrate. (Buffer was 20 mM sodium 1-heptanesulfonate and 10 mM Na_2HPO_4, pH adjusted to 6.0 with phosphoric acid.)

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Supelcosil LC-ABZ
Column: 250 × 4.6 5 μm Supelcosil LC-ABZ
Mobile phase: MeCN:buffer 25:75 (Buffer was 4.45 g sodium 1-heptanesulfonate and 9.5 g $Na_3PO_4.12H_2O$ in 750 mL water, adjust pH to 2.5 with 5 M phosphoric acid, make up to 1 L with water.)
Flow rate: 1
Injection volume: 20
Detector: UV 365

CHROMATOGRAM

Retention time: 5.5

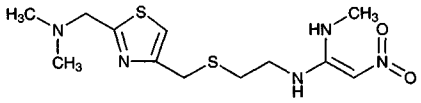
Internal standard: nitrofurazone

OTHER SUBSTANCES
Extracted: furazolidone

KEY WORDS
cow; muscle; nitrofurazone is IS

REFERENCE
Hormazábal,V.; Yndestad,M. Simple and rapid method of analysis for furazolidone in meat tissues by high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 1871–1877.

Nizatidine



Molecular formula: $C_{12}H_{21}N_5O_2S_2$
Molecular weight: 331.46
CAS Registry No.: 76963-41-2
Merck Index: 6758
Lednicer No.: 4 95

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 100 μL Plasma + 100 μL 75 μM IS + 100 μL 5 M NaOH + 5 mL dichloromethane, shake for 10 min, centrifuge at 2000 rpm for 10 min. Evaporate 4 mL of the organic phase to dryness under a stream of nitrogen. Dissolve residue in 100 μL mobile phase, inject a 50 μL aliquot. Tissue. Homogenize 500 mg liver with saline on ice for 1 min. Add 100 μL 75 μM IS, 100 μL 0.5 M NaOH, and 5 mL dichloromethane, shake for 10 min, centrifuge at 3000 rpm for 10 min. Evaporate 3 mL of the organic phase to dryness under a stream of nitrogen. Dissolve residue in 100 μL mobile phase, pass through a Ministar-RC 15 cartridge (Sartorius, Germany), inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 Senshu Pak ODS-1031 (Senshu Sciences, Japan)
Column: 250 × 4.6 Senshu Pak ODS -1251 (Senshu Sciences, Japan)
Mobile phase: MeCN:water 5:95 containing 5 mM NaH_2PO_4 and 5 mM tetramethylammonium chloride
Flow rate: 1.5
Injection volume: 50
Detector: UV 228

CHROMATOGRAM
Internal standard: cimetidine
Limit of detection: 50-100 μg/mL (sic)

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Takedomi,S.; Matsuo,H.; Yamano,K.; Yamamoto,K.; Iga,T.; Sawada,Y. Quantitative prediction of the interaction of midazolam and histamine H_2 receptor antagonists in rats, *Drug Metab.Dispos.*, **1998**, *26*, 318–323.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject

a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

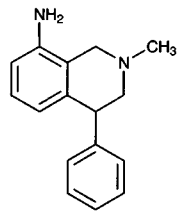
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 316.4

CHROMATOGRAM
Retention time: 3.302

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Nomifensine



Molecular formula: $C_{16}H_{18}N_2$
Molecular weight: 238.33
CAS Registry No.: 24526-64-5, 32795-47-4 (maleate)
Merck Index: 6768
Lednicer No.: 4 146

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 240

CHROMATOGRAM
Retention time: 4.87
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: bulk

Sample preparation: Mix 8 mg nomifensine maleate with 200 μL trifluoroacetic anhydride, stir at room temperature for 10 min, evaporate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 2 mL mobile phase, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 3.6 5 μm Spherisorb silica

Mobile phase: Chloroform:MeOH:water 100:3:0.15 containing 2 mM (+)-camphor-10-sulfonic acid

Flow rate: 1

Injection volume: 5

Detector: UV 254

CHROMATOGRAM

Retention time: 16 (D), 18 (L)

Limit of quantitation: 25 ng

KEY WORDS

derivatization; chiral; normal phase

REFERENCE

Tsujiyama,T.; Tsuchiya,M.; Hamachi,Y.; Kuriki,T.; Fukunaga,T.; Suzuki,N. Ion-pair chromatographic separation of nomifensine maleate enantiomers, *Anal.Sci.*, **1989**, *5*, 285–288.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.7

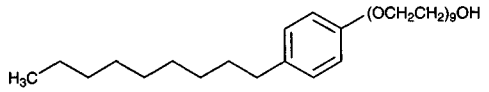
OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Nonoxynol-9



Molecular formula: $C_{33}H_{60}O_{10}$
Molecular weight: 616.83
CAS Registry No.: 26027-38-3
Merck Index: 6772

SAMPLE

Matrix: blood, urine, vaginal fluid
Sample preparation: Urine. Centrifuge at 4000 rpm for 5 min, inject a 20 μL aliquot of the supernatant. Serum. Mix serum with an equal volume of THF, centrifuge at 4000 rpm for 5 min, remove an aliquot of the supernatant and mix it with an equal volume of THF, centrifuge at 4000 rpm for 5 min, inject a 20 μL aliquot of the supernatant. Vaginal fluid. Vortex swab with 1 mL MeCN, let stand at room temperature for 15 min, weigh, vortex, remove swab, centrifuge at 4000 rpm for 5 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4.6 10 μm R-SIL-amine (Alltech)
Mobile phase: THF:MeCN 95:5
Flow rate: 1
Injection volume: 20
Detector: F ex 275 em 575

CHROMATOGRAM

Retention time: 4
Limit of detection: 460 ng (vaginal fluid), 230 ng/mL (urine), 1.01 μg/mL (serum)

KEY WORDS

place a 2 μm filter at the column inlet

REFERENCE

Beck,G.J.; Kossak,D.; Saxena,S.J. A simple, sensitive assay for the spermicide nonoxynol-9 in biological fluids by high-performance liquid chromatography, *J.Pharm.Sci.*, **1990**, *79*, 1029–1031.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 0.2% solution, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 75 PLgel 5 mixed-C (polymer Laboratories) in series with 300 × 78 Ultrastyragel 100 Å styrene-divinylbenzene(Waters)
Mobile phase: THF
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: RI

KEY WORDS

SEC

REFERENCE

Ysambertt,F.; Cabrera,W.; Marquez,N.; Salager,J.L. Analysis of ethoxylated nonylphenol surfactants by high-performance size-exclusion chromatography (HPSEC), *J.Liq.Chromatogr.*, **1995**, *18*, 1157–1171.

SAMPLE

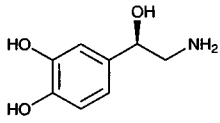
Matrix: vaginal lavage fluid
Sample preparation: 500 μL Vaginal lavage fluid (water) + 500 μL 25 μg/mL 4-octylphenol in mobile phase, vortex briefly, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm R-SIL-Amine (NH2) (Alltech) (Change 2 μm filter in front of column before each run.)
Mobile phase: MeCN:THF 2:98
Flow rate: 1
Injection volume: 20
Detector: F ex 227 em 612

CHROMATOGRAM
Retention time: 5.7
Internal standard: 4-octylphenol (4.0)
Limit of detection: 480 ng/mL
Limit of quantitation: 3.125 μg/mL

REFERENCE
McPherson,J.L.; Nichols,J.H.; Barditch-Crovo,P.; Hamzeh,F.M. Determination of the spermicide nonoxynol-9 in vaginal lavage by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *677*, 204–208.

Norepinephrine



Molecular formula: $C_8H_{11}NO_3$
Molecular weight: 169.18
CAS Registry No.: 51-41-2, 69815-49-2 (bitartrate monohydrate), 51-40-1 (bitartrate)
Merck Index: 6788

SAMPLE
Matrix: blood
Sample preparation: 20 mL Whole blood + 1 mL 20 mg/mL EDTA solution containing 10 mg/mL sodium metabisulfite, mix, centrifuge at 4° at 4000 g for 10 min. Remove the plasma and add concentrated perchloric acid until the concentration of perchloric acid is 400 mM, mix, let stand in the cold for 15 min, centrifuge at 4° at 20000 g for 20 min. Adjust pH of 2 mL supernatant to 7.0 ± 0.2 with 500 mM KOH, add 400 μL 875 μg/mL o-phthalaldehyde in pH 10.40 ± 0.02 buffer (containing 2-mercaptoethanol ?), add 2 g NaCl, add 2 mL ethyl acetate, shake for 1 min, centrifuge at 3400 g, repeat the extraction. Combine the organic layers and add them to 2 mL 35 mM pH 10.0 ± 0.1 Na_2HPO_4 buffer, shake for 1 min, centrifuge at 3400 g, discard the aqueous layer, wash the ethyl acetate layer again with phosphate buffer. Reduce the ethyl acetate volume to 100 μL under a stream of nitrogen, inject a 10-50 μL aliquot.

HPLC VARIABLES
Guard column: Co:Pell ODS
Column: 300 × 4 10 μm μBondapak phenyl
Mobile phase: Gradient. MeCN:25 mM pH 5.10 NaH_2PO_4 buffer 25:75 for 15 min then MeOH:25 mM pH 5.10 NaH_2PO_4 buffer 45:55 (step gradient).
Column temperature: 26
Flow rate: 1.5
Injection volume: 10-50
Detector: F ex 340 em 480

CHROMATOGRAM
Retention time: 11
Internal standard: tyramine (44)
Limit of detection: 0.5 ng/mL

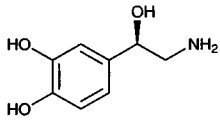
OTHER SUBSTANCES
Extracted: dopamine, serotonin

HPLC VARIABLES
Column: 250 × 4.6 10 μm R-SIL-Amine (NH2) (Alltech) (Change 2 μm filter in front of column before each run.)
Mobile phase: MeCN:THF 2:98
Flow rate: 1
Injection volume: 20
Detector: F ex 227 em 612

CHROMATOGRAM
Retention time: 5.7
Internal standard: 4-octylphenol (4.0)
Limit of detection: 480 ng/mL
Limit of quantitation: 3.125 μg/mL

REFERENCE
McPherson,J.L.; Nichols,J.H.; Barditch-Crovo,P.; Hamzeh,F.M. Determination of the spermicide nonoxynol-9 in vaginal lavage by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *677*, 204–208.

Norepinephrine



Molecular formula: $C_8H_{11}NO_3$
Molecular weight: 169.18
CAS Registry No.: 51-41-2, 69815-49-2 (bitartrate monohydrate), 51-40-1 (bitartrate)
Merck Index: 6788

SAMPLE
Matrix: blood
Sample preparation: 20 mL Whole blood + 1 mL 20 mg/mL EDTA solution containing 10 mg/mL sodium metabisulfite, mix, centrifuge at 4° at 4000 g for 10 min. Remove the plasma and add concentrated perchloric acid until the concentration of perchloric acid is 400 mM, mix, let stand in the cold for 15 min, centrifuge at 4° at 20000 g for 20 min. Adjust pH of 2 mL supernatant to 7.0 ± 0.2 with 500 mM KOH, add 400 μL 875 μg/mL o-phthalaldehyde in pH 10.40 ± 0.02 buffer (containing 2-mercaptoethanol ?), add 2 g NaCl, add 2 mL ethyl acetate, shake for 1 min, centrifuge at 3400 g, repeat the extraction. Combine the organic layers and add them to 2 mL 35 mM pH 10.0 ± 0.1 Na_2HPO_4 buffer, shake for 1 min, centrifuge at 3400 g, discard the aqueous layer, wash the ethyl acetate layer again with phosphate buffer. Reduce the ethyl acetate volume to 100 μL under a stream of nitrogen, inject a 10-50 μL aliquot.

HPLC VARIABLES
Guard column: Co:Pell ODS
Column: 300 × 4 10 μm μBondapak phenyl
Mobile phase: Gradient. MeCN:25 mM pH 5.10 NaH_2PO_4 buffer 25:75 for 15 min then MeOH:25 mM pH 5.10 NaH_2PO_4 buffer 45:55 (step gradient).
Column temperature: 26
Flow rate: 1.5
Injection volume: 10-50
Detector: F ex 340 em 480

CHROMATOGRAM
Retention time: 11
Internal standard: tyramine (44)
Limit of detection: 0.5 ng/mL

OTHER SUBSTANCES
Extracted: dopamine, serotonin

KEY WORDS
plasma; whole blood; pig; derivatization

REFERENCE
Davis,T.P.; Gehrke,C.W.,Jr.; Williams,C.H.; Gehrke,C.W.; Gerhardt,K.O. Pre-column derivatization and high-performance liquid chromatography of biogenic amines in blood of normal and malignant hyperthermic pigs, *J.Chromatogr.*, **1982**, *228*, 113–122.

SAMPLE
Matrix: blood
Sample preparation: Chill 20 mL whole blood in ice water, add 1 mL reagent, invert several time, centrifuge at 4° at 4000 g for 10 min. Remove the plasma and make it 0.4 M in perchloric acid by adding concentrated perchloric acid, mix, let stand at 4° for 15 min, centrifuge at 4° at 20000 g for 20 min. Remove a 2 mL aliquot and adjust the pH to 7.0 ± 0.2 with 0.5 M KOH, add 400 μL reagent, add 2 g NaCL, add 2 mL ethyl acetate, shake for 1 min, centrifuge at 3400 g, repeat the extraction. Combine the organic layers, add 2 mL 35 mM pH 10.0 ± 0.1 Na_2HPO_4 buffer, shake for 1 min, centrifuge at 3400 g, repeat the wash, evaporate the ethyl acetate layer to 100 μL with a stream of nitrogen, inject a 10-50 μL aliquot. (Reagent was 875 μg/mL o-phthalaldehyde and 2-mercaptoethanol in pH 10.40 ± 0.2 potassium borate buffer.)

HPLC VARIABLES
Guard column: Co:Pell ODS
Column: 300 × 4 10 μm μBondapak phenyl
Mobile phase: Gradient. MeCN:25 mM pH 5.10 NaH_2PO_4 25:75 for 15 min, then MeOH:25 mM pH 5.10 NaH_2PO_4 45:55 for 35 min (step gradient).
Column temperature: 26
Flow rate: 1.5
Injection volume: 10-50
Detector: F ex 340 em 480

CHROMATOGRAM
Retention time: 12
Limit of detection: <0.5 ng/mL

OTHER SUBSTANCES
Extracted: dopamine, histamine, octopamine, serotonin, tyramine

KEY WORDS
pig; whole blood; derivatization

REFERENCE
Davis,T.P.; Gehrke,C.W.,Jr.; Williams,C.H.; Gehrke,C.W.; Gerhardt,K.O. Pre-column derivatization and high-performance liquid chromatography of biogenic amines in blood of normal and malignant hyperthermic pigs, *J.Chromatogr.*, **1982**, *228*, 113–122.

SAMPLE
Matrix: blood
Sample preparation: Plasma. Prepare a SPE column by adding 500 μL of a 20% suspension of 19-40 μm Toyopak SP (strong cation-exchange sulfopropyl resin, Na^+ (Toyo Soda)) in water to a 35 × 6 column, wash with two 1 mL portions of 2 M LiOH, wash with two 5 mL portions of water, wash with two 1 mL portions of EtOH:12 M HCl 90:10, wash with two 5 mL portions of water, wash with three 1 mL portions of buffer. 500 μL Plasma + 25 μL 10 nM isoproterenol + 500 μL buffer, mix, add to the SPE column, wash with two 5 mL portions of water, wash with 1 mL MeCN:water 50:50, elute with 300 μL 600 μM potassium ferricyanide in 600 mM KCl:MeCN 50:50, add 50 μL reagent to the eluate, heat at 37° for 40 min, cool in ice-water, inject a 100 μL aliquot. Urine. 10 μL Urine + 1 mL MeCN:500 mM KCl 60:40 + 10 μL 500 nM isoproterenol + 10 μL 75 mM potassium hexacyanoferrate(III) + 100 μL reagent, heat at 37° for 40 min, inject a 100 μL aliquot (J. Chromatogr. 1986, 380, 229). (Prepare buffer by mixing 8 volumes 250 mM LiOH in 200 mM phosphoric acid with 1 volume 200 mM phosphoric acid, pH 5.8. Prepare reagent by dissolving 212 mg 1,2-diphenylethylenediamine in 10 mL 100 mM HCl, pH 6.7.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm TSK-gel ODS-120T (Toyo Soda)
Mobile phase: MeCN:MeOH:50 mM pH 7.0 Tris-HCl buffer 50:10:40 (Wash with MeCN:MeOH: water 50:10:40 for 15 min at the end of each day.)
Flow rate: 1
Injection volume: 100
Detector: F ex 345 em 485 (plasma), F ex 350 em 480 (urine)

CHROMATOGRAM
Retention time: 3
Internal standard: isoproterenol (8)
Limit of detection: 7 pM

OTHER SUBSTANCES
Extracted: dopamine, epinephrine

KEY WORDS
derivatization; plasma; SPE

REFERENCE
Mitsui,A.; Nohta,H.; Ohkura,Y. High-performance liquid chromatography of plasma catecholamines using 1,2-diphenylethylenediamine as precolumn fluorescence derivatization reagent, *J.Chromatogr.*, **1985**, *344*, 61–70.

SAMPLE
Matrix: blood
Sample preparation: Prepare a 20 × 5 polypropylene column packed with CM-Sephadex preswollen in water, wash with 5 mL 2 M HCl, wash with 10 mL water, wash with 10 mL 100 mM pH 7 phosphate buffer. 1 mL Plasma + 30 μL 80 ng/mL N-methyldopamine, apply to column, wash with 5.5 mL water (A), elute with 3 mL 0.5 M perchloric acid. Collect eluate, add 2 mL 1.5 M pH 9.3 Tris buffer containing 60 mM EDTA, add 20 mg alumina, vortex for 2 min, discard supernatant, add 2 mL water to alumina, mix, centrifuge at 3000 g for 3 min, repeat water wash, remove as much water as possible, elute catecholamines from alumina with 100 μL 100 mM acetic acid with vortexing for 2 min, centrifuge, inject 25 μL aliquot of supernatant. (Wash water A contains levodopa, carbidopa, DOPAC, and O-methyldopa.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:MeOH:25 mM sodium acetate 4:4:92 containing 0.2 mM 1-octanesulfonic acid and 0.3 mM disodium EDTA, pH was adjusted to pH 3 with acetic acid
Flow rate: 0.9
Injection volume: 10
Detector: E, ESA Coulochem 5100 A, 5010 A analytical cell, first electrode +0.25 V, second electrode -0.30 V

CHROMATOGRAM
Retention time: 8
Internal standard: N-methyldopamine (16)
Limit of detection: 0.5 ng/mL

OTHER SUBSTANCES
Simultaneous: epinephrine, dopamine

KEY WORDS
plasma; SPE

REFERENCE
Betto,P.; Ricciarello,G.; Giambenedetti,M.; Lucarelli,C.; Ruggeri,S.; Stocchi,F. Improved high-performance liquid chromatographic analysis with double detection system for L-dopa, its metabolites and carbidopa in plasma of parkinsonian patients under L-dopa therapy, *J.Chromatogr.*, **1988**, *459*, 341–349.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 250 μL 1 ng/mL α-methylnorepinephrine + 1 mL buffer + 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol, shake for 2 min, centrifuge at 20° at 1000 g for 5 min, freeze in acetone/dry ice. Remove the organic phase and add it to 2 mL 1-octanol and 200 μL 80 mM acetic acid, shake, centrifuge at 20° at 1000 g for 5 min, freeze in acetone/dry ice. Discard the organic phase, thaw the aqueous phase and add it to 1 mL 10 mM HCl, 1 mL buffer, and 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol, shake for 2 min, centrifuge at 20° at 1000 g for 5 min, freeze in acetone/dry ice. Remove the organic phase and add it to 2 mL 2 M pH 8.6 ammonia/ammonium chloride buffer containing 13.4 mM EDTA, shake, freeze in dry ice/acetone. Remove the organic layer and add it to 2 mL 1-octanol and 150 μL 80 mM acetic acid, shake, centrifuge at 20° at 1000 g for 5 min, freeze in dry ice/acetone, discard the organic layer. Thaw the aqueous layer and add it to 250 μL MeCN, 50 μL 1.75 M pH 7.05 bicine, and 100 μL 100 mM 1,2-diphenylethylenediamine in 100 mM HCl, add 20 μL 20 mM potassium ferricyanide in water, heat at 37° in the dark for 1 h, keep at 20° in the dark, inject a 100 μL aliquot. (Buffer was 2 M pH 8.6 ammonia/ammonium chloride buffer containing 8.9 mM diphenylborate-ethanolamine complex and 13.4 mM EDTA. Stir buffer with 45 g/L activated alumina for 2 h before use. Wash 1-octanol with 80 mM acetic acid. Recrystallize 1,2-diphenylethylenediamine from toluene:light petroleum (bp 60-80°) 10:90, dry overnight at 60°.)

HPLC VARIABLES
Column: 100 × 4.6 3 μm Cp MicroSpher C18 (Chrompack)
Mobile phase: MeCN:MeOH:50 mM pH 7.0 sodium acetate buffer 40:8:50
Flow rate: 1
Injection volume: 100
Detector: F ex 350 em 480

CHROMATOGRAM
Retention time: 2
Internal standard: α-methylnorepinephrine (3)
Limit of detection: 2 pg/mL

OTHER SUBSTANCES
Extracted: dihydroxybenzylamine, dopamine, epinephrine, isoproterenol

KEY WORDS
plasma; derivatization; comparison with electrochemical detection

REFERENCE
van der Hoorn,F.A.J.; Boomsma,F.; Man in 't Veld,A.J.; Schalekamp,M.A.D.H. Determination of catecholamines in human plasma by high-performance liquid chromatography: comparison between a new method with fluorescence detection and an established method with electrochemical detection, *J.Chromatogr.*, **1989**, *487*, 17–28.

SAMPLE
Matrix: blood
Sample preparation: Pack a 65 × 15 SPE column with 50 mg WA-4 alumina (Sigma). Add 500 μL plasma to the SPE column, add 1 mL buffer, rotate for 15 min, wash three times with water (aspirating to dryness each time), centrifuge to dryness, add 200 μL 100 mM pH 1.2 perchloric acid, mix, let stand for 15 min, centrifuge the SPE column at 1000 g for 3 min, inject an aliquot of the effluent. (Buffer was 45 g Tris and 5 g EDTA in 200 mL water, pH adjusted to 8.6 with concentrated HCl.)

HPLC VARIABLES
Guard column: 50 × 4.6 5 μm reversed-phase
Column: 250 × 4.6 5 μm ODS Spherisorb
Mobile phase: Buffer contained 1.4% monochloroacetic acid, 0.47% NaOH, and 0.075% EDTA, finally pH adjusted to 3.0 with NaOH or monochloroacetic acid and 6 mg% sodium octylsulfate added.
Column temperature: 35
Flow rate: 1

Injection volume: 100
Detector: E, Bioanalytical Systems LC-4B, TL-5 transducer with a glassy carbon electrode, +650 mV, 1 nA, Ag/AgCl reference electrode

OTHER SUBSTANCES
Extracted: epinephrine, dopamine

KEY WORDS
plasma; rabbit; human; SPE

REFERENCE
Ganhao,M.F.; Hattingh,J.; Hurwitz,M.L.; Pitts,N.I. Evaluation of a simple plasma catecholamine extraction procedure prior to high-performance liquid chromatography and electrochemical detection, *J.Chromatogr.*, **1991**, *564*, 55–66.

SAMPLE
Matrix: blood
Sample preparation: Plasma. 1 mL Plasma + 125 μL 2 ng/mL α-methylnorepinephrine + 1 mL buffer + 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol, shake for 2 min, centrifuge at 1000 g for 5 min, freeze in dry ice/acetone. Remove the organic phase and add it to 2 mL 1-octanol (saturated with 80 mM acetic acid) and 200 μL 80 mM acetic acid, shake, centrifuge at 1000 g for 5 min. Freeze the aqueous layer and remove the organic layer. Add 1 mL 10 mM HCl, 1 mL buffer, and 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol to the aqueous phase. Shake, centrifuge, freeze, remove the organic layer and add it to 2 mL 2 M pH 8.6 ammonia-ammonium chloride buffer containing 13.4 mM EDTA (but no complex). Freeze, remove the organic layer and add it to 2 mL 1-octanol and 150 μL 80 mM acetic acid, shake, centrifuge at 1000 g for 5 min. Freeze, remove the organic layer and add the aqueous layer to 200 μL MeCN, 50 μL 1.75 M pH 6.95 bicine buffer containing 1% EDTA, 100 μL 100 mM 1,2-diphenylethylenediamine in 100 mM HCl, and 20 μL 20 mM potassium ferricyanide in water. Heat at 37° in the dark for 1 h, inject a 75 μL aliquot (keep it in the dark in the autosampler). Urine. 100 μL Urine + 1 mL 10 mM HCl + 125 μL 40 ng/mL α-methylnorepinephrine + 1 mL buffer + 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol, shake for 2 min, centrifuge at 1000 g for 5 min, freeze in dry ice/acetone. Remove the organic phase and add it to 2 mL 1-octanol (saturated with 80 mM acetic acid) and 200 μL 80 mM acetic acid, shake, centrifuge at 1000 g for 5 min. Freeze the aqueous layer and remove the organic layer. Add 1 mL 10 mM HCl, 1 mL buffer, and 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol to the aqueous phase. Shake, centrifuge, freeze, remove the organic layer and add it to 2 mL 1-octanol and 150 μL 80 mM acetic acid, shake, centrifuge at 1000 g for 5 min. Freeze, remove the organic layer and add the aqueous layer to 200 μL MeCN, 50 μL 1.75 M pH 6.95 bicine buffer containing 1% EDTA, 100 μL 100 mM 1,2-diphenylethylenediamine in 100 mM HCl, and 20 μL 20 mM potassium ferricyanide in water. Heat at 37° in the dark for 1 h, inject a 50 μL aliquot (keep it in the dark in the autosampler). (Buffer was a 2 M pH 8.6 ammonia-ammonium chloride buffer containing 8.9 mM diphenyl borate-ethanolamine complex and 13.4 mM EDTA.)

HPLC VARIABLES
Column: 100 × 4.6 3 μm PhaseSep C18 ODS2
Mobile phase: Gradient. A was MeCN:MeOH:50 mM pH 7.0 sodium acetate buffer 20:4:76. B was MeCN:MeOH:50 mM pH 7.0 sodium acetate buffer 60:10:30. A:B 40:60 for 3 min, go to 0:100 over 0.5 min, stay at 0:100 for another 4.5 min. (After the last sample flush column with 60 mL MeCN:MeOH:water 70:10:20.)
Flow rate: 1
Injection volume: 50-75
Detector: F ex 350 em 480

CHROMATOGRAM
Retention time: 2
Internal standard: α-methylnorepinephrine (2.5)
Limit of detection: 0.3-0.6 pg

OTHER SUBSTANCES
Simultaneous: epinephrine, dopamine, epinine

Interfering: α-methyldopa

KEY WORDS
plasma; derivatization

REFERENCE
Boomsma,F.; Alberts,G.; van der Hoorn,F.A.J.; Man in 't Veld,A.J.; Schalekamp,M.A.D.H. Simultaneous determination of free catecholamines and epinine and estimation of total epinine and dopamine in plasma and urine by high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.*, **1992**, *574*, 109–117.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 5 mg alumina + 10 μL 10 μM IS in 10 mM perchloric acid + 100 μL pH 8.7 Tris-HCl buffer, stir for 10 min, centrifuge at 3000 g for 1 min, discard the supernatant. Wash the alumina with two 500 μL portions of water, add 100 μL 100 mM perchloric acid, mix for 1 min, centrifuge at 3000 g for 1 min, inject a 50 μL aliquot of the supernatant. (Heat 20 g alumina (WA-4, Sigma) with 200 mL 2 M HCl at 100° for 1 h with gentle mixing, decant the supernatant, wash with twenty 200 mL portions of water, filter (Toyo Roshi No. 2 paper), dry at 120° overnight.)

HPLC VARIABLES
Column: 150 × 4.6 catecholpak (JASCO)
Mobile phase: MeCN:50 mM pH 3.20 potassium acetate:50 mM pH 3.20 potassium phosphate buffer 3:92.15:4.85 containing 1 mM sodium hexanesulfonate
Column temperature: 40
Flow rate: 0.5
Injection volume: 50
Detector: Chemiluminescence (Kenko filter Y-46) following post-column reaction. The column effluent mixed with reagent 1 pumped at 0.25 mL/min and the mixture flowed through a 15 m × 0.5 mm i.d. knitted PTFE coil at 80°. The effluent from the coil mixed with reagent 2 pumped at 1.4 mL/min and this mixture flowed to the detector. (Reagent 1 was 105 mM ethylenediamine (semiconductor grade) and 175 mM imidazole in MeCN:EtOH 90:10. Reagent 2 was 0.25 mM bis[4-nitro-2-(3,6,9-trioxadecyloxycarbonyl)phenyl] oxalate (Wako), 150 mM hydrogen peroxide, and 110 mM trifluoroacetic acid in dioxane:ethyl acetate 50:50 (Caution! Dioxane is a carcinogen!).)

CHROMATOGRAM
Retention time: 14
Internal standard: 3,4-dihydroxybenzylamine (17)
Limit of detection: 1 fmole

OTHER SUBSTANCES
Extracted: dopamine, epinephrine

KEY WORDS
human; rat; plasma; SPE; post-column reaction

REFERENCE
Higashidate,S.; Imai,K. Determination of femtomole concentrations of catecholamines by high-performance liquid chromatography with peroxyoxalate chemiluminescence detection, *Analyst*, **1992**, *117*, 1863–1868.

SAMPLE
Matrix: blood
Sample preparation: Filter (Ultrafree-MC with 10000 molecular mass cut-off, Millipore) 100 μL plasma while centrifuging at 15000 g for 15 min. Mix 50 μL ultrafiltrate and 10 μL 140 ng/mL 3-methoxytyramine in Ringer solution, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 150 × 1 5 μm Inertsil-2 ODS

Mobile phase: MeCN:THF:water 6:0.8:93.2 containing 0.48 g/L sodium 1-octanesulfonate, 2 g/L NaH_2PO_4, 8.82 g/L sodium citrate, 10 mg/L EDTA, and 1 mL/L diethylamine, pH adjusted to 3.2 with concentrated orthophosphoric acid.
Flow rate: 0.06
Injection volume: 5
Detector: E, Bioanalytical Systems BAS-4C, glassy carbon working electrodes, upstream +0.75 V, downstream +0.05 V (measuring electrode), Ag/AgCl reference electrode

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CHROMATOGRAM
Retention time: 2.5
Internal standard: 3-methoxytyramine (11)
Limit of detection: 0.2-0.5 pg

OTHER SUBSTANCES
Extracted: dopamine, epinephrine, 3,4-dihydroxyphenylacetic acid, serotonin, 5-hydroxyindoleacetic acid, homovanillic acid

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KEY WORDS
plasma; microbore; rat; ultrafiltrate

REFERENCE
Cheng,F.-C.; Yang,L.-L.; Kuo,J.-S.; Yang,M.C.M.; Yu,P.-C. Rapid assay of the monoamine content in small volumes of rat plasma, *J.Chromatogr.B*, **1994**, *653*, 9–16.

SAMPLE
Matrix: blood, food, peptides, plants, tissue
Sample preparation: Hydrolyze peptide with 6 M HCl containing 0.2% 3,3'-thiodipropionic acid at 110° for 24 h, evaporate to dryness, reconstitute with 50-200 μL 0.1% HCl containing 0.2% 3,3'-thiodipropionic acid. Homogenize (Ultra-Turrax) 0.1-1 g food, tissue, plant material, lyophilized plasma, or lyophilized tissue in 10 mL 250 nM IS in 100 mM HCl containing 0.2% 3,3'-thiodipropionic acid at 20000 rpm for 2 min, sonicate for ≤30 min, centrifuge at 5000 g for 20 min, discard fat layer, filter (Millipore ultrafiltration insert (MW cutoff 5000) prewashed with 200 μL 100 mM HCl containing 0.2% 3,3'-thiodipropionic acid) 3 mL supernatant while centrifuging at 3500 g for 1 h. Mix 20 μL deproteinized sample (or 10 μL peptide hydrolysate) with 180 μL buffer, vortex, add 200 μL reagent, mix, heat at 70° for 15 min with mixing at 1 min and 12 min, cool in an ice bath for 5 min, centrifuge at 10000 g for 10 s, add 400 μL diluent, mix thoroughly, centrifuge at 15000 g for 5 min, inject a 10 μL aliquot of the supernatant. (Prepare buffer by dissolving 630 mg sodium bicarbonate in 40 mL water, adjusting pH to 8.6 with NaOH, and making up to 50 mL with water. Prepare reagent by sonicating 40 mg dabsyl chloride in 10 mL acetone for 10 min, then filtering into brown vials and storing at -20°. Prepare diluent by mixing 50 mL MeCN, 25 mL EtOH, and 25 mL mobile phase A.)

HPLC VARIABLES
Guard column: present but not specified
Column: 150 × 3.9 4 μm Novapak C18
Mobile phase: Gradient. A was DMF:9 mM NaH_2PO_4 containing 0.16% triethylamine, adjusted to pH 6.55 with phosphoric acid. B was MeCN:water 80:20. A:B 92:8 for 2 min, to 80:20 over 5 min (Waters convex curve 5), to 65:35 over 28 min (Waters concave curve 7), to 50:50 over 10 min, to 0:100 over 21 min, maintain at 0:100 for 11 min, return to initial conditions over 0.5 min, re-equilibrate for 12.5 min.
Column temperature: 50
Flow rate: 1
Injection volume: 10
Detector: UV 436

CHROMATOGRAM
Retention time: 68.49
Internal standard: norleucine (40.90), norvaline (35.06)

OTHER SUBSTANCES
Extracted: amino acids dopamine, epinephrine, histamine, taurine

KEY WORDS
rinse glass and plasticware with 70% EtOH and water and dry before use; derivatization; cheese; meat; sausage; fish; plasma

REFERENCE
Krause,I.; Bockhardt,A.; Neckermann,H.; Henle,T.; Klostermeyer,H. Simultaneous determination of amino acids and biogenic amines by reversed-phase high-performance liquid chromatography of the dabsyl derivatives, *J.Chromatogr.A*, **1995**, *715*, 67–79.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. Add 1 mL serum to 100 mg activated aluminum oxide suspended in 1 mL pH 8.7 Tris-HCl buffer, stir, let stand for 10 min. Discard the supernatant and wash the solid three times with 5 mL portions of water, wash the solid with 3 mL MeOH, dry under reduced pressure, elute with 3 mL 4 M acetic acid. Evaporate the eluate to dryness under reduced pressure, reconstitute the residue with 90 μL water, inject a 10 μL aliquot. Urine. 5 mL Urine + 5.3 mL 2 M HCl, heat at 100° for 20 min, cool to room temperature, add 1 mL 50 mM disodium EDTA, adjust the pH to 8.5 with dilute ammonia, add 500 mg 200 mesh aluminum oxide (Wako), shake for 10 min, filter, wash the solid with 10 mL water, elute with 5 mL 300 mM acetic acid, inject an aliquot of the eluate.

HPLC VARIABLES
Column: 250 × 3.6 10-25 μm Hitachi 3011 C resin
Mobile phase: 50 mM K_2HPO_4 containing 0.05% phosphoric acid
Column temperature: 45
Flow rate: 0.6
Injection volume: 10
Detector: F ex 383 em 486 following post-column reaction. The column effluent mixed with 1% 2-cyanoacetamide in water pumped at 0.5 mL/min and with buffer pumped at 1 mL/min and the mixture flowed through a 5 m × 0.5 mm ID PTFE coil at 100 ± 1° to the detector. (Buffer was 600 mM boric acid containing 750 mM KOH.)

CHROMATOGRAM
Retention time: 6
Limit of detection: 0.11 pmole

OTHER SUBSTANCES
Extracted: dopamine, epinephrine

KEY WORDS
post-column reaction; serum; SPE

REFERENCE
Honda,S.; Takahashi,M.; Araki,Y.; Kakehi,K. Postcolumn derivatization of catecholamines with 2-cyanoacetamide for fluorimetric monitoring in high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *274*, 45–52.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a 150 μL Toyopak IC-SP S (sulfopropyl resin, H^+ form) SPE cartridge (Tosoh) with 10 mL water. Plasma. 700 μL Plasma + 50 μL 700 nM 3,4-dihydroxybenzylamine + 350 μL 2 M perchloric acid, mix, centrifuge at 4° at 1000 g for 15 min. Remove a 700 μL aliquot of the supernatant and add it to 30 μL 2 M potassium carbonate, centrifuge at 4° at 1000 g for 5 min. Add a 500 μL aliquot of the supernatant to the SPE cartridge, wash with 1 mL water, wash with 500 μL EtOH:water 50:50, wash with 5 mL water, elute with 500 μL 2 M sodium perchlorate, filter (0.2 μm), inject a 50 μL aliquot of the filtrate. Urine. Acidify urine collected over 24 h with 10 mL 6 M HCl. 500 μL Urine + 25 μL 10 μM 3,4-dihydroxybenzylamine + 25 μL 40 μM ferulic acid + 500 μL 1 M perchloric acid, mix, centrifuge at 4° at 1000 g for 15 min. Remove a 700 μL aliquot of the supernatant and add it to 30 μL 2 M potassium carbonate, centrifuge at 4° at 1000 g for 5 min, add a 500 μL aliquot of the supernatant to the SPE cartridge, wash with 1.5 mL water, wash with 500 μL EtOH:water 50:50,

wash with 5 mL water, elute with 500 μL 2 M sodium perchlorate, filter (0.2 μm), inject a 50 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 150 × 4.6 5 μm TSK-gel ODS-80TM (Tosoh)
Mobile phase: Gradient. A was buffer. B was MeCN:MeOH:buffer 8:12:80, pH 3.1. A:B 100:0 for 4 min, to 60:40 over 8 min, to 0:100 over 2 min, maintain at 0:100 for 16 min, return to initial conditions (step gradient), re-equilibrate for 20 min. Buffer was 60 mM pH 3.1 citric acid containing 32 mM Na_2HPO_4, 1.7 mM sodium hexanesulfonate, and 0.1 mM disodium EDTA (J. Chromatogr. 1989, 467, 237).
Flow rate: 1
Injection volume: 50
Detector: F ex 345 em 480 following post-column reaction. The column effluent passed through a Hitachi 655A electrochemical detector with carbon cloth electrodes; working electrode at +0.68 V versus reference electrode (200 mM equimolar mixture of potassium hexacyanoferrate(II) and potassium hexacyanoferrate(III) containing 200 mM potassium nitrate and 200 mM KOH). The effluent from the electrochemical detector mixed with 20 mM meso-1,2-diphenylethylenediamine in 50 mM HCl pumped at 0.4 mL/min and with 1 M glycine containing 490 mM KOH and 3 mM potassium hexacyanoferrate(III) pumped at 0.4 mL/min. This mixture flowed through a 10 m × 0.47 mm ID coil at 80° to the detector (J. Chromatogr. 1989, 467, 237).

CHROMATOGRAM

Retention time: 6
Internal standard: 3,4-dihydroxybenzylamine (12.5)
Limit of detection: 0.6 nM

OTHER SUBSTANCES

Extracted: dopamine, epinephrine, levodopa, metanephrine, 3-methoxytyramine

KEY WORDS

post-column reaction; plasma; SPE

REFERENCE

Nohta,H.; Yamaguchi,E.; Ohkura,Y.; Watanabe,H. Measurement of catecholamines, their precursor and metabolites in human urine and plasma by solid-phase extraction followed by high-performance liquid chromatography with fluorescence derivatization, *J.Chromatogr.*, **1989**, *493*, 15–26.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 3 mL ice-cold MeOH:500 mM perchloric acid 98:2, centrifuge at 4° at 4000 g for 3 min. Remove 200 μL of the supernatant and add it to 100 μL 80 ng/mL N-methyldopamine, evaporate to dryness under vacuum, reconstitute in 200 μL mobile phase, inject a 5-20 μL aliquot. Urine. 1 mL Urine + 50 mL water, inject a 10 μL aliquot. (To deconjugate adjust pH to 1, flush with nitrogen, heat in a boiling water bath for 1 h, dilute with 50 mL water, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeOH:13 mM sodium acetate containing 0.5 mM sodium 1-octanesulfonate and 0.5 mM disodium EDTA 14:86, pH 3.10
Flow rate: 1
Injection volume: 5-20
Detector: E, ESA Model 5100 A Coulochem, Model 5011 A analytical cell, first electrode +0.40 V, second electrode -0.30 V

CHROMATOGRAM

Retention time: 5
Internal standard: N-methyldopamine (11)

OTHER SUBSTANCES

Extracted: methyldopa, epinephrine, dopamine, dihydroxyphenylacetic acid, 3-O-methylmethyldopa, homovanilic acid

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Lucarelli,C.; Betto,P.; Ricciarello,G.; Grossi,G. High-performance liquid chromatographic determination of L-3-(3,4-dihydroxyphenyl)-2-methylalanine (α-methyldopa) in human urine and plasma, *J.Chromatogr.*, **1991**, *541*, 285–296.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Toyopak IC-SP S sulfopropyl resin, H^+ form, SPE cartridge (Tosoh) with 10 mL water and 2 mL 200 mM pH 5.0 sodium phosphate buffer. Plasma. 700 μL Plasma + 30 μL 700 nM isoproterenol + 50 μL 7 μM 3,4-dihydroxyphenylpropanoic acid + 350 μL 2 M perchloric acid, mix, centrifuge at 4° at 1000 g for 15 min. Remove a 700 μL aliquot of the supernatant and adjust the pH to 1.5-2.0 with about 150 μL 2 M potassium carbonate, centrifuge at 4° at 1000 g for 5 min, add the supernatant to the SPE cartridge, wash with 10 mL water, elute with 300 μL MeOH:2 M sodium perchlorate 7:93, filter (cellulose acetate membrane), inject a 100 μL aliquot of the filtrate. Urine. Collect human urine for 24 h in the presence of 10 mL 6 M HCl. 500 μL Urine + 10 μL 15 μM isoproterenol + 25 μL 800 μM 3,4-dihydroxyphenylpropanoic acid + 500 μL 1 M perchloric acid, mix, centrifuge at 4° at 1000 g for 15 min. Remove a 700 μL aliquot of the supernatant and adjust the pH to 1.5-2.0 with about 130 μL 2 M potassium carbonate, centrifuge at 4° at 1000 g for 5 min, add the supernatant to the SPE cartridge, wash with 1.5 mL water, wash with 500 μL EtOH:water 50:50, wash with 5 mL water, elute with 500 μL 1.5 M KCl in MeOH:100 mM HCl 7:93, filter (cellulose acetate membrane), inject a 100 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm TSK-gel ODS-80TM (Tosoh)
Mobile phase: MeOH:buffer 7:93 (Buffer was 30 mM pH 2.5 citrate buffer containing 0.4 mM sodium octanesulfonate.)
Flow rate: 0.8
Injection volume: 100
Detector: F ex 350 em 480 following post-column reaction. The column effluent mixed with reagent A pumped at 0.3 mL/min and the mixture flowed through a 3 m × 0.5 mm ID stainless steel coil at 90°. The effluent from this coil mixed with reagent B pumped at 0.3 mL/min and the mixture flowed through a 10 m × 0.5 mm ID stainless steel coil at 90° and through a 1 m × 0.5 mm ID stainless steel cooling coil to the detector (Anal. Sci. 1991, 7, 257). (Reagent A was 10 mM sodium periodate containing 3 mM potassium ferricyanide. Reagent B was 30 mM meso-1,2-diphenylethylenediamine in EtOH:water 70:30 containing 130 mM sodium methylate.)

CHROMATOGRAM
Retention time: 16
Internal standard: isoproterenol (60)
Limit of detection: 0.4-0.5 nM

OTHER SUBSTANCES
Extracted: dopamine, epinephrine, levodopa, metanephrine, 3-methoxytyramine, normetanephrine

KEY WORDS
post-column reaction; plasma; SPE

REFERENCE
Jeon,H.-K.; Nohta,H.; Ohkura,Y. High-performance liquid chromatographic determination of catecholamines and their precursor and metabolites in human urine and plasma by postcolumn derivatization involving chemical oxidation followed by fluorescence reaction, *Anal.Biochem.*, **1992**, *200*, 332–338.

SAMPLE
Matrix: blood, urine
Sample preparation: 2 mL Plasma or 1 mL urine + dihydroxybenzylamine + 20 mg Sigma WA4 alumina + 200 μL 1 M pH 8.6 Tris-EDTA buffer, mix for 10 min, discard plasma. Wash the

alumina three times with 3 mL water and dry it. Add 125 μL 500 mM phosphoric acid, after 1 min inject a 100 μL aliquot. (Ann. Clin. Biochem. 1985, 22, 194-203)

HPLC VARIABLES
Column: 250 × 4.5 5 μm Ultratechsphere
Mobile phase: Per liter 75 mmol citric acid, 58.5 mmol NaH_2PO_4, 0.2 mmol disodium EDTA, and 4.4 mmol heptanesulfonic acid, pH adjusted to 3.4, made up to a final volume of 2 L, add 200 mL MeOH
Flow rate: 1
Injection volume: 100
Detector: E, ESA Coulochem conditioning cell +0.35 V, first electrode +0.05 V, second electrode -0.35 V

CHROMATOGRAM
Retention time: 6.00
Internal standard: dihydroxybenzylamine (10.53)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Simultaneous: levodopa, metanephrine, epinephrine, 3-methoxytyrosine, normetanephrine, dihydroxyphenylacetic acid, dopamine

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KEY WORDS
plasma

REFERENCE
Dutton,J.; Copeland,L.G.; Playfer,J.R.; Roberts,N.B. Measuring L-dopa in plasma and urine to monitor therapy of elderly patients with Parkinson disease treated with L-dopa and a dopa decarboxylase inhibitor, *Clin.Chem.*, **1993**, *39*, 629–634.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 2.8

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: cell cultures
Sample preparation: Centrifuge at 20000 g for 5 min, dilute the supernatant 10-50-fold, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 125 × 3 3 μm Nucleosil 100C18
Mobile phase: MeOH:buffer 5:95, pH adjusted to 3.0 with 5 M NaOH (Buffer was 50 mM citric acid containing 30 mM phosphoric acid, 0.75 mM octylsulfate, and 0.5 mM EDTA.)
Column temperature: 40
Flow rate: 0.7
Injection volume: 30
Detector: E, Waters 460, glassy carbon electrode

CHROMATOGRAM
Limit of detection: 10 nM

OTHER SUBSTANCES
Extracted: epinephrine

REFERENCE
Ghindilis,A.L.; Michael,N.; Makower,A. A new sensitive and simple method for detection of catecholamines from adrenal chromaffin cells, *Pharmazie*, **1995**, *50*, 599–600.

SAMPLE
Matrix: dialysate
Sample preparation: Mix 30 μL dialysate + 5 μL 200 mM perchloric acid, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 80 × 4.6 3 μm HR-80 C18 (ESA)
Mobile phase: MeCN:MeOH:buffer 15:13:72 (Buffer was 75 mM NaH_2PO_4, 1.5 mM sodium dodecyl sulfate, 100 μL/L triethylamine, and 20 μm EDTA, adjusted to pH 5.6.)
Column temperature: 25
Flow rate: 1
Injection volume: 20
Detector: E, ESA Coulochem II, Model 5014 Microdialysis Cell with E1 -175 mV and E2 +175 mV, Pd reference electrode, Model 5020 Guard Cell EGC +300 mV

CHROMATOGRAM
Retention time: 2.83
Limit of detection: 400 fg

OTHER SUBSTANCES
Extracted: dopamine, epinephrine, serotonin

KEY WORDS
rat; brain; pharmacokinetics; use PEEK tubing and sample loop

REFERENCE
Gariepy,K.C.; Bailey,B.; Yu,J.; Maher,T.; Acworth,I.N. Simultaneous determination of norepinephrine, dopamine, and serotonin in hippocampal microdialysis samples using normal bore high performance liquid chromatography: Effects of dopamine receptor agonist stimulation and euthanasia, *J.Liq.Chromatogr.*, **1994**, *17*, 1541–1556.

SAMPLE
Matrix: dialysate
Sample preparation: 20 μL Dialysate (Ringer's solution) + 50 μL 80 pg/mL 3,4-dihydroxybenzylamine in 2% acetic acid + 5 mg acid-washed alumina + 1 mL 1 M pH 8.6 Tris buffer containing 0.2% disodium EDTA, shake for 15 min, wash the alumina 3 times with water. Place

the alumina in an Ultrafree C3 microfilter tube (Millipore), dry by centrifuging at 600 g for 5 min, elute with 60 μL 2% acetic acid, inject a 50 μL aliquot of the eluate.

HPLC VARIABLES
Guard column: 5 × 4 AC-ODS (Eicom)
Column: 150 × 2.1 Eicompak CA-50DS (Eicom)
Mobile phase: MeOH:100 mM pH 6.1 phosphate buffer 10:90 containing 600 μg/mL sodium 1-octanesulfonate
Column temperature: 25
Flow rate: 0.25
Injection volume: 50
Detector: E, Eicom ECD-300, graphite electrode + 400 mV, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 7
Internal standard: 3,4-dihydroxybenzylamine (14)
Limit of detection: 5 pg/mL

KEY WORDS
SPE; cat

REFERENCE
Yamazaki,T.; Akiyama,T.; Shindo,T. Routine high-performance liquid chromatographic determination of myocardial interstitial norepinephrine, *J.Chromatogr.B*, **1995**, *670*, 328–331.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Dissolve powdered tablets in 10 mM HCl, filter if necessary, inject an aliquot. Injections, solutions. Dilute with 10 mM HCl, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Partisil-5 ODS-3
Mobile phase: MeOH:buffer 30:70 (Buffer was 10 mM sodium 1-octanesulfonate in 0.2% acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 10.5
Limit of detection: 27 ng

OTHER SUBSTANCES
Simultaneous: epinephrine, levonordefrin, isoproterenol, phenylephrine, metaraminol, impurities

KEY WORDS
tablets; injections; ophthalmic solutions; inhalation solutions

REFERENCE
Smela,M.J.,Jr.; Stromberg,R. Liquid chromatographic determination of six sympathomimetic drugs in dosage forms, *J.Assoc.Off.Anal.Chem.*, **1991**, *74*, 289–291.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Grind tablets, weigh out a portion, dissolve in 50 mL mobile phase, sonicate, filter (No. 4 sintered glass plate), dilute, inject an aliquot. Capsules. Dissolve 10 capsules (without opening) in 100 mL mobile phase, sonicate, inject an aliquot. Injections, ampules, sprays. Dilute, inject an aliquot.

HPLC VARIABLES
Column: 120 × 4.6 Spherisorb C18 ODS-2
Mobile phase: Isopropanol:buffer 5:95 (Buffer was 100 mM sodium dodecyl sulfate containing 25 mM Na_2HPO_4, pH adjusted to 3.0 with HCl.)
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: k' 3.1
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Simultaneous: carbidopa, dopamine, epinephrine, hydrochlorothiazide, isoproterenol, levodopa, methyldopa, 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: perfusate
Sample preparation: 30 μL Perfusate (artificial CSF) + 10 μL 200 mM perchloric acid. Mix a 25 μL aliquot with 12.5 μL reagent, let stand for 2 min, inject an aliquot. (Prepare a stock solution by dissolving 27 mg o-phthalaldehyde in 1 mL MeOH, add 5 μL β-mercaptoethanol, add 9 mL 100 mM pH 9.3 sodium tetraborate containing 10 μM EDTA. This solution is good for 5 days in a sealed amber bottle at room temperature. Prepare the working reagent by diluting 1 mL of the stock solution with 3 mL 100 mM pH 9.3 sodium tetraborate containing 10 μM EDTA, allow to stand for 24 h before use.)

HPLC VARIABLES
Column: two columns 150 × 4.6 5 μm M.S. Gel C18 (ESA)
Mobile phase: MeOH:buffer 8:92 adjusted to pH 3.0 with phosphoric acid (Buffer was 54 mM NaH_2PO_4 containing 1.24 mM sodium heptanesulfonate.)
Column temperature: 33
Flow rate: 1.2
Detector: E, ESA Coulochem Electrode Array System Model 5500, detector temp 33°, oxidation potential 70 mV

CHROMATOGRAM
Retention time: 2.51
Limit of quantitation: 0.5 ng/mL

OTHER SUBSTANCES
Extracted: apomorphine, dopamine, hydralazine, isoproterenol, methoxamine, morphine, phenylephrine

KEY WORDS
rat; derivatization

REFERENCE
Acworth,I.N.; Yu,J.; Ryan,E.; Gariepy,K.C.; Gamache,P.; Hull,K.; Maher,T. Simultaneous measurement of monoamine, amino acid, and drug levels, using high performance liquid chromatography and coulometric array technology: application to in vivo microdialysis perfusate analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 685–705.

SAMPLE
Matrix: perfusate
Sample preparation: Perfusate + 20 μL 25 pM dihydroxybenzylamine + 15 mg activated alumina + pH 8.3-8.5 Tris buffer containing 1% EDTA, mix. Remove the alumina and wash it with water, add 100 μL 200 mM perchloric acid, centrifuge, store supernatant on ice, inject a 20 μL aliquot.

HPLC VARIABLES
Column: Lichrospher 60 RP-select B C18
Mobile phase: MeCN:buffer 1.5:98.5 (Buffer was 100 mM NaH_2PO_4 containing 1.4 mM sodium 1-octanesulfonate and 0.2 mM EDTA, pH 2.5.)
Flow rate: 0.8
Injection volume: 20
Detector: E, BAS LC3A, 0.8 V

CHROMATOGRAM
Retention time: 10
Internal standard: dihydroxybenzylamine (16)
Limit of detection: 20 fmole

KEY WORDS
SPE

REFERENCE
Forray,M.I.; Andrés,M.E.; Bustos,G.; Gysling,K. Regulation of endogenous noradrenaline release from the bed nucleus of stria terminalis, *Biochem.Pharmacol.*, **1995**, *49*, 687–692.

SAMPLE
Matrix: solutions
Sample preparation: Dilute a few μL of a <1 mM solution to 20 μL with 50 mM pH 8.0 phosphate or borate buffer, add 10 μL 2 mg/mL (?) fluorescamine in acetone with vigorous shaking, inject an aliquot.

HPLC VARIABLES
Column: 500 × 3 Hitachi 3011 gel glass column
Mobile phase: MeOH:100 mM pH 8.0 Tris-HCl buffer 70:30
Flow rate: 0.72
Detector: F primary filter Corning No. 7-51, secondary filter No. 4-7116

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: dopamine
Also analyzed: 3-methoxytyramine, normetanephrine

KEY WORDS
derivatization

REFERENCE
Imai,K. Fluorimetric assay of dopamine, norepinephrine and their 3-O-methyl metabolites by using fluorescamine, *J.Chromatogr.*, **1975**, *105*, 135–140.

SAMPLE
Matrix: solutions
Sample preparation: 200 μL 5 μg/mL Amine solution + 300 μL 100 mM pH 8.0 phosphate buffer + 300 μL 200 μg/mL fluorescamine in acetone + 200 μL water, mix, saturate with NaCl, add 500 μL ethyl acetate, shake for 1 min, inject a 50 μL aliquot of the organic phase.

HPLC VARIABLES
Column: 250 × 2 10 μm LiChrosorb Si 60-10

Mobile phase: Benzene:dioxane:acetic acid 76:22:2 (Caution! Benzene and dioxane are carcinogens!)
Flow rate: 0.5
Injection volume: 50
Detector: F ex 325-385 (filter) em 451

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: dopamine

KEY WORDS
derivatization; normal phase

REFERENCE
Schwedt,G. Hochdruck-Flüssigkeits-chromatographische Analyse der Katecholamine Dopamin und Noradrenalin als Fluorescaminderivate, *J.Chromatogr.*, **1976**, *118*, 429–432.

SAMPLE
Matrix: solutions
Sample preparation: Dilute with 5% dextrose, inject a 15 μL aliquot.

HPLC VARIABLES
Column: Waters microparticulate C18
Mobile phase: MeOH:350 mM acetic acid and 5 mM sodium heptanesulfonate 15:85
Flow rate: 1.6-2.0
Injection volume: 15
Detector: F ex 285 em 315

CHROMATOGRAM
Retention time: 4.75

OTHER SUBSTANCES
Interfering: dopamine

REFERENCE
Williams,D.A.; Fung,E.Y.Y.; Newton,D.W. Ion-pair high-performance liquid chromatography of terbutaline and catecholamines with aminophylline in intravenous solutions, *J.Pharm.Sci.*, **1982**, *71*, 956–958.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 250 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 5-C18
Mobile phase: 50 mM Potassium perchlorate containing 250 μL/L 3% copper acetate in water and 10 g/L sodium acetate, pH adjusted to 4.45 with acetic acid
Flow rate: 1
Injection volume: 250
Detector: F ex 400 em 500 following post-column reaction. The column effluent flowed through the reactor then through a 3.5 m × 0.8 mm ID coil of PTFE tubing at 30°. The effluent from the coil mixed with reducing solution pumped at 1 (?) mL/min and this mixture flowed through a 2 m × 0.8 mm ID PTFE coil at 30° to the detector. (Prepare the reactor as follows. Dissolve 75.3 g manganese nitrate in 500 mL water, add 50 g 18-35 mesh silica gel (Macherey-Nagel), stir vigorously, slowly add 31.6 g potassium permanganate in 500 mL water, stir for 30 min, filter (500 μm sieve), wash until no permanganate color is left, dry in a desiccator, pack in a 50 × 2.1 stainless steel tube. The reducing solution was 266 g NaOH, 13.4 g anhydrous sodium sulfite, and 9 mL 2-mercaptoethanol in 1 L water. Note that some Nucleosil 5-C18 column packings do not give separation at pH 4.45. In this case it is necessary to use 50 mM perchloric

acid as mobile phase and mix the column effluent with pH 4.4 sodium acetate buffer before it enters the reactor.)

CHROMATOGRAM
Retention time: 7
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Extracted: epinephrine

KEY WORDS
post-column reaction

REFERENCE
Rüter,J.; Kurz,U.P.; Neidhart,B. Solid phase reactors as an analytical tool in the determination of urinary noradrenaline and adrenaline, *J.Liq.Chromatogr.*, **1985**, *8*, 2475–2496.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid,

progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (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: 4.90

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, 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
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 100 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 150 × 4 5 μm Crownpak CR(+) immobilized crown ether
Mobile phase: 0.1% pH 1.9 Perchloric acid
Column temperature: 25
Flow rate: 1
Detector: UV 210

CHROMATOGRAM
Retention time: 3.88, 4.18

OTHER SUBSTANCES
Simultaneous: octopamine

KEY WORDS
chiral; comparison with capillary electrophoresis

REFERENCE
Nishi,H.; Nakamura,K.; Nakai,H.; Sato,T. Separation of enantiomers and isomers of amino compounds by capillary electrophoresis and high-performance liquid chromatography utilizing crown ethers, *J.Chromatogr.A*, **1997**, *757*, 225–235.

SAMPLE
Matrix: tissue
Sample preparation: Prepare a 70 × 5 SPE column of Sephadex G 10 in a Pasteur pipette, wash with 3 mL 20 mM ammonia and 3 mL 10 mM formic acid. Homogenize up to 150 mg rat brain in 1 mL 100 mM perchloric acid, centrifuge at 4000 g at 4° for 15 min, add 500 μL of the supernatant to the SPE column, wash with 2.5 mL 10 mM formic acid, elute with 1 mL 10 mM formic acid followed by 1.5 mL 5 mM Na_2HPO_4, inject an aliquot of the eluate.

HPLC VARIABLES
Column: Nucleosil 5 C18
Mobile phase: pH 6.5 Buffer prepared from 200 mM Na_2HPO_4 and 100 mM citric acid
Flow rate: 0.7
Injection volume: 200
Detector: E, rotating disc electrode, 500 mV

CHROMATOGRAM
Retention time: 4
Limit of detection: 0.05 nmole/g

OTHER SUBSTANCES
Extracted: uric acid

KEY WORDS
rat; brain; SPE

REFERENCE
Westerink,B.H.C.; Mulder,T.B.A. Determination of picomole amounts of dopamine, noradrenaline, 3,4-dihydroxyphenylacetic acid, homovanillic acid, and 5-hydroxyindolacetic acid in nervous tissue after one-step purification on Sephadex G-10, using high-performance liquid chromatography with a novel type of electrochemical detection, *J.Neurochem.*, **1981**, *36*, 1449–1462.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (glass potters) with 5 volumes of 100 mM perchloric acid containing 1.9 mM sodium bisulfite, centrifuge at 10000 g at 4° for 30 min. Filter (0.22 μm) the supernatant, add dihydroxybenzylamine, inject a 5-20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 Hypersil H5 ODS
Mobile phase: EtOH:buffer 2:98 (Buffer was 13.8 g NaH_2PO_4, 60 mg disodium EDTA, and 20 mg 1-octanesulfonic acid in 1 L water, pH adjusted to 3.70 with phosphoric acid.)
Flow rate: 1
Injection volume: 5-20
Detector: E, Unicam PU 4022, 70 mV

CHROMATOGRAM
Retention time: 5
Internal standard: dihydroxybenzylamine

OTHER SUBSTANCES
Extracted: dopamine, epinephrine

KEY WORDS
adrenal; fetal

REFERENCE
Garciá,J.C.; Blanco,L.; McPherson,M.; Leiva,A.; Maciás,R. High-performance liquid chromatographic determination of norepinephrine, epinephrine and dopamine in human foetal adrenal gland, *J.Chromatogr.B*, **1994**, *656*, 77–80.

SAMPLE
Matrix: urine
Sample preparation: Acidify urine with 1% (v/v) 6 M HCl. 6-10 mL Swine urine or 1-2.5 mL rat urine, centrifuge at 4000 g for 30 min, add 200 ng 3,4-dihydroxybenzylamine hydrobromide and 15 mL 1g/L EDTA, adjust to pH 6.45-6.55 with HCl or NaOH. Add the mixture to a cation-exchange resin SPE cartridge (Bio-Rad), wash twice with 10 mL water and with 5 ml water, elute with 8 mL 10 g/L boric acid. Dilute boric acid eluate with an equal volume of mobile phase, inject a 60 μL aliquot. (Procedure for determining methoxycatecholamines is also described.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Kromasil C8
Mobile phase: MeOH:buffer 15:85 (Mobile phase was 300 mL MeOH, 1.5 mL 200 mg/mL 1-octanesulfonic acid, 100 mL 1 M sodium acetate, and about 1 L water. The pH was adjusted to pH 3.8 with citric acid and made up to 2 L with water.)
Flow rate: 0.6
Injection volume: 60
Detector: E, Bioanalytical Systems, glassy carbon electrode + 650 mV, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 7.28
Internal standard: 3,4-dihydroxybenzylamine hydrobromide (10.31)
Limit of detection: 40 pg

OTHER SUBSTANCES
Extracted: dopamine, epinephrine

KEY WORDS
pig; rat; SPE; pharmacokinetics

REFERENCE
Hay,M.; Mormède,P. Determination of catecholamines and methoxycatecholamines excretion patterns in pig and rat urine by ion-exchange liquid chromatography with electrochemical detection, *J.Chromatogr.B*, **1997**, *703*, 15–23.

SAMPLE
Matrix: urine
Sample preparation: Acidify urine by adding 1% 6 M HCl. 5 mL Acidified urine + 1 mL 7.5% disodium EDTA, adjust pH to 8.5 with 1 M NaOH, add 250 mg alumina (previously treated with 2 M HCl), shake for 5 min, decant the supernatant, wash the alumina three times with 5 mL portions of water. Place the alumina in a 4 mm ID glass column, elute with 250 mM acetic acid in water, collect 2.5 mL eluate, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 1000 × 2.1 Zipax SCX (DuPont)
Mobile phase: MeCN:50 mM NaH_2PO_4 5:95
Column temperature: 40
Flow rate: 0.8
Injection volume: 100
Detector: F ex 400 em 490 following post-column reaction. The column effluent mixed with the reagent pumped at 0.4 mL/min and the mixture flowed through a 10 m × 0.5 mm PTFE coil at 75 ± 0.1° to the detector. (Reagent was 500 mM borate buffer adjusted to pH 9.7 with NaOH.)

CHROMATOGRAM
Retention time: 5.5
Limit of quantitation: 0.25 ng

OTHER SUBSTANCES
Extracted: epinephrine

KEY WORDS
post-column reaction; SPE

REFERENCE
Nimura,N.; Ishida,K.; Kinoshita,T. Novel post-column derivatization method for the fluorimetric determination of norepinephrine and epinephrine, *J.Chromatogr.*, **1980**, *221*, 249–255.

SAMPLE
Matrix: urine
Sample preparation: 1 mL Urine + 2 mL water + 1 mL 500 μM EDTA in 1 mM HCl + 1 mL 2 M pH 8.5 phosphate buffer + 20 μL 100 μM 3,4-dihydroxybenzylamine + 50 mg alumina, vortex for 3 min, discard the supernatant, wash the alumina 3 times with 5 mL portions of water, elute by washing the alumina twice with 200 μL portions of 100 mM phosphoric acid for 30 s each time. Combine the acidic layers and add them to 560 μL 400 mM pH 9.0 borate buffer, add 20 μL 50 mM NaCN, add 20 μL 5 mM naphthalene-2,3-carboxaldehyde in MeOH, mix thoroughly, let stand at room temperature for 20 min, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm ODS-120T (Toyo Soda)
Mobile phase: MeCN:THF:10 mM pH 2.5 phosphate buffer 38:6:56
Flow rate: 1
Injection volume: 5
Detector: F ex 420 em 483

CHROMATOGRAM
Retention time: 18
Internal standard: 3,4-dihydroxybenzylamine (15.5)
Limit of detection: 20 fmole

OTHER SUBSTANCES
Extracted: dopamine

KEY WORDS
derivatization; SPE

REFERENCE
Kawasaki,T.; Higuchi,T.; Imai,K.; Wong,O.S. Determination of dopamine, norepinephrine, and related trace amines by prechromatographic derivatization with naphthalene-2,3-dicarboxaldehyde, *Anal.Biochem.*, **1989**, *180*, 279–285.

SAMPLE
Matrix: urine
Sample preparation: Add disodium EDTA and sodium metabisulfite to urine. 100 μL Urine + 2 mL water, vortex, add 1 mL reagent 1, add 5 mL reagent 2, shake vigorously for 2 min, centrifuge at 2000 g for 2 min, freeze in dry ice/acetone. Remove the organic layer and add it to 200 μL 80 mM acetic acid and 2 mL n-octanol saturated with acetic acid, shake vigorously for 2 min, centrifuge at 2000 g for 2 min, freeze in dry ice/acetone until the aqueous layer is just solid, remove the organic layer. Thaw out the aqueous layer and add it to 1 mL reagent 1 and 5 mL reagent 2, shake vigorously for 2 min, centrifuge at 2000 g for 2 min, freeze in dry ice/acetone. Remove the organic layer and add it to 200 μL 80 mM acetic acid and 2 mL n-octanol saturated with acetic acid, shake vigorously for 2 min, centrifuge at 2000 g for 2 min, freeze in dry ice/acetone until the aqueous layer is just solid, remove the organic layer. Thaw out the aqueous layer and add 100 μL Bicine buffer, 250 μL MeCN, and 100 μL 100 mM 1,2-diphenylethylenediamine in 100 mM HCl, vortex, add 20 μL 20 mM potassium ferricyanide, vortex, heat at 37° for 40 min, cool to room temperature, inject a 100 μL aliquot. (Prepare reagent 1 by dissolving 214 g ammonium chloride and 10 g disodium EDTA in 2 L water, adjust pH to 8.3-8.5 with concentrated ammonium hydroxide, add 4.0 g diphenylborate-ethanolamine complex, stir for several hours until a clear solution is obtained. Prepare reagent 2 by dissolving 2.5 g tetraoctylammonium bromide and 10 mL n-octanol (saturated with acetic acid) in 1 L n-heptane. Prepare Bicine buffer by dissolving 14.3 g Bicine (N,N-bis(2-hydroxyethyl)glycine) and 359 mg anhydrous sodium acetate in 45 mL water, stir overnight until dissolved, adjust pH to 7.30 with concentrated NaOH, make up to 50 mL with water. Note that concentration of 1,2-diphenylethylenediamine is not given in paper. Other authors have used 100 mM (J.Chromatogr. 1989, 487, 17; 1992, 574, 109; 1992, 583, 236).)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. A was MeCN:MeOH:50 mM pH 7.0 sodium acetate buffer 40:10:50. B was MeCN:MeOH:50 mM pH 7.0 sodium acetate buffer 50:10:40. A:B 75:25 for 1 min, to 10:90 over 7 min, return to initial conditions over 1 min.
Flow rate: 1
Injection volume: 100
Detector: F ex 365 em 418 (cutoff filter)

CHROMATOGRAM
Retention time: 3
Limit of detection: <0.4 nM

OTHER SUBSTANCES
Extracted: dopamine, epinephrine
Simultaneous: isoproterenol

KEY WORDS
derivatization; protect from light

REFERENCE
Moleman,P.; van Dijk,J. Determination of urinary norepinephrine and epinephrine by liquid chromatography with fluorescence detection and pre-column derivatization, *Clin.Chem.*, **1990**, *36*, 732–736.

SAMPLE
Matrix: urine

Sample preparation: Add 10-15 mL 6 M HCl to a 24 h volume of urine. 2 mL 3 M Tris buffer containing 30 mM EDTA + 500 μL 10 μM dihydrobenzylamide in 100 mM perchloric acid + 2 mL 3 M tris buffer containing 30 mM EDTA + 100 μL 5 M NaOH + 4 mL acidified urine, mix, add to a 1 mL SPE column containing 200 mg alumina (70-230 mesh-ASTM (Touzart et Matignon) at 3 mL/min, wash with 9 mL water at 12 mL/min, force through 0.5 mL air, elute with 1 mL 150 mM perchloric acid at 0.75 mL/min, mix eluate, inject a 100 μL aliquot. (The pH of the Tris buffer is such that the pH of the mixture applied to the SPE column is 7.75-8.0.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil 100/C18
Mobile phase: MeOH:buffer 36:64 (Buffer was 75 mM NaH_2PO_4, 0.15 mM EDTA, and 6 mM sodium heptanesulfonate, pH 3.96.)
Flow rate: 1.25
Injection volume: 100
Detector: F ex 280 em 310

CHROMATOGRAM
Retention time: 6.7
Internal standard: dihydrobenzylamide hydrobromide (9.6)
Limit of detection: 10 nM

OTHER SUBSTANCES
Extracted: dopamine, epinephrine
Simultaneous: levodopa, methyldopa

KEY WORDS
SPE

REFERENCE
Said,R.; Robinet,D.; Barbier,C.; Sartre,J.; Huguet,C. Fully automated high-performance liquid chromatographic assay for the analysis of free catecholamines in urine, *J.Chromatogr.*, **1990**, *530*, 11–18.

SAMPLE
Matrix: urine
Sample preparation: 100 μL Urine + 125 μL 218.6 nM α-methylnorepinephrine in 10 mM HCl + 1 mL 10 mM HCl + 1 mL reagent + 5 mL 4.6 mM tetraoctylammonium bromide in n-heptane: 1-octanol 99:1, shake for 2 min, centrifuge at 20° at 1000 g for 5 min, freeze in dry ice/acetone. Remove the organic phase and add it to 2 mL 1-octanol saturated with 80 mM acetic acid and 200 μL 80 mM acetic acid, shake, centrifuge at 20° at 1000 g for 5 min, freeze in dry ice/acetone. Discard the organic layer and add 1 mL 10 mM HCl to the aqueous layer, add 2 mL 1-octanol saturated with 80 mM acetic acid, add 150 μL 80 mM acetic acid, shake, centrifuge at 20° at 1000 g for 5 min, freeze in dry ice/acetone. Discard the organic layer and add 200 μL MeCN and 50 μL buffer to the aqueous layer, add 100 μL 100 mM 1,2-diphenylethylenediamine in 100 mM HCl, add 20 μL 20 mM potassium ferricyanide in water, heat at 37° in the dark for 1 h, inject a 50 μL aliquot. (Reagent was 8.9 mM diphenylborate-ethanolamine complex in 2 M pH 8.6 ammonia/ammonium chloride buffer containing 13.4 mM EDTA. Buffer was 1.75 M pH 7.05 bicine in water containing 1% EDTA. Recrystallize 1,2-diphenylethylenediamine from toluene:light petroleum (bp 60-80°) 10:90, dry overnight at 60°.)

HPLC VARIABLES
Column: 100 × 4.6 3 μm Cp MicroSpher C18 (Chrompack)
Mobile phase: MeCN:MeOH:50 mM pH 7.0 sodium acetate 40:8:50 (At the end of the day flush column with 60 mL MeCN:MeOH:water 70:10:20.)
Flow rate: 1
Injection volume: 50
Detector: F ex 350 em 480

CHROMATOGRAM
Retention time: 2
Internal standard: α-methylnorepinephrine (Janssen, Beerse, Belgium) (3)
Limit of quantitation: 5.3 nM

OTHER SUBSTANCES
Extracted: dopamine, epinephrine

KEY WORDS
derivatization; protect from light

REFERENCE
van der Hoorn,F.A.J.; Boomsma,F.; Man in 't Veld,A.J.; Schalekamp,M.A.D.H. Improved measurement of urinary catecholamines by liquid-liquid extraction, derivatization and high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.*, **1991**, *563*, 348–355.

SAMPLE
Matrix: urine
Sample preparation: Condition a 100 mg Bakerbond C-18 SPE cartridge with 2 mL MeOH and 2 mL buffer I. Heat urine at 50°, centrifuge, remove a 500 μL aliquot and add it to 1 mL buffer II (?), add 20 μL 860 ng/mL dihydroxybenzylamine, shake for 5 min, add a 1 mL aliquot to the SPE cartridge, wash with 2 mL buffer I, wash with 1 mL MeOH:buffer I 50:50, wash with 500 μL water, elute with 2 mL 1 M acetic acid, inject a 20 μL aliquot. (Buffer I was 200 mM ammonium chloride containing 0.05% EDTA and 0.4% tetrabutylammonium iodide, pH adjusted to 8.0 ± 0.1. Buffer II was 2 M ammonium chloride containing 0.5% EDTA and 1.2% tetrabutylammonium iodide, pH adjusted to 8.0 ± 0.1.)

HPLC VARIABLES
Column: 150 × 3 5 μm Separon SGX C-18 (Tessek)
Mobile phase: MeOH:buffer 5:95-7:93 (Buffer was 50 mM pH 3.0 ± 0.1 phosphate buffer containing 50 mM EDTA, 1 mM sodium octanesulfonate, and 1 mM NaCl.)
Injection volume: 20
Detector: E, AMOR 400 mV (SunChrom)

CHROMATOGRAM
Internal standard: dihydroxybenzylamine
Limit of detection: 3 ng/mL

OTHER SUBSTANCES
Extracted: dopamine, epinephrine

KEY WORDS
SPE

REFERENCE
Brandsteterova,E.; Krajnak,K.; Skacani,I. HPLC analysis of urinary catecholamines using affinity SPE procedure, *Pharmazie*, **1995**, *50*, 825–826.

SAMPLE
Matrix: urine
Sample preparation: Adjust urine to pH 3.0 with 6 M HCl, centrifuge at 1600 g for 10 min. 900 μL Supernatant + 100 μL 2.5 μM N-methyldopamine, mix, inject a 100 μL aliquot on to column A and elute to waste with mobile phase A, after 5 min elute the contents of column A on to column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 35 × 4 TSK-precolumn-CA 2 (Tosoh); B 150 × 4.6 mixed mode (C18/cation exchange) (Alltech)
Mobile phase: A 15 mM pH 6.0 citric acid/trisodium citrate buffer; B 200 mM pH 6.0 citric acid/trisodium citrate buffer
Column temperature: 35
Flow rate: 0.7
Injection volume: 100
Detector: E, Mitsubishi 8 channel, 150 mV

CHROMATOGRAM
Retention time: 10.5
Internal standard: N-methyldopamine (30 mV) (20)
Limit of detection: 1 nM

OTHER SUBSTANCES
Extracted: dopamine (30 mV), epinephrine (150 mV), metanephrine (380 mV), 3-methoxytyramine (340 mV), normetanephrine (380 mV), serotonin (150 mV)

KEY WORDS
column-switching

REFERENCE
Mashige,F.; Matsushima,Y.; Miyata,C.; Yamada,R.; Kanazawa,H.; Sakuma,I.; Takai,N.; Shinozuka,N.; Ohkubo,A.; Nakahara,K. Simultaneous determination of catecholamines, their basic metabolites and serotonin in urine by high-performance liquid chromatography using a mixed-mode column and an eight-channel electrochemical detector, *Biomed.Chromatogr.*, **1995**, *9*, 221–225.

SAMPLE
Matrix: urine
Sample preparation: Condition a 10 × 2 20 mg 15-25 μm PLRP-S polymer-based SPE cartridge (Spark Holland) with 1 mL MeOH, with 0.5 mL water, and with 1.5 mL 200 mM pH 8.5 ammonia/ammonium chloride buffer containing 0.05% EDTA. Collect 24 h urine with 10 mL 6 M HCl, final pH 1-3. Dilute 4-fold with buffer. Inject a 200 μL aliquot onto the SPE cartridge, wash with 1 mL 200 mM pH 8.5 ammonia/ammonium chloride buffer containing 0.05% EDTA, wash with 250 μL MeOH:200 mM pH 8.5 ammonia/ammonium chloride buffer 20:80, wash with water at 1 mL/min for 2.25 min. Elute the contents of the SPE cartridge onto column A with the mobile phase for 30 s then remove the SPE cartridge from the circuit, elute column A with mobile phase onto column B, after 1.25 min elute column A to waste with mobile phase and elute column B with mobile phase, monitor the effluent from column B. (Buffer was 2 M pH 8.5 ammonia/ammonium chloride containing 0.5% EDTA, 0.1% diphenylborate, and 18 ng/mL dihydroxybenzylamine.)

HPLC VARIABLES
Column: A 30 × 4.6 C18 (Brownlee); B 250 × 4.6 5 μm Ultrasphere IP C18
Mobile phase: MeCN:MeOH:buffer 15:8:100, apparent pH adjusted to 3.2 with 1.5 M orthophosphoric acid (Buffer was 50 mM KH_2PO_4 containing 1 mM sodium heptane sulphate and 0.07 mM EDTA.)
Flow rate: 0.8
Injection volume: 200
Detector: E, ESA Model 5100A, Model 5021 conditioning cell, Model 5011 analytical cell, oxidizing electrode +350 mV, screen electrode +100 mV, quantifying electrode -300 mV

CHROMATOGRAM
Retention time: 6
Internal standard: dihydroxybenzylamine (8)
Limit of detection: 1.3 ng/mL

OTHER SUBSTANCES
Extracted: epinephrine, dopamine

KEY WORDS
SPE; column-switching

REFERENCE
Pastoris,A.; Cerutti,L.; Sacco,R.; De Vecchi,L.; Sbaffi,A. Automated analysis of urinary catecholamines by high-performance liquid chromatography and on-line sample pretreatment, *J.Chromatogr.B*, **1995**, *664*, 287–293.

SAMPLE
Matrix: urine

Sample preparation: Acidify urine to pH 2.0-3.5 with 5 M HCl, centrifuge at 7000 g for 10 min, inject a 10-500 μL aliquot on to column A and elute to waste with mobile phase A, after 10 min backflush the contents of column A on to column B with mobile phase B, elute with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A nitrophenylboronic acid modified copolymer (U.S. Patent 4 767 529 (Chem.Abs. 1988, 108, 71698t)); B 53 × 4.6 1.5 μm MICRA NPS RP-18 (MICRA Scientific, Northbrook)
Mobile phase: A 20 mM $(NH_4)_2HPO_4$ containing 10 mM EDTA, adjusted to pH 8.7 with 25% ammonia solution; B 10 mM NaH_2PO_4 containing 0.1 mM dodecanesulfonic acid, adjusted to pH 2.5 with orthophosphoric acid
Flow rate: A 0.5; B from 0.2 to 0.5 over 2 min, maintain at 0.5
Injection volume: 10-500
Detector: F ex 275 em 330

CHROMATOGRAM

Retention time: 4
Limit of detection: 2.43 pmole
Limit of quantitation: 5.57 pmole

OTHER SUBSTANCES

Extracted: dopamine, epinephrine

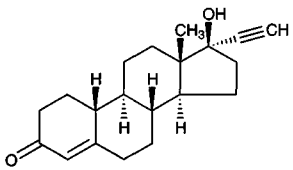
KEY WORDS

column-switching

REFERENCE

Rudolphi,A.; Boos,K.-S.; Seidel,D. Coupled-column HPLC analysis of free urinary catecholamines using restricted access affinity precolumn and micro-particulate nonporous silica analytical column, *Chromatographia*, **1995**, *41*, 645–650.

Norethindrone



Molecular formula: $C_{20}H_{26}O_2$
Molecular weight: 298.43
CAS Registry No.: 68-22-4, 51-98-9 (acetate)
Merck Index: 6790
Lednicer No.: 1 164, 165; 2 145

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30

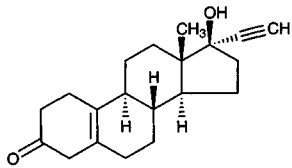
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 240.5

CHROMATOGRAM
Retention time: 24.038

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Norethynodrel



Molecular formula: $C_{20}H_{26}O_2$
Molecular weight: 298.43
CAS Registry No.: 68-23-5
Merck Index: 6791
Lednicer No.: 1 168

SAMPLE
Matrix: solutions

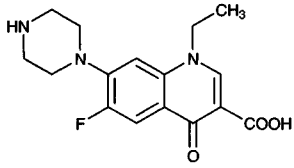
HPLC VARIABLES
Column: 50 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeOH:THF:water 10:20:70
Flow rate: 2
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 6.2 (norethynodrel acetate)

OTHER SUBSTANCES
Simultaneous: ethinyl estradiol, norethindrone, norethindrone acetate, norgestrel

REFERENCE
Supelco Catalog, **1994**, p. 779.

Norfloxacin



Molecular formula: $C_{16}H_{18}FN_3O_3$
Molecular weight: 319.34
CAS Registry No.: 70458-96-7, 100587-52-8 (norfloxacin succinil)
Merck Index: 6793
Lednicer No.: 4 141, 143

SAMPLE
Matrix: aqueous humor, blood, tissue

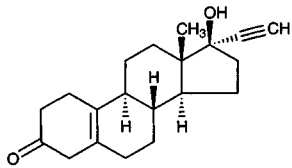
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 240.5

CHROMATOGRAM
Retention time: 24.038

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Norethynodrel



Molecular formula: $C_{20}H_{26}O_2$
Molecular weight: 298.43
CAS Registry No.: 68-23-5
Merck Index: 6791
Lednicer No.: 1 168

SAMPLE
Matrix: solutions

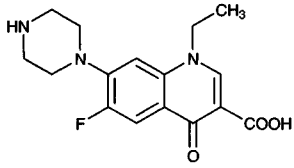
HPLC VARIABLES
Column: 50 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeOH:THF:water 10:20:70
Flow rate: 2
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 6.2 (norethynodrel acetate)

OTHER SUBSTANCES
Simultaneous: ethinyl estradiol, norethindrone, norethindrone acetate, norgestrel

REFERENCE
Supelco Catalog, **1994**, p. 779.

Norfloxacin



Molecular formula: $C_{16}H_{18}FN_3O_3$
Molecular weight: 319.34
CAS Registry No.: 70458-96-7, 100587-52-8 (norfloxacin succinil)
Merck Index: 6793
Lednicer No.: 4 141, 143

SAMPLE
Matrix: aqueous humor, blood, tissue

Sample preparation: Homogenize sample in 1 M HCl, add MeCN, centrifuge, add an equal volume of dichloromethane, centrifuge, inject a 50-100 μL aliquot of the aqueous supernatant.

HPLC VARIABLES
Guard column: Corasil C18
Column: μBondapak C18
Mobile phase: MeCN:25 mM phosphoric acid adjusted to pH 3.0 with tetrabutylammonium hydroxide 9.5:90.5
Flow rate: 1.5
Injection volume: 50-100
Detector: F ex 272 em 370 (cut-off)

CHROMATOGRAM
Retention time: 6.5
Limit of detection: 0.1 ng/g

KEY WORDS
serum; rabbit; human; cornea

REFERENCE
Bron,A.M.; Péchinot,A.; Garcher,C.; Guyonnet,G.; Kazmierczak,A. Ocular penetration of topically applied norfloxacin 0.3% in the rabbits and in humans, *J.Ocul.Pharmacol.*, **1992**, *8*, 241–246.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 50 μL 400 μg/mL IS in water, vortex for 30 s. Add 500 μL MeCN, vortex for 1 min. Centrifuge at 6000 rpm for 10 min. Evaporate the supernatant to 200 μL at 40° under a stream of nitrogen, vortex for 30 s. Inject a 30-80 μL aliquot.

HPLC VARIABLES
Column: 100 × 8.0 4 μm Radial-pak Novapak C18
Mobile phase: MeCN:buffer 14:86 (Buffer was 2 g citric acid, 2 g sodium acetate, and 1 mL triethylamine in 1 L water.)
Flow rate: 2.5
Injection volume: 30-80
Detector: F ex 330 em 440

CHROMATOGRAM
Retention time: 4.0
Internal standard: acebutolol (7.4)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: pefloxacin
Simultaneous: ciprofloxacin, lomefloxacin, ofloxacin

KEY WORDS
serum; pharmacokinetics

REFERENCE
Abanmi,N.; Zaghloud,I.; El Sayed,N.; al-Khamis,K.I. Determination of pefloxacin and its main active metabolite in human serum by high-performance liquid chromatography, *Ther.Drug Monit.*, **1996**, *18*, 158–163.

SAMPLE
Matrix: blood
Sample preparation: Add 20 μL MeOH:0.1% trifluoroacetic acid 15:85 and 5 μL (sic) MeCN to 300 μL plasma. Centrifuge at 600 g for 10 min. Evaporate the supernatant under nitrogen at 40° for 30 min. Reconstitute the residue in 200 μL MeOH:0.1% trifluoroacetic acid 15:85. Inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 12.5 × 4 Zorbax RX-C18
Column: 150 × 4.6 5 μm Zorbax SB-C8
Mobile phase: MeCN:water:trifluoracetic acid 19:81:0.02
Flow rate: 1
Injection volume: 50
Detector: UV 279

CHROMATOGRAM
Retention time: 3.8-3.9
Internal standard: norfloxacin

OTHER SUBSTANCES
Extracted: ciprofloxacin, enrofloxacin

KEY WORDS
cat; plasma; norfloxacin is IS

REFERENCE
Kordick,D.L.; Papich,M.G.; Breitschwerdt,E.B. Efficacy of enrofloxacin or doxycycline for treatment of Bartonella henselae or Bartonella clarridgeiae infection in cats, *Antimicrob.Agents Chemother.*, **1997**, *41*, 2448–2455.

SAMPLE
Matrix: blood
Sample preparation: Filter 1 mL plasma using a micropartition system (MPS-1, Amicon, MA) while centrifuging at 2000 g for 20 min at 10°, inject an aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb ODS-2 endcapped
Mobile phase: MeCN:buffer 11:89 containing 5 mM tetrabutylammonium sulfate, adjusted to pH 2.5 with 1 M NaOH (Buffer was 100 mM citric acid containing 200 mM ammonium perchlorate.)
Column temperature: 37
Flow rate: 1.2
Detector: UV 272

CHROMATOGRAM
Retention time: 8.07
Internal standard: pipemidic acid (4.14)

OTHER SUBSTANCES
Simultaneous: pefloxacin

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Zlotos,G.; Bücker,A.; Kinzig-Schippers,M.; Sorgel,F.; Holzgrabe,U. Plasma protein binding of gyrase inhibitors, *J.Pharm.Sci.*, **1998**, *87*, 215–220.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Plasma + 1 mL 100 mM pH 7.0 K_2HPO_4 adjusted to pH 7.0 with 85% orthophosphoric acid + 100 μL 300 μg/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 μL MeOH:50 mM NaOH 2:1, vortex, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm 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: 6.5
Internal standard: nalidixic acid (5.0)

OTHER SUBSTANCES
Extracted: fenbufen, felbinac
Interfering: ofloxacin, 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: 500 μL Serum + 250 μL 10% trichloroacetic acid, vortex for 10 s, centrifuge at >700 g for 10 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 8 μBondapak 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 278 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: 100 μL Plasma + 100 μL pH 7.4 phosphate buffer + 50 μL 10 μg/mL β-hydroxypropyltheophylline in pH 7.4 phosphate buffer + 5 mL chloroform:isopropanol 80:20, 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 Sherisorb 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 KH_2PO_4 + 5.94 g $Na_2HPO_4.2H_2O$ + 1.49 g tetrabutylammonium hydrogen sulfate per L.)
Flow rate: 1.3
Injection volume: 25
Detector: UV 280

CHROMATOGRAM
Retention time: 5.5
Internal standard: β-hydroxypropyltheophylline
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Simultaneous: theophylline, enoxacin, ciprofloxacin

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: 150 μL Plasma + 10 μL 100 μg/mL enoxacin in water + 75 μL 10% trichloroacetic acid + 600 μL chloroform, vortex for 5 min, centrifuge at 13800 g for 10 min. Remove 500 μL of the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 150 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 80 × 4.6 5 μm Zorbax C8
Mobile phase: MeOH:0.01% trifluoroacetic acid 25:75
Flow rate: 1.2
Injection volume: 50
Detector: F ex 280 em 418

CHROMATOGRAM
Retention time: 6.9
Internal standard: enoxacin (5.8)
Limit of quantitation: 25 ng/mL

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Hussain,M.S.; Chukwumaeze-Obiajunwa,V.; Micetich,R.G. Sensitive high-performance liquid chromatographic assay for norfloxacin utilizing fluorescence detection, *J.Chromatogr.B*, **1995**, *663*, 379–384.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 1 mL 2.6 μg/mL N-ethylnorfloxacin in chloroform, vortex, centrifuge at 11000-12000 g for 1 min. Remove the organic layer and evaporate it to dryness under a stream of air at 60°, reconstitute the residue in 200 μL mobile phase, inject a 25-50 μL aliquot.

HPLC VARIABLES
Column: 40 × 3.2 3 μm RP-18 Spheri-3
Mobile phase: MeCN:10 mM pH 2.5 NaH_2PO_4 containing 1 mM triethylamine 11:89
Column temperature: 30
Flow rate: 1
Injection volume: 25-50
Detector: UV 279

CHROMATOGRAM
Retention time: 1.9
Internal standard: N-ethylnorfloxacin (Heat 3.2 g norfloxacin, 1.5 g triethylamine, and 12-20 mmoles ethyl iodide in 40 mL DMF at 80-90° with stirring for 2 h, concentrate to dryness,

recrystallize from chloroform/benzene to give N-ethylnorfloxacin (mp 251-3°) (J.Med.Chem. 1980, 23, 1358)) (2.9)
Limit of detection: 20 ng/mL

KEY WORDS
serum

REFERENCE
Wallis,S.C.; Charles,B.G.; Gahan,L.R. Rapid and economical high-performance liquid chromatographic method for the determination of norfloxacin in serum using a microparticulate C18 guard cartridge, *J.Chromatogr.B*, **1995**, *674*, 306–309.

SAMPLE
Matrix: blood, dialysate
Sample preparation: 100 μL Plasma or dialysate + 400 μL MeOH, vortex, centrifuge, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: strong cation exchange
Mobile phase: MeCN:100 mM pH 3 citrate buffer 20:80
Detector: F ex 278 em 440

CHROMATOGRAM
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: pefloxacin

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Rose,T.F.; Bremner,D.A.; Collins,J.; Ellis-Pegler,R.; Isaacs,R.; Richardson,R.; Small,M. Plasma and dialysate levels of pefloxacin and its metabolites in CAPD patients with peritonitis, *J.Antimicrob.Chemother.*, **1990**, *25*, 657–664.

SAMPLE
Matrix: blood, formulations
Sample preparation: Blood. Centrifuge 200 μL fresh blood at 3000 rpm for 10 min. Inject an aliquot of the plasma. Formulations. Completely dissolve 50 mg sample in 20 mL MeOH, sonicate. Filter insoluble material and adjust filtrate to 50 mL with MeOH. Inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 4.6 Cosmosil 5C18 (Nacalai Tesque, Japan)
Mobile phase: MeCN:solution 1:6.5 (Solution was 1 g sodium acetate trihydrate, 2 g citric acid monohydrate and 1 mL triethylamine in 1 L water (?).)
Column temperature: 40
Flow rate: 1.5
Injection volume: 100
Detector: UV 277

CHROMATOGRAM
Internal standard: p-nitrophenylacetic acid

KEY WORDS
freeze-dried formulations; plasma; rat; egg albumin; olive oil

REFERENCE
Tsuji,Y.; Kakegawa,H.; Miyataka,H.; Nishiki,M.; Matsumoto,H.; Satoh,T. Pharmaceutical properties of freeze-dried formulations of egg albumin, several drugs and olive oil, *Biol.Pharm.Bull.*, **1996**, *19*, 636–640.

SAMPLE

Matrix: blood, tissue

Sample preparation: Plasma. Mix 500 μL plasma with 5 μg IS, add 4 mL dichloromethane and 100 μL pH 7.4 phosphate buffer, agitate for 10 min, centrifuge at 5300 g for 10 min. Collect 3.5 mL organic phase. Add 4 mL dichloromethane to the aqueous phase again, agitate, centrifuge. Combine the organic phases, evaporate at 60°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot. Tissue. Mix 500 μL epiploic-fat and 4 mL dichloromethane and keep at 4°, add 5 μg IS, mix by using an automatic grinder (Ultra Turrax, Ika-Werk, Stauffen, Germany). Collect the mixture, centrifuge at 5300 g for 10 min. Add 4 mL 100 mM NaOH to the dichloromethane, agitate for 10 min, centrifuge at 5300 g for 5 min. Eliminate the organic phase, adjust the aqueous phase to pH 7.4 with concentrated trichloroacetic acid, add 4 mL dichloromethane, agitate for 10 min and centrifuge at 5300 g for 5 min. Evaporate the organic phase at 60°. Reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 25 × 4 5 μm 100 RP-18 Lichrosphere
Column: 125 × 4 5 μm 100 RP-18 endcapped Lichrosphere
Mobile phase: MeCN:pH 4.8 citrate buffer 85:15
Flow rate: 1
Injection volume: 20
Detector: F ex 330 em 418

CHROMATOGRAM

Internal standard: 4844P (pefloxacin analog)
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES

Extracted: pefloxacin

KEY WORDS

epiploic-fat; plasma; pharmacokinetics; fat

REFERENCE

Jacoberger,B.; Ubeaud,G.; Freys,G.; Pottecher,T.; Jung,L.; Koffel,J.C. Concentrations of pefloxacin in plasma and tissue after administration as surgical prophylaxis, *Antimicrob.Agents Chemother.*, **1998**, *42*, 425–427.

SAMPLE

Matrix: blood, tissue

Sample preparation: Tissue. Homogenize 100-250 mg tissue with 5 mL 500 mM pH 7.0 phosphate buffer, remove a 1 mL aliquot, add 100 μL 10 μg/mL IS, mix, add 10 mL chloroform: isopentanol 90:10, shake for 15 min, centrifuge , repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of air at 60°, reconstitute the residue in 100 μL 1% ammonia, inject a 25 μL aliquot. Plasma. 250-500 μL Plasma + 100 μL 10 μg/mL IS + 1 mL 500 mM pH 7.0 sodium phosphate buffer, mix, add 10 mL chloroform:isopentanol 90:10, shake for 15 min, centrifuge, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of air at 60°, reconstitute the residue in 100 μL 1% ammonia, inject a 25 μL aliquot.

HPLC VARIABLES

Column: 100 × 5 10 μm Nucleosil C18
Mobile phase: MeCN:water 15:85 containing 2 g/L sodium acetate trihydrate, 2 g/L citric acid monohydrate, and 1 mL/L triethylamine
Flow rate: 2
Injection volume: 25
Detector: F ex 330 em 440

CHROMATOGRAM

Retention time: 2.8
Internal standard: 1-allyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-methyl-1-piperazinyl)quinoline-3-carboxylic acid (Roger Bellon Laboratories) (6.6)
Limit of detection: 30 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, pefloxacin

KEY WORDS
plasma; prostate

REFERENCE
Montay,G.; Tassel,J.P. Improved high-performance liquid chromatographic determination of pefloxacin and its metabolite norfloxacin in human plasma and tissue, *J.Chromatogr.*, **1985**, *339*, 214–218.

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 μL aliquot of supernatant. Urine. Dilute urine 1:10 to 1:100 with 30 mM phosphoric acid, centrifuge, inject a 20 μL aliquot of supernatant. Tissue (lung, gut). Cut tissue with a scalpel, homogenize with 1-3 mL buffer, centrifuge at 9600 g for 5 min three times, inject a 20 μL aliquot. Tissue (chondral). Cut tissue with a scalpel, homogenize with 3-6 mL buffer in an ice bath for 2-3 min, centrifuge at 9600 g for 5 min four or five times, inject a 100 μL aliquot. Dilute human pleural samples with buffer, centrifuge, inject a 20 μL aliquot. (Buffer was 66.6 mM K_2HPO_4 adjusted to pH 7.40 with KH_2PO_4.)

HPLC VARIABLES
Column: 200 × 4 5 μm Nucleosil C18
Mobile phase: MeOH: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: 4
Limit of detection: 2.5 ng/mL

OTHER SUBSTANCES
Simultaneous: ofloxacin, ciprofloxacin

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 500 μL MeCN to 500 μL plasma or urine diluted with water, vortex vigorously for 20 s, centrifuge at 4000 rpm for 2 min, mix a 250 μL aliquot of the supernatant with 500 μL 100 mM perchloric acid containing 20 mM triethylamine, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 80 × 4.0 3 μm Nucleosil 3C18
Mobile phase: MeOH:100 mM perchloric acid containing 20 mM triethylamine 30:70
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: F ex 300 em 450

CHROMATOGRAM
Retention time: 2.09

OTHER SUBSTANCES
Noninterfering: acyclovir, ampicillin, amoxicillin, clavulanic acid, doxycycline, erythromycin, lansoprazole, metronidazole, minocycline, omeprazole, penicillin V, trimethoprim

KEY WORDS
plasma

REFERENCE
Mascher,H.J.; Kikuta,C. Determination of norfloxacin in human plasma and urine by high-performance liquid chromatography and fluorescence detection, *J.Chromatogr.A*, **1998**, *812*, 381–385.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Wash a C18 Sep-Pak cartridge with 1 mL 4% methanolic phosphoric acid and 10 mL water. Add 1 mL plasma to the cartridge, wash with 6 mL water, elute with 1 mL 4% methanolic phosphoric acid then with 1 mL water. Combine the eluates, make up to 2 mL with water, inject a 50 μL aliquot. Urine. Dilute 100 μL urine with 900 μL mobile phase and directly inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: μBondapak C18-Corasil
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:MeCN:water 300:50:700 + 1.74 g K_2HPO_4 + 20 mg sodium heptanesulfonate, pH adjusted to 3 with phosphoric acid
Flow rate: 2
Injection volume: 10 (urine), 50 (plasma)
Detector: F ex 285 em 440

CHROMATOGRAM
Retention time: 4
Limit of detection: 500 ng/mL (urine), 20 ng/mL (plasma)

KEY WORDS
plasma; SPE

REFERENCE
Gutzler,F.; de Vries,J.X. Bestimmung von Norfloxacin in Plasma und Urin durch Hochdruckflüssigkeitschromatographie, *Fortschr.Antimikr.Antineoplast.Chemother.*, **1984**, *3*, 673–677.

SAMPLE
Matrix: blood, urine
Sample preparation: Dilute with one or more volumes of water, filter (0.6 μm)

HPLC VARIABLES
Column: 200 × 4 5 μm 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: 2.8
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Noninterfering: trimethoprim, sulfamethoxazole, netilmicin, metronidazole, penicillin G, cloxacillin, doxycycline, cefuroxime, erythromycin, salicylic acid, digoxin, furosemide, acetaminophen, prednisolone, warfarin, dextropropoxyphene
Interfering: ciprofloxacin

KEY WORDS
serum

REFERENCE
Nilsson-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: Plasma. 250 μL Plasma + 250 μL 10 μg/mL IS in water + 750 μL MeOH, stir, centrifuge at 2000 rpm for 5 min, inject a 50 μL aliquot of the supernatant. Urine. 500 μL Urine + 3.5 mL 8 μg/mL IS in water, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 Nucleosil C8
Mobile phase: MeCN:water:triethylamine:formic acid 11:87.5:0.1:1 containing 0.2% sodium acetate and 0.1% formic acid
Flow rate: 1
Injection volume: 50
Detector: F ex 280 em 450

CHROMATOGRAM
Internal standard: 1-ethyl-6-chloro-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid (RP 41983)
Limit of quantitation: 500 ng/mL (urine), 100 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: pefloxacin

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Humbert,G.; Brumpt,I.; Montay,G.; Le Liboux,A.; Frydman,A.; Borsa-Lebas,F.; Moore,N. Influence of rifampin on the pharmacokinetics of pefloxacin, *Clin.Pharmacol.Ther.*, **1991**, *50*, 682–687.

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, 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 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 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 280 em 445

CHROMATOGRAM
Retention time: 11.7
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: 0.2 ng/mL

OTHER SUBSTANCES
Simultaneous: ciprofloxacin, ofloxacin (determine at 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: perfusate

HPLC VARIABLES
Column: 250 × 4.6 Spheris C18 (Phase Separations)
Mobile phase: MeCN:15 mM tetrabutylammonium iodide 5:95
Flow rate: 1
Detector: UV 275

CHROMATOGRAM
Internal standard: pipemidic acid

REFERENCE
Lin,H.-H.; Hsu,L.-R.; Wu,P.-C.; Tsai,Y.-H. Increased norfloxacin skin permeability for fatty alcohol propylene glycol (FAPG) ointment by optimized process of preparation: Behavior of stearic acid in stratum corneum lipids, *Biol.Pharm.Bull.*, **1995**, *18*, 1560–1565.

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: 5.9

OTHER SUBSTANCES
Simultaneous: ciprofloxacin, 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: 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 280

CHROMATOGRAM
Retention time: 8.6

OTHER SUBSTANCES
Simultaneous: ciprofloxacin, 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
Sample preparation: Filter (0.45 μm) a solution in MeCN:water 10:90, inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: MeCN:buffer 7:93 (Buffer was 25 mM phosphoric acid adjusted to pH 3.89 with 100 mM tetrabutylammonium hydroxide.)
Flow rate: 1
Injection volume: 10
Detector: UV 280

CHROMATOGRAM
Retention time: 8.8

OTHER SUBSTANCES
Simultaneous: ciprofloxacin, enoxacin, fleroxacin, ofloxacin (UV 295), pipemidic acid

REFERENCE
Barbosa,J.; Bergés,R.; Sanz-Nebot,V. Solvatochromic parameter values and pH in aqueous-organic mixtures used in liquid chromatography. Prediction of retention of a series of quinolones, *J.Chromatogr.A*, **1996**, *719*, 27–36.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax) eye tissue with 3 mL 50 mM pH 5.8 sodium phosphate-citrate buffer and IS, centrifuge. Add the supernatant to 7 mL chloroform, agitate, centrifuge at 1000 g for 10 min. Remove the lower organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL mobile phase, inject a 5-20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 3 μm Nucleosil C8
Mobile phase: MeCN:water 26:74 containing 2 g/L sodium acetate trihydrate, 2 g/L citric acid monohydrate, 4 mL/L triethylamine, and 2 mL/L formic acid, pH 4.8
Flow rate: 1
Injection volume: 5-20
Detector: UV 280

CHROMATOGRAM
Retention time: 2.19
Internal standard: 1-allyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-methyl-1-piperazinyl)quinoline-3-carboxylic acid (?) 4662 P (Roger-Bellon Laboratories) (2.95)
Limit of detection: 5 ng

OTHER SUBSTANCES
Extracted: pefloxacin

KEY WORDS
rabbit; eye; pharmacokinetics

REFERENCE
Cochereau-Massin,I.; Bauchet,J.; Faurisson,F.; Vallois,J.M.; Lacombe,P.; Pocidalo,J.J. Ocular kinetics of pefloxacin after intramuscular administration in albino and pigmented rabbits, *Antimicrob.Agents Chemother.*, **1991**, *35*, 1112–1115.

SAMPLE
Matrix: urine
Sample preparation: Dilute with water, inject an aliquot.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeOH:MeCN:100 mM pH 5.75 phosphate buffer 24.1:2.6:73.3
Flow rate: 1
Detector: F ex 275 em 415

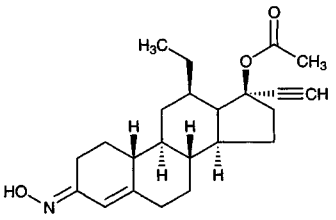
CHROMATOGRAM
Limit of quantitation: 3.13 μg/mL

OTHER SUBSTANCES
Extracted: pefloxacin

KEY WORDS
pharmacokinetics

REFERENCE
Jaehde,U.; Sörgel,F.; Stephan,U.; Schunack,W. Effect of an antacid containing magnesium and aluminum on absorption, metabolism, and mechanism of renal elimination of pefloxacin in humans, *Antimicrob.Agents Chemother.*, **1994**, *38*, 1129–1133.

Norgestimate



Molecular formula: $C_{23}H_{31}NO_3$
Molecular weight: 369.50
CAS Registry No.: 35189-28-7
Merck Index: 6796

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 μL MeOH, inject an aliquot.

HPLC VARIABLES
Guard column: 18 mm long (Brownlee)
Column: 300 × 3.6 10 μm μBondapak C18
Mobile phase: MeOH:water 80:20
Flow rate: 1
Detector: UV 254 or RIA

CHROMATOGRAM
Retention time: 9.3, 9.8 (syn and anti)

OTHER SUBSTANCES
Extracted: metabolites, norgestrel

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: culture 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 × 3.9 Techopak 10 C18 (HPLC Technology)
Mobile phase: MeOH:water 70:30
Flow rate: 1.5
Detector: UV 240, radioactivity

CHROMATOGRAM
Retention time: 13.5, 15.5 (racemate)

OTHER SUBSTANCES
Extracted: metabolites, norgestrel

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: 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: ethinylestradiol, degradation products

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: 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 incubation with 5 mL diethyl ether, proceed as before.

HPLC VARIABLES
Guard column: on-line guard column
Column: 100 × 8 μBondapak radial compression module
Mobile phase: MeOH:water 70:30
Flow rate: 1.5
Injection volume: 100
Detector: UV 240

CHROMATOGRAM
Retention time: 17.5

OTHER SUBSTANCES
Simultaneous: 3-ketonorgestimate, 17-deacetylnorgestimate, norgestrel

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: 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: levonorgestrel

REFERENCE
Killinger,J.; Hahn,D.W.; Phillips,A.; Hetyei,N.S.; McGuire,J.L. The affinity of norgestimate for uterine progestogen 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

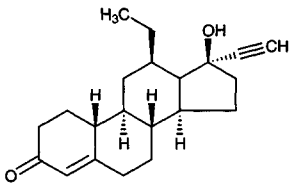
CHROMATOGRAM
Retention time: 15, 18 (syn and anti)

OTHER SUBSTANCES
Simultaneous: norgestrel, 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.

Norgestrel



Molecular formula: $C_{21}H_{28}O_2$
Molecular weight: 312.45
CAS Registry No.: 797-63-7, 797-64-8 ((-) form), 6533-00-2
Merck Index: 6797
Lednicer No.: 1 167, 2 151, 3 84

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 241.7

CHROMATOGRAM
Retention time: 21.565

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: 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, add 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: solutions

HPLC VARIABLES
Column: 200 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:water 50:50
Column temperature: 37
Flow rate: 2.0
Detector: UV 243

CHROMATOGRAM
Retention time: 4.07

REFERENCE
Kim,D.-D.; Kim,J.L.; Chien,Y.W. Mutual hairless rat skin permeation-enhancing effect of ethanol/water system and oleic acid, *J.Pharm.Sci.*, **1996**, *85*, 1191–1195.

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.; Hetyei,N.S.; McGuire,J.L. The affinity of norgestimate for uterine progestogen receptors and its direct action on the uterus, *Contraception*, **1985**, *32*, 311–319.

SAMPLE
Matrix: solutions
Sample preparation: Direct injection

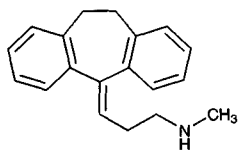
HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil C-18 ODS-3
Mobile phase: MeCN:water 50:50
Flow rate: 2
Detector: UV 243

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.

Nortriptyline



Molecular formula: $C_{19}H_{21}N$
Molecular weight: 263.38
CAS Registry No.: 72-69-5, 894-71-3 (HCl)
Merck Index: 6812
Lednicer No.: 1 151

SAMPLE
Matrix: bile, blood, gastric contents, tissue, urine
Sample preparation: Chop 5-g tissue and homogenize (Ultra Turrax T25) at 8500, 9500, 13500, 20500, and 24000 rpm for 1 min each. Add homogenate to 20 mL water. Dilute blood, urine, gastric contents, and bile four times with water. Mix 4 mL sample with 100 μL 400 μg/mL IS and 2 mL 500 mM NaOH, vortex briefly, add 4 mL heptane:isoamyl alcohol 98.5:1.5 and mix for 15 min (Spiramix 10, Denley, UK). Separate the organic layer, add 4 mL heptane:isoamyl alcohol 98.5:1.5 to extraction sample, mix. Combine the organic layers and extract them with 2 mL 50 mM sulfuric acid. Make the acid layer alkaline with 1 mL 1.0 M pH 9.0 carbonate/bicarbonate buffer and mix with 2 mL toluene:isoamyl alcohol 85:15 for 15 min. Evaporate the organic layer to dryness, reconstitute the residue in 100 μL MeOH and inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Apex II ODS

Column: 150 × 4.6 5 μm Apex II OD
Mobile phase: MeCN:pH 3 phosphate buffer:n-nonylamine 40-50:60:0.12
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 3.70
Internal standard: doxepin (2.99)

OTHER SUBSTANCES
Extracted: amitriptyline

KEY WORDS
liver; lung; muscle; urine; pericardial fluid

REFERENCE
Pounder,D.J.; Adams,E.; Fuke,C.; Langford,A.M. Site to site variability of postmortem drug concentrations in liver and lung, *J.Forensic Sci.*, **1996**, *41*, 927–932.

SAMPLE
Matrix: blood
Sample preparation: Add 250 μL 2 M sodium carbonate to 500 μL plasma. Add 100 μL 1 μg/mL IS in MeOH, extract with 10 mL n-hexane. Shake for 30 min and centrifuge at 3000 g for 10 min. Cool in a dry ice-acetone bath. Add 200 μL 0.3% phosphoric acid to upper organic layer. Shake for 10 min and centrifuge at 3000 g for 10 min. Separate the organic layer. Inject a 100 μL aliquot of the acidic aqueous layer.

HPLC VARIABLES
Column: 250 × 4.6 5 μm C18 Symmetry (Waters Millipore, USA)
Mobile phase: MeCN:67 mM potassium phosphate buffer adjusted to pH 3.0 with phosphoric acid 35:65 (After each chromatographic session wash the column with 200 mL MeCN:water 50:50.)
Flow rate: 1.2
Injection volume: 100
Detector: UV 226, UV 254, UV 400

CHROMATOGRAM
Retention time: 10.45
Internal standard: clovoxamine (6.5)
Limit of quantitation: 5 ng/mL (UV 226, UV 400 nm); 7 ng/mL (UV 254)

OTHER SUBSTANCES
Extracted: metabolites, amitriptyline, clomipramine, desipramine, fluoxetine imipramine, maprotiline
Simultaneous: amineptine, carbamazepine, chlordiazepoxide, chlorpromazine, clonazepam, clorazepate, clozapine, cyamemazine, desmethylmaproptiline, desmethylvenlafaxine, doxepin, flunitrazepam, fluvoxamine, haloperidol, levomepromazine, lorazepam, loxapine, mianserine, sulpiride, trimipramine, venlafaxine, viloxazine, zolpidem, zopiclone
Noninterfering: diazepam, valproic acid

KEY WORDS
plasma

REFERENCE
Aymard,G.; Livi,P.; Pham,Y.T.; Diquet,B. Sensitive and rapid method for the simultaneous quantification of five antidepressants with their respective metabolites in plasma using high-performance liquid chromatography with diode-array detection, *J.Chromatogr.B*, **1997**, *700*, 183–189.

SAMPLE
Matrix: blood

Sample preparation: Condition a 1 mL 30 mg Oasis HLB SPE cartridge with 1 mL MeOH and 1 mL water. Acidify (?) mL serum with 20 μL phosphoric acid, vortex for 5 s, add to the SPE cartridge, wash with 1 mL MeOH :water 5:95, elute with 1 mL MeOH. Evaporate the eluate to dryness at 40° under a stream of nitrogen. Reconstitute the residue with 200 μL MeOH:20 mM pH 7 phosphate buffer 20:80, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 3.9 Sentry
Column: 150 × 3.9 5 μm Symmetry C18 (Waters)
Mobile phase: MeOH:20 mM pH 7 potassium phosphate 70:30
Column temperature: 35
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 8
Internal standard: nordoxepin (4.9)

OTHER SUBSTANCES
Simultaneous: amitriptyline, doxepin

KEY WORDS
pig; serum; SPE

REFERENCE
Cheng,Y.-F.; Phillips,D.J.; Neue,U.; Bean,L. Solid-phase extraction for the determination of tricyclic antidepressants in serum using a novel polymeric extraction sorbent, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2461–2473.

SAMPLE
Matrix: blood, microsomal incubations
Sample preparation: Vortex 1 mL plasma or microsomal incubation with 200 μL 1 μg/mL desipramine and 100 μL 5 M NaOH for 10 s, add 5 mL butan-1-ol:hexane 2:98, vortex for 1 min, centrifuge at 2000 g and 4° for 5 min, evaporate the organic phase to dryness at 40° using a vacuum vortex evaporator, reconstitute the residue in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 5 μm Nova-Pak C18
Mobile phase: MeCN:buffer 30:70 (Buffer was water containing 1% triethylamine, adjusted to pH 3 with orthophosphoric acid.)
Flow rate: 2
Injection volume: 50
Detector: UV 240

CHROMATOGRAM
Retention time: 8.5
Internal standard: desipramine (6.3)
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES
Extracted: amitriptyline
Noninterfering: diazepam, furafylline, hydroxyamitriptyline, hydroxynortriptyline, quinidine, mephenytoin, triacetyloleandomycin
Interfering: ketoconazole

KEY WORDS
human; liver; rat; plasma

REFERENCE
Ghahramani,P.; Lennard,M.S. Quantitative analysis of amitriptyline and nortriptyline in human plasma and liver microsomal preparations by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *685*, 307–313.

SAMPLE
Matrix: blood, tissue, urine
Sample preparation: Serum, urine. 500 μL Serum or urine + 100 μL 2 μg/mL diazepam + 200 μL 20% sodium carbonate + 500 μL water + 3 mL n-hexane:isoamyl alcohol 98.5:1.5, mix for 2 min, centrifuge at 1200 g for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, inject a 10 μL aliquot. Tissue. Homogenize 1 g sample with 9 mL 100 mM HCl and 100 μL 20 μg/mL diazepam, centrifuge at 15000 g for 10 min. Add 500 μL 20% sodium carbonate and 4 mL n-hexane:isoamyl alcohol 98.5:1.5 to 1 mL of the supernatant, mix for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, filter by microconcentrator (Microcon-30, Grace). Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 2 μm TSK gel Super-Octyl (A) or 100 × 4.6 5 μm Hypersil MOS-C8 (B), (Yokogawa, Japan)
Mobile phase: MeOH:20 mM pH 7 KH_2PO_4 60:40
Flow rate: 0.6
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 7.0 (A), 9.2 (B)
Internal standard: diazepam (4.4, A)
Limit of quantitation: 50 ng/mL(serum, urine), 500 ng/mL (tissue)

OTHER SUBSTANCES
Extracted: amitriptyline, amoxapine, clomipramine, desipramine, dothiepin, doxepin, imipramine, maprotiline, melitracen, mianserin
Noninterfering: barbital, carbamazepine, ethosuximide, hexobarbital, lofepramine, pentobarbital, phenobarbital, phenytoin, primidone, sulpiride, trimethadione, trimipramine

KEY WORDS
serum; brain; liver

REFERENCE
Tanaka,E.; Terada,M.; Nakamura,T.; Misawa,S.; Wakasugi,C. Forensic analysis of eleven cyclic antidepressants in human biological samples using a new reversed-phase chromatographic column of 2 μm porous microspherical silica gel, *J.Chromatogr.B*, **1997**, *692*, 405–412.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 500 μL 3 M ammonia solution and 7 mL n-pentane:isopropanol 95:5 to 2 mL plasma or urine, shake in an overhead shaker for 20 min, let stand for 10 min. Transfer the upper organic layer to a tube containing 1 mL 100 mM HCl, shake for 20 min, let stand for 5 min. Aspirate the organic phase to waste, wash the remaining aqueous layer with 3 mL pentane by shaking for 10 min. Add 500 μL 3 M ammonia solution and 6 mL a-pentane: isopropanol 95:5 to the washed aqueous layer, shake for 20 min, evaporate the organic layer under a stream of nitrogen at 65°, reconstitute the residue with 160 μL mobile phase, inject 60 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.5 3 μm Spherisorb silica
Mobile phase: MeOH:hexane:nonylamine 5:95:0.3
Flow rate: 1
Injection volume: 60
Detector: UV 254

CHROMATOGRAM
Retention time: 10
Internal standard: nortriptyline

OTHER SUBSTANCES
Extracted: N-desmethyldoxepin, doxepin

KEY WORDS
dog; human; plasma; normal phase; nortriptyline is IS

REFERENCE
Yan,J.; Hubbard,J.W.; McKay,G.; Midha,K.K. Stereoselective and simultaneous measurement of cis- and trans-isomers of doxepin and N-desmethyldoxepin in plasma or urine by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *691*, 131–138.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 206.4

CHROMATOGRAM
Retention time: 15.603

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: serum
Sample preparation: 1 mL Serum + 500 μL 750 mM pH 10 sodium bicarbonate/carbonate buffer + 50 μL IS in EtOH:water 50:50 + 8 mL heptane:isoamyl alcohol 98:2, shake at 250 cycles/min for 5 min, centrifuge at 1500 g for 10 min, freeze in dry ice/EtOH. Remove the organic layer and add it to 150 μL 22 mM pH 2.5 KH_2PO_4/phosphoric acid buffer, shake at 250 cycles/min for 5 min, centrifuge at 1500 g for 10 min, freeze in dry ice/EtOH. Discard the organic layer, inject a 65 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 250 × 4.6 Supelco C18

Mobile phase: MeCN:buffer 45:55 (Buffer was 44 mM KH_2PO_4 containing 1.5 mL/L triethylamine, adjusted to pH 2.5 with phosphoric acid.)
Flow rate: 1.5
Injection volume: 65
Detector: UV 240

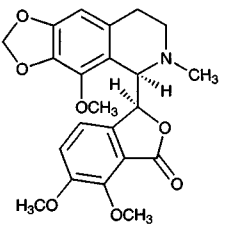
CHROMATOGRAM
Retention time: 9.73
Internal standard: 1-(3-(dimethylamino)propyl)-1-(p-chlorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile (LU 10-202) (Lundbeck, Copenhagen) (8.33)

OTHER SUBSTANCES
Extracted: metabolites, citalopram, amitriptyline
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.

Noscapine



Molecular formula: $C_{22}H_{23}NO_7$
Molecular weight: 413.43
CAS Registry No.: 128-62-1, 6035-40-1 (dl-form), 912-60-7 (HCl)
Merck Index: 6815

SAMPLE
Matrix: blood
Sample preparation: Condition a C18 SPE cartridge with 2 mL MeOH, 2 mL water, and 1 mL 50 mM pH 7.4 sodium phosphate buffer at 1.5 mL/min. Centrifuge rapidly thawed plasma samples. Combine 1 mL plasma with 40 µL 903 ng/mL papaverine free base and 2 mL 50 mM pH 7.4 sodium phosphate buffer, vortex. Add 2.75 mL diluted plasma at a rate of 0.36 ml/min to the SPE cartridge, wash with 1 mL 50 mM pH 7.4 sodium phosphate buffer and 2 mL water. Dry by passing 5 mL of air through the SPE cartridge at 5 mL/min. Elute with 1.5 mL MeCN, evaporate the eluate to dryness under nitrogen at 30°. Reconstitute with 200 µL MeCN, inject a 50 µL aliquot.

HPLC VARIABLES
Column: 125 × 4.0 5 µm Nucleosil 120-5-C18
Mobile phase: MeCN:25 mM pH 4.5 sodium phosphate buffer 40:60
Column temperature: 25
Flow rate: 1.0
Injection volume: 50
Detector: UV 211

CHROMATOGRAM
Retention time: 4.99
Internal standard: papaverine (3.98)
Limit of detection: 0.9 ng/mL

Limit of quantitation: 7.2 ng/mL

KEY WORDS
plasma; SPE

REFERENCE
Chollet,D.F.; Ruols,C.; Arnera,V. Determination of noscapine in human plasma using solid-phase extraction and high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *701*, 81–85.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 213.4

CHROMATOGRAM
Retention time: 12.827

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.2

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquin-

amide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

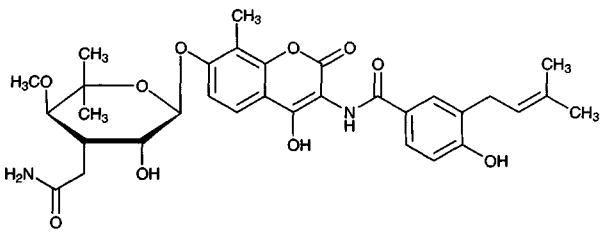
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, dil-

tiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Novobiocin



Molecular formula: $C_{31}H_{36}N_2O_{11}$
Molecular weight: 612.63
CAS Registry No.: 303-81-1, 1476-53-5 (Na salt), 4309-70-0 (Ca salt)
Merck Index: 6818

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL buffer + 4.5 mL MTBE:isopropanol 97.5:2.5, shake at 70 strokes/min for 5 min, centrifuge at 10-15° at 1100 g for 10 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 20-25°, reconstitute the residue in 0.5 mL mobile phase, vortex, sonicate for 5 min, inject a 100 µL aliquot. (Buffer was 400 mL 1 M KH_2PO_4, 400 mL K_2HPO_4 solution, and 250 g KCl, adjust pH to 6.5.)

HPLC VARIABLES

Column: 100 × 8 5 µm Nova-Pak C18 Radial-Pak
Mobile phase: MeOH:2-methoxyethanol:buffer 80:5:15 (Buffer was 4.33 g sodium lauryl sulfate and 2 mL 1 M phosphoric acid in 150 mL water, pH 2.8.)
Flow rate: 1.8

Injection volume: 100
Detector: UV 330

CHROMATOGRAM
Retention time: 3.0
Internal standard: novobiocin

OTHER SUBSTANCES
Extracted: coumermycin A1

KEY WORDS
dog; plasma; novobiocin is IS

REFERENCE
Strojny,N.; Conzentino,P.; de Silva,J.A. Determination of coumermycin A1 in plasma by reversed-phase high-performance liquid chromatographic analysis, *J.Chromatogr.*, **1985**, *342*, 145–158.

SAMPLE
Matrix: blood
Sample preparation: 0.5-1 mL Plasma + 10 μL 5 mM prednisone in MeOH + 5 mL MeOH, vortex for 10 s, centrifuge at 2000 g for 10 min, inject a 15 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: CN Guard-Pak
Column: 150 × 4.6 5 μm 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: 18.1
Internal standard: prednisone (5.5)
Limit of detection: 5 μM

KEY WORDS
plasma

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: 100 μL Serum + 5 μL 4 mM mitomycin C in MeCN + 400 μL MeCN, vortex, centrifuge at 12000 g for 2 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 15 × 3.2 NewGuard RP18
Column: 220 × 4.6 5 μm Spheri-5 RP18
Mobile phase: MeCN:10 mM phosphoric acid 80:20
Flow rate: 0.8
Injection volume: 50
Detector: UV 340

CHROMATOGRAM
Retention time: 4.53
Internal standard: mitomycin C (6.66)
Limit of quantitation: 1 μg/mL

KEY WORDS
serum

REFERENCE
Zuhowski,E.G.; Gutheil,J.C.; Egorin,M.J. Rapid and sensitive high-performance liquid chromatographic assay for novobiocin in human serum, *J.Chromatogr.B*, **1994**, *655*, 147–152.

SAMPLE
Matrix: blood, milk, tissue
Sample preparation: Blend 10 g tissue with 30 mL 200 mM $(NH_4)H_2PO_4$. Dilute each 1 mL of milk or serum with 3 mL 200 mM $(NH_4)H_2PO_4$. Add 10 mL Tissue homogenate, diluted milk, or diluted serum to 10 (muscle, milk, serum) or 20 (liver, kidney) mL MeOH, swirl vigorously, let stand for 5 min, filter (paper), refilter if not clear, to each 3 mL of filtrate from liver or kidney add 1 mL water, inject a 200 μL aliquot.

HPLC VARIABLES
Guard column: Supelcosil LC-18-DB
Column: 150 × 4.6 Supelcosil LC-18-DB
Mobile phase: Gradient. MeCN:MeOH:10 mM phosphoric acid 0:50:50 for 1 min, to 80:0:20 over 19 min, return to initial conditions. (At the end of each day flush the column with the final mobile phase, store in this mobile phase.)
Flow rate: 1 (at end of run set flow rate to 2 mL/min for 5 min then 1 mL/min for 5 min)
Injection volume: 200
Detector: UV 340

CHROMATOGRAM
Retention time: 21
Limit of detection: 10 ppb

KEY WORDS
serum; cow; muscle; liver; kidney

REFERENCE
Moats,W.A.; Leskinen,L. Determination of novobiocin residues in milk, blood, and tissues by liquid chromatography, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 776–778.

SAMPLE
Matrix: formulations
Sample preparation: Add 10 mL THF to the peanut oil formulation, make up to 100 mL with mobile phase, sonicate, shake at high speed for 5 min, centrifuge at 2000 g for 5 min, inject a 20 μL aliquot of the clear supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm LiChrosorb SI-100
Mobile phase: Butyl chloride:THF:MeOH:acetic acid 88:5:4:3 (Butyl chloride was 50% water saturated prepared by mixing equal volumes of water-saturated butyl chloride and anhydrous butyl chloride.)
Flow rate: 1
Injection volume: 20
Detector: UV 340

CHROMATOGRAM
Retention time: 16
Internal standard: prednisone (UV 254) (10)
Limit of detection: 10 ng

OTHER SUBSTANCES
Simultaneous: impurities, degradation products

KEY WORDS
stability-indicating; normal phase; oils

REFERENCE
Tsuji,K.; Rahn,P.D.; Kane,M.P. High-performance liquid chromatographic method for the determination of novobiocin, *J.Chromatogr.*, **1982**, *235*, 205–214.

SAMPLE
Matrix: milk
Sample preparation: 10 g Milk + 30 mL 200 mM ammonium phosphate, shake. Remove a 10 mL aliquot and add it to 10 mL MeOH, swirl vigorously, swirl occasionally for 5 min, filter (paper), refilter if necessary, inject a 1 mL aliquot.

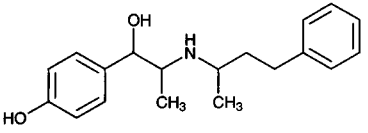
HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelcosil LC-18-DB
Column: 150 × 4.6 5 μm Supelcosil LC-18-DB
Mobile phase: Gradient. MeOH:5 mM phosphoric acid 50:50 for 1 min, to MeCN:MeOH:5 mM phosphoric acid 80:0:20 over 20 min, maintain at MeCN:MeOH:5 mM phosphoric acid 80:0:20 for 5 min, re-equilibrate at initial conditions for 6 min.
Flow rate: 1 (re-equilibrate at 2 mL/min for 5 min and 1 mL/min for 1 min)
Injection volume: 1000
Detector: UV 340

CHROMATOGRAM
Retention time: 23-25
Limit of detection: <0.05 ppm

KEY WORDS
cow

REFERENCE
Reeves,V.B. Liquid chromatographic procedure for the determination of novobiocin residues in bovine milk: Interlaboratory study, *J.AOAC Int.*, **1995**, *78*, 55–58.

Nylidrin



Molecular formula: $C_{19}H_{25}NO_2$
Molecular weight: 299.41
CAS Registry No.: 447-41-6, 849-55-8 (HCl)
Merck Index: 6830
Lednicer No.: 1 69

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 500 μL buffer, mix briefly, add 50-75 mg resin, mix at 10-25 rpm for 30 min, discard the supernatant, wash twice with 1 mL buffer, add 500 μL 50 mg/mL KOH in MeOH:water 50:50, mix for 30 min, inject a 20 μL aliquot of the eluate. (Buffer was 100 mM citric acid:200 mM Na_2HPO_4 29:71, pH 6.5 (McIlvaine buffer) Wash 20-50 mesh Dowex HCR-S resin twice with water and allow it to equilibrate in buffer).

HPLC VARIABLES
Guard column: 25 × 4.6 5 μm Spherisorb ODS-I
Column: 250 × 4.6 5 μm Spherisorb ODS-I
Mobile phase: MeCN:MeOH:buffer 30:18:52 containing 1.8 mM octanesulfonic acid (Buffer was 30 mM KH_2PO_4 adjusted to pH 3.0 with concentrated orthophosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: E, Gynotek M20, glassy carbon working electrode 950 mV, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 11

Internal standard: nylidrin

OTHER SUBSTANCES
Extracted: isoxsuprine

KEY WORDS
horse; plasma; nylidrin is IS; SPE

REFERENCE
Hashem,A.; Lubczyk,B. Determination of isoxsuprine in equine plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1991**, *563*, 216–223.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

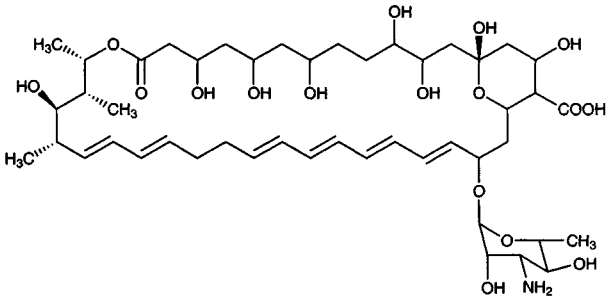
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasox-

izole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Nystatin



Molecular formula: $C_{47}H_{75}NO_{17}$
Molecular weight: 926.11
CAS Registry No.: 1400-61-9
Merck Index: 6834

SAMPLE
Matrix: CSF
Sample preparation: Condition a BakerBond C18 SPE cartridge with 3 mL MeOH and 3 mL 100 mM pH 9 carbonate buffer. Add 1 mL CSF to the SPE cartridge, wash with 2 mL 100 mM pH 9 carbonate buffer, air dry for 2 min, elute with two 500 μL aliquots of MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute with 200 μL MeOH, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeCN:10 mM pH 5 EDTA 35:65
Flow rate: 0.5
Injection volume: 100
Detector: UV 410

CHROMATOGRAM
Retention time: 8.5
Internal standard: nystatin

OTHER SUBSTANCES
Extracted: amphotericin B

KEY WORDS
dog; human; SPE; nystatin is IS

REFERENCE

Liu,H.; Davoudi,H.; Last,T. Determination of Amphotericin B in cerebrospinal fluid by solid-phase extraction and liquid chromatography, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1395–1400.

SAMPLE
Matrix: bulk

HPLC VARIABLES
Column: 500 × 1 7-8 μm Zorbax B.P. Sil
Mobile phase: MeOH:DMF:water:acetic acid 72:25:3:0.4

Flow rate: 0.125
Injection volume: 1
Detector: UV 308

CHROMATOGRAM
Retention time: 13

KEY WORDS
microbore; normal phase

REFERENCE
Milhaud,J.; Gareil,P.; Rosset,R. Separation of filipin and nystatin complexes by semi-preparative and microbore high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *358*, 284–287.

SAMPLE
Matrix: bulk
Sample preparation: Make up a 10% solution in DMF then dilute to 20 mg/mL with MeOH: water 66:34, inject a 200 μL aliquot.

HPLC VARIABLES
Column: 500 × 9 10 μm Partisil ODS 2
Mobile phase: MeOH:water:acetic acid 66:33:1
Flow rate: 8
Injection volume: 200
Detector: UV 308

CHROMATOGRAM
Retention time: 22

KEY WORDS
semi-preparative

REFERENCE
Milhaud,J.; Gareil,P.; Rosset,R. Separation of filipin and nystatin complexes by semi-preparative and microbore high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *358*, 284–287.

SAMPLE
Matrix: bulk

HPLC VARIABLES
Column: 300 × 4.5 μBondapak C18
Mobile phase: MeOH:water 70:30
Flow rate: 2
Detector: UV 280

CHROMATOGRAM
Retention time: 10

REFERENCE
Mehta,R.T.; Hopfer,R.L.; Gunner,L.A.; Juliano,R.L.; Lopez-Berestein,G. Formulation, toxicity, and antifungal activity in vitro of liposome-encapsulated nystatin as therapeutic agent for systemic candidiasis, *Antimicrob.Agents Chemother.*, **1987**, *31*, 1897–1900.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve in mobile phase to a concentration of 1 mg/mL.

HPLC VARIABLES
Column: 250 × 4 7 μm LiChrosorb RP-18

Mobile phase: MeOH:water 90:10 + 1% formic acid, pH 3.5
Flow rate: 1
Injection volume: 10
Detector: UV 340

CHROMATOGRAM
Retention time: 6.3

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE
Sauer,B.; Matusch,R. High-performance liquid chromatographic separations of nystatin and their influence on the antifungal activity, *J.Chromatogr.A*, **1994**, *672*, 247–253.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 5 μm ODS-Hypersil
Mobile phase: MeOH:DMF:10 mM pH 7.0 Tris in water 56:9.6:34.4
Detector: UV 305

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Egodage,K.L.; Haslam,J.S.; Rajewski,R.A.; Stella,V.J. Correlation and validation to the USP bioassay of a RP-HPLC assay for nystatin (Abstract 3373), *Pharm.Res.*, **1997**, *14*, S587.

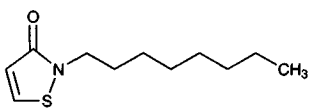
SAMPLE
Matrix: solutions
Sample preparation: Dissolve in DMSO to 10 mg/mL, dilute 1:20 with MeOH.

HPLC VARIABLES
Column: 250 × 4.6 10 μm μBondapak C18
Mobile phase: MeCN:50 mM phosphate buffer (pH 3.5-8.1) 30:70 to 35:65
Flow rate: 0.4-2
Detector: UV 313

OTHER SUBSTANCES
Simultaneous: amphotericin A

REFERENCE
Aszalos,A.; Bax,A.; Burlinson,N.; Roller,P.; McNeal,C. Physico-chemical and microbiological comparison of nystatin, amphotericin A and amphotericin B, and structure of amphotericin A, *J.Antibiot.(Tokyo)*, **1985**, *38*, 1699–1713.

Octhilinone



Molecular formula: $C_{11}H_{19}NOS$
Molecular weight: 213.34
CAS Registry No.: 26530-20-1
Merck Index: 6853

SAMPLE
Matrix: blood, urine

Mobile phase: MeOH:water 90:10 + 1% formic acid, pH 3.5
Flow rate: 1
Injection volume: 10
Detector: UV 340

CHROMATOGRAM
Retention time: 6.3

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE
Sauer,B.; Matusch,R. High-performance liquid chromatographic separations of nystatin and their influence on the antifungal activity, *J.Chromatogr.A*, **1994**, *672*, 247–253.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 5 μm ODS-Hypersil
Mobile phase: MeOH:DMF:10 mM pH 7.0 Tris in water 56:9.6:34.4
Detector: UV 305

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Egodage,K.L.; Haslam,J.S.; Rajewski,R.A.; Stella,V.J. Correlation and validation to the USP bioassay of a RP-HPLC assay for nystatin (Abstract 3373), *Pharm.Res.*, **1997**, *14*, S587.

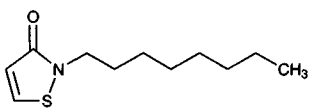
SAMPLE
Matrix: solutions
Sample preparation: Dissolve in DMSO to 10 mg/mL, dilute 1:20 with MeOH.

HPLC VARIABLES
Column: 250 × 4.6 10 μm μBondapak C18
Mobile phase: MeCN:50 mM phosphate buffer (pH 3.5-8.1) 30:70 to 35:65
Flow rate: 0.4-2
Detector: UV 313

OTHER SUBSTANCES
Simultaneous: amphotericin A

REFERENCE
Aszalos,A.; Bax,A.; Burlinson,N.; Roller,P.; McNeal,C. Physico-chemical and microbiological comparison of nystatin, amphotericin A and amphotericin B, and structure of amphotericin A, *J.Antibiot.(Tokyo)*, **1985**, *38*, 1699–1713.

Octhilinone



Molecular formula: $C_{11}H_{19}NOS$
Molecular weight: 213.34
CAS Registry No.: 26530-20-1
Merck Index: 6853

SAMPLE
Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

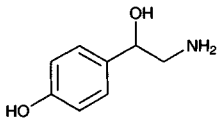
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 3.112

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Octopamine



Molecular formula: $C_8H_{11}NO_2$
Molecular weight: 153.18
CAS Registry No.: 104-14-3, 876-04-0 (D-(-)), 770-05-8 (DL HCl)
Merck Index: 6856
Lednicer No.: 5 23

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 100 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 150 × 4 5 μm Crownpak CR(+) immobilized crown ether
Mobile phase: 0.1% pH 1.9 Perchloric acid
Column temperature: 25
Flow rate: 1
Detector: UV 210

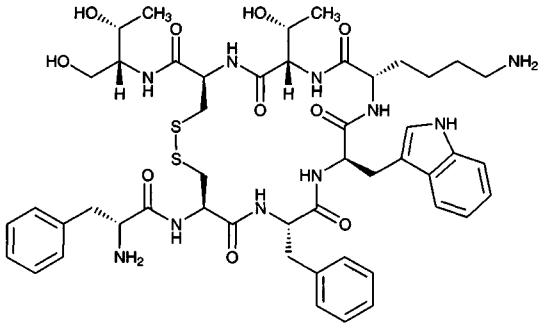
CHROMATOGRAM
Retention time: 4.64, 4.95

OTHER SUBSTANCES
Simultaneous: norepinephrine

KEY WORDS
chiral; comparison with capillary electrophoresis

REFERENCE
Nishi,H.; Nakamura,K.; Nakai,H.; Sato,T. Separation of enantiomers and isomers of amino compounds by capillary electrophoresis and high-performance liquid chromatography utilizing crown ethers, *J.Chromatogr.A*, **1997**, *757*, 225–235.

Octreotide



Molecular formula: $C_{49}H_{66}N_{10}O_{10}S_2$
Molecular weight: 1019.26
CAS Registry No.: 83150-76-9
Merck Index: 6859

SAMPLE
Matrix: bile, blood, feces, urine
Sample preparation: Hydrolyze pooled plasma with 5 mg Subtilisin A at 50° for 1 h. Lyophilize feces and extract twice with 4 volumes MeOH. Lyophilize urine and bile and reconstitute with water.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm RP-18 (Brownlee)
Column: 100 × 4.6 5 μm RP-18 (Brownlee)
Mobile phase: Gradient. MeCN:water:trifluoroacetic acid from 0:99.8:0.2 to 99.8:0:0.2 over 40 min.
Flow rate: 1.5
Detector: UV 210 or radioactivity

CHROMATOGRAM
Retention time: 45

KEY WORDS
rat; plasma

REFERENCE
Lemaire,M.; Azria,M.; Dannecker,R.; Marbach,P.; Schweitzer,A.; Maurer,G. Disposition of sandostatin, a new synthetic somatostatin analogue, in rats, *Drug Metab.Dispos.*, **1989**, *17*, 699–703.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 2.6 5 μm Bakerbond C18
Mobile phase: Gradient. A was MeCN:water:1 M tetramethylammonium hydroxide 10:88:2 adjusted to pH 4.5 with concentrated orthophosphoric acid. B was MeCN:water:1 M tetramethylammonium hydroxide 60:38:2 adjusted to pH 4.5 with concentrated orthophosphoric acid. A:B from 100:0 to 0:100 over 14 min.
Flow rate: 1.3
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 11.5

OTHER SUBSTANCES
Simultaneous: degradation products, des-threninol

KEY WORDS
protect from light; injections

REFERENCE
Stiles,M.L.; Allen,L.V.,Jr.; Resztak,K.E.; Prince,S.J. Stability of octreotide acetate in polypropylene syringes, *Am.J.Hosp.Pharm.*, **1993**, *50*, 2356–2358.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb RP18
Mobile phase: MeCN:buffer 31:69, pH 4.7 (Buffer was 20 mM tetramethylammonium hydroxide adjusted to pH 4.7 with phosphoric acid.)
Column temperature: 40
Flow rate: 1.2
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 18

OTHER SUBSTANCES
Simultaneous: derivatives

REFERENCE
Kuhn,R.; Morin,C.; Erni,F. A simple model describing the retention behavior of octreotide and its glycosylated derivatives in reversed phase HPLC, *Chromatographia*, **1995**, *41*, 516–520.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 30 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Spheri-5 C18
Mobile phase: MeCN:water:1 M tetramethylammonium hydroxide pentahydrate 33:65:2, adjusted to pH 4.5 with phosphoric acid
Flow rate: 0.8
Injection volume: 30
Detector: UV 280

CHROMATOGRAM
Retention time: 6.2

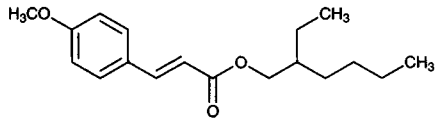
OTHER SUBSTANCES
Simultaneous: degradation products (UV 210)

KEY WORDS
injections

REFERENCE
Ripley,R.G.; Ritchie,D.J.; Holstad,S.G. Stability of octreotide acetate in polypropylene syringes at 5 and -20°C, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 1910–1911.

Octyl methoxycinnamate

Molecular formula: $C_{18}H_{26}O_3$
Molecular weight: 290.40
CAS Registry No.: 5466-77-3
Merck Index: 6864



SAMPLE

Matrix: formulations
Sample preparation: 1-1.5 g sun-screen lotion + 50 mL isopropanol, dissolve. Remove a 5 mL aliquot and make up to 50 mL with mobile phase, filter (0.45 μm) inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm C8 Hypersil
Mobile phase: Isopropanol:buffer 10:90 (Buffer was 100 mM sodium dodecyl sulfate (electrophoresis grade) containing 0.3% triethylamine adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 254, UV 300

CHROMATOGRAM

Retention time: 19.40

OTHER SUBSTANCES

Simultaneous: 2-ethylhexyl p-dimethylaminobenzoate, oxybenzone, methyl paraben, propyl paraben

KEY WORDS

lotion

REFERENCE

Tomasella,F.P.; Zuting,P.; Love,L.J. Determination of sun-screen agents in cosmetic products by micellar liquid chromatography, *J.Chromatogr.*, **1991**, *587*, 325–328.

SAMPLE

Matrix: formulations
Sample preparation: Weigh out lotion, add 20 mL EtOH, heat to 60°, stir for 30 min at room temperature, make up to 25 mL, stir for 5 min, centrifuge at 14500 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Column: 200 × 4.6 5 μm Ultrasphere C8
Mobile phase: Gradient. MeOH:1% aqueous acetic acid from 80:20 to 100:0 over 10 min, maintain at 100:0 for 2 min, re-equilibrate for 4 min.
Column temperature: 25
Flow rate: 1
Detector: UV 325

CHROMATOGRAM

Retention time: 8.7

OTHER SUBSTANCES

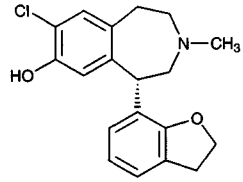
Simultaneous: benzophenone-3, butylmethoxydibenzoylmethane, impurities

KEY WORDS

lotion; sunscreen; an isomer forms on exposure to light

REFERENCE
Meijer,J.; Lodén,M. Stability analysis of three UV-filters using HPLC, *J.Liq.Chromatogr.*, **1995**, *18*, 1821–1832.

Odapipam



Molecular formula: $C_{19}H_{20}ClNO_2$
Molecular weight: 329.83
CAS Registry No.: 131796-63-9

SAMPLE
Matrix: microsomal incubations
Sample preparation: Condition a 500 mg C18 Bond-Elut SPE cartridge with MeOH and water. Mix 2 mL microsomal incubation with 4.5 mL ice-cold MeOH, centrifuge at 4000 g for 10 min, dilute the supernatant with 15 mL water. Add a 2 mL aliquot to the SPE cartridge, wash with 2 mL water, elute with 2 mL MeOH:25% aqueous ammonia 96:4, evaporate the eluate to dryness under reduced pressure, reconstitute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 3 μm ChromSpher Si (Chrompack)
Mobile phase: n-Heptane:2-propanol:water:25% ammonia 90:10:0.2:0.1
Detector: UV 280; MS, VG TRIO 1000, particle beam interface at 50°, helium at 25-30 psi, ion source 200°, positive ionization mode, electron current 150 μA, electron energy 70 eV

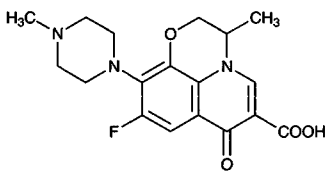
CHROMATOGRAM
Retention time: 2.3

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
normal phase; rat; liver; SPE

REFERENCE
Andersen,J.V.; Hansen,K.T. Normal-phase liquid chromatography-particle-beam mass spectrometry in drug metabolism studies of the dopamine receptor antagonist Odapipam and the muscarine M1 receptor agonist Xanomeline, *Xenobiotica*, **1997**, *27*, 901–912.

Ofloxacin



Molecular formula: $C_{18}H_{20}FN_3O_4$
Molecular weight: 361.37
CAS Registry No.: 82419-36-1
Merck Index: 6865
Lednicer No.: 4 141-145

SAMPLE
Matrix: aqueous humor, blood
Sample preparation: Aqueous humor. Inject a 10 μL aliquot directly. Plasma. Condition a 3 mL C18 SPE cartridge (Varian) with two 3 mL portions of MeCN and 3 mL buffer. Add 2 mL 625 ng/mL ciprofloxacin in buffer to 500 μL of plasma, mix, add to the SPE cartridge. Wash with 3 mL buffer. Remove moisture with vacuum (200 mbar) for 10 min. Elute with two 500 μL

portions of MeCN:buffer 40:60. Vortex the eluate, inject a 10 μL aliquot. (Buffer was 100 mM Tris adjusted to pH 5.0 with HCl).

HPLC VARIABLES
Column: 300 × 4.6 5 μm endcapped ODS-Hypersil
Mobile phase: MeCN:DMF:10 mM NaH_2PO_4 15:6:79, adjusted to pH 3.0 with 85% phosphoric acid
Flow rate: 1
Injection volume: 10
Detector: UV 285

CHROMATOGRAM
Retention time: 10.2
Internal standard: ciprofloxacin (12.0)
Limit of detection: 80 ng/mL (aqueous humor), 310 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: cefotaxime

KEY WORDS
plasma; SPE

REFERENCE
Kraemer,H.-J.; Gehrke,R.; Breithaupt,A.; Breithaupt,H. Simultaneous quantification of cefotaxime, desacetylcefotaxime, ofloxacine and ciprofloxacine in ocular aqueous humor and in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *700*, 147–153.

SAMPLE
Matrix: bile, blood, perfusate
Sample preparation: Intestinal perfusate. Centrifuge before analysis. Plasma. Mix 200 μL plasma with 200 μL 100 mM pH 6.8 phosphate buffer, add 4 mL dichloromethane, shake at 100 cycles/min for 10 min. Remove the organic layer and dry it under nitrogen at 40°, reconstitute with 200 μL mobile phase, inject a 20 μL aliquot (J. Chromatogr. 1988, 434, 320). Bile. Mix 100 μL bile with 900 μL pH 7.0 phosphate buffer, add 500 μL MeOH. Shake for 1 min and centrifuge at 1000 g for 5 min. Inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: Bovine Serum Albumin (Macherey Nagel)
Mobile phase: 200 mM Potassium dihydrogen phosphate containing 5 mM N,N-dimethyloctylamine, adjusted to pH 8.0 with KOH
Flow rate: 1
Injection volume: 20
Detector: F ex 298 em458

CHROMATOGRAM
Retention time: 6 (S-(-)), 7.8 (R-(+))
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Also analyzed: cefoperazone, ciprofloxacin, ofloxacin, quinidine, verapamil

KEY WORDS
chiral; intestinal efflux; pharmacokinetics; plasma

REFERENCE
Rabbaa,L.; Dautrey,S.; Colas-Linhart,N.; Carbon,C.; Farinotti,R. Intestinal elimination of ofloxacin enantiomers in the rat: Evidence of a carrier-mediated process, *Antimicrob.Agents Chemother.*, **1996**, *40*, 2126–2130.

SAMPLE
Matrix: blood

Sample preparation: Mix 1 mL blood with 1 mL 0.25 mM Triton, vortex for 30 s, add 4 mL 6% trichloroacetic acid. Vortex for 30 s, centrifuge at 2000 g for 10 min, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm C18
Mobile phase: MeCN:buffer 10:90 (Buffer was 1 L 25 mM phosphoric acid and 15 mL 40% tetrabutyl ammonium hydrogen sulfate, adjusted to pH 3.0 with 66.6 mM phosphate buffer.)
Flow rate: 2
Injection volume: 100
Detector: F ex 330 em 450

CHROMATOGRAM
Retention time: 5
Limit of detection: 30 ng/mL (plasma), 50 ng/mL (blood)

KEY WORDS
plasma; pharmacokinetics; rabbit

REFERENCE
Colino,C.I.; García Turiño,A.; Sanchez Navarro,A.; Lanao,J.M. A comparative study of ofloxacin and ciprofloxacin erythrocyte distribution, *Biopharm.Drug Dispos.*, **1998**, *19*, 71–77.

SAMPLE
Matrix: blood
Sample preparation: Mix equal volumes serum and MeOH. Centrifuge sample, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4 Spherisorb 5 ODS II
Mobile phase: MeCN:water:phosphoric acid 5:94.6:0.32 adjusted to pH 3.0 with tetrabutylammonium hydroxide
Column temperature: 50
Flow rate: 2
Injection volume: 20
Detector: F ex 310 em 489

CHROMATOGRAM
Limit of detection: 80 ng/mL

KEY WORDS
serum; pharmacokinetics

REFERENCE
Bethell,D.B.; Day,N.P.; Dung,N.M.; McMullin,C.; Loan,H.T.; Tam,D.T.; Minh,L.T.; Linh,N.T.; Dung,N.Q.; Vinh,H.; MacGowan,A.P.; White,L.O.; White,N.J. Pharmacokinetics of oral and intravenous ofloxacin in children with multidrug-resistant typhoid fever, *Antimicrob.Agents Chemother.*, **1996**, *40*, 2167–2172.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Whole blood or 500 μL plasma, vortex for 30 s with 1 mL 250 μM Triton, add 4 mL 6% trichloroacetic acid. Vortex for 30 s, centrifuge at 2000 g for 10 min. Inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 10 μm C18
Mobile phase: MeCN:buffer 10:90 (Buffer was mixture of 1 L 25 mM phosphoric acid and 15 mL 40% tetrabutylamonium hydrogen sulfate, adjusted to pH 3 with 66.6 mM phosphate buffer.)
Flow rate: 2
Injection volume: 100
Detector: F ex 330 em 450

CHROMATOGRAM
Retention time: 5
Limit of detection: 30 ng/mL (plasma), 50 ng/mL (blood)

KEY WORDS
plasma; whole blood; pharmacokinetics

REFERENCE
Colino,C.I.; García Turiño,A.; Sanchez Navarro,A.; Lanao,J.M. A comparative study of ofloxacin and ciprofloxacin erythrocyte distribution, *Biopharm.Drug Dispos.*, **1998**, *19*, 71–77.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Blood or 500 μL plasma, vortex for 30 s with 1 mL 250 μM Triton, add 4 mL 6% trichloroacetic acid. Vortex for 30 s, centrifuge at 2000 g for 10 min. Inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 10 μm C18
Mobile phase: MeCN:buffer 10:90 (Buffer was mixture of 1 L 25 mM phosphoric acid and 15 mL 40% tetrabutylamonium hydrogen sulfate, adjusted to pH 3 with 66.6 mM phosphate buffer.)
Flow rate: 2
Injection volume: 100
Detector: F ex 277 em 445

CHROMATOGRAM
Retention time: 9
Limit of detection: 30 ng/mL (plasma), 50 ng/mL (blood)

KEY WORDS
plasma; whole blood; pharmacokinetics

REFERENCE
Colino,C.I.; García Turiño,A.; Sanchez Navarro,A.; Lanao,J.M. A comparative study of ofloxacin and ciprofloxacin erythrocyte distribution, *Biopharm.Drug Dispos.*, **1998**, *19*, 71–77.

SAMPLE
Matrix: blood
Sample preparation: Filter 1 mL plasma using a micropartition system (MPS-1, Amicon, MA) while centrifuging at 2000 g for 20 min at 10°, inject an aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb ODS-2 endcapped
Mobile phase: MeCN:buffer 13:87 containing 5 mM tetrabutylammonium sulfate, adjusted to pH 2.5 with 1 M NaOH (Buffer was 100 mM citric acid containing 200 mM ammonium perchlorate.)
Column temperature: 37
Flow rate: 1
Detector: UV 295

CHROMATOGRAM
Retention time: 8.54
Internal standard: pipemic acid (4.67)

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Zlotos,G.; Bücker,A.; Kinzig-Schippers,M.; Sorgel,F.; Holzgrabe,U. Plasma protein binding of gyrase inhibitors, *J.Pharm.Sci.*, **1998**, *87*, 215–220.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 295

CHROMATOGRAM

Retention time: 8.648

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: formulations

Sample preparation: Dilute a 25 mg/mL levofloxacin injection with an infusion fluid to a concentration of 100 μg/mL. Inject a 20 μL aliquot. (Infusion fluids were 0.9% NaCl, 5% dextrose, 5% dextrose and 0.9% NaCl, 5% dextrose and lactated Ringer's injections, 5% sodium bicarbonate, Plasma-Lyte 56 and 5% dextrose, 5% dextrose, 0.45% NaCl, and 0.15% KCl, 1/6 M sodium lactate, sterile water, and 20% mannitol.)

HPLC VARIABLES

Column: 250 × 4.6 Zorbax SB-Phenyl

Mobile phase: MeCN:MeOH:94 mM KH_2PO_4:trifluoroacetic acid 15:5:80:0.3

Column temperature: 45

Flow rate: 1

Injection volume: 25

Detector: UV 294

CHROMATOGRAM

Retention time: 15

OTHER SUBSTANCES

Simultaneous: degradation products

KEY WORDS

injections; stability indicating; for levofloxacin

REFERENCE

Williams,N.A.; Bornstein,M.; Johnson,K. Stability of levofloxacin in intravenous solutions in polyvinyl chloride bags, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 2309–2313.

SAMPLE
Matrix: growth medium
Sample preparation: 500 μL Sample + 500 μL 100 μg/mL IS in cold (4°) MeCN, vortex, centrifuge at 3000 *g* for 5 min. Remove a 500 μL aliquot of the supernatant, filter (0.45 μm Acrodisc syringe filter), inject a 30 μL aliquot. (Protect all specimens from light.)

HPLC VARIABLES
Guard column: C18 5U (Alltech)
Column: 150 × 4.6 7 μm Adsorbosphere HS C18 7U
Mobile phase: MeCN:20 mM pH 3.0 phosphate buffer 35:65 containing 0.2% triethylamine and 0.2% sodium dodecyl sulfate, adjusted to pH 3.0 with 85% phosphoric acid
Flow rate: 1.75
Injection volume: 30
Detector: UV 280

CHROMATOGRAM
Retention time: 4.15
Internal standard: sparfloxacin (7.09)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Also analyzed: ciprofloxacin, clinafloxacin, levofloxacin, sparfloxacin, temafloxacin, trovafloxacin

KEY WORDS
Mueller-Hinton broth

REFERENCE
Wright,D.H.; Herman,V.K.; Konstantinides,F.N.; Rotschafer,J.C. Determination of quinolone antibiotics in growth media by reversed-phase high-performance liquid chromatography, *J.Chromatogr.B*, **1998**, *709*, 97–104.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 4 μm NovaPak C18
Mobile phase: MeCN:MeOH:buffer:acetic acid 2.5:10:86.5:1 containing 20 mM triethylamine (The pH 2.7 buffer was 0.4% diammonium hydrogen phosphate in water containing 0.4% (?) tetrabutylammonium hydrogen sulfate.)
Flow rate: 1
Detector: UV 279

CHROMATOGRAM
Retention time: 11.6

OTHER SUBSTANCES
Extracted: ciprofloxacin, enrofloxacin

REFERENCE
Cester,C.C.; Toutain,P.L. A comprehensive model for enrofloxacin to ciprofloxacin transformation and disposition in dog, *J.Pharm.Sci.*, **1997**, *86*, 1148–1155.

SAMPLE
Matrix: solutions
Sample preparation: Filter (0.45 μm) a solution in MeCN:water 10:90, inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 RP-18

Mobile phase: MeCN:buffer 7:93 (Buffer was 25 mM phosphoric acid adjusted to pH 3.89 with 100 mM tetrabutylammonium hydroxide.)
Flow rate: 1
Injection volume: 10
Detector: UV 295

CHROMATOGRAM
Retention time: 8.8

OTHER SUBSTANCES
Simultaneous: ciprofloxacin (UV 280), enoxacin (UV 280), fleroxacin (UV 280), norfloxacin (UV 280), pipemidic acid (UV 280)

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.

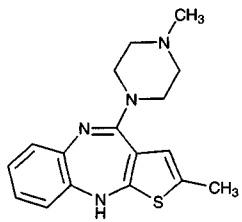
SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 20 mm long Supelguard LC-18S (Supelco)
Column: 250 × 4.6 Suplecosil 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.

Olanzapine

Molecular formula: $C_{17}H_{20}N_4S$
Molecular weight: 312.44
CAS Registry No.: 132539-06-1
Merck Index: 6959



SAMPLE
Matrix: blood
Sample preparation: Condition a Certify mixed bed (RP and ion exchange) SPE cartridge with MeOH and phosphate buffer. Add serum, wash with 1 M acetic acid, wash with MeOH, elute with 3% ammonium hydroxide in ethyl acetate. Evaporate the eluate, reconstitute, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 Microsorb CN
Mobile phase: MeCN:MeOH:50 mM pH 6.5 sodium phosphate buffer 5:28:67
Column temperature: 37
Flow rate: 1.5

CHROMATOGRAM
Retention time: 9.6
Internal standard: clozapine (12.5)

Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Simultaneous: imipramine
Also analyzed: haloperidol, risperidone
Interfering: paroxetine

KEY WORDS
SPE; serum

REFERENCE
Prieto,I.V.; Hoffman,D.W. HPLC monitoring of olanzapine (Abstract 131), *Ther.Drug Monit.*, **1997**, *19*, 580.

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL Plasma with 100 μL 100 ng/mL IS in MeCN, add 500 μL saturated sodium carbonate, mix. Add 7 mL pentane:dichloromethane 85:15, shake for 10 min, centrifuge at 18° for 10 min. Evaporate the supernatant to dryness under a slow stream of nitrogen at 60°. Dissolve the residue in 150 μL MeCN, inject a 30-120 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere CN
Mobile phase: MeCN:MeOH:130 mM pH 6.8 ammonium acetate 86:6:8
Column temperature: 40
Flow rate: 0.8
Injection volume: 30-120
Detector: E, ESA Coulochem model 5100A, model 5020 guard cell +1 V, model 5011 dual electrode analytical cell, electrode 1 +300 mV, electrode 2 +930 mV

CHROMATOGRAM
Retention time: 14.6
Internal standard: 2-ethyl analog of olanzapine LY170222 (Lilly Research Labs) (13)
Limit of detection: 250 pg/mL

OTHER SUBSTANCES
Simultaneous: acetaminophen, benztropine, chlorpromazine, clonazepam, clozapine, fluphenazine, ibuprofen, lorazepam, perphenazine, pseudoephedrine, risperidone, spiperone, sulpiride, trifluoperazine, trihexyphenidyl

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Aravagiri,M.; Ames,D.; Wirshing,W.C.; Marder,S.R. Plasma level monitoring of olanzapine in patients with schizophrenia: Determination by high-performance liquid chromatography with electrochemical detection, *Ther.Drug Monit.*, **1997**, *19*, 307–313.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 200 μL Microsomal incubation + 200 μL cold MeCN, mix, centrifuge in a microfuge at maximum speed for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm YMC basic column (YMC, USA)
Mobile phase: MeCN:MeOH:75 mM pH 7 sodium phosphate buffer 20:30:50
Column temperature: 40
Flow rate: 1
Detector: E, ESA Coulochem Dual Electrode Detector, guard cell model 5020 + 300 mV, analytical cell model 5014, detector 1 + 0.0 V, detector 2 + 300 mV

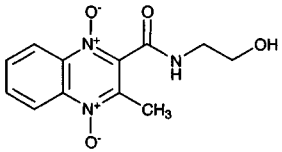
CHROMATOGRAM
Retention time: 12
Internal standard: LY170222 (2-ethylolanzapine)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
liver; human

REFERENCE
Ring,B.J.; Catlow,J.; Lindsay,T.J.; Gillespie,T.; Roskos,L.K.; Cerimele,B.J.; Swanson,S.P.; Hamman,M.A.; Wrighton,S.A. Identification of the human cytochromes P450 responsible for the in vitro formation of the major oxidative metabolites of the antipsychotic agent olanzapine, *J.Pharmacol.Exp.Ther.*, **1996**, *276*, 658–666.

Olaquindox



Molecular formula: $C_{12}H_{13}N_3O_4$
Molecular weight: 263.25
CAS Registry No.: 23696-28-8
Merck Index: 6960

SAMPLE
Matrix: feed
Sample preparation: Grind and sieve feed with a Moulinex blender. Weigh out 10 g and add it to 20 mL DMF and 60 mL carbon tetrachloride, stir magnetically at 500 rpm at 60° for 30 min, cool, filter (100 μm glass), wash the residue with a little carbon tetrachloride. Remove 25 mL of the filtrate and add it to 45 mL water, stir vigorously for 2 min, centrifuge at 320 g for 5 min, inject an aliquot of the aqueous layer.

HPLC VARIABLES
Column: 250 × 4.1 10 μm Versapack C18 (Alltech)
Mobile phase: Gradient. MeOH:water 15:85 for 4 min, to 50:50 over 2 min, maintain at 50:50 for 4 min, return to initial conditions over 2 min.
Flow rate: 1.5
Injection volume: 20
Detector: UV 262

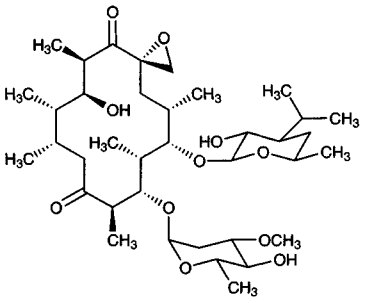
CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Extracted: carbadox (UV 305 nm)

KEY WORDS
protect from light

REFERENCE
dos Ramos,F.J.; da Silveira,I.N.; de Graaf,G. Column liquid chromatographic determination of carbadox and olaquindox in feeds, *J.Chromatogr.*, **1991**, *558*, 125–130.

Oleandomycin



Molecular formula: $C_{35}H_{61}NO_{12}$
Molecular weight: 687.87
CAS Registry No.: 3922-90-5, 6696-47-5 (HCl), 7060-74-4 (phosphate)
Merck Index: 6962

SAMPLE

Matrix: blood, tissue
Sample preparation: Homogenize (Physcotron) liver with 4 volumes of ice-cold saline. Put 200 μL plasma or liver homogenate into the tube. Add 2 mL MTBE and 5 μL 1 M NaOH and shake mechanically for 5 min. Centrifuge at 1500 g for 10 min, transfer the upper layer into a glass tube and evaporate it to dryness under dry nitrogen. Rinse the inner wall of the tube with 200 μL MeOH and evaporate to dryness. Dissolve the residue in 30 μL MeOH and inject a 10 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Cosmosil 5-C18 (Nacalai Tesque)
Mobile phase: MeCN:100 mM pH 6.6 sodium acetate buffer 50:50
Flow rate: 0.6
Injection volume: 10
Detector: E, BAS LC-4C, 1.1 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 8.5

OTHER SUBSTANCES

Extracted: erythromycin

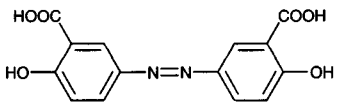
KEY WORDS

rat; plasma; liver; pharmacokinetics; oleandomycin is IS

REFERENCE

Hanada,E.; Ohtani,H.; Kotaki,H.; Sawada,Y.; Iga,T. Determination of erythromycin concentrations in rat plasma and liver by high-performance liquid chromatography with amperometric detection, *J.Chromatogr.B*, **1997**, *692*, 478–482.

Olsalazine



Molecular formula: $C_{14}H_{10}N_2O_6$
Molecular weight: 302.24
CAS Registry No.: 15722-48-2, 6054-98-4 (Na salt)
Merck Index: 6976
Lednicer No.: 4 42

SAMPLE

Matrix: blood, urine
Sample preparation: Serum. Add disodium-3,5'-azo-bis-(6-hydroxybenzoate) to serum, treat with proteinase K (0.5 mg/mL protein) for 10 min, add tetrabutylammonium hydrogen sulfate buffered to pH 6.5, add dichloromethane, agitate for 30 min, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in mobile phase, inject an aliquot.

Urine. Add 2,4-dihydroxybenzoic acid to urine, add perchloric acid, add diethyl ether, shake for 10 min, freeze. Remove the organic layer and add it to pH 7.4 phosphate buffer, extract, inject an aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: 30 × 4 30-40 μm Perisorb RP-18
Column: 250 × 4 10 μm Nucleosil C18
Mobile phase: MeOH:buffer 52:48 (Buffer was pH 7.4 phosphate buffer containing 20 mM tetrabutylammonium hydrogen sulfate.)
Detector: UV 365

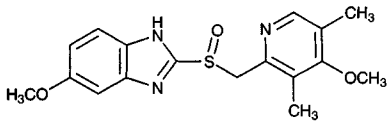
CHROMATOGRAM
Retention time: k' 5.2
Internal standard: disodium-3,5'-azo-bis-(6-hydroxybenzoate), 2,4-dihydroxybenzoic acid
Limit of quantitation: 1 μM urine, 0.5 μM (serum)

OTHER SUBSTANCES
Extracted: olsalazine sulfate

KEY WORDS
serum; pharmacokinetics

REFERENCE
Ryde,E.M.; Ahnfelt,N.-O. The pharmacokinetics of olsalazine sodium in healthy volunteers after a single i.v. dose and after oral doses with and without food, *Eur.J.Clin.Pharmacol.*, **1988**, *34*, 481–488.

Omeprazole



Molecular formula: $C_{17}H_{19}N_3O_3S$
Molecular weight: 345.42
CAS Registry No.: 73590-58-6
Merck Index: 6977
Lednicer No.: 4 133

SAMPLE
Matrix: blood
Sample preparation: Extract 500 μL plasma with dichloromethane containing 50 μL 1.0 M dibasic sodium phosphate and 100 μL 25 μg/mL IS. Centrifuge and aspirate aqueous layer to waste. Evaporate organic layer under a stream of nitrogen, reconstitute residue in 200 μL MeCN:20 mM pH 8.0 phosphate buffer 25:75. Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Zorbax SB C18
Mobile phase: Gradient. MeCN:20 mM pH 7.5 phosphate buffer from 30:70 to 40:60 over 10 min. (Mobile phase was contained 0.1% triethylamine.)
Injection volume: 10
Detector: UV 302, to UV 280 at 6.4 min

CHROMATOGRAM
Retention time: 5.7
Internal standard: carbamazepine (7.3)

KEY WORDS
plasma

REFERENCE
Sarich,T.; Kalhorn,T.; Magee,S.; Al-sayegh,F.; Adams,S.; Slattery,J.; Goldstein,J.; Nelson,S.; Wright,J. The effect of omeprazole pretreatment on acetaminophen metabolism in rapid and slow metabolizers of S-mephenytoin, *Clin.Pharmacol.Ther.*, **1997**, *62*, 21–28.

SAMPLE
Matrix: blood
Sample preparation: Add 10 μL 72 μg/mL flunitrazepam in MeOH to 1 mL plasma, shake briefly, add 3 mL toluene:isoamyl alcohol 95:5, vortex at 1000 rpm for 90 s, centrifuge at 2600 g for 10 min. Evaporate a 2.5 mL aliquot of the upper organic layer to dryness under nitrogen at 40°, reconstitute with 100 μL mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.6 5 μm Nucleosil 120-5 C18
Column: 250 × 4 5 μm Nucleosil 120-5 C 18
Mobile phase: MeOH:buffer 47:53 (Buffer was 100 mM Na_2HPO_4 adjusted to pH 7.8 with orthophosphoric acid.)
Column temperature: 37
Flow rate: 1.2
Injection volume: 25
Detector: UV 302

CHROMATOGRAM
Retention time: 10.1
Internal standard: flunitrazepam (11.4)
Limit of quantitation: 9.7 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE
Macek,J.; Ptácek,P.; Klíma,J. Determination of omeprazole in human plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *689*, 239–243.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

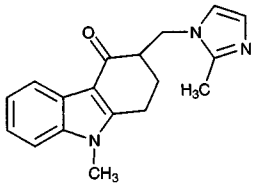
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 14.065

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Ondansetron



Molecular formula: $C_{18}H_{19}N_3O$
Molecular weight: 293.37
CAS Registry No.: 99614-02-5, 116002-70-1, 99614-01-4 (HCl dihydrate), 103639-04-9 (HCl dihydrate)
Merck Index: 6979
Lednicer No.: 5 164

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: MeCN:20 mM KH_2PO_4 43:57
Flow rate: 1.8
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 9.6

OTHER SUBSTANCES
Simultaneous: idarubicin

KEY WORDS
0.9% NaCl; injections

REFERENCE
Zhang,H.; Ye,L.; Stewart,J.T. HPLC determination of idarubicin-etoposide and idarubicin-ondansetron mixtures in 0.9% sodium chloride injection USP, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 979–988.

SAMPLE
Matrix: formulations
Sample preparation: If necessary, dilute injection 1:9 with mobile phase (for 50 mL admixtures) and 1:4 (for 100 mL admixtures), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 60 RP-Select B
Mobile phase: MeCN:buffer 25:75 (Buffer was 20 mM KH_2PO_4 adjusted to pH 6.0 with NaOH solution.)
Injection volume: 20
Detector: UV 241

CHROMATOGRAM
Retention time: 12.5

OTHER SUBSTANCES
Simultaneous: dexamethasone

KEY WORDS
5% dextrose; 0.9% sodium chloride; injections; stability-indicating

REFERENCE
Evrard,B.; Ceccato,A.; Gaspard,O.; Delattre,L.; Delporte,J.-P. Stability of ondansetron hydrochloride and dexamethasone sodium phosphate in 0.9% sodium chloride injection and in 5% dextrose injection, *Am.J.Health-Syst.Pharm.*, **1997**, *54*, 1065–1068.

SAMPLE
Matrix: solutions
Sample preparation: Add 380 μg propofol and 83 μg ondansetron hydrochloride to 0.9% sodium chloride, shake vigorously for 2 min, make up to 10 mL with 0.9% sodium chloride, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4.6 10 μm T-Bondapak phenyl
Mobile phase: MeCN:buffer 50:50 (Buffer was 10 mM KH_2PO_4 adjusted to pH 4.0 with 10% phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 268

CHROMATOGRAM
Retention time: 8.5
Limit of detection: 61 ng/mL

OTHER SUBSTANCES
Simultaneous: propofol

REFERENCE
King,D.T.; Stewart,J.T.; Venkateshwaran,T.G. HPLC determination of propofol-thiopental sodium and propofol-ondansetron mixtures, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2285–2294.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a solution in 0.9% sodium chloride.

HPLC VARIABLES
Column: 220 × 4.6 5 μm underivatized silica (Brownlee Silica Applied Biosystems, Inc.,San Jose)
Mobile phase: MeOH:buffer 40:60 (Buffer was 10 mM KH_2PO_4 adjusted to pH 4.0 with 10% phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6.7
Limit of detection: 47 ng/mL

OTHER SUBSTANCES
Simultaneous: meperidine, morphine (UV 233)

REFERENCE
Venkateshwaran,T.G.; Stewart,J.T.; King,D.T. HPLC determination of morphine-ondansetron and meperidine-ondansetron mixtures in 0.9% sodium chloride injection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1329–1338.

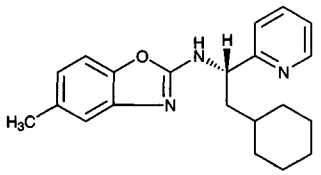
SAMPLE
Matrix: solutions

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 detection

KEY WORDS
14C labeled

REFERENCE
Collett,A.; Sims,E.; Walker,D.; He,Y.-L.; Ayrton,J.; Rowland,M.; Warhurst,G. Comparison of HT29-18-C_1 and Caco-2 cell lines as models for studying intestinal paracellular drug absorption, *Pharm.Res.*, **1996**, *13*, 216–221.

Ontazolast



Molecular formula: $C_{21}H_{25}N_3O$
Molecular weight: 335.45
CAS Registry No.: 147432-77-7

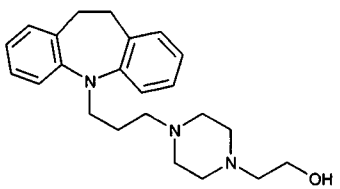
SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax LC-8
Mobile phase: MeCN:MeOH:50 mM pH 6 phosphate buffer 1:3:8
Flow rate: 2
Detector: UV 245

CHROMATOGRAM
Retention time: 7
Internal standard: BIRM 390 BS (10.5)
Limit of quantitation: 5 ng/mL

REFERENCE
Hauss,D.J.; Fogal,S.E.; Ficorilli,J.V.; Price,C.A.; Roy,T.; Jayaraj,A.A.; Keirns,J.J. Lipid-based delivery systems for improving the bioavailability and lymphatic transport of a poorly water-soluble LTB4 inhibitor, *J.Pharm.Sci.*, **1998**, *87*, 164–169.

Opipramol



Molecular formula: $C_{23}H_{29}N_3O$
Molecular weight: 363.50
CAS Registry No.: 315-72-0, 909-39-7 (2.HCl)
Merck Index: 6985

SAMPLE
Matrix: blood
Sample preparation: Deproteinize 100 μL plasma with 100 μL MeCN containing 2.0 mg/L IS. Inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 4 × 4 Superspher

Column: 125 × 4 Superspher 60 select B
Mobile phase: MeCN:0.07% orthophosphoric acid 20:80
Column temperature: 30
Flow rate: 1.2
Injection volume: 50
Detector: UV 210, UV 255

CHROMATOGRAM
Retention time: 4.1
Internal standard: methylphenyl-phenylhydantion (7.8)
Limit of detection: 50 μg/L

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE
Lappenberg-Pelzer,M. Identification and determination of opipramol metabolites in plasma and urine, *J.Anal.Toxicol.*, **1998**, *22*, 215–219.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 256

CHROMATOGRAM
Retention time: 7.62
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acen-

ocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 255.8

CHROMATOGRAM

Retention time: 14.163

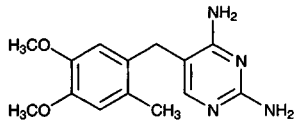
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Ormetoprim



Molecular formula: $C_{14}H_{18}N_4O_2$
Molecular weight: 274.32
CAS Registry No.: 6981-18-6
Lednicer No.: 2 302

SAMPLE

Matrix: blood, tissue
Sample preparation: Plasma. 200 µL Plasma + 90 µL 24% trichloroacetic acid in MeOH + 10 µL 10 µg/mL sulfamethoxazole in MeOH, vortex for 30 s, centrifuge at 14000 g for 5 min, inject a 50 µL aliquot of the supernatant. Muscle. Homogenize 1 g muscle in 1.5 mL MeOH:buffer 20:80, add 50 µL 10 µg/mL sulfamethoxazole in MeOH, mix thoroughly for 1 min, centrifuge at 14000 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was 25 mM NaH_2PO_4 containing 15 mM sodium 1-heptanesulfonate adjusted to pH 2.8 with 5 M phosphoric acid.)

HPLC VARIABLES

Guard column: 20 × 4.6 40 µm ODS-Hypersil
Column: 150 × 4.6 3 µm ODS-Hypersil C18
Mobile phase: MeCN:buffer:triethylamine 20:80:0.02 (Buffer was 25 mM NaH_2PO_4 containing 15 mM sodium 1-heptanesulfonate adjusted to pH 2.8 with 5 M phosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: UV 270

CHROMATOGRAM

Retention time: 12.5
Internal standard: sulfamethoxazole (8)
Limit of detection: 50 ng/g (muscle), 30 ng/mL (plasma)

OTHER SUBSTANCES

Extracted: sulfadimethoxine

KEY WORDS

plasma; muscle; fish; salmon

REFERENCE

Samuelsen,O.B. Simultaneous determination of ormethoprim and sulphadimethoxine in plasma and muscle of Atlantic salmon *(Salmo salar)*, *J.Chromatogr.B*, **1994**, *660*, 412–417.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a solution in MeCN:MeOH:100 mM pH 4.5 acetate buffer 12.5:12.5:75, 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: 8

OTHER SUBSTANCES

Simultaneous: sulfamethoxazole, trimethoprim

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: tissue
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL water. 5 g Fish + 1 mL 100 μg/mL carbamazepine diol in MeOH + 15 mL MeCN + 500 μL 50% trichloroacetic acid, homogenize (Brinkmann Polytron PT 10/35) at medium speed for 30 s, centrifuge at 4° at 7800 g for 25 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 5 mL water, vortex for 30 s, filter (13 mm dia. 8 μm Membra-Fil (Nucleopore)), add the filtrate to the SPE cartridge, elute with 5 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 1 mL mobile phase, vortex for 30 s, inject a 20 μL aliquot. (Flush injection valve with 1 mL mobile phase between analyses.)

HPLC VARIABLES
Guard column: 15 × 3.2 NewGuard RP-18
Column: 250 × 4.6 5 μm Ultrasphere
Mobile phase: MeCN:MeOH:100 mM pH 4.0 phosphate buffer 17:10:73
Flow rate: 1
Injection volume: 20
Detector: UV 280

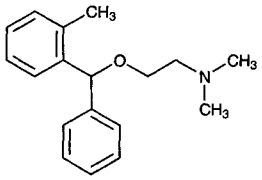
CHROMATOGRAM
Retention time: 5.5
Internal standard: carbamazepine diol (10.5)
Limit of quantitation: 0.2 ppm

OTHER SUBSTANCES
Extracted: sulfadimethoxine
Simultaneous: sulfacetamide, sulfadiazine, sulfamerazine, sulfamethazine, sulfisoxazole

KEY WORDS
fish; salmon; SPE; pharmacokinetics

REFERENCE
Walisser,J.A.; Burt,H.M.; Valg,T.A.; Kitts,D.D.; McErlane,K.M. High-performance liquid chromatographic analysis of Romet-30 in salmon following administration of medicated feed, *J.Chromatogr.*, **1990**, *518*, 179–188.

Orphenadrine



Molecular formula: $C_{18}H_{23}NO$
Molecular weight: 269.39
CAS Registry No.: 83-98-7, 4682-36-4 (citrate)
Merck Index: 7007
Lednicer No.: 1 42

SAMPLE
Matrix: blood
Sample preparation: Add 300 μL MeCN to 100 μL plasma, vortex, centrifuge for 2 min. Remove the supernatant and add it to 300 μL pH 5.9 sodium phosphate buffer and 3 mL hexane and vortex for 45 s. Centrifuge at 2500 g for 3 min. Evaporate the supernatant under a stream of nitrogen and reconstitute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 ODS

Mobile phase: MeCN:MeOH:25 mM potassium phosphate 25:25:50 containing 0.75 mL/L 2 M sulfuric acid and 0.25 mL/L triethylamine
Flow rate: 1.0
Detector: UV 250

CHROMATOGRAM
Retention time: 5.7
Internal standard: orphenadrine

OTHER SUBSTANCES
Extracted: ethopropazine

KEY WORDS
plasma; rat; pharmacokinetics; orphenadrine is IS

REFERENCE
Padovani,P.K.; Timby,D.M.; Wright,M.R.; Kapil,R.P. Quantitative analysis of DMP 851 in rat and dog plasma by liquid-liquid extraction and reverse-phase high performance liquid chromatography with ultraviolet detection (Abstract 3318), *Pharm.Res.*, **1997**, *14*, S568.

SAMPLE
Matrix: blood
Sample preparation: 10 mL Plasma or whole blood + 1 mL 1 M NaOH, extract twice with 10 mL hexane for 30 min. Remove the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL 100 mM HCl, add 5 mL chloroform, vortex for 1 min, centrifuge. Remove a 4.5 mL aliquot of the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot. (It is implied, but not explicitly stated in the paper, that this extraction procedure works for this compound.)

HPLC VARIABLES
Column: 10 μm Micropak CN (Varian)
Mobile phase: MeCN:20 mM ammonium acetate 90:10
Flow rate: 2.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 8.2
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: acetophenazine, amitriptyline, benztropine, butaperazine, carphenazine, fluphenazine, haloperidol, imipramine, mesoridazine, nortriptyline, piperacetazine, promazine, promethazine, thioridazine, thiothixene, trifluoperazine, triflupromazine, trihexyphenidyl, trimeprazine
Interfering: chlorpromazine

KEY WORDS
plasma; whole blood

REFERENCE
Curry,S.H.; Brown,E.A.; Hu,O.Y.-P.; Perrin,J.H. Liquid chromatographic assay of phenothiazine, thioxanthene and butyrophenone neuroleptics and antihistamines in blood and plasma with conventional and radial compression columns and UV and electrochemical detection, *J.Chromatogr.*, **1982**, *231*, 361–376.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 3.6

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 cellulose tris(3,5-dimethylphenylcarbamate)
Mobile phase: Hexane:isopropanol 90:10
Flow rate: 0.5
Detector: UV

CHROMATOGRAM
Retention time: k' 0.56 (of first (+) enantiomer)

KEY WORDS
chiral; α 1.89

REFERENCE
Okamoto,Y.; Aburatani,R.; Hatano,K.; Hatada,K. Optical resolution of racemic drugs by chiral HPLC on cellulose and amylose tris(phenylcarbamate) derivatives, *J.Liq.Chromatogr.*, **1988**, *11*, 2147–2163.

SAMPLE
Matrix: solutions

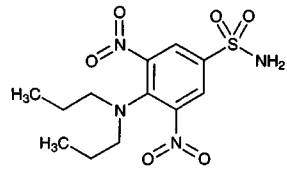
HPLC VARIABLES
Column: 150 × 3.9 5 μm Spherisorb C8
Mobile phase: MeCN:buffer 60:40 (Buffer was 1.5 mL triethylamine in 1 L water adjusted to pH 3.0 with 85% phosphoric acid.)
Flow rate: 1.5
Detector: UV 199

CHROMATOGRAM
Retention time: k' 3.90

OTHER SUBSTANCES
Simultaneous: hyoscyamine, bromocriptine, benztropine, biperiden
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.

Oryzalin



Molecular formula: $C_{12}H_{18}N_4O_6S$
Molecular weight: 346.36
CAS Registry No.: 19044-88-3
Merck Index: 7015

SAMPLE
Matrix: blood, microsomal incubations
Sample preparation: Condition a Bond Elut C18 SPE cartridge with 3 mL MeCN and 3 mL water. Plasma. Add 500 μL plasma to the SPE cartridge, dry with vacuum. Elute with three 200 μL portions of MeCN. Inject a 10-50 μL aliquot of the eluate. Microsomal incubations. Centrifuge microsomal incubation at 12000 g. Add 900 μL portion of the supernatant to the SPE cartridge, dry with vacuum. Elute with three 200 μL portions of MeCN. Inject a 10-50 μL aliquot of the eluate.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Adsorbosphere HS C18
Mobile phase: MeCN:water 53:47
Flow rate: 2
Injection volume: 10-50
Detector: UV 254; MS, Finnigan TSQ tandem mass, APCI, vaporizer 450°, corona discharge current 5 μA, CID, neutral argon -31 eV

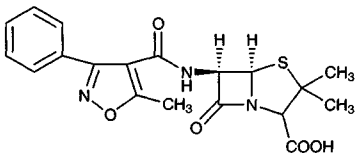
CHROMATOGRAM
Retention time: 4.7
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
SPE; plasma; mouse; rat; liver; pharmacokinetics

REFERENCE
Dvorakova,K.; Dorr,R.T.; Gallegos,A.; McClure,T.; Powis,G. Pharmacokinetic studies of the herbicide and antitumor compound oryzalin in mice, *J.Chromatogr.B*, **1997**, *696*, 275–281.

Oxacillin



Molecular formula: $C_{19}H_{19}N_3O_5S$
Molecular weight: 401.44
CAS Registry No.: 66-79-5, 7240-38-2 (Na salt monohydrate), 1173-88-2 (Na salt)
Merck Index: 7036
Lednicer No.: 1 413

SAMPLE
Matrix: blood
Sample preparation: 400 µL Serum + 400 µL MeCN, vortex for 10 s, shake slowly for 15 min, centrifuge at 3000 g for 10 min. Remove the supernatant and add it to 4 mL dichloromethane, vortex for 10 s, shake for 15 min, centrifuge at 3000 g for 10 min, inject a 50 µL aliquot of the upper aqueous layer.

HPLC VARIABLES
Column: µBondapak C18
Mobile phase: MeCN:water:200 mM ammonium acetate 28:62:10, pH 5.6
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 5.7
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: cloxacillin, dicloxacillin, methicillin, nafcillin
Noninterfering: amdinocillin (mecillinam), amikacin, amoxicillin, ampicillin, carbenicillin, cefachmandole, cefazolin, ceforanide, cefatoxamine, cefoxitin, cephalexin, cephaloridine, cephalothin, cephradine, cepharin, chloramphenicol, clindamycin, co-trimoxazole, fluorocytosine, gentamicin, metronidazole, moxalactam, penicillin, piperacillin, sulfamethoxazole, theophylline, ticarcillin, tobramycin, trimethoprim, vancomycin

KEY WORDS
serum

REFERENCE
Rudrik,J.T.; Bawdon,R.E. Determination of penicillinase-resistant penicillins in serum using high-pressure liquid chromatography, *J.Liq.Chromatogr.*, **1981**, *4*, 1525–1545.

SAMPLE
Matrix: blood, milk
Sample preparation: Milk. Adjust 5 mL milk to pH 6.3 with 100 mM HCl, deproteinize with 10 mL MeCN. Centrifuge at 1932 g for 20 min and extract the aqueous phase with two 5 mL

portions of chloroform for 20 min (Caution! Chloroform is a carcinogen!). Centrifuge at 1932 g for 20 min. Evaporate the organic phase to dryness, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot. Serum. Adjust 2.5 mL serum to pH 6.3 with 100 mM HCl, deproteinize with 10 mL MeCN. Centrifuge at 1932 g for 20 min and extract the aqueous phase with two 5 mL portions of dichloromethane for 20 min. Centrifuge at 1932 g for 20 min. Evaporate the organic phase to dryness, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot

HPLC VARIABLES
Column: 15 × 3.9 4 μm Nova Pack C18
Mobile phase: MeCN:20 mM KH_2PO_4 21:79, pH 5
Flow rate: 1.2
Injection volume: 100
Detector: UV 225

CHROMATOGRAM
Retention time: 3.8
Internal standard: oxacillin

OTHER SUBSTANCES
Extracted: cloxacillin

KEY WORDS
serum; cow; oxacillin is IS

REFERENCE
Pérez,B.; Prats,C.; Castells,E.; Arboix,M. Determination of cloxacillin in milk and blood of dairy cows by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *698*, 155–160.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma, serum. 100 μL Plasma or serum + cloxacillin + 100 μL 500 mM pH 2.2 citric acid buffer + 20 μL 500 mM HCl + 2.5 mL dichloromethane, extract. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in mobile phase, inject an aliquot. Urine. Dilute urine with water, inject an aliquot.

HPLC VARIABLES
Guard column: 50 × 2.1 ODS pellicular
Column: 250 × 4.6 5 μm Lichrosorb RP-8
Mobile phase: MeCN:20 mM pH 6.6 sodium acetate 34:100
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 7
Internal standard: cloxacillin (9)
Limit of detection: 400 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, dicloxacillin, flucloxacillin

KEY WORDS
plasma; serum

REFERENCE
Thijssen,H.H.W. Analysis of isoxazolyl penicillins and their metabolites in body fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *183*, 339–345.

SAMPLE
Matrix: blood, urine

Sample preparation: Plasma. 1 mL Plasma + 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. Make up 5-100 μL urine 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 NaH_2PO_4 5:95; B MeCN:33 mM NaH_2PO_4 20:80
Injection volume: 500
Detector: UV 210

CHROMATOGRAM

Internal standard: oxacillin

OTHER SUBSTANCES

Extracted: penicillin V

KEY WORDS

plasma; column-switching; ultrafiltrate; oxacillin is IS

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: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 14.76

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Blend tablets and capsules with water in a high-speed blender for 5 min, filter, dilute with mobile phase, inject a 20 μL aliquot. Dilute oral suspensions and injections with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 70 mm long Co:Pell ODS
Column: 300 × 4.6 10 μm Chromegabond C18 (E.S. Industries)
Mobile phase: MeCN:MeOH:10 mM KH_2PO_4 19:11:70
Flow rate: 1
Injection volume: 20
Detector: UV 225

CHROMATOGRAM
Retention time: 10.0
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Simultaneous: amoxicillin, ampicillin, cloxacillin, dicloxacillin, methicillin, nafcillin, 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: Mix 10 mL milk with 2 mL 100 mM tetraethylammonium chloride, add 40 mL MeCN slowly with continual stirring, let stand for 10 min, decant the supernatant through a plug of glass wool. Collect 40 mL filtrate, add 2 mL buffer, evaporate to 1-2 mL under reduced pressure at 40-50°, dilute to 4 mL with water, filter (0.45 μm PVDF). Inject a 2 mL aliquot onto a 150 × 4.6 5 μm Supelcosil LC-18 column, elute with MeCN:10 mM KH_2PO_4 0:100 for 3 min, to 60:40 over 37 min at 1 mL/min, collect a 1.5-2 mL aliquot containing the compound (ca. 27.0 min), evaporate to <1 mL under reduced pressure, make up to 1 mL with water, inject an aliquot. (Prepare the buffer by mixing 10 mM KH_2PO_4 and 10 mM Na_2HPO_4 in a 5:1 ratio, pH 6.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-18-DB
Mobile phase: MeCN:buffer 38:62 (Buffer was 2 mM phosphoric acid containing 8 mM potassium dihydrogen phosphate.)
Flow rate: 1
Injection volume: 200
Detector: UV 215

REFERENCE
Moats,W.A.; Romanowski,R.D. Multiresidue determination of β-lactam antibiotics in milk and tissues with the aid of high-performance liquid chromatographic fractionation for clean up, *J.Chromatogr.A*, **1998**, *812*, 237–247.

SAMPLE
Matrix: milk
Sample preparation: Condition a 6 mL 500 mg Bond Elut C18 SPE cartridge with 10 mL MeOH, 10 mL water, 5 mL 2% NaCl, and 5 mL 100 mM pH 8 phosphate extraction buffer. Add 30 mL 100 mM pH 8 phosphate extraction buffer to 5 mL milk, add 1.65 mL 1 M sulfuric acid to reach pH 4.0-4.5, vortex for 30 s, centrifuge at 2400 g for 10 min, add 600 μL 5 M NaOH to the supernatant to reach pH 8, vortex, centrifuge at 2400 g for 5 min. Add the supernatant

to a reservoir attached to the SPE cartridge, pull through the SPE cartridge at 3 mL/min, remove the reservoir and elute with 1 mL MeCN:water 40:60. Add 500 μL derivatizing reagent to the eluate, vortex, heat at 65° for 10 min, cool to room temperature (protect from light), inject a 100 μL aliquot of the derivatized sample. (Prepare the 100 mM pH 8 phosphate extraction buffer as follows. Dissolve 15.6 g K_2HPO_4 dihydrate in 800 mL water, adjust pH to 8 with 10 M NaOH, make up to 1 L. Prepare the derivatizing reagent as follows. Weigh out 13.78 g 1,2,4-triazole, add 60 mL water, stir, add 10 mL 100 mM mercuric chloride solution, mix, adjust pH to 9.0 ± 0.5 with 5 M NaOH, dilute to 100 mL with water.)

HPLC VARIABLES

Column: 150 × 3.9 5 μm Symmetry C8 (Waters)

Mobile phase: MeCN:MeOH:buffer 37:5:58 (Prepare the 100 mM pH 6.5 phosphate buffer containing 15 mM thiosulfate and 30 mM tetrabutylammonium hydrogen sulfate as follows. Weigh 4.969 g anhydrous NaH_2PO_4, 10.139 g Na_2HPO_4 dihydrate, 3.894 g sodium thiosulfate pentahydrate, and 10.186 g tetrabutylammonium hydrogen sulfate, dissolve in 800 mL water, adjust pH to 6.5 with 5 M NaOH, dilute to 1 L with water, mix thoroughly, filter under vacuum (0.45 μm).)

Flow rate: 1

Injection volume: 100

Detector: UV 340

CHROMATOGRAM

Retention time: 10

Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Extracted: cloxacillin, dicloxacillin

KEY WORDS

derivatization; SPE

REFERENCE

Verdon,E.; Couedor,P. Determination of isoxazolylpenicillins residues in milk by ion-pair reversed-phase high-performance liquid chromatography after precolumn derivatization, *J.Chromatogr.B*, **1998**, *705*, 71–78.

SAMPLE

Matrix: milk

Sample preparation: 50 g Milk + 2 drops penicillinase (Difco Laboratories), let stand 1 h at 37°, add 50 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 centrifuging at 1000 g for 10 min, filter dichloromethane extracts through 30 g anhydrous sodium sulfate, evaporate to dryness under reduced pressure at 35°, if water remains add 5-10 mL MeOH to flask and complete evaporation. Dissolve residue in 1 mL 10% acetic acid, add 0.5 mL 0.08% dansyl hydrazine in 10% acetic acid, let stand 90 min to overnight in the dark, transfer reaction mixture to a separatory funnel with three 25 mL portions of dichloromethane, add 5 mL 2 M HCl, shake for 1 min, wash organic layer with 5 mL 5% $NaHCO_3$ solution, filter through 10-20 g anhydrous sodium sulfate. Extract acid aqueous layer again with 25 mL dichloromethane. Combine dichloromethane layers and evaporate to dryness at 35° under reduced pressure. Dissolve residue in 2 mL IS solution, inject a 20 μL aliquot. (Prepare IS solution by dissolving 10 μL benzaldehyde in 100 mL dichloromethane, evaporate 1 mL to dryness under reduced pressure, dissolve residue in 1 mL 10% acetic acid, add 0.5 mL 0.08% dansyl hydrazine in 10% acetic acid, let stand 90 min to overnight in the dark, transfer reaction mixture to a separatory funnel with three 25 mL portions of dichloromethane, add 5 mL 2 M HCl, shake for 1 min, wash organic layer with 5 mL 5% $NaHCO_3$ solution, filter through 10-20 g anhydrous sodium sulfate. Extract acid aqueous layer again with 25 mL dichloromethane. Combine dichloromethane layers and evaporate to dryness at 35° under reduced pressure. Dissolve residue in 100 mL MeCN then dilute an aliquot 1:4 with MeCN.)

HPLC VARIABLES
Column: 250 × 4 10 μm Lichrosorb RP-18
Mobile phase: MeCN:water 58:42
Flow rate: 1
Injection volume: 20
Detector: F ex 254 em 500 filter

CHROMATOGRAM
Retention time: 7.21
Internal standard: benzaldehyde (derivatized) (12.18)
Limit of detection: 5 ng/g

OTHER SUBSTANCES
Extracted: penicillin G, phenethicillin, methicillin, cloxacillin, dicloxacillin, nafcillin
Interfering: penicillin V, phenethicillin

KEY WORDS
derivatization

REFERENCE
Munns,R.K.; Shimoda,W.; Roybal,J.E.; Vieira,C. Multiresidue method for determination of eight neutral β-lactam penicillins in milk by fluorescence-liquid chromatography, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 968–971.

SAMPLE
Matrix: milk
Sample preparation: Add 2 volumes MeCN to milk, stand 5 min, decant aqueous portion, suction filter, extract with an equal volume of 1:1 methylene chloride:hexane, centrifuge aqueous phase at 3000 rpm for 10 min. Dilute 3:1 with 20 mM sodium acetate buffer and filter (0.2 μm nylon). Inject 50 μL onto column with mobile phase A, run mobile phase A for 30 min and elute to waste. After 30 min switch to mobile phase B and elute through detector.

HPLC VARIABLES
Column: 100 × 8 Radial-Pak 10 μm μBondapak C18
Mobile phase: A 20 mM sodium acetate buffer; B Gradient. MeCN:MeOH:20 mM sodium acetate buffer from 15:10:75 to 30:0:70 over 15 min and hold at 30:0:70
Flow rate: A 3; B 2
Injection volume: 50
Detector: E, Waters 464 pulsed electrochemical detector using a thin layer cell with a Ag/AgCl reference electrode. E1 = 1300 mV for 0.166 s, E2 = 1500 mV for 0.166 s, E3 = -200 mV for 0.333 s.

CHROMATOGRAM
Retention time: 13.4
Limit of detection: 0.2 ppm

OTHER SUBSTANCES
Extracted: penicillin V, ampicillin, methicillin, penicillin G, cloxacillin, nafcillin, dicloxacillin

KEY WORDS
column-switching

REFERENCE
Kirchmann,E.; Earley,R.L.; Welch,L.E. The electrochemical detection of penicillins in milk, *J.Liq.Chromatogr.*, **1994**, *17*, 1755–1772.

SAMPLE
Matrix: milk
Sample preparation: Condition a Bond Elut C8 SPE cartridge with 5 mL MeOH and 5 mL water. 20 mL Milk + 20 mL buffer, heat at 60° for 20 min or until milk curdles, centrifuge for 10 min, add the supernatant to the SPE cartridge, wash with two 2.5 mL portions of water,

elute with 2.5 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, extract the residue with three 100 μL portions of 50 mM pH 6.0 potassium phosphate buffer, filter (0.2 μm), inject an aliquot of the filtrate. (Buffer was 545 mL 100 mM citric acid, 455 mL 200 mM Na_2HPO_4, and 74.4 g EDTA, adjust to pH 4.5 with ammonium hydroxide, make up to 2 L with water.)

HPLC VARIABLES
Column: 250 × 4.6 10 μm Lichrosorb RP-8
Mobile phase: MeOH:50 mM pH 6.0 potassium phosphate buffer 35:65
Flow rate: 1
Injection volume: 200
Detector: UV 210 or Charm II assay

CHROMATOGRAM
Retention time: 33.10

OTHER SUBSTANCES
Extracted: ampicillin, ceftiofur, cephapirin, cloxacillin, dicloxacillin, nafcillin, penicillin G
Simultaneous: amoxicillin

KEY WORDS
SPE

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: milk
Sample preparation: Condition a Bond Elut C8 SPE cartridge with 5 mL MeOH and 5 mL water. 20 mL Milk + 20 mL buffer, heat at 60° for 20 min or until milk curdles, centrifuge for 10 min, add the supernatant to the SPE cartridge, wash with two 2.5 mL portions of water, elute with 2.5 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, extract the residue with three 100 μL portions of 50 mM pH 6.0 potassium phosphate buffer, filter (0.2 μm), inject an aliquot of the filtrate. (Buffer was 545 mL 100 mM citric acid, 455 mL 200 mM Na_2HPO_4, and 74.4 g EDTA, adjust to pH 4.5 with ammonium hydroxide, make up to 2 L with water.)

HPLC VARIABLES
Column: 250 × 4.6 10 μm Lichrosorb RP-8
Mobile phase: MeOH:50 mM pH 6.0 potassium phosphate buffer 35:65
Flow rate: 1
Injection volume: 200
Detector: UV 210 or Charm II assay

CHROMATOGRAM
Retention time: 33.10

OTHER SUBSTANCES
Extracted: ampicillin, ceftiofur, cephapirin, cloxacillin, dicloxacillin, nafcillin, penicillin G
Simultaneous: amoxicillin

KEY WORDS
SPE

REFERENCE
Zomer,E.; Quintana,J.; Saul,S.; Charm,S.E. LC-Receptograms: A method for identification and quantitation of β-lactams in milk by liquid chromatography with microbial receptor assay, *J.AOAC Int.*, **1995**, *78*, 1165–1172.

SAMPLE

Matrix: milk

Sample preparation: Condition a 500 mg tC18 SPE cartridge (Waters) with 20 mL MeOH, 20 mL water, and 10 mL 2% NaCl. Centrifuge 30 mL milk at 1500 g for 10 min. Dilute a 10 mL portion of the defatted milk with 20 mL water, add 200 μL 2 μg/mL penicillin V in pH 9.0 buffer, add 6 mL 170 mM sulfuric acid, add 5.6 mL 5% sodium tungstate, shake vigorously for 1 min, allow to stand for 5 min, check that the pH is in the range 4.6-4.8 (if it is outside this range start again using a different volume of sodium tungstate solution), centrifuge at 1500 g for 10 min, adjust the pH of the supernatant to 8.1-8.2 with 5 M and 0.1 M NaOH, filter (glass fiber) the clear liquid phase. Pass the filtrate through the SPE cartridge at 2 mL/min, wash with 2 mL water, dry by pulling air through the cartridge for 1 min, elute with 2 mL MeCN. Add 150 μL pH 9.0 buffer to the eluate and evaporate to about 100 μL under a stream of nitrogen at 45-50°, add 400 μL pH 9.0 buffer, add 75 μL reagent I, vortex for 30 s, let stand at room temperature for 10 min, use 500 μL water to transfer the mixture to a separatory funnel, add 20 mL dichloromethane, add 5 mL pH 2.45 buffer, shake for 1 min, let stand for no more than 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure at 35-40°, dissolve the residue in 500 μL pH 9.0 buffer, add 75 μL reagent I, vortex for 30 s, let stand at room temperature for 10 min, add 450 μL reagent II, vortex for 1 min, heat at 55 ± 1° for 30 min, cool, filter (0.45 μm), inject a 150 μL aliquot. (Prepare pH 9.0 buffer by dissolving 0.34 g KH_2PO_4 in water, adjusting the pH to 9.0 with NaOH, and making up to 100 mL with water. Prepare pH 2.45 buffer by dissolving 2.72 g KH_2PO_4 in water, adjusting the pH to 2.45 with phosphoric acid, and making up to 100 mL with water. Prepare reagent 1 by dissolving 1.13 g benzoic anhydride in MeCN, make up to 25 mL with MeCN. Prepare reagent II by dissolving 6.905 g 1,2,4-triazole in 30 mL water and adding 5 mL 26 mM mercuric chloride in water, adjust pH to 9.0 ± 0.05 with 5 M NaOH, make up to 50 mL. Prepare reagents I and II 1-4 h before use. Silanize glassware with dichlorodimethylsilane.)

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18

Mobile phase: Gradient. A as MeCN:buffer 10:90. B was MeCN:buffer 30:70. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 13 min, return to initial conditions over 2 min, re-equilibrate at initial conditions for 5 min. (Prepare buffer by dissolving 9.938 g Na_2HPO_4, 17.938 g $NaH_2PO_4.H_2O$, and 4.964 g sodium thiosulfate in water, make up to 2 L with water, pH 6.5.)

Column temperature: 30

Flow rate: 1

Injection volume: 150

Detector: UV 323

CHROMATOGRAM

Retention time: 34

Internal standard: penicillin V (28.5)

Limit of detection: 1.4 ng/mL

Limit of quantitation: 1.9 ng/mL

OTHER SUBSTANCES

Extracted: amoxicillin, ampicillin, cloxacillin, dicloxacillin, penicillin G

KEY WORDS

derivatization; cow; SPE

REFERENCE

Sorensen,L.K.; Rasmussen,B.M.; Boison,J.O.; Keng,L. Simultaneous determination of six penicillins in cows' raw milk by a multiresidue high-performance liquid chromatographic method, *J.Chromatogr.B*, **1997**, *694*, 383–391.

SAMPLE

Matrix: perfusate

Sample preparation: 200 μL Perfusate + 300 μL MeCN, mix, centrifuge for 5 min, inject a 15 μL aliquot.

HPLC VARIABLES

Guard column: 50 × 3.6 LiChrosorb RP-2

Column: Chemcosorb 5-ODS-H (Chemco, Japan)
Mobile phase: MeOH:pH 5.2 acetate buffer 50:50
Column temperature: 40
Flow rate: 1
Injection volume: 15
Detector: UV 230

CHROMATOGRAM
Limit of quantitation: 2 μg/mL

KEY WORDS
rat; liver; pharmacokinetics

REFERENCE
Yasui,H.; Yamaoka,K.; Fukuyama,T.; Nakagawa,T. Effect of liver intoxication by carbon tetrachloride in hepatic local disposition of oxacillin using moment characteristics as index, *Drug Metab.Dispos.*, **1995**, *23*, 779–785.

SAMPLE
Matrix: perfusate
Sample preparation: Inject a 15 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Chemcosorb 5-ODS-H (Chemco, Osaka)
Mobile phase: MeOH:acetate buffer 50:50, pH 5.2
Flow rate: 1
Injection volume: 15
Detector: UV 220

CHROMATOGRAM
Limit of quantitation: 3 μg/mL

KEY WORDS
rat

REFERENCE
Ohata,Y.; Yamaoka,K.; Yasui,H.; Nakagawa,T. Consideration on moments of outflow profile in liver perfusion system with change in perfusate flow rate using oxacillin as model drug, *Biol.Pharm.Bull.*, **1996**, *19*, 83–87.

SAMPLE
Matrix: solutions
Sample preparation: React the antibiotic, triethylamine, and 1-(2,5-dihydroxyphenyl)-2-bromoethanone in a 1:2:4 molar ratio in DMF at 45° for 2 h (use dibenzo-18-crown-6 to make the sodium salt soluble), inject a 10 μL aliquot. (Preparation of 1-(2,5-dihydroxyphenyl)-2-bromoethanone is as follows. Stir 27.6 g 1,4-dimethoxybenzene and 28 mL bromoacetyl bromide at 0°, add 53.4 g aluminum bromide over 10 min (an exothermic reactions ensues), let stand at room temperature for 12 h, add 100 mL 48% HBr, add 100 g ice, stir for 1 h, extract twice with 200 mL portions of diethyl ether. Combine the extracts and wash them 3 times with 200 mL portions of water, dry over 40 g anhydrous magnesium sulfate, evaporate to dryness, recrystallize the product 3 times from EtOH to yield 1-(2,5-dihydroxyphenyl)-2- bromoethanone monobromoacetate (mp 105-107°). Dissolve 11 g 1-(2,5-dihydroxyphenyl)-2-bromoethanone monobromoacetate in 200 mL warm dry MeOH saturated with HBr, stir for 18 h, add 200 mL water, cool to -10°. Collect the yellow solid and dry it under vacuum at 50° for 48 h, recrystallize from toluene:heptane 50:50 then toluene to obtain 1-(2,5-dihydroxyphenyl)-2-bromoethanone as yellow needles (mp 117-119°).)

HPLC VARIABLES
Column: 250 × 4 7 μm RP-18 LiChrocart (Merck)
Mobile phase: MeOH:100 mM pH 6.5 sodium acetate 58:42
Flow rate: 1

Injection volume: 10
Detector: E, Bioanalytical Systems Model LC4B, glassy carbon electrode 0.8 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 19

OTHER SUBSTANCES
Extracted: carbenicillin, cephapirin, cloxacillin, dicloxacillin, hetacillin, methicillin, nafcillin, penicillin G

KEY WORDS
derivatization

REFERENCE
Munns,R.K.; Roybal,J.E.; Shimoda,W.; Hurlbut,J.A. 1-(4-Hydroxyphenyl)-, 1-(2,4-dihydroxyphenyl)- and 1-(2,5-dihydroxyphenyl)-2-bromoethanones: new labels for determination of carboxylic acids by high-performance liquid chromatography with electrochemical and ultraviolet detection, *J.Chromatogr.*, **1988**, *442*, 209–218.

SAMPLE
Matrix: solutions
Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate 25:75
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 220

OTHER SUBSTANCES
Also analyzed: penicillin V

REFERENCE
Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax) 25 g tissue with 25 mL MeCN for 1 min, add 5 mL 500 mM pH 2.2 phosphate buffer while the homogenizer is still running, add 65 mL MeCN, homogenize for 1 min, centrifuge at 4000 g for 10 min. Remove the supernatant and add it to 7 g NaCl and 50 mL dichloromethane, shake for 2 min, allow to stand for 30 min. Remove the upper organic layer and add it to 5 g anhydrous sodium sulfate, shake for 30 s, filter through a cotton-wool plug, evaporate to about 4 mL under reduced pressure at 30°, add 3 mL dichloromethane, evaporate to about 4 mL, add 3 mL light petroleum, evaporate to about 0.5 mL, Suspend this residue with sonication in three 3 mL portions of light petroleum and place these fractions in a separate tube, rinse the original tube with 2 mL pH 7 phosphate buffer. Add the phosphate buffer rinse to the light petroleum extracts, vortex for 30 s, centrifuge, remove the aqueous layer. Extract the light petroleum layer with 2 mL pH 7 phosphate buffer and with two 1.5 mL portions of pH 7 phosphate buffer, combine all the aqueous phase, centrifuge, inject a 200 μL aliquot on to column A and elute to waste with mobile phase B, after 15 min elute to waste with mobile phase C at 2 mL/min, after 10 min elute the contents of column A on to column B with mobile phase D, after 2 min remove column A from the circuit, elute column B with mobile phase D, monitor the effluent from column B. (Wash column A with mobile phase A at 2 mL/min for 7 min, with mobile phase A at 1 mL/min for 5 min, with mobile phase B at 2 mL/min for 8 min, and with mobile phase B at 1 mL/min for 6 min.)

HPLC VARIABLES
Column: A 4 × 4 5 μm LiChrospher 100 RP-18e; B 250 × 4 5 μm LiChrospher 100 RP-18e
Mobile phase: A MeCN:water 50:50; B 20 mM pH 7 phosphate buffer; C MeCN:20 mM pH 3 phosphate buffer 10:90; D MeCN:200 mM pH 3.0 phosphate buffer 35:65 containing 2 mM disodium EDTA
Column temperature: 35
Flow rate: 1 (except where indicated)
Injection volume: 200
Detector: E, Merck Model L3500, glassy carbon working electrode +0.65 V, stainless-steel auxiliary electrode, Ag/AgCl reference electrode following post-column reaction. The column effluent flowed through a 10 m × 0.3 mm ID woven PTFE coil illuminated by a UV 254 low-pressure mercury lamp to the detector.

CHROMATOGRAM
Retention time: 7.1
Limit of detection: 2.7 ng

OTHER SUBSTANCES
Extracted: cloxacillin, dicloxacillin, penicillin V, penicillin G

KEY WORDS
post-column reaction; post-column photochemical derivatization; cow; muscle; column-switching

REFERENCE
Lihl,S.; Rehorek,A.; Petz,M. High-performance liquid chromatographic determination of penicillins by means of automated solid-phase extraction and photochemical degradation with electrochemical detection, *J.Chromatogr.A*, **1996**, *729*, 229–235.

SAMPLE
Matrix: urine
Sample preparation: Filter (0.45 μm), inject a 5 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 5 LiChrosorb RP-2
Column: 250 × 4.6 LiChrosorb RP-18
Mobile phase: MeOH:30 mM pH 5.6 acetate buffer 33:66
Flow rate: 1.5
Injection volume: 5
Detector: UV 254

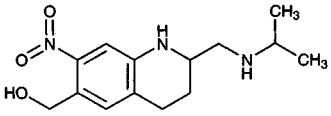
CHROMATOGRAM
Retention time: 28

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; human; pharmacokinetics

REFERENCE
Murai,Y.; Nakagawa,T.; Yamaoka,K.; Uno,T. High performance liquid chromatographic analysis and pharmacokinetic investigation of oxacillin and its metabolites in man, *Chem.Pharm.Bull.(Tokyo)*, **1981**, *29*, 3290–3297.

Oxamniquine



Molecular formula: $C_{14}H_{21}N_3O_3$
Molecular weight: 279.34
CAS Registry No.: 21738-42-1
Merck Index: 7051
Lednicer No.: 2 372

SAMPLE
Matrix: microsomal incubations
Sample preparation: Basify 1 mL microsomal incubation with 2 drops of 5 M NaOH, add 4 mL diethyl ether, vortex for 30 s, centrifuge at 3000 rpm for 5 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 30°, reconstitute the residue in 200-500 μL mobile phase, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES
Column: 100 × 4 Chiral-AGP α1-acid glycoprotein (ChromTech)
Mobile phase: MeCN:10 mM pH 5.80 sodium phosphate buffer 0.6:99.4
Flow rate: 0.9
Injection volume: 20
Detector: UV 246

CHROMATOGRAM
Retention time: 2.3 (L), 3.4 (D)
Limit of detection: 2.3 ng (d), 0.3 ng (l)

KEY WORDS
chiral; rat; mouse; liver

REFERENCE
Noctor,T.A.G.; Fell,A.F.; Kaye,B. High-performance liquid chromatographic resolution of oxamniquine enantiomers: application to in vitro metabolism studies, *Chirality*, **1990**, *2*, 269–274.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 100 × 5 Enantiopac α1-glycoprotein (AGP) (LKB)
Mobile phase: Isopropanol:10 mM phosphate buffer containing 100 mM NaCl 0.5:99.5, pH 5.85
Injection volume: 20
Detector: UV 260

CHROMATOGRAM
Retention time: 8 (L), 12.5 (D)
Limit of detection: 0.5 ng

KEY WORDS
chiral

REFERENCE
Fell,A.F.; Noctor,T.A.G.; Mama,J.E.; Clark,B.J. Computer-aided optimisation of drug enantiomer separation in chiral high-performance liquid chromatography, *J.Chromatogr.*, **1988**, *434*, 377–384.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 100 × 4.6 5 μm Chiral-AGP (ChromTech)

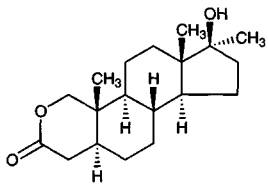
Mobile phase: Isopropanol:buffer 99.5:0.5 (Buffer was 10 mM NaH_2PO_4 containing 100 mM NaCl, pH 5.85.)
Flow rate: 1
Injection volume: 20
Detector: UV 246

CHROMATOGRAM
Retention time: 8, 11 (enantiomers)

KEY WORDS
chiral

REFERENCE
Abushoffa,A.M.; Clark,B.J. Resolution of the enantiomers of oxamniquine by capillary electrophoresis and high-performance liquid chromatography with cyclodextrins and heparin as chiral selectors, *J.Chromatogr.A*, **1995**, *700*, 51–58.

Oxandrolone



Molecular formula: $C_{19}H_{30}O_3$
Molecular weight: 306.45
CAS Registry No.: 53-39-4
Merck Index: 7054
Lednicer No.: 1 174

SAMPLE
Matrix: formulations
Sample preparation: Oils. 1 mL Sample + 25 mL MeOH:water 90:10, shake vigorously for 5 min, centrifuge, inject a 10 μL aliquot of the supernatant. Tablets. Grind a tablet to a fine powder, add 25 mL MeOH, sonicate for 5-10 min, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate. Suspensions (aqueous). Make up 5 mL to 50 mL with MeOH, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:water 75:25
Flow rate: 1.5
Injection volume: 10
Detector: UV 210

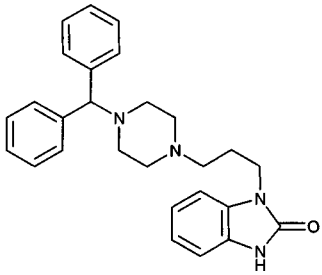
CHROMATOGRAM
Retention time: 4.7
Limit of detection: 5 μg/mL

OTHER SUBSTANCES
Simultaneous: methandrostenolone, nandrolone, norgestrel, testosterone, dehydroepiandrosterone (UV 210), mibolerone, methyltestosterone, methandriol (UV 210), norethindrone acetate, calusterone, mesterolone (UV 210), norethandrolone, trenbolone acetate, benzyl benzoate, nandrolone acetate, testosterone acetate, stanozolol, oxymetholone, nandrolone propionate, methenolone acetate, testosterone propionate, aspirin, caffeine, formebolone, benzyl alcohol, testolactone, cortisone
Interfering: fluoxymesterone, norethindrone, boldenone, ethisterone

KEY WORDS
oils; tablets; suspensions

REFERENCE
Walters,M.J.; Ayers,R.J.; Brown,D.J. Analysis of illegally distributed anabolic steroid products by liquid chromatography with identity confirmation by mass spectrometry or infrared spectrophotometry, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 904–926.

Oxatomide



Molecular formula: $C_{27}H_{30}N_4O$
Molecular weight: 426.56
CAS Registry No.: 60607-34-3
Merck Index: 7058
Lednicer No.: 3 173

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

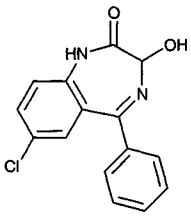
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 205.2

CHROMATOGRAM
Retention time: 15.797

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Oxazepam



Molecular formula: $C_{15}H_{11}ClN_2O_2$
Molecular weight: 268.72
CAS Registry No.: 604-75-1
Merck Index: 7059
Lednicer No.: 1 366

SAMPLE

Matrix: bile, blood, gastric contents, tissue, urine
Sample preparation: Chop 5-g tissue and homogenize (Ultra Turrax T25) at 8500, 9500, 13500, 20500, and 24000 rpm for 1 min each. Add homogenate to 20 mL water. Dilute blood, urine, gastric contents, and bile four times with water. Mix 4 mL sample with 10 μL 1 mg/mL prazepam and 1 mL pH 7.4 phosphate buffer, vortex briefly, add 4 mL diethyl ether and mix for 15 min (Spiramix 10, Denley, UK). Separate the organic layer, add 4 mL diethyl ether to extraction sample, mix. Evaporate combined organic layers to dryness under a stream of dry air at 50°. Purify extracts by partition between 1 mL MeCN and 2 mL heptane, separate MeCN layer, evaporate it to dryness, reconstitute the residue in 100 μL MeOH and inject a 20 μL aliquot of the solution.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Apex II ODS
Column: 150 × 4.6 5 μm Apex II ODS
Mobile phase: MeCN:MeOH:10 mM phosphoric acid:10 mM Na_2HPO_4 40:20:36:4
Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM

Retention time: 3.8
Internal standard: prazepam (14.5)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES

Extracted: diazepam, nitrazepam, temazepam

KEY WORDS

liver; lung; muscle; urine; pericardial fluid

REFERENCE

Pounder,D.J.; Adams,E.; Fuke,C.; Langford,A.M. Site to site variability of postmortem drug concentrations in liver and lung, *J.Forensic Sci.*, **1996**, *41*, 927–932.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Plasma + 100 μL 1 μg/mL loxapine in isopropanol:diethylamine 99.9:0.1 + 250 μL 25% potassium carbonate containing 0.1% diethylamine + 5 mL hexane: isoamyl alcohol 97:3, vortex for 30 s, centrifuge at 500 g for 3 min. Remove the organic layer and add it to 100 μL 250 mM HCl, vortex for 30 s, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: 50 × 4.6 40 μm C8 (Supelco)
Column: 250 × 4.6 5 μm Supelcosil C8
Mobile phase: MeCN:water:diethylamine:85% phosphoric acid 53.3:45.1:1:0.4, pH adjusted to 7.2 with NaOH or phosphoric acid
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: k' 2.44
Internal standard: loxapine (k' 7.18)

OTHER SUBSTANCES
Extracted: amitriptyline, chlorpromazine, desipramine, desmethldiazepam, desmethylchlordiazepoxide, diazepam, doxepin, haloperidol, imipramine, nortriptyline, thiothixene
Noninterfering: molindone, perphenazine, trifluoperazine
Interfering: chlordiazepoxide, desmethyldoxepin, fluphenazine

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: 1 mL Serum + 2 mL water + 50 μL 3.2 μg/mL estazolam in MeOH + 2 mL 100 mM NaOH, mix gently, add 8 mL diethyl ether, shake for 15 min, centrifuge at 2500 rpm for 5 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, vortex for 30 s, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 50 × 4.6 Shim-pack FLC-C8 (Shimadzu)
Mobile phase: MeOH:buffer 53:47 (Buffer was 5 mM Na_2HPO_4 adjusted to pH 6.0 with phosphoric acid.)
Flow rate: 0.6
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 5
Internal standard: estazolam (4)
Limit of detection: 8 ng/mL

OTHER SUBSTANCES
Extracted: diazepam, temazepam, nordiazepam, triazolam
Simultaneous: sulpride, bromazepam, nitrazepam, flunitrazepam
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
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 × 3.2 5 μm Brownlee ODS; B 250 × 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: 25
Limit of detection: 30 ng/mL

OTHER SUBSTANCES
Simultaneous: temazepam, nordiazepam, diazepam

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: Inject 100-200 μL plasma onto column A with mobile phase A and elute to waste, after 5 min backflush the contents of column A onto column B with mobile phase B, after 5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Wash column A with MeCN:water 60:40 at 1 mL/min for 6 min then re-equilibrate with pH 7.5 buffer for 10 min.

HPLC VARIABLES
Column: A 45 × 4 12 μm TSK-gel G 3 PW (Tosohass); B 75 × 4.6 Ultrasphere ODS C18 3 μm
Mobile phase: A 50 mM pH 7.5 phosphate buffer; B Gradient. A was MeCN. B was 65 mM KH_2PO_4 + 1% diethylamine adjusted to pH 5.4 with phosphoric acid. A:B 22:78 for 5 min, to 25:75 over 10 min, to 60:40 over 15 min.
Flow rate: 1
Injection volume: 100-200
Detector: UV 230

CHROMATOGRAM
Retention time: 21.5

OTHER SUBSTANCES
Extracted: alprazolam, bromazepam, chlordiazepoxide, clobazam, clonazepam, clorazepate, clotiazepam, desmethylclobazam, desmethyldiazepam, diazepam, flunitrazepam, loflazepate, lorazepam, medazepam, nitrazepam, prazepam, temazepam, tetrazepam, tofisopam, triazolam
Noninterfering: carbamazepine, phenytoin, ethosuximide, phenobarbital, primidone, valproic acid
Interfering: estazolam

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: Rock 5 mL whole blood + 10 mL water + 8.5 mL Na_2WO_4 in a 50 mL stoppered tube for 1 min, add 6 mL $NiCl_2$, rock for 5 min, add 15 mL 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. (Na_2WO_4 prepared by mixing 10 g $Na_2WO_4.2H_2O$ in 38 mL of 2 M NaOH and 2.5 g of $NaHCO_3$ and making up to 100 mL. $NiCl_2$ was 17% w/v $NiCl_2$ in water.)

HPLC VARIABLES
Column: 200 × 4.6 5 μm Hypersil C8
Mobile phase: A = MeCN; B = 20 mM n-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: 13
Limit of detection: 0.40 ppm

OTHER SUBSTANCES
Extracted: bromazepam, clonazepam, diazepam, flunitrazepam, flurazepam, medazepam, nitrazepam
Also analyzed: buprenorphine, caffeine, cocaine, codeine, diamorphine, ethylmorphine, lidocaine, methaqualone, morphine, naloxone, noscapine, papaverine, pentazocine, procaine

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: 200 μL Plasma + 500 μL 23 μg/mL acetophenone in MeCN, vortex for 20 s, decant, filter (13 mm diameter, 0.2 μm), inject a 100 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 20 × 2 Pellicular ODS (Whatman)
Column: 250 × 4.6 Ultracarb 7 ODS 30 (Phenomenex)
Mobile phase: MeCN:water:glacial acetic acid 45:55:0.5
Flow rate: 1
Injection volume: 100
Detector: UV 238

CHROMATOGRAM
Retention time: 6.8
Internal standard: acetophenone (9.1)
Limit of detection: 40 ng/mL
Limit of quantitation: 240 ng/mL

KEY WORDS
rat; mouse; plasma; pharmacokinetics

REFERENCE
Yuan,J.; Goehl,T.J.; Hong,L.; Clark,J.; Murrill,E.; Moore,R. Toxicokinetics of oxazepam in rats and mice, *J.Pharm.Sci.*, **1994**, *83*, 1373–1379.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min.

Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 229

CHROMATOGRAM

Retention time: 4.34
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood

Sample preparation: 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, diazepam, fluvoxamine, lorazepam

KEY WORDS
serum

REFERENCE
Vandenberghe,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, CSF, gastric contents, urine
Sample preparation: 200 μL Serum, urine, CSF, or gastric fluid + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES
Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM
Retention time: 12.21
Internal standard: heptanophenone (19)

OTHER SUBSTANCES
Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, 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, 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 SPE cartridge, wash with 2 mL MeCN:water 20:80, dry for 3 to 4 min, elute with four 200 μL aliquots of MeOH, evaporate combined eluates under nitrogen and take up residue in 100 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 Lichrocart Lichrosorb RP 18-5
Column: 5 μm 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: 2.09
Internal standard: climazolam (5.62)
Limit of quantitation: 5ng/mL

OTHER SUBSTANCES
Simultaneous: temazepam

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: Whole blood, stomach contents. 1 mL Whole blood or stomach contents + IS + 500 μL 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 μL initial mobile phase, vortex, centrifuge, inject a 50 μL aliquot. Tissue. Cut 1 g tissue into small pieces, make up to 5 mL with water, homogenize (Ultraturrax). 1 mL Homogenate + IS + 500 μL 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 μL initial mobile phase, vortex, centrifuge, inject a 50 μL aliquot. Urine. 1 mL Urine + IS + 250 μL 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 μL initial mobile phase, vortex, centrifuge, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 2.1 pellicular reverse phase (Chrompack)
Column: 100 × 3 5 μm 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: 9
Internal standard: camazepam (10.5), clotiazepam (11)
Limit of detection: 15 ng/mL

Limit of quantitation: 45 ng/mL

OTHER SUBSTANCES
Extracted: nitrazepam, bromazepam, flunitrazepam, diazepam, nordiazepam
Simultaneous: clonazepam, triazolam, alprazolam, brotizolam, chlordiazepoxide, loprazolam, cloxazolam, prazepam, flurazepam, medazepam, lormetazepam

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, milk
Sample preparation: 500 µL Plasma or milk + 25 µL 5 µg/mL flurazepam in water:MeCN 2.5: 97.5 + 500 µL 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 × 4 5 µm LiChrospher 60 RP-select B
Column: 125 × 4 5 µm LiChrospher 60 RP-select B
Mobile phase: MeCN: 10 mM KH_2PO_4 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: 3.8
Internal standard: flurazepam (3.0)

OTHER SUBSTANCES
Extracted: diazepam, nordiazepam, 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, urine
Sample preparation: Blood. Condition a Bond-Elut C18 SPE cartridge with 2 mL MeOH and 30 mL water. Mix 1 mL serum with 4 mL water and 10 µL 40 µM IS in EtOH. Adjust to pH 4.0 with 250 mM sulfuric acid. Add to the SPE cartridge, wash with 10 mL water, dry under vacuum, elute with 5 mL MeOH, evaporate the eluate to dryness under a stream of nitrogen, dissolve the residue in 200 µL MeOH, inject a 20 µL aliquot. Urine. Mix 100 µL urine with 50 µL 40 µM IS in EtOH, dilute to 1 mL with water, inject a 20 µL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 µm LiChrospher 100 RP 18
Mobile phase: MeCN:isopropanol:25% orthophosphoric acid:water 18:7.5:1.2:73.3 (?), pH 2.05
Injection volume: 20

Detector: UV 230

CHROMATOGRAM
Retention time: 27.9
Internal standard: diazepam (21.7)
Limit of detection: 10 nM
Limit of quantitation: 40 nM

OTHER SUBSTANCES
Extracted: metabolites, glucuronides

KEY WORDS
SPE; serum; sheep; pharmacokinetics

REFERENCE
Mawa,R.; Mis,D.; Gagnieu,M.C.; Grancher,D.; Petit-Ramel,M.; Bressolle,F.; Vallon,J.J. Simple high-performance liquid chromatographic separation of oxazepam and its diastereoisomeric glucuronides in serum. Applications in a pharmacokinetic study in sheep, *J.Chromatogr.B*, **1996**, *677*, 331–338.

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 bufferto 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 to 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
Injection volume: 20
Detector: UV 228

CHROMATOGRAM
Retention time: 2
Internal standard: prazepam (7)
Limit of detection: 79 ng/mL

OTHER SUBSTANCES
Also analyzed: diazepam, temazepam, nordazepam, brotizolam, adinazolam, midazolam

KEY WORDS
plasma; SPE.

REFERENCE
Casas,M.; Berrueta,L.A.; Gallo,B.; Vicente,F. Solid-phase extraction of 1,4-benzodiazepines from biological fluids, *J.Pharm.Biomed.Anal.*, **1993**, *11*, 277–284.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 228.7

CHROMATOGRAM
Retention time: 16.745

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: feces, urine
Sample preparation: Urine. Centrifuge at 1000 rpm for 10 min, inject an aliquot of the supernatant. Feces. Homogenize (Kinematica polytron) feces in 100 mM pH 6.8 sodium acetate buffer. 1 mL Homogenate + 3 mL MeCN, vortex vigorously, centrifuge at 1000 rpm for 10 min, pass through a C18 Sep-Pak.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Microsorb C18
Mobile phase: Gradient. MeOH:10 mM pH 6.5 dibutylamine phosphate from 15:85 to 100:0 over 23 min.
Flow rate: 1.2
Detector: UV or radioactivity

CHROMATOGRAM
Retention time: 21.5

KEY WORDS
rat; radiolabeled

REFERENCE
Griffin,R.J.; Burka,L.T. Metabolism and elimination of oxazepam in F344 rats, *Drug Metab.Dispos.*, **1995**, *23*, 232–239.

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 NaH_2PO_4 + 30 mL diethylamine.)
Flow rate: 2

Injection volume: 30
Detector: UV 214

CHROMATOGRAM
Internal standard: protriptyline (4)

OTHER SUBSTANCES
Extracted: amitriptyline, desipramine, diazepam, dothiepin, flunitrazepam, haloperidol, imipramine, imipramine, nitrazepam, nortriptyline, temazepam

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: 2.5 mL Microsomal incubation + 2.5 mL acetone, add 30 μL diazepam in MeOH, add 2.5 mL chloroform, centrifuge. Remove the organic layer and evaporate it to dryness under reduced pressure at 40°, reconstitute the residue in 100 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 6.2 7 μm Zorbax silica
Mobile phase: Hexane:dichloromethane:isopropanol 77:20:3
Flow rate: 2
Detector: UV 232

CHROMATOGRAM
Retention time: 37
Internal standard: diazepam (12)

OTHER SUBSTANCES
Extracted: halazepam

KEY WORDS
human; liver; normal phase; pharmacokinetics

REFERENCE
Lu,X.-L.; Guengerich,F.P.; Yang,S.K. Stereoselective metabolism of prazepam and halazepam by human liver microsomes, *Drug Metab.Dispos.*, **1991**, *19*, 637–642.

SAMPLE
Matrix: reaction mixtures
Sample preparation: Irradiate MeCN solutions with UV light, inject a 20-100 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:THF:60 mM pH 5.8 phosphate buffer 22:2:76
Column temperature: 20
Flow rate: 1
Injection volume: 20-100
Detector: UV 265

CHROMATOGRAM
Retention time: 8.25
Limit of detection: 0.6 ng

OTHER SUBSTANCES
Extracted: desmethylchlordiazepoxide, demoxepam, desmethyldiazepam, chlordiazepoxide

REFERENCE
Soentjens-Werts,V.; Dubois,J.G.; Atassi,G.; Hanocq,M. High-performance liquid chromatographic determination of chlordiazepoxide, its metabolites and oxaziridines generated after UV irradiation, *J.Chromatogr.A*, **1994**, *662*, 255–262.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 5 μm ChiraDex, LichroCART
Mobile phase: MeOH:pH 2.8 phosphate buffer 40:60
Column temperature: 15
Flow rate: 0.5
Detector: UV 220

CHROMATOGRAM
Retention time: 8.37, 11.40 (enantiomers)

KEY WORDS
chiral

REFERENCE
Cabrera,K.; Jung,M.; Fluck,M.; Schurig,V. Determination of enantiomerization barriers by computer simulation of experimental elution profiles obtained by high-performance liquid chromatography on a chiral stationary phase, *J.Chromatogr.A*, **1996**, *731*, 315–321.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 30 × 2.1 Spheri-5 RP-8
Column: 220 × 2.1 Spheri-5 RP-8
Mobile phase: Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95: % for 2 min, to 0:100 over 15 min, maintain at 0:100 for 5 min.
Column temperature: 50
Flow rate: 0.5
Detector: UV 200

CHROMATOGRAM
Retention time: 11.3

OTHER SUBSTANCES
Simultaneous: chlordiazepoxide, desalkylflurazepam, diazepam, flurazepam, norchlordiazepoxide, nordiazepam, prazepam

REFERENCE
Rainin Catalog 1991-2, p. 3.26.

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: 5.063

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, oxycodone, phenylpropanolamine, prilocaine, procaine, terfenadine

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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 MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN: water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 18.1

OTHER SUBSTANCES
Simultaneous: acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, diflunisal, doxapram, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, testosterone, testosterone propionate, tranylcypromine, tripelennamine

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KEY WORDS
details for purification of triethylamine in paper

REFERENCE
Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 30 × 2.1 Spheri-5 RP-8
Column: 220 × 2.1 Spheri-5 RP-8
Mobile phase: Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95: 5 for 2 min, to 0:100 over 15 min (?), maintain at 0:100 for 5 min.
Column temperature: 50
Flow rate: 0.5
Detector: UV 200

CHROMATOGRAM
Retention time: 11.5

OTHER SUBSTANCES
Simultaneous: norchlordiazepoxide, chlordiazepoxide, nordiazepam, desalkylflurazepam, diazepam, flurazepam, prazepam
Also analyzed: amitriptyline, amphetamine, chlorpromazine, desipramine, desmethyldoxepin, diethylpropion, doxepin, ephedrine, fenfluramine, imipramine, mesoridazine, methamphetamine, nortriptyline, phentermine, phenylpropanolamine, promazine, thioridazine, thiothixene, trifluoperazine

REFERENCE
Rainin Catalog, C1-94, **1994**, p. 7.24.

SAMPLE
Matrix: solutions
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 90:10
Column temperature: 80
Injection volume: 0.2
Detector: UV 254

CHROMATOGRAM
Retention time: 19.1 (second peak)

KEY WORDS
derivatization; subcritical fluid chromatography; chiral; density of mobile phase 0.65 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

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 223, 310

CHROMATOGRAM
Retention time: 5.2
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, 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 5 μm LiChroCART ChiraDex (β-cyclodextrin chemically bonded to silica) (Merck)
Mobile phase: MeOH:10 mM pH 2.8 NaH_2PO_4 40:60
Column temperature: 5
Flow rate: 0.5
Detector: UV 220

CHROMATOGRAM
Retention time: 9, 14 (enantiomers)

KEY WORDS
chiral

REFERENCE
Cabrera,K.; Lubda,D. Influence of temperature on chiral high-performance liquid chromatographic separations of oxazepam and Prominal on chemically bonded β-cyclodextrin as stationary phase, *J.Chromatogr.A*, **1994**, *666*, 433–438.

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: 9

OTHER SUBSTANCES
Simultaneous: bromazepam, nitrazepam, flunitrazepam, clobazam, chlordiazepoxide, lorazepam, tofisopam, chlorazepate, diazepam

KEY WORDS
conditions are optimized

REFERENCE
Guillaume,Y.; Guinchard,C. Study and optimization of column efficiency in HPLC: Comparison of two methods for separating ten benzodiazepines, *J.Liq.Chromatogr.*, **1994**, *17*, 1443–1459.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200

mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 10 μm Chiralpak OD (Daicel)
Mobile phase: Carbon dioxide:EtOH:diethylamine 79.5:20:0.5
Column temperature: 10
Flow rate: 2
Detector: UV 220

CHROMATOGRAM
Retention time: 8, 8.9 (enantiomers)

KEY WORDS
SFC; chiral; pressure 350 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

HPLC VARIABLES
Column: 62 × 2 packed with chiral packing (Prepare packing by dissolving 4-chloro-3-methylphenylcarbamate cellulose in THF, coat on Nucleosil 1000-7, dry at 60° for 3 h under reduced pressure.)
Mobile phase: Hexane:isopropanol 90:10
Flow rate: 0.1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 18.30

KEY WORDS
narrow-bore; chiral; α 1.25

REFERENCE
Chankvetadze,B.; Chankvetadze,L.; Sidamonidze,S.; Yashima,E.; Okamoto,Y. Enantioseparation of some chiral pharmaceuticals using narrow-bore liquid chromatography, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 695–699.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: 10 × 3 not otherwise specified
Column: 100 × 4 Chiral-AGP (ChromTech)
Mobile phase: MeCN:10 mM pH 7.0 phosphate buffer 5:95
Flow rate: 0.9
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 8, 10 (enantiomers)

KEY WORDS
chiral

REFERENCE
Fitos,I.; Visy,J.; Simonyi,M.; Hermansson,J. Separation of enantiomers of benzodiazepines on the Chiral-AGP column, *J.Chromatogr.A*, **1995**, *709*, 265–273.

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: 7.2

OTHER SUBSTANCES
Simultaneous: bromazepam, chlordiazepoxide, clobazam, clorazepate, diazepam, flunitrazepam, lorazepam, nitrazepam, tofisopam

REFERENCE
Guillaume,Y.; Guinchard,C. Marked difference between acetonitrile/water and methanol/water mobile phase systems on the thermodynamic behavior of benzodiazepines in reversed phase liquid chromatography, *Chromatographia*, **1995**, *41*, 84–87.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova pack C18
Mobile phase: MeOH:water 52:48
Column temperature: 48
Flow rate: 0.8
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6.5

OTHER SUBSTANCES
Simultaneous: bromazepam, chlordiazepoxide, clobazam, clorazepate, diazepam, flunitrazepam, lorazepam, nitrazepam, 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: 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: 6.00 (A), 4.52 (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, chlordi-

azepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, 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
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 × 4.6 column containing riboflavin binding proteins (Prepare as follows. Add riboflavin to saturate protein of egg yolk, homogenize with 3 volumes buffer, centrifuge, add the supernatant to a 500 × 30 column of DEAE-cellulose (Whatman) equilibrated with buffer, wash extensively with buffer to remove bound protein, elute riboflavin binding proteins (RFBP) with buffer containing 200 mM NaCl (RFBP has intense yellow color, absorption at 455 nm). Purify RFBP on a Sephadex G-100 column with 50 mM pH 7.5 Tris-HCl buffer as eluent, remove the bound riboflavin by extensive dialysis at pH 3.0. Add 4.5 g N,N-disuccinylimidyl carbonate to 3 g Nucleosil 5NH2 slurried in MeCN, filter, wash with MeCN, wash with 50 mM pH 7.5 phosphate buffer. Suspend 300 mg RFBP in 50 mM phosphate buffer, add the activated silica, mix gently for 2 h using a rotary evaporator, filter, wash with sterile water, wash with isopropanol:water 1:2, pack in a 100 × 4.6 column.) (Buffer was 100 mM pH 5.3 sodium acetate.)
Mobile phase: EtOH:50 mM pH 5.5 KH_2PO_4 5:95
Flow rate: 0.8
Injection volume: 20
Detector: UV

CHROMATOGRAM
Retention time: k' 3.92

OTHER SUBSTANCES
Simultaneous: bepridil, manidipine, nicardipine
Interfering: lorazepam

KEY WORDS
chiral; α = 4.55

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' 9.90

KEY WORDS
chiral; α 1.27

REFERENCE
Oliveros,L.; Lopez,P.; Minguillon,C.; Franco,P. Chiral chromatographic discrimination ability of a cellulose 3,5-dimethylphenylcarbamate/10-undecenoate mixed derivative fixed on several chromatographic matrices, *J.Liq.Chromatogr.*, **1995**, *18*, 1521–1532.

SAMPLE
Matrix: urine
Sample preparation: 500 μL Urine + N-ethylnordiazepam + chlorpheniramine + 100 μL buffer, centrifuge at 11000 g for 30 s, inject a 500 μL aliquot onto column A with mobile phase A, after 0.6 min backflush column A with mobile phase A to waste for 1.6 min, elute column A with 250 μL mobile phase B, with 200 μL mobile phase C, and with 1.15 mL mobile phase D. Elute column A to waste until drugs start to emerge then elute onto column B. Elute column B to waste until drugs started to emerge, then elute onto column C. When all the drugs have emerged from column B remove it from the circuit, elute column C with mobile phase D, monitor the effluent from column C. Flush column A with 7 mL mobile phase E, with mobile phase D, and mobile phase A. Flush column B with 5 mL mobile phase E then with mobile phase D. (Buffer was 6 M ammonium acetate adjusted to pH 8.0 with 2 M KOH.)

HPLC VARIABLES
Column: A 10 × 2.1 12-20 μm PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 × 3.2 11 μm Aminex A-28 (Bio-Rad); C 25 × 3.2 5 μm C8 (Phenomenex) + 150 × 4.6 5 μm silica (Macherey-Nagel)
Mobile phase: A 0.1% pH 8.0 potassium borate buffer; B 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid; C MeCN:buffer 40:60 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); D MeCN:buffer 33:67 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); E MeCN:buffer 70:30 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)
Column temperature: ambient (column A), 40 (columns B and C)
Flow rate: A 5; B-E 1
Injection volume: 500
Detector: UV 210, UV 235

CHROMATOGRAM
Retention time: k' 1.1

Internal standard: N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)
Limit of detection: 300 ng/mL

OTHER SUBSTANCES
Extracted: diazepam, phenylpropanolamine, phentermine, amphetamine, phenmetrazine, lidocaine, ephedrine, pentazocine, methamphetamine, desipramine, nortriptyline, diphenhydramine, methadone, imipramine, flurazepam, amitriptyline, morphine, codeine, hydromorphone, hydrocodone, caffeine
Interfering: cotinine, benzoylecgonine, secobarbital, phenobarbital, 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.

SAMPLE
Matrix: urine
Sample preparation: Adjust pH of 2.5 mL urine to 5 with dilute HCl, add 400 μL 200 mM pH 5 acetate buffer, add 50 μL β-glucuronidase, heat at 45° for 5 h, cool, adjust pH to 8-9 with 25% NaOH, add to an Extrelut 3 SPE cartridge, let stand for 10 min, elute with 15 mL chloroform:isopropanol 90:10. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 2 mL 100 mM pH 9.2 borate buffer. Extract this solution twice with 3 mL diethyl ether, evaporate the combined organic phases to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm Lichrospher 100 RP8
Column: 250 × 4 5 μm Lichrospher 100 RP8
Mobile phase: MeCN:buffer 45:55 (Buffer was 1.361 g KH_2PO_4 in 950 mL, add 1.3 mL methanesulfonic acid, adjust pH to 3.5 with 5 M KOH, make up to 1 L with water.)
Flow rate: 1
Injection volume: 20
Detector: UV 234

CHROMATOGRAM
Retention time: 5.2
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: desmethyldiazepam
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, trifluprazine, trimethoprim, trimipramine

KEY WORDS
SPE

REFERENCE
Ferrara,S.D.; Tedeschi,L.; Frison,G.; Castagna,F. Solid-phase extraction and HPLC-UV confirmation of drugs of abuse in urine, *J.Anal.Toxicol.*, **1992**, *16*, 217–222.

SAMPLE
Matrix: urine

Sample preparation: Condition a 100 mg Bond-Elut C2 SPE cartridge with MeOH and 10 mM pH 6.0 phosphate buffer. 5 mL Urine + 1250 U β-glucuronidase, adjust pH to 5.0 with HCl, heat at 37° for 24 h. Add 5 μg nordiazepam, buffer to pH 6.0 with 500 μL 100 mM pH 6.0 phosphate buffer, add to the SPE cartridge, wash with 3 volumes of water, wash with 1 mL MeOH:water 25:75, wash with 1 mL water, elute with 1 mL MeOH:water 60:40. Evaporate the eluate to dryness, reconstitute in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 35 × 4.6 5 μm Ultrabase C18 (Scharlau)
Mobile phase: MeOH:water 60:40
Flow rate: 0.5
Injection volume: 20
Detector: F ex 364 em 469 following post-column reaction. The effluent from the column mixed with acetic acid pumped at 1.1 mL/min and the mixture flowed through a 15 m × 0.5 mm i.d. coil of PTFE tubing at 100° to the detector

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CHROMATOGRAM
Retention time: 6
Internal standard: nordiazepam (8.5)
Limit of detection: 4 ng/mL
Limit of quantitation: 10 ng/mL

KEY WORDS
post-column reaction; SPE

REFERENCE
Berrueta,L.A.; Gallo,B.; Vicente,F. Analysis of oxazepam in urine using solid-phase extraction and high-performance liquid chromatography with fluorescence detection by post-column derivatization, *J.Chromatogr.*, **1993**, *616*, 344–348.

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 mLL 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.

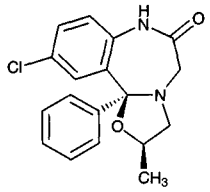
HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 RP-18(e)
Mobile phase: MeOH:water:triethylamine 30:70:0.1 adjusted to pH 5.5 with phosphoric acid
Flow rate: 0.7
Injection volume: 60
Detector: UV 240

CHROMATOGRAM
Retention time: 7.0
Internal standard: prazepam (17.0)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Extracted: desmethyldiazepam, diazepam, temazepam
Simultaneous: amitriptyline, caffeine, carbamazepine, chlordiazepoxide, chlorpromazine, clonazepam, desipramine, flunitrazepam, flurazepam, haloperidol, imipramine, levomepromazine, maprotiline, nitrazepam, nortriptyline, perphenazine, phenobarbital, phenytoin, sulpride, thioridazine, triazolam
Interfering: mianserin

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.

Oxazolam



Molecular formula: $C_{18}H_{17}ClN_2O_2$
Molecular weight: 328.80
CAS Registry No.: 27167-30-2
Merck Index: 7061

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 20 μL 20 μg/mL IS + 200 μL 1 M potassium carbonate + 3 mL chloroform, mix for 2 min, centrifuge at 1200 g for 5 min, aspirate aqueous phase. Evaporate the organic phase under a stream of nitrogen at 40°. Dissolve the residue in 100 μL mobile phase, inject a 20 μL aliquot. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES
Column: 100 × 4.6 2 μm TSK gel Super-ODS (A) or 100 × 4.6 5 μm Hypersil ODS-C18 (B)
Mobile phase: MeCN:5 mM pH 6 NaH_2PO_4 45:55
Flow rate: 0.65
Injection volume: 20
Detector: UV 254

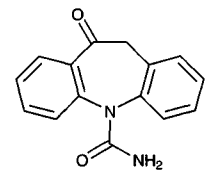
CHROMATOGRAM
Retention time: 14.6 (A), 46.9 (B)
Internal standard: diazepam (29.8 (A), 77.5 (B))
Limit of quantitation: 5 ng/mL (A)

OTHER SUBSTANCES
Extracted: bromazepam, chlordiazepoxide, clonazepam, estazolam, etizolam, flutazolam, haloxazolam, lorazepam, nitrazepam, triazolam
Simultaneous: alprazolam
Noninterfering: barbital, carbamazepine, cloxazolam, ethosuximide, hexobarbital, mexazolam, oxazepam, pentobarbital, phenobarbital, phenytoin, primidone, trimethadione

KEY WORDS
serum

REFERENCE
Tanaka,E.; Terada,M.; Misawa,.; Wakasugi,C. Simultaneous determination of twelve benzodiazepines in human serum using a new reversed-phase chromatographic column on a 2-μm porous microspherical silica gel, *J.Chromatogr.B*, **1996**, *682*, 173–178.

Oxcarbazepine



Molecular formula: $C_{15}H_{12}N_2O_2$
Molecular weight: 252.27
CAS Registry No.: 28721-07-5
Merck Index: 7063

SAMPLE
Matrix: blood
Sample preparation: 250 μL Plasma + 2 μg 10-methoxycarbamazepine + 25 μL 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 μL MeCN, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.9 10 μm LiChrosorb RP8
Mobile phase: MeCN:water 32:68
Flow rate: 1.8
Injection volume: 10
Detector: UV 215

CHROMATOGRAM
Retention time: 4.8
Internal standard: 10-methoxycarbamazepine (9.3)
Limit of detection: 500 nM
Limit of quantitation: 2 μM

OTHER SUBSTANCES
Extracted: metabolites, carbamazepine, 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-hydroxycarbazepine, *J.Chromatogr.*, **1990**, *528*, 473–479.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond Elut C18 SPE cartridge with 2 column volumes of MeCN and 2 column volumes of water. Dilute plasma with two volumes 2 μg/mL 10,11-dihydrocarbamazepine in 67 mM pH 5 Sorensen's phosphate buffer. Add 500 μL water then 300 μL diluted plasma to the SPE cartridge, let stand for 2 min, wash with 1 column volume of water, wash with 1 column volume of MeCN:water 5:95, elute with 250 μL acetone. Evaporate the eluate to dryness, reconstitute the residue in 500 μL mobile phase, inject a 75 μL aliquot.

HPLC VARIABLES
Guard column: 5 μm LiChrospher C18
Column: 100 × 5 4 μm Nova-Pak C18 radial compression
Mobile phase: MeCN:MeOH:water 13:25:62
Flow rate: 1.2
Injection volume: 75
Detector: UV 214

CHROMATOGRAM
Retention time: 6.2
Internal standard: 10,11-dihydrocarbamazepine (13.8)
Limit of detection: 50-100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: carbamazepine, chlormethiazole edisylate, clonazepam, diazepam, ethoxyzolamide, nitrazepam, phenobarbital, phenytoin, prednisolone, prednisone
Noninterfering: acetaminophen, acetazolamide, ampicillin, aspirin, caffeine, cefuroxime, chlorothiazide, theophylline, vitamin K

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Hartley,R.; Green,M.; Lucock,M.D.; Ryan,S.; Forsythe,W.I. Solid phase extraction of oxcarbazepine and its metabolites from plasma for analysis by high performance liquid chromatography, *Biomed.Chromatogr.*, **1991**, *5*, 212–215.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL 50 mg Bond-Elut C18 SPE cartridge with 1 mL MeOH and 1 mL water. 100 μL Plasma + 100 μL water + 10 μL 198 μM IS in MeOH:water 50:50, vortex for a few s, add to the SPE cartridge, wash with 2 mL 20 mM K_2HPO_4, wash with 2 mL MeOH:water 5:95, elute with 250 μL MeOH, add 950 μL water to the eluate, mix, inject a 500 μL aliquot.

HPLC VARIABLES
Guard column: 33 × 4.6 37-53 μm pellicular ODS (Whatman)
Column: 40 × 4.6 3 μm Hypersil ODS
Mobile phase: MeCN:MeOH:10 mM pH 5.0 KH_2PO_4 9:11:80
Flow rate: 2
Injection volume: 500
Detector: UV 210

CHROMATOGRAM
Retention time: 9.5
Internal standard: 5,6-dihydro-11-oxo-11H-dibenz[b,e]azepine-5-carboxamide (Ciba-Geigy) (14.0)
Limit of quantitation: 200 nM

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: carbamazepine, phenobarbital, phenytoin
Noninterfering: valproic acid

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Rouan,M.C.; Decherf,M.; Le Clanche,V.; Lecaillon,J.B.; Godbillon,J. Automated microanalysis of oxcarbazepine and its monohydroxy and transdiol metabolites in plasma by liquid chromatography, *J.Chromatogr.B*, **1994**, *658*, 167–172.

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 2 μg/mL carbamazepine in 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 × 4.6 10 μm Chiralcel OD + 250 × 4.6 10 μm Chiralcel ODH
Mobile phase: n-Hexane:EtOH 70:30
Column temperature: 40 (second column only)
Flow rate: 0.9
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 28.9

Internal standard: carbamazepine (21.9)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: phenytoin, valproic acid
Interfering: phenobarbital

KEY WORDS
serum; chiral (for metabolites)

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 × 4 5 μm LiChrospher 100 RP-18
Column: 125 × 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: 14.49
Limit of detection: 12 ng/mL
Limit of quantitation: 55 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, carbamazepine

KEY WORDS
serum

REFERENCE
Pienimaki,P.; Fuchs,S.; Isojarvi,J.; Vahakangas,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 + 100 μL MeCN, centrifuge, inject a 5 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 4.6 3.5 μm 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 μM

OTHER SUBSTANCES
Extracted: carbamazepine, carbamazepine epoxide, hydroxycarbamazepine, lamotrigine, phenobarbital, phenytoin
Also analyzed: ibuprofen, naproxen, trimethoprim

KEY WORDS
plasma

REFERENCE
Lessing,U.; Vielemeyer,O.; Heilmann,P.; Schöneshöfer,M. Routine determination of serum primidone levels with a fully automated liquid chromatographic method: Comparison with an immuno-assay-technique (Abstract 100), *Ther.Drug Monit.*, **1995**, *17*, 408.

SAMPLE
Matrix: blood, tissue
Sample preparation: Dialyze with artificial CSF (brain) or physiological saline (blood, liver) using a 20 kDa cut-off membrane (CMA-10). Mix 40 μL dialysate with 25 μL 500 ng/mL carbamazepine in artificial CSF, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 2.1 Spheri 5-ODS
Column: 220 × 2.1 5 μm Spheri-5 ODS
Mobile phase: MeCN:buffer 22:78 (Buffer was 50 mM KH_2PO_4 adjusted to pH 6.5 with 5 M NaOH.)
Flow rate: 0.4
Injection volume: 50
Detector: UV 220

CHROMATOGRAM
Retention time: 10.2
Internal standard: carbamazepine (20.6)
Limit of detection: 10 ng/mL
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; brain; liver; narrow-bore; dialysate

REFERENCE
Van Belle,K.; De Koster,V.; Sarre,S.; Ebinger,G.; Michotte,Y. Narrow-bore liquid chromatographic assay for oxcarbazepine and its major metabolite in rat brain, liver and blood microdialysates, *J.Chromatogr.B*, **1994**, *657*, 149–154.

SAMPLE
Matrix: dialysate
Sample preparation: 10 μL Dialysate + 2.5 μL 1 μg/mL carbamazepine-10,11-epoxide in water, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 0.8 3 μm Hypersyl C18 BDS
Mobile phase: MeCN:water 27:73
Flow rate: 0.025
Injection volume: 10
Detector: UV 220

CHROMATOGRAM
Retention time: 4.5
Internal standard: carbamazepine-10,11-epoxide (4)
Limit of detection: 1 ng/mL

Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; microcolumn

REFERENCE
Van Belle,K.; Verfaillie,I.; Ebinger,G.; Michotte,Y. Liquid chromatographic assay using a microcolumn coupled to a U-shaped optical cell for high-sensitivity ultraviolet absorbance detection of oxcarbazepine and its major metabolite in microdialysates, *J.Chromatogr.B*, **1995**, *672*, 97–102.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 0.8 3 μm Hypersil C18 BDS
Mobile phase: MeCN:water 27:73
Flow rate: 0.025
Injection volume: 10
Detector: UV 220 (8 mm path length, quartz ball lens)

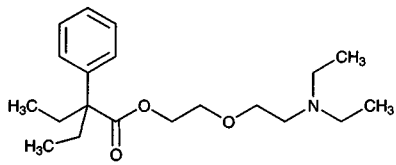
CHROMATOGRAM
Retention time: 4.5
Limit of detection: 1 ng/mL
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
microcolumn

REFERENCE
Van Belle,K.; Ebinger,G.; Michotte,Y. An LC assay using a microcolumn coupled to a U-shaped optical cell for high sensitivity UV absorbance detection of oxcarbazepine and its major metabolite in microdialysates, *Biomed.Chromatogr.*, **1995**, *9*, 277–278.

Oxeladin



Molecular formula: $C_{20}H_{33}NO_3$
Molecular weight: 335.49
CAS Registry No.: 468-61-1, 52432-72-1
Merck Index: 7064
Lednicer No.: 1 90

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2

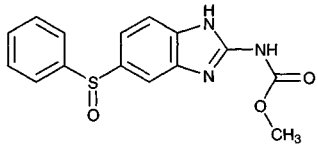
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 3.6

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Oxfendazole



Molecular formula: $C_{15}H_{13}N_3O_3S$
Molecular weight: 315.35
CAS Registry No.: 53716-50-0
Merck Index: 7069
Lednicer No.: 3 353

SAMPLE
Matrix: abomasal fluid, blood, duodenal fluid, rumen fluid

Sample preparation: 4 mL Plasma, rumen fluid, abomasal fluid, or duodenal fluid + 4 mL pH 7.4 phosphate buffer + 20 mL ether, shake on a rotary mixer for 10 min, remove 16 mL of the ether layer, add 20 mL ether, shake on a rotary mixer for 10 min, remove 20 mL of the ether layer. Combine the ether layers and evaporate them under a stream of nitrogen at 60° to dryness, reconstitute in 50 μL MeOH, sonicate, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 100 × 8 ODS Hypersil 10
Mobile phase: MeOH:50 mM ammonium carbonate 65:35
Flow rate: 1.5
Injection volume: 5
Detector: UV 292

CHROMATOGRAM
Retention time: 3
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: albendazole, thiabendazole, cambendazole, mebendazole, oxibendazole, fenbendazole, parbendazole

KEY WORDS
plasma; sheep

REFERENCE
Bogan,J.A.; Marriner,S. Analysis of benzimidazoles in body fluids by high-performance liquid chromatography, *J.Pharm.Sci.*, **1980**, *69*, 422–423.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 100 μL 10 μg/mL albendazole in MeOH + 200 μL 500 mM ammonium hydroxide (to adjust pH to 11) + 200 mg NaCl + 5 mL distilled diethyl ether, roll for 15 min, remove 4 mL supernatant, repeat extraction, remove 5 mL supernatant. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 60 μL MeOH, sonicate for 2 min, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: present but not specified
Column: 100 × 5 Nucleosil 5C18
Mobile phase: MeCN:1% acetic acid 43:57
Flow rate: 0.9
Injection volume: 20
Detector: UV 292

CHROMATOGRAM
Retention time: 0.9
Internal standard: albendazole (1.6)
Limit of detection: 12.5 ng/mL

OTHER SUBSTANCES
Extracted: febantel, fenbendazole, oxfendazole sulfone

KEY WORDS
plasma; sheep

REFERENCE
Landuyt,J.; Debackere,M.; Delbeke,F.; McKellar,Q. A high performance liquid chromatographic method for the determination of febantel and its major metabolites in lamb plasma, *Biomed.Chromatogr.*, **1993**, 7, 78–81.

SAMPLE
Matrix: feed

Sample preparation: 20 g Pulverized feed + 100 mL MeOH:glacial acetic acid 90:10, shake on a gyrorotary shaker at 45° for 30 min, cool to room temperature, , centrifuge an aliquot at 1000 rpm for 5 min. 10 mL Supernatant + 4 mL reagent, mix thoroughly, let stand for 5 min, make up to 25 mL with water, mix thoroughly, centrifuge at 1000 rpm. 15 mL Supernatant + 100 mL phosphate solution, adjust pH to 8 with 3.2 mL 1 M NaOH, mix well, add 50 mL dichloromethane, shake gently, repeat extraction twice more with 40 mL portions of dichloromethane. Filter extracts through anhydrous sodium sulfate, add 15 mL 20 μg/mL 2-guanadinobenzimidazole in MeOH to the filtrate, evaporate to dryness under a stream of nitrogen using a steam bath, reconstitute the residue in 10 mL mobile phase, sonicate for 5 min, filter (Millipore polyvic 2 μm), inject 100 μL of the filtrate. (Reagent was 100 g zinc acetate dihydrate in 300 mL water, stir until dissolved, add 1.5 mL glacial acetic acid, make up to 500 mL with water. Phosphate solution was 35 g K_2HPO_4 + 50 g NaCl in 1 L water.)

HPLC VARIABLES

Column: 250 × 4.6 10 μm Partisil PSX-SCX
Mobile phase: MeCN:10 mM phosphoric acid:10 mM KH_2PO_4 50:25:25
Flow rate: 1.5
Injection volume: 100
Detector: UV 254

CHROMATOGRAM

Retention time: 10
Internal standard: 2-guanadinobenzimidazole (39)
Limit of detection: 0.012%

OTHER SUBSTANCES

Simultaneous: degradation products

KEY WORDS

stability-indicating

REFERENCE

Shah,G.; Bradley,D.; Shek,E. Liquid chromatographic determination of oxfendazole in swine feeds, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 707–714.

SAMPLE

Matrix: formulations
Sample preparation: 1 g Paste + 75 mL MeOH, sonicate for 15 min, shake for 1 min, sonicate for 5 min, shake for 30 s, cool to room temperature, make up to 100 mL with MeOH, centrifuge an aliquot at 2000 rpm for 10 min. Mix 2 mL supernatant with 2 mL 200 μg/mL methyl paraben in MeOH, make up to 25 mL with MeCN:water:phosphoric acid 20:80:1, filter (Millipore 0.6 μm polyvic), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: 70 × 2.1 CO:PELL ODS
Column: 250 × 4.6 Partisil-5 ODS-3
Mobile phase: MeCN:buffer 20:80 (Buffer was 720 mL 1.38 g/L $NaH_2PO_4.H_2O$ + 80 mL 1.42 g/L Na_2HPO_4.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 200

CHROMATOGRAM

Retention time: 21.79
Internal standard: methyl paraben (15.71)

OTHER SUBSTANCES

Simultaneous: degradation products, trichlorfon

KEY WORDS

horse; paste

REFERENCE
Fleitman,J.; Neu,D.; Benjamin,E. Analysis of pharmaceutical dosage forms for oxfendazole: II. Simultaneous liquid chromatographic determination of oxfendazole and trichlorfon in equine paste, *J.Assoc.Off.Anal. Chem.*, **1986**, *69*, 24–28.

SAMPLE
Matrix: milk
Sample preparation: 50 g Milk + 1 mL 1 μg/mL IS in 100 mM HCl, swirl, add 150 mL acetone, shake mechanically for 10 min, centrifuge. Remove the acetone extract and add it to 10 mL pH 8 phosphate buffer and 300 mL chloroform, shake for 10 min, let stand for 10 min. Dry the chloroform layer briefly over 80 g anhydrous sodium sulfate, filter through glass wool, rinse flask with 50 mL chloroform:acetone 2:1, filter rinse through glass wool. Combine filtrates and evaporate them to an oily residue under reduced pressure at 37°, take up the residue in 50 mL hexane and 50 mL MeCN, shake for 5 min, wash the MeCN layer twice with 25 mL portions of hexane. Evaporate the MeCN layer and take up the residue in 50 mL ethyl acetate. Extract the ethyl acetate layer with three 10 mL portions of 200 mM HCl. Combine the aqueous layers and wash them with two 10 mL portions of hexane. Add 6 mL 1 M NaOH to the aqueous layer, adjust the pH to 8 with 300 mg sodium bicarbonate, extract twice with 25 mL portions of ethyl acetate. Dry the ethyl acetate extracts over 5 g anhydrous sodium sulfate, evaporate to dryness, reconstitute in 10 mL ethyl acetate, filter (0.5 μm Millipore fluoropore FHLP 1300), evaporate the filtrate to dryness, reconstitute in 200 μL MeOH, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: Co:Pell ODS
Column: 300 × 4 μBondapak C18
Mobile phase: MeCN:water 24.5:75.5
Flow rate: 2
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 8.5
Internal standard: [5-(4-methylsulfinylphenoxy)-1H-benzimidazol-2-yl]carbamate (Syntex) (11)
Limit of quantitation: 5 ng/g

KEY WORDS
pharmacokinetics

REFERENCE
Tsina,I.W.; Matin,S.B. Determination of oxfendazole in cow milk by reversed-phase high-performance liquid chromatography, *J.Pharm.Sci.*, **1981**, *70*, 858–860.

SAMPLE
Matrix: milk
Sample preparation: Condition a 3 mL Bond Elut silica SPE cartridge with 2 mL dichloromethane. 10 g Milk + 5 mL 1 M sodium carbonate, mix, add 150 mL ethyl acetate, add 1 mL 10 mg/mL BHT in ethyl acetate, blend (tissuemizer) at high speed for 5 min, add 10 g anhydrous sodium sulfate, blend for 1 min, let settle for 2-3 min, filter (No. 41 paper), add another 150 mL ethyl acetate to the sodium sulfate, blend for 2 min, filter. Combine the filtrates and evaporate them to dryness under vacuum. Rinse out flask with two 10 mL portions of hexane and two 10 mL portions of 1 M phosphoric acid. Combine rinses, shake vigorously for 2 min, extract the hexane layer with two 10 mL portions of 1 mL phosphoric acid. Combine all the aqueous layers and wash them with 5 mL hexane, adjust the pH to 8-9 by slowly adding about 9 mL 10 M KOH (use an ice bath), add 50 mL ethyl acetate, shake vigorously for 2 min, repeat extraction. Filter the organic layers through 40 g anhydrous sodium sulfate, wash the sodium sulfate with 25 mL ethyl acetate. Combine the organic layers, add 200 μL 10 mg/mL BHT in ethyl acetate, evaporate to dryness under vacuum. Take up the residue in two 3 mL portions of dichloromethane and add them to the SPE cartridge, wash with 5 mL dichloromethane, elute with 5 mL dichloromethane:MeOH 75:25. Evaporate the eluate to dryness under nitrogen, reconstitute in 1 mL mobile phase, vortex, filter (0.2 μm), inject an aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 pellicular C18 (Alltech)

Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:buffer 53:47 (Buffer was 1.15 g $(NH_4)H_2PO_4$ in 950 mL water, adjust pH to 7.0 with dilute ammonia, make up to 1 L with water.)
Flow rate: 1
Injection volume: 50
Detector: UV 298

CHROMATOGRAM
Retention time: 10
Limit of detection: 0.5 ppb

OTHER SUBSTANCES
Extracted: thiabendazole

KEY WORDS
cow; SPE

REFERENCE
Tai,S.S.; Cargile,N.; Barnes,C.J. Determination of thiabendazole, 5-hydroxythiabendazole, fenbendazole, and oxfendazole in milk, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 368–373.

SAMPLE
Matrix: premix
Sample preparation: Weigh out premix containing 67.5 mg oxfendazole, add 50 mL acetone, shake gently on a mechanical shaker for 2 h, make up to about 190 mL with MeOH, sonicate for 15 min, cool to room temperature, shake well, make up to 250 mL with MeOH, centrifuge an aliquot at 2000 rpm for 10 min. Mix 5 mL supernatant with 2 mL 240 μg/mL methyl paraben in MeOH, make up to 25 mL with MeCN:water:phosphoric acid 20:80:1, filter (Millipore 0.6 μm polyvic), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 70 × 2.1 CO:PELL ODS
Column: 250 × 4.6 Partisil-5 ODS-3
Mobile phase: MeCN:buffer 20:80 (Buffer was 720 mL 1.38 g/L $NaH_2PO_4.H_2O$ + 80 mL 1.42 g/L Na_2HPO_4.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 20
Internal standard: methyl paraben (15)
Limit of quantitation: 10 ng

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Fleitman,J.; Neu,D.; Visor,G. Analysis of pharmaceutical dosage forms for oxfendazole: I. Reverse phase liquid chromatographic determination of oxfendazole in swine premix, *J.Assoc.Off.Anal.Chem.*, **1986**, *69*, 20–24.

SAMPLE
Matrix: tissue
Sample preparation: Wash 22 g bulk 40 μm 18% load end-capped C18 material (Analytichem) in a syringe barrel with 100 mL hexane, with 100 mL dichloromethane, and with 100 mL MeOH and dry under vacuum aspiration. Gently blend 2 g C18 material, 0.5 g liver, and 10 μL 40 μg/mL mebendazole in DMF in a glass pestle for 1 min until homogeneous in appearance. Place in a 10 mL syringe barrel plugged with filter paper (Whatman No. 1), cover with filter paper, compress to 4.5 mL, place a 100 μL pipette tip on the barrel to restrict flow, wash with 8 mL hexane, elute with 8 mL MeCN. Pass the eluate through 0.5 g activated alumina (EM Science Type F-20 80-200 mesh) between filter paper in a 10 mL syringe barrel (wash column

with 4 mL MeCN just before use). Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH and 400 μL 17 mM phosphoric acid, sonicate for 5-10 min, centrifuge at 17000 g for 5 min, filter the supernatant (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm Micro Pak ODS (Varian)
Mobile phase: MeCN:17 mM phosphoric acid 40:60
Column temperature: 45
Flow rate: 1
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: 6
Internal standard: mebendazole (9)
Limit of detection: 100 ng/g

OTHER SUBSTANCES
Extracted: albendazole, thiafendazole, fenbendazole

KEY WORDS
matrix solid-phase dispersion; liver

REFERENCE
Long,A.R.; Malbrough,M.S.; Hsieh,L.C.; Short,C.R.; Barker,S.A. Matrix solid phase dispersion isolation and liquid chromatographic determination of five benzimidazole anthelmintics in fortified beef liver, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 860–863.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 2.8 mL 500 mg 40 μm 60 Å Bond Elut silica SPE cartridge with 2 mL dichloromethane. 10 g Minced tissue + 5 mL 1 M sodium carbonate + 150 mL ethyl acetate + 1 mL 10 mg/mL BHT in ethyl acetate, blend (Waring) at high speed for 5 min, add 80 g anhydrous sodium sulfate, blend at low speed for 1 min. Decant the organic layer and filter it (No. 41 paper), add 150 mL acetone to material remaining in blender, blend at low speed for 2-3 min, filter, wash solid with 10 mL EtOH. Combine all the filtrates and evaporate them to dryness under vacuum at 30-35° (beware of bumping). Rinse out flask with two 10 mL portions of hexane and two 10 mL portions of 1 M phosphoric acid, combine rinses, shake vigorously for 2 min, allow to separate for 10 min, extract the hexane layer twice more with 10 mL portions of 1 M phosphoric acid. Combine all the aqueous layers and wash them with 10 mL hexane, adjust the pH of the aqueous layer to 8.5 ± 1.0 by slowly adding about 9 mL 10 M KOH while using an ice bath. Extract twice with 50 mL ethyl acetate (2 min shaking), pass ethyl acetate layers through 40 g anhydrous sodium sulfate, wash the sodium sulfate with 25 mL ethyl acetate. Combine the ethyl acetate layers, add 200 μL 10 mg/mL BHT in ethyl acetate, evaporate to dryness under vacuum at 30-35° (beware of bumping). Rinse out flask with three 3 mL portions of dichloromethane, add rinses to the SPE cartridge, wash with 5 mL dichloromethane, elute with 5 mL dichloromethane:MeOH 75:25. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase, vortex, filter (0.2 μm), inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 Brownlee RP-18 Spheri-10 MPLC
Column: 250 × 4.6 5 μm C18 (Alltech)
Mobile phase: MeOH:buffer 53:47 (Buffer was 1.15 g $(NH_4)H_2PO_4$ in 950 mL water, adjust pH to 7.0 with dilute ammonia, make up to 1 L with water.)
Flow rate: 1
Injection volume: 50
Detector: UV 298

CHROMATOGRAM
Retention time: 8.5

OTHER SUBSTANCES
Extracted: thiabendazole, mebendazole
Simultaneous: chloramphenicol
Noninterfering: amprolium, chlortetracycline, erythromycin, levamisole, morantel, oxytetracycline, phenothiazine, sulfadimethoxine, sulfamethazine, sulfaquinoxaline

KEY WORDS
cow; liver; SPE

REFERENCE
LeVan,L.W.; Barnes,C.J. Liquid chromatographic method for multiresidue determination of benzimidazoles in beef liver and muscle: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1991**, *74*, 487–493.

SAMPLE
Matrix: tissue
Sample preparation: 3 g Pulverized tissue + 7 mL water, homogenize (Silverson) for 1 min, add 20 mL MeOH, sonicate for 15 min, centrifuge at 2000 g at 5° for 10 min. Remove 10 mL of the supernatant and add it to 8 mL light petroleum (bp 40-60°), shake gently for 30 s, centrifuge. Add the aqueous phase to 14 mL 500 mM NaH_2PO_4 and 8 mL diethyl ether:ethyl acetate 60:40, shake gently for 30 s, centrifuge, repeat the extraction with 5 mL and 3 mL portions of diethyl ether:ethyl acetate 60:40. Combine the upper organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 μL MeCN:water 50:50, sonicate for 5 min, inject a 50 μL aliquot.

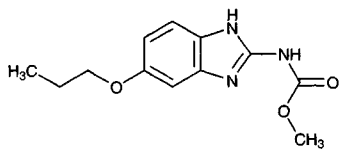
HPLC VARIABLES
Column: 125 × 4 LiChrosorb RP18
Mobile phase: MeCN:THF:water 30:10:60 containing 50 mM ammonium acetate
Flow rate: 1
Injection volume: 50
Detector: MS, Vestec Model 201A, thermospray, positive-ion chemical ionization, electron beam 250 μA, electron multiplier 2000 V, source block 250°, tip heater 250°, lens assembly 150°, vaporizer probe 10° below take-off point, m/z 316

CHROMATOGRAM
Retention time: 3
Limit of detection: 100 ng/g

KEY WORDS
sheep; muscle; liver; pharmacokinetics; LC-MS

REFERENCE
Blanchflower,W.J.; Cannavan,A.; Kennedy,D.G. Determination of fenbendazole and oxfendazole in liver and muscle using liquid chromatography-mass spectrometry, *Analyst*, **1994**, *119*, 1325–1328.

Oxibendazole



Molecular formula: $C_{12}H_{15}N_3O_3$
Molecular weight: 249.27
CAS Registry No.: 20559-55-1
Merck Index: 7070
Lednicer No.: 2 352

SAMPLE
Matrix: abomasal fluid, blood, duodenal fluid, rumen fluid
Sample preparation: 4 mL Plasma, rumen fluid, abomasal fluid, or duodenal fluid + 4 mL pH 7.4 phosphate buffer + 20 mL ether, shake on a rotary mixer for 10 min, remove 16 mL of the ether layer, add 20 mL ether, shake on a rotary mixer for 10 min, remove 20 mL of the ether

layer. Combine the ether layers and evaporate them under a stream of nitrogen at 60° to dryness, reconstitute in 50 μL MeOH, sonicate, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 100 × 8 ODS Hypersil 10
Mobile phase: MeOH:50 mM ammonium carbonate 65:35
Flow rate: 1.5
Injection volume: 5
Detector: UV 292

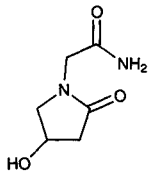
CHROMATOGRAM
Retention time: 5.3
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: albendazole, oxfendazole, cambendazole, thiabendazole, mebendazole, fenbendazole, parbendazole

KEY WORDS
plasma; sheep

REFERENCE
Bogan,J.A.; Marriner,S. Analysis of benzimidazoles in body fluids by high-performance liquid chromatography, *J.Pharm.Sci.*, **1980**, *69*, 422–423.

Oxiracetam



Molecular formula: $C_6H_{10}N_2O_3$
Molecular weight: 158.16
CAS Registry No.: 62613-82-5
Merck Index: 7076

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 35% perchloric acid, vortex for 15 s, sonicate for 10 min, centrifuge at 12000 rpm for 10 min. Remove a 500 μL aliquot of the supernatant and add it to 500 μL mobile phase, vortex for 15 s, centrifuge at 12000 rpm for 10 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Guard column: 15 × 3.2 PRP (Brownlee)
Column: 300 × 7.8 Aminex Ion-Exclusion HPX 874 (Bio-Rad)
Mobile phase: MeCN:0.05% sulfuric acid 12:88
Flow rate: 0.6
Injection volume: 40
Detector: UV 210

CHROMATOGRAM
Retention time: 13.8
Internal standard: oxiracetam

OTHER SUBSTANCES
Extracted: pidotimod

KEY WORDS
plasma; oxiracetam is IS

layer. Combine the ether layers and evaporate them under a stream of nitrogen at 60° to dryness, reconstitute in 50 μL MeOH, sonicate, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 100 × 8 ODS Hypersil 10
Mobile phase: MeOH:50 mM ammonium carbonate 65:35
Flow rate: 1.5
Injection volume: 5
Detector: UV 292

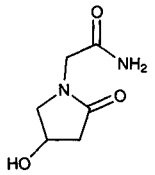
CHROMATOGRAM
Retention time: 5.3
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: albendazole, oxfendazole, cambendazole, thiabendazole, mebendazole, fenbendazole, parbendazole

KEY WORDS
plasma; sheep

REFERENCE
Bogan,J.A.; Marriner,S. Analysis of benzimidazoles in body fluids by high-performance liquid chromatography, *J.Pharm.Sci.*, **1980**, *69*, 422–423.

Oxiracetam



Molecular formula: $C_6H_{10}N_2O_3$
Molecular weight: 158.16
CAS Registry No.: 62613-82-5
Merck Index: 7076

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 35% perchloric acid, vortex for 15 s, sonicate for 10 min, centrifuge at 12000 rpm for 10 min. Remove a 500 μL aliquot of the supernatant and add it to 500 μL mobile phase, vortex for 15 s, centrifuge at 12000 rpm for 10 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Guard column: 15 × 3.2 PRP (Brownlee)
Column: 300 × 7.8 Aminex Ion-Exclusion HPX 874 (Bio-Rad)
Mobile phase: MeCN:0.05% sulfuric acid 12:88
Flow rate: 0.6
Injection volume: 40
Detector: UV 210

CHROMATOGRAM
Retention time: 13.8
Internal standard: oxiracetam

OTHER SUBSTANCES
Extracted: pidotimod

KEY WORDS
plasma; oxiracetam is IS

REFERENCE
Dal Bo,L.; Broccali,G.P.; Silingardi,S.; Coppi,G. A new HPLC method for pidotimod plasma levels determination, *Boll.Chim.Farm.*, **1993**, *132*, 126–128.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg phenylboronic acid SPE cartridge (Analytichem) with one column volume of 100 mM pH 8.5 potassium phosphate buffer. Condition a silica SPE cartridge with one column volume of MeCN. 200 μL Plasma + 50 μL water + 50 μL 25 μg/mL IS in water + 500 μL MeCN, vortex, centrifuge at 8800 g for 10 min. Remove the supernatant and add it to 500 μL 100 mM pH 8.5 potassium phosphate buffer, vortex, add to the phenylboronic acid SPE cartridge. Collect the eluate and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute with 1 mL MeOH, sonicate, vortex, centrifuge at 1500 g for 5 min. Evaporate the supernatant to dryness under a stream of nitrogen at 50°, reconstitute the residue in 200 μL anhydrous pyridine, add 100 μL n-propyl isocyanate, vortex, heat at 50° for 1 h, evaporate to dryness under a stream of nitrogen. Reconstitute the residue in two 500 μL aliquots of MeCN, add to the silica SPE cartridge, wash with 1 mL MeCN, elute with 1 mL MeOH:MeCN 50:50. Evaporate the eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 200 μL water, inject a 10-25 μL aliquot.

HPLC VARIABLES
Column: 250 × 2 5 μm Ultrasphere octadecylsilica
Mobile phase: Gradient. MeOH:50 mM pH 6.0 acetate buffer 10:90 for 5 min, to 20:80 over 4 min, maintain at 20:80 for 1 min, to 50:50 over 1 min, maintain at 50:50 for 5 min, return to initial conditions over 1 min, re-equilibrate for 14 min.
Column temperature: 50
Flow rate: 0.3
Injection volume: 10-25
Detector: F ex 340 em 455 following post-column reaction. The column effluent mixed with the reagent pumped at 0.2 mL/min and the mixture flowed through a reaction coil (1 mL volume, ABI Analytical PCRS Model 520) at 90° to the detector. (Reagent was prepared by adding 2 mL 2 mg/mL o-phthalaldehyde in MeOH and 80 μL 3-mercaptopropionic acid to 1 L 2 g/L NaOH in water, filter (0.45 μm), use within 48 h.)

CHROMATOGRAM
Retention time: 10.9
Internal standard: 4-hydroxy-2-oxo-1-pyrrolidinepropionamide (ISF 2839) (12.9)
Limit of detection: 20 ng/mL
Limit of quantitation: 40 ng/mL

KEY WORDS
plasma; SPE; derivatization; post-column reaction

REFERENCE
Simpson,R.C.; Boppana,V.K.; Hwang,B.Y.-H.; Rhodes,G.R. Determination of oxiracetam in human plasma by reversed-phase high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.*, **1993**, *631*, 227–232.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 250 μL Plasma + 4.2 mL MeCN:water 100:0.4, mix, add 10 μL 50 μg/mL IS in water, add 800 μL dichloromethane, vortex for a few s, centrifuge at 2000 g for 5 min, inject a 1 mL aliquot of the supernatant. Urine. 10 μL Urine + 4.2 mL MeCN:water 100:0.4, mix, add 40 μL 50 μg/mL IS in water, add 800 μL dichloromethane, vortex for a few s, inject a 1 mL aliquot of the supernatant onto column A and elute to waste with mobile phase A, after 4 min divert effluent containing oxiracetam and IS onto column B, after 1.5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. (Flush column A with MeCN:water 50:50 for 7.5 min, re-equilibrate with mobile phase A for 5 min.)

HPLC VARIABLES
Column: A 100 × 4.7 5 μm Lichrosorb NH2; B 300 × 4.7 5 μm Nucleosil NH2

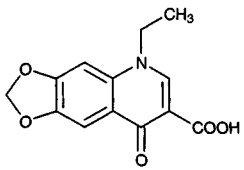
Mobile phase: A MeCN:water 95:5; B MeCN:water 90:10
Flow rate: A 2; B 1
Injection volume: 1000
Detector: UV 200

CHROMATOGRAM
Retention time: 15
Internal standard: 2-(2-oxo-1-pyrrolidine)acetamidoacetamide (CGP 14 998) (13)
Limit of quantitation: 12 μg/mL (urine), 240 ng/mL (plasma)

KEY WORDS
plasma; column-switching; heart-cut; pharmacokinetics

REFERENCE
Lecaillon,J.B.; Souppart,C.; Le Duigou,F.; Dubois,J.P. Determination of oxiracetam in plasma and urine by column-switching high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *497*, 223–230.

Oxolinic acid



Molecular formula: $C_{13}H_{11}NO_5$
Molecular weight: 261.23
CAS Registry No.: 14698-29-4
Merck Index: 7079
Lednicer No.: 2 370

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond-Elut C18 SPE cartridge with two 3 mL portions of MeOH and 3 mL of 0.05% orthophosphoric acid. Centrifuge plasma at 500 g for 5 min. Mix 200 μL centrifuged plasma with 200 μL 10 μg/mL IS in buffer, let stand for 1 h. Add 3 mL buffer, vortex for 1 min. Add the mixture to the SPE cartridge under low vacuum, wash with 1 mL water and 500 μL 0.05% orthophosphoric acid solution, dry under vacuum. Elute with six 250 μL portions of MeCN. Evaporate the combined eluates to dryness under a stream of nitrogen at 40°. Reconstitute the residue with 200 μL buffer, vortex, sonicate, let stand for 30 min, vortex again. Inject a 50 μL aliquot. (Buffer was 1/15 M pH 8.0 phosphate buffer.)

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChroSpher 100 RP-18 end-capped
Column: 125 × 4 5 μm LiChroSpher 100 RP-18 end-capped
Mobile phase: MeCN:DMF:20 mM pH 2.3 orthophosphoric acid 10:30:60
Flow rate: 0.8
Injection volume: 50
Detector: UV 340

CHROMATOGRAM
Retention time: 5.77
Internal standard: nalidixic acid (9.66)
Limit of detection: 14 ng/mL
Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES
Noninterfering: 2-phenoxyethanol

KEY WORDS
plasma; sea bass; SPE; fish

REFERENCE
Loussouarn,S.; Pouliquen,H.; Armand,F. High-performance liquid chromatographic determination of oxolinic acid in the plasma of seabass (Dicentrarchus labrax) anaesthetized with 2-phenoxyethanol, *J.Chromatogr.B*, **1997**, *698*, 251–259.

SAMPLE
Matrix: blood
Sample preparation: Filter 1 mL plasma using a micropartition system (MPS-1, Amicon, MA) while centrifuging at 2000 g for 20 min at 10°, inject an aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb ODS-2 endcapped
Mobile phase: MeCN:buffer 30:70 containing 5 mM tetrabutylammonium sulfate, adjusted to pH 2.5 with 1 M NaOH (Buffer was 100 mM citric acid containing 200 mM ammonium perchlorate.)
Column temperature: 37
Flow rate: 0.9
Detector: UV 263

CHROMATOGRAM
Retention time: 6.82
Internal standard: difloxacin (4.09)

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Zlotos,G.; Bücker,A.; Kinzig-Schippers,M.; Sorgel,F.; Holzgrabe,U. Plasma protein binding of gyrase inhibitors, *J.Pharm.Sci.*, **1998**, *87*, 215–220.

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood. Filter serum through a 0.45 μm syringe filter with a cellulose acetate membrane, inject a 50 μL aliquot of the filtrate. Tissue. Add 1 mL MeCN:THF 95:5 to 1 g muscle, homogenize with a Pencil Mixer (Iuchi, Japan) for 2 min, centrifuge at 1500 g for 5 min, filter the supernatant through a syringe filter unit, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μL Hisep shielded hydrophobic phase precolumn (Supelco)
Column: 150 × 4.6 5 μL Hisep shielded hydrophobic phase (Supelco)
Mobile phase: MeCN:buffer 15:85 (Buffer was 50 mM citric acid:200 mM pH 2.5 Na_2HPO_4 buffer containing 10 mM tetra-*n*-butyl ammonium bromide 85:15.)
Flow rate: 1
Injection volume: 20-50
Detector: UV 265

CHROMATOGRAM
Retention time: 14
Limit of detection: 50 ng/mL (serum), 100 ng/mL (muscle)

OTHER SUBSTANCES
Extracted: sulfamonomethoxine, miloxacin

KEY WORDS
fish; muscle; serum

REFERENCE
Ueno,R.; Aoki,T. High-performance liquid chromatographic method for the rapid and simultaneous determination of sulfamonomethoxine, miloxacin and oxolinic acid in serum and muscle of cultured fish, *J.Chromatogr.B*, **1996**, *682*, 179–181.

SAMPLE

Matrix: tissue

Sample preparation: Homogenize 1 g tissue with 5 mL acetone, centrifuge, decant and save the supernatant, repeat the extraction. Add 2 mL acetone, 3 mL hexane, and 6 mL 3% NaCl to the combined supernatants, extract, centrifuge, discard the hexane layer. Add the extract to 25 mL chloroform (Caution! Chloroform is a carcinogen!), mix, separate the phases, add 2.5 mL 100 mM pH 9.0 Na_3PO_4 buffer and one drop 1 M NaOH to the chloroform phase, mix, separate the phases, discard the chloroform layer. Wash the aqueous layer with 2.5 mL chloroform, centrifuge the aqueous layer. Dialyze a 740 μL aliquot of the supernatant (in 2 portions) against 3.9 mL 20 mM pH 5.0 sodium phosphate buffer pumped at 0.6 mL/min using a Cuprophan 15000 MW cut-off cellulose acetate membrane. After washing column A with 500 μL MeCN:water 50:50 and 500 μL 20 mM pH 5.0 Na_3PO_4 buffer the dialysate flowed through column A to waste. Wash column A with 500 μL 20 mM pH 5.0 sodium phosphate buffer, elute the contents of column A onto column B with mobile phase, elute with mobile phase, monitor the effluent from column B. (Wash the donor channel with 2 mL 0.01% Triton X-100 in 20 mM pH 5.0 sodium phosphate buffer. Wash the acceptor channel with 3 mL 20 mM pH 5.0 sodium phosphate buffer. Regenerate the membrane with 2 mL 100 mM pH 9.0 sodium phosphate buffer (donor channel) and 3 mL 20 mM pH 5.0 sodium phosphate buffer (acceptor channel).)

HPLC VARIABLES

Column: A 5.8 × 4.6 Hypersil ODS; B 150 × 4.6 PLRP-S (Polymer Labs., UK)
Mobile phase: MeCN:THF:20 mM pH 5.0 Na_3PO_4 buffer 20:15:65
Flow rate: 0.6
Detector: F ex 318 em 364

CHROMATOGRAM

Retention time: 7
Limit of detection: 2.5 ng/g

OTHER SUBSTANCES

Extracted: flumequine

KEY WORDS

column switching; chicken; liver; dialysis

REFERENCE

Eng,G.Y.; Maxwell,R.J.; Cohen,E.; Piotrowski,E.G.; Fiddler,W. Determination of flumequine and oxolinic acid in fortified chicken tissue using on-line dialysis and high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.A*, **1998**, *799*, 349–354.

SAMPLE

Matrix: urine

Sample preparation: Make up 1 mL urine to 25 mL with deionized water. Adjust to pH 2.5-3 with HCl, extract with 25 mL chloroform. Separate the organic layer, dry the organic phase with sodium sulfate, evaporate it to dryness. Dissolve the residue in 3 mL MeCN, dilute to 10mL with water, filter (0.45 μm). Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeCN:400 μM oxalic acid in water 28:72
Flow rate: 2.0
Injection volume: 20
Detector: F ex 260 em 360

CHROMATOGRAM

Retention time: 2.15
Limit of detection: 10.8 ng/mL

OTHER SUBSTANCES

Extracted: nalidixic acid

REFERENCE
Durán Merá,I.; Galeano Díaz,T.; Rodríguez Cáceres,M.I.; Salinas López,F. Determination of the chemotherapeutic quinolonic and cinolonic derivatives in urine by high-performance liquid chromatography with ultraviolet and fluorescence detection in series, *J.Chromatogr.A*, **1997**, *787*, 119–127.

SAMPLE
Matrix: urine
Sample preparation: Make up 1 mL urine to 25 mL with mobile phase, filter (0.45 μm). Inject a 20 μL aliquot.

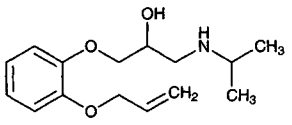
HPLC VARIABLES
Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeCN:400 μM oxalic acid in water 28:72
Flow rate: 2.0
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 2.15
Limit of detection: 1.09 μg/mL

OTHER SUBSTANCES
Simultaneous: cinoxacin, nalidixic acid, pipemidic acid, piromidic acid

REFERENCE
Durán Merá,I.; Galeano Díaz,T.; Rodríguez Cáceres,M.I.; Salinas López,F. Determination of the chemotherapeutic quinolonic and cinolonic derivatives in urine by high-performance liquid chromatography with ultraviolet and fluorescence detection in series, *J.Chromatogr.A*, **1997**, *787*, 119–127.

Oxprenolol



Molecular formula: $C_{15}H_{23}NO_3$
Molecular weight: 265.35
CAS Registry No.: 6452-71-7, 6452-73-9 (HCl)
Merck Index: 7086
Lednicer No.: 1 117

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 25 μL 25 ng/mL propranolol + 100 μL 1 M NaOH + 5 mL dichloromethane, shake, centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 100 μL dichloromethane, vortex for 5 s, add 10 μL 0.01% S-(+)-1-(1-naphthyl)ethyl isocyanate, heat at 37° for 2 h, add 20 μL tert-butylamine, evaporate under a stream of nitrogen, reconstitute with 50 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 240 × 4.6 5 μm Spherisorb C18 ODS
Mobile phase: MeOH:THF:200 mM pH 3.6 acetate buffer 51:14:35
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: F ex 226 em 333

CHROMATOGRAM
Retention time: 15.8 (S-(-)), 17.8 (R-(+))
Internal standard: propranolol (25.2 (R-(+)), 28.3 (S-(-))

Limit of detection: 2.5 ng/mL

OTHER SUBSTANCES
Noninterfering: metabolites

KEY WORDS
plasma; chiral; derivatization; pharmacokinetics

REFERENCE
Laethem,M.E.; Rosseel,M.T.; Wijnant,P.; Belpaire,F.M. Chiral high-performance liquid chromatographic determination of oxprenolol in plasma, *J.Chromatogr.*, **1993**, *621*, 225–229.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 273

CHROMATOGRAM
Retention time: 5.18
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; metha-

done; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: 100 µL Plasma or urine + 25 µL 25 ng/mL propranolol + 100 µL 1 M NaOH + 5 mL dichloromethane, shake, centrifuge at 1000 g for 10 min (J.Chromatogr. 1993, 621, 225). Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 50 µL 0.02% S(+)-1-(1-naphthyl)ethylisocyanate in hexane:chloroform 50:50, vortex for 30 min, evaporate, reconstitute with 50 µL mobile phase, inject a 35 µL aliquot.

HPLC VARIABLES

Column: Lichrosorb DIOL
Mobile phase: Hexane:chloroform:MeOH 90:10:0.38
Injection volume: 35
Detector: F ex 226 em 340

CHROMATOGRAM

Internal standard: propranolol
Limit of quantitation: 10 ng/mL

KEY WORDS

plasma; chiral; derivatization; pharmacokinetics

REFERENCE

Laethem,M.E.; Lefebvre,R.A.; Belpaire,F.M.; Vanhoe,H.L.; Bogaert,M.G. Stereoselective pharmacokinetics of oxprenolol and its glucuronides in humans, *Clin.Pharmacol.Ther.*, **1995**, *57*, 419–424.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 12.017

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Lichrosorb RP C18
Mobile phase: MeOH:water 55:45 pH 4.5
Flow rate: 1.0 for 4 min, then 2.0
Injection volume: 10
Detector: UV 260

CHROMATOGRAM
Retention time: 4.35

OTHER SUBSTANCES
Simultaneous: acebutolol, nifedipine

REFERENCE
el Walily,A.F.M. Analysis of nifedipine--acebutolol hydrochloride binary combination in tablets using UV-derivative spectroscopy, capillary gas chromatography and high performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 21–30.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.1

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, 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, fenflura-

mine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 3.85

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, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

SAMPLE

Matrix: solutions
Sample preparation: Mix a 100 μL of a 10 μM solution in MeCN:water:triethylamine 50:50:0.1 with 100 μL 1 mM (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in MeCN, heat in the dark at 65° for 1.5 h, inject an aliquot. (Synthesis of (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL

water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene from EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate: benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ TLC plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei. Cool a solution of 16.4 g (S)-(-)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane: acetone 95:5 to obtain (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65°, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry over anhydrous magnesium sulfate, evaporate to obtain (3R)-3-azido-1-(phenylmethyl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3R)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the minimum amount of EtOH to obtain (3R)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). 3R-(+)-aminopyrrolidine is also reported to be available from Tokyo Kasei. Add 100 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to a stirred solution of 200 mg 3R-(+)-aminopyrrolidine in 20 mL MeCN at 0-10°, stir at room temperature for 30 min, remove the MeCN by evaporation under reduced pressure, dissolve the residue in 50 mL 5% HCl, wash 3 times with 50 mL portions of ethyl acetate, adjust the pH of the aqueous solution to 13-14 with 5% NaOH, extract 6 times with 50 mL portions of ethyl acetate. Combine the organic layers and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane to obtain (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as orange crystals (mp 96-98°) (Analyst 1992, 117, 727). Add 100 μL thiophosgene in 10 mL benzene (Caution! Benzene is a carcinogen!) to 100 mg (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in 100 mL acetone, reflux for 1 h, remove the solvent by evaporation under reduced pressure, suspend the residue in 100 mL water, extract 4 times with 25 mL portions of benzene. Combine the extracts and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane:benzene 1:2 to obtain (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as yellow crystals (mp 160-170° d) (Analyst 1995, 120, 385).)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-80A

Mobile phase: MeCN:water:trifluoroacetic acid 62:38:0.1
Column temperature: 40
Flow rate: 1
Detector: F ex 460 em 550

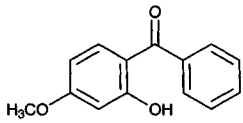
CHROMATOGRAM
Retention time: 10.2, 12.0 (enantiomers)
Limit of detection: 15-16 fmole

OTHER SUBSTANCES
Also analyzed: alprenolol, propranolol

KEY WORDS
derivatization; chiral

REFERENCE
Toyo'oka,T.; Toriumi,M.; Ishii,Y. Enantioseparation of β-blockers labelled with a chiral fluorescent reagent, R(-)-DBD-PyNCS, by reversed-phase liquid chromatography, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1467–1476.

Oxybenzone



Molecular formula: $C_{14}H_{12}O_3$
Molecular weight: 228.25
CAS Registry No.: 131-57-7
Merck Index: 7088

SAMPLE
Matrix: blood, formulations
Sample preparation: Plasma. Add 200 μL MeCN to 100 μL plasma or 2% bovine serum albumin in pH 7.2 phosphate buffer, centrifuge at 10000 g for 10 min, inject an aliquot of the supernatant. Formulations. Keep about 5 mL aliquot of lotion or cream in full sunlight at 17-34° during the day, dilute a 100 mg sample to 100 mL with MeOH:water 50:50, dilute a 200 μL aliquot of the diluted sample to 10 mL with MeOH, inject an aliquot.

HPLC VARIABLES
Column: 100 × 8 4 μL Nova Pak C18 RCM
Mobile phase: MeOH:water 88:12
Flow rate: 1
Injection volume: 10
Detector: UV 315

CHROMATOGRAM
Retention time: 4.8
Limit of detection: 10 ng/mL
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Simultaneous: Escalol 507, Parsol MCX, Parsol 1789, octylsalicylate

KEY WORDS
cream; bovine serum albumin; lotion; plasma; spray

REFERENCE
Jiang,R.; Hayden,C.G.; Prankerd,R.J.; Roberts,M.S.; Benson,H.A. High-performance liquid chromatographic assay for common sunscreening agents in cosmetic products, bovine serum albumin solution and human plasma, *J.Chromatogr.B*, **1996**, *682*, 137–145.

SAMPLE
Matrix: formulations
Sample preparation: Lotion. Weigh out lotion equivalent to about 90 mg oxybenzone, add 7 mL water, add MeOH slowly with vigorous shaking until total volume was 100 mL. Remove a 10 mL aliquot and make up to 100 mL with MeOH, filter (paper), discard first 5 mL of filtrate. Mix 4 mL filtrate and 1 mL 200 μg/mL sulfathiazole in MeOH, make up to 10 mL with MeOH, filter (0.45 μm), inject a 20 μL aliquot. Lipstick. Weigh out an amount equivalent to 25-90 mg oxybenzone, add 10 mL chloroform, add MeOH slowly with vigorous shaking until total volume was 100 mL. Remove a 10 mL aliquot and make up to 100 mL with MeOH, filter (paper), discard first 5 mL of filtrate. Mix 4 mL filtrate and 1 mL 200 μg/mL sulfathiazole in MeOH, make up to 10 mL with MeOH, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 40 × 4.6 25-37 μm Co:Pell ODS
Column: 250 × 4.6 10 μm Partisil PXS ODS-2
Mobile phase: MeCN:MeOH 10:90
Flow rate: 0.7
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 5.7
Internal standard: sulfathiazole (3.9)
Limit of quantitation: 20 ng

OTHER SUBSTANCES
Simultaneous: padimate-O, propyl paraben

KEY WORDS
lotion; lipstick; sun-screen

REFERENCE
Tan,H.S.I.; Sih,R.; Moseley,S.E.; Lichtin,J.L. Assay of mixtures of padimate-O and oxybenzone in sunscreen formulations by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *291*, 275–282.

SAMPLE
Matrix: formulations
Sample preparation: 1-1.5 g sun-screen lotion + 50 mL isopropanol, dissolve. Remove a 5 mL aliquot and make up to 50 mL with mobile phase, filter (0.45 μm) inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm C8 Hypersil
Mobile phase: Isopropanol:buffer 10:90 (Buffer was 100 mM sodium dodecyl sulfate (electrophoresis grade) containing 0.3% triethylamine adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 254, UV 300

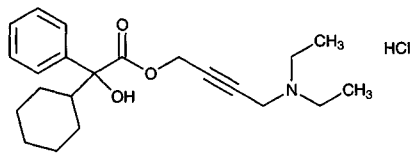
CHROMATOGRAM
Retention time: 3.92

OTHER SUBSTANCES
Simultaneous: 2-ethylhexyl p-dimethylaminobenzoate, octyl methoxycinnamate, methyl paraben, propyl paraben

KEY WORDS
lotion

REFERENCE
Tomasella,F.P.; Zuting,P.; Love,L.J. Determination of sun-screen agents in cosmetic products by micellar liquid chromatography, *J.Chromatogr.*, **1991**, *587*, 325–328.

Oxybutynin chloride



Molecular formula: $C_{22}H_{32}ClNO_3$
Molecular weight: 393.95
CAS Registry No.: 1508-65-2, 5633-20-5 (free base)
Merck Index: 7089
Lednicer No.: 1 93

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 100 μL 1 μg/mL dicyclomine in water + 1 mL MeCN, vortex, allow to stand for 10 min, add 200 μL 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 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 100 mm long 5 μm 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

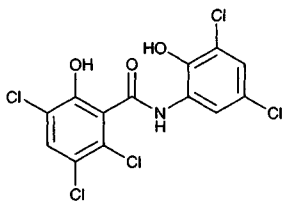
CHROMATOGRAM
Retention time: 17
Internal standard: dicyclomine (23)
Limit of detection: 0.5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

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.

Oxyclozanide



Molecular formula: $C_{13}H_6Cl_5NO_3$
Molecular weight: 401.46
CAS Registry No.: 2277-92-1
Merck Index: 7092

SAMPLE
Matrix: formulations
Sample preparation: Dissolve sample in MeOH containing 10% formic acid, dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil C18
Mobile phase: MeOH:buffer 19:81, pH 3.9 (Buffer was prepared by dissolving 6.6 g dibasic ammonium phosphate in 1 L water and adjusting to pH 3.9 with phosphoric acid.)
Flow rate: 1
Detector: UV 254

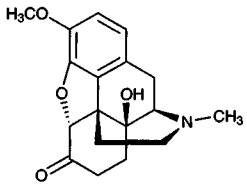
CHROMATOGRAM
Retention time: 11.1

OTHER SUBSTANCES
Simultaneous: albendazole, fenbendazole, niclosamide

KEY WORDS
tablets; powder; liquid formulations;

REFERENCE
van Tonder,E.C.; de Villiers,M.M.; Handford,J.S.; Malan,C.E.P.; Du Preez,J.L. Simple, robust and accurate high-performance liquid chromatography method for the analysis of several anthelmintics in veterinary formulations, *J.Chromatogr.A*, **1996**, *729*, 267–272.

Oxycodone



Molecular formula: $C_{18}H_{21}NO_4$
Molecular weight: 315.37
CAS Registry No.: 76-42-6, 124-90-3 (HCl), 64336-55-6 (terephthalate)
Merck Index: 7093
Lednicer No.: 1 290

SAMPLE
Matrix: blood
Sample preparation: Make plasma containing IS alkaline and extract it with MTBE. Evaporate the organic layer to dryness under nitrogen, reconstitute the residue in 100 μL MeOH:water 90:10, inject an aliquot.

HPLC VARIABLES
Column: Zorbax SB-C18
Mobile phase: MeOH:10mM pH 4.0 ammonium formate 40:60
Flow rate: 1
Detector: MS, Sciex API IIIplus tandem mass, positive ion mode, 316.0/298.0 parent/product ions

CHROMATOGRAM
Retention time: 5
Internal standard: nalmefene (6.9)
Limit of quantitation: 250 pg/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE
Kaisershot,C.; Pierce,A.; Talaat,R. Quantitative analysis of oxycodone and its metabolites noroxycodone and oxymorphone in human plasma by high-performance liquid chromatography with ionspray tandem mass spectrometry (Abstract 2129), *Pharm.Res.*, **1997**, *14*, S262.

SAMPLE
Matrix: blood
Sample preparation: Condition a Certify SPE cartridge (Varian) with 2 mL MeOH and 2 mL 100 mM pH 8.0 phosphate buffer. Add 50 μL 5.96 μM IS in water to 500 μL plasma. Add 1 mL 100 mM pH 8.0 phosphate buffer, vortex for 10 min, add to the SPE cartridge using a gentle vacuum. Wash with 5 mL 100 mM pH 8.0 phosphate buffer, 1 mL water, 2 mL 100 mM pH 4.0 acetate buffer, and 2 mL MeOH. Dry cartridge under vacuum, wash with 200 μL butyl chloride:isopropanol 80:20, dry under vacuum. Elute with 1.2 mL butyl chloride:isopropanol 80:20, evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase. Inject a 150 μL aliquot.

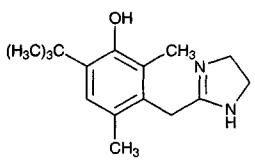
HPLC VARIABLES
Guard column: GuardPak C18 μBondapak
Column: 100 × 8 NovaPak C18
Mobile phase: MeCN:MeOH:13.3 mM pH 7.5 phosphate buffer 2:23:75 containing 40 mg/L cetyltrimethylammonium bromide
Column temperature: 23
Flow rate: 1
Injection volume: 150
Detector: E, Waters Model 460, working electrode 1.10-1.25 V, reference electrode KCl

CHROMATOGRAM
Retention time: 9.6
Internal standard: codeine hydrochloride (14.7)
Limit of quantitation: 14.2 nM

KEY WORDS
plasma; SPE

REFERENCE
Wright,A.W.E.; Lawrence,J.A.; Iu,M.; Cramond,T.; Smith,M.T. Solid-phase extraction method with high-performance liquid chromatography and electrochemical detection for the quantitative analysis of oxycodone in human plasma, *J.Chromatogr.B*, **1998**, *712*, 169–175.

Oxymetazoline



Molecular formula: $C_{16}H_{24}N_2O$
Molecular weight: 260.38
CAS Registry No.: 1491-59-4, 2315-02-8 (HCl)
Merck Index: 7100
Lednicer No.: 1 242

SAMPLE
Matrix: blood
Sample preparation: 300 μL Whole blood + 50 ng IS, vortex, let stand for 30 min, add 900 μL 100 mM sodium carbonate, extract with 3 mL diethyl ether. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in 100 μL MeCN:5 mM ammonium acetate 50:50, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 2.1 5 μm 8 nm pore size Zorbax Rx-C18
Mobile phase: MeCN:5 mM pH 6.5 ammonium acetate 60:40
Flow rate: 0.4
Injection volume: 20
Detector: MS, PE-Sciex API III tandem quadrupole, articulated ionspray, 20:1 post-column split, SIM, m/z 261, 270, nebulizer gas air at 42 psi, curtain gas nitrogen 1.2 L/min, orifice 70 V, open collision cell (Q2)

CHROMATOGRAM
Retention time: 1.5
Internal standard: nonadeutero oxymetazoline (deuterium all on t-butyl group)
Limit of quantitation: 1 ng/g

KEY WORDS
whole blood; LC-MS; rat; pharmacokinetics

REFERENCE
Hayes,F.J.; Baker,T.R.; Dobson,R.L.M.; Tsueda,M.S. Rapid liquid chromatographic-mass spectrometric assay for oxymetazoline in whole rat blood, *J.Chromatogr.A*, **1995**, *692*, 73–81.

SAMPLE
Matrix: formulations
Sample preparation: Dilute nasal solution 10-fold with water, filter (0.45 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 125 × 4 5 μm Aluspher RP-select B (Merck)
Mobile phase: Gradient. MeCN:1 mM NaOH from 10:90 to 80:20 over 25 min.
Column temperature: 25
Flow rate: 1.2
Injection volume: 10
Detector: UV 224 or UV 283

CHROMATOGRAM
Retention time: 13.5
Limit of detection: 1 ng (UV 224)

OTHER SUBSTANCES
Simultaneous: ephedrine, naphazoline, xylometazoline

KEY WORDS
nasal solution

REFERENCE
De Orsi,D.; Gagliardi,L.; Cavazzutti,G.; Mediati,M.G.; Tonelli,D. Simultaneous determination of ephedrine and 2-imidazolines in pharmaceutical formulations by reversed-phase HPLC, *J.Liq.Chromatogr.*, **1995**, *18*, 3233–3242.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.3

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine,

buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a solution in MeOH:water 40:60, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.1 RSIL C18 (RSL, Eke, Belgium)
Mobile phase: MeOH:water 40:60 containing 20 mM sodium 1-octanesulfonate and 10 mM N,N-dimethyloctylamine, pH adjusted to 3.0 with orthophosphoric acid
Column temperature: 25
Flow rate: 1
Injection volume: 10
Detector: UV 220

CHROMATOGRAM

Retention time: 30

OTHER SUBSTANCES

Simultaneous: degradation products, antazoline, coumazoline, lidocaine, naphazoline, prednisolone, sulfadimidine, sulfanilamide, sulfathiazole, tenaphtoxaline, tetrahydrozoline, tolazoline, tramazoline, xylometazoline

REFERENCE

De Schutter,J.A.; Van den Bossche,W.; De Moerloose,P. Stability-indicating analysis of tetryzoline hydrochloride in pharmaceutical formulations by reversed-phase ion-pair liquid chromatography, *J.Chromatogr.*, **1987**, *391*, 303–308.

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

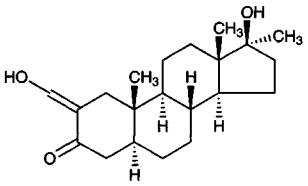
CHROMATOGRAM
Retention time: 10.42 (A), 5.82 (B)

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, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, 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
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.

Oxymetholone



Molecular formula: $C_{21}H_{32}O_3$
Molecular weight: 332.48
CAS Registry No.: 434-07-1
Merck Index: 7101
Lednicer No.: 1 173

SAMPLE

Matrix: formulations
Sample preparation: Oils. 1 mL Sample + 25 mL MeOH:water 90:10, shake vigorously for 5 min, centrifuge, inject a 10 μL aliquot of the supernatant. Tablets. Grind a tablet to a fine powder, add 25 mL MeOH, sonicate for 5-10 min, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate. Suspensions (aqueous). Make up 5 mL to 50 mL with MeOH, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:water 75:25
Flow rate: 1.5
Injection volume: 10
Detector: UV 240

CHROMATOGRAM

Retention time: 16.6
Limit of detection: 5 μg/mL

OTHER SUBSTANCES

Simultaneous: nandrolone propionate, methenolone acetate, testosterone propionate, aspirin, caffeine, formebolone, benzyl alcohol, testolactone, cortisone, fluoxymesterone, norethindrone, oxandrolone (UV 210), boldenone, ethisterone, methandrostenolone, nandrolone, norgestrel, testosterone, dehydroepiandrosterone (UV 210), mibolerone, methyltestosterone, methandriol (UV 210), norethindrone acetate, calusterone, mesterolone (UV 210), norethandrolone, benzyl benzoate, trenbolone acetate, nandrolone acetate
Interfering: testosterone acetate, stanozolol

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: solutions
Sample preparation: Inject a 5 μL aliquot of a 10 μg/mL solution in MeOH.

HPLC VARIABLES

Column: 75 × 4.6 3 μm Ultrasphere ODS
Mobile phase: MeCN:10 mM ammonium acetate buffer 45:55
Flow rate: 0.5
Injection volume: 5
Detector: UV 280

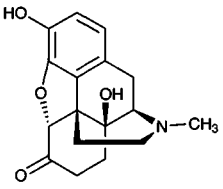
CHROMATOGRAM

Retention time: 14.010

OTHER SUBSTANCES
Simultaneous: boldenone (UV 254), epimethandienone (UV 254), epitestosterone (UV 254), fluoxymesterone (UV 254), 6β-hydroxymethandienone (UV 254), methandienone (UV 254), norethindrone (UV 254), trenbolone (UV 254)

REFERENCE
Barrón,D.; Pascual,J.A.; Segura,J.; Barbosa,J. Prediction of LC retention of steroids using solvatochromic parameters, *Chromatographia*, **1995**, *41*, 573–580.

Oxymorphone



Molecular formula: $C_{17}H_{19}NO_4$
Molecular weight: 301.34
CAS Registry No.: 76-41-5, 357-07-3 (HCl)
Merck Index: 7103
Lednicer No.: 1 290

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 20 μL 1 μg/mL IS + 200 μL 3.5% sodium carbonate, extract with 4 mL dry ether. Extract the organic layer with 200 μL 20 mM phosphoric acid. Inject an aliquot of the phosphoric acid solution.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax C8
Mobile phase: MeCN:MeOH:EDTA:70 mM KH_2PO_4 6:10:0.02:83.98
Flow rate: 1
Detector: E, LCB4, oxidation potential 900 mV

CHROMATOGRAM
Retention time: 6.5
Internal standard: nalorphine (12.5)

KEY WORDS
plasma; rat

REFERENCE
Hussain,M.A.; Aungst,B.J. Intranasal absorption of oxymorphone, *J.Pharm.Sci.*, **1997**, *86*, 975–976.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 1 M sodium bicarbonate + 200 μL 400 ng/mL hydromorphone hydrochloride + 15 mL diethyl ether, rotate for 20 min, centrifuge at 800 g for 15 min. Remove the organic phase and add it to 200 μL 17 mM phosphoric acid, mix vigorously for 15 s, centrifuge for 5 min. Remove the aqueous phase and evaporate it to dryness under a stream of air at 45°, reconstitute the residue in 200 μL 17 mM phosphoric acid, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 25 × 4.6 5 μm Hi-Chrom reversible octyl (Regis)
Mobile phase: MeCN:30 mM pH 4 KH_2PO_4:0.3% sodium octanesulfonate 15:75:9
Flow rate: 1.5
Injection volume: 100
Detector: E, BAS LC4B, glassy carbon working electrode 0.9 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 7.0

Internal standard: hydromorphone hydrochloride (8.9)
Limit of quantitation: 1.34 ng/mL

KEY WORDS
rat; plasma; pharmacokinetics

REFERENCE
Lam,G.; Williams,R.M.; Whitney,C.C. Electrochemical determination of oxymorphone in rat plasma by ion-pair reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *413*, 309–314.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

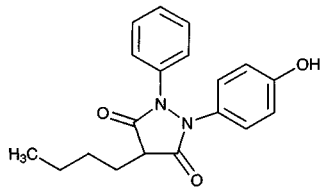
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine,

thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Oxyphenbutazone



Molecular formula: $C_{19}H_{20}N_2O_3$
Molecular weight: 324.38
CAS Registry No.: 129-20-4, 7081-38-1 (H2O)
Merck Index: 7106
Lednicer No.: 1 236

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

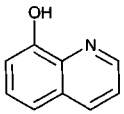
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, ox-

ytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Oxyquinoline



Molecular formula: C_9H_7NO
Molecular weight: 145.16
CAS Registry No.: 148-24-3, 134-31-6 (sulfate)
Merck Index: 4890

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 PRP-1 in a PEEK column (Hamilton)
Mobile phase: Gradient. MeCN:10 mM pH 5.0 acetate buffer 5:95 for 3 min, to 90:10 over 27 min (Waters curve no. 5). (At the beginning of each day flush column with 0.01% EDTA for 30 min, equilibrate at initial conditions for 30 min.)
Detector: UV 254

CHROMATOGRAM

Retention time: 20.9

OTHER SUBSTANCES

Simultaneous: metabolites, glucuronide, quinoline, 5-hydroxyquinoline

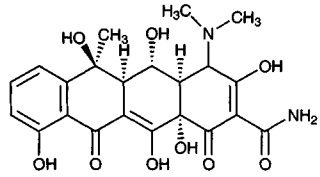
KEY WORDS

use PEEK tubing; metal-free system

REFERENCE

Nagiel-Ostaszewski,I.; Vavrek,M.T.; Weisburger,J.H. Separation of hydroxyquinolines by high-performance liquid chromatography, *Xenobiotica*, **1991**, *21*, 751–754.

Oxytetracycline



Molecular formula: $C_{22}H_{24}N_2O_9$
Molecular weight: 460.44
CAS Registry No.: 79-57-2, 6153-64-6 (dihydate), 2058-46-0 (HCl), 15251-48-6 (Ca salt)
Merck Index: 7111
Lednicer No.: 1 212

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 18:82
Flow rate: 2
Injection volume: 80
Detector: UV 270

CHROMATOGRAM
Retention time: 3.2
Limit of detection: 120 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
Sample preparation: 250 µL Plasma + 50 µL 12 µg/mL tetracycline in 10 mM HCl + 1 mL buffer, vortex vigorously, add 6 mL ethyl acetate, add 500 µL 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 µL mobile phase and 1 mL hexane, vortex, centrifuge at 9800 g for 5 min, inject a 100 µL aliquot of the lower aqueous layer. (Buffer was 3 M NaH_2PO_4 containing 1 M sodium sulfite, pH ca. 5.4.)

HPLC VARIABLES
Column: 125 × 4 5 µm 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: 3.5
Internal standard: tetracycline (4.5)
Limit of detection: 5 ng/mL

KEY WORDS
plasma; cow; pharmacokinetics

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: Filter (Advantec 0.45 μm cellulose acetate), inject a 100 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Hisep shielded hydrophobic phase (Supelco)
Column: 150 × 4.6 5 μm Hisep shielded hydrophobic phase (Supelco)
Mobile phase: MeOH:200 mM oxalic acid 10:90, pH adjusted to 7.0 with 28% aqueous ammonia
Flow rate: 1
Injection volume: 100
Detector: UV 360

CHROMATOGRAM

Retention time: 3.8
Limit of detection: 50 ng/mL

KEY WORDS

serum; chicken; pig; cow; fish; trout; direct injection

REFERENCE

Ueno,R.; Uno,K.; Aoki,T. Determination of oxytetracycline in blood serum by high-performance liquid chromatography with direct injection, *J.Chromatogr.*, **1992**, *573*, 333–335.

SAMPLE

Matrix: blood

Sample preparation: 100 μL Serum + 200 μL 24% trichloroacetic acid in MeOH + 300 μL mobile phase buffer (A), vortex for 1 min, centrifuge at 2000 g for 15 min, inject 50 μL of the supernatant.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Capcell C18 type SG-120 (Shiseido)
Mobile phase: MeOH:buffer 30:70 (Buffer (A) was 100 mM pH 6.5 sodium acetate containing 35 mM calcium chloride and 25 mM disodium ethylenediamine tetraacetate.)
Column temperature: 30 ± 0.2
Flow rate: 1
Injection volume: 50
Detector: F ex 390 em 512

CHROMATOGRAM

Retention time: 6
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Also analyzed: tetracycline, chlortetracycline

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: 200 μL Serum + 50 μL water + 250 μL 500 mM trichloroacetic acid, vortex for 30 s, centrifuge at 10000 g for 10 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: Nova-Pak phenyl guard
Column: 4 μm Nova-Pak phenyl radial compression
Mobile phase: MeCN:MeOH:100 mM oxalic acid 11:9:80 adjusted to pH 2.7 with 1 M HCl
Flow rate: 2
Injection volume: 50
Detector: UV 350

CHROMATOGRAM

Retention time: 13.5-14
Internal standard: oxytetracycline

OTHER SUBSTANCES

Extracted: minocycline

KEY WORDS

serum; oxytetracycline is IS

REFERENCE

Birmingham,K.; Vaughan,L.M.; Strange,C. Rapid serum minocycline assay for pleurodesis monitoring using high-performance liquid chromatography with radial compression, *Ther.Drug Monit.*, **1995**, *17*, 268–272.

SAMPLE

Matrix: blood, urine
Sample preparation: Serum. 500 μL Serum + 50 μL 6% ascorbic acid in water + 50 μL demeclocycline in MeOH/100 mM HCl + 1 mL buffer, mix for 30 s, add 6 mL ethyl acetate, rotate for 10 min, centrifuge at 3000 rpm for 6 min. Remove the organic layer and add it to 100 μL 0.2% ascorbic acid in MeOH, evaporate to dryness under vacuum while vortexing, reconstitute the residue in 200 μL mobile phase, mix, filter, keep in ice, inject a 20 μL aliquot. Urine. 100 μL Urine + 50 μL 6% ascorbic acid in water + 50 μL demeclocycline in MeOH/100 mM HCl + 400 μL buffer, mix for 30 s, add 3 mL ethyl acetate, rotate for 10 min, centrifuge at 3000 rpm for 6 min. Remove the organic layer and add it to 100 μL 0.2% ascorbic acid in MeOH, evaporate to dryness under vacuum while vortexing, reconstitute the residue in 200 μL mobile phase, mix, filter, keep in ice, inject a 20 μL aliquot. (Buffer was 27.6 g NaH_2PO_4 + 25.2 g sodium sulfite in 100 mL water, pH 6.1.)

HPLC VARIABLES

Column: 100 × 2 5 μm Lichrosorb RP8
Mobile phase: MeCN:100 mM citric acid 24:76
Flow rate: 0.5
Injection volume: 20
Detector: UV 350

CHROMATOGRAM

Retention time: 2
Internal standard: demeclocycline (4)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Extracted: tetracycline, chlortetracycline, methacycline, doxycycline

KEY WORDS

serum

REFERENCE

De Leenheer,A.P.; Nelis,H.J.C.F. Doxycycline determination in human serum and urine by high-performance liquid chromatography, *J.Pharm.Sci.*, **1979**, *68*, 999–1002.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Bulk. Prepare a 10-100 μg/mL solution in buffer, inject an aliquot. Capsules, tablets. Prepare a 1 mg/mL solution of capsule contents or crushed tablets in buffer, sonicate for 10 min, filter (0.45 μm), dilute with buffer, inject an aliquot. Injections. 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: 17

OTHER SUBSTANCES
Simultaneous: impurities, tetracycline

KEY WORDS
capsules; tablets; injections

REFERENCE
Bryan,P.D.; Stewart,J.T. Chromatographic analysis of selected tetracyclines from dosage forms and bulk drug substance using polymeric columns with acidic mobile phases, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 675–692.

SAMPLE
Matrix: eggs, milk, tissue
Sample preparation: Muscle. Homogenize (Ultra-Turrax) 5 g muscle, 200 μL 125 μg/mL tetracycline in 10 mM HCl, 50 mL 50 mM HCl, and 10 mL hexane for 2 min, sonicate for 3 min, centrifuge at 1920 g for 5 min. Dialyze (Cuprophan cellulose dialysis membrane, 15 kD cutoff) a 530 μL aliquot of the aqueous layer against seven 875 μL aliquots of recipient solution. Pump the recipient solution into the recipient channel at 1.7 mL/min, allow to remain stationary for 36 s, then pump onto column A. Finally, backflush the contents of column A onto the analytical column with mobile phase. After 2 min remove column A from the circuit and flush it with 2 mL recipient solution. Flush the recipient channel with 2 mL recipient solution, flush the donor channel with 2 mL 0.01% Triton X-100. (Recipient solution was 20 mM pH 5 phosphate buffer containing 5 mM sodium heptanesulfonate.) Liver. Homogenize (Ultra-Turrax) 2 g liver, 200 μL 25 μg/mL tetracycline in 10 mM HCl, 20 mL 10 mM HCl, and 10 mL hexane:dichloromethane 25:75 for 1 min, sonicate for 3 min, centrifuge at 1920 g for 5 min. Dialyze (Cuprophan cellulose dialysis membrane, 15 kD cutoff) a 530 μL aliquot of the aqueous layer against seven 875 μL aliquots of recipient solution. Pump the recipient solution into the recipient channel at 1.7 mL/min, allow to remain stationary for 36 s, then pump onto column A. Finally, backflush the contents of column A onto the analytical column with mobile phase. After 2 min remove column A from the circuit and flush it with 2 mL recipient solution. Flush the recipient channel with 2 mL recipient solution, flush the donor channel with 2 mL 0.01% Triton X-100. (Recipient solution was 20 mM pH 5 phosphate buffer containing 5 mM sodium heptanesulfonate.) Eggs. 2 g Homogenized whole egg + 100 μL 50 μg/mL tetracycline in 10 mM HCl + 2 mL 0.9% NaCl + 600 μL 10% sodium azide (Caution! Sodium azide is highly toxic and may be carcinogenic!), shake manually for 10 s. Dialyze (Cuprophan cellulose dialysis membrane, 15 kD cutoff) a 530 μL aliquot against seven 875 μL aliquots of recipient solution. Pump the recipient solution into the recipient channel at 1.7 mL/min, allow to remain stationary for 36 s, then pump onto column A. Finally, backflush the contents of column A onto the analytical column with mobile phase. After 2 min remove column A from the circuit and flush it with 2 mL recipient solution. Flush the recipient channel with 2 mL recipient solution, flush the donor channel with 2 mL 0.01% Triton X-100 containing 18 g/L NaCl. (Recipient solution was 20 mM pH 5 phosphate buffer containing 5 mM sodium heptanesulfonate and 16.5 g/L NaCl.) Milk. 5 mL Milk + 100 μL 50 μg/mL tetracycline in 10 mM HCl + 1 mL 100 mM EDTA, shake manually for 10 s, centrifuge for 10 min, freeze at -20° for 10 min, remove the decreamed milk solution. Dialyze (Cuprophan cellulose dialysis membrane, 15 kD cutoff) a 530 μL aliquot of the decreamed milk

solution against seven 875 μL aliquots of recipient solution. Pump the recipient solution into the recipient channel at 1.7 mL/min, allow to remain stationary for 36 s, then pump onto column A. Finally, backflush the contents of column A onto the analytical column with mobile phase. After 2 min remove column A from the circuit and flush it with 2 mL recipient solution. Flush the recipient channel with 2 mL recipient solution, flush the donor channel with 2 mL 0.01% Triton X-100. (Recipient solution was 20 mM pH 5 phosphate buffer containing 10 mM sodium heptanesulfonate.)

HPLC VARIABLES

Column: A 10 × 2 36 μm Dynospheres polystyrene beads (Dyno Particles, Lillestrom, Norway); B 150 × 4.6 5 μm PLRP-S (Polymer Labs)

Mobile phase: MeCN:buffer 23:77 (Buffer was 20 mM orthophosphoric acid containing 5 (muscle, liver, eggs) or 10 (milk) mM sodium heptanesulfonate.)

Flow rate: 0.7

Detector: F ex 358 em 460 following post-column reaction. The column effluent mixed with 2 M NaOH pumped at 0.15 mL/min and the mixture flowed through a knitted 10 m × 0.3 mm ID reaction coil irradiated at 366 nm to the detector.

CHROMATOGRAM

Retention time: 8

Internal standard: tetracycline (11)

Limit of detection: 4 ng/g (chicken muscle), 3 ng/g (cow muscle), 8 ng/g (salmon liver), 1 ng/g (eggs), 1 ng/mL (milk)

KEY WORDS

post-column photochemical reaction; column-switching; dialysis; salmon; cow; chicken; muscle; liver

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REFERENCE

Agasoster,T. Automated determination of oxytetracycline residues in muscle, liver, milk and egg by on-line dialysis and post-column reaction detection HPLC, *Food Addit.Contam.*, **1992**, *9*, 615–622.

SAMPLE

Matrix: eggs, tissue

Sample preparation: Prepare a metal chelate affinity chromatography (MCAC) column by adding 1.5 mL of thoroughly mixed Chelating Sepharose Fast-Flow suspension in EtOH:water 20:80 (Pharmacia) to a 150 × 10 glass column, allow to drain, wash with three 2 mL portions of water, add 2 mL 10 mM copper(II) sulfate in water, wash with two 2 mL portions of water. Condition an SBD-RPS extraction membrane (3M Company, St. Paul, MN) with 2 mL MeOH and 2 mL 100 mM HCl. Add 20 mL 100 mM pH 4.0 sodium succinate buffer to 3 g pig kidney, pig muscle, cow liver, or whole chicken egg, vortex for 1 min and shake for 10 min on a horizontal shaker. Add 20 mL MeOH, sonicate for 5 min and centrifuge at 2666 g for 10 min at 4°. Filter the supernatant through a Whatman 541 filter paper. Add the clear supernatant to the MCAC column. Wash sequentially with 2 mL 100 mM sodium succinate buffer, 2 mL water, 2 mL MeOH, 2 mL water, and with 500 μL McIlvaine-EDTA-NaCl buffer. Elute with 3 mL McIlvaine-EDTA-NaCl buffer and adjust the eluate to pH 1.3 with 400 μL 4 M HCl. Add the eluate directly to the extraction membrane to prevent crystallization of EDTA. Wash the membrane with 1 mL 100 mM HCl and elute with four 250 μL portions of MeOH:25% ammonia 97:3, evaporate the eluate to dryness under the nitrogen at 40°. Reconstitute the dry residue with 250 μL 10 mM oxalic acid in water, vortex, sonicate. Inject a 100 μL aliquot. (The sodium succinate buffer was 100 mM succinic acid, pH adjusted to 4.0 with 10 M NaOH. Prepare the McIlvaine buffer by dissolving 12.9 g citric acid monohydrate and 10.9 g Na_2HPO_4 in 1 L water. The McIlvaine-EDTA-NaCl buffer was 100 mM EDTA and 500 mM NaCl in McIlvaine buffer. Protect all solutions from light.)

HPLC VARIABLES

Guard column: 5 × 3.0 PLRP-S (Polymer Laboratories)

Column: 250 × 4.6 8 μ PLRP-S (Polymer Laboratories)

Mobile phase: Gradient. A was 10 mM oxalic acid in water adjusted to pH 2.0 with 4 M HCl. B was MeCN. A:B from 85:15 to 60:40 over 16 min.

Flow rate: 1

Injection volume: 100

Detector: F ex 406 em 515 following post-column reaction. The column effluent mixed with reagent pumped at 1 mL/min and the mixture flowed through a 600 μL reaction coil to the detector. (Reagent was 5% zirconyl chloride octahydrate in water stored at 4°.)

CHROMATOGRAM

Retention time: 10
Limit of detection: 0.61 ng/g (pig kidney), 0.42 ng/g (pig muscle), 0.68 ng/g (cow liver), 0.21 ng/g (chicken egg)
Limit of quantitation: 2 ng/g (pork kidney)

OTHER SUBSTANCES

Extracted: chlortetracycline, doxycycline, tetracycline
Also analyzed: demeclocycline

KEY WORDS

cow; liver; pig; kidney; muscle; chicken; metal chelate affinity chromatography; MCAC; SPE; post-column reaction

REFERENCE

Croubels,S.M.; Vanoosthuyze,K.E.I.; Van Peteghem,C.H. Use of metal chelate affinity chromatography and membrane-based ion-exchange as clean-up procedure for trace residue analysis of tetracyclines in animal tissues and egg, *J.Chromatogr.B*, **1997**, *690*, 173–179.

SAMPLE

Matrix: eggs, tissue
Sample preparation: Condition an Anagel-TSK Chelate-SPW column with 25 μL 50 mg/mL copper sulfate in water and 500 μL. Homogenize 2 g sliced chicken liver with 1.2 mL 1 M pH 4 citrate buffer and 12 mL ethyl acetate for 1 min. Homogenize 2 g sliced tissue with 1.2 mL 1 M pH 5 citrate buffer and 12 mL ethyl acetate for 1 min. Shake 2 g blended egg with 1.2 mL 1 M pH 5 citrate buffer and 12 mL ethyl acetate for 15 min. Centrifuge the mixture at 11000 rpm for 10 min, decant the supernatant, reextract the residue with two 12 mL portions of ethyl acetate. Add 10 g anhydrous sodium sulfate to the combined supernatants, swirl, let stand for 5-10 min, filter (Whatman 1PS phase-separating filter paper). Evaporate the filtrate to dryness or to an oily residue on a rotary evaporator under reduced pressure at 40°, reconstitute the residue in 2 mL MeOH by vortexing, filter (0.2 μm syringe filter). Add 1.5 mL of the filtrate to the Anagel column at 0.36 mL/min, wash with 500 μL water, 500 μL MeOH, and 500 μL water. Elute the contents of the Anagel column onto the analytical column with mobile phase A, after 11 min remove the Anagel column from the circuit, elute column B using gradient elution of mobile phase A:B, monitor the effluent from column B. (Prepare 1 M pH 4 or 5 citrate buffer as follows: dissolve 192 g citric acid in approximately 800 mL water, adjust pH value with 1 M NaOH and make up to 1 L with water.)

HPLC VARIABLES

Guard column: 5 × 3 PLRP-S
Column: 150 × 4.6 5 μm Polymer Labs PLRP-S
Mobile phase: Gradient. A:B 100:0 for 11 min, to 0:100 in 10 min, maintain at 0:100 for 10 min. A was buffer. B was MeCN:MeOH:buffer 25:10:65. (Buffer was 100 mM KH_2PO_4 containing 10 mM citric acid, and 10 mM EDTA).
Flow rate: 1
Injection volume: 1500
Detector: UV 350

CHROMATOGRAM

Retention time: 24.5
Limit of detection: 3 ng/g

OTHER SUBSTANCES

Extracted: tetracycline, chlortetracycline, demeclocycline

KEY WORDS

chicken; egg; metal chelate affinity chromatography; muscle; liver; salmon; trout; venison; SPE

REFERENCE
Cooper,A.D.; Stubbings,G.W.F.; Kelly,M.; Tarbin,J.A.; Farrington,W.H.H.; Shearer,G. Improved method for the on-line metal chelate affinity chromatography-high-performance liquid chromatographic determination of tetracycline antibiotics in animal products, *J.Chromatogr.A*, **1998**, *812*, 321–326.

SAMPLE
Matrix: feed
Sample preparation: Condition a C18 Extract-Clean SPE cartridge (Alltech) with 5 mL MeOH, 5 mL water, and 5 mL 10 mM pH 3.0 oxalic acid buffer. Homogenize 1 g feed in 3 mL ethyl acetate, add 100 μL 200 μg/mL tetracycline in MeOH, add 2 mL 10 mM pH 3.0 EDTA, evaporate the ethyl acetate under reduced pressure. Wash the aqueous phase into a vial with 1 mL 10 mM EDTA, add 10 IU lipase (EC 3.1.4.3, Sigma), let stand at room temperature in the dark overnight, centrifuge at 5000 g for 5 min, add the supernatant to the SPE cartridge, wash with two 5 mL portions of n-hexane, elute with 8 mL ethyl acetate:MeCN 6:2. Evaporate the eluate to dryness under vacuum at 40°, reconstitute the residue in 2 mL mobile phase, inject a 50-100 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:THF:10 mM pH 3.0 oxalic acid buffer 15:3:82
Flow rate: 1
Injection volume: 50-100
Detector: UV 365

CHROMATOGRAM
Retention time: 9
Internal standard: tetracycline (5)
Limit of detection: 10 ng/g

KEY WORDS
SPE

REFERENCE
Touraki,M.; Rigas,P.; Pergandas,P.; Kastritsis,C. Determination of oxytetracycline in the live fish feed Artemia using high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1995**, *663*, 167–171.

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' 0.39

OTHER SUBSTANCES
Simultaneous: anhydrotetracycline, chlortetracycline, demeclocycline, doxycycline, epianhydrotetracycline, quatrimycin, rolitetracycline, tetracycline

KEY WORDS
ointment

REFERENCE

Lingeman,H.; van Munster,H.A.; Beynen,J.H.; Underberg,W.J.; Hulshoff,A. High-performance liquid chromatographic analysis of basic compounds on non-modified silica gel and aluminium oxide with aqueous solvent mixtures, *J.Chromatogr.*, **1986**, *352*, 261–274.

SAMPLE

Matrix: honey

Sample preparation: Prepare a 100 mg Baker 10 C18 cartridge by washing with MeOH, water, and 10 mL saturated aqueous Na_2EDTA. Dissolve 5 g honey in 20 mL 100 mM pH 4.0 Na_2EDTA-McIlvaine buffer, filter, apply to cartridge, wash with 20 mL water, air dry under vacuum for 5 min. Condition a Baker 10 COOH cartridge with ethyl acetate. Elute contents of C18 cartridge onto COOH cartridge with 50 mL ethyl acetate. Wash COOH cartridge with 10 mL MeOH, elute with 10 mL mobile phase, inject 100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Bakerbond C8
Mobile phase: MeOH:MeCN:10 mM aqueous oxalic acid 1:1.5:3
Flow rate: 1
Injection volume: 100
Detector: UV 350

CHROMATOGRAM

Retention time: 3.3
Limit of detection: 0.02 ppm

OTHER SUBSTANCES

Simultaneous: tetracycline, doxycycline, chlortetracycline

KEY WORDS

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: honey

Sample preparation: Condition a 500 mg Baker-10 C18 SPE cartridge with 10 mL MeOH, 10 mL water, and 10 mL saturated aqueous disodium EDTA. Condition a 500 mg Baker-10 COOH cartridge with MeOH:ethyl acetate 10:90. Dissolve 25 g honey in 50 mL 100 mM pH 4.0 disodium EDTA-McIlvaine buffer, filter. Add the filtrate to the C18 SPE cartridge, wash with 20 mL water, wash with 400 μL ethyl acetate, air dry under vacuum for 5 min, elute with 50 mL MeOH:ethyl acetate 10:90. Add a 5 mL aliquot to the COOH SPE cartridge, wash with 5 mL MeOH (?), elute with 10 mL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 75 × 4.6 3 μm Chemcosorb 3C8 (Chemco)
Mobile phase: MeCN:MeOH:10 mM aqueous oxalic acid 3:2:16, pH 3.0
Flow rate: 1
Injection volume: 100
Detector: UV 350

CHROMATOGRAM

Retention time: 2
Limit of detection: 0.1 ppm

OTHER SUBSTANCES

Extracted: chlortetracycline, demeclocycline (demethylchlortetracycline), doxycycline, methacycline, minocycline, tetracycline

KEY WORDS
SPE

REFERENCE
Oka,H.; Ikai,Y.; Kawamura,N.; Uno,K.; Yamada,M.; Harada,K.; Suzuki,M. Improvement of chemical analysis of antibiotics. XII. Simultaneous analysis of seven tetracyclines in honey, *J.Chromatogr.*, **1987**, *400*, 253–261.

SAMPLE
Matrix: milk
Sample preparation: 2 mL Milk + 4 mL buffer, filter (Amicon CF-25 ultrafiltration membrane) while centrifuging at 20° at 1000 g for 1 h, suspend solids in 2 mL buffer and repeat filtration for 40 min. Combine filtrates and inject a 500 μL aliquot as soon as possible. (Buffer (McIlvaine) was prepared by mixing 625 mL 28.41 g/L Na_2HPO_4 and 1 L 21.01 g/L citric acid monohydrate. The buffer was also 100 mM in disodium EDTA and the final pH was 4.0 ± 0.1.)

HPLC VARIABLES
Column: 150 × 3.9 Novapak C18
Mobile phase: Gradient. MeCN:MeOH:10 mM oxalic acid 0:0:100 for 1 min, to 22:8:70 over 5 min, maintain at 22:8:70 for 10 min, re-equilibrate at 0:0:100 at 1.5 mL/min for 5 min and at 1 mL/min for 1 min. (Flush daily with 10 column volumes of water. Store column in MeOH: water 60:40, flush with water before use.)
Column temperature: 30
Flow rate: 1
Injection volume: 500
Detector: UV 360

CHROMATOGRAM
Retention time: 10.3
Limit of detection: 7.9 ng/mL
Limit of quantitation: 14.1 ng/mL

OTHER SUBSTANCES
Extracted: chlortetracycline, tetracycline

KEY WORDS
cow; protect from light; ultrafiltrate

REFERENCE
Thomas,M.H. Simultaneous determination of oxytetracycline, tetracycline, and chlortetracycline in milk by liquid chromatography, *J.Assoc.Off.Anal.Chem.*, **1989**, *72*, 564–567.

SAMPLE
Matrix: milk
Sample preparation: Place 22 g 40 μm, 18% load, end-capped bulk C18 material (Analytichem) in a 50 mL syringe barrel, wash with 2 column volumes hexane, dichloromethane, and MeOH, vacuum aspirate until dry. 2 g Bulk C18 material + 50 mg disodium EDTA + 50 mg oxalic acid + 500 μL milk + 10 μL MeOH, blend gently in a glass mortar and pestle for 30 s, place the mixture in a 10 mL plastic syringe barrel plugged with a piece of filter paper. Compress column volume to 4.5 mL, add a 100 μL pipette tip on the column outlet to restrict the flow. Wash with 8 mL hexane, remove excess hexane with positive pressure, elute with 8 mL MeCN:ethyl acetate 75:25. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 500 μL mobile phase, sonicate for 5-10 min, centrifuge at 17000 g for 5 min, filter the supernatant (0.45 μm), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 300 × 4 10 μm Micro Pak ODS
Mobile phase: MeCN:10 mM oxalic acid in water 30:70
Flow rate: 1
Injection volume: 20
Detector: UV 365

CHROMATOGRAM
Retention time: 4.1
Limit of detection: 2 ng

OTHER SUBSTANCES
Extracted: chlortetracycline, tetracycline

KEY WORDS
cow; matrix solid-phase dispersion

REFERENCE
Long,A.R.; Hsieh,L.C.; Malbrough,M.S.; Short,C.R.; Barker,S.A. Matrix solid-phase dispersion (MSPD) isolation and liquid chromatographic determination of oxytetracycline, tetracycline, and chlortetracycline in milk, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 379–384.

SAMPLE
Matrix: milk
Sample preparation: Prepare a column as follows. Swirl Chelating Sepharose Fast Flow resin (Pharmacia) in its bottle, add it to a polypropylene column to give a bed volume of 1.0-1.2 mL, wash 3 times with 2 mL portions of water, wash with 2 mL 10 mM copper sulfate, wash with two 2 mL portions of water. Centrifuge 5 mL milk at 10° at 1500 g for 15 min, remove the lower layer and add it to 10 mL succinate buffer, mix, centrifuge at 1500 g for 30 min, add the supernatant to the column. Wash with 2 mL succinate buffer, wash with 2 mL water, wash with 2 mL MeOH, wash with 2 mL water, wash with 700 μL citrate/phosphate buffer (be careful not to disturb bed), elute with 2.5 mL citrate/phosphate buffer (column is white and eluate is blue). Filter (Amicon Centricon 30, MW 30000 cut-off; pre-washed by centrifuging with 2 mL water) while centrifuging at 5000 g for 30-90 min, inject a 600 μL aliquot of the ultrafiltrate. (Prepare succinate buffer by dissolving 11.8 g succinic acid in 980 mL water, adjust pH to 4.0 with 10 M NaOH, make up to 1 L. Prepare the citrate/phosphate buffer by dissolving 12.9 g citric acid monohydrate, 10.9 g Na_2HPO_4, 37.2 g disodium EDTA dihydrate, and 29.2 g NaCl in 1 L water.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm PLRP-S (Polymer Labs)
Mobile phase: Gradient. MeCN:MeOH:10 mM oxalic acid 0:0:100 for 1 min, to 22:8:70 over 5 min, maintain at 22:8:70 for 11 min, return to initial conditions.
Flow rate: 1
Injection volume: 600
Detector: UV 355

CHROMATOGRAM
Retention time: 12.8
Limit of detection: 0.42 ng/mL
Limit of quantitation: 0.83 ng/mL

OTHER SUBSTANCES
Extracted: chlortetracycline, demeclocycline, doxycycline, methacycline, minocycline, tetracycline
Noninterfering: chloramphenicol, gentian violet, hydromycin B, ivermectin, spectinomycin, sulfa drugs

KEY WORDS
cow; SPE; ultrafiltrate

REFERENCE
Carson,M.C. Simultaneous determination of multiple tetracycline residues in milk using metal chelate affinity chromatography, *J.AOAC Int.*, **1993**, *76*, 329–334.

SAMPLE
Matrix: milk

Sample preparation: 5 mL Milk + 1 mL 1 M HCl, mix, add 24 mL MeCN slowly with swirling over 30 s, let stand for 5 min, decant the clear supernatant through a plug of glass wool. 15 mL Filtrate + 15 mL dichloromethane + 30 mL hexane, mix, collect the aqueous layer. Extract the organic layer with 1 mL water. Combine the aqueous layers, make up to 4 mL with water, filter (13 mm, 0.45 μm, PVDF), inject a 1000 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: 5 μm PLRP-S 100 Å polystyrene divinylbenzene (Polymer Laboratories)
Column: 150 × 4.6 5 μm PLRP-S 100 Å polystyrene divinylbenzene (Polymer Laboratories)
Mobile phase: Gradient. MeCN:buffer 20:80 for 3 min, to 38:62 over 22 min, maintain at 38:62 for 5 min, return to initial conditions for 1 min, re-equilibrate for 9 min. (Buffer was 3.94 g potassium oxalate + 3.61 g oxalic acid + 1.22 g sodium decanesulfonate in 1 L water, pH 2.30.)
Flow rate: 1
Injection volume: 1000
Detector: UV 365

CHROMATOGRAM

Retention time: 16.5
Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: chlortetracycline, tetracycline

REFERENCE

White,C.R.; Moats,W.A.; Kotula,K.L. Optimization of a liquid chromatographic method for determination of oxytetracycline, tetracycline, and chlortetracycline in milk, *J.AOAC Int.*, **1993**, *76*, 549–554.

SAMPLE

Matrix: milk
Sample preparation: Prepare a column by adding 1.5 mL of thoroughly mixed Chelating Sepharose Fast-Flow suspension in EtOH:water 20:80 (Pharmacia) to a 150 × 10 glass column, allow to drain, wash with three 2 mL portions of water, add 2 mL 10 mM copper(II) sulfate in water, wash with two 2 mL portions of water. Centrifuge 10 mL milk at 2100 g for 5 min, decant the skimmed milk, rinse the tube with two 1 mL portions of water. Add 10 mL pH 4.0 buffer to the milk and rinses, sonicate for 3 min, filter (Whatman 541 paper) the supernatant. Add the filtrate to the column, wash with 2 mL pH 4.0 buffer, wash with 2 mL water, wash with 2 mL MeOH, wash with 2 mL water, add 700 μL EDTA buffer to the column, elute with 3 mL EDTA buffer, add 20 μL 25 μg/mL demeclocycline hydrochloride in MeOH to the eluate, inject a 100 μL aliquot. (Prepare pH 4.0 buffer by adjusting 100 mM succinic acid to pH 4.0 with 10 M NaOH. Prepare EDTA buffer by dissolving 12.9 g citric acid monohydrate, 10.9 g Na_2HPO_4, 29.2 g NaCl, and 100 mmoles EDTA in 1 L water.)

HPLC VARIABLES

Guard column: 5 × 3 PLRP-S (Polymer Laboratories)
Column: 250 × 4.6 5 μm 100 Å PLRP-S (Polymer Laboratories)
Mobile phase: MeCN:MeOH:buffer 15:10:60 (Buffer was 10 mM oxalic acid adjusted to pH 2.0 with 4 M HCl.)
Flow rate: 1
Injection volume: 100
Detector: F ex 406 em 515 following post-column reaction. The column effluent mixed with reagent pumped at 1 mL/min and the mixture flowed through a 600 μL reaction coil to the detector. (Reagent was 5% zirconyl chloride octahydrate in water.)

CHROMATOGRAM

Retention time: 4.9
Internal standard: demeclocycline (8.3)
Limit of detection: 1 ng/mL

OTHER SUBSTANCES

Extracted: chlortetracycline, tetracycline

KEY WORDS

protect from light; cow; post-column reaction; SPE; complexation

REFERENCE
Croubels,S.; Van Peteghem,C.; Baeyens,W. Sensitive spectrofluorimetric determination of tetracycline residues in bovine milk, *Analyst*, **1994**, *119*, 2713–2716.

SAMPLE
Matrix: milk, tissue
Sample preparation: Wash column A with 20 mL 5 mg/mL aqueous copper sulfate and column B with 100 mL acetone, 100 mL MeOH, and 100 mL water. Sonicate 10 mL milk or 10 g sliced tissue with 40 mL pH 4.0 succinate buffer for 3 min. (Buffer was 5 g succinic anhydride in 1 L water adjusted to pH 4.0 with 1 M NaOH.) Homogenize for 2 min (Ultra-Turrax), centrifuge at 12000 and 24000 rpm for 5 min, filter the supernatant through a Whatman 541 filter paper. Repeat extraction with 40 mL and 20 mL portions of succinate buffer, load the combined filtrates onto column A. Wash column A with 10 mL water, 30 mL MeOH, and two 10 mL portions of water. Elute tetracycline fraction with 40 mL pH 4.0 succinate buffer containing 100 mM disodium EDTA. After elution wash column A with 10 mL pH 4.0 succinate buffer containing 100 mM disodium EDTA. Load 50 mL combined eluate from column A onto column B, wash with two 100 mL portions of water and elute with 100 mL redistilled MeOH, discard the first 10 mL eluate. Evaporate eluate to a small volume on rotary evaporator at 40° and transfer to pear-shaped flask with three 2 mL portions of redistilled MeOH. Add 100 μL 5% β-mercaptopropionic acid in MeOH, evaporate MeOH under reduced pressure at 40°, reconstitute the residue in 500 μL mobile phase, vortex for 15 s and sonicate for 30 s, inject a 10 μL aliquot. (Column A was a 200 × 20 chelating Sepharose column. Prepare column as follows. Thoroughly mix 5 mL chelating Sepharose suspension (Pharmacia AB), place it in a 200 × 20 glass column, let settle to a 15 mm bed height. Remove liquid excess and load the column by passing 20 mL 5 mg/mL copper sulfate through it. Vortex the column after first 10 mL to remove bubbles, then pass 15 mL pH 4.0 succinate buffer through the column. Wash the column with 20 mL water after use, store in MeOH:water 20:80 at 4°. Column B was a 200 × 20 Amberlite XAD-2 resin column. Prepare as follows. Wash Amberlite resin sequentially with 100 mL MeOH and 100 mL water, place the resin in a 200 × 2 0 glass column to 100 mm bed height.)

HPLC VARIABLES
Guard column: 10 × 2.1 30-40 μm Lichrosorb RP8
Column: 200 × 3 Lichrosorb RP8
Mobile phase: MeCN:10 mM oxalic acid 50:50
Flow rate: 0.4
Injection volume: 10
Detector: UV 350

CHROMATOGRAM
Retention time: 6
Limit of quantitation: 10 ng/g

OTHER SUBSTANCES
Extracted: chlortetracycline, tetracycline

KEY WORDS
cow; kidney; milk; pig; sheep; muscle; trout; SPE

REFERENCE
Farrington,W.H.; Tarbin,J.; Bygrave,J.; Shearer,G. Analysis of trace residues of tetracyclines in animal tissues and fluids using metal chelate affinity chromatography/HPLC, *Food Addit.Contam.*, **1991**, *8*, 55–64.

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: 7

OTHER SUBSTANCES
Simultaneous: tetracycline, chlortetracycline

REFERENCE
Moats,W.A. Effect of the silica support of bonded reversed-phase columns on chromatography of some antibiotic compounds, *J.Chromatogr.*, **1986**, *366*, 69–78.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 1 μg/mL solution in 10 mM HCl.

HPLC VARIABLES
Guard column: present but not specified
Column: 150 × 4.6 5 μm PLRP-S styrene-divinylbenzene copolymer (Polymer Laboratories)
Mobile phase: Gradient. MeCN:50 mM pH 2.0 oxalate buffer 15:85, for 3 min to 60:40 over 17 min, maintain at 60:40 for 5 min, return to initial conditions over 1 min, re-equilibrate for 9 min. (After use flush with water for 10 min, store in MeCN:water 60:40.)
Flow rate: 1
Detector: UV 355

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: chlortetracycline, tetracycline

REFERENCE
White,C.R.; Moats,W.A.; Kotula,K.L. Comparative study of high performance liquid chromatographic methods for the determination of tetracycline antibiotics, *J.Liq.Chromatogr.*, **1993**, *16*, 2873–2890.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, 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, dox-

apram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: tissue

Sample preparation: Condition a 500 mg Bond-Elut C18 SPE cartridge with MeOH and water. Blend 5 g homogenized tissue with 20, 20, and 10 mL portions of 100 mM pH 4.0 Na_2EDTA McIlvaine buffer, centrifuge at 1000 g for 10 min. Add the filtrate to the SPE cartridge, wash with 20 mL water, elute with 7 mL 10 mM oxalic acid in MeOH, dilute to 10 mL with water, inject an aliquot.

HPLC VARIABLES

Guard column: 50 × 4.6 Inertsil C8
Column: 250 × 4.6 Inertsil C8
Mobile phase: MeCN:THF:100 mM pH 3.0 ammonium acetate buffer 12.5:15:72.5
Flow rate: 0.6
Detector: UV

CHROMATOGRAM

Retention time: 10.5
Limit of detection: 40 ng/mL

OTHER SUBSTANCES

Extracted: degradation products

KEY WORDS

SPE; muscle; pig

REFERENCE
Fedeniuk,R.W.; Ramamurthi,S.; McCurdy,A.R. Application of reversed-phase liquid chromatography and prepacked C18 cartridges for the analysis of oxytetracycline and related compounds, *J.Chromatogr.B*, **1996**, *677*, 291–297.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 6 mL 500 mg C18 SPE cartridge with 20 mL MeOH and 20 mL water. Mix 5 g tissue with 20 mL buffer, homogenize for 30 s, rinse probe twice with 2 mL buffer into the centrifuge tube. Shake for 10 min at high speed and centrifuge at 2500 g for 10 min. Remove the supernatant. Add 20 mL buffer to the tissue plug, shake for 10 min at high speed and centrifuge at 2500 g for 10 min. Remove the supernatant and repeat all steps as described above with 10 mL buffer. Combine the supernatants from all three extractions, centrifuge at 2500 g for 20 min, filter (GF/B paper). Rinse the centrifuge tube twice with 2 mL portions of buffer and filter. Add the filtrate to the SPE cartridge, rinse the flask twice with buffer and add the rinses to the SPE cartridge, wash with 20 mL water, dry the cartridge by drawing air through it, elute with 6 mL 1.26 g/L oxalic acid dihydrate in MeOH, dilute the eluate to 10 mL with water, filter, inject a 60 μL aliquot. (Prepare the buffer (McIlvaine-EDTA buffer) as follows. Mix 1 L 21.0 g/L citric acid monohydrate with 625 mL 28.4 g/L disodium hydrogen phosphate, adjust pH to 4.0 with 100 mM HCl or 100 mM NaOH, add 60.5 g disodium EDTA dihydrate, mix until the solid dissolves.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm C8
Mobile phase: MeCN:MeOH:10 mM oxalic acid 15:20:65
Flow rate: 1.5
Injection volume: 10-60
Detector: UV 350

CHROMATOGRAM
Retention time: 5
Limit of detection: 1.5 ng

OTHER SUBSTANCES
Extracted: chlortetracycline, tetracycline

KEY WORDS
SPE; pig; muscle; cow

REFERENCE
MacNeil,J.D.; Martz,V.K.; Korsrud,G.O.; Salisbury,C..C.; Oka,H.; Epstein,R.L.; Barnes,C.J. Chlortetracycline, oxytetracycline, and tetracycline in edible animal tissues, liquid chromatographic method: Collaborative study, *J.AOAC Int.*, **1996**, *79*, 405–417.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 6 mL 500 mg Bond-Elut C8 SPE cartridge with 6 mL MeOH, 6 mL water, and 2 mL buffer A. Condition a 6 mL SPE cartridge containing 3 g wet XAD-2 resin with 10 mL MeOH, 10 mL water, and 2 mL buffer B. Homogenize (Ultra-Turrax) 2 g tissue with 20 mL succinate buffer for 1 min, centrifuge at 30 897 g for 15 min, filter (Whatman No. 1 paper) the supernatant, dilute 12 mL filtrate with 6 mL buffer B. For sheep liver add the diluted filtrate to the C8 SPE cartridge, wash with 10 mL buffer A, wash with 2 mL water, elute with 6 mL MeOH. For cow kidney add the diluted filtrate to the XAD-2 cartridge, wash with 14 mL buffer A, wash with 2 mL water, elute with 6 mL MeOH. Inject 25 μL 50 mg/mL copper sulfate and 500 μL water onto column A then load 1.5 mL of the eluate from the SPE cartridge at 0.36 mL/min onto column A. Wash to waste with 500 μL water, 500 μL MeOH, and 500 μL water then elute the contents of column A onto column B with mobile phase A. After 11 min remove column A from the circuit and elute column B with a linear gradient of A:B from 100:0 to 0:100 over 10 min, maintain at 0:100 for 10 min, re-equilibrate to 100:0. Monitor the effluent from column B. (Buffer A was 100 mM KH_2PO_4 containing 3 g/L pentanesulfonic acid. Succinate buffer was 60 g succinic acid in 1 L water adjusted to pH 4.0 with

1 M NaOH. Buffer B was 37.2 g disodium EDTA and 3 g pentanesulfonic acid in 1 L succinate buffer.)

HPLC VARIABLES
Column: A 10 × 6 10 μm Anagel-TSK-Chelate-SPW (Anachem); B 5 × 3 5 μm Polymer Labs. PLRP-S + 150 × 4.6 5 μm Polymer Labs. PLRP-S
Mobile phase: A was buffer. B was MeCN:MeOH:buffer 25:10:65. (Buffer was 100 mM KH_2PO_4 containing 10 mM citric acid and 10 mM EDTA.)
Injection volume: 1500
Detector: UV 350

CHROMATOGRAM
Retention time: 22
Limit of detection: 10 μg/kg

OTHER SUBSTANCES
Extracted: chlortetracycline, demeclocycline, tetracycline

KEY WORDS
SPE; sheep; cattle; liver; kidney; column-switching

REFERENCE
Stubbings,G.; Tarbin,J.A.; Shearer,G. On-line metal chelate affinity chromatography clean-up for the high-performance liquid chromatographic determination of tetracycline antibiotics in animal tissues, *J.Chromatogr.B*, **1996**, *679*, 137–145.

SAMPLE
Matrix: tissue
Sample preparation: Fill a disposable polypropylene column (Bio-Rad Econo-Pac column) with Chelating Sepharose Fast Flow (Pharmacia) and condition it with 10 mL water, 1.5 mL 100 mM copper sulfate, and 100 mL water. Condition a 6 mL SupelClean ENVI-Chrom P SPE cartridge with 2 mL MeOH and 5 mL water. Homogenize 10 g tissue with 20-30 mL 100 mM pH 4 succinic acid buffer. Centrifuge the homogenate at 2000 g at 10° for 15-20 min. Add the supernatant to the metal chelate affinity column, wash sequentially with 5 mL 500 mM NaCl, 10 mL water, 10 mL MeOH, 10 mL water, and 3 mL McIlvaine buffer, discard the clear effluent. Elute with 8 mL McIlvaine-EDTA-NaCl buffer. Add the eluate to the SPE cartridge under gravity, rinse the column with 2.5 mL water, add the rinse to the SPE cartridge. Wash the SPE cartridge with 2.5 mL water. Dry the SPE cartridge by drawing air through it for 2-3 min. Elute with 5 mL MeOH. Evaporate the eluate to dryness under nitrogen at 40-50°, dissolve the residue in 1 mL water. Inject a 100 μL aliquot. (McIlvaine buffer was 500 mM NaCl and 100 mM EDTA (Carson, M.C. J. AOAC Int. 1993, 76, 329).)

HPLC VARIABLES
Column: 150 × 3.9 5 μm PLRP-S (Polymer Labs, USA)
Mobile phase: MeOH:5 mM oxalic acid 58:42
Flow rate: 0.5
Injection volume: 100
Detector: MS, HP 5989, NICI, high energy dynode, HP 59980B particle beam interface 60°, helium sheath 40-45 p.s.i., source 250°, quadrupole 100°, source pressure 1 Torr with methane reagent gas, m/z 378-483

CHROMATOGRAM
Retention time: 4.93
Limit of detection: 100 ng/g

OTHER SUBSTANCES
Extracted: chlortetracycline, demeclocycline, doxycycline, minocycline, tetracycline

KEY WORDS
metal chelate affinity chromatography; shrimp; SPE

REFERENCE
Carson,M.C.; Ngoh,M.A.; Hadley,S.W. Confirmation of multiple tetracycline residues in milk and oxytetracycline in shrimp by liquid chromatography-particle beam mass spectrometry, *J.Chromatogr.B*, **1998**, *712*, 113–128.

SAMPLE
Matrix: tissue
Sample preparation: Wash 20 g Amberlite XAD-2 resin (20-50 mesh) with 100 mL portions of MeOH, acetone and water before packing into a 150 × 15 glass column. Wash the packed column with 500 mL portions of MeOH and water, wash with 30 mL 1 M HCl. Macerate 25 g minced fish with 100 mL extractant for 30 s (Ultra Turrax homogenizer), filter through Buchner funnel (Whatman No1, 90 mm diameter), reextract the residue with 50 mL extractant, filter, wash the solid with 25 mL extractant. Add the extract to the Amberlite XAD-2 resin column at 4 mL/min, wash with 75 mL water containing 100 μL 1 M HCl. Elute with 75 mL MeOH and collect the eluate in a receiver containing 100 μL 0.2% ascorbic acid in MeOH. Evaporate eluate on a rotary evaporator at 36°, dissolve the residue in 1 mL mobile phase, inject a 50-100 μL aliquot. (Prepare extractant as follows. Mix 85 mL concentrated HCl and 80 mL 60% perchloric acid, add this mixture to distilled water to give a total volume of 2 L.

HPLC VARIABLES
Column: 200 × 4.6 5 μm Hypersil SAS or 150 × 4.6 5 μm Hypersil SAS
Mobile phase: MeCN:buffer 30:70 (Mobile phase was 340 mL 100 mM citric acid, 5 mL 100 mM trisodium citrate, and 5 mL 100 mM Na_2EDTA made up to 500 mL with MeCN.)
Flow rate: 2
Injection volume: 50-100
Detector: UV 370

CHROMATOGRAM
Retention time: 2.7
Limit of detection: 5 ng/g

OTHER SUBSTANCES
Simultaneous: chlortetracycline, furazolidone, tetracycline

KEY WORDS
fish; muscle; SPE

REFERENCE
Murray,J.; McGill,A.S.; Hardy,R. Development of a method for the determination of oxytetracycline in trout, *Food Addit.Contam.*, **1987**, *5*, 77–83.

SAMPLE
Matrix: tissue
Sample preparation: Condition a Sep-pak C18 SPE cartridge with MeOH and water. Blend 10 g muscle or 2 g liver with 30 mL 1 M HCl and 2 mL 50% trifluoroacetic acid, centrifuge, blend the residue with 15 mL 1 M HCl and 1 mL 50% trifluoroacetic acid using a high speed blender. Centrifuge at 3000 rpm for 10 min, filter the supernatants and add to the SPE cartridge, elute with 10 mL MeOH. Add 1 mL 0.1% dithiothreitol to the eluate, evaporate to about 500 μL under nitrogen at 40°, adjust the volume to 1 mL with 1 M HCl, filter (Millipore 0.45 μm), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Shandon ODS Hypersil C18
Mobile phase: MeCN:dimethylformamide:buffer 19:6:18 (The buffer was water containing 5 g diammonium hydrogen phosphate and 5 mL diethanolamine in a total volume of 180 mL.)
Column temperature: 30
Flow rate: 1
Injection volume: 70
Detector: UV 365

CHROMATOGRAM
Retention time: 5.0

Limit of quantitation: 5 ng/mL (muscle), 10 ng/g (liver)

KEY WORDS
fish; liver; muscle; SPE

REFERENCE
Nordlander,I.; Johnsson,H.; Osterdahl,B. Oxytetracycline residues in rainbow trout analysed by a rapid HPLC method, *Food Addit.Contam.*, **1987**, *4*, 291–296.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 3 mL 500 mg Bond Elut C18 SPE cartridge with saturated aqueous disodium EDTA. Blend 5 g tissue with two 20 mL portions and one 10 mL portion of 100 mM pH 4.0 disodium EDTA-McIlvaine buffer at high speed, centrifuge at 850 g for 5 min each time. Combine the supernatants, centrifuge at 850 g for 15 min, filter. Add the filtrate to the SPE cartridge, wash with 20 mL water, air-dry by aspiration for 5 min, elute with 10 mL ethyl acetate followed by 20 mL MeOH:ethyl acetate 5:95, evaporate the eluate to dryness under reduced pressure at 30°, dissolve the residue in 100 μL water, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 2 μm TSK Gel Super Octyl (Tosoh)
Mobile phase: MeCN:0.05% aqueous trifluoroacetic acid 20:80
Flow rate: 0.5
Injection volume: 50
Detector: MS, Finnigan MAT TSQ 7000 Triple-Stage Quadrupole, electrospray voltage 4.5 kV, gas sheath flow 483 kPa nitrogen, collision gas argon, collision offset -25 V, m/z 461

CHROMATOGRAM
Retention time: 3.9

OTHER SUBSTANCES
Extracted: chlortetracycline, doxycycline, tetracycline

KEY WORDS
cow; kidney; liver; muscle; SPE

REFERENCE
Oka,H.; Ikai,Y.; Ito,Y.; Hayakawa,J.; Harada,K.-; Suzuki,M.; Odani,H.; Maeda,K. Improvement of chemical analysis of antibiotics. XXIII. Identification of residual tetracyclines in bovine tissues by electrospray high-performance liquid chromatography-tandem mass spectrometry, *J.Chromatogr.B*, **1997**, *693*, 337–344.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax T25) 5 g tissue, 200 μL 125 μg/mL tetracycline in 10 mM HCl, 50 mL 50 mM HCl, and 10 mL hexane for 2 min, sonicate for 3 min, centrifuge at 1920 g for 5 min. Dialyze (Cuprophan cellulose dialysis membrane, 15 kD cutoff) a 530 μL aliquot of the aqueous layer against seven 875 μL aliquots of recipient solution. Pump the recipient solution into the recipient channel at 1.7 mL/min, allow to remain stationary for 36 s, then pump onto column A. Finally, backflush the contents of column A onto the analytical column with mobile phase. After 2 min remove column A from the circuit and flush it with 2 mL recipient solution. Flush the recipient channel with 2 mL recipient solution, flush the donor channel with 2 mL 0.01% Triton X-100. (Recipient solution was 20 mM pH 5 phosphate buffer containing 5 mM sodium heptanesulfonate.)

HPLC VARIABLES
Column: A 10 × 2 36 μm Dynospheres polystyrene beads (Dyno Particles, Lillestrom, Norway); B 150 × 4.6 5 μm PLRP-S (Polymer Labs)
Mobile phase: MeCN:buffer 23:77 (Buffer was 20 mM orthophosphoric acid containing 5 mM sodium heptanesulfonate.)
Flow rate: 0.7

Detector: F ex 358 em 460 following post-column reaction. The column effluent mixed with 2 M NaOH pumped at 0.15 mL/min and the mixture flowed through a knitted 10 m × 0.3 mm ID reaction coil irradiated at 366 nm to the detector.

CHROMATOGRAM
Retention time: 8
Internal standard: tetracycline (10)
Limit of detection: 5 ng/g

KEY WORDS
post-column reaction; post-column photochemical derivatization; muscle; fish; salmon; column-switching; dialysis

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REFERENCE
Agasoster,T.; Rasmussen,K.E. On-line dialysis, liquid chromatography and post-column reaction detection of oxytetracycline in salmon muscle extracts, *J.Pharm.Biomed.Anal.*, **1992**, *10*, 349–354.

SAMPLE
Matrix: tissue
Sample preparation: Prepare an affinity column by filling a 10 mL column with 5 mL chelating Sepharose, allow to settle, wash with 20 mL 0.5% copper(II) sulfate solution, eliminate air bubbles by agitation, wash with 15 mL 50 mM pH 4 succinate buffer, do not allow to dry. Condition an Analytichem Bond Elut C18 SPE cartridge with 10 mL MeOH and 10 mL water, do not allow to dry. Homogenize 4 g minced kidney with 40 mL 50 mM pH 4 succinate buffer, sonicate for 10 min, centrifuge at 9000 rpm for 10 min, filter the supernatant through paper, repeat the extraction. Combine the supernatants and pass them through the affinity column at 5-7 mL/min, wash with 10 mL water, wash with 30 mL MeOH, wash with 20 mL water, elute with 50 mL 50 mM pH 4 succinate buffer containing 3.7% Titriplex III (ethylenedinitrilotetracacetic acid, disodium salt dihydrate). Add the eluate to the SPE cartridge at 5-7 mL/min, wash with 10 mL water, dry with air aspiration for 10 min, elute with 5 mL MeOH:MeCN 1:1, evaporate the eluate at 40° under a stream of nitrogen, dissolve the residue in 500 μL mobile phase, inject an aliquot. Protect from light through process. (The affinity columns may be reused 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 (SIC) 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: 4.5
Limit of quantitation: 10 ng/g

OTHER SUBSTANCES
Simultaneous: tetracycline, chlortetracycline, demethylchlortetracycline, methacycline, doxycycline

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KEY WORDS
kidney; SPE

REFERENCE
Degroodt,J.M.; Wyhowski de Bukanski,B.; Srebrnik,S. Multiresidue analysis of tetracyclines in kidney by HPLC and photodiode array detection, *J.Liq.Chromatogr.*, **1993**, *16*, 3515–3529.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 500 mg Separcol SI C18 SPE cartridge (Anapron) with 2 mL MeOH and 4 mL buffer. Homogenize 5 g muscle with 20 mL buffer and 3 mL n-hexane:di-

chloromethane 1:3 at 4°, centrifuge at 2400 g at 4° for 30 min, remove the supernatant, repeat homogenization with 10 mL buffer. Combine the supernatants, slowly add with constant stirring a volume of 1 g/mL trichloroacetic acid in water equal to 10% of the supernatant volume, stir for another min, keep in ice for 15 min, filter through paper, add to SPE cartridge at no more than 10 mL/min, wash with 2 mL water, elute with 4 mL 10 mM oxalic acid in MeOH, inject a 10 μL aliquot. (Buffer was 15 g $Na_2HPO_4.2H_2O$ + 13 g citric acid monohydrate + 3.72 g EDTA in 1 L water, pH 4.)

HPLC VARIABLES
Guard column: 5 μm LiChrospher 100 RP-18 guard column
Column: 250 × 4 5 μm HP Spherisorb ODS 2
Mobile phase: MeOH:MeCN:10 mM aqueous oxalic acid 20:35:45
Flow rate: 1
Injection volume: 10
Detector: UV 360

CHROMATOGRAM
Retention time: 5.5
Limit of detection: 50 ng/g

OTHER SUBSTANCES
Simultaneous: tetracycline, chlortetracycline

KEY WORDS
muscle; cow; pig; SPE

REFERENCE
Sokol,J.; Matisova,E. Determination of tetracycline antibiotics in animal tissues of food-producing animals by high-performance liquid chromatography using solid-phase extraction, *J.Chromatogr.A*, **1994**, *669*, 75–80.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 500 mg Bond Elut cyclohexyl (CH) SPE cartridge with 10 mL MeOH and 10 mL water. Powder (domestic food blender) frozen kidney or muscle. Homogenize (Silverson Machines) 5 g powdered tissue and 45 mL 100 mM glycine in 1 M HCl for 1 min, add 5 g ammonium sulfate, shake for 30 s, let stand for 10 min, centrifuge at 2000 rpm for 15 min, filter (glass wool) the supernatant, repeat the extraction with 50 mL 100 mM glycine in 1 M HCl. Combine the filtrates and centrifuge an aliquot at 2200 rpm for 10 min, add a 20 mL aliquot of the supernatant to the SPE cartridge, wash with 10 mL water, elute with 7 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 65°, reconstitute the residue in 500 μL MeCN:20 mM oxalic acid 20:80, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: Chromspher C8 (Chrompack)
Column: 200 × 3 5 μm Chromspher C8 glass column (Chrompack)
Mobile phase: Gradient. A was MeCN. B was MeCN:20 mM oxalic acid 10:90. A:B from 10:90 to 20:80 over 2 min, maintain at 20:80 for 8 min, to 25:75 over 1 min, maintain at 25:75 for 9 min, return to initial conditions over 5 min, re-equilibrate for 10 min.
Flow rate: 0.4
Injection volume: 50
Detector: F ex 390 em 490 following post-column reaction. The column effluent mixed with 750 mM aluminum chloride (degas by sonication, store in a brown bottle) pumped at 0.6 mL/min and flowed through a 13.7 m × 0.3 mm i.d. PTFE column at 60° to the detector.

CHROMATOGRAM
Retention time: 10.7
Limit of detection: 100 ng/g (kidney), 30 ng/g (muscle)

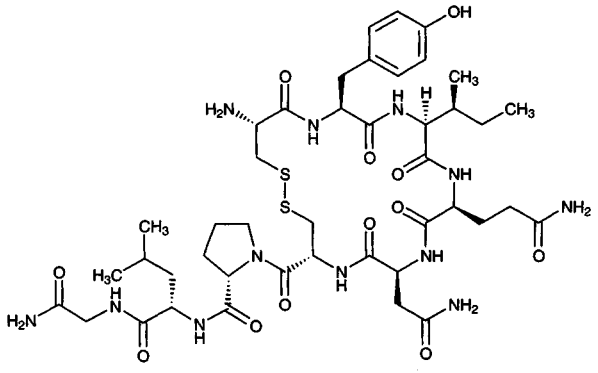
OTHER SUBSTANCES
Extracted: chlortetracycline, tetracycline

KEY WORDS
pig; cow; poultry; kidney; muscle; SPE; post-column reaction; complexation

REFERENCE
McCracken,R.J.; Blanchflower,W.J.; Haggan,S.A.; Kennedy,D.G. Simultaneous determination of oxytetracycline, tetracycline and chlortetracycline in animal tissues using liquid chromatography, post-column derivatization with aluminium, and fluorescence detection, *Analyst*, **1995**, *120*, 1763–1766.

Oxytocin

Molecular formula: $C_{43}H_{66}N_{12}O_{12}S_2$
Molecular weight: 1007.20
CAS Registry No.: 50-56-6
Merck Index: 7114



SAMPLE
Matrix: blood, tissue
Sample preparation: Condition a Sep-Pak ODS SPE cartridge with MeOH. Homogenize 500 mg tissue with 6 mL 100 mM pH 7.4 Tris buffer. Acidify a 2 mL aliquot of plasma or tissue homogenate with 200 µL 1 M HCl, add to the SPE cartridge, elute with 3 mL MeOH over 3 min, elute with 2 mL over 1 min. Evaporate the eluate to dryness under a stream of air at 37°, reconstitute the residue in 200 µL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES
Column: 280 × 5 Dynamax 300-A C8 (Rainin)
Mobile phase: MeCN:water 20:80 containing 0.1% trifluoroacetic acid, 50 mM heptanesulfonic acid and 30 mM triethylamine, pH adjusted to 2.5 with Na_2HPO_4
Flow rate: 1
Injection volume: 20
Detector: UV 200-400

CHROMATOGRAM
Retention time: 4.58
Limit of detection: 1 ng

OTHER SUBSTANCES
Extracted: lypressin, arginine vasopressin

KEY WORDS
pig; plasma; SPE; heart

REFERENCE
Rao,P.S.; Weinstein,G.S.; Wilson,D.W.; Rujikarn,N.; Tyras,D.H. Isocratic high-performance liquid chromatography-photodiode-array detection method for determination of lysine- and arginine-vasopressins and oxytocin in biological samples, *J.Chromatogr.*, **1991**, *536*, 137–142.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 100 µL aliquot.

HPLC VARIABLES
Column: 250 × 3 10 μm RP 8 (Merck)
Mobile phase: MeCN:pH 7 phosphate buffer 20:80
Flow rate: 1.47
Injection volume: 100
Detector: F ex 390 em 470 following post-column reaction. The column effluent mixed with 300 μg/mL fluorescamine in MeCN pumped at 0.16 mL/min and the mixture flowed through a 4.4 m × 0.25 mm ID coil to the detector.

CHROMATOGRAM
Retention time: 6
Limit of detection: 9 ng

KEY WORDS
post-column reaction; injections

REFERENCE
Frei,R.W.; Michel,L.; Santi,W. Post-column fluorescence derivatization of peptides. Problems and potential in high-performance liquid chromatography, *J.Chromatogr.*, **1976**, *16*, 665–677.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 50 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:buffer 35:65 containing 0.05% sodium tetradecyl sulfate (Buffer was 0.83 mM phosphoric acid adjusted to pH 5.0 with triethylamine. Use a 50 × 4.6 5-25 μm LiChroprep Si 60 column before the injector. Wash column with MeCN:83 mM phosphoric acid 40:60 after use.)
Flow rate: 2.5
Injection volume: 50
Detector: UV

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: ephedrine, ergonovine (ergometrine)

KEY WORDS
injections

REFERENCE
Pask-Hughes,R.A.; Corran,P.H.; Calam,D.H. Assay of the combined formulation of ergometrine and oxytocin by high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *214*, 307–315.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 6.5 mL aliquot of a solution in 5% dextrose or lactated Ringer's solution with 5% dextrose onto column A at 0.8 mL/min and elute to waste with 15 mM pH 3 sodium phosphate, after 12.5 min backflush the contents of column A onto column B with the mobile phase, after 2 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B.

HPLC VARIABLES
Column: A Alltech C18 guard cartridge; B Alltech C18 guard cartridge + 125 × 4.6 5 μm Partisphere C18
Mobile phase: MeCN:0.1%phosphoric acid 21:79
Flow rate: 1.3
Injection volume: 5000-7000

Detector: F ex 250 em 418 (cutoff filter). The column effluent mixed with 0.02% fluorescamine in MeCN pumped at 0.3 mL/min and buffer pumped at 0.7 mL/min and the mixture flowed through a 1 mL coil to the detector. (Prepare buffer by mixing 10 g KH_2PO_4, 100 mL MeCN, and 800 mL water, adjust pH to 8.1 with 10 M NaOH, make up to 1 L with water, add 3 mL 30% Brij-35.)

CHROMATOGRAM
Retention time: 12
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
post-column reaction; column-switching; siliconize glassware with Surfasil (Pierce); injections

REFERENCE
Brown,D.S.; Jenke,D.R. Determination of trace levels of oxytocin in pharmaceutical solutions by high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *410*, 157–168.

SAMPLE
Matrix: media
Sample preparation: Condition a 3 mL C18 SPE cartridge (J.T. Baker) with 1 column volume MeOH and 2 column volumes water. 1 mL Supernatant from culture media + 500 μL 50 mM HCl, vortex briefly, centrifuge at 1000 g for 5 min. Mix the supernatant with an equal volume of Dulbecco's PBS and add to the SPE cartridge, wash with 1 column volume water, wash with 2 column volumes 1.5% acetic acid (pH 4.8), wash with 1 column volume water, dry under vacuum for 5 min, elute with 1.5 mL MeOH, evaporate the eluate to dryness under reduced pressure, reconstitute with 1 mL water, add to an immunoaffinity column at 4°, wash with 2 column volumes refrigerated water, wash with 2 column volumes refrigerated 1 M NaCl, wash with 2 column volumes refrigerated water, wash with 2 column volumes refrigerated MeCN, elute with 1.5 mL MeOH, evaporate the eluate to dryness under reduced pressure, reconstitute in 200 μL mobile phase, inject a 20 μL aliquot. (Water should be polished using a Sep-Pak C18 SPE cartridge and should be at least 15 MΩ/cc. Prepare immunoaffinity column as follows. Covalently link 1 mg purified (procedure given in paper) oxytocin Ab to 200 mg CNBr-activated Sepharose 4B, pack a 100 × 10 column with antibody-conjugated Sepharose 4B:unmodified Sepharose 4B 20:80. Column can be regenerated with 1 column volume MeOH and used at least 25 times.)

HPLC VARIABLES
Column: 70 × 4.6 Ultrasphere XL ODS C18
Mobile phase: MeOH:20 mM pH 5.7 sodium phosphate buffer 40:60 (Recirculate solvent.)
Column temperature: 30
Flow rate: 0.5
Injection volume: 20
Detector: E, Environmental Sciences Association Model 5200 Coulochem II, Model 5010 porous graphite analytical cell, screening electrode 525 mV, analysis electrode 775 mV

CHROMATOGRAM
Retention time: 9.72
Limit of detection: 40 pg

KEY WORDS
SPE; immunoaffinity

REFERENCE
Kukucka,M.A.; Misra,H.P. Determination of oxytocin in biological samples by isocratic high-performance liquid chromatography with coulometric detection using C18 solid-phase extraction and polyclonal antibody-based immunoaffinity column purification, *J.Chromatogr.B*, **1994**, *653*, 139–145.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18 or 300 × 4 10 μm Nucleosil C18
Mobile phase: Gradient. MeCN:buffer from 25:75 to 40:60 over 45 min. (Buffer was water adjusted to pH 2.1 with trifluoroacetic acid.)
Flow rate: 1
Injection volume: 10-950
Detector: UV 220 or RIA

CHROMATOGRAM
Retention time: 17.50

OTHER SUBSTANCES
Simultaneous: saralasin, vasopressin, peptides
Interfering: bradykinin

REFERENCE
Unger,T.; Moursi,M.; Ganten,D.; Hermann,K.; Lang,R.E. Antihypertensive action of the converting enzyme inhibitor perindopril (S9490-3) in spontaneously hypertensive rats: comparison with enalapril (MK421) and ramipril (Hoe498), *J.Cardiovasc.Pharmacol.*, **1986**, *8*, 276–285.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 5 Nova-Pak C18
Mobile phase: Gradient. MeCN:10 mM pH 5.0 acetate buffer 15:85 for 10 min, then to 18:82 over 5 min, maintain at 18:82 for 25 min.
Flow rate: 1
Detector: UV 214, UV 280

CHROMATOGRAM
Retention time: 22.0

OTHER SUBSTANCES
Simultaneous: lypressin, arginine vasopressin

KEY WORDS
hippopotamus

REFERENCE
Rouille,Y.; Chauvet,M.T.; Chauvet,J.; Acher,R.; Hadley,M.E. The distribution of lysine vasopressin (lysipressin) in placental mammals: a reinvestigation of the Hippopotamidae (*Hippopotamus amphibius*) and Tayassuidae (*Tayassu angulatus*) families, *Gen.Comp.Endocrinol.*, **1988**, *71*, 475–483.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil SCX
Mobile phase: MeOH:20 mM pH 5.0 KH_2PO_4 10:90
Flow rate: 0.5
Injection volume: 20
Detector: UV 209

CHROMATOGRAM
Retention time: 18
Limit of detection: 900 ng/mL

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Wang,H.P.; Pácaková,V.; Stulík,K.; Barth,T. Ion-exchange high-performance liquid chromatographic analysis of the products of the enzymatic degradation of oxytocin, *J.Chromatogr.*, **1990**, *519*, 244–249.

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: 7.5

OTHER SUBSTANCES
Simultaneous: angiotensin I, angiotensin II, bradykinin, insulin, leucin enkephalin, lysozyme, melittin, methionine enkephalin

REFERENCE
Supelco Catalog, **1992**, p. 104.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Kromasil (Alltech)
Mobile phase: MeCN:pH 7 phosphate buffer 20:80
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 6.5

OTHER SUBSTANCES
Simultaneous: vasopressin

REFERENCE
Supelco Catalog, **1993**, p. 525.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Vydac 228TP
Mobile phase: Gradient. A was 0.25% trifluoroacetic acid in water. B was 0.25% trifluoroacetic acid in MeCN:water 70:30. A:B from 95:5 to 0:100 over 30 min.
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Simultaneous: angiotensin I, angiotensin II, bradykinin, eledosin, insulin, lysozyme, myoglobin, neurotensin, ovalbumin

REFERENCE
Supelco Catalog, **1993**, p. 581.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.5 Kromasil-5-C8 (Eka Nobel)
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in MeCN:water 10:90. B was 0.1% trifluoroacetic acid in MeCN:water 90:10. A:B from 100:0 to 75:25 over 8 min, to 25:75 over 12 min
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 7.5

OTHER SUBSTANCES
Simultaneous: angiotensin I, angiotensin II, bradykinin, leukin enkephalin, insulin, lysozyme, melittin, methionine enkephalin

REFERENCE
Bodman Chromatography Catalog, **1994**, p. 62.

SAMPLE
Matrix: solutions

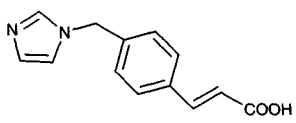
HPLC VARIABLES
Column: 35 × 4.6 TSKgel octadecyl-NPR (A) or 50 × 4.6 TSKgel Super-ODS (B)
Mobile phase: Gradient. MeCN:13 mM perchloric acid from 0:100 to 50:50 in 10 min (A) or MeCN:13 mM perchloric acid from 10:90 to 50:50 in 10 min (B)
Flow rate: 1.5 (A) or 2 (B)
Detector: UV 220

CHROMATOGRAM
Retention time: 3 (A), 2.5 (B)

OTHER SUBSTANCES
Simultaneous: α-endorphin, bombesin, leu-enkephalin, gamma-endorphin, somatostatin

REFERENCE
Moriyama,H.; Anegayama,M.; Komiya,K.; Kato,Y. Characterization of a new reversed-phase chromatographic column on a 2-μm porous microspherical silica gel, *J.Chromatogr.A*, **1995**, *691*, 81–89.

Ozagrel



Molecular formula: $C_{13}H_{12}N_2O_2$
Molecular weight: 228.25
CAS Registry No.: 82571-53-7
Merck Index: 7115

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 400 μL MeOH, mix well, centrifuge at 10000 rpm for 2 min. Filter the supernatant and inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 4.6 5 μm TSK-Gel ODS-80 TM (Toyo Soda)
Mobile phase: MeCN:10 mM pH 4.0 acetate buffer 15:85
Flow rate: 1
Detector: UV 274

KEY WORDS
rabbit; plasma; pharmacokinetics

REFERENCE
Zheng,N.X.; Sato,H.; Adachi,I.; Kanamoto,I.; Horikoshi,I. Pharmacokinetic and pharmacodynamic studies of a thromboxane synthetase inhibitor, ozagrel, in rabbits, *Biol.Pharm.Bull.*, **1995**, *18*, 1738–1743.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 25:75 containing100 mM KCl
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 270

REFERENCE
Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.