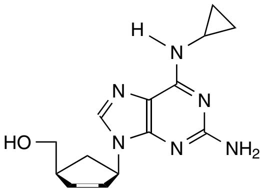
Abacavir



Molecular formula: $C_{14}H_{18}N_6O$
Molecular weight: 286.33
CAS Registry No: 136470-78-5 (base), 188062-50-2 (sulfate)
Merck Index: 13,1

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL 100 mg Bond Elut-C SPE cartridge with 1 mL MeOH and 1 mL 100 mM pH 7.0 ammonium acetate buffer. Heat plasma at 58° for 1 h to inactivate HIV. Vortex 800 μL plasma with 300 μL 2 μg/mL hexobarbital in 25 mM pH 7.0 ammonium acetate buffer for 30 s and centrifuge at 18 000 g for 5 min. Add 1 mL of the supernatant to the SPE cartridge, wash with 1 mL 100 mM pH 7.0 ammonium acetate buffer, suck dry for 1 min, elute with 800 μL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 40° and reconstitute the residue with 100 μL mobile phase. Vortex for 30 s, centrifuge at 18 000 g for 3 min, and inject an 80 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 3.9 5 μm Polarity dC18 (Waters)
Column: 150 × 3.9 5 μm Polarity dC18 (Waters)
Column temperature: 40
Mobile phase: Gradient. A was 10 mM pH 6.5 ammonium acetate buffer. B was 10 mM pH 6.5 ammonium acetate buffer:MeCN:MeOH 20:50:30. A:B 96:4 for 15 min, to 36:64 over 15 min, maintain at 36:64 for 3 min, re-equilibrate at initial conditions for 7 min.
Flow rate: 1.1
Injection volume: 80
Detector: UV 269 for 11 min, UV 250 for 3 min, UV 271 for 10 min, UV 230 for 9 min

CHROMATOGRAM

Retention time: 25.1
Internal standard: hexobarbital (30.6)
Limit of quantitation: 10.0 ng/mL

OTHER SUBSTANCES

Extracted: didanosine (13.6), lamivudine (8.6), nevirapine (27.3), stavudine (15.7), zalcitabine (5.9), zidovudine (23.8)
Noninterfering: tenofovir

KEY WORDS

plasma; SPE

REFERENCE

Rezk, N.L.; Tidwell, R.R.; Kashuba, A.D.M. Simultaneous determination of six HIV nucleoside analogue reverse transcriptase inhibitors and nevirapine by liquid chromatography with ultraviolet absorbance detection, *J.Chromatogr.B*, **2003**, *791*, 137–147.

SAMPLE

Matrix: blood

Sample preparation: Condition a 100 mg Dual Zone C18 SPE cartridge (Diazem) with 2 mL MeOH and 2 mL water. Dilute 500 μL serum with 1 mL water, add to the SPE cartridge, wash with 500 μL water, elute with 1 mL MeOH. Evaporate the eluate to dryness with vortexing under reduced pressure at 40° and reconstitute the residue with 300 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES

Column: two 150 × 4.6 3 μm Luna C18 columns in series
Column temperature: 60
Mobile phase: Gradient. MeCN:water from 5:95 to 45:55 over 20 min.
Flow rate: 0.85
Injection volume: 10
Detector: UV 250

CHROMATOGRAM

Retention time: 17
Limit of detection: 75 ng/mL

OTHER SUBSTANCES

Extracted: didanosine (10.5, LOD 120 ng/mL), lamivudine (9.5, LOD 260 ng/mL), stavudine (11.5, LOD 40 ng/mL), zalcitabine (7.5, LOD 440 ng/mL), zidovudine (16, LOD 30 ng/mL)

KEY WORDS

SPE; serum

REFERENCE

Simon, V.A.; Thiam, M.D.; Lipford, L.C. Determination of serum levels of thirteen human immunodeficiency virus-suppressing drugs by high-performance liquid chromatography, *J.Chromatogr.A*, **2001**, *913*, 447–453.

SAMPLE

Matrix: blood
Sample preparation: Mix 300 μL plasma with 75 μL 20% perchloric acid for 30 s, centrifuge at 1300 g for 15 min, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 3.8 Symmetry C18 (Waters)
Column: 100 × 4.6 3.5 μm Symmetry C18 (Waters)
Column temperature: 41 ± 2
Mobile phase: MeCN:25 mM pH 7.0 phosphate buffer 15:85
Flow rate: 1
Injection volume: 100
Detector: UV 285

CHROMATOGRAM

Retention time: 4.8
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES

Simultaneous: didanosine, folic acid, ganciclovir, lamivudine, nevirapine, pyrazinamide, ranitidine, rifampin, stavudine, sulfamethoxazole, trimethoprim, zidovudine
Noninterfering: adefovir, amprenavir, delavirdine, efavirenz, fluconazole, indinavir, itraconazole, methadone, nelfinavir, oxazepam, pyrimethamine, rifampin, ritonavir, saquinavir, zalcitabine

KEY WORDS

plasma

REFERENCE

Veldkamp, A.I.; Sparidans, R.W.; Hoetelmans, R.M.W.; Beijnen, J.H. Quantitative determination of abacavir (1592U89), a novel nucleoside reverse transcriptase inhibitor, in human plasma using isocratic reversed-phase high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1999**, *736*, 123–128.

SAMPLE

Matrix: blood

Sample preparation: Centrifuge plasma at 4000 g for 20 min using a Centrifree micropartition device (Amicon), inject a 100 μL aliquot of the ultrafiltrate.

HPLC VARIABLES

Column: 250 × 4.6 Adsorbsphere C18

Mobile phase: Gradient. A was MeCN:water 80:20. B was 50 mM ammonium acetate containing 0.1% triethylamine adjusted to pH 5.5. A:B from 0:100 to 50:50 over 30 min, re-equilibrate at initial conditions for 10 min.

Flow rate: 1

Injection volume: 100

Detector: UV 260, UV 285

CHROMATOGRAM

Retention time: 23

OTHER SUBSTANCES

Extracted: carbovir (20)

KEY WORDS

rat; pharmacokinetics; plasma

REFERENCE

Daluge, S.M.; Good, S.S.; Faletto, M.B.; Miller, W.H.; St.Clair, M.H.; Boone, L.R.; Tisdale, M.; Parry, N.R.; Reardon, J.E.; Dornsife, R.E.; Averett, D.R.; Krenitsky, T.A. 1592U89, a novel carbocyclic nucleoside analog with potent, selective anti-human immunodeficiency virus activity, *Antimicrob.Agents Chemother.*, **1997**, *41*, 1082–1093.

SAMPLE

Matrix: CSF, urine

Sample preparation: Centrifuge CSF or urine at 12 000 g for 5 min, dilute a 75 μL aliquot to 750 μL with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 × 3.2 5 μm Kromasil C18 (Phenomenex)

Mobile phase: Gradient. MeOH:25 mM pH 4.0 ammonium acetate buffer from 5:95 to 50:50 over 30 min, re-equilibrate at initial conditions for 10 min.

Flow rate: 0.7

Detector: UV 295

CHROMATOGRAM

Retention time: 25.5

Limit of quantitation: 62 ng/mL (CSF), 629 ng/mL (urine)

OTHER SUBSTANCES

Extracted: metabolites, abacavir 5′-glucuronide, abacavir 5′-carboxylate

REFERENCE

Ravitch, J.R.; Moseley, C.G. High-performance liquid chromatographic assay for abacavir and its two major metabolites in human urine and cerebrospinal fluid, *J.Chromatogr.*, **2001**, *762*, 165–173.

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Fung, E.N.; Cai, Z.; Burnette, T.C.; Sinhababu, A.K. Simultaneous determination of Ziagen and its phosphorylated metabolites by ion-pairing high-performance liquid chromatography-tandem mass spectrometry, *J.Chromatogr.B*, **2001**, *754*, 285–295. [LC-MS]

Sparidans, R.W.; Hoetelmans, R.M.W.; Beijnen, J.H. Liquid chromatographic assay for simultaneous determination of abacavir and mycophenolic acid in human plasma using dual spectrophotometric detection, *J.Chromatogr.B*, **2001**, *750*, 155–161.

Thomas, S.A.; Bye, E.; Segal, M.B. Transport characteristics of the anti-human immunodeficiency virus nucleoside analog, abacavir, into brain and cerebrospinal fluid, *J.Pharmacol.Exp.Ther.*, **2001**, *298*, 947–953.

Yuen, G.J.; Lou, Y.; Thompson, N.F.; Otto, V.R.; Allsup, T.L.; Mahony, W.B.; Hutman, H.W. Abacavir/lamivudine/zidovudine as a combined formulation tablet: Bioequivalence compared with each component administered concurrently and the effect of food on absorption, *J.Clin.Pharmacol.*, **2001**, *41*, 277–288.

Aymard, G.; Legrand, M.; Trichereau, N.; Diquet, B. Determination of twelve antiretroviral agents in human plasma sample using reversed-phase high-performance liquid chromatography, *J.Chromatogr.B*, **2000**, *744*, 227–240. [for amprenavir; efavirenz; indinavir; nelfinavir; ritonavir; saquinavir; abacavir; didanosine; lamivudine; stavudine; nevirapine; zidovudine]

McDowell, J.A.; Lou, Y.; Symonds, W.S.; Stein, D.S. Multiple-dose pharmacokinetics and pharmacodynamics of abacavir alone and in combination with zidovudine in human immunodeficiency virus-infected adults, *Antimicrob.Agents Chemother.*, **2000**, *44*, 2061–2067.

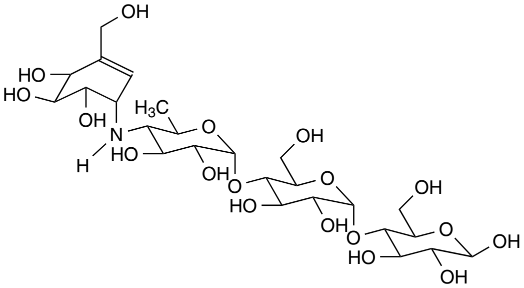
Kumar, P.N.; Sweet, D.E.; McDowell, J.A.; Symonds, W.; Lou, Y.; Hetherington, S.; LaFon, S. Safety and pharmacokinetics of abacavir (1592U89) following oral administration of escalating single doses in human immunodeficiency virus type 1-infected adults, *Antimicrob.Agents Chemother.*, **1999**, *43*, 603–608.

McDowell, J.A.; Chittick, G.E.; Ravitch, J.R.; Polk, R.E.; Kerkering, T.M.; Stein, D.S. Pharmacokinetics of [^{14}C]abacavir, a human immunodeficiency virus type 1 (HIV-1) reverse transcriptase inhibitor, administered in a single oral dose to HIV-1-infected adults: a mass balance study, *Antimicrob.Agents Chemother.*, **1999**, *43*, 2855–2861.

Wang, L.H.; Chittick, G.E.; McDowell, J.A. Single-dose pharmacokinetics and safety of abacavir (1592U89), zidovudine, and lamivudine administered alone and in combination in adults with human immunodeficiency virus infection, *Antimicrob.Agents Chemother.*, **1999**, *43*, 1708–1715.

Acarbose

Molecular formula: $C_{25}H_{43}NO_{18}$
Molecular weight: 645.60
CAS Registry No: 56180-94-0
Merck Index: 13, 18



SAMPLE

Matrix: formulations

Sample preparation: Powder tablet, extract 3 times with 5 mL aliquots of water with sonication for 15 min with vortexing at 5 min intervals each time, centrifuge at 2750 g for 5 min, combine supernatants, make up to 20 mL with water. Dilute a 50 µL aliquot to 1 mL with MeOH, filter (0.2 µM), inject a 20 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Nucleosil-NH2
Mobile phase: MeOH:dichloromethane 65:35
Flow rate: 1
Injection volume: 20
Detector: ELSD, nebulizing gas air at 2.5 bar and 4 L/min, solvent evaporated at 40°

CHROMATOGRAM

Retention time: 4.1
Limit of detection: 5 µg/mL
Limit of quantitation: 15 µg/mL

OTHER SUBSTANCES

Simultaneous: sucrose (3.5)

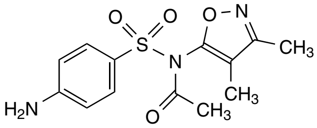
KEY WORDS

comparison with capillary electrophoresis; tablets

REFERENCE

Cherkaoui, S.; Daali, Y.; Christen, P.; Veuthey, J.-L. Development and validation of liquid chromatography and capillary electrophoresis methods for acarbose determination in pharmaceutical tablets, *J.Pharm.Biomed.Anal.*, **1998**, *18*, 729–735.

Acetyl sulfisoxazole



Molecular formula: $C_{13}H_{15}N_3O_4S$

Molecular weight: 309.35

CAS Registry No: 80-74-0

Merck Index: 13, 9041

SAMPLE

Matrix: formulations

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

HPLC VARIABLES

Column: 300 × 4 10 μm μBondapak C18

Mobile phase: MeCN:water 40:60

Flow rate: 1.5

Injection volume: 5

Detector: UV 254

CHROMATOGRAM

Retention time: 7

Internal standard: benzanilide (11)

OTHER SUBSTANCES

Simultaneous: sulfanilamide (2.5), sulfisoxazole (3)

Noninterfering: erythromycin ethylsuccinate

KEY WORDS

oral suspensions

REFERENCE

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

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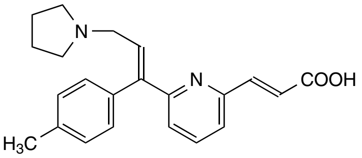
Suber, R.L.; Edds, G.T. High performance liquid chromatographic determinations of sulfonamides by ionic suppression, *J.Liq.Chromatogr.*, **1980**, *3*, 257–268. [for sulfanilamide; sulfaguanidine; sulfamerazine; sulfamethazine; sulfapyridine; sulfisoxazole; *N*-acetylsulfisoxazole; sulfathiazole; in plasma]

Acrivastine



Molecular formula: $C_{22}H_{24}N_2O_2$

Molecular weight: 348.44

CAS Registry No: 87848-99-5

Merck Index: 13, 129

SAMPLE

Matrix: blood

Sample preparation: Mix 1 mL whole blood with 20 μL 1 μg/mL dibenzepin in MeOH:water 50:50, add 300 μL pH 11 tris buffer, mix, add 500 μL butyl acetate, vortex for 2 min, centrifuge. Preserve the aqueous layer (A). Remove the organic layer and add it to 75 μL 10 mM ammonium acetate buffer containing 0.1% formic acid (pH 3.2), evaporate (?). Add 75 μL MeCN, sonicate for 5 min, centrifuge at 5000 rpm for 5 min, keep the extract as B. Add 20 μL 1 μg/mL enalapril in MeOH:water 50:50 and 120 mg NaCl to the aqueous layer (A), mix, add 500 μL pH 3 phosphate buffer, add 600 μL 8.5% phosphoric acid, add 5 mL dichloromethane:isopropanol 95:5, shake at 250 cycles/min in a bench-top shaker for 30 min, centrifuge at 5000 rpm for 5 min. Remove the lower organic layer and evaporate it to dryness under a stream of air at 45°. Reconstitute the residue with 150 μL initial mobile phase, sonicate, centrifuge, combine with extract B, inject a 30 μL aliquot. (Sample preparation from Gergov,M.; Robson,J.N.; Ojanperä,I.; Heinonen,O.P.; Vuori,E. Simultaneous screening and quantitation of 18 antihistamine drugs in blood by liquid chromatography ionspray tandem mass spectrometry. *Forensic Sci.Inter.* **2001**, *121*, 108–115.)

HPLC VARIABLES

Guard column: 40 mm long 4 μm Purospher RP-18 LiChro Cart 4-4

Column: 100 × 2.1 4 μm Genesis C18 (Jones Chromatography)

Column temperature: 35

Mobile phase: Gradient. MeCN:buffer from 20:80 to 100:0 over 10 min, maintain at 0:100 for 3 min, re-equilibrate at initial conditions for 5 min. (Buffer was 10 mM ammonium acetate containing 0.1% formic acid (pH 3.2).

Flow rate: 0.2

Injection volume: 30

Detector: MS, PE Sciex API 365 triple stage quadrupole LC-MS-MS, PE Sciex Turbo Ion Spray interface, positive ion mode, needle voltage 5.2 kV, nebulizer gas air at 60 psi, curtain gas nitrogen at 40 psi, collision cell gas nitrogen at 40 psi, turbo ionspray heater 375°, heater gas flow 7 L/min

CHROMATOGRAM

Retention time: 5.7

Internal standard: dibenzepin, enalapril

Limit of detection: <20 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol (3.8, LOD 0.1 μg/mL), acetaminophen (2.5, LOD <5 μg/mL), alprazolam (6.1, LOD <0.02 μg/mL), alprenolol (5.4, LOD 0.01 μg/mL), amantadine (3.4, LOD 0.1 μg/mL), amiloride (2.0, LOD 0.1 μg/mL), aminophenazone (2.8, LOD <5 μg/mL), amiodarone (10.2, LOD 0.05 μg/mL), amitriptyline (6.6, LOD <0.02 μg/mL), astemizole (5.8, LOD <0.02 μg/mL), atenolol (1.7, LOD 0.30 μg/mL), azacyclonol (5.1, LOD 0.02 μg/mL), benzhexol (6.6, LOD <0.02 μg/mL), benzoylecgonine (3.3, LOD 0.01 μg/mL), betaxolol (5.5, LOD 0.01 μg/mL), biperidine (6.2, LOD <0.02 μg/mL), bisoprolol (5.0, LOD <0.02 μg/mL), brompheniramine (5.3, LOD 0.002 μg/mL), bupivacaine (5.1, LOD <0.02 μg/mL), buprenorphine (5.9, LOD 0.01 μg/mL), buspirone (5.1, LOD 0.002 μg/mL), caffeine (2.8, LOD 1 μg/mL), carbamazepine

(6.1, LOD <0.02 μg/mL), carbinoxamine (5.1, LOD 0.002 μg/mL), carisoprodol (6.7, LOD <5 μg/mL), carvedilol (6.2, LOD <0.02 μg/mL), celiprolol (4.3, LOD 0.05 μg/mL), cetirizine (6.3, LOD 0.05 μg/mL), chlorcyclizine (6.6, LOD <0.02 μg/mL), chlordiazepoxide (5.7, LOD <0.02 μg/mL), chlormezanone (5.8, LOD <5 μg/mL), chloroquine (2.7, LOD 0.02 μg/mL), chlorpheniramine (5.1, LOD 0.002 μg/mL), chlorpromazine (7.0, LOD 0.02 μg/mL), chlorpropamide (6.7, LOD <5 μg/mL), chlorprothixene (7.0, LOD <0.02 μg/mL), cinnarizine (7.9, LOD <0.02 μg/mL), citalopram (5.7, LOD <0.02 μg/mL), clemastine (7.7, LOD 0.02 μg/mL), clobazam (7.3, LOD <0.02 μg/mL), clobutinol (5.3, LOD 0.02 μg/mL), clomethiazole (6.2, LOD 0.5 μg/mL), clomipramine (7.1, LOD <0.02 μg/mL), clonazepam (6.6, LOD <0.02 μg/mL), clonidine (2.8, LOD 0.1 μg/mL), clozapine (5.6, LOD <0.02 μg/mL), cocaine (4.6, LOD <0.02 μg/mL), codeine (2.5, LOD 0.1 μg/mL), coumatetralyl (8.4, LOD 0.05 μg/mL), cyclizine (5.8, LOD <0.02 μg/mL), dextropropoxyphene (6.6, LOD 0.05 μg/mL), demoxepam (5.8, LOD 0.02 μg/mL), dextromethorphan (5.5, LOD <0.02 μg/mL), diazepam (8.1, LOD 0.02 μg/mL), diltiazem (5.8, LOD <0.02 μg/mL), diphenhydramine (5.7, LOD <0.02 μg/mL), dipyridamole (5.4, LOD 0.005 μg/mL), disopyramine (4.4, LOD <0.02 μg/mL), dixyrazine (6.8, LOD 0.005 μg/mL), doxapram (4.8, LOD <0.02 μg/mL), doxepin (5.9, LOD <0.02 μg/mL), dronabinol (12.3, LOD 0.05 μg/mL), ebastine (9.6, LOD 0.005 μg/mL), embutramide (6.7, LOD 0.005 μg/mL), ergotamine (5.5, LOD 0.005 μg/mL), ethenzamide (5.0, LOD 0.05 μg/mL), ethylmorphine (3.2, LOD 0.05 μg/mL), ethylparathion (9.7, LOD <5 μg/mL), etodroxizine (6.4, LOD <0.02 μg/mL), felodipine (9.6, LOD 0.02 μg/mL), fenazepam (7.5, LOD <0.02 μg/mL), fenfluramine (5.3, LOD <0.02 μg/mL), fenkamfamine (5.1, LOD <0.02 μg/mL), fentanyl (5.5, LOD <0.02 μg/mL), fexofenadine (6.3, LOD <0.02 μg/mL), flecainide (5.9, LOD <0.02 μg/mL), fluconazole (4.0, LOD 0.1 μg/mL), flumazenil (5.2, LOD <0.02 μg/mL), flunitrazepam (7.1, LOD 0.002 μg/mL), fluoxetine (6.8, LOD 0.1 μg/mL), flupentixol (7.5, LOD 0.18 μg/mL), fluvoxamine (6.3, LOD 0.02 μg/mL), glibenclamide (8.5, LOD <0.02 μg/mL), glipizide (6.8, LOD <0.05 μg/mL), haloperidol (6.1, LOD <0.02 μg/mL), histapyrrodine (6.3, LOD 0.02 μg/mL), hydrocodone (3.0, LOD 0.05 μg/mL), hydroxychloroquine (2.4, LOD <0.3 μg/mL), hydroxyzine (6.3, LOD <0.02 μg/mL), imipramine (6.4, LOD 0.05 μg/mL), indomethacin (8.6, LOD 0.05 μg/mL), isoniazid (2.2, LOD 3 μg/mL), isradipine (8.6, LOD 0.05 μg/mL), ketamine (3.6, LOD <0.05 μg/mL), ketobemidone (3.3, LOD <0.05 μg/mL), ketoprofen (7.3, LOD 0.1 μg/mL), ketorolac (6.2, LOD 0.05 μg/mL), labetalol (4.9, LOD 0.05 μg/mL), lamotrigine (4.0, LOD 0.1 μg/mL), levocabastine (5.8, LOD 0.01 μg/mL), levomepromazine (6.5, LOD 0.02 μg/mL), lidocaine (3.7, LOD <0.05 μg/mL), loratadine (9.3, LOD 0.002 μg/mL), lorazepam (6.6, LOD 0.02 μg/mL), lormetazepam (7.4, LOD <0.02 μg/mL), LSD (4.7, LOD <0.02 μg/mL), malathion (8.9, LOD 10 μg/mL), maprotiline (6.4, LOD <0.02 μg/mL), MDMA (3.3, LOD 0.02 μg/mL), meclozine (8.5, LOD <0.02 μg/mL), medazepam (6.3, LOD <0.02 μg/mL), meloxicam (7.1, LOD 0.01 μg/mL), melperone (5.0, LOD <0.02 μg/mL), meperidine (4.7, LOD <0.02 μg/mL), mepivacaine (3.7, LOD <0.02 μg/mL), meprobamate (4.9, LOD 0.1 μg/mL), mesoridazine (5.4, LOD <0.02 μg/mL), methamphetamine (3.3, LOD 0.05 μg/mL), methadone (6.7, LOD <0.02 μg/mL), methylparathion (8.6, LOD 10 μg/mL), methylphenidate (4.2, LOD <0.02 μg/mL), metoclopramide (3.8, LOD <0.02 μg/mL), metoprolol (4.1, LOD 0.02 μg/mL), metronidazole (2.6, LOD 1 μg/mL), mexiletine (4.4, LOD 0.05 μg/mL), mianserin (5.7, LOD <0.02 μg/mL), midazolam (5.9, LOD <0.02 μg/mL), mirtazapine (4.4, LOD <0.02 μg/mL), mizolastine (5.5, LOD 0.01 μg/mL), moclobemide (3.7, LOD 0.05 μg/mL), molindone (4.0, LOD <0.02 μg/mL), monoacetylmorphine (2.7, LOD 0.1 μg/mL), morphine (2.0, LOD 0.1 μg/mL), nicotine (2.2, LOD 0.05 μg/mL), nifedipine (7.5, LOD 0.02 μg/mL), nikethamide (3.6, LOD <0.02 μg/mL), nitrazepam (6.5, LOD <0.02 μg/mL), nizatidine (1.7, LOD 1 μg/mL), nomifensine (4.6, LOD <0.02 μg/mL), nortriptyline (6.4, LOD <0.02 μg/mL), norverapamil (6.2, LOD 1 μg/mL), noscapine (5.0, LOD <0.02 μg/mL), olanzapine (3.0, LOD 0.05 μg/mL), ondansetron (4.6, LOD <0.02 μg/mL), orphenadrine (6.1, LOD <0.02 μg/mL), oxazepam (6.3, LOD <0.02 μg/mL), oxcarbazepine (5.3, LOD 0.02 μg/mL), oxprenolol (4.7, LOD 0.02 μg/mL), oxycodone (2.8, LOD 0.05 μg/mL), papaverine (4.8, LOD <0.02 μg/mL), paroxetine (6.2, LOD 0.02 μg/mL), pemoline (3.3, LOD 0.05 μg/mL), pentazocine (5.0, LOD <0.02 μg/mL), pentifylline (7.3, LOD <5 μg/mL), pentoxyverine (6.6, LOD <0.02 μg/mL), perphenazine (6.9, LOD 0.002 μg/mL), phenazone (3.9, LOD

0.05 μg/mL), phencyclidine (5.3, LOD 0.05 μg/mL), pheniramine (4.1, LOD 0.02 μg/mL), phenylbutazone (9.0, LOD <5 μg/mL), phenylpropanolamine (2.5, LOD 0.3 μg/mL), phenytoin (6.1, LOD 0.05 μg/mL), pindolol (3.3, LOD 0.05 μg/mL), piroxicam (6.6, LOD 0.02 μg/mL), pitofenone (5.4, LOD <0.02 μg/mL), pizotifen (6.5, LOD <0.02 μg/mL), practolol (1.8, LOD 0.1 μg/mL), prazosin (4.1, LOD 0.05 μg/mL), prilocaine (3.8, LOD <0.02 μg/mL), primidone (4.0, LOD <5 μg/mL), procainamide (2.2, LOD 0.05 μg/mL), prochlorperazine (7.5, LOD 0.02 μg/mL), promazine (6.2, LOD <0.02 μg/mL), promethazine (6.0, LOD 0.05 μg/mL), propafenone (6.3, LOD <0.02 μg/mL), propranolol (5.4, LOD 0.02 μg/mL), propyphenazone (6.6, LOD 0.50 μg/mL), pseudoephedrine (2.6, LOD 1 μg/mL), quinine (4.2, LOD 0.02 μg/mL), ranitidine (1.8, LOD 0.1 μg/mL), risperidone (4.9, LOD <0.02 μg/mL), rocurone (3.8, LOD 0.1 μg/mL), ropivacaine (4.6, LOD <0.02 μg/mL), salicylamide (4.2, LOD <5 μg/mL), selegiline (4.1, LOD 0.05 μg/mL), sertindole (7.2, LOD <0.02 μg/mL), sertraline (6.8, LOD 0.02 μg/mL), sulindac (6.5, LOD 0.02 μg/mL), simazine (6.0, LOD 0.1 μg/mL), sincocaine (6.5, LOD <0.02 μg/mL), sisapride (5.9, LOD <0.02 μg/mL), sotalol (2.1, LOD 0.1 μg/mL), strychnine (5.3, LOD 0.05 μg/mL), sulpiride (1.9, LOD 0.1 μg/mL), sulthiame (4.1, LOD 0.05 μg/mL), temazepam (7.2, LOD <0.02 μg/mL), terbutaline (2.3, LOD 0.1 μg/mL), terfenadine (8.1, LOD 0.002 μg/mL), terodiline (6.7, LOD <0.02 μg/mL), tetracaine (5.7, LOD <0.02 μg/mL), tetrahydrozoline (3.6, LOD 0.1 μg/mL), theobromine (2.3, LOD <5 μg/mL), theophylline (2.4, LOD <5 μg/mL), thioridazine (7.5, LOD 0.02 μg/mL), timolol (3.8, LOD 0.05 μg/mL), thiothixene (6.7, LOD 0.02 μg/mL), tolbutamide (7.1, LOD <5 μg/mL), toremifene (8.7, LOD 0.02 μg/mL), tramadol (4.2, LOD 0.02 μg/mL), trazodone (5.2, LOD <0.02 μg/mL), triamterene (3.2, LOD 0.1 μg/mL), triazolam (6.7, LOD 0.002 μg/mL), trimeprazine (6.4, LOD <0.02 μg/mL), trimethoprim (3.1, LOD 0.05 μg/mL), trimipramine (6.7, LOD <0.02 μg/mL), venlafaxine (4.9, LOD 0.02 μg/mL), verapamil (6.5, LOD <0.02 μg/mL), warfarin (7.9, LOD <0.02 μg/mL), yohimbine (4.5, LOD <0.02 μg/mL), zolpidem (4.7, LOD <0.02 μg/mL), zopiclone (4.0, LOD 0.1 μg/mL)

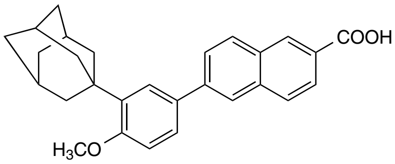
KEY WORDS

whole blood

REFERENCE

Gergov, M.; Ojanperä, I.; Vuori, E. Simultaneous screening for 238 drugs in blood by liquid chromatography-ionspray tandem mass spectrometry with multiple-reaction monitoring, *J.Chromatogr.B*, **2003**, *795*, 41–53.

Adapalene



Molecular formula: $C_{28}H_{28}O_3$
Molecular weight: 412.52
CAS Registry No: 106685-40-9
Merck Index: 13, 150

SAMPLE

Matrix: formulations
Sample preparation: Inject an aliquot of a 0.1% gel.

HPLC VARIABLES

Column: 250 × 4 ODS-RP18 (Merck)
Mobile phase: MeCN:THF:water:trifluoroacetic acid 43:36:21:0.02
Flow rate: 1
Detector: UV 270

CHROMATOGRAM

Retention time: 6.1

OTHER SUBSTANCES

Noninterfering: tretinoin

KEY WORDS

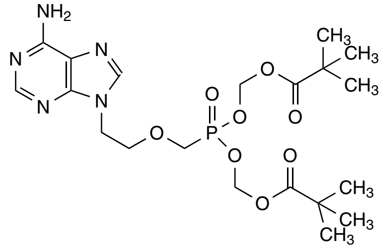
gel

REFERENCE

Martin, B.; Meunier, C.; Montels, D.; Watts, O. Chemical stability of adapalene and tretinoin when combined with benzoyl peroxide in presence and in absence of visible light and ultraviolet radiation, *Br.J.Dermatol.*, **1998**, *139* (Suppl. 52), 8–11.

Adefovir dipivoxil

Molecular formula: $C_{20}H_{32}N_5O_8P$
Molecular weight: 501.47
CAS Registry No: 142340-99-6
Merck Index: 13, 151



SAMPLE

Matrix: blood

Sample preparation: Mix 100 μL plasma with 200 μL 0.1% trifluoroacetic acid in MeCN. Evaporate the supernatant to dryness under reduced pressure at room temperature. Reconstitute with 0.34% chloroacetaldehyde in 100 mM pH 4.5 sodium acetate, vortex, centrifuge. Heat the supernatant at 95° for 40 min, evaporate to dryness, reconstitute with 100 μL 25 mM pH 6.0 potassium phosphate buffer containing 5 mM tetrabutylammonium hydrogen phosphate, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 Brownlee RP-18 Newguard
Column: 150 × 4.6 Zorbax RX-C18
Column temperature: 35
Mobile phase: Gradient. A was MeCN:25 mM pH 6.0 potassium phosphate buffer containing 5 mM tetrabutylammonium hydrogen phosphate 2:98. B was MeCN:25 mM pH 6.0 potassium phosphate buffer containing 5 mM tetrabutylammonium hydrogen phosphate 65:35. A:B 100:0 for 2 min, to 0:100 over 13 min, re-equilibrate at initial conditions for 10 min. (Only adefovir is detected in blood. However, the method is reported to distinguish between adefovir and adefovir dipivoxil.)
Flow rate: 1.5
Injection volume: 50
Detector: F ex 236 em 420

KEY WORDS

derivatization; dog; pharmacokinetics; plasma

REFERENCE

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

SAMPLE

Matrix: blood

Sample preparation: Vortex 200 μL plasma with 50 μL 20% trichloroacetic acid in water, centrifuge at 1300 g for 15 min. Remove 150 μL of the supernatant and mix it with 50 μL 160 mM chloroacetaldehyde in water containing 2 M sodium acetate, vortex, close the tube, heat at 98° for 30 min, cool to 2°, vortex, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 3 R3 (Chrompack)
Column: 150 × 4.6 5 μm Chromspher C8
Column temperature: 40 ± 2
Mobile phase: MeCN:buffer 10:90 (Buffer was 10 mM pH 7.0 sodium phosphate buffer containing 2 mM tetrabutylammonium hydrogen sulfate.)
Flow rate: 1.5

Injection volume: 20
Detector: F ex 254 em 425

CHROMATOGRAM

Retention time: 4.5
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Extracted: adefovir (4)

KEY WORDS

derivatization; plasma

REFERENCE

Sparidans, R.W.; Veldkamp, A.; Hoetelmans, R.M.W.; Beijnen, J.H. Improved and simplified liquid chromatographic assay for adefovir, a novel antiviral drug, in human plasma using derivatization with chloroacetaldehyde, *J.Chromatogr.B*, **1999**, *736*, 115–121.

Adrenocorticotropic hormone

CAS Registry No: 9002-60-2

Merck Index: 13, 136

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL Analytichem weak cation-exchange (carboxymethylhydrogen form, CBA) SPE cartridge with 1 mL 1% trifluoroacetic acid in MeOH, 1 mL MeOH, and 2 mL water. Add 1 mL plasma to the SPE cartridge, rinse the tube with 1 mL water, add the rinse to the SPE cartridge, wash with 1 mL 1% trifluoroacetic acid in water, wash with 2 mL water, wash with 2 mL MeOH, elute with 2 mL 1% trifluoroacetic acid in MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH:buffer 50:50, inject a 5−75 μL aliquot. (Buffer was 5.7 g monochloroacetic acid, 2.0 g NaOH, and 0.2 g disodium EDTA in 1 L water, pH 3.2.) (The procedure was not necessarily validated for this compound.)

HPLC VARIABLES

Column: 250 × 2 5 μm Ultrasphere octyl

Column temperature: 60

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. (The buffer was 5.7 g monochloroacetic acid, 2.0 g NaOH, and 0.2 g disodium EDTA in 1 L water, pH 3.2.)

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 ID reaction coil at 70° to the detector.

CHROMATOGRAM

Retention time: 45

Limit of detection: 100 fmole

OTHER SUBSTANCES

Simultaneous: angiotensin I, angiotensin II, angiotensin III, atrial natriuretic peptide, bombesin, bradykinin, gonadorelin (LHRH), somatoliberin, vasopressin

KEY WORDS

plasma; post-column reaction; SPE

REFERENCE

Rhodes, G.R.; Boppana, V.K. High-performance liquid chromatographic analysis of arginine-containing peptides in biological fluids by means of a selective post-column reaction with fluorescence detection, *J.Chromatogr.*, **1988**, *444*, 123−131.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: Gradient. A was 0.08% trifluoroacetic acid. B was MeCN:0.08% trifluoroacetic acid 70:30. A:B from 70:30 to 50:50 over 30 min.

Flow rate: 1
Detector: UV 206

CHROMATOGRAM
Retention time: 25

OTHER SUBSTANCES
Simultaneous: adrenocorticotropic hormone fragments, melanotropin

KEY WORDS
human

REFERENCE
McDermott, J.R.; Smith, A.I.; Biggins, J.A.; Al-Noaemi, M.C.; Edwardson, J.A. Characterization and determination of neuropeptides by high-performance liquid chromatography and radioimmunoassay, *J.Chromatogr.*, **1981**, *222*, 371–379.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in 100 mM NaH_2PO_4 adjusted to pH 2.1 with orthophosphoric acid, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 Aquapore RP 300 (Kontron)
Mobile phase: Gradient. A was 100 mM NaH_2PO_4 adjusted to pH 2.1 with orthophosphoric acid. B was MeOH. A:B from 90:10 to 35:65 over 180 min.
Flow rate: 1
Injection volume: 100
Detector: UV 225

CHROMATOGRAM
Retention time: 145

OTHER SUBSTANCES
Simultaneous: adrenocorticotropin hormone fragments, lipotropic hormone and fragments, melanotropin, endorphins, prolactin, somatropin, menotropins

KEY WORDS
pig

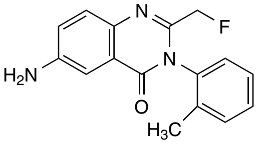
REFERENCE
Richter, W.O.; Schwandt, P. Separation of neuropeptides by HPLC: evaluation of different supports, with analytical and preparative applications to human and porcine neurophysins, β-lipotropin, adrenocorticotropic hormone, and β-endorphin, *J.Neurochem.*, **1985**, *44*, 1697–1703.

ANNOTATED BIBLIOGRAPHY
Capp, M.W.; Simonian, M.H. Separation of the major adrenal steroids by reversed-phase high-performance liquid chromatography, *Anal.Biochem.*, **1985**, *147*, 374–381.

Janssen, P.S.; van Nispen, J.W.; Hamelinck, R.L.; Melgers, P.A.; Goverde, B.C. Application of reversed-phase HPLC in some critical peptide separations, *J.Chromatogr.Sci.*, **1984**, *22*, 234–238.

Smith, A.I.; McDermott, J.R. High-performance liquid chromatography of neuropeptides using radially compressed polythene cartridges, *J.Chromatogr.*, **1984**, *306*, 99–108.

Afloqualone



Molecular formula: $C_{16}H_{14}FN_3O$

Molecular weight: 283.30

CAS Registry No: 56287-74-2

Merck Index: 13, 183

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: Chiralpak AS
Column temperature: 50
Mobile phase: Hexane:EtOH 95:5
Flow rate: 1.3
Detector: UV 254

CHROMATOGRAM

Retention time: 30, 35 (enantiomers)

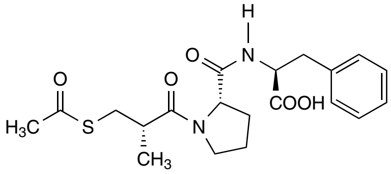
KEY WORDS

chiral

REFERENCE

Application Guide for Chiral Column Selection, Second Edition; Chiral Technologies: Exton PA, **1995**, p. 43.

Alacepril



Molecular formula: $C_{20}H_{26}N_2O_5S$
Molecular weight: 406.50
CAS Registry No: 74258-86-9
Merck Index: 13, 200

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 10 μm Cosmosil 5C18-MS
Column temperature: 50
Mobile phase: Gradient. MeCN:10 mM pH 2.5 potassium phosphate buffer 0:100 for 2 min, to 75:25 over 7.5 min, maintain at 75:25 for 5.5 min. Isocratic. MeCN:buffer 40:60
Flow rate: 1.5
Detector: UV (wavelength not specified)

CHROMATOGRAM

Retention time: 10.9 (gradient) or 4.1 (isocratic)

OTHER SUBSTANCES

Simultaneous: acetaminophen (7.9), ampicillin (7.9), aspirin (10.0), caffeine (8.5), carbenicillin (9.5), cefotiam (7.2), chlorpromazine (10.8), cromolyn (8.9), enalapril (9.9), loperamide (11.6), ofloxacin (8.3), procainamide (7.4), procaine (7.9), propranolol (9.6), sultamicillin tosylate (8.3), tegafur (8.4), temocapril (12.3), theophylline (8.0), tulobuterol (8.9) (gradient retention times; isocratic conditions may differ)

REFERENCE

Sugiyama, T.; Matsuyama, R.; Usui, S.; Katagiri, Y.; Hirano, K. Selection of mobile phases in high-performance liquid chromatographic determination for medicines, *Biol.Pharm.Bull.*, **2000**, *23*, 274–278.

SAMPLE

Matrix: enzyme reactions
Sample preparation: Mix 40 μL enzyme reaction mixture with 200 μL MeCN, add 200 μL of a 20 μg/mL solution of *n*-propyl paraben, centrifuge, inject a 30 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4.6 Cosmosil 5-C18 MS
Column temperature: 50
Mobile phase: MeCN:10 mM pH 2.5 potassium phosphate buffer 40:60
Flow rate: 1.5
Injection volume: 30
Detector: UV 220

CHROMATOGRAM

Internal standard: *n*-propyl paraben

OTHER SUBSTANCES

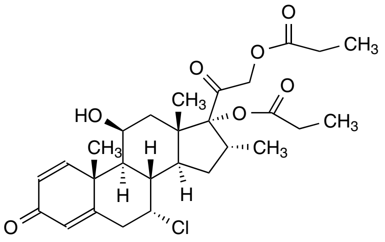
Extracted: deacetylalacepril

REFERENCE

Usui, S.; Kubota, M.; Iguchi, K.; Kiho, T.; Sugiyama, T.; Katagiri, Y.; Hirano, K. Sialic acid 9-*O*-acetylesterase catalyzes the hydrolyzing reaction from alacepril to deacetylalacepril, *Pharm.Res.*, **2003**, *20*, 1309–1316.

Alclometasone 17,21-dipropionate

Molecular formula: $C_{28}H_{37}ClO_7$
Molecular weight: 521.05
CAS Registry No: 66734-13-2
Merck Index: 13, 219



SAMPLE

Matrix: formulations

Sample preparation: Condition a 3 mL 500 mg Megabond MF C18 SPE cartridge (Varian) with 3 mL MeOH and 3 mL water. Sonicate 1 g cosmetic with 10 mL MeOH or MeOH:dichloromethane 10:90 (depending on what appears visually to give best solubility) at 40° for 10 min, centrifuge, collect the clear supernatant. Add 5 mL of the supernatant to the SPE cartridge, wash with 4 mL acetone:water 20:80, wash with 1 mL *n*-hexane, elute with 4 mL diethyl ether. Evaporate the eluate to dryness under reduced pressure, reconstitute the residue with 5 mL (or more) MeOH, inject a 10 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 µm endcapped Purospher RP-18
Column temperature: 25
Mobile phase: Isocratic. MeCN:water 60:40. Gradient. MeCN:water from 25:75 to 90:10 over 30 min, maintain at 90:10 for 10 min.
Flow rate: 1
Injection volume: 10
Detector: UV 239

CHROMATOGRAM

Retention time: k′ 2.55 (isocratic); 21.0 min (gradient)
Limit of detection: 300 ng/mL

OTHER SUBSTANCES

Simultaneous: amcinonide (isocratic k′ 3.18; gradient retention time (min) 22.6; LOD 0.1 µg/mL), betamethasone (isocratic k′ 0.18; gradient retention time (min) 11.8; LOD 0.1 µg/mL), betamethasone-17-acetate (isocratic k′ 0.73; gradient retention time (min) 15.4; LOD 0.3 µg/mL), betamethasone-17-benzoate (isocratic k′ 2.04; gradient retention time (min) 20.6; LOD 0.3 µg/mL), betamethasone-17-propionate-21-stearate (isocratic k′ >13; gradient retention time (min) >35; LOD 0.5 µg/mL), betamethasone-17-propionate-21-butyrate (isocratic k′ 5.91; gradient retention time (min) 26.1; LOD 0.4 µg/mL), betamethasone-17-valerate-21-acetate (isocratic k′ 4.41; gradient retention time (min) 23.1; LOD 0.4 µg/mL), betamethasone-17-valerate (isocratic k′ 2.32; gradient retention time (min) 21.4; LOD 0.3 µg/mL), betamethasone-17,21-dipropionate (isocratic k′ 4.00; gradient retention time (min) 24.2; LOD 0.4 µg/mL), betamethasone-17,21-diacetate (isocratic k′ 1.81; gradient retention time (min) 20.5; LOD 0.3 µg/mL), betamethasone-17,21-divalerate (isocratic k′ 10.82; gradient retention time (min) 28.0; LOD 0.4 µg/mL), betamethasone-21-acetate (isocratic k′ 0.77; gradient retention time (min) 15.6; LOD 0.3 µg/mL), betamethasone propionate (isocratic k′ 0.82; gradient retention time (min) 17.1; LOD 0.3 µg/mL), clobetasol propionate (isocratic k′ 3.41; gradient retention time (min) 23.4; LOD 0.1 µg/mL), clobetasone butyrate (isocratic k′ 5.45; gradient retention time (min) 26.3; LOD 0.1 µg/mL), cortisone (isocratic k′ 0.18; gradient retention time (min) 11.1; LOD 0.6 μg/mL), cortisone acetate (isocratic k′ 0.73; gradient retention time (min) 15.2; LOD 0.6 μg/mL), dehydrocorticosterone (isocratic k′ 4.27; gradient retention time (min) 22.3; LOD 0.5 μg/mL), deoxymethasone (isocratic k′ 0.64; gradient retention time (min) 14.2; LOD 0.2 μg/mL), dexamethasone (isocratic k′ 0.27; gradient retention time (min) 11.9; LOD 0.1 μg/mL), dexamethasone-21-acetate (isocratic k′ 0.91; gradient retention time (min) 16.1; LOD 0.2 μg/mL), dexamethasone isonicotinate (isocratic k′ 1.05; gradient retention time (min) 17.7; LOD 0.4 μg/mL), dexamethasone pivalate (isocratic k′ 3.45; gradient retention time (min) 24.1; LOD 0.3 μg/mL), dexamethasone valerate (isocratic k′ 3.00; gradient retention time (min) 21.6; LOD 0.3 μg/mL), diflucortolone valerate (isocratic k′ 4.73; gradient retention time (min) 23.3; LOD 0.3 μg/mL), fludrocortisone acetate (isocratic k′ 0.59; gradient retention time (min) 14.1; LOD 0.3 μg/mL), flumethasone pivalate (isocratic k′ 2.68; gradient retention time (min) 21.2; LOD 0.3 μg/mL), fluocinolone acetonide (isocratic k′ 0.91; gradient retention time (min) 13.4; LOD 0.3 μg/mL), fluocinonide (isocratic k′ 1.45; gradient retention time (min) 20.5; LOD 0.1 μg/mL), fluocortin butyl ester (isocratic k′ 5.59; gradient retention time (min) 24.6; LOD 0.3 μg/mL), fluocortolone caproate (isocratic k′ 6.59; gradient retention time (min) 25.1; LOD 0.3 μg/mL), fluocortolone pivalate (isocratic k′ 4.50; gradient retention time (min) 23.6; LOD 0.3 μg/mL), fluorometholone (isocratic k′ 0.59; gradient retention time (min) 14.4; LOD 0.1 μg/mL), 9-α-fluoroprednisolone (isocratic k′ 0.18; gradient retention time (min) 10.0; LOD 0.1 μg/mL), 9-α-fluoroprednisolone acetate (isocratic k′ 0.50; gradient retention time (min) 13.9; LOD 0.2 μg/mL), flurandrenolide (isocratic k′ 0.50; gradient retention time (min) 13.5; LOD 0.1 μg/mL), halcinonide (isocratic k′ 1.64; gradient retention time (min) 20.6; LOD 0.1 μg/mL), hydrocortisone (isocratic k′ 0.18; gradient retention time (min) 10.0; LOD 0.4 μg/mL), hydrocortisone-17-butyrate (isocratic k′ 1.09; gradient retention time (min) 17.7; LOD 0.6 μg/mL), hydrocortisone-21-acetate (isocratic k′ 0.77; gradient retention time (min) 15.3; LOD 0.6 μg/mL), hydrocortisone pivalate (isocratic k′ 2.27; gradient retention time (min) 20.4; LOD 0.8 μg/mL), methylprednisolone (isocratic k′ 0.55; gradient retention time (min) 13.5; LOD 0.1 μg/mL), mometasone furoate (isocratic k′ 3.05; gradient retention time (min) 22.0; LOD 0.2 μg/mL), prednisolone-21-acetate (isocratic k′ 0.60; gradient retention time (min) 13.6; LOD 0.2 μg/mL), prednisolone acetonide (isocratic k′ 0.50; gradient retention time (min) 13.0; LOD 0.3 μg/mL), prednisolone pivalate (isocratic k′ 2.05; gradient retention time (min) 19.7; LOD 0.3 μg/mL), triamcinolone (isocratic k′ 0.14; gradient retention time (min) 7.2; LOD 0.1 μg/mL), triamcinolone acetonide (isocratic k′ 0.50; gradient retention time (min) 13.9; LOD 0.2 μg/mL), triamcinolone diacetate (isocratic k′ 0.45; gradient retention time (min) 13.9; LOD 0.3 μg/mL)

KEY WORDS

cosmetics; SPE

REFERENCE

Gagliardi, L.; De Orsi, D.; Del Giudice, M.R.; Gatta, F.; Porrà, R.; Chimenti, P.; Tonelli, D. Development of a tandem thin-layer chromatography-high-performance liquid chromatography method for the identification and determination of corticosteroids in cosmetic products, *Anal.Chim.Acta*, **2002**, *457*, 187–198.

SAMPLE

Matrix: formulations

Sample preparation: Mix 1 g of a topical product, 5 mL 100 mM pH 4 citrate buffer saturated with NaCl, and 10 mL ethyl acetate, shake vigorously by hand to ensure that no large clumps stick to the tube, mix with the tube on its side on an oscillating shaker for 15 min. Remove the upper ethyl acetate layer and wash with citrate buffer as before. Dry the ethyl acetate over anhydrous sodium sulfate and evaporate to dryness. Dissolve the residue in a mixture of 10 mL heptane and 5 mL MeCN:water 90:10. Remove the upper heptane layer and extract it with 2 mL MeCN:water 90:10. Combine the MeCN/water layers and evaporate them to dryness, dissolve the residue in 300 μL MeOH, filter (0.45 μm nylon), inject a 5 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm Brownlee NewGuard C18
Column: 75 × 4.6 3.5 μm Symmetry C18 (Waters)
Mobile phase: Gradient. MeCN:water 18:82 for 2 min, to 82:18 over 12 min, maintain at 82:18 for 3 min, re-equilibrate at initial conditions for 12 min.
Flow rate: 1
Injection volume: 5
Detector: UV 240

CHROMATOGRAM

Retention time: 10.93
Limit of detection: 0.001%

OTHER SUBSTANCES

Simultaneous: amcinonide (10.90), beclomethasone 17,21-dipropionate (11.90), betamethasone (6.52), betamethasone 21-acetate (8.47), betamethasone 17-benzoate (10.28), betamethasone 17,21-dipropionate (11.42), betamethasone 17-valerate (10.25), budesonide (8.55, 8.69 (epimers)), clobetasol 17-propionate (11.06), cortisone (5.62), cortisone 21-acetate (8.07), dexamethasone (6.57), dexamethasone 21-acetate (8.68), desonide (6.99), desoximetasone (7.60), desoxycorticosterone acetate (10.90), desoxycorticosterone pivalate (14.45), diflorasone 17,21-diacetate (9.81), fluocinolone acetonide (7.38), fluocinonide (9.79), flurandrenolide (7.36), fludrocortisone 21-acetate (7.77), fluorometholone (7.67), flumethasone 21-pivalate (11.20), flunisolide (7.14), fluprednisolone (5.46), fluticasone 17-propionate (11.19), halcinonide (10.72), halobetasol propionate (10.98), hydrocortisone (5.50), hydrocortisone 21-acetate (7.65), hydrocortisone 17-butyrate (8.66), hydrocortisone 21-cypionate (12.54), hydrocortisone 17-valerate (9.53), mometasone 17-furoate (11.24), methylprednisolone (6.31), methylprednisolone 21-acetate (8.34), meprednisone (6.55), prednisolone (5.37), prednisolone 21-acetate (7.47), prednisolone 21-tebuate (11.01), paramethasone 21-acetate (8.64), prednicarbate (10.72), prednisone (5.46), triamcinolone (4.15), triamcinolone acetonide (7.04), triamcinolone 16,21-diacetate (7.49), triamcinolone hexacetonide (13.12)

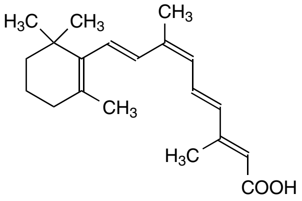
KEY WORDS

body wash, cream, gel, lotion, shampoo, spray

REFERENCE

Reepmeyer, J.C. Screening for corticosteroids in topical pharmaceuticals by HPLC with a scanning ultraviolet detector, *J.Liq.Chromatogr.Rel.Technol.*, **2001**, *24*, 693–709.

Alitretinoin



Molecular formula: $C_{20}H_{28}O_2$
Molecular weight: 300.43
CAS Registry No: 5300-03-8
Merck Index: 13, 244
[9-cis-retinoic acid]

SAMPLE

Matrix: blood
Sample preparation: 1 mL plasma + 50 μL 500 μg/mL IS in MeOH:MeCN 50:50 + 1 mL 1 M pH 6.0 phosphate buffer, mix, add 6 mL MTBE, shake on a horizontal shaker for 10 min, freeze the aqueous layer in a dry ice/acetone bath. Decant the organic layer and evaporate it to dryness under nitrogen at 25°, reconstitute the residue with 200 μL MeOH, add 100 μL 5 mM ammonium acetate, centrifuge at 13 000 g for 3 min, inject a 100 μL aliquot. (Use silanized glassware. Process under yellow light.)

HPLC VARIABLES

Guard column: 10 × 2 5 μm Hypersil BDS C18
Column: 100 × 4.6 3 μm Microsorb Short One C18 (Rainin)
Column temperature: 36
Mobile phase: Gradient. A was 5 mM pH 2.7 ammonium acetate/acetic acid buffer. B was 1% acetic acid in MeOH. A:B 30:70 for 6.5 min, to 20:80 over 0.5 min, to 11:80 over 14.4 min, to 30:70 over 0.5 min, maintain at 30:70 for 10 min.
Flow rate: 1
Injection volume: 100
Detector: UV 348

CHROMATOGRAM

Retention time: 21
Internal standard: all-*trans*-3,7-dimethyl-9-(2,4,6-trimethylphenyl)-2,4,6,8-nonatetraenoic acid (Ro 11–5036) (19)
Limit of detection: 2.5 ng/mL

OTHER SUBSTANCES

Extracted: isotretinoin (19.5), tretinoin (21.5), vitamin A (20.5)

KEY WORDS

plasma

REFERENCE

Dzerk, A.M.; Carlson, A.; Loewen, G.R.; Shirley, M.A.; Lee, J.W. A HPLC method for the determination of 9-cis retinoic acid (ALRT1057) and its 4-oxo metabolite in human plasma, *J.Pharm.Biomed.Anal.*, **1998**, *16*, 1013–1019.

SAMPLE

Matrix: blood, food, formulations, tissue
Sample preparation: Serum. Extract one volume (20–100 μL) serum with three volumes isopropanol:dichloromethane 2:1 containing about 6 nM IS and 1 mM butylated hydroxytoluene (BHT, antioxidant), add glacial acetic acid (1 μL/20 μL serum). Vortex for 30 s, centrifuge for 1 min, inject a 20–70 μL aliquot of the supernatant. Tissue, food. Homogenize 100–200 mg human or rat liver, 200 mg–2 g other tissues, or 2–5 g pulp of fruits and fresh vegetables with 3–5 mL isopropanol:dichloromethane 2:1, make up to 10 mL with isopropanol:dichloromethane 2:1. Vortex for 1 min, keep under argon

at −20° overnight, vortex for 1 min, return to the freezer. On the third day, vortex the mixture, centrifuge or filter. Evaporate the supernatant or filtrate to dryness in a rotary evaporator. Dissolve the residue in 200 μL isopropanol:dichloromethane 2:1, inject a 20−40 μL aliquot. Multivitamin tablets. Grind tablet to a powder, add 10 mL isopropanol:dichloromethane 2:1. Vortex for 1 min, keep under argon at −20° overnight, vortex for 1 min, return to the freezer. On the third day, vortex the mixture, centrifuge about 500 μL solution, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: C18 (Upchurch)
Column: 100 × 4.6 3 μm Microsorb MV
Mobile phase: Gradient. A was MeOH:water 75:25 containing 10 mM ammonium acetate. B was MeOH:dichloromethane 80:20. A:B from 100:0 to 0:100 over 15 min, maintain at 0:100 for 15−20 min, to 100:0 over 5 min, re-equilibrate at initial conditions for 10 min.
Flow rate: 0.8
Injection volume: 50
Detector: UV 340

CHROMATOGRAM

Retention time: 10.2
Internal standard: retinyl acetate (13.8)

OTHER SUBSTANCES

Extracted: *β*-carotene (27.1), isotretinoin (9.9), all-trans retinal (13.8), all-trans retinyl palmitate (24.1), all-trans retinyl stearate (26.4), tretinoin (10.5), vitamin A (12.9), vitamin E (18.7),

KEY WORDS

human, ketchup, liver, mango, multivitamin tablets, papaya, rat, serum, spinach, tomato

REFERENCE

Barua, A.B.; Olson, J.A. Reversed-phase gradient high-performance liquid chromatographic procedure for simultaneous analysis of very polar to nonpolar retinoids, carotenoids and tocopherols in animal and plant samples, *J.Chromatogr.B*, **1998**, *707*, 69−79.

SAMPLE

Matrix: formulations
Sample preparation: Capsules. Cut open 10 capsules, sonicate three times at 30° for 5 min with 40 mL portions of MeCN:EtOH:1% acetic acid 70:20:10, centrifuge at 3500 rpm for 6 min. Filter the supernatants, combine, make up to 250 mL. Dilute a 1 mL aliquot to 10 mL with mobile phase, filter (nylon 0.45 μm), inject an aliquot. Gel. Sonicate a portion with 8 mL mobile phase for 1 min, centrifuge at 3500 rpm for 10 min. Filter the supernatant and make up to 10 mL. Dilute a 1 mL aliquot to 5 mL with mobile phase, filter (nylon 0.45 μm), inject an aliquot. Cream. Sonicate an aliquot twice for 5 min with 4 mL portions of MeCN:EtOH:1% acetic acid 70:20:10, centrifuge at 3500 rpm for 6 min. Filter the supernatants, combine, make up to 10 mL with MeCN:EtOH:1% acetic acid 70:20:10. Dilute a 2 mL aliquot to 5 mL with mobile phase, filter (nylon 0.45 μm), inject an aliquot.

HPLC VARIABLES

Column: 250 × 3.2 Phenomenex Prodigy 5ODS
Column temperature: 32 ± 2
Mobile phase: MeCN:EtOH:1% acetic acid 68:8:24
Flow rate: 0.4
Injection volume: 20
Detector: F ex 350 em 520

CHROMATOGRAM

Retention time: 31
Limit of detection: 11.09 pmole (S/N = 3)

OTHER SUBSTANCES

Simultaneous: isotretinoin (28.5), tretinoin (33)

KEY WORDS

avoid exposure to light, use amber-colored glassware, capsules, cream, gel

REFERENCE

Gatti, R.; Gioia, M.G.; Cavrini, V. Analysis and stability study of retinoids in pharmaceuticals by LC with fluorescence detection, *J.Pharm.Biomed.Anal.*, **2000**, *23*, 147–159.

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Disdier, B.; Bun, H.; Placidi, M.; Durand, A. Excretion of oral 9-cis-retinoic acid in the rat, *Drug Metab.Dispos.*, **1996**, *24*, 1279–1281.

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

Gatti, R.; Gioia, M.G.; Di Pietra, A.M.; Cini, M. Determination of retinoids in galenicals by column liquid chromatography with fluorescence and diode-array detection, *J.Chromatogr.A*, **2001**, *905*, 345–350.

Marchetti, M.-N.; Sampol, E.; Bun, H.; Scoma, H.; Lacarelle, B.; Durand, A. In vitro metabolism of three major isomers of retinoic acid in rats. Intersex and interstrain comparison, *Drug Metab.Dispos.*, **1997**, *25*, 637–646.

Miyagi, M.; Yokoyama, H.; Shiraishi, H.; Matsumoto, M.; Ishii, H. Simultaneous quantification of retinol, retinal, and retinoic acid isomers by high-performance liquid chromatography with a simple gradiation, *J.Chromatogr.B*, **2001**, *757*, 365–368.

Rühl, R.; Schweigert, F.J. Automated solid-phase extraction and liquid chromatographic method for retinoid determination in biological samples, *J.Chromatogr.B*, **2003**, *798*, 309–316.

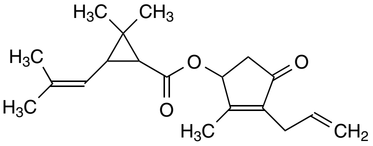
Shih, T.-W.; Lin, T.-H.; Shealy, Y.F.; Hill, D.L. Nonenzymatic isomerization of 9-cis-retinoic acid catalyzed by sulfhydryl compounds, *Drug Metab.Dispos.*, **1997**, *25*, 27–32.

Van Merris, V.; Meyer, E.; De Wasch, K.; Burvenich, C. Simple quantification of endogenous retinoids in bovine serum by high-performance liquid chromatography – diode-array detection, *Anal.Chim.Acta*, **2002**, *468*, 237–244.

Wyss, R.; Bucheli, F. Determination of endogenous levels of 13-cis-retinoic acid (isotretinoin), all-trans-retinoic acid (tretinoin) and their 4-oxo metabolites in human and animal plasma by high-performance liquid chromatography with automated column switching and ultraviolet detection, *J.Chromatogr.B*, **1997**, *700*, 31–47.

Yamakoshi, Y.; Fukasawa, H.; Yamauchi, T.; Waki, H.; Kadowaki, T.; Shudo, K.; Kagechika, H. Determination of endogenous levels of retinoic acid isomers in type II diabetes mellitus patients. Possible correlation with HbA1c values, *Biol.Pharm.Bull.*, **2002**, *25*, 1268–1271.

Allethrin



Molecular formula: $C_{19}H_{26}O_3$
Molecular weight: 302.41
CAS Registry No: 584-79-2
Merck Index: 13, 256

SAMPLE

Matrix: fruit, vegetables

Sample preparation: Prepare a cleanup column by placing 4 g Florisil, 1 g activated charcoal, and a 20 mm layer of anhydrous sodium sulfate in a 400 × 10 glass column, wash with 40 mL toluene, wash with 40 mL toluene:MeCN 99:1. Homogenize 25 g chopped fruit or vegetable with 70 mL MeOH at high speed for 3 min, filter, homogenize solid with 30 mL MeOH, and filter. Combine the filtrates and add them to 60 mL toluene and 300 mL 10% NaCl in water, shake well for 3 min, let layers separate. Dry the organic layer by passing it through 20 g anhydrous sodium sulfate in a 20 mm diameter column, concentrate to about 5 mL under reduced pressure at 80°, add to the cleanup column, elute with 40 mL toluene:MeCN 99:1. Evaporate the eluate just to dryness under reduced pressure at 80°, reconstitute with 1 mL MeOH, inject an aliquot. (Reflux activated charcoal (20–40 mesh) with 1 M HCl for 4 h, wash with water until the washings are neutral, dry at 95–100° (*J.Assoc.Off.Anal.Chem.* **1983**, *66*, 1013). Heat 60–100 mesh Florisil at 200° for 24 h, cool, add 4% water, mix thoroughly, store in a sealed jar (*J.Assoc.Off.Anal.Chem.* **1983**, *66*, 1003).)

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Column temperature: 50
Mobile phase: Gradient. MeCN:water from 62:38 to 78:22 over 32 min (Waters curve 6).
Flow rate: 1.5
Detector: UV 206

CHROMATOGRAM

Retention time: 11.55
Limit of detection: 50 ng/g

OTHER SUBSTANCES

Simultaneous: cypermethrin (21.05–22.08), permethrin (24.60, 27.01), tetramethrin (13.08)

KEY WORDS

apple, cabbage, cucumber; peach, pear, tomato, SPE

REFERENCE

Pang, G.-F.; Chao, Y.-Z.; Liu, X.-S.; Fan, C.-L. Multiresidue liquid chromatographic method for simultaneous determination of pyrethroid insecticides in fruits and vegetables, *J.AOAC Int.*, **1995**, *78*, 1474–1480.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: Two 250 × 4 Phase 3019 columns in series (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH 500:30:0.15

Flow rate: 0.8
Detector: UV 230

CHROMATOGRAM
Retention time: 34, 36, 37, 39, 40, 42, 44, 46 (isomers)

REFERENCE
Phenomenex Catalogue, Phenomenex: Torrance CA, **1994**, p. 1035.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 50 × 4 40 μm pellicular material
Column: 250 × 4.6 5 μm Ultrasphere octadecylsilica
Mobile phase: MeOH:water 80:20
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k′ 4.68 (cis), k′ 5.32 (trans)

OTHER SUBSTANCES
Also analyzed: cyfluthrin (baythroid) (k′ 7.41 (cis, S), k′ 7.77 (trans, R), k′ 8.01 (cis, S), k′ 8.73 (trans, R)), permethrin (k′ 14.9 (trans), k′ 19.5 (cis)), resmethrin (k′ 13.5 (cis), k′ 15.0 (trans)), tetramethrin (k′ 4.05 (cis), k′ 4.68 (trans))

REFERENCE
Abidi, S.L. Column selectivity in high-performance liquid chromatography of substituted *gem*-dimethylcyclopropanes, *J.Chromatogr.*, **1986**, *368*, 59–76.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Cyclobond I cyclodextrin-modified silica (Astec)
Mobile phase: MeCN:water 22:78
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 7 (cis isomers), 9.5 (1R,trans, αS), 10.5 (1S,trans, αR), 13 (1R,trans, αR), 15 (1S,trans, αS)

KEY WORDS
comparison with GC

REFERENCE
Kutter, J.P.; Class, T.J. Diastereoselective and enantioselective chromatography of the pyrethroid insecticides allethrin and cypermethrin, *Chromatographia*, **1992**, *33*, 103–112.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 0.1–1 mg/mL solution in hexane.

HPLC VARIABLES

Guard column: 5 μm Spherisorb NH2
Column: 250 × 4.6 Pirkle ionic type 1-A column (Technicol)
Mobile phase: Hexane:isopropanol 99.85:0.15
Flow rate: 0.8
Detector: UV 230

OTHER SUBSTANCES

Also analyzed: cypermethrin, fenpropathrin, fenvalerate, tetramethrin

KEY WORDS

chiral

REFERENCE

Lisseter, S.G.; Hambling, S.G. Chiral high-performance liquid chromatography of synthetic pyrethroid insecticides, *J.Chromatogr.*, **1991**, *539*, 207–210.

SAMPLE

Matrix: urine
Sample preparation: Add 4 g solid NaCl, 3.5 mL MeCN, and 5 mL saturated NaCl solution to 5 mL MeCN, shake for 1 min. Remove the MeCN layer and extract the aqueous layer with 1 mL MeCN. Combine the MeCN layers and adjust to a known volume (0.5–1 mL), mix, filter (0.45 μm), inject a 40 μL aliquot.

HPLC VARIABLES

Column: 150 × 3 3 μm Luna C18(2) (Phenomenex)
Column temperature: 30
Mobile phase: Gradient. MeCN:water 10:90 for 1 min, to 90:10 over 30 min, maintain at 90:10 for 4 min, to 100:0 over 1 min, maintain at 100:0 for 10 min, return to initial conditions over 1 min.
Flow rate: 0.5
Injection volume: 40
Detector: UV 235

CHROMATOGRAM

Retention time: 31.8
Limit of detection: 5 ng/mL

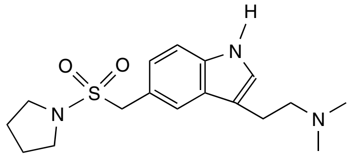
OTHER SUBSTANCES

Extracted: bifenthrin (37, LOD 5 ng/mL), cyfluthrin (34.3, LOD 5 ng/mL), fenvalerate (35.3, LOD 2 ng/mL), *cis*-permethrin (35.7, LOD 5 ng/mL), *trans*-permethrin (36.3, LOD 5 ng/mL), phenothrin (36.4, LOD 5 ng/mL), *m*-phenoxybenzyl alcohol (21, LOD 5 ng/mL), pyrethrin I (29.6, LOD 4 ng/mL), pyrethrin II (33.7, LOD 40 ng/mL), resmethrin (35.2, LOD 5 ng/mL), tetramethrin (31.4, LOD 5 ng/mL)

REFERENCE

Loper, B.L.; Anderson, K.A. Determination of pyrethrin and pyrethroid pesticides in urine and water matrices by liquid chromatography with diode array detection, *J.AOAC Int.*, **2003**, *86*, 1236–1240.

Almotriptan



Molecular formula: $C_{17}H_{25}N_3O_2S$
Molecular weight: 335.47
CAS Registry No: 154323-57-6
Merck Index: 13, 301

SAMPLE

Matrix: blood, urine
Sample preparation: Condition a C2 SPE cartridge (Baker) with 2 mL MeCN and 2 mL water. Dilute 500 μL plasma or 100 μL urine with 1 mL water containing IS, mix, add to the SPE cartridge, wash with 750 μL MeCN:water 30:70, wash with 250 μL water, elute with mobile phase over 1 min (straight onto column (?)).

HPLC VARIABLES

Guard column: Guardpak μBondapak CN
Column: 150 × 4 5 μm Spherisorb ODS-2
Mobile phase: MeCN:50 mM pH 4.0 sodium phosphate buffer:triethylamine 20:80:0.2
Flow rate: 1
Detector: UV 227

CHROMATOGRAM

Retention time: 6.5
Internal standard: 4-[3-(2-aminoethyl)-1H-indol-5-ylmethylsulfonyl]piperazine-1-carboxylic acid ethyl ester (10)
Limit of quantitation: 1 ng/mL (plasma), 50 ng/mL urine

KEY WORDS

plasma, SPE

REFERENCE

Jansat, J.M.; Costa, J.; Salvà, P.; Fernandez, F.J.; Martinez-Tobed, A. Absolute bioavailability, pharmacokinetics, and urinary excretion of the novel antimigraine agent almotriptan in healthy male volunteers, *J.Clin.Pharmacol.*, **2002**, *42*, 1303–1310.

SAMPLE

Matrix: microsomal incubations
Sample preparation: Mix 500 μL microsomal incubation with 1 mL 200 mM pH 4 sodium acetate buffer, centrifuge, inject an aliquot.

HPLC VARIABLES

Guard column: GuardPak μBondapak CN
Column: 300 × 3.9 10 μm μBondapak
Mobile phase: Gradient. A:B from 80:20 to 40:60 over 30 min. A was buffer. B was MeCN:buffer 80:20. Buffer was 10 mM orthophosphoric acid containing 0.1% triethylamine, adjusted to pH 6.5 with NaOH.
Flow rate: 1
Detector: UV 227

CHROMATOGRAM

Retention time: 25

OTHER SUBSTANCES

Extracted: metabolites

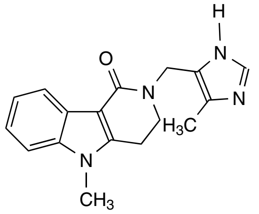
KEY WORDS

human, liver

REFERENCE

Salva, M.; Jansat, J.M.; Martinez-Tobed, A.; Palacios, J.M. Identification of the human liver enzymes involved in the metabolism of the antimigraine agent almotriptan, *Drug Metab.Dispos.*, **2003**, *31*, 404–411.

Alosetron



Molecular formula: $C_{17}H_{18}N_4O$

Molecular weight: 294.35

CAS Registry No: 122852-42-0, 122852-69-1 (HCl)

Merck Index: 13, 305

SAMPLE

Matrix: blood

Sample preparation: Condition a 100 mg LRC Bond Elut ethyl (C2) SPE cartridge with 1 mL isopropanol and 1 mL buffer. Mix 1.1 mL plasma or serum with 1 mL buffer containing 10 ng/mL IS, vortex, add 2 mL to the SPE cartridge, wash with 2 mL buffer, dry with nitrogen for 30 s, wash with 2 mL MeCN, elute with two 2 mL aliquots of MeCN:buffer 90:10. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue with 300 μL mobile phase, vortex, inject a 200 μL aliquot. (The buffer was 10 mM ammonium acetate adjusted to pH 4.0 with glacial acetic acid.)

HPLC VARIABLES

Guard column: 15 × 4.6 7 μm Spherisorb cyanopropyl
Column: 100 × 4.6 5 μm Spheri cyanopropyl (Brownlee)
Column temperature: 45
Mobile phase: MeOH:THF:10 mM pH 4.0 ammonium acetate buffer 24:6:70
Flow rate: 0.5
Injection volume: 200
Detector: F ex 295 em 370

CHROMATOGRAM

Retention time: 10.1
Internal standard: GR87442, 6-fluoroalosetron (Glaxo) (13.7)
Limit of quantitation: 0.1 ng/mL

OTHER SUBSTANCES

Noninterfering: amitriptyline, carbamazepine, carmustine, chlorpromazine, cimetidine, cisplatin, cyclophosphamide, dexamethasone, diazepam, digoxin, etoposide, furosemide, haloperidol, ibuprofen, imipramine, indomethacin, methotrexate, phenobarbital, phenytoin, propranolol, ranitidine, theophylline, triazolam, warfarin

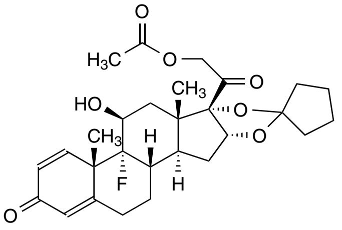
KEY WORDS

plasma; serum; SPE

REFERENCE

Lloyd, T.L.; Gupta, S.K.; Gooding, A.E.; Alianti, J.R. Determination of alosetron in human plasma or serum by high-performance liquid chromatography with robotic sample preparation, *J.Chromatogr.B*, **1996**, *678*, 261–267.

Amcinonide



Molecular formula: $C_{28}H_{35}FO_7$
Molecular weight: 502.57
CAS Registry No: 51022-69-6
Merck Index: 13, 387

SAMPLE

Matrix: formulations

Sample preparation: Condition a 3 mL 500 mg Megabond MF C18 SPE cartridge (Varian) with 3 mL MeOH and 3 mL water. Sonicate 1 g cosmetic with 10 mL MeOH or MeOH:dichloromethane 10:90 (depending on what appears visually to give the best solubility) at 40° for 10 min, centrifuge, collect the clear supernatant. Add 5 mL of the supernatant to the SPE cartridge, wash with 4 mL acetone:water 20:80, wash with 1 mL *n*-hexane, elute with 4 mL diethyl ether. Evaporate the eluate to dryness under reduced pressure, reconstitute the residue with 5 mL (or more) MeOH, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm endcapped Purospher RP-18
Column temperature: 25
Mobile phase: Isocratic.MeCN:water 60:40. Gradient. MeCN:water from 25:75 to 90:10 over 30 min, maintain at 90:10 for 10 min.
Flow rate: 1
Injection volume: 10
Detector: UV 239

CHROMATOGRAM

Retention time: k′ 3.18 (isocratic); 22.6 min (gradient)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Simultaneous: alclometasone dipropionate (isocratic k′ 2.55; gradient retention time (min) 21.0; LOD 0.3 μg/mL), betamethasone (isocratic k′ 0.18; gradient retention time (min) 11.8; LOD 0.1 μg/mL), betamethasone-17-acetate (isocratic k′ 0.73; gradient retention time (min) 15.4; LOD 0.3 μg/mL), betamethasone-17-benzoate (isocratic k′ 2.04; gradient retention time (min) 20.6; LOD 0.3 μg/mL), betamethasone-17-propionate-21-stearate (isocratic k′ >13; gradient retention time (min) >35; LOD 0.5 μg/mL), betamethasone-17-propionate-21-butyrate (isocratic k′ 5.91; gradient retention time (min) 26.1; LOD 0.4 μg/mL), betamethasone-17-valerate-21-acetate (isocratic k′ 4.41; gradient retention time (min) 23.1; LOD 0.4 μg/mL), betamethasone-17-valerate (isocratic k′ 2.32; gradient retention time (min) 21.4; LOD 0.3 μg/mL), betamethasone-17,21-dipropionate (isocratic k′ 4.00; gradient retention time (min) 24.2; LOD 0.4 μg/mL), betamethasone-17,21-diacetate (isocratic k′ 1.81; gradient retention time (min) 20.5; LOD 0.3 μg/mL), betamethasone-17,21-divalerate (isocratic k′ 10.82; gradient retention time (min) 28.0; LOD 0.4 μg/mL), betamethasone-21-acetate (isocratic k′ 0.77; gradient retention time (min) 15.6; LOD 0.3 μg/mL), betamethasone propionate (isocratic k′ 0.82; gradient retention time (min) 17.1; LOD 0.3 μg/mL), clobetasol propionate (isocratic k′ 3.41; gradient retention time (min) 23.4; LOD 0.1 μg/mL), clobethasone butyrate (isocratic k′ 5.45; gradient retention time (min) 26.3; LOD 0.1 μg/mL), cortisone (isocratic k′ 0.18; gradient retention time (min) 11.1; LOD 0.6 μg/mL), cortisone acetate (isocratic k′ 0.73; gradient retention time (min) 15.2; LOD 0.6 μg/mL), dehydrocorticosterone (isocratic k′ 4.27; gradient retention time (min) 22.3; LOD 0.5 μg/mL), deoxymethasone (isocratic k′ 0.64; gradient retention time (min) 14.2; LOD 0.2 μg/mL), dexamethasone (isocratic k′ 0.27; gradient retention time (min) 11.9; LOD 0.1 μg/mL), dexamethasone-21-acetate (isocratic k′ 0.91; gradient retention time (min) 16.1; LOD 0.2 μg/mL), dexamethasone isonicotinate (isocratic k′ 1.05; gradient retention time (min) 17.7; LOD 0.4 μg/mL), dexamethasone pivalate (isocratic k′ 3.45; gradient retention time (min) 24.1; LOD 0.3 μg/mL), dexamethasone valerate (isocratic k′ 3.00; gradient retention time (min) 21.6; LOD 0.3 μg/mL), diflucortolone valerate (isocratic k′ 4.73; gradient retention time (min) 23.3; LOD 0.3 μg/mL), fludrocortisone acetate (isocratic k′ 0.59; gradient retention time (min) 14.1; LOD 0.3 μg/mL), flumethasone pivalate (isocratic k′ 2.68; gradient retention time (min) 21.2; LOD 0.3 μg/mL), fluocinolone acetonide (isocratic k′ 0.91; gradient retention time (min) 13.4; LOD 0.3 μg/mL), fluocinonide (isocratic k′ 1.45; gradient retention time (min) 20.5; LOD 0.1 μg/mL), fluocortin butyl ester (isocratic k′ 5.59; gradient retention time (min) 24.6; LOD 0.3 μg/mL), fluocortolone caproate (isocratic k′ 6.59; gradient retention time (min) 25.1; LOD 0.3 μg/mL), fluocortolone pivalate (isocratic k′ 4.50; gradient retention time (min) 23.6; LOD 0.3 μg/mL), fluorometholone (isocratic k′ 0.59; gradient retention time (min) 14.4; LOD 0.1 μg/mL), 9-α-fluoroprednisolone (isocratic k′ 0.18; gradient retention time (min) 10.0; LOD 0.1 μg/mL), 9-α-fluoroprednisolone acetate (isocratic k′ 0.50; gradient retention time (min) 13.9; LOD 0.2 μg/mL), flurandrenolide (isocratic k′ 0.50; gradient retention time (min) 13.5; LOD 0.1 μg/mL), halcinonide (isocratic k′ 1.64; gradient retention time (min) 20.6; LOD 0.1 μg/mL), hydrocortisone (isocratic k′ 0.18; gradient retention time (min) 10.0; LOD 0.4 μg/mL), hydrocortisone-17-butyrate (isocratic k′ 1.09; gradient retention time (min) 17.7; LOD 0.6 μg/mL), hydrocortisone-21-acetate (isocratic k′ 0.77; gradient retention time (min) 15.3; LOD 0.6 μg/mL), hydrocortisone pivalate (isocratic k′ 2.27; gradient retention time (min) 20.4; LOD 0.8 μg/mL), methylprednisolone (isocratic k′ 0.55; gradient retention time (min) 13.5; LOD 0.1 μg/mL), mometasone furoate (isocratic k′ 3.05; gradient retention time (min) 22.0; LOD 0.2 μg/mL), prednisolone-21-acetate (isocratic k′ 0.60; gradient retention time (min) 13.6; LOD 0.2 μg/mL), prednisolone acetonide (isocratic k′ 0.50; gradient retention time (min) 13.0; LOD 0.3 μg/mL), prednisolone pivalate (isocratic k′ 2.05; gradient retention time (min) 19.7; LOD 0.3 μg/mL), triamcinolone (isocratic k′ 0.14; gradient retention time (min) 7.2; LOD 0.1 μg/mL), triamcinolone acetonide (isocratic k′ 0.50; gradient retention time (min) 13.9; LOD 0.2 μg/mL), triamcinolone diacetate (isocratic k′ 0.45; gradient retention time (min) 13.9; LOD 0.3 μg/mL).

KEY WORDS

cosmetics; SPE

REFERENCE

Gagliardi, L.; De Orsi, D.; Del Giudice, M.R.; Gatta, F.; Porrà, R.; Chimenti, P.; Tonelli, D. Development of a tandem thin-layer chromatography-high-performance liquid chromatography method for the identification and determination of corticosteroids in cosmetic products, *Anal.Chim.Acta*, **2002**, *457*, 187–198.

SAMPLE

Matrix: formulations

Sample preparation: Mix 1 g of a topical product, 5 mL 100 mM pH 4 citrate buffer saturated with NaCl, and 10 mL ethyl acetate, shake vigorously by hand to ensure that no large clumps stick to the tube, mix with the tube on its side on an oscillating shaker for 15 min. Remove the upper ethyl acetate layer and wash with citrate buffer as before. Dry the ethyl acetate over anhydrous sodium sulfate and evaporate to dryness. Dissolve the residue in a mixture of 10 mL heptane and 5 mL MeCN:water 90:10. Remove the upper heptane layer and extract it with 2 mL MeCN:water 90:10. Combine the MeCN/water layers and evaporate them to dryness, dissolve the residue in 300 μL MeOH, filter (0.45 μm nylon), inject a 5 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm Brownlee NewGuard C18

Column: 75 × 4.6 3.5 μm Symmetry C18 (Waters)

Mobile phase: Gradient. MeCN:water 18:82 for 2 min, to 82:18 over 12 min, maintain at 82:18 for 3 min, re-equilibrate at initial conditions for 12 min.
Flow rate: 1
Injection volume: 5
Detector: UV 240

CHROMATOGRAM

Retention time: 10.90
Limit of detection: 0.001%

OTHER SUBSTANCES

Extracted:
Simultaneous: alclometasone 17,21-dipropionate (10.93), beclomethasone 17,21-dipropionate (11.90), betamethasone (6.52), betamethasone 21-acetate (8.47), betamethasone 17-benzoate (10.28), betamethasone 17,21-dipropionate (11.42), betamethasone 17-valerate (10.25), budesonide (8.55, 8.69 (epimers)), clobetasol 17-propionate (11.06), cortisone (5.62), cortisone 21-acetate (8.07), dexamethasone (6.57), dexamethasone 21-acetate (8.68), desonide (6.99), desoximetasone (7.60), desoxycorticosterone acetate (10.90), desoxycorticosterone pivalate (14.45), diflorasone 17,21-diacetate (9.81), fluocinolone acetonide (7.38), fluocinonide (9.79), flurandrenolide (7.36), fludrocortisone 21-acetate (7.77), fluorometholone (7.67), flumethasone 21-pivalate (11.20), flunisolide (7.14), fluprednisolone (5.46), fluticasone 17-propionate (11.19), halcinonide (10.72), halobetasol propionate (10.98), hydrocortisone (5.50), hydrocortisone 21-acetate (7.65), hydrocortisone 17-butyrate (8.66), hydrocortisone 21-cypionate (12.54), hydrocortisone 17-valerate (9.53), mometasone 17-furoate (11.24), methylprednisolone (6.31), methylprednisolone 21-acetate (8.34), meprednisone (6.55), prednisolone (5.37), prednisolone 21-acetate (7.47), prednisolone 21-tebuate (11.01), paramethasone 21-acetate (8.64), prednicarbate (10.72), prednisone (5.46), triamcinolone (4.15), triamcinolone acetonide (7.04), triamcinolone 16,21-diacetate (7.49), triamcinolone hexacetonide (13.12)

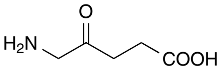
KEY WORDS

body wash, cream, gel, lotion, shampoo, spray

REFERENCE

Reepmeyer, J.C. Screening for corticosteroids in topical pharmaceuticals by HPLC with a scanning ultraviolet detector, *J.Liq.Chromatogr.Rel.Technol.*, **2001**, *24*, 693–709.

Aminolevulinic acid



Molecular formula: $C_5H_9NO_3$

Molecular weight: 131.13

CAS Registry No: 106-60-5

Merck Index: 13, 445

SAMPLE

Matrix: blood, tissue

Sample preparation: Deproteinize plasma by adding perchloric acid to a final concentration of 800 mM. Neutralize the supernatant by adding solid sodium bicarbonate until a pH of ca. 7.6 is reached. Homogenize tissue with 3 volumes of 10 mM pH 7.2 HEPES buffer containing 250 mM sucrose and 500 mM EDTA, centrifuge at 800 g for 5 min. Mix 10 μL sample with 5 μL reagent and 35 μL water, let stand at room temperature for 1 min, inject a 20 μL aliquot. (Prepare the reagent by dissolving 27 mg *o*-phthalaldehyde in 500 μL MeOH, add 5 mL 100 mM sodium tetraborate, add 20 μL mercaptoethanol, mix.)

HPLC VARIABLES

Column: 150 × 3.9 4 μm C18 (Waters)

Mobile phase: MeCN:50 mM pH 7.0 phosphate buffer 10:90 containing 2.4 mM EDTA

Flow rate: 1

Injection volume: 20

Detector: E, Shimadzu LECD 6A, glassy carbon working electrode at +0.45 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 44.6

Limit of detection: 50 nM

Limit of quantitation: 100 nM

KEY WORDS

brain; derivatization; human; liver; plasma; rat

REFERENCE

Costa, C.A.; Trivelato, G.C.; Demasi, M.; Bechara, E.J.H. Determination of 5-aminolevulinic acid in blood plasma, tissues and cell cultures by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.B*, **1997**, *695*, 245–250.

SAMPLE

Matrix: blood, urine

Sample preparation: 50 μL Plasma or urine + 3.5 mL reagent + 450 μL 10% formaldehyde, vortex for 3 s, heat at 100° for 10 min, cool in an ice bath, filter (0.8 μm, plasma samples only), inject a 10 (urine) or 20 (plasma) μL aliquot. (Prepare the reagent by mixing 15 mL acetylacetone, 10 mL EtOH, and 75 mL water.)

HPLC VARIABLES

Column: 150 × 4.6 Shim-pack CLC-ODS (Shimadzu)

Column temperature: 40

Mobile phase: MeOH:water:acetic acid 50:50:1

Flow rate: 0.7

Injection volume: 10–20

Detector: F ex 370 em 460

CHROMATOGRAM
Retention time: 6.1
Limit of detection: 3 ng/mL

KEY WORDS
derivatization; plasma; protect from light

REFERENCE
Oishi, H.; Nomiyama, H.; Nomiyama, K.; Tomokuni, K. Fluorometric HPLC determination of delta-aminolevulinic acid (ALA) in the plasma and urine of lead workers: biological indicators of lead exposure, *J.Anal.Toxicol.*, **1996**, *20*, 106–110.

SAMPLE
Matrix: urine
Sample preparation: Centrifuge urine at 1000 g and store at −20°. 20 µL Urine + 5 mL acetylacetone:EtOH:4 g/L NaCl in water 15:10:75 + 450 µL 9.3% formaldehyde solution, mix, boil for 15 min, cool with water, store sample in the dark at 15° until injection, inject a 50 µL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 µm TSK-80 TM (Tosoh)
Column temperature: 40
Mobile phase: Gradient. A was MeCN:MeOH:water:acetic acid 10:35:54:1. B was MeCN. A:B 100:0 for 7.5 min, to 50:50 over 1.5 min, return to initial conditions over 2 min, re-equilibrate for 2 min.
Flow rate: 0.8
Injection volume: 50
Detector: F ex 246 em 458

CHROMATOGRAM
Retention time: 7.3
Limit of detection: 10 ng/mL

KEY WORDS
derivatization; protect from light; improved version of A. Okayama et al. *Clin.Chem.* **1990**, *36*, 1494.

REFERENCE
Endo, Y.; Okayama, A.; Endo, G.; Ueda, T.; Nakazono, N.; Horiguchi, S. Improvement of urinary δ-aminolevulinic acid determination by HPLC and fluorescence detection using condensing reaction with acetylacetone and formaldehyde, *Jap.J.Ind.Health*, **1994**, *36*, 49–56.

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Dalton, J.T.; Meyer, M.C.; Golub, A.L. Pharmacokinetics of aminolevulinic acid after oral and intravenous administration to dogs, *Drug Metab.Dispos.*, **1999**, *27*, 432–435. [derivatization]

Ho, J.; Guthrie, R.; Tieckelmann, H. Detection of δ-aminolevulinic acid, porphobilinogen and porphyrins related to heme biosynthesis by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *375*, 57–63. [derivatization]

Ho, J.W. Micro assay for urinary δ-aminolevulinic acid and porphobilinogen by high-performance liquid chromatography with pre-column derivatization, *J.Chromatogr.*, **1990**, *527*, 134–139.

Kondo, M.; Kimura, H.; Maekubo, T.; Tomita, T.; Senda, M.; Urata, G.; Kajiwara, M. Direct injection method for quantitation of δ-aminolevulinic acid in urine by high-performance liquid chromatography, *Chem.Pharm.Bull.*, **1992**, *40*, 1948–1950. [derivatization]

Lim, C.K.; Rideout, J.M.; Samson, D.M. Determination of 5-aminolaevulinic acid and porphobilinogen by high-performance liquid chromatography, *J.Chromatogr.*, **1979**, *185*, 605–611.

Meisch, H.U.; Reinle, W.; Wolf, U. Determination of 5-aminolevulinic acid in biological samples by high-performance liquid chromatography, *Anal.Biochem.*, **1985**, *149*, 29–34.

Minder, E.I. Measurement of 5-aminolevulinic acid by reversed phase HPLC and fluorescence detection, *Clin.Chim.Acta*, **1986**, *161*, 11–18. [derivatization]

Miyajima, K.; Hirata, M.; Yoshida, T.; Kosaka, H.; Okayama, A. Study on measurement of delta-aminolevulinic acid in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *654*, 165–169.

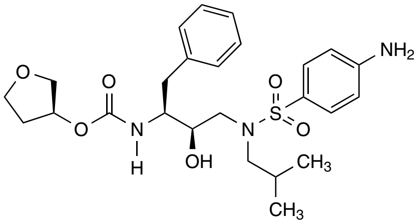
Okayama, A. Fluorimetric determination of urinary δ-aminolevulinic acid by high-performance liquid chromatography and post-column derivatization, *J.Chromatogr.*, **1988**, *426*, 365–369.

Okayama, A.; Fujii, S.; Miura, R. Optimized fluorometric determination of urinary delta-aminolevulinic acid by using pre-column derivatization, and identification of the derivative, *Clin.Chem.*, **1990**, *36*, 1494–1497.

Tomokuni, K.; Ichiba, M.; Hirai, Y.; Hasegawa, T. Optimized liquid-chromatographic method for fluorometric determination of urinary delta-aminolevulinic acid in workers exposed to lead, *Clin.Chem.*, **1987**, *33*, 1665–1667.

Amprenavir

Molecular formula: $C_{25}H_{35}N_3O_6S$
Molecular weight: 505.64
CAS Registry No: 161814-49-9
Merck Index: 13, 594



SAMPLE

Matrix: blood

Sample preparation: Condition a C18 SPE cartridge (Baker) with 3 mL MeOH and 3 mL water. Do not allow to run dry. Add 1 mL plasma to the SPE cartridge, wash with 2 mL water, suck dry for 1 min, elute with 2.6 mL MeOH. Evaporate a 1 mL aliquot of the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue with 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: GuardPak μBondapak C18

Column: 250 × 4.6 5 μm Symmetry C18

Column temperature: 37

Mobile phase: MeCN:40 mM disodium hydrogen phosphate containing 4% octanesulfonic acid 50:50. (At the end of each session, wash column with MeOH:water 50:50 and MeCN:water 80:20.)

Flow rate: 1.3

Injection volume: 100

Detector: UV 261 for 9 min, UV 241 for 11 min, UV 254 for 12 min

CHROMATOGRAM

Retention time: 5.6

Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES

Extracted: efavirenz (15.2, LOQ 50 ng/mL), indinavir (4.8, LOQ 50 ng/mL), nelfinavir (19.2, LOQ 50 ng/mL), ritonavir (12.8, LOQ 50 ng/mL), saquinavir (16.8, LOQ 5 ng/mL)

Noninterfering: abacavir, acebutolol, acetaminophen, acetylcysteine, acyclovir, albendazole, alimemazine, alizapride, amikacin, amiodarone, amphotericin B, ampicillin, aspirin, bepridil, buprenorphine, butobarbital, caffeine, calcium folinate, captopril, carbamazepine, carbutamide, chloroquine, ciprofloxacin, clindamycin, clofazimine, clofibrate, clonazepam, clonidine, cloxacillin, clozapine, cocaine, codeine, cyamemazine, dantrolene, dexamethasone, dextropropoxyphene, diazepam, diclofenac, didanosine, digoxin, dihydroergotamine, diltiazem, doxycycline, ethambutol, flecainide, fluconazole, fluoxetine, fluvoxamine, foscarnet, furosemide, ganciclovir, gentamicin, glibenclamide, granisetron, halofantrine, haloperidol, hydrocortisone, imipramine, indomethacin, interferon alfa, isoniazid, itraconazole, josamycin, ketoconazole, lamivudine, levomepromazine, lidocaine, loperamide, loratadine, losartan, mefloquine, meprobamate, methadone, methylprednisolone, metoclopramide, metronidazole, mianserin, moclobemide, morphine, nevirapine, nifedipine, niflumic acid, nitrofurantoin, omeprazole, paroxetine, pentamidine, phenobarbital, phenytoin, piracetam, prazosin, prednisolone, prednisone, primidone, propranolol, quinidine, quinine, ranitidine, ribavirin, rifabutin, rifampin, roxithromycin, salicylic acid, simvastatin, stavudine, sulfadiazine, sulfamethoxazole, sulpiride, thalidomide, theophylline, trimethoprim, valproic acid, venlafaxine, vigabatrin, viloxazine, zidovudine, zolpidem, zopiclone

Interfering: delavirdine, flunitrazepam

KEY WORDS

plasma; SPE

REFERENCE

Aymard, G.; Legrand, M.; Trichereau, N.; Diquet, B. Determination of twelve antiretroviral agents in human plasma sample using reversed-phase high-performance liquid chromatography, *J.Chromatogr. B*, **2000**, *744*, 227–240.

SAMPLE

Matrix: blood

Sample preparation: Mix 250 µL plasma with 50 µL MeOH, add 100 µL 2 µg/mL IS in MeOH, add 250 µL 1 M NaOH, add 3 mL hexane:ethyl acetate 50:50, shake at high speed for 25 min, centrifuge at 3000 g for 15 min. Evaporate the organic layer to dryness under a stream of air, reconstitute the residue with 1 mL initial mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES

Guard column: 10 × 2.1 Symmetry Shield

Column: 30 × 2.1 3.5 µm Symmetry C18

Mobile phase: Gradient. MeCN:5 mM pH 3.25 acetate buffer from 25:75 to 80:20 over 4 min using a nonlinear gradient (not specified).

Flow rate: 0.35

Injection volume: 20

Detector: MS, PE Sciex API 3000, turbo ionspray source, column effluent split 1:1 before entering source

CHROMATOGRAM

Retention time: 2.7

Internal standard: Abbott A-86093 (3.2)

Limit of detection: 380 pg/mL

Limit of quantitation: 16.3 ng/mL

OTHER SUBSTANCES

Extracted: indinavir (2.0, LOQ 16.3 ng/mL, LOD 1.5 ng/mL), lopinavir (3.1, LOQ 16.3 ng/mL, LOD 750 pg/mL), nelfinavir (2.5, LOQ 16.3 ng/mL, LOD 330 pg/mL), ritonavir (2.9, LOQ 51.2 ng/mL, LOD 650 pg/mL), saquinavir (2.4, LOQ 16.3 ng/mL, LOD 780 pg/mL)

KEY WORDS

plasma

REFERENCE

Frerichs, V.A.; DiFrancesco, R.; Morse, G.D. Determination of protease inhibitors using liquid chromatography-tandem mass spectrometry, *J.Chromatogr.B*, **2003**, *787*, 393–403.

SAMPLE

Matrix: blood

Sample preparation: Mix 1 mL plasma with 200 µL 10 µg/mL IS in water, add 200 µL 100 mM NaOH, mix, add 4 mL diethyl ether, shake for 5 min, centrifuge at 2500 rpm for 5 min. Evaporate the organic layer to dryness under a stream of nitrogen, reconstitute the residue with 200 µL initial mobile phase, inject a 100 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Stability RP18 (CIL, France)

Mobile phase: Gradient. MeCN:50 mM pH 5.65 phosphate buffer from 36:64 to 64:36 over 25 min, to 80:20 (step gradient), maintain at 80:20 for 10 min, re-equilibrate at initial conditions for 5 min.
Flow rate: 1.5
Injection volume: 100
Detector: UV 240 for 5 min, UV 215 for 22 min, UV 260 for rest of the run

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CHROMATOGRAM

Retention time: 11.2
Internal standard: JR051012 (Janssen Cilag) (28.2)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES

Extracted: efavirenz (19.9), indinavir (8.5), lopinavir (18.9), nelfinavir (24.1), nevirapine (3.3), ritonavir (17.6), saquinavir (16.7)
Noninterfering: acetaminophen, amineptine, amphotericin B, aspirin, bromazepam, buspirone, citalopram, clobazam, diazepam, didanosine, fluconazole, flunitrazepam, fluvoxamine, hydroxyitraconazole, isoniazid, itraconazole, lamivudine, loprazolam, lorazepam, metronidazole, minalcipram, nordiazepam, omeprazole, paroxetine, pyrimethamine, rifampin, sertraline, stavudine, sulfadiazine, trimethoprim, venlafaxine, zalcitabine, zidovudine, zolpidem, zopiclone

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KEY WORDS

plasma

REFERENCE

Titier, K.; Lagrange, F.; Péhourcq, F.; Edno-Mcheik, L.; Moore, N.; Molimard, M. High-performance liquid chromatographic method for the simultaneous determination of the six HIV-protease inhibitors and two non-nucleoside reverse transcriptase inhibitors in human plasma, *Ther.Drug Monit.*, **2002**, *24*, 417–424.

SAMPLE

Matrix: microsomal incubations
Sample preparation: Extract 100 μL incubation mixture twice with 5 mL MTBE. Evaporate the organic layer to dryness, reconstitute the residue with 100 μL MeCN, inject a 30 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 Beckman ODS Ultrasphere
Column temperature: 45
Mobile phase: Gradient. A was 0.1% formic acid in water. B was 0.1% formic acid in MeCN. A:B 100:0 for 1 min, to 30:70 over 3 min, to 5:95 over 3 min, maintain at 5:95 for 3 min, to 100:0 over 1 min.
Flow rate: 0.35
Injection volume: 30
Detector: MS, Hewlett-Packard 5989B, electrospray ionization, selected ion monitoring, m/z 506.6

CHROMATOGRAM

Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES

Also analyzed: astemizole, indinavir, ketoconazole, methadone, nelfinavir, rifabutin, rifampin, ritonavir, saquinavir, terfenadine, trimethoprim

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KEY WORDS

human; liver; rat

REFERENCE

Decker, C.J.; Laitinen, L.M.; Bridson, G.W.; Raybuck, S.A.; Tung, R.D.; Chaturvedi, P.R. Metabolism of amprenavir in liver microsomes: role of CYP3A4 inhibition for drug interactions, *J.Pharm.Sci.*, **1998**, *87*, 803–807.

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Cociglio, M.; Hillaire-Buys, D.; Peyrière, H.; Alric, R. Performance analysis of a rapid HPLC determination with the solvent demixing extraction of HIV antiproteases and efavirenz in plasma, *J.Chromatogr.Sci.*, **2003**, *41*, 80–86. [efavirenz; indinavir; amprenavir; ritonavir; saquinavir; nelfinavir]

Dailly, E.; Thomas, L.; Kergueris, M.F.; Jolliet, P.; Bourin, M. High-performance liquid chromatographic assay to determine the plasma levels of HIV-protease inhibitors (amprenavir, indinavir, nelfinavir, ritonavir and saquinavir) and the non-nucleoside reverse transcriptase inhibitor (nevirapine) after liquid-liquid extraction, *J.Chromatogr.B*, **2001**, *758*, 129–135.

Droste, J.A.H.; Verweij-van Wissen, C.P.W.G.M.; Burger, D.M. Simultaneous determination of the HIV drugs indinavir, amprenavir, saquinavir, ritonavir, lopinavir, nelfinavir, the nelfinavir hydroxymetabolite M8, and nevirapine in human plasma by reversed-phase high-performance liquid chromatography, *Ther.Drug Monit.*, **2003**, *25*, 393–399.

Edwards, J.E.; Brouwer, K.R.; McNamara, P.J. GF120918, a P-glycoprotein modulator, increases the concentration of unbound amprenavir in the central nervous system in rats, *Antimicrob.Agents Chemother.*, **2002**, *46*, 2284–2286.

Faux, J.; Venisse, N.; Le Moal, G.; Dupuis, A.; Bouquet, S. Simultaneous determination of six HIV protease inhibitors, one metabolite, and two non-nucleoside reverse transcriptase inhibitors in human plasma by isocratic reversed-phase liquid chromatography after solid-phase extraction, *Chromatographia* **2003**, *58*, 421–426. [amprenavir; indinavir; lopinavir; nelfinavir; ritonavir; saquinavir; efavirenz; nevirapine; prazepam]

Gao, W.; Kishida, T.; Kimura, K.; Kageyama, M.; Sumi, M.; Yoshikawa, Y.; Shibata, N.; Takada, K. Sensitive and simultaneous determination of HIV protease inhibitors in rat biological samples by liquid chromatography-mass spectrometry, *Biomed.Chromatogr.*, **2002**, *16*, 267–273. [indinavir; amprenavir; saquinavir; nelfinavir]

Goujard, C.; Vincent, I.; Meynard, J.-L.; Choudet, N.; Bollens, D.; Rousseau, C.; Demarles, D.; Gillotin, C.; Bidault, R.; Taburet, A.-M. Steady-state pharmacokinetics of amprenavir coadministered with ritonavir in human immunodeficiency virus type 1-infected patients, *Antimicrob.Agents Chemother.*, **2003**, *47*, 118–123.

Gunawan, S.; Griswold, M.P.; Kahn, D.G. Liquid chromatographic-tandem mass spectrometric determination of amprenavir (agenerase) in serum/plasma of human immunodeficiency virus type-1 infected patients receiving combination antiretroviral therapy, *J.Chromatogr.A*, **2001**, *914*, 1–4.

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Justesen, U.S.; Pedersen, C.; Klitgaard, N.A. Simultaneous quantitative determination of the HIV protease inhibitors indinavir, amprenavir, ritonavir, lopinavir, saquinavir, nelfinavir and the nelfinavir active metabolite M8 in plasma by liquid chromatography, *J.Chromatogr.B*, **2003**, *783*, 491–500.

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Leibenguth, P.; Le Guellec, C.; Besnier, J.-M.; Bastides, F.; Macé, M.; Gaudet, M.-L.; Autret-Leca, E.; Paintaud, G. Therapeutic drug monitoring of HIV protease inhibitors using high-performance liquid chromatography with ultraviolet or photodiode array detection, *Ther.Drug Monit.*, **2001**, *23*, 679–688. [indinavir; saquinavir; lopinavir; ritonavir; nelfinavir; amprenavir; carbamazepine]

Marzolini, C.; Telenti, A.; Buclin, T.; Biollaz, J.; Decosterd, L.A. Simultaneous determination of the HIV protease inhibitors indinavir, amprenavir, saquinavir, ritonavir, nelfinavir and the non-nucleoside reverse transcriptase inhibitor efavirenz by high-performance liquid chromatography after solid-phase extraction, *J.Chromatogr.B*, **2000**, *740*, 43–58.

Marzolini, C.; Béguin, A.; Telenti, A.; Schreyer, A.; Buclin, T.; Biollaz, J.; Decosterd, L.A. Determination of lopinavir and nevirapine by high-performance liquid chromatography after solid-phase extraction: application for the assessment of their transplacental passage at delivery, *J.Chromatogr.B*, **2002**, *774*, 127–140. [lopinavir; nevirapine; amprenavir; efavirenz; clozapine]

Pereira, A.S.; Kenney, K.B.; Cohen, M.S.; Eron, J.J.; Tidwell, R.R.; Dunn, J.A. Determination of amprenavir, a HIV-1 protease inhibitor, in human seminal plasma using high-performance liquid chromatography-tandem mass spectrometry, *J.Chromatogr.B*, **2002**, *766*, 307–317.

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Poirier, J.M.; Robidou, P.; Jaillon, P. Simultaneous determination of the six HIV protease inhibitors (amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, and saquinavir) plus M8 nelfinavir metabolite and the nonnucleoside reverse transcription inhibitor efavirenz in human plasma by solid-phase extraction and column liquid chromatography, *Ther.Drug Monit.*, **2002**, *24*, 302–309.

Polk, R.E.; Brophy, D.F.; Israel, D.S.; Patron, R.; Sadler, B.M.; Chittick, G.E.; Symonds, W.T.; Lou, Y.; Kristoff, D.; Stein, D.S. Pharmacokinetic interaction between amprenavir and rifabutin or rifampin in healthy males, *Antimicrob.Agents Chemother.*, **2001**, *45*, 502–508.

Proust, V.; Toth, K.; Hulin, A.; Taburet, A.-M.; Gimenez, F.; Singlas, E. Simultaneous high-performance liquid chromatographic determination of the antiretroviral agents amprenavir, nelfinavir, ritonavir, saquinavir, delavirdine and efavirenz in human plasma, *J.Chromatogr.B*, **2000**, *742*, 453–458.

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Sparidans, R.W.; Hoetelmans, R.M.W.; Beijnen, J.H. Sensitive liquid chromatographic assay for amprenavir, a human immunodeficiency virus protease inhibitor, in human plasma, cerebrospinal fluid and semen, *J.Chromatogr.B*, **2000**, *742*, 185–192.

Tran, J.Q.; Petersen, C.; Garrett, M.; Hee, B.; Kerr, B.M. Pharmacokinetic interaction between amprenavir and delavirdine: evidence of induced clearance by amprenavir, *Clin.Pharmacol.Ther.*, **2002**, *72*, 615–626.

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Tribut, O.; Arvieux, C.; Michelet, C.; Chapplain, J.-M.; Allain, H.; Bentué-Ferrer, D. Simultaneous quantitative assay of six HIV protease inhibitors, one metabolite, and two non-nucleoside reverse transcriptase inhibitors in human plasma by isocratic reversed-phase liquid chromatography, *Ther.Drug Monit.*, **2002**, *24*, 554–562. [nevirapine; efavirenz; indinavir; amprenavir; nelfinavir; ritonavir; lopinavir; saquinavir]

Turner, M.L.; Reed-Walker, K.; King, J.R.; Acosta, E.P. Simultaneous determination of nine antiretroviral compounds in human plasma using liquid chromatography, *J.Chromatogr.B*, **2003**, *784*, 331–341. [indinavir; nelfinavir; saquinavir; ritonavir; amprenavir; delavirdine; efavirenz; lopinavir]

van Heeswijk, R.P.G.; Hoetelmans, R.M.W.; Harms, R.; Meenhorst, P.L.; Mulder, J.W.; Lange, J.M.A.; Beijnen, J.H. Simultaneous quantitative determination of the HIV protease inhibitors amprenavir, indinavir, nelfinavir, ritonavir and saquinavir in human plasma by ion-pair high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1998**, *719*, 159–168.

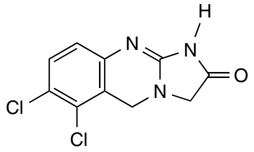
Veronese, L.; Rautaureau, J.; Sadler, B.M.; Gillotin, C.; Petite, J.-P.; Pillegand, B.; Delvaux, M.; Masliah, C.; Fosse, S.; Lou, Y.; Stein, D.S. Single-dose pharmacokinetics of amprenavir, a human immunodeficiency virus type 1 protease inhibitor, in subjects with normal or impaired hepatic function, *Antimicrob.Agents Chemother.*, **2000**, *44*, 821–826.

Villani, P.; Feroggio, M.; Gianelli, L.; Bartoli, A.; Montagna, M.; Maserati, R.; Regazzi, M.B. Antiretrovirals: simultaneous determination of five protease inhibitors and three nonnucleoside transcriptase inhibitors in human plasma by a rapid high-performance liquid chromatography-mass spectrometry assay, *Ther.Drug Monit.*, **2001**, *23*, 380–388. [saquinavir; indinavir; ritonavir; nelfinavir; amprenavir; nevirapine; delavirdine; efavirenz]

Yamada, H.; Kotaki, H.; Nakamura, T.; Iwamoto, A. Simultaneous determination of the HIV protease inhibitors indinavir, amprenavir, saquinavir, ritonavir and nelfinavir in human plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **2001**, *755*, 85–89.

Yu, L.; Bridgers, A.; Polli, J.; Vikers, A.; Long, S.; Roy, A.; Winnike, R.; Coffin, M. Vitamin E-TPGS increases absorption flux of an HIV protease inhibitor by enhancing its solubility and permeability, *Pharm.Res.*, **1999**, *16*, 1812–1817.

Anagrelide



Molecular formula: $C_{10}H_7Cl_2N_3O$
Molecular weight: 256.09
CAS Registry No: 68475-42-3
Merck Index: 13, 629

SAMPLE

Matrix: blood, urine
Sample preparation: Mix 2 mL plasma or urine with 2 mL 200 mM pH 7.0 phosphate buffer, extract twice with 10 mL portions of ethyl acetate. Evaporate the organic layer to dryness under a stream of nitrogen, reconstitute the residue with 60 μL DMSO, mix, sonicate, inject a 40 μL aliquot.

HPLC VARIABLES

Guard column: 40 mm long μBondapak phenyl corasil
Column: 300 × 3.9 μBondapak phenyl
Mobile phase: MeCN:10 mM pH 4 sodium acetate buffer 25:75 for 10 min, DMSO for 8 min, return to original mobile phase
Flow rate: 2.5 for 13 min, 1 for 5 min, 2.5 for rest of the run
Injection volume: 40
Detector: UV 254; Radioactivity (^{14}C)

CHROMATOGRAM

Retention time: 6–8

KEY WORDS

plasma; radiolabeled

REFERENCE

Gaver, R.C.; Deeb, G.; Pittman, K.A.; Smyth, R.D. Disposition of anagrelide, an inhibitor of platelet aggregation, *Clin.Pharmacol.Ther.*, **1981**, *29*, 381–386.

Anakinra

Molecular weight: 17 000
CAS Registry No: 143090-92-0
Merck Index: 13, 5022

SAMPLE

Matrix: blood, tissue
Sample preparation: Inject a 50 μL aliquot of plasma or tissue homogenate supernatant.

HPLC VARIABLES

Guard column: 40 × 6 Spherogel TSK PWHR (Beckman)
Column: 300 × 7.8 5 μm Progel-TSK G2000 SWXL (Supelco)
Mobile phase: 10 mM pH 6.5 citrate buffer containing 140 mM NaCl and 0.5 mM EDTA
Flow rate: 0.5
Injection volume: 50
Detector: UV; Radioactivity (^{35}S); ELISA

CHROMATOGRAM

Retention time: 20

KEY WORDS

brain; gut; heart; kidney; liver; lung; muscle; plasma; rat; spleen

REFERENCE

Kim, D.C.; Reitz, B.; Carmichael, D.F.; Bloedow, D.C. Kidney as a major clearance organ for recombinant human interleukin-1 receptor antagonist, *J.Pharm.Sci.*, **1995**, *84*, 575–580.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 100 μL aliquot of a 2–5 mg/mL solution in 10 mM pH 6.5 citrate buffer containing 140 mM NaCl and 0.5 mM EDTA.

HPLC VARIABLES

Column: 75 × 7.5 Bio-Gel SP-5-PW (Bio-Rad)
Mobile phase: Gradient. A:B from 99:1 to 40:60 over 60 min. A was 20 mM pH 5.5 2-(*N*-morpholino)ethanesulfonic acid monohydrate. B was 20 mM pH 5.5 2-(*N*-morpholino)ethanesulfonic acid monohydrate containing 1.0 M NaCl.
Flow rate: 0.5
Injection volume: 100
Detector: UV 280

REFERENCE

Nahata, M.C.; Morosco, R.S.; Sabados, B.K.; Weber, T.R. Stability and compatibility of anakinra with intravenous cimetidine hydrochloride or famotidine in 0.9% sodium chloride injection, *J.Clin.Pharm. Ther.*, **1995**, *20*, 97–99.

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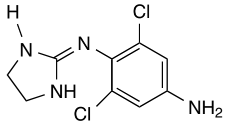
Chang, B.S.; Reeder, G.; Carpenter, J.F. Development of a stable freeze-dried formulation of recombinant human interleukin-1 receptor antagonist, *Pharm.Res.*, **1996**, *13*, 243–249.

Nahata, M.C.; Morosco, R.S.; Sabados, B.K.; Weber, T.R. Stability and compatibility of anakinra with ceftriaxone sodium injection in 0.9% sodium chloride or 5% dextrose injection, *J.Clin.Pharm.Ther.*, **1997**, *22*, 167–169.

Apraclonidine



Molecular formula: $C_9H_{10}Cl_2N_4$
Molecular weight: 245.11
CAS Registry No: 66711-21-5
Merck Index: 13, 756

SAMPLE

Matrix: solutions
Sample preparation: Inject a 50 μL aliquot of a solution in glutathione bicarbonated Ringer's solution (pH 7.4).

HPLC VARIABLES

Column: 150 × 4 5 μm Ultrasphere ODS
Mobile phase: MeCN:water 20:80 to 60:40 (?) containing 5 mM sodium heptanesulfonic acid at pH 3.5
Flow rate: 1–1.5
Injection volume: 50
Detector: UV 254

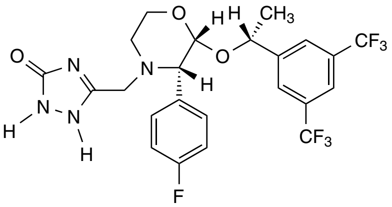
OTHER SUBSTANCES

Simultaneous: clonidine

REFERENCE

Chien, D.S.; Homsy, J.J.; Gluchowski, C.; Tang-Liu, D.D.-S. Corneal and conjunctival/scleral penetration of *p*-aminoclonidine, AGN 190342, and clonidine in rabbit eyes, *Current Eye Res.*, **1990**, *9*, 1051–1059.

Aprepitant



Molecular formula: $C_{23}H_{21}F_7N_4O_3$
Molecular weight: 534.43
CAS Registry No: 170729-80-3

SAMPLE

Matrix: blood, tissue

Sample preparation: Mix 200 μL plasma with 20 ng IS and 1.7 mL water, add 500 μL MeCN, add to a 500 mg Bond Elut C18 SPE cartridge, wash with 6 mL water, elute with 3 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue with 300 μL mobile phase, inject an aliquot. Alternatively, mix 50 μL plasma or brain homogenate with 5 ng IS and 100 μL MeCN, vortex, centrifuge at 3000 g for 10 min, inject a 5–25 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 50 × 4.6 5 μm Spherisorb C8
Mobile phase: MeCN:10 mM ammonium acetate:formic acid 55:45:0.1
Flow rate: 1
Injection volume: 5–25
Detector: MS, Sciex API III+, heated nebulizer interface, dwell time 450 ms, m/z 535 to 179

CHROMATOGRAM

Retention time: 1.5
Internal standard: desfluoroaprepitant (m/z 535 to 161) (1.5)

KEY WORDS

brain; ferret; plasma; SPE

REFERENCE

Huskey, S.-E.W.; Dean, B.J.; Bakhtiar, R.; Sanchez, R.I.; Tattersall, F.D.; Rycroft, W.; Hargreaves, R.; Watt, A.P.; Chicchi, G.G.; Keohane, C.; Hora, D.F.; Chiu, S.-H.L. Brain penetration of aprepitant, a substance P receptor antagonist, in ferrets, *Drug Metab.Dispos.*, **2003**, *31*, 785–791.

SAMPLE

Matrix: blood, tissue

Sample preparation: Mix 3 mL plasma with 6 mL MeCN, centrifuge at 3000 g, evaporate the supernatant to dryness under a stream of nitrogen, reconstitute the residue with 1 mL MeOH:water 40:60, inject a 250–400 μL aliquot of the supernatant. Homogenize the brain with 3 volumes of water. Vortex 10 mL homogenate with 90 mL MeCN, sonicate for 5 min, centrifuge at 3000 g for 10 min, re-extract the pellet with 10 mL MeOH. Combine the organic layers and add to a Bond Elut C18 SPE cartridge equipped with an Acrodisc glass filter, elute with 5 mL MeOH:MeCN:water 50:25:25. Collect all the cartridge effluent and evaporate to dryness under a stream of nitrogen, reconstitute the residue with 5 mL MeOH, vortex, sonicate, centrifuge. Evaporate the supernatant to dryness under a stream of nitrogen, reconstitute the residue with 1 mL MeOH:water 40:60, inject a 400 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX-C8

Mobile phase: Gradient. A:B 65:35 to 20:80 over 40 min. A was 10 mM ammonium acetate. B was MeCN:MeOH 92.8:7.2 containing 7.2 mM ammonium acetate. (Alternatively, A 10 mM ammonium acetate in water containing 0.1% trifluoroacetic acid and B MeCN:MeOH 92.8:7.2 containing 7.2 mM ammonium acetate and 0.1% trifluoroacetic acid with the same gradient.)
Flow rate: 1
Injection volume: 250–400
Detector: Radioactivity (^{14}C)

CHROMATOGRAM

Retention time: 26
Internal standard: desfluoroaprepitant (m/z 535 to 161) (1.5)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

brain; ferret; plasma; SPE

REFERENCE

Huskey, S.E.W.; Dean, B.J.; Bakhtiar, R.; Sanchez, R.I.; Tattersall, F.D.; Rycroft, W.; Hargreaves, R.; Watt, A.P.; Chicchi, G.G.; Keohane, C.; Hora, D.F.; Chiu, S.H.L. Brain penetration of aprepitant, a substance P receptor antagonist, in ferrets, *Drug Metab.Dispos.*, **2003**, *31*, 785–791.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 1 μL aliquot of a solution in MeOH:water 10:90.

HPLC VARIABLES

Column: 20 × 2 5 μm DASH BetaBasic C8 (ThermoHypersil Keystone)
Mobile phase: Gradient. A was MeCN:water:formic acid 5:95:0.1. B was MeCN:water: formic acid 95:5:0.1. A:B 100:0 for 0.2 min, to 0:100 over 1.5 min.
Flow rate: 1.5
Injection volume: 1
Detector: MS, PE Sciex API-3000, turbo ionspray, electrospray 4500 V, ring 290 V, orifice 60 V, drying gas 400°, 20% of column effluent entered the detector, m/z 535.3–277

CHROMATOGRAM

Retention time: 1.4

OTHER SUBSTANCES

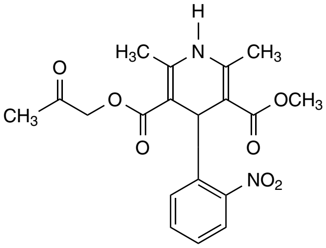
Simultaneous: amitriptyline (m/z 278.3–233) (1.1), diclofenac (m/z 296.1–215) (1.35), enoxacin (m/z 321.2–234) (0.7), fenofibrate (m/z 360.9–233) (1.6), finasteride (m/z 373.2–317) (1.2), indinavir (m/z 614.4–421) (0.93), pioglitazone (357.2–134) (0.87), raloxifene (m/z 474.1–112) (0.97)

REFERENCE

Romanyshyn, L.A.; Tiller, P.R. Ultra-short columns and ballistic gradients: considerations for ultra-fast chromatographic liquid chromatographic-tandem mass spectrometric analysis, *J.Chromatogr.A*, **2001**, *928*, 41–51.

Aranidipine

Molecular formula: $C_{19}H_{20}N_2O_7$
Molecular weight: 388.37
CAS Registry No: 86780-90-7
Merck Index: 13, 772



SAMPLE

Matrix: blood

Sample preparation: Add 20 ng nifedipine and 500 μL 100 mM pH 9.0 borate buffer to 1 mL plasma, vortex for 10 s, add 6 mL toluene, shake mechanically for 10 min, centrifuge at 1000 g for 15 min. Evaporate the organic layer to dryness under a stream of nitrogen at 40°, reconstitute the residue with 200 μL mobile phase, inject a 50 μL aliquot. (Carry out all steps under yellow fluorescent lighting.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-2
Column temperature: 40
Mobile phase: MeOH:360 mM sodium perchlorate 45:55
Flow rate: 0.8
Injection volume: 50
Detector: E, BAS LC-4B/17AT, +0.92 V versus Ag/AgCl

CHROMATOGRAM

Retention time: 16
Internal standard: nifedipine (26)
Limit of quantitation: 2.5 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

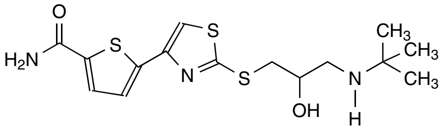
KEY WORDS

dog; pharmacokinetics; plasma

REFERENCE

Iida, Y.; Kinouchi, Y.; Takeichi, Y.; Imai, T.; Otagiri, M. Simultaneous determination of a new dihydropyridine calcium antagonist (MPC-1304) and its metabolite in dog plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1991**, *571*, 277–282.

Arotinolol



Molecular formula: $C_{15}H_{21}N_3O_2S_3$
Molecular weight: 371.55
CAS Registry No: 68377-92-4, 68377-91-3 (HCl)
Merck Index: 13, 797

SAMPLE

Matrix: blood

Sample preparation: Add 100 μL 10 mg/mL IS in water to 500 μL plasma, make up to 1 mL with water, add 100 μL 3 M pH 9 ammonium acetate, vortex vigorously for 2 min, centrifuge at 3000 g for 10 min. Extract the aqueous layer three times with 1 mL portions of ether, evaporate the extracts to dryness under reduced pressure, reconstitute the residue with 100 μL 100 mM HCl, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Chirobiotic T (Advanced Separation Technologies)
Mobile phase: MeOH:acetic acid:triethylamine 100:0.1:0.1
Flow rate: 0.8
Detector: UV 317

CHROMATOGRAM

Retention time: 17.25 (S-(+)), 20.06 (R-(−))
Internal standard: labetalol hydrochloride (21.98, 23.43 (enantiomers))
Limit of detection: 50 ng/mL
Limit of quantitation: 100 ng/mL

KEY WORDS

chiral; plasma

REFERENCE

Aboul-Enein, H.Y.; Hefnawy, M.M. Enantioselective determination of arotinolol in human plasma by HPLC using teicoplanin chiral stationary phase, *Biomed.Chromatogr.*, **2003**, *17*, 453–457.

SAMPLE

Matrix: blood, urine

Sample preparation: Condition a 1 mL C18 Bakerbond SPE cartridge with MeOH. Mix 1 mL plasma with 50 μL 5 μg/mL alpiropride in water. Mix 100 μL pure or diluted urine with 250 μL blank plasma and 100 μL 5 μg/mL alpiropride in water. Add the sample to the SPE cartridge, wash three times with 1 mL portions of water, wash three times with 1 mL portions of *n*-hexane:diethyl ether 50:50, elute with two 1 mL portions of chloroform:triethylamine 90:10 (Caution! Chloroform is a carcinogen!). Evaporate the eluate to dryness under reduced pressure, reconstitute the residue with 150 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 4.6 7 μm C18
Column: 250 × 4.6 5 μm ODS Hypersil
Column temperature: 25
Mobile phase: MeCN:MeOH:buffer 12.5:12.5:75 (The buffer was 67 mM pH 5.6 phosphate buffer containing 0.6 mM tetrabutylammonium chloride.)
Flow rate: 1.2
Injection volume: 100
Detector: F ex 310 em 395; UV 310

CHROMATOGRAM

Retention time: 10.0
Internal standard: alpiropride (4.7)
Limit of detection: 0.11 ng/mL (plasma), 11 ng/mL (urine)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

pharmacokinetics; plasma; SPE

REFERENCE

Moulin, A.; Maillet, E.; Truffer, D.; Dufour, A. High performance liquid chromatographic determination of arotinolol and AC 623, its main metabolite in biological samples, *J.Liq.Chromatogr.*, **1992**, *15*, 151–164.

SAMPLE

Matrix: blood, urine
Sample preparation: Condition a 1 mL C18 Bakerbond SPE cartridge with MeOH. Mix 1 mL plasma with 50 µL 5 µg/mL alpiropride in water. Mix 100 µL pure or diluted urine with 250 µL blank plasma and 100 µL 5 µg/mL alpiropride in water. Add the sample to the SPE cartridge, wash three times with 1 mL portions of water, wash three times with 1 mL portions of *n*-hexane:diethyl ether 50:50, elute with two 1 mL portions of chloroform:triethylamine 90:10 (Caution! Chloroform is a carcinogen!). Evaporate the eluate to dryness under reduced pressure, reconstitute the residue with 150 µL mobile phase, inject a 100 µL aliquot.

HPLC VARIABLES

Guard column: 15 × 4.6 7 µm diol
Column: 200 × 4.6 5 µm Lichrosorb diol
Column temperature: 25
Mobile phase: Dichloromethane containing 10 mM Z-glycyl-L-proline:MeOH 100:1
Flow rate: 2
Injection volume: 100
Detector: F ex 320 em 425

CHROMATOGRAM

Retention time: 12 (R-(−)), 15 (S-(+))
Internal standard: alpiropride (7.5)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

chiral; plasma; SPE

REFERENCE

Moulin, A.; Truffer, D.; Maillet, E.; Dufour, A. High performance liquid chromatographic determination of the optical isomers of arotinolol and AC 623, its main metabolite, in biological samples, *J.Liq.Chromatogr.*, **1992**, *15*, 165–181.

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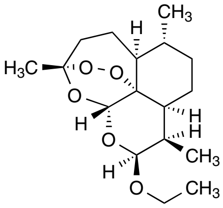
Nakamura, K.; Fujima, H.; Kitagawa, H.; Wada, H.; Makino, K. Preparation and chromatographic characteristics of a chiral-recognizing perphenylated cyclodextrin column, *J.Chromatogr.A*, **1995**, *694*, 111−118. [ibuprofen; chlorpheniramine; acetylpheneturide; alprenolol; arotinolol; atenolol; benzoin; biperiden; bunitrolol; chlormezanone; chlorphenesin; eperisone; flavanone; oxprenolol; phenylethyl alcohol; phenylethylamine; pindolol; proglumide; propranolol; trihexyphenidyl]

Arteether



Molecular formula: $C_{17}H_{28}O_5$
Molecular weight: 312.40
CAS Registry No: 75887-54-6
Merck Index: 13, 822

SAMPLE

Matrix: blood

Sample preparation: Add 5 µL 10 µg/mL artemisinin in MeOH to 200 µL serum, vortex, add 2 mL hexane, vortex for 1 min, centrifuge at 1000 g for 5 min, freeze in liquid nitrogen. Repeat the extraction. Combine the organic layers and evaporate to dryness, reconstitute the residue with 40 µL MeOH, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 300 × 4.6 5 µm Ultracarb 5 ODS 20 (Phenomenex)
Mobile phase: MeOH:100 mM sodium acetate 80:20
Flow rate: 1
Injection volume: 20
Detector: MS, Quattro II triple quadrupole, electrospray, nebulizing gas nitrogen 10 L/h, curtain gas nitrogen 250 L/h, ESI capillary at 3.5 kV, cone voltage 52 V, positive mode, m/z 335 [M + Na]+, one tenth of column effluent was allowed into MS

CHROMATOGRAM

Retention time: 1.73 (α), 2.81 (β)
Internal standard: artemisinin (m/z 305) (1.02)
Limit of detection: 5 ng/mL
Limit of quantitation: 20 ng/mL

KEY WORDS

rat; serum

REFERENCE

Rajanikanth, M.; Madhusudanan, K.P.; Gupta, R.C. Liquid chromatographic-mass spectrometric method for the determination of α-, β-arteether in rat serum, *J.Chromatogr.B*, **2003**, *783*, 391–399.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 µm IBO-SIL C18 (Phenomenex)
Mobile phase: MeOH:water 80:20
Flow rate: 0.9
Detector: UV 260

REFERENCE

Illapakurthy, A.C.; Sabnis, Y.A.; Avery, B.A.; Avery, M.A.; Wyandt, C.M. Interaction of artemisinin and its related compounds with hydroxypropyl-β-cyclodextrin in solution state: experimental and molecular-modeling studies, *J.Pharm.Sci.*, **2003**, *92*, 649–655.

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Baker, J.K.; Yarber, R.H.; Hufford, C.D.; Lee, I.-S.; ElSohly, H.N.; McChesney, J.D. Thermospray mass spectroscopy/high performance liquid chromatographic identification of the metabolites formed from arteether using a rat liver microsome preparation, *Biomed.Environ.Mass Spectrom.*, **1989**, *18*, *337–351*.

Baker, J.K.; McChesney, J.D.; Chi, H.T. Decomposition of arteether in simulated stomach acid yielding compounds retaining antimalarial activity, *Pharm.Res.*, **1993**, *10*, 662–666.

Benakis, A.; Schopfer, C.; Paris, M.; Plessas, C.; Karayannakos, P.E.; Dondas, I.; Kotsarelis, D.; Plessas, S.T.; Skalkeas, G. Pharmacokinetics of arteether in dog, *Eur.J.Drug Metab.Pharmacokinet.*, **1991**, *16*, 325–328.

Chi, H.T.; Ramu, K.; Baker, J.K.; Hufford, C.D.; Lee, I.S.; Zeng, Y.-L.; McChesney, J.D. Identification of the in vivo metabolites of the antimalarial arteether by thermospray high-performance liquid chromatography/mass spectrometry, *Biol.Mass Spectrom.*, **1991**, *20*, 609–628.

Idowu, O.R.; Edwards, G.; Ward, S.A.; Orme, M.L'E.; Breckenridge, A.M. Determination of arteether in blood plasma by high-performance liquid chromatography with ultraviolet detection after hydrolysis with acid, *J.Chromatogr.*, **1989**, *493*, 125–136.

Leo, K.U.; Grace, J.M.; Li, Q.; Peggins, J.; Mitchell, A.L.; Aguilar, T.; Brewer, T.G. Effects of *Plasmodium berghei* infection on arteether metabolism and disposition, *Pharmacology*, **1997**, *54*, 276–284.

Leskovac, V.; Theoharides, A.D. Hepatic metabolism of artemisinin drugs – I. Drug metabolism in rat liver microsomes, *Comp.Biochem.Physiol.C*, **1991**, *99*, 383–390. [arteether; dihydroartemisinin]

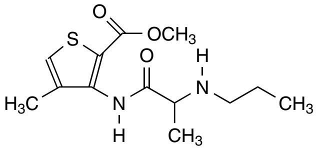
Li, Q.G.; Brueckner, R.P.; Peggins, J.O.; Trotman, K.M.; Brewer, T.G. Arteether toxicokinetics and pharmacokinetics in rats after 25 mg/kg/day single and multiple doses, *Eur.J.Drug Metab.Pharmacokinet.*, **1999**, *24*, 213–223.

Melendez, V.; Peggins, J.O.; Brewer, T.G.; Theoharides, A.D. Determination of the antimalarial arteether and its deethylated metabolite dihydroartemisinin in plasma by high-performance liquid chromatography with reductive electrochemical detection, *J.Pharm.Sci.*, **1991**, *80*, 132–138.

Rajanikanth, M.; Madhusudanan, K.P.; Gupta, R.C. An HPLC-MS method for simultaneous estimation of α,β-arteether and its metabolite dihydroartemisinin, in rat plasma for application to pharmacokinetic study, *Biomed.Chromatogr.*, **2003**, *17*, 440–446.

Ramu, K.; Baker, J.K. Identification of the glucuronides of the hydroxylated metabolites of the antimalarial arteether in rat plasma and urine by thermospray high-performance liquid chromatography/mass spectrometry, *J.Pharm.Sci.*, **1997**, *86*, 915–920.

Articaine



Molecular formula: $C_{13}H_{20}N_2O_3S$

Molecular weight: 284.38

CAS Registry No: 23964-58-1, 23964-57-0 (HCl)

Merck Index: 13, 1884

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 50 μL 2 μg/mL etidocaine in water + 100 μL 1 M NaOH + 3 mL heptane:ethyl acetate 90:10, shake for 2 min, centrifuge at 1200 g for 10 min. Remove the organic phase and add it to 50 μL 50 mM sulfuric acid, shake for 2 min, centrifuge at 1200 g for 5 min. Remove the aqueous phase and add it to 820 μg sodium acetate, inject a 40 μL aliquot. (The sodium acetate was measured out by adding 50 μL 200 mM sodium acetate in MeOH to the tube and evaporating the MeOH.)

HPLC VARIABLES

Column: 250 × 4 10 μm μBondapak C18

Column temperature: 30

Mobile phase: MeCN:10 mM sodium dihydrogen phosphate 7:93, adjusted to pH 2.1

Flow rate: 1

Injection volume: 40

Detector: UV 205

CHROMATOGRAM

Retention time: 19

Internal standard: etidocaine (10)

Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: mepivacaine (15)

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 4 mm SDB-RPS SPE disk cartridge (3M Empore) with 500 μL MeOH, 500 μL air, 500 μL water, and 1 mL air. Mix 1 mL serum with 50 μL perchloric acid, let stand for 10 min, mix, centrifuge at 16 000 g for 10 min. Add 800 μL to the cartridge followed by 2 mL air. Wash with 800 μL 0.5% phosphoric acid in MeOH:water 20:80, push through 1.5 mL air, wash with 700 μL water, push through 2 mL air, elute with 500 μL MeOH containing 1% ammonia, push through 1.2 mL air. Evaporate the eluate to dryness under a stream of air at 70° and reconstitute the residue with 50 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES

Column: 125 × 3 5 μm Nucleosil 50-5 endcapped RP-8

Column temperature: 35
Mobile phase: MeCN:buffer 12:88 (Buffer was 880 mL 20 mM potassium dihydrogen phosphate containing 500 μL phosphoric acid, pH 3.)
Flow rate: 1
Injection volume: 40
Detector: UV 274

CHROMATOGRAM

Retention time: 9.5
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Extracted: articainic acid (3.5)

KEY WORDS

serum; SPE

REFERENCE

Richter, K.; Oertel, R. Solid-phase extraction and high-performance liquid chromatographic determination of articaine and its metabolite articainic acid in human serum, *J.Chromatogr.B*, **1999**, *724*, 109–115.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 10 μm Chiralcel OD
Mobile phase: *n*-Hexane:isopropanol 80:20
Flow rate: 0.4
Injection volume: 5
Detector: UV 274

CHROMATOGRAM

Retention time: 12, 14 (enantiomers)

KEY WORDS

chiral

REFERENCE

Rustichelli, C.; Ferioli, V.; Gamberini, G.; Stancanelli, R. Enantiomeric separation of local anaesthetic drug by HPLC on chiral stationary phases, *Chromatographia*, **2001**, *54*, 731–736.

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Chankvetadze, B.; Chankvetadze, L.; Sidamonidze, S.; Yashima, E.; Okamoto, Y. High performance liquid chromatography enantioseparation of chiral pharmaceuticals using tris(chloro-methylphenylcarbamate)s of cellulose, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 1295–1303. [oxazepam; oxazolam; hexobarbital; phenobarbital; thiopental; lorazepam; lopirazepam; camazepam; cloxazolam; ketazolam; pindolol; propranolol; sotalol; alprenolol; bupranolol; acebutolol; penbutolol; toliprolol; enilconazole; econazole; miconazole; bifonazole; ornidazole; bayleton; metomidate; nisoldipine; nimodipine; isradipine; nicardipine; triadimefon; pheniramine; doxylamine; chlorphenoxamine; carbinoxamine; azelastine; mequitazine; paramethadione; norgestrel; tesicam; mesuximide; metofoline; tramadol; clofedanol; lofexidine; clenbuterol; piprozolin; etozolin; doxapram; chlormezanone; aminoglutethimide; articaine; etidocaine; nefopam]

Oertel, R.; Richter, K.; Weile, K.; Gramatte, T.; Berndt, A.; Feller, K. A simple method for the determination of articaine and its metabolite articainic acid in dentistry: application to a comparison of articaine and lidocaine concentrations in alveolus blood, *Methods Find.Exp.Clin.Pharmacol.*, **1993**, *15*, 541–547.

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. [metoprolol; talinolol; celiprolol; tiracizine; triamterene; ajmaline; articaine; lamotrigine]

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. [cocaine; benzoylecgonine; procaine; *p*-aminobenzoic acid; butacaine; tetracaine; articaine; prilocaine; *o*-toluidine; lidocaine; monoethylglycine xylidide; bupivacaine; pipecolylxylidene; etidocaine; dibucaine; caffeine; amphetamine; ephedrine; epinephrine; morphine; monoacetylmorphine; diamorphine; ethylmorphine; codeine; acetylcodeine; fluorescence detection; UV detection; SPE]

Vree, T.B.; Baars, A.M.; van Oss, G.E.; Booij, L.H. High-performance liquid chromatography and preliminary pharmacokinetics of articaine and its 2-carboxy metabolite in human serum and urine, *J.Chromatogr.*, **1988**, *424*, 440–444.

Asparaginase

Molecular weight: ca. 136 000
CAS Registry No: 9015-68-3
Merck Index: 13, 841

SAMPLE

Matrix: reaction mixtures
Sample preparation: Adjust to pH 7 with 2 M NaOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 10 μm HEMA-BIO 1000 (Tessek, Prague) (hydroxyethyl methacrylate−type column)
Mobile phase: 100 mM Potassium dihydrogen phosphate adjusted to pH 6.9 with 2 M NaOH
Flow rate: 0.8
Injection volume: 20
Detector: UV 210

CHROMATOGRAM

Retention time: 2.1
Limit of detection: 0.51 U/mL

REFERENCE

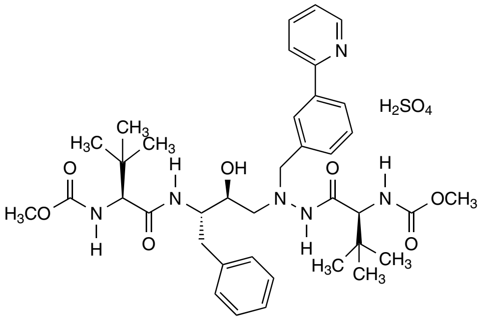
Barek, J.; Cvacka, J.; Zima, J.; De Méo, M.; Laget, M.; Michelon, J.; Castegnaro, M. Chemical degradation of wastes of antineoplastic agents amsacrine, azathioprine, asparaginase and thiotepa, *Ann.Occup.Hyg.*, **1998**, *42*, 259−266.

Atazanavir sulfate

Molecular formula: $C_{38}H_{52}N_6O_7.H_2O_4S$

Molecular weight: 802.93

CAS Registry No: 229975-97-7



SAMPLE

Matrix: blood

Sample preparation: Condition each well of a 3M Empore C2-SD 96 well plate with 250 μL MeOH and 500 μL 0.1% acetic acid, do not allow to go dry. Add 50 μL 200 ng/mL IS in MeOH:water 60:40 to 200 μL MeOH:water 60:40 containing 5 million cells, sonicate for 10 min, centrifuge at 2600 g for 10 min. Evaporate the supernatant to dryness, reconstitute with 50 μL MeOH, add 200 μL water, add 250 μL 0.1% acetic acid, mix, add to a well on the SPE plate, allow to pass through under vacuum over 2 min, wash with 500 μL 0.1% acetic acid, dry under vacuum for 2 min, elute twice with 200 μL portions of MeCN:MeOH 50:50, pulling to dryness after each portion. Evaporate the eluate to dryness under a stream of nitrogen at 60° over ca. 40 min, reconstitute the residue with 200 μL mobile phase, vortex, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 2 5 μm YMC Basic

Column: 50 × 2 5 μm YMC Basic

Mobile phase: MeCN:MeOH:water:88% formic acid 30:30:40:0.025

Flow rate: 0.25

Injection volume: 20

Detector: MS, Sciex API 3000 turbo ionspray, electrospray, positive mode at 400°, m/z 705 to 335, IonSpray 4600 V, declustering potential 56 V, entrance potential −10 V, focusing potential 220 V, TurboIon gas nitrogen 8 L/min, collision energy 42 V, collision cell exit potential 24 V, dwell time 500 ms, pause time 5 ms

CHROMATOGRAM

Retention time: <4

Internal standard: $^{13}C_6$-atazanavir

Limit of quantitation: 5 fmole/million cells

KEY WORDS

peripheral blood mononuclear cells; SPE

REFERENCE

Jemal, M.; Rao, S.; Gatz, M.; Whigan, D. Liquid chromatography-tandem mass spectrometric quantitative determination of the HIV protease inhibitor atazanavir (BMS-232632) in human peripheral blood mononuclear cells (PBMC): practical approaches to PBMC preparation and PBMC assay design for high-throughput analysis, *J.Chromatogr.B*, **2003**, *795*, 273–289.

SAMPLE

Matrix: blood

Sample preparation: Condition each well of a10 mg Oasis HLB 96 well SPE plate with 1 mL MeOH and 1 mL 0.1% acetic acid. Add 40 μL 5 μg/mL IS in water and 300 μL 0.1%

acetic acid to 250 μL plasma, mix, add to a well of the SPE plate, wash with 500 μL 0.1% acetic acid, wash with 500 μL MeOH:water 20:80, elute with 300 μL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue with 500 μL MeCN:MeOH:10 mM pH 5.5 ammonium acetate 30:30:40, inject a 15 μL aliquot.

HPLC VARIABLES

Column: 33 × 4.6 3 μm Uptisphere HDO C18 (Interchim)

Mobile phase: Gradient. MeCN:5 mM ammonium acetate 50:50 for 0.5 min, to 60:40 over 0.1 min, maintain at 60:40 for 1.7 min, return to initial conditions over 0.1 min, re-equilibrate for 2.1 min.

Flow rate: 0.8

Injection volume: 15

Detector: MS, Micromass Quattro Ultima, atmospheric pressure electrospray ionization, column effluent split 1:20 before entering MS, positive ion mode, capillary sprayer voltage 3.2 kV, sample cone voltage 80 V, source temperature 100°, desolvation temperature 350°, nebulizing gas nitrogen, cone gas nitrogen at 37 L/h, desolvation gas nitrogen at 500 L/h, collision gas argon at 2.6 μbar, collision energy was set at 40 eV, resolution set at 0.7 mass units at half height for the first and third quadrupoles.

CHROMATOGRAM

Retention time: 2.3

Internal standard: $^{13}C_6$-atazanavir

Limit of quantitation: 1 ng/mL

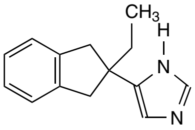
KEY WORDS

plasma; SPE

REFERENCE

Schuster, A.; Burzawa, S.; Jemal, M.; Loizillon, E.; Couerbe, P.; Whigan, D. Quantitative determination of the HIV protease inhibitor atazanavir (BMS-232632) in human plasma by liquid chromatography-tandem mass spectrometry following automated solid-phase extraction, *J.Chromatogr.B*, **2003**, *788*, 377–386.

Atipamezole



Molecular formula: $C_{14}H_{16}N_2$
Molecular weight: 212.29
CAS Registry No: 104054-27-5
Merck Index: 13, 866

SAMPLE

Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with water, MeOH, and 100 mM ammonium acetate. 5 mL Plasma + 250 ng detomidine, add to the SPE cartridge, wash with 100 mM ammonium acetate, elute with MeOH:100 mM ammonium acetate 75:25. Evaporate the eluate to dryness under reduced pressure, reconstitute the residue in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Hitachi gel #3056
Mobile phase: MeOH:100 mM ammonium acetate 65:35
Flow rate: 1
Injection volume: 50
Detector: MS, Hitachi M-1000, APCI interface, drift voltage 21 V, nebulizer 260°, vaporizer 399°, multiplier voltage 1500 VF, m/z 213

CHROMATOGRAM

Retention time: 8
Internal standard: detomidine (m/z 187) (6.5)
Limit of quantitation: 1–2 ng/mL

OTHER SUBSTANCES

Extracted: medetomidine (7.5, m/z 201), midazolam (10.5, m/z 326)

KEY WORDS

pharmacokinetics; pig; plasma; SPE

REFERENCE

Kanazawa, H.; Nishimura, R.; Sasaki, N.; Takeuchi, A.; Takai, N.; Nagata, Y.; Matsushima, Y. Determination of medetomidine, atipamazole and midazolam by liquid chromatography-mass spectrometry, *Biomed.Chromatogr.*, **1995**, *9*, 188–191.

SAMPLE

Matrix: blood
Sample preparation: Mix 200 μL 250 mM NaOH with 500 μL plasma, add 6 mL dichloromethane, mix gently for 10 min, centrifuge at 1700 g for 10 min. Evaporate 4 mL of the lower organic layer to dryness under a stream of nitrogen, reconstitute the residue with 100 μL 50 mM pH 3.2 phosphate buffer, vortex for 1.5 min, centrifuge at 1700 g for 10 min, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Supelguard LC-DP
Column: 250 × 4.6 5 μm Supelcosil LC-DP
Mobile phase: MeCN:50 mM phosphate buffer:triethylamine 27:73:0.05, adjusted to pH 3.2
Flow rate: 1
Injection volume: 50

Detector: UV 215

CHROMATOGRAM

Retention time: 16.2
Limit of detection: 5 ng/mL

OTHER SUBSTANCES

Extracted: medetomidine (14.6)

KEY WORDS

pharmacokinetics; plasma; reindeer

REFERENCE

Ranheim, B.; Horsberg, T.E.; Nymoen, U.; Soli, N.E.; Tyler, N.J.; Arnemo, J.M. Reversal of medetomidine-induced sedation in reindeer (*Rangifer tarandus tarandus*) with atipamezole increases the medetomidine concentration in plasma, *J.Vet.Pharmacol.Ther.*, **1997**, *20*, 350–354.

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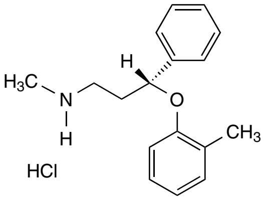
Kanazawa, H.; Nagata, Y.; Matsushima, Y.; Takai, N.; Uchiyama, H.; Nishimura, R.; Takeuchi, A. Liquid chromatography-mass spectrometry for the determination of medetomidine and other anaesthetics in plasma, *J.Chromatogr.*, **1993**, *631*, 215–220.

Atomoxetine hydrochloride

Molecular formula: $C_{17}H_{21}NO.HCl$

Molecular weight: 291.82

CAS Registry No: 82248-59-7



SAMPLE

Matrix: blood

Sample preparation: Add 500 μL plasma to a Varian SDB-XC SPE cartridge, wash with 1 mL MeOH:water 15:85, elute with 750 μL MeCN containing 0.1% trifluoroacetic acid. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue with 100 μL MeCN, mix with 25 μL water, inject an aliquot.

HPLC VARIABLES

Column: 100 × 4.6 5 μm Brownlee Spheri-5 ODS

Mobile phase: MeCN:water 85:15 containing 5 mM ammonium acetate, 0.2% formic acid, and 0.03% trifluoroacetic acid

Flow rate: 1

Detector: MS, PE Sciex API III, MS/MS, positive atmospheric pressure chemical ionization, heated nebulizer interface, m/z 256 to 44

CHROMATOGRAM

Limit of quantitation: 0.25 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

dog; plasma; rat; SPE

REFERENCE

Mattiuz, E.L.; Ponsler, G.D.; Barbuch, R.J.; Wood, P.G.; Mullen, J.H.; Shugert, R.L.; Li, Q.; Wheeler, W.J.; Kuo, F.; Conrad, P.C.; Sauer, J.-M. Disposition and metabolic fate of atomoxetine hydrochloride: Pharmacokinetics, metabolism, and excretion in the Fischer 344 rat and beagle dog, *Drug Metab.Dispos.*, **2003**, *31*, 88–97.

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. Mix 3 mL MeCN with 1.5 mL plasma, centrifuge, evaporate the supernatant to dryness under a stream of nitrogen, reconstitute the residue with 200 μL MeCN:water 10:90, inject an aliquot. Urine. Lyophilize urine, reconstitute with MeCN:water 10:90 to one-tenth original volume, vortex, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Zorbax Eclipse XDB-C18

Column temperature: 30

Mobile phase: Gradient. MeCN:50 mM ammonium acetate from 10:90 to 60:40 over 30 min. (Use 25 mM ammonium acetate for MS detector.)

Flow rate: 1

Detector: Radioactivity (^{14}C); MS, Finnigan TSQ 700 or TSQ 7000, positive electrospray, collision gas argon, 0.2 mL/min of column effluent entered MS

CHROMATOGRAM

Retention time: 20

OTHER SUBSTANCES

Extracted: metabolites

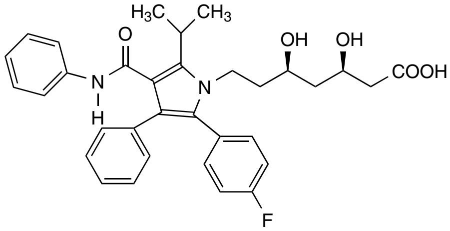
KEY WORDS

plasma

REFERENCE

Sauer, J.-M.; Ponsler, G.D.; Mattiuz, E.L.; Long, A.J.; Witcher, J.W.; Thomasson, H.R.; Desante, K.A. Disposition and metabolic fate of atomoxetine hydrochloride: the role of CYP2D6 in human disposition and metabolism, *Drug Metab.Dispos.*, **2003**, *31*, 98–107.

Atorvastatin



Molecular formula: $C_{33}H_{35}FN_2O_5$
Molecular weight: 558.64
CAS Registry No: 134523-00-5
Merck Index: 13, 868

SAMPLE

Matrix: blood

Sample preparation: Mix 500 µL serum with IS, acidify to pH 6 with sodium acetate buffer, extract with MTBE, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue, inject an aliquot.

HPLC VARIABLES

Column: YMC Basic

Mobile phase: Gradient. A:B from 70:30 to 45:55 over 1 min, maintain at 45:55 for 0.5 min, return to initial conditions over 0.1 min, maintain at 70:30 for 1.9 min. A was MeOH:water:88% formic acid 5:95:0.0043. B was MeCN:MeOH:88% formic acid 95:5:0.0043.

Detector: MS, Finnigan TSQ-7000, electrospray, m/z 559-440

CHROMATOGRAM

Internal standard: deuterated atorvastatin

Limit of quantitation: 500 pg/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

pharmacokinetics; serum

REFERENCE

Kantola, T.; Kivistö, K.T.; Neuvonen, P.J. Effect of itraconazole on the pharmacokinetics of atorvastatin, *Clin.Pharmacol.Ther.*, **1998**, *64*, 58–65.

SAMPLE

Matrix: formulations

Sample preparation: Shake 10 tablets with 50 mL MeCN:THF:50 mM pH 4 ammonium citrate buffer 27:20:53 at 450 rpm for 1 h, make up to 100 mL with the same solution, filter, dilute a 2 mL aliquot to 10 mL, inject an aliquot.

HPLC VARIABLES

Guard column: 4 × 3 5 µm C18 Luna (Phenomenex)

Column: 250 × 4.6 5 µm C18 Luna (Phenomenex)

Mobile phase: Gradient. MeCN:THF:20 mM pH 4.0 ammonium acetate buffer from 25:5:70 to 70:5:25 over 50 min, maintain at 70:5:25 for 10 min.

Flow rate: 1

Injection volume: 100

Detector: UV 248

CHROMATOGRAM

Retention time: 30

Limit of detection: 13 ng/mL
Limit of quantitation: 130 ng/mL

OTHER SUBSTANCES

Simultaneous: impurities

KEY WORDS

tablets

REFERENCE

Ertürk, S.; Aktas, E.S.; Ersoy, L.; Fiçicioglu, S. An HPLC method for the determination of atorvastatin and its impurities in bulk drug and tablets, *J.Pharm.Biomed.Anal.*, **2003**, *33*, 1017–1023.

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Al-Rawithi, S.; Hussein, R.F.; Alzahrani, A. Sensitive assay for the determination of fluvastatin in plasma utilizing high-performance liquid chromatography with fluorescence detection, *Ther.Drug Monit.*, **2003**, *25*, 88–92. [atorvastatin is IS]

Jacobsen, W.; Kuhn, B.; Soldner, A.; Kirchner, G.; Sewing, K.-F.; Kollman, P.A.; Benet, L.Z.; Christians, U. Lactonization is the critical first step in the disposition of the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor atorvastatin, *Drug Metab.Dispos.*, **2000**, *28*, 1369–1378. [microsomal incubations]

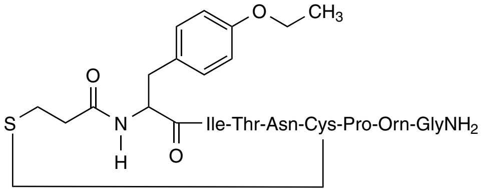
Mazzu, A.L.; Lasseter, K.C.; Shamblen, E.C.; Agarwal, V.; Lettieri, J.; Sundaresen, P. Itraconazole alters the pharmacokinetics of atorvastatin to a greater extent than either cerivastatin or pravastatin, *Clin.Pharmacol.Ther.*, **2000**, *68*, 391–400. [SPE]

Miao, X.-S.; Metcalfe, C.D. Determination of cholesterol-lowering statin drugs in aqueous samples using liquid chromatography-electrospray ionization tandem mass spectrometry, *J.Chromatogr.A*, **2003**, *998*, 133–141. [atorvastatin; lovastatin; pravastatin; simvastatin]

Prueksaritanont, T.; Tang, C.; Qiu, Y.; Mu, L.; Subramanian, R.; Lin, J.H. Effects of fibrates on metabolism of statins in human hepatocytes, *Drug Metab.Dispos.*, **2002**, *30*, 1280–1287. [cerivastatin; simvastatin; atorvastatin; rosuvastatin; pravastatin]

Atosiban

Molecular formula: $C_{43}H_{67}N_{11}O_{12}S_2$
Molecular weight: 994.20
CAS Registry No: 90779-69-4
Merck Index: 13, 869



SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: Kromasil C8 pre-column
Column: 100 × 2.1 KR 100-5 C8 1572 (Hichrom)
Mobile phase: MeCN:water:triethylammonium phosphate 27:72.9:0.1
Flow rate: 0.2
Detector: UV 190

REFERENCE

Lundin, S.; Svedman, P.; Höglund, P.; Jönsson, K.; Broeders, A.; Melin, P. Absorption of an oxytocin antagonist (antocin) and a vasopressin analogue (dDAVP) through a standardized skin erosion in volunteers, *Pharm.Res.*, **1995**, *12*, 2024–2029.

SAMPLE

Matrix: solutions
Sample preparation: Inject an aliquot of a solution in mobile phase.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Spherisorb NH_2 (Before use flush column with isopropanol at 60° (to remove hexane-shipping solvent) and then with aqueous trifluoroacetic acid (pH 2.0) at 75° (to protonate amino groups).)
Column temperature: 40
Mobile phase: MeCN:water 92.35:7.65 containing 2.5 mM ammonium acetate and 250 mM sodium perchlorate
Flow rate: 0.5–1.2
Detector: UV 210

CHROMATOGRAM

Retention time: 230 (0.5 mL/min), 130 (1.2 mL/min)

OTHER SUBSTANCES

Simultaneous: impurities

REFERENCE

Oyler, A.R.; Armstrong, B.L.; Cha, J.Y.; Zhou, M.X.; Yang, Q.; Robinson, R.I.; Dunphy, R.; Burinsky, D.J. Hydrophilic interaction chromatography on amino-silica phases complements reversed-phase high-performance liquid chromatography and capillary electrophoresis for peptide analysis, *J.Chromatogr.A*, **1996**, *724*, 378–383.