Abacavir

Molecular formula: C$_{14}$H$_{18}$N$_{6}$O
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 18000 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 18000 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**

**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**

**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, iraconazole, methadone, nelfinavir, oxazepam, pyrimethamine, rifampin, ritonavir, saquinavir, zalcitabine

**KEY WORDS**
- plasma
REFERENCE

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

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

ANNOTATED BIBLIOGRAPHY
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
Acetyl sulfisoxazole

**Molecular formula:** C₁₃H₁₅N₃O₄S

**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


### ANNOTATED BIBLIOGRAPHY

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_{2}O_{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 °C. 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 I on 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), 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), carboxamine (5.1, LOD 0.002 µg/mL), carisoprodol (6.7, LOD <5 µg/mL), carvedilol (6.2, LOD <0.02 µg/mL), cefepime (4.3, LOD 0.05 µg/mL), cetirizine (6.3, LOD 0.05 µg/mL), chlorcyclizine (6.6, LOD <0.02 µg/mL), clomipramine (7.1, LOD 0.005 µg/mL), clofibric acid (7.0, LOD 0.02 µg/mL), clofibrate (6.7, LOD <0.02 µg/mL), clomipramine (7.0, LOD <0.02 µg/mL), clonazepam (5.6, LOD <0.02 µg/mL), codeine (2.5, LOD 0.1 µg/mL), coumarin (8.4, LOD 0.05 µg/mL), cyclidine (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), dextromethorphan (5.5, LOD <0.02 µg/mL), diphenhydramine (5.7, LOD <0.02 µg/mL), dipyriridamole (5.4, LOD 0.005 µg/mL), disopyramide (4.4, LOD <0.02 µg/mL), doxycycline (6.8, LOD 0.005 µg/mL), doxapram (4.8, LOD <0.02 µg/mL), doxepin (5.9, LOD <0.02 µg/mL), elabast (9.6, LOD 0.005 µg/mL), embutramide (6.7, LOD 0.005 µg/mL), ergotamine (5.5, LOD 0.005 µg/mL), ethanesidade (5.0, LOD 0.05 µg/mL), ethanomorphine (3.2, LOD 0.05 µg/mL), ethylparathion (9.7, LOD <5 µg/mL), etodaxine (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), fenxamifime (5.1, LOD <0.02 µg/mL), fentanyl (5.5, LOD <0.02 µg/mL), fenoxadine (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 <0.025 µg/mL), flunitrazepam (7.1, LOD 0.002 µg/mL), flutroxetin (5.3, LOD 0.01 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), histapyrroline (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), ketobemidine (3.3, LOD <0.05 µg/mL), ketoprofen (7.3, LOD 0.1 µg/mL), ketorolac (6.2, LOD 0.05 µg/mL), ketobemidine (3.3, LOD <0.05 µg/mL), ketobemidine (3.3, LOD <0.05 µg/mL), ketorolac (6.2, LOD 0.05 µg/mL), ketoprofen (7.3, LOD 0.1 µg/mL), lamotrigine (4.0, LOD 0.1 µg/mL), levocastabine (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), lorsetazepam (7.4, LOD <0.02 µg/mL), LSD (4.7, LOD <0.02 µg/mL), malathion (8.9, LOD 10 µg/mL), meprobamate (4.9, LOD 0.1 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), mephenylparahex (8.6, LOD 10 µg/mL), methylphenidate (4.2, LOD <0.02 µg/mL), metoclopramide (3.8, LOD <0.02 µg/mL), metaprolol (4.1, LOD 0.2 µg/mL), metonidazole (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 (5.3, LOD <0.01 µg/mL), mofobemide (3.7, LOD 0.05 µg/mL), molindone (4.0, LOD <0.02 µg/mL), monoanacetil Morphine (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 0.01 µg/mL), nomifensine (4.6, LOD <0.02 µg/mL), nortriptyline (6.4, LOD <0.02 µg/mL), norvepamipil (6.2, LOD 0.01 µg/mL), noscapine (5.0, LOD <0.02 µg/mL), oxazepam (6.1, LOD <0.02 µg/mL), oxazepam (6.3, LOD <0.02 µg/mL), oxcarbazepine (5.3, LOD 0.02 µg/mL), oxphenyl (4.7, LOD 0.02 µg/mL), oxydodone (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), pentryliflone (7.3, LOD <0.05 µg/mL), pentoxyverine (6.6, LOD <0.02 µg/mL), perphenazine (6.9, LOD 0.002 µg/mL), phenoxyne (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), pizotifen (6.5, LOD < 0.02 µg/mL), prochlorperazine (7.5, LOD 0.02 µg/mL), promazine (6.2, LOD < 0.02 µg/mL), promethazine (6.0, LOD 0.02 µg/mL), propafenone (6.3, 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), thiourea (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), triazolam (6.7, LOD 0.002 µg/mL), trimipramine (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**
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
Adefovir dipivoxil

Molecular formula: \( \text{C}_{20}\text{H}_{32}\text{N}_5\text{O}_8\text{P} \)
Molecular weight: 501.47
CAS Registry No: 142340-99-6
Merck Index: 13, 151

SAMPLE
Matrix: blood
Sample preparation: Mix 100 \( \mu \text{L} \) plasma with 200 \( \mu \text{L} \) 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\°C for 40 min, evaporate to dryness, reconstitute with 100 \( \mu \text{L} \) 25 mM pH 6.0 potassium phosphate buffer containing 5 mM tetrabutylammonium hydrogen phosphate, inject a 50 \( \mu \text{L} \) aliquot.

HPLC VARIABLES
Guard column: 15 \( \times \) 3.2 Brownlee RP-18 Newguard
Column: 150 \( \times \) 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

SAMPLE
Matrix: blood
Sample preparation: Vortex 200 \( \mu \text{L} \) plasma with 50 \( \mu \text{L} \) 20\% trichloroacetic acid in water, centrifuge at 1300 g for 15 min. Remove 150 \( \mu \text{L} \) of the supernatant and mix it with 50 \( \mu \text{L} \) 160 mM chloroacetaldehyde in water containing 2 M sodium acetate, vortex, close the tube, heat at 98\°C for 30 min, cool to 2\°C, vortex, inject a 20 \( \mu \text{L} \) aliquot.

HPLC VARIABLES
Guard column: 10 \( \times \) 3 R3 (Chrompack)
Column: 150 \( \times \) 4.6 5 \( \mu \text{m} \) Chromspher C8
Column temperature: 40 \( \pm \) 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
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°C  
**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 mL/min  
**Injection volume:** 5–75 µL  
**Detector:** 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°C 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**  

**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

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in 100 mM NaH₂PO₄ adjusted to pH 2.1 with orthophosphoric acid, inject a 100 µL aliquot.

HPLC VARIABLES
Column: 250 × 4 Aquapore RP 300 (Kontron)
Mobile phase: Gradient. A was 100 mM NaH₂PO₄ adjusted to pH 2.1 with orthophosphoric acid. B was MeOH. A:B from 90:10 to 35:65 over 180 min.
Flow rate: 1
Injection volume: 100
Detector: UV 225

CHROMATOGRAM
Retention time: 145

OTHER SUBSTANCES
Simultaneous: adrenocorticotropic 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
Afloqualone

Molecular formula: $C_{16}H_{14}FN_{3}O$
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
Alacepril

Molecular formula: \( C_{20}H_{26}N_{2}O_{5}S \)
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

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 Cosmostil 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
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), clobetasol butyrate (isocratic k' 5.45; gradient retention time (min) 26.3; LOD 0.1 µg/mL), cortisone (isocratic k' 0.18; gradient retention time
Mix 1 g of a topical product, 5 mL 100 mM pH 4 citrate buffer. Sample preparation:

Matrix:

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.

KEY WORDS

cosmetics; SPE

REFERENCE


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.
Alclometasone 17,21-dipropionate

**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), dexamethasone 21-acetate (8.07), dexamethasone 21-acetate (8.68), desonide (6.99), desoximetasone (7.60), desoxycorticosterone acetate (10.90), desoxycorticosterone pivalate (14.45), diflorsone 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**

Alitretinoin

Molecular formula: C20H28O2
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 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


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 x 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


### 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 x 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

ANNOTATED BIBLIOGRAPHY
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 x 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. (Refux 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 x 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**

**SAMPLE**
Matrix: solutions

**HPLC VARIABLES**
Column: Two 250 x 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

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 50 × 4 40 µm pellicular material
Column: 250 × 4.6 5 µm Ultrasphere octadeysilica
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

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, fenpropatrin, fenvalerate, tetramethrin

KEY WORDS
chiral

REFERENCE

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
Almotriptan

**Molecular formula:** C_{17}H_{25}N_{3}O_{2}S

**Molecular weight:** 335.47

**CAS Registry No:** 154323-57-6

**Merck Index:** 13, 301

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**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 (?)).

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**HPLC VARIABLES**

**Guard column:** Guardpak µ Bondapak 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

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**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

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**KEY WORDS**

plasma, SPE

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**REFERENCE**


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**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.

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**HPLC VARIABLES**

**Guard column:** GuardPak µ Bondapak CN

**Column:** 300 × 3.9 10 µm µ Bondapak

**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

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**CHROMATOGRAM**

**Retention time:** 25

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**OTHER SUBSTANCES**

**Extracted:** metabolites
KEY WORDS
human, liver

REFERENCE
Alosetron

Molecular formula: C₁₇H₁₈N₄O
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°C, 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
Amcinonide

Molecular formula: C28H35FO7
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-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), clobetasol 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...
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)

**REFERENCE**  

**KEY WORDS**  
cosmetics; SPE
Amcinonide

**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

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**CHROMATOGRAM**

**Retention time:** 10.90

**Limit of detection:** 0.001%

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**OTHER SUBSTANCES**

**Extracted:**

**Simultaneous:** alclometasone 17,21-dipropionate (10.93), beclometasone 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), diflucorsone 17,21-diacetate (9.81), flucinolone acetonide (7.38), flucinonide (9.79), flurandrenolide (7.36), fludrocortisone 21-acetate (7.77), flurometholone (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 (5.62), 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-tebaxone (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).

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**KEY WORDS**

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

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**REFERENCE**

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 $\alpha$-phthalaldehyde in 500 µL MeOH, add 5 mL 100 mM sodium tetraborate, add 20 µL mercaptoethanol, mix.)

HPLC VARIABLES
Column: $150 \times 3.9$ 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

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: $150 \times 4.6$ 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
Aminolevulinic acid

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

KEY WORDS
derivatization; plasma; protect from light

REFERENCE

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

REFERENCE

ANNOTATED BIBLIOGRAPHY
Aminolevulinic acid


Amprenavir

**Molecular formula:** C_{25}H_{35}N_{3}O_{6}S

**Molecular weight:** 505.64

**CAS Registry No:** 161814-49-9

**Merck Index:** 13, 594

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**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.

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**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

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**CHROMATOGRAM**

**Retention time:** 5.6

**Limit of quantitation:** 25 ng/mL

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**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, acemaminophen, acetylcysteine, acyclovir, albendazole, alimemazine, alizapride, amikacin, amiodarone, amphotericin B, ampicillin, aspirin, bepridil, buprenorphine, butobarbital, caffeine, calcium folinate, captopril, carbamazepine, carbamylamide, chloroquine, ciprofloxacin, clindamycin, clofazimine, clofibrate, clonazepam, cioridazine, clozapine, cocaine, codeine, cymemazine, dantrolene, dexamethasone, dextropropoxyphene, diazepam, diclofenac, didanosine, digoxin, dihydroergotamine, diltiazem, doxycycline, ethambutol, flecainide, fluconazole, furosemide, fosarnet, furosemide, ganliclovir, gentamicin, glibenclamide, granisetron, halofantrine, haloperidol, hydrocortisone, imipramine, indomethacin, interferon alpha, isoniazid, itraconazole, josamycin, ketocoazol, lamivudine, levomepromazine, lidocaine, loperamide, loratadine, lornazepam, metoflamine, mepropamate, methadone, methylprednisolone, metoclopramide, metronidazole, mianserin, moclobemide, morphine, nevirapine, nifedipine, niflumic acid, nitrofurantoin, omeprazole, paroxetine, pentamidine, phenobarbital, phenoxytoin, piroxicam, prazosin, prednisolone, pridamnol, primidone, propranolol, quinidine, quinine, ranitidine, ribavirin, rizabutin, rifampin, roxithromycin, salicylic acid, simvastatin, stavudine, sulfadiazine, sulfamethoxazole, sulpiride, thalidomide, theophylline, trimethoprim, valproic acid, venlafaxine, vigabatrin, vloxazine, zidovudine, zolpidem, zopiclone

**Interfering:** delavirdine, flunitrazepam
KEY WORDS
plasma; SPE

REFERENCE

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

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

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, aminopterin, 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

KEY WORDS
plasma

REFERENCE

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

KEY WORDS
human; liver; rat
Amprenavir 39

REFERENCE

ANNOTATED BIBLIOGRAPHY
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Faux, J.; Venisse, N.; Le Moul, 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, J.Chromatographia 2003, 58, 421–426. [amprenavir; indinavir; lopinavir; ritonavir; saquinavir; efavirenz; nevirapine; prazepam]


Tribout, O.; Arvieux, C.; Michelet, C.; Chaplain, 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; delavirdine; saquinavir]


Anagrelide

Molecular formula: C₁₀H₇Cl₂N₃O
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 µ Bondapak 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 (¹⁴C)

CHROMATOGRAM
Retention time: 6–8

KEY WORDS
plasma; radiolabeled

REFERENCE
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 (35S); ELISA

### CHROMATOGRAM

**Retention time:** 20

### KEY WORDS

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

### REFERENCE


### 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


### ANNOTATED BIBLIOGRAPHY

Apraclonidine

Molecular formula: C₉H₁₀Cl₂N₄
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
Aprepitant

Molecular formula: C23H21F7N4O3
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

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 (14C)

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

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

HPLC VARIABLES
Column: 20 × 25 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
Aranidipine

Molecular formula: C₁₉H₂₀N₂O₇
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
Arotinolol

Molecular formula: C₁₅H₂₁N₃O₂S₃
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

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
Arotinolol

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

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
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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; acetylsalicylic acid; alpenolol; arotinolol; atenolol; benzoin; biperiden; bunitrolol; chloromezamine; chlorphenesin; eperisone; flavone; oxprenolol; phenylethyl alcohol; phenylethyamine; pindolol; proglumide; propranolol; trihexyphenidyl]
Arteether

**Molecular formula:** C17H28O5  
**Molecular weight:** 312.40  
**CAS Registry No:** 75887-54-6  
**Merck Index:** 13,822

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**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.

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**HPLC VARIABLES**  
**Column:** 300 × 4.65 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

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**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

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**KEY WORDS**  
rat; serum

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**REFERENCE**  

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**SAMPLE**  
**Matrix:** solutions

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**HPLC VARIABLES**  
**Column:** 250 × 4.65 µm IBO-SIL C18 (Phenomenex)  
**Mobile phase:** MeOH:water 80:20  
**Flow rate:** 0.9  
**Detector:** UV 260

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**REFERENCE**  
ANNO TED BIBLIOGRAPHY
Articaine

Molecular formula: C_{13}H_{20}N_{2}O_{3}S
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 Bondapak 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

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°C 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

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

ANNOTATED BIBLIOGRAPHY
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Rop, P.P.; Grimaldi, F.; Bresson, M.; Fornaris, M.; Viala, A. Liquid chromatographic analysis of cocaine, benzoylcegonine, local anaesthetic agents and some of their metabolites in biological fluids, *J.Liq.Chromatogr.*, **1993**, *16*, 2797–2811. [cocaine; benzoylcegonine; procaine; p-aminobenzoic acid; butacaine; tetracaine; articaine; prilocaine; o-toluidine; lidocaine; monoethylglycine xylidide; bupivacaine; piperoclyxilidene; etidocaine; dibucaine; caffeine; amphetamine; ephedrine; epinephrine; morphine; monoacetylmorphine; diamorphine; ethylmorphine; codeine; acetyldcodeine; fluorescence detection; UV detection; SPE]

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
Atazanavir sulfate

**Molecular formula:** C₃₈H₅₂N₆O₇.H₂O₄S  
**Molecular weight:** 802.93  
**CAS Registry No:** 229975-97-7

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**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.

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**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, Turbolon gas nitrogen 8 L/min, collision energy 42 V, collision cell exit potential 24 V, dwell time 500 ms, pause time 5 ms

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**CHROMATOGRAM**  
**Retention time:** <4  
**Internal standard:** ¹³C₆-atazanavir  
**Limit of quantitation:** 5 fmole/million cells

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**KEY WORDS**  
peripheral blood mononuclear cells; SPE

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**REFERENCE**  

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**SAMPLE**  
**Matrix:** blood  
**Sample preparation:** Condition each well of a 10 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 µ bar, 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:** 13C6-atazanavir

**Limit of quantitation:** 1 ng/mL

**KEY WORDS**

plasma; SPE

**REFERENCE**

Atipamezole

Molecular formula: C14H16N2
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°, vapor-izer 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

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

ANNOTATED BIBLIOGRAPHY
Atomoxetine hydrochloride

**Molecular formula:** C₁₇H₂₁NO.HCl  
**Molecular weight:** 291.82  
**CAS Registry No:** 82248-59-7

![Molecular structure of atomoxetine hydrochloride]

**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 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**  

**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 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**

Atorvastatin

Molecular formula: C33H35FN2O5
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

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 x 3 5 µm C18 Luna (Phenomenex)
Column: 250 x 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

ANNOTATED BIBLIOGRAPHY

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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]

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

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