MONOGRAPHS

ABACAVIR

CHEMISTRY

Abacavir is an antiretroviral. Its chemical name is {(1*S*,4*R*)-4-[2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl] cyclopent-2-enyl}methanol. Other names include Abakavir and Ziagen. Its molecular formula is $C_{14}H_{18}N_6O$, with a molecular weight of 286.3 and a CAS number of 136470-78-5.

METHODS

Assay 1 Dogan-Topal et al. [1] reported an HPLC method for the simultaneous determination of abacavir, efavirenz, and valganciclovir in human serum. An Agilent Technologies 1100 series liquid chromatograph was equipped with a model G1315B diode-array detector (DAD) and model G1329 ALS autosampler. The stationary phase was a Waters Spherisorb column (250 × 4.6 mm, 5 μm particle size). The mobile phase consisted of acetonitrile, methanol, and monobasic potassium phosphate buffer (pH 5.0) (40 : 20 : 40, vol/vol/vol) and was isocratically delivered at 1.0 mL/min. The runtime was 15 min. The injection volume was 20 μL.

Stock solutions of abacavir, efavirenz, valganciclovir, and fluvastatin (internal standard) at 1.0 mg/mL each were prepared in methanol–water (50 : 50, vol/vol). Working solutions were prepared by diluting stock solutions with methanol–water and were stored at −20°C. Standards were prepared by spiking drug-free serum with working solutions. A standard or serum sample (1.0 mL) was mixed with 1.0 mL of acetonitrile, vortexed for 5 min, and centrifuged at 5000 *g* for 10 min. The supernatant was collected and assayed. Retention times for abacavir, efavirenz, valganciclovir, and fluvastatin were 4.1, 11.6, 3.4, and 5.5 min, respectively.

A calibration curve for abacavir was constructed in the concentration range of 50–30,000 ng/mL. The correlation coefficient was 0.999. The coefficient of variation of the assay was 0.3%. Recovery of the drug from serum was better than 98.8%. Limit of detection and limit of quantification were 3.80 and 12.68 ng/mL, respectively. No interference was found from endogenous substances.

Assay 2 Verweij-van Wissen et al. [2] developed an HPLC-UV method for the simultaneous determination of abacavir, didanosine, lamivudine, stavudine, and zidovudine in patient plasma samples. The liquid chromatograph consisted of ThermoElectron model P4000 solvent delivery pump, model AS3000 autosampler, model UV2000 programmable wavelength UV detector, and a ChromJet integrator. The stationary phase was a Waters SymmetryShield RP18 column (150 × 4.6 mm, 3.5 μm particle size) coupled with a SymmetryShield RP18 guard column (20 × 3.9 mm, 3.5 μm particle size). The column temperature was set at 30°C. Solvent A was a mixture of 20 mM potassium acetate buffer (pH 4.60) and acetonitrile (95 : 5, vol/vol), and solvent B consisted of 20 mM potassium acetate buffer (pH 4.60) and acetonitrile (76 : 24, vol/vol). The mobile phase was delivered at 100% A from 0 to 10 min, decreased linearly to 0% from 10 to 24 min, returned to 100% A from 24 to 26 min, and reequilibrated at 100% A for an additional 9 min. The flow rate was 1.0 mL/min. UV detection was performed at 260 nm.

A stock solution of these drugs (0.5 mg/mL each) was prepared in methanol/water (1 : 9). Standards were prepared by fortifying blank human plasma with the stock solution. All solutions were stored at −20°C. Waters Oasis MAX solid-phase extraction (SPE) columns were conditioned with 0.5 mL of methanol followed by 0.25 mL of water. A plasma sample (0.5 mL) and an aliquot (0.5 mL) of water were loaded onto a preconditioned SPE column, drawn through under vacuum, washed with 0.15 mL of water 2 times, dried under vacuum for 5 min, eluted by 0.25 mL of methanol/water (80 : 20, vol/vol) twice, evaporated to dryness at 40°C under a stream of nitrogen, reconstituted in 0.2 mL of acetonitrile/water (5 : 95, vol/vol), vortexed for 20 s, centrifuged for 5 min, and assayed. The injection volume was 25 μL. Under these conditions, retention times for lamivudine, didanosine, stavudine, zidovudine, and abacavir were about 4.3, 5.3, 6.2, 18.7, and 21.3 min, respectively.

Calibration curves for abacavir were linear over the concentration range of 0.015–5 mg/L. Average recovery for abacavir was 101.0%. The accuracy ranged from 97% to 100%. Intraday and interday coefficients of variation were less than 1.9% and 2.3%, respectively. The limit of quantification was 0.015 mg/L.

This assay was free of interference from acetaminophen, acyclovir, amphotericin B, amoxycillin, amprenavir, atovaquone, caffeine, calcium folinate, carbamazepine, clarithromycin, clindamycin, clofazimine, dapsone, domperidone, efavirenz, erythromycin, ethambutol, famotidine, fluconazole, ganciclovir, indinavir, isoniazid, itraconazole, ketoconazole, lidocaine, lopinavir, methadone, nelfinavir, nevirapine, ofloxacin, oxazepam, pentamidine, phenobarbital, phenytoin, pyrazinamide, pyrimethamine, rifabutin, rifampicine, ritonavir, saquinavir, sulfamethoxazole, sulfametrol, tenofovir, trimethoprim, valproic acid, zalcitabine, and its metabolites. Over 1500 patient samples were analyzed using this assay in pharmacokinetic studies.

Assay 3 Aymard et al. [3] developed an HPLC method for simultaneous determination of 12 antiretroviral drugs in human plasma. For the analysis of amprenavir, indinavir, nelfinavir, ritonavir, saquinavir, and efavirenz, a ThermoQuest liquid chromatographic system equipped with an isocratic P1000 pump, AS3000 autosmpler, PC1000 integrator, UV1000 variable-wavelength UV detector, and a Waters column heater was used. The stationary phase was a Waters Symmetry C_{18} column (250 × 4.6 mm, 5 μm particles size) protected by a Waters Guard-Pak μBondapak C_{18} precolumn. The column temperature was maintained

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at 37°C. The mobile phase was composed of 0.04 M dibasic sodium phosphate buffer with 4% (vol/vol) 0.25 M octanesulfonic acid and acetonitrile (50 : 50, vol/vol) and was delivered at 1.3 mL/min. UV detection was carried out at 261 nm between 0 and 9 min, at 241 nm between 9 and 20 min, and at 254 nm between 20 and 32 min. The injection volume was 100 μL.

For the determination of abacavir, didanosine, lamivudine, stavudine, zidovudine, delavirdine, and nevirapine, a liquid chromatographic system consisting of three Beckman model 114M pumps, a Waters model WISP 717 Plus autosampler, a model 481 variable-wavelength UV detector, a Shimadzu RF551 fluorescence monitor, two Lea Switch I&T switch valves, a Cil Cluzeau Croco-Cil column heater, and a Beckman System Gold 2 integrator was used. The stationary phase was a Waters SymmetryShield C_{18} column (250 × 4.6 mm, 5 μm particle size) protected by an UpChurch filter (2 μm). The column temperature was maintained at 30°C. The mobile phase consisted of monobasic potassium phosphate buffer with 1% (vol/vol) 0.25 M octanesulfonic acid and acetonitrile. Mobile phase 1 contained 5% (vol/vol) acetonitrile and was delivered at 1 mL/min from 0 to 12 min, mobile phase 2 contained 20% (vol/vol) acetonitrile and was delivered at 1 mL/min from 12 to 35 min, and mobile phase 3 contained 70% (vol/vol) acetonitrile and was delivered at 1.2 mL/min from 35 to 40 min. UV detection was performed at 260 nm. The fluorescence detector for delavirdine was set at an excitation wavelength of 305 nm and emission wavelength of 425 nm, respectively. The injection volume was 150 μL.

Stock solutions of abacavir, didanosine, lamivudine, and stavudine at 1 mg/mL were prepared in water and stored at 4°C. Stock solutions of zidovudine, delavirdine, efavirenz, amprenavir, indinavir, nelfinavir, ritonavir, and saquinavir at 1 mg/mL and neviapine at 5 mg/mL were prepared in methanol and stored at −20°C. Working solutions of didanosine, stavudine, and zidovudine at 100 μg/mL were prepared by diluting stock solutions with water. Working solutions of saquinavir and efavirenz at 500 μg/mL were prepared by diluting stock solutions with methanol. All working solutions were stored at 4°C. Standards were prepared by spiking drug-free human plasma with working solutions. A plasma sample or standard (1 mL) was loaded onto a J. T. Baker C_{18} extraction column that was preconditioned with 3 mL of methanol followed with 3 mL of distilled water, drawn through the column under pressure, washed with 2 mL of distilled water, dried under vacuum for 1 min, and eluted with 2.6 mL of methanol. The eluate was divided into two aliquots of 1 and 1.6 mL. These two solutions were evaporated to dryness at 40°C under a gentle stream of nitrogen. One residue was reconstituted with 200 μL of water for the analysis of abacavir, didanosine, lamivudine, stavudine, zidovudine, and nevirapine. The other residue was reconstituted with 150 μL of mobile phase for the analysis of amprenavir, indinavir, nelfinavir, ritonavir, saquinavir, and efavirenz. Retention times for indinavir, amprenavir, ritonavir, efavirenz, saquinavir, nelfinavir, lamivudine, didanosine, stavudine, zidovudine, abacavir, and nevirapine were 4.8, 5.6, 12.9, 15.2, 16.8, 29.2, 8.5, 9.6, 11.1, 17.4, 20.9, and 27.9 min, respectively.

A calibration curve for abacavir was constructed in the concentration range of 20–2,000 ng/mL. The correlation coefficient was greater than 0.998. Within-day and between-day coefficients of variation were less than 9.5% and 9.9%, respectively. The limit of quantification was 20 ng/mL. There was no interference with the analysis of amprenavir from the following coadministered drugs except sulpiride: acebutolol, acetaminophen, acetylcysteine, acetylsalicylic acid, acyclovir, albendazole, alimemazine, alizapride, amikacin, amiodarone, amphotericin B, ampicillin, bepridil, buprenorphine, butobarbital, caffeine, calcium folinate, captopril, carbamazepine, carbutamide, chloroquine, ciprofloxacin, clindamycin, clofazimine, clofibrate, clonazepam, clonidine, cloxacillin, clozapine, cocaine, codeine, cortisol, cyamemazine, dantrolene, dexamethasone, dextropropoxyphene, diazepam, diclofenac, digoxin, dihydroergotamine, diltiazem, doxycycline, ethambutol, flecainide, fluconazole, flunitrazepam, fluoxetine, fluvoxamine, foscarvir, furosemide, ganciclovir, gentamicin, glibenclamide, granisetron, halofantrine, haloperidol, imipramine, indomethacin, interferon alfa, isoniazid, itraconazole, josamycin, ketoconazole, levomepromazine, lidocaine, loperamide, loratadine, losartan, mefloquine, meprobamate, methadone, methylprednisolone, metoclopramide, metronidazole, mianserin, moclobemide, morphine, nifedipine, niflumic acid, nitrofurantoin, omeprazole, paroxetine, pentamidine, phenobarbital, phenytoin, piracetam, prazosin, prednisolone, prednisone, primidone, propranolol, quinidine, quinine, ranitidine, ribavirin, rifabutine, rifampicin, roxithromycin, salicylic acid, simvastatin, sulfadiazine, sulfamethoxazole, thalidomide, theophylline, trimethoprim, valproic acid, venlafaxine, vigabatrin, viloxazine, zolpidem, and zopiclone.

More than 500 plasma samples were assayed on each column without significant loss of resolution.

Assay 4 Saux et al. [4] reported the simultaneous determination of abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, and zidovudine in human plasma by high-performance liquid chromatography with tandem mass spectrometry. A ThermoFinnigan Accela liquid chromatograph was coupled with a ThermoFinnigan TSQ Quantum Discovery Max triple quadrupole mass spectrometer through a heated-electrospray ionization (HESI) interface. The stationary phase was a Waters Atlantis T3 column (100 × 2.1 mm, 3 μm particle size). The column temperature was maintained at 40°C and autosampler temperature, at 10°C. Solvent A was 0.05% formic acid in water, and solvent B was 0.05% formic acid in methanol. The mobile phase was delivered at 0.250 mL/min at 5% B from 0 to 3 min, linearly increased from 5% to 40% B from 3 to 8 min, and kept at 5% B from 8 to 14 min. The injection volume was 10 μL.

The mass spectrometer was operated in the positive mode: source temperature 50°C, capillary temperature 270°C, capillary voltage 5 kV, nebulizing gas (nitrogen) 35 psi (lb/in.^{2}), auxiliary gas (nitrogen) 30 units, and collision gas (argon) 1.5 mTorr. Abacavir was monitored in the selected-reaction monitoring (SRM) mode: m/z 287.1 → 190.0 at collision energy 20 V and tube lens 120 V. 6-β-Hydroxytheophyline (internal standard) was observed

in SRM mode: m/z 225.1 → 181.3 at collision energy 18 V and tube lens 110 V.

Stock solutions of abacavir and tenofovir at 1000 μg/mL each were prepared in water and didanosine, emtricitabine, lamivudine, stavudine, and zidovudine at 1000 μg/mL in methanol. Working solutions of zidovudine, stavudine, and abacavir (40 μg/mL each), lamivudine, didanosine, and emtricitabine (20 μg/mL each), and tenofovir (10 μg/mL) were prepared by diluting stock solutions with water. A stock solution of 6-β-hydroxytheophyline (internal standard) at 1000 μg/mL was prepared in water and diluted to 2 μg/mL with water. An aliquot (100 μL) of drug-free human plasma was spiked with 50 μL of a working solution, whereas a plasma sample (100 μL) with 50 μL of water, mixed with 50 μL of the internal standard, protein-precipitated with 500 μL of acetonitrile, vortexed for 30 s, allowed to stand at room temperature for 15 min, and centrifuged at 2200 *g* for 10 min at ambient temperature. The supernatant was collected, evaporated to dryness at 35°C under a stream of nitrogen, reconstituted in 500 μL of water, and centrifuged at 2200 *g* for 10 min. The supernatant was assayed. Retention times for abacavir and the internal standard were 8.36 and 8.21 min, respectively.

A calibration curve for abacavir was constructed in the concentration range of 0.020–4.000 μg/mL. The correlation coefficient was 0.995. Within-day and between-day coefficients of vaiation were less than 4.62% and 10.00%, respectively. The recovery ranged from 83.8% to 90.6%.

The observed ion suppression did not influence quantitation of the analytes. No interference was found from endogenous substances or other drugs such as tipranavir, darunavir, fosamprenavir, ritonavir, lopinavir, saquinavir, atazanavir, indinavir, efavirenz, nevirapine, etravirine, enfivirtide, raltegravir, voriconazole, posaconazole, caspofungin, amphotericin B, fluconazole, ceftazidime, ceftriaxone, cefotaxime, ciprofloxacin, perfloxacin, rifampicin, rifabutin, and isoniazid. More than 1000 samples were analyzed for therapeutic drug monitoring using this method.

Assay 5 Notari et al. [5] developed an HPLC-UV assay for the simultaneous analysis of 16 anti-HIV drugs—abacavir, amprenavir, atazanavir, didanosine, efavirenz, emtricitabine, indinavir, lamivudine, lopinavir, nelfinavir, nevirapine, ritonavir, saquinavir, stavudine, zalcitabine, and zidovudine—in human plasma. A Waters liquid chromatograph consisting of a Waters model 600 pump, model 717 Plus autosampler, and model 2487 UV–visible detector was used. The stationary phase was a Waters Symmetry C_{18} column (250 × 4.6 mm, 5 μm particle size) protected by a Waters Sentry guard column (20 × 3.9 mm) of the same packing material. The mobile phase consisted of 0.01 M monobasic potassium phosphate buffer, and acetonitrile and was delivered at 1.0 mL/min in a gradient mode. The percentage of the phosphate buffer was decreased from 94% to 40% in 10 min, maintained at 40% phosphate buffer for 10 min, further decreased to 0% in 5 min, and returned to 94% phosphate buffer in 10 min. The total runtime was 40 min. UV detections were performed at 240 and 260 nm. The injection volume was 20 μL.

Stock solutions of 16 drugs at 1.0 mg/mL each were individually prepared in methanol. Working solutions were prepared by diluting stock solutions in methanol and were stored at 4°C. Standards were prepared by spiking drug-free human plasma with working solutions. An aliquot of 600 μL of plasma or standard was mixed with 100 μL of methanol, vortexed for 1 min, and centrifuged at 13,000 rpm (rev/min) for 6 min. The supernatant was diluted with 1 mL of water; loaded onto a Waters Oasis HLB cartridge (30 mg/1 mL), which was preconditioned with 1 mL of methanol followed by 1 mL of water; washed with 1 mL of 5% methanol in water; eluted with 550 μL of 0.01 monobasic potassium phosphate buffer, followed by 2 mL of absolute methanol; evaporated to dryness at 36°C under a steam of nitrogen; reconstituted with 100 μL of methanol; and assayed. Under these conditions, retention times (in minutes) were 4.1 (lamivudine), 6.2 (zalcitabine), 7.8 (emtricitabine), 8.6 (didanosine), 9.7 (stavudine), 15.1 (abacavir), 16.2 (zidovudine), 16.6 (nevirapine), 18.1 (indinavir), 19.2 (saquinavir), 19.9 (amprenavir), 21.1 (nelfinavir), 23.1 (ritonavir), 24.5 (lopinavir), 28.4 (efavirenz), and 32.0 (atazanavir).

Calibration curves for abacavir were constructed over the range from 0.025 to 10 μg/mL. Correlation coefficients were 0.9959. The recovery in plasma was 93.6%. The accuracy expressed as the relative percentage error was 8.8%. Intraday and interday coefficients of variation were 8.9% and 9.9%, respectively. The limit of quantification was 0.025 μg/mL. This assay was used routinely at the researchers' institute for therapeutic drug monitoring in HIV-infected patients.

Assay 6 Rezk et al. [6] reported the simultaneous determination of zalcitabine, lamivudine, didanosine, stavudine, zidovudien, abacavir, and nevirapine in human plasma by HPLC with UV detection. An Agilent series 1100 liquid chromatograph consisting of a binary pump, a degasser, an autosampler, and a UV photodiode-array detector was used. The stationary phase was a Waters Polarity dC_{18} column (150 × 3.9 mm, 5 μm particle size) with a guard column (20 × 3.9 mm, 5 μm particle size) of the same packing material. The column temperature was maintained at 40°C. Mobile phase A was 10 mM ammonium acetate buffer adjusted to pH 6.5 with diluted acetic acid. Mobile phase B was a mixture of 200 mL of mobile phase A, 500 mL of acetonitrile, and 300 mL of methanol. The mobile phase was delivered at 1.1 mL/min in a gradient mode. The mobile phase B was delivered at 4% for 15 min, linearly increased to 64% over next 15 min, held at 64% for 3 min, and pumped at 4% for another 7 min. UV detection was performed at 269 nm from 0 to 11 min, at 250 nm from 11 to 14 min, at 271 nm from 14 to 24 min, and at 230 nm from 24 to 33 min. The injection volume was 80 μL.

Stock solutions of zalcitabine, didanosine, and stavudine at 1.0 mg/mL each were separately prepared in water. Stock solutions of lamivudine, abacavir, zidovudine, and nevirapine at 1.0 mg/mL were individually prepared in a mixture of methanol and water (60 : 40). Working solutions were prepared by diluting stock solutions with water. Standards were prepared by spiking human plasma

with working solutions. A stock solution of hexobarbital at 1.0 mg/mL was prepared in acetonitrile. A working solution of hexobarbital at 2.0 μg/mL was prepared by diluting stock solution with 25 mM ammonium acetate buffer (pH 7.0) and was used as an internal standard. An aliquot of 1.0 mL of plasma sample or standard was spiked with internal standard; loaded onto a Varian Bond Elut C_{18} solid-phase extraction cartridge (100 mg/1 mL), which was preconditioned with 1 mL of methanol followed with 1 mL of 100 mM ammonium acetate buffer (pH 7.0); passed through the cartridge; washed with 1 mL of 100 mM ammonium acetate buffer (pH 7.0); dried under vacuum for 1 min, eluted with 800 μL of methanol; evaporated to dryness at 40°C under a stream of nitrogen; reconstituted with 100 μL of mobile phase; vortexed for 30 s; and centrifuged at 18,000 *g* for 3 min. The supernatant was separated and assayed. Under these conditions, retention times for zalcitabine, lamivudine, didanosine, stavudine, zidovudine, abacavir, nevirapine, and hexobarbital were 5.9, 8.6, 13.6, 15.7, 23.8, 25.1, 27.3, and 30.6 min, respectively.

Calibration curves for abacavir were constructed over the range from 10 to 10,000 ng/mL. Correlation coefficients were greater than 0.998. The accuracy was 101%. Intraassay and interassay coefficients of variation were less than 7% and 7.6%, respectively. The limit of quantification was 10 ng/mL. There were no interferences from either endogenous compounds in plasma or drugs such as indinavir, amprenavir, saquinavir, nelfinavir, ritonavir, lopinavir, delavirdine, efavirenz, tenofovir, and atorvastatin.

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ABECARNIL

CHEMISTRY

Abecarnil is a β-carboline compound and is studied as an anxiolytic and anticonvulsant. Its chemical name is isopropyl 6-(benzyloxy)-4-(methoxymethyl)-9*H*-pyrido(3,4-b)indole-3-carboxylate. Other names include Abecarnilo and ZK112119. Its molecular formula is $C_{24}H_{24}N_2O_4$, with a molecular weight of 404.5 and a CAS number of 111841-85-1.

METHOD

Assay 1 Krause et al. [1, 2] described the determination of abecarnil by HPLC with fluorescence detection. A liquid chromatographic system was composed of a Waters model 6000A pump, model 710B WISP autosampler, and a Kratos model MS970 fluorescence detector. The stationary phase was a Spherisorb ODS II column (125 × 4.6 mm, 5 μm particle size). The mobile phase consisted of methanol and 0.01 M ammonium carbonate buffer (70 : 30, vol/vol) and was isocratically delivered at 1.5 mL/min. Fluorescence detector was set at an excitation wavelength of 295 nm and an emission wavelength of 418 nm. The injection volume was 200 μL.

5-Benzyloxyabecarnil was used as an internal standard. Plasma or urine (0.5 mL) was mixed with 2.5 mL of diethylether, shaken for 15 min, centrifuged at room temperature at 1500 *g* for 10 min, and frozen. The organic phase (top layer) was collected, evaporated to dryness under a stream of nitrogen, reconstituted with 250 μL of the mobile phase, and assayed.

A linear calibration curve for abecarnil was constructed in the concentration range of 0.1–200 ng/injection. The recovery of the drug from plasma was about 90%. The between-day (interday) coefficient of variation was less than 11%. The limit of detection was 0.2–0.5 ng/mL. No interference was found from endogenous substances.

A similar method was used by Karara et al. [3].

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ACAMPROSATE CALCIUM

CHEMISTRY

Acamprosate calcium is used in treatment of alcohol dependence. Its chemical name is calcium 3-acetamido-1-propanesulfate. Other names include Acamprosatum

Calcicum, Campral, and Zulex. Its molecular formula is $C_{10}H_{20}CaN_2O_8S_2$, with a molecular weight of 400.5 and a CAS number of 77337-73-6. Acamprosate calcium is a white powder. Acamprosate calcium is freely soluble in water and practically insoluble in ethanol and dichloromethane.

METHODS

Assay 1 Luo et al. [1] developed a LC-MS/MS method for the determination of acamprosate calcium in human plasma. A Shimazdu 10AVP liquid chromatograph included model LC10ADVP pump, model SIL-HTc autosampler, and model CTO10ASVP column oven. The stationary phase was a Phenomenex Gemini C_{18} column (50 × 3.0 mm, 3 μm particle size). The column temperature was maintained at 50°C. The mobile phase consisted of 10 mM ammonium acetate and methanol (95 : 5), adjusted to pH 7.4 with ammonia. The flow rate was 0.2 mL/min.

An API3000 tandem mass spectrometer coupled to the liquid chromatograph was operated in a negative electrospray ionization mode. Acamprosate calcium was monitored in multiple reaction monitoring mode: m/z 180 → 80. The operating parameters were as follows: nebulizer gas 13, curtain gas 10, core energy −55 V, capillary energy −3.6 kV, collision energy −32 V, and source temperature 450°C.

Acamprosate calcium stock solution 200 mg/L was prepared in water and stored at 4°C. Working solutions were prepared by diluting the stock solution with water. Standards were prepared by spiking the blank human plasma with working solutions. Patient plasma or standards (150 μL) were mixed with 500 μL of acetonitrile and centrifuged at 16°C at 10,000 rpm for 8 min. Supernatants (500 μL) were collected, evaporated to dryness at 50°C in a water bath under a stream of air, reconstituted in 100 μL of the mobile phase, mixed with 1.0 mL of dichloromethane, and centrifuged at 16°C at 2000 rpm for 3 min. Supernatants were collected and assayed. The injection volume was 15 μL. Under these conditions, the retention time of acamprosate calcium was 2.4 min.

A calibration curve for acamprosate calcium was constructed in the range from 2 to 2048 μg/L. The correlation coefficient was 0.9999. Within-day and between-day coefficients of variation were less than 4.0% and 11.6%, respectively. Recoveries of acaprosate calcium in plasma ranged from 83.6% to 94.4%. The limit of detection was 2.0 μg/L.

Assay 2 Girault et al. [2] described the determination of acamprosate calcium (calcium acetylhomotaurinate) in human plasma and urine by gas chromatography–mass spectrometry (GC-MS). A Hewlett-Packard 5985B gas chromatograph–mass spectrometer was utilized. The injection port was maintained at 320°C. The oven temperature was programmed from 240°C to 310°C at 10°C/min and held at 310°C for additional 2 min. Helium was used as the carrier gas. The stationary phase was a Chrompack fused-silica capillary column (25 m × 0.35 mm) wall-coated with an OV1701 liquid phase. The film thickness and inner side diameter of the capillary column were 0.2 μm and 0.25 mm, respectively.

The mass spectrometer was set in the negative-ion chemical ionization mode and operated at an electron energy of 100 eV, an emission current of 300 μA, and an ion-source temperature of 150°C. 4-Acetylaminobutane sulfonic acid was used as an internal standard. The drug and internal standard were monitored in a single-ion monitoring (SIM) mode: m/z = 424 and 317 for the pentafluorobenzoyl di-*n*-butylamide derivative of acamprosate calcium and m/z = 438 and 311 for the derivative of internal standard.

Stock solutions of acamprosate calcium at 1 μg/mL and internal standard at 10 μg/mL were individually prepared in water and stored in the dark at 4°C. Standards in plasma were prepared by spiking 1 mL of the blank human plasma with 50 μL of internal standard and desired amouts of the stock solution of the drug. Standards in urine were prepared by fortifying 50 μL of blank human urine with 50 μL of internal standard and various amounts of the drug stock solution. The drug was extracted from plasma and urine and derivatized as described below. A plasma sample or standard (1 mL) was mixed with 1 mL of acetonitrile, vortexed for 20 s, and centrifuged at 1600 *g* for 15 min. The supernatant was collected, extracted with 5 mL of methylene chloride for 10 min, and centrifuged for 15 min. The aqueous layer was removed and hydrolyzed with 0.2 mL of 10 M hydrochloric acid at 100°C for 2 h. A urine sample or standard (50 μL) was hydrolyzed directly. The hydrolyzed solution was applied to a J. T. Baker C_{18} disposable solid-phase extraction cartridge that was preconditioned with 1 mL of methanol followed by 1 mL of distilled water. The eluate was collected and alkalized with 5 M sodium hydroxide solution. This solution was mixed with 20 μL of pentafluorobenzoyl chloride, adjusted to pH 3 with 2 M hydrochloric acid, washed twice with 3 mL diethyl ether, and centrifuged. The aqueous layer was collected, mixed with 0.1 mL of 10% tetrabutylammonium hydrogensulfate solution and 5 mL of methylene chloride at room temperature for 10 min, and centrifuged for 10 min. The organic layer was collected, evaporated to dryness at 45°C under a gentle stream of nitrogen, redisolved in 50 μL of thionyl chloride, incubated at 80°C for 30 min, mixed with 0.2 mL of 2 M di-*n*-butylamine in acetonitrile, acidified with 1 mL of 20% phosphoric acid, extracted with 4 mL of pentane, and centrifuged. The organic layer was collected, evaporated to dryness under a stream of nitrogen, reconstituted in 0.3 mL of ethyl acetate, and assayed. The injection volume was 1 μL. Under these conditions, retention times of acamprosate calcium and the internal standard were 5.5 and 6.2 min, respectively.

A calibration curve for acamprosate calcium was constructed daily in the concentration range of 3.12–800 ng/mL. The correlation coefficient was greater than 0.9999. The coefficient of variation of the assay was less than 7.9%. There was no interference with the analysis of the drug from endogenous compounds. This method was applied to the pharmacokinetic study of acamprosate calcium in 24 healthy volunteers.

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ACEBUTOLOL HYDROCHLORIDE

CHEMISTRY

Acebutolol is a cardioselective β-blocker. Its chemical name is (±)-3′-acetyl-4′-(2-hydroxy-3-isopropylaminopropoxy)-butyranilide hydrochloride. Other names include Abutol, Prent, and Sectral. Its molecular formula is $C_{18}H_{28}N_2O_4 \cdot HCl$, with a molecular weight of 372.9 and a CAS number of 34381-68-5. Acebutolol hydrochloride is a white or almost white crystalline powder. Acebutolol hydrochloride has solubilities of 200 mg/mL in water and 70 mg/mL in alcohol. It is very slightly soluble in acetone and dichloromethane and practically insoluble in ether. Acebutolol has an apparent pK_a of 9.4 in water.

METHODS

Assay 1 Umezawa et al. [1] described the simultaneous determination of four ß-blockers, acebutolol, labetalol, metoprolol, and propranolol in human plasma, using LC-MS/MS. An Agilent 1100 series system consisting of a model G1315A diode-array detector, model G1313A autosampler, and model G1322A vacuum membrane degasser was utilized. The stationary phase was a Shodex MSpak GF310 4B column (50 × 4.6 mm, 6 μm particle size) protected by a 2-μm SUMIPAX PG-ODS inline filter. Solvent A was 10 mM ammonium acetate aqueous solution and solvent B, acetonitrile. The mobile phase was delivered at 0.55 mL/min in a gradient mode at 100% A from 0 to 3 min, decreased to 0% A in 1 min, and kept at 0% A from 4 to 9.5 min. The column was reequilibrated at 100% A for 5.5 min.

An Applied Biosystems/MDS SCIEX API2000 triple quadrupole mass spectrometer equipped with a TurboIonSpray ion source was operated in the positive mode: TurboIonSpray temperature 490°C, ion source voltage 3 kV, ring voltage 390 V, nebulizer gas (high-purity air) 20 psi, heater gas (high-purity air) 80 psi, curtain gas (high-purity nitrogen) 40 psi, orifice voltage 41 V for acebutolol, 25 V for labetalol, 40 V for metoprolol, 51 V for propranolol, and 40 V for pindolol, collision gas (nitrogen) 4, collision energy −29 eV for acebutolol, −22 eV for labetalol, −25 eV for metoprolol, −25 eV for propranolol, and −25 eV for pindolol. Quantification was performed in selective-reaction monitoring (SRM) mode using ion transitions at m/z 337 → 116 for acebutolol, m/z 329 → 311 for labetalol, m/z 268 → 116 for metoprolol, m/z 260 → 116 for propranolol, and m/z 249 → 116 for pindolol, respectively.

Stock solutions of acebutolol, labetalol, metoprolol, propranolol, and pindolol (as internal standard) at 1 mg/mL were separately prepared in methanol. Working solutions were prepared by diluting stock solutions with 10 mM ammonium acetate in water. Calibrators were prepared by spiking drug-free plasma with working solution. An aliquot of 1 mL of a plasma sample or calibrator was mixed with 3 mL of 13.3 mM ammonium acetate aqueous solution and centrifuged at 9000 rpm for 10 min. The supernatant was filtered through a 0.2-μm Whatman GD/X syringe filter (13 mm) and assayed. The injection volume was 100 μL. Under these conditions, retention times of labetalol, metoprolol, acebutolol, propranolol, and pindolol were 6.6, 6.9, 7.2, 7.8, and 7.9 min, respectively.

Calibration curves for acebutolol were constructed in the range from 10 to 1000 ng/mL. Correlation coefficients were greater than 0.9992. The effect of ion suppression on the analysis of acebutolol was less than 29.8%. The recovery of acebutolol from plasma ranged from 74.4 to 89.9%. The accuracy ranged from 94.0% to 111%. Intraday and interday coefficients of variation were less than 2.9% and 5.6%, respectively. The limit of detection was 1 ng/mL.

Assay 2 Vieno et al. [2] developed an LC-MS/MS method for the detection of acebutolol, atenolol, metoprolol, sotalol, carbamazepine, ciprofloxacin, ofloxacin, and norfloxacin in drinking water, surface water, and sewage treatment plant water. An Agilent 1100 series system consisting of a binary pump, vacuum degasser, autosampler, and a thermostated column oven was used. The stationary phase was an Agilent Zorbax XDB C_{18} column (50 × 2.1 mm, 5 μm particle size) protected by an Agilent narrowbore guard column (12.5 × 2.1 mm, 5 μm particle size). The column temperature was maintained at 30°C. The mobile phase consisted of acetonitrile and 1% acetic acid in water and was delivered at 250 μL/min in a gradient mode from 3% to 28% acetonitrile in 12 min and to 53% acetonitrile in another 5 min, kept at 53% acetonitrile for 1 min, and then returned to the initial condition in 1 min. The column was equilibrated at 3% acetonitrile for 8 min.

A Micromass Quattro Micro triple quadrupole mass spectrometer equipped with an electrospray ionization interface was operated in positive mode: desolvation gas 640 L/h, nebulizing gas 30 L/h, collision gas (argon) 2.8×10^{-3} mbar, source temperature 120°C, and desolvation temperature 325°C. Cone voltage (V) and collision energy (eV) were 28 and 20 for acebutolol, 30 and 23 for atenololol, 25 and 15 for metoprolol, 30 and 23 for sotalol, 25 and 15 for alprenolol (internal standard), 29 and 18 for carbamazepine, 35 and 21 for dihydro-carbamazepine (internal standard), 30 and 17 for ciprofloxacin, 28 and 16 for norfloxacin, 29 and 18 for ofloxacin, and 28 and 18 for enrofloxacin (internal standard). Quantification was performed in multiple-reaction monitoring (MRM) mode using ion transitions at m/z 336.8 → 116.0 for acebutolol, m/z 267.0 → 144.9 for atenolol, m/z 267.9 → 190.9 for metoprolol, m/z 254.8 → 132.9 for sotalol, m/z 249.9 → 172.9 for alprenolol, m/z 237.0 → 193.9 for carbamazepine, m/z 239.0 → 193.9 for dihydrocarbamazepine, m/z 331.9 → 287.9 for ciprofloxacin, m/z 319.8 → 275.9 for norfloxacin,

m/z 361.8 → 317.9 for ofloxacin, and m/z 359.9 → 315.9 for enrofloxacin, respectively.

Stock solutions of drugs were prepared in methanol, except that antibiotics were prepared in a mixture of methanol and 0.01 M hydrochloric acid (1 : 1, vol/vol) and stored at −18°C. Working solutions were prepared daily by diluting stock solutions with the same solvents. Standards were prepared by spiking noncontaminated groundwater with working solutions and internal standards. A sample (100, 250, 500, and 1000 mL for sewage influent, sewage effluent, surface water, and groundwater, respectively) was adjusted to pH 10.0 with 2 M sodium hydroxide solution, spiked with 500 ng of the internal standards, and filtered through a 0.45 μm Schleicher & Schuell GF6 filter that was previously washed with *n*-hexane, acetone, methanol, and water. It was then loaded onto a Waters Oasis HLB solid-phase extraction cartridge (3 mL, 60 mg) by means of polytetrafluoroethylene (PTFE) (Teflon) tubes at flow rates of 2, 5, 10, and 20 mL/min (sewage influent water, sewage effluent water, surface water, and groundwater, respectively), which was preconditioned sequentially with 2 mL of *n*-hexane, 2 mL of acetone, 10 mL of methanol, and 10 mL of noncontaminated groundwater (pH adjusted to 10.0); pulled through the cartridge; washed with 2 mL of 5% methanol in 2% aqueous ammonium hydroxide; dried with a stream of nitrogen for 30 min; eluted with 4 × 1 mL of methanol; evaporated to near dryness under a stream of nitrogen; reconstituted with 20 μL of methanol and 480 μL of 1% acetic acid; and assayed. Under these conditions, retention times of sotalol, atenolol, norfloxacin, ofloxacin, ciprofloxacin, enrofloxacin, acebutolol, metoprolol, alprenolol, carbamazepine, and dihydrocarbamazepine were 3.3, 4.4, 9.8, 9.8, 10.2, 10.9, 11.1, 11.2, 15.4, 17.6, and 17.8 min, respectively.

Calibration curves for acebutolol were constructed in the range from 0.82 to 6000 μg/L. Correlation coefficients were greater than 0.996. Average recoveries of acebutolol from groundwater and tapwater, surface water, sewage effluent water, and sewage influent water were 93%, 105%, 78%, and 64%, respectively. Limits of quantification in drinking (potable) water, surface water, sewage effluent water, and sewage influent water were 0.4, 0.8, 2.1, and 6.4 ng/L, respectively.

This LC-MS/MS method was successfully applied for the determination of acebutolol in sewage influent water, sewage effluent water, and their recipient rivers.

Assay 3 Delamoye et al. [3] developed an HPLC method for simultaneous determination of 13 ß-blockers and one metabolite: atenolol, sotalol, diacetolol, carteolol, nadolol, pindolol, acebutolol, metoprolol, celiprolol, oxprenolol, labetalol, propranolol, tertatolol, and betaxolol. A Spectra liquid chromatographic system consisting of a model P1000XR quaternary gradient pump, model AS3000 autoinjector with a 100-μL loop, and model 6000LP photodiode-array detector was used. The stationary phase was a ThermoHypersil Hypurity C_{18} column (250 × 4.6 mm, 5 μm particle size) protected by a C_{18} precolumn (4 × 4.4 mm, 5 μm particle size). The column temperature was maintained at 35°C. UV detection was performed at 220 nm. The injection volume was 80 μL.

Stock solutions of these compounds at 1.0 g/L each were prepared in methanol. Working solutions were prepared by diluting stock solutions with methanol. A stock solution of medroxalol at 50.0 mg/L in methlanol was used as an internal standard. These stock and working solutions were stored at −20°C. An aliquot of 1 mL of plasma, standard, or control was spiked with 20 μL of the internal standard, mixed with 500 μL of 1 M sodium carbonate (pH 9.7), extracted with 7 mL of chloroform–pentanol–diethyl ether (6 : 2 : 1, vol/vol/vol), shaken for 15 min, and centrifuged at 3000 *g* for 5 min. The organic phase was collected, mixed with 250 μL of 0.05 M phosphoric acid (pH 2.1), shaken for 10 min, and centrifuged at 3000 *g* for 5 min. The aqueous phase was collected and assayed. Under these conditions, retention times for atenolol, sotalol, diacetolol, carteolol, nadolol, pindolol, acebutolol, metoprolol, celiprolol, oxprenolol, medroxalol, labetalol, propranolol, tertatolol, and betaxolol were 5.1, 5.6, 7.9, 9.2, 9.9, 10.5, 14.5, 15.4, 18.8, 20.4, 21.2, 21.8, 24.6, 25.1, and 25.9 min, respectively.

Calibration curves for acebutolol were constructed over the range from 25 to 1000 ng/mL. The mean correlation coefficient was 0.999. The mean accuracy was 100.1% at 100 ng/mL. The mean recovery ranged from 90 to 113%. Intraday and interday coefficients of variation were 6.27% and 6.60%, respectively. Limits of detection and quantification were 6 and 25 ng/mL, respectively.

Assay 4 Lee et al. [4] developed an LC-MS/MS method for the simultaneous determination of 12 ß-blockers and $ß_2$-agonists in sewage samples. A Waters 2695 separation module was used. The stationary phase was an Agilent Zorbax SB C_8 column (150 × 2.1 mm, 3.5 μm particle size) protected by a SB C_8 guard column(12.5 × 2.1 mm, 5 μm particle size). The column temperature was maintained at 35°C. Mobile phase A was a mixture of water, acetonitrile, and formic acid (94.5 : 5.0 : 0.5, vol/vol/vol), and mobile phase B was a mixture of acetonitrile and formic acid (99.5 : 0.5, vol/vol). The mobile phase was delivered in a gradient mode from 100% A to 75% A in 13 min, held at 75% A for 13 min, and then pumped at 100% A for another 14 min. The flow rate was 0.2 mL/min. The injection volume was 10 μL. The total runtime of an injection was 40 min.

A Micromass Quattro Ultima triple quadrupole mass spectrometer equipped with an electrospray ionization source was operated in the positive-ion mode. The major parameters were set as follows: nebulizer gas (nitrogen) 50 L/h, desolvation gas (nitrogen) 500 L/h, source temperature 120°C, desolvation temperature 350°C, capillary voltage 3.45 kV, cone energy 60 V, and collision energy 17 kV. Acebutolol was analyzed in the multiple-reaction monitoring (MRM) mode at ion transitions m/z 337 → 116 for quantitation and m/z 337 → 260 for confirmation.

Individual stock solutions of acebutolol, alprenolol, atenolol, bisoprolol, clenbuterol, fenoterol, labetalol, metoprolol, nadolol, pindolol, propranolol, sotalol, terbutaline, and timolol at 1000 μg/mL were prepared in acetonitrile or methanol. A stock solution of salbutamol was prepared at 500 μg/mL. These stock solutions were stored at −20°C. Working solutions were prepared by mixing and diluting

these stock solutions with mobile phase B. An aliquot of 250 mL of sewage sample was filtered through a 1.2-μm GF/C filter (90 mm i.d.) with a layer of Celite; acidified to pH 3 with 1 M hydrochloric acid; loaded onto a Waters Oasis MCX cartridge (6 mL, 150 mg, 30 μm) at a flow rate of 10–15 mL/min, which was preconditioned with 6 mL of methanol followed by 10 mL of water at pH 3; dried for 10 min under vacuum; washed with 100 mL of water at pH 3 followed by 6 mL of methanol; eluted with 8 mL of a mixture of dichloromethane, 2-propanol, and ammonium hydroxide (78 : 20 : 2, vol/vol/vol); evaporated to dryness at 40°C under a stream of nitrogen; reconstituted in 1.0 mL of mobile phase B; filtered through a 0.45-μm nylon syringe filter; and assayed. Under these conditions, retention times for acebutolol, alprenolol, atenolol, bisoprolol, labetalol, metoprolol, nadolol, pindolol, propranolol, sotalol, timolol, clenbuterol, fenoterol, salbutamol, and terbutaline were 17.36, 24.32, 8.96, 21.77, 22.11, 18.02, 13.60, 14.15, 23.87, 7.85, 17.02, 17.91, 11.50, 6.97, and 6.53 min, respectively.

Calibration curves for acebutolol were constructed over the range from 50 to 500 pg/μL. The mean recovery of the drug from water ranged from 88% to 95%. The limit of detection was 9 ng/L.

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ACECLOFENAC

CHEMISTRY

Aceclofenac is a NSAID (nonsteroidal anti-inflammatory drug). Its chemical name is [*o*-(2,6-dichloroanilino) phenyl]acetate glycolic acid ester. Other names include Aceclofar, Aceclofenaco, Aceclofenacum, Beofenac, and Preservex. Its molecular formula is $C_{16}H_{13}Cl_2NO_4$, with a molecular weight of 354.2 and a CAS number of 89796-99-6. Aceclofenac occurs as a white or almost white crystalline powder. Aceclofenac is practically insoluble in water. It is soluble in alcohol and freely soluble in acetone. Aceclofenac should be stored in airtight containers and protected from light.

METHODS

Assay 1 Jin et al. [1] reported an HPLC method for the determination of aceclofenac in human plasma. A Shimadzu LC10A system equipped with a model SPD10A UV detector was used. The stationary phase was a Shimadzu ODS column (150 × 4.6 mm, 5 μm particle size). The mobile phase consisted of methanol and 0.1 M ammonium acetate aqueous solution (pH 6.0) (7 : 3, vol/vol) and was isocratically delivered at 1.0 mL/min. UV detection was performed at 275 nm and 0.005 AUFS (absorbance units full scale). The injection volume was 20 μL.

An aliquot of 0.5 mL of a plasma sample was spiked with 75 μL of 1 M hydrochloric acid, vortexed, mixed with 3.5 mL of ether, shaken for 3 min, and centrifuged at 3000 rpm for 10 min. An aliquot of 3 mL of the supernatant was collected, evaporated to dryness at 37°C under a stream of nitrogen, reconstituted with 0.2 mL of mobile phase, and assayed. Under these conditions, the retention time of aceclofenac was about 6.9–8.4 min.

A calibration curve for aceclofenac was constructed in the concentration range from 0.05 to 40.0 mg/L. The correlation coefficient was 0.9999. The average recovery of aceclofenac from plasma was 82.5%. Intraday and interday coefficients of variation were less than 7.1% and 9.3%, respectively. The limit of quantification was 0.05 mg/L. The analysis of aceclofenac was not affected by endogenous compounds in plasma.

Assay 2 Lee et al. [2] described the simultaneous determination of aceclofenac and diclofenac in human plasma by narrowbore HPLC using a column-switching technique. A Shiseido Nanospace SI-1 series liquid chromatograph consisting of two model 2001 pumps, model 2002 UV–visible detector, model 2003 autosampler, model 2004 column oven, model 2012 high-pressure switching valve, and model 2009 degassing unit was utilized. The stationary phase was a Phenomenex Luna 2 phenylhexyl narrowbore column (100 × 2 mm, 3 μm particle size). The column temperature was maintained at 30°C. The mobile phase consisted of acetonitrile and 0.02 M potassium phosphate buffer (pH 7) (33 : 67, vol/vol) and was delivered at 0.2 mL/min. UV detection was performed at 278 nm.

Stock solutions of aceclofenac and diclofenac at 1 mg/mL were prepared in methanol. Standards were prepared by spiking drug-free human plasma with stock solutions. An aliquot of 100 μL of a plasma sample was filtered through a 0.2-μm membrane filter and introduced onto a Capcell Pak MF Ph1 precolumn (20 × 4 mm) to remove proteins using a mixture of acetonitrile and 0.1 M potassium phosphate buffer (pH 7) (14 : 86, vol/vol) at 0.5 mL/min from 0 to 6.0 min. The valve was switched to a Capcell Pak C_{18} UG120 column (35 × 2 mm) to concentrate drugs from 6.0 to 8.8 min. From 8.8 to 17 min, the valve was switched to the analytical column to separate drugs using the mobile phase at 0.2 mL/min. Under these conditions, retention times of diclofenac and aceclofenac were about 13 and 14.3 min, respectively (estimated from the published chromatogram).

A calibration curve for aceclofenac was constructed in the range from 50 to 10,000 ng/mL. The correlation

coefficient was 0.999. The mean recovery of aceclofenac from plasma was 90.5%. Intraday and interday coefficients of variation were 2.8% and 3.0%, respectively. The limit of detection was 10 ng/mL.

Assay 3 Zinellu et al. [3] evaluated a capillary electrophoresis method for the simultaneous determination of aceclofenac and diclofenac in human plasma. A Beckman MDQ capillary electrophoresis system equipped with diode-array detector was utilized. The uncoated fused-silica capillary had 75 μm i.d. and 40 cm in length (30 cm to the detection window). The injection was made at 3.5 kPa for 15 s under vacuum, and the injection volume was 112 nL. The run buffer was 300 mM sodium borate aqueous solution containing 200 mM *N*-methyl-D-glucamine, adjusted to pH 8.9 with 5 M sodium hydroxide solution. The separation of drugs was carried out at 30 kV (140 μA), 25°C, and normal polarity. UV detection was performed at 290 nm.

A plasma was mixed with an equal volume of 200 μL of acetonitrile, vortexed, centrifuged at 3000 *g* for 5 min, and directly injected onto the capillary. Under these conditions, migration times of aceclofenac and diclofenac were about 2.8 and 3.1 min, respectively.

Calibration curves for acecelofenac were constructed in the range from 2.5 to 40 mg/L. Correlation coefficients were greater than 0.999. Intraday and interday coefficients of variation were 4.2% and 5.8%, respectively. Limits of detection and quantification were 0.03 and 0.1 mg/L, respectively.

Assay 4 Hinz et al. [4] reported the simultaneous analysis of aceclofenac and its metabolites in human plasma by HPLC. A liquid chromatographic system equipped with a Jasco model PU980 gradient pump and a Spectra Physics model Spectra 100 detector was used. The stationary phase was a Machery–Nagel Nucleosil 120-5 C_{18} column protected by a C_{18} precolumn. The column temperature was maintained at 30°C. Solvent A was a mixture of 0.005 M phosphate buffer and acetonitrile (20 : 80, vol/vol) and solvent B, a mixture of 0.01 M phosphate buffer and acetonitrile (88 : 12, vol/vol), where 0.01 M phosphate buffer was prepared by dissolving 7.1 g of dibasic sodium phosphate dodecahydrate and 6.8 g of monobasic potassium phosphate in 5 L of distilled water. The mobile phase was delivered at 1 mL/min in a gradient mode as follows:

Time (min)	%A	%B
0	4	96
24	4	96
25	12	88
40	12	88
41	15	85
60	15	85
61	4	96
76	4	96

UV detection was performed at 282 nm. The injection volume was 100 μL.

Stock solutions of aceclofenac and its metabolites, diclofenac, 4′-hydroxyaceclofenac, and 4′-hydroxydiclofenac, were prepared in acetonitrile and stored at −80°C. Standards were prepared by spiking blank human plasma with stock solutions. Ketoprofen at 0.05 mg/mL in acetonitrile − 0.01 M phosphate buffer solution (pH 2.5) (95 : 5, vol/vol) was used as an internal standard. An aliquot of 1 mL of a plasma sample or standard was mixed with 0.5 mL of 40 mg/mL sodium fluoride solution and 0.1 mL internal standard, extracted with 5 mL of *n*-hexane/diethylether (50 : 50, vol/vol), shaken for 30 min, and centrifuged at 4000 rpm for 10 min. The organic layer was collected; evaporated to dryness under a stream of nitrogen; reconstituted in 120 μL of a mixture (pH 2.5) containing 72% 0.01 M phosphate buffer, 15% acetonitrile, 10% methanol, and 3% tetrahydrofuran; and assayed. Under these conditions, retention times of aceclofenac, diclofenac, 4′-hydroxy-aceclofenac, 4′-hydroxy-diclofenac, and ketoprofen were 69.1, 60.9, 46.9, 28.4, and 21.2 min, respectively.

Linear relationships were observed over the concentration ranges from 10 to 10,000 ng/mL for aceclofenac, 4′-hydroxyaceclofenac, and diclofenac and from 25 to 10,000 ng/mL for 4′-hydroxydiclofenac. Correlation coefficients were greater than 0.996. Intraday coefficients of variation were less than 9.9%, 10.9%, 6.3%, and 6.3% for aceclofenac, 4′-hydroxyaceclofenac, diclofenac, and 4′-hydroxydiclofenac, respectively. Interday coefficients of variation were less than 2.7%, 9.3%, 4.2%, and 5.6% for aceclofenac, 4′-hydroxyaceclofenac, diclofenac, and 4′-hydroxydiclofenac, respectively.

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ACEMETACIN

CHEMISTRY

Acemetacin is a NSAID. Its chemical name is *O*-[(1-*p*-chlorobenzoyl-5-methoxy-2-methylindol-3-yl)acetyl] glycolic acid. Other names include Acemetacinum, Bay-f-4975, Emflex, Rantudil, and TVX-1322. Its molecular formula is $C_{21}H_{18}ClNO_6$, with a molecular weight of 415.8 and a CAS number of 53164-05-9.

METHODS

Assay 1 Shi et al. [1] reported an HPLC method for simultaneous determination of acemetacin and its metabolite, indomethacin, in human plasma. A Shimadzu liquid chromatographic system consisted of a model SPD10AD pump, model SPD10A UV detector, model SIL10A autosampler, model CBM10A integrator, and a Sys-tee column heater. The stationary phase was a Kromasil C_{18} column (150 $\times$ 4.6 mm, 5 μm particle size) protected by a frit (0.5 μm). The column temperature was maintained at 30°C. The mobile phase consisted of 0.02 M monobasic sodium phosphate buffer (pH 4.5), acetonitrile, and methanol (400 : 300 : 300) and was isocratically delivered at 1.0 mL/min. UV detection was carried out at 254 nm. The injection volume was 40 μL.

Stock solutions of acemetacin and indomethacin at 1000 μg/mL were prepared in methanol and stored at 4°C. Working solutions were prepared by diluting stock solutions with 50% methanol in water. Flurbiprofen at 1000 μg/mL in methanol was used as an internal standard and stored at 4°C. Standards of acemetacin and indomethacin were prepared by fortifying the blank human plasma with working solutions. A standard or plasma sample (0.5 mL) was mixed with 0.5 mL of 0.02 M monobasic sodium phosphate buffer; vortexed for 1 min, centrifuged at 10,000 rpm for 10 s; loaded onto a Varian Bond Elut C_2 disposable cartridge (100 mg, 1 mL) that was preconditioned sequentially with 1 mL of mobile phase, 1 mL $\times$ 2 of methanol, and 1 mL of water; drawn through the cartridge under vacuum; washed with 1 mL of 0.02 M monobasic sodium phosphate buffer and then with 0.1 mL of mobile phase; dried under vacuum for 5 min; eluted with 0.25 mL of mobile phase; mixed with 25 μL of internal standard (4 μg/mL in mobile phase); vortexed for 1 min, and assayed. Retention times for acemetacin, flurbiprofen, and indomethacin were 5.88, 7.10, and 9.19 min, respectively. There was no interference from endogeneous compounds in plasma.

A calibration curve for acemetacin was constructed in the concentration range of 20–1000 ng/mL. The correlation coefficient for acemetacin was 0.9990. The recovery of acemetacin from plasma ranged from 89.5% to 91.5%. Intraassay and interassay coefficients of variation for acemetacin were less than 3.9% and 4.7%, respectively. The limit of detection was 20 ng/mL.

A calibration curve for indomethacin was also obtained in the concentration range of 20–1000 ng/mL. The correlation coefficient was 0.9980. The recovery of indomethacin from plasma ranged from 73.2% to 76.9%. Intraassay and interassay coefficients of variation for indomethacin were 7.3% and 9.0%, respectively. The limit of detection was 20 ng/mL.

Assay 2 Ban et al. [2] developed an HPLC method for the pharmacokinetic study of acemetacin in human plasma. A Waters 2690 Alliance separation module equipped with a Waters 2487 (dual-wavelength) absorbance detector was used. The stationary phase was a Shiseido Capcell Pak C_{18} reversed-phase column (150 $\times$ 4.6 mm, 5 μm particle size). The mobile phase consisted of 20 mM potassium phosphate buffer (pH 2.9) and acetonitrile (60 : 40, vol/vol) and was isocratically delivered at 1 mL/min. UV detection was performed at 254 nm. The injection volume was 80 μL.

Stock solutions of acemetacin, its metabolite (indometacin), and flurbiprofen (internal standard) at 10 mg/mL were prepared in methanol. Working solutions were prepared by diluting stock solutions with mobile phase. Stock solutions were stored at −20°C. Standards were prepared by spiking blank human plasma with working solutions. An aliquot of 1 mL of a plasma sample or standard was spiked with 20 μL of the internal standard at 25 μg/mL, mixed with 1 mL of 100 mM potassium phosphate buffer (pH 2.0), extracted with 9 mL of ethyl acetate, vortexed for 5 s, and centrifuged at 3000 *g* for 10 min. The organic layer was collected, evaporated to dryness under a stream of nitrogen at 30°C, reconstituted with 125 μL of mobile phase, and assayed. Under these conditions, retention times of flurbiprofen, indometacin, and acemetacin were 21.0, 25.1, and 27.3 min, respectively.

Linear calibration curves for acemetacin and indometacin were constructed over the range from 100 to 400 ng/mL. Correlation coefficients were 0.9998 for acemetacin and 0.9999 for indometacin. The average accuracy for acemetacin ranged from 98.1% to 107.5%. Intraday and interday coefficients of variation for acemetacin were less than 16.6% and 12.3%, respectively. The average accuracy for indometacin ranged from 97.7% to 102.5%. Intraday and interday coefficients of variation were less than 15.8% and 19.5%, respectively. Limit of quantification was 100 ng/mL for both compounds.

Assay 3 Hu et al. [3] described the determination of acemetacin and indometacin in human serum by HPLC. A Shimadzu system equipped with a model LC10A pump, model SPD6AS UV detector, and model CR2AS integrator was utilized. The stationary phase was a Spherisorb C_8 column (250 $\times$ 4.6 mm, 5 μm particle size). The mobile phase consisted of acetate buffer (pH 4.6), methanol, and acetonitrile (55 : 5 : 40, vol/vol/vol) and was isocratically delivered at 1.0 mL/min. UV detection was performed at 254 nm and 0.005 AUFS. The injection volume was 20 μL.

Standards were prepared in blank human serum, and the final concentraton of tolbutamide as internal standard was 0.5 mg/L. An aliquot of 0.4 mL of a plasma sample or standard was spiked with 20 μL of the internal standard, mixed with 0.2 mL of acetate buffer (pH 4.6), vortexed for 10 s, extracted with 3 mL of diethyl ether, vortexed for 2 min, and centrifuged at 4000 rpm for 10 min. A portion of 5 mL of the organic layer was collected, evaporated to dryness at 45°C under a stream of nitrogen, reconstituted with 0.2 mL of mobile phase, and assayed. Under these conditions, retention times of acemetacin, indometacin, and tolbutamide were 5.5, 7.5, and 8.8 min, respetively.

Linear calibration curves for acemetacin were constructed over the range from 12.5 μg/L to 1.6 mg/L. Correlation coefficients were greater than 0.9996. Intraday and interday coefficients of variation were 3.6% and 5.6%, respectively. The average recovery was 78.3%. Limits of detection and quantification were 0.25 ng and 6.2 μg/L, respectively.

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ACENOCOUMAROL

CHEMISTRY

Acenocoumarol is an oral anticoagulant. Its chemical name is (*RS*)-4-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl] coumarin. Other names include Acenocumarin, Acenocumarol, G-23350, Sinthrome, and Sintrom. Its molecular formula is $C_{19}H_{15}NO_6$, with a molecular weight of 353.3 and a CAS number of 152-72-7. Acenocoumarol occurs as an almost white to buff-colored odorless or almost odorless powder. It is practically insoluble in water and ether and slightly soluble in alcohol and chloroform. The drug dissolves in aqueous solutions of alkali hydroxides.

METHOD

Assay 1 Rentsch et al. [1] reported a normal-phase HPLC assay for the stereospecific determination of *R*- and *S*-acenocoumarol and *R*- and *S*-phenprocoumon in human plasma. A Varian liquid chromatograph consisting of a model 9010 pump, model 9100 autosampler with a 100-μL loop, and model 9050 UV–visible detector was utilized. The stationary phase was a Merck LiChroCART S,S-Whelk-01 chiral column (250 × 4.0 mm, 5 μm particle size) protected by a Merck LiChrosper 100 DIOL guard column (4 × 4 mm, 5 μm particle size). Eluent A was a mixture of *n*-hexane and ethanol (90 : 10, vol/vol) containing 0.5% acetic acid, and eluent B was a mixture of *n*-hexane and ethanol (60 : 40, vol/vol) containing 0.5% acetic acid. The mobile phase was delivered at 1.0 mL/min from 5% B to 50% B in 20 min, increased to 100% B in another 5 min, and was maintained at 100% B for additional 10 min. UV detection was performed at 310 nm.

Warfarin (both *R*- and *S*-warfarin) at 10 μg/mL in 0.05 M sodium hydroxide aqueous solution was used as an internal standard. Standards were prepared by spiking blank human plasma with *R*- and *S*-acenocoumarol and *R*- and *S*-phenprocoumon stock solutions. An aliquot of 1 mL of a plasma sample, control, or standard was spiked with 100 μL of internal standard, adjusted to pH 3.5 with 1 M hydrochloric acid, extracted with 5 mL of toluene for 20 min, and centrifuged at 1000 *g* for 5 min. The organic phase was collected, evaporated to dryness, reconstituted in 150 μL of eluent A, and assayed. Under these conditions, retention times of *S*-phenprocoumon, *R*-phenprocoumon, *R*-warfarin, *S*-warfarin, *R*-acenocoumarol, and *S*-acenocoumarol were 9.5, 11.5, 13.5, 17, 24, and 29 min, respectively.

Linear calibration curves for *R*- and *S*-acenocoumarol were constructed over the range from 15 to 2000 μg/L. Correlation coefficients were greater than 0.9998. Within-day (intraday) and between-day (interday) coefficients of variation were 4.6% and 7.8% for *R*-acenocoumarol and 4.7% and 6.1% for *S*-acenocoumarol, respectively. Average recoveries were better than 86.3% for *R*-acenocoumarol and 84.3% for *S*-acenocoumarol. The limits of detection and quantification were 5 and 15 μg/L, respectively, for both compounds. This assay was free of interference from the following drugs: alprazolam, amitriptyline, bupivacaine, carbamazepine, chlordiazepoxide, citalopram, clobazam, clomipramine, clonazepam, clozapine, desipramine, diazepam, diclofenac, diphenhydramine, doxepin, fentanyl, flunitrazepam, flupentixol, fluphenazine, flurazepam, fluvoxamine, haloperidol, imipramine, lamotrigine, levomepromazine, lidocaine, lorazepam, maprotiline, mefenamic acid, mepivacaine, mianserin, midazolam, nefazodone, nordazepam, nortriptyline, olanzapine, opipramol, oxcarbazepine, penfluridol, phenobarbital, pipamperone, promazine, quinine, ranitidine, sertraline, *S*-ibuprofen, sotalol, temazepam, thiopental, thioridazine, tolfenamic acid, topiramate, trazodone, triazolam, trimethoprim, trimipramine, venlafaxine, zolpidem, and zuclopenthixol. Carbamazepine coeluted with *S*-warfarin.

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ACETAMINOPHEN

CHEMISTRY

Acetaminophen is a synthetic nonopiate derivative of *p*-aminophenol. Its chemical name is *N*-(4-hydroxyphenyl) acetamide. Other names include Paracetamol, Tempra, and Tylenol. Its molecular formula is $C_8H_9NO_2$, with a molecular weight of 151.2 and a CAS number of 103-90-2. Acetaminophen is a white, crystalline powder with a slightly bitter taste. It is soluble in boiling water and freely soluble in alcohol. Acetaminophen has a pK_a of 9.51.

METHODS

Assay 1 Johnson and Plumb [1] compared HPLC with monolithic column and UPLC with UPLC column coupled with QTof mass spectrometer in the determination of metabolites of acetaminophen in human urine.

The Waters Acquity ultra-high-permformance liquid chromatography (UPLC) system was used. The stationary

phases were a Merck ChromSpeed monolithic column (50 × 4.6 mm) and a Waters Acquity column (50 × 2.1 mm, 1.7 μm). The column temperature was maintained at 40°C. Solvent A was 0.1% formic acid in water, and solvent B was acetonitrile. The mobile phase was delivered in a linear gradient of 0 to 40% B in 10 min. The flow rate was 500 μL/min for the UPLC column and 2 mL/min for the monolithic column, respectively.

The Waters QTof micro mass spectrometer was operated in positive-ion mode: nebulization gas (300 L/h, 250°C), cone gas (0 L/h), source temperature (120°C), capillary voltage (3200 V), cone voltage (30 V), collision gas (argon at 5.3×10^{-5} Torr), collision energies (5 and 25 eV), acquisition rate (0.3 s), and interscan delay (0.1 s). Leucine–enkephalin was used as the lock mass (m/z 556.2771) with a lockspray frequency of 5 s.

Urine was collected 1 h after two acetaminophen tablets (500 mg each) were taken orally by a volunteer and stored at −20°C. Before injection, urine samples were diluted 1 : 5 in distilled water. The injection volume was 10 μL. The column eluent was split to the mass spectrometer at 150 μL/min. Ion chromatograms were extracted at m/z 232 for acetaminophen sulfate, m/z 271 for acetaminophen-*S*-cysteine conjugate, m/z 328 for acetaminophen–glucuronide, and $m/z = 427$ for acetaminophen–glutathione conjugate, respectively. Retention times were 2.0 and 3.9 min for acetaminophen–glucuronide and acetaminophen–glutathione, respectively.

Assay 2 Makino et al. [2] described the simultaneous determination of ibuprofen, acetaminophen, indomethacin, and salicylic acid in human serum by capillary-zone electrophoresis and micellar electrokinetic chromatography. The Agilent HP^{3D} CE system equipped with a diode-array UV detector was utilized. The separation of drugs was carried out on an Agilent fused-silica extended light path capillary (64.5 cm × 50 μm internal diameter). The internal diameter of the so-called bubble cell capillary was 150 μm and the distance between the inlet end and the detector was 56 cm. The electrophoresis running buffer was 100 mM boric acid (pH 8.8), which was filtered through a 0.45-μm Millipore type HV filter and degassed before use. The fused-silica capillary was rinsed with running buffer for 4 min before each analysis. Injections were carried out by the vacuum system at 50 mmHg for 8 s. A constant voltage of 30 kV was applied at 25°C. UV detection was performed at 200 nm.

3-Isobutyl-1-methylxanthine at 50 μg/mL in acetonitrile was used as an internal standard. An aliquot of 100 μL of serum samples was mixed with 200 μL of the internal standard solution, vigorously mixed for 30 s, and centrifuged at 13400 *g* for 5 min. The supernatant was assayed. Under these conditions, migration times for acetaminophen, internal standard, indomethacin, ibuprofen, and salicylic acid were about 3.1, 3.5, 4.0, 4.4, and 5.4 min, respectively (estimated from the published electropherogram).

Calibration curves for acetaminophen were constructed in the therapeutic-to-toxic range with correlation coefficients greater than 0.999. The limit of detection was 4 μg/mL. Intraday and interday coefficients of variation of the migration time were 0.17–0.95% and 1.14–2.02%, respectively. Intraday and interday coefficients of variation of the relative peak area were 0.20–21.59% and 5.05–20.41%, respectively.

Assay 3 Baranowska et al. [3] developed an HPLC method for simultaneous determination of imipenem, paracetamol, dipyrone, vancomycin, amikacin, fluconazole, cefazolin, prednisolone, dexamethasone, furosemide, and ketoprfen in human urine. The Merck–Hitachi liquid chromatographic system consisting of a model L6200A "intelligent" pump, model L7480 diode-array detector, a model 7360 fluorescence detector, and a Rheodyne injector with a 20-μL loop was utilized. The stationary phase was a Merck LiChroCART Purospher C_{18}e analytical column (125 × 3 mm, 5 μm particle size) protected with a precolumn (4 × 4 mm, 5 μm particle size) of the same packing material. Solvent A was 0.05% trifluoroacetic acid in water; solvent B, methanol; and solvent C, acetonitrile. The mobile phase was delivered in a gradient mode as follows:

Time (min)	%A	%B	%C	Flow Rate (mL/min)
0	92	6	2	0.75
10	50	42	8	0.65
20	25	55	20	0.60
25	92	6	2	0.75

UV detections were performed at 300, 243, 259, 210, 210, 274, 242, 242, 234, and 254 nm for imipenem, paracetamol, dipyrone, vancomycin, fluconazole, cefazolin, prednisolone, dexamethasone, furosemide, and ketoprofen, respectively. Amikacin after derivatization was monitored by a fluorescence detector at an excitation wavelength of 355 nm and an emission wavelength of 415 nm. The runtime of an injection was 25 min. Under these conditions, retention times were 4.0, 4.9, 6.7, 8.1, 9.5, 10.0, 10.9, 13.3, 14.1, 16.0, and 19.0 min for imipenem, paracetamol, dipyrone, vancomycin, amikacin, fluconazole, cefazolin, prednisolone, dexamethasone, furosemide, and ketoprofen in human urine, respectively.

Stock solutions of these drugs at 1 mg/mL were separately prepared in water/methanol (50 : 50, vol/vol) and stored at −18°C. Working solutions containing these drugs were prepared by mixing individual stock solutions and diluting them with water/methanol (90 : 10, vol/vol). Standards were prepared by spiking drug-free human urine with working stock solutions. An aliquot of 0.75 mL of a urine sample or standard was adjusted to pH 8.0 with 1.5 M sodium hydroxide, mixed with 1.5 mL of acetonitrile and 1.5 mL of methanol, filled to the 10-mL mark with water, shaken for 1 min, and centrifuged at 22°C at 6500 rpm for 15 min. The supernatant was collected and assayed. The injection volume was 20 μL.

A calibration curve for paracetamol was constructed in the range of 0.5–45 μg/mL. The correlation coefficient was 0.9996. The recovery of paracetamol from urine ranged from 97.1% to 103.6%. The coefficient of variation for the assay was less than 6.9%. The accuracy in the relative percentage error was less than 3.6%. Limits of detection and quantification were 0.13 and 0.42 μg/mL, respectively.

Assay 4 Nagaralli et al. [4] reported the analysis of cetirizine hydrochloride and paracetamol in human plasma by HPLC. A Shimadzu liquid chromatograph consisting of a model LC10AT solvent pump, model SPD10AVP detector, and a Hamilton 702μR injector with a 25-μL loop was used. The stationary phase was a CLC C_{18} column (250 × 4.6 mm, 5 μm particle size) protected by a CLC ODS guard column (40 × 4.6 mm). The mobile phase consisted of acetonitrile and water (55 : 45, vol/vol) and was isocratically delivered at 0.8 mL/min. UV detection was performed at 230 nm. The injection volume was 20 μL.

Stock solutions of cetirizine and paracetamol at 1 mg/mL were separately prepared in mobile phase. Working standard solutions were prepared by diluting stock solutions with mobile phase. Nimesulide at 1 mg/mL in mobile phase was used as an internal standard. An aliquot of plasma or standard was spiked with internal standard solution, extracted with ether, evaporated to dryness at 40°C under a gentle stream of nitrogen, reconstituted in mobile phase, and assayed. Under these conditions, retention times for paracetamol, cetirizine, and nimesulide were 2.39, 3.50, and 5.88 min, respectively.

Calibration curves for paracetamol were constructed over the range from 2.0 to 39 μg/mL. Correlation coefficients were 0.9978. The mean accuracy expressed as the relative percentage error was 6.5%. Intraday and interday coefficients of variation were less than 2.4% and 2.3%, respectively. Limits of detection and quantification were 0.208 and 0.715 μg/mL, respectively. There was no interference with this assay from the following drugs: ibuprofen, chlorpheniramine maleate, amoxicillin, cloxacillin, pseudoephedrine hydrochloride, cefadroxil, methdilazine hydrochloride, diclofenac sodium, and ciprofloxacin.

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ACETAZOLAMIDE

CHEMISTRY

Acetazolamide is an antiglaucoma drug. Its chemical name is 5-acetamido-1,3,4-thiadiazole-2-sulfonamide. Other names include Acetazolam, Acetazolamid, Acetazolamida, Diamox, and Glaupax. Its molecular formula is $C_4H_6N_4O_3S_2$, with a molecular weight of 222.2 and a CAS number of 59-66-5. Acetazolamide occurs as a white to faintly yellowish-white, odorless, crystalline powder. It is very slightly soluble in water and in alcohol.

METHOD

Assay 1 Jin et al. [1] reported a solid phase extraction and reversed-phase (RP)-HPLC screening procedure for bumetanide, spironolactone, amiloride, acetazolamide, hydrochlorothiazide, chlorothiazide, dichlorphenamide, furosemide, pemoline, triamterene, benzthiazide, bendroflumethiazide, ethacrynic acid, probenecid, and canrenone in urine. An HP1090 series system equipped with a diode-array detector was used. The stationary phase was a LiChrosorb RP18 column (200 × 4.6 mm, 5 μm particle size). The column temperature was maintained at 40°C. The mobile phase consisted of monobasic sodium phosphate buffer (pH 3) (A) and acetonitrile (B), where the phosphate buffer was prepared by dissolving 6.9 g of monobasic sodium phosphate and 1.305 g of ethanolamine in 1000 mL of water and adjusted to pH 3 with phosphoric acid. The mobile phase was initially delivered at 1 mL/min at 15% B from 0 to 3 min, increased to 33% B from 3 to 9.5 min, to 40% B from 9.5 to 9.8 min, and to 80% B from 9.8 to 20 min. UV detections were performed at 216, 230, and 275 nm. The injection volume was 10 μL.

Stock solutions of bumetanide, spironolactone, amiloride, acetazolamide, hydrochlorothiazide, chlorothiazide, dichlorphenamide, furosemide, pemoline, triamterene, benzthiazide, bendroflumethiazide, ethacrynic acid, probenecid, and canrenone at 1 mg/mL were individually prepared in methanol and stored at 4°C. Standards were prepared by spiking drug-free urine with stock solutions. An aliquot of 2 mL of urine or standard was centrifuged. The supernatant was loaded onto a laboratory-made XAD-2 (100–200 μm) solid-phase extraction column that was preconditioned with 5 mL of water, washed with water to remove water-soluble materials, eluted with 4 mL of ethyl acetate–ether (1 : 1), evaporated to dryness, reconstituted with 300 μL of methanol, and assayed. Under these conditions, retention times for amiloride, acetazolamide, hydrochlorothiazide, caffeine, pemoline, triamterene, dichlorphenamide, chlorothiazide, furosemide, benzthiazide, bendroflumethiazide, ethacrynic acid, bumetanide, probenecid, spironolactone, and canrenone were 3.5, 4.2, 5.6, 6.2, 7.0, 7.9, 10.2, 10.7, 13.5, 14.6, 15.4, 15.8, 16.1, 16.4, 17.8, and 18.4 min, respectively.

The mean recovery of acetazolamide from urine was 81%. The limit of detection was 1.5 μg/mL when the drug was monitored at 216 nm.

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ACONITINE

CHEMISTRY

Aconitine is a common ingredient in traditional Chinese remedies. Its chemical name is 8-acetoxy-3,11,18-trihydroxy-16-ethyl-1,6,19-trimethoxy-4-methoxymethylaconitan-10-yl benzoate. Its molecular formula is $C_{34}H_{47}NO_{11}$, with a molecular weight of 645.7 and a CAS number of 302-27-2.

METHOD

Assay 1 Wang et al. [1] developed a sensitive and simple HPLC method for the simultaneous determination of aconitine, mesaconitine, and hypaconitine in whole-blood and urine samples. A Waters system consisted of a model 600E pump, model 717 Plus autosampler, and a model 996 PDA detector. The stationary phase was a Waters XTerra RP18 column (150 × 4.6 mm, 5 μm particle size) coupled with a guard column (20 × 3.0 mm). The column temperature was maintained at 30°C and the autosampler, at 4°C. Solvent A was 10 mM ammonium hydrogen carbonate aqueous buffer, which was adjusted to pH 10.05 with concentrated ammonia, and solvent B was acetonitrile. The mobile phase was delivered at 100% A from 0 to 10 min and 50% A, from 11 to 25 min. The flow rate was 1 mL/min. UV detection was performed at 237 nm.

A standard stock solution containing aconitine, mesaconitine, and hypaconitine was prepared in acetonitrile and stored at 0°C. Standard working solutions containing aconitine (0.055–11 μg/mL), hypaconitine (0.055–11 μg/mL), and mesaconitine (0.060–12 μg/mL) were prepared from the stock solution. Standards in whole blood or in urine were prepared by spiking whole blood (1 mL) or urine (4 mL) with 50 μL of standard working solutions. Blood samples (1 mL each) were mixed with 20 μL concentrated phosphoric acid, diluted with 2 mL of deionized water, and centrifuged at 8000 *g* for 20 min. The supernatants were collected and applied onto Waters Oasis MCX solid-phase extraction cartridges that were preconditioned with methanol and deionized water. For urine, samples (4 mL each) were mixed with 40 μL of 5 M hydrochloric acid, filtered through 0.45-μm cellulose membrane filter, and applied onto Oasis MCX cartrideges. The cartridges were washed with 0.1 M hydrochloric acid, followed by 2% acetic acid in methanol. Alkaloids were eluted with a solution containing 5% concentrated ammonia in 70% methanol, evaporated to dryness at 40°C, reconstituted in 1 mL of the mobile phase, and assayed. The injection volume was 30 μL. Under these conditions, retention times of mesaconitine, aconitine, and hypaconitine were about 18.0, 20.8, and 22.8 min, respectively (estimated from the published chromatogram).

Calibration curves for aconitine were constructed in the concentrations ranging from 2.6 to 524 ng/mL with correlation coefficients of 0.9979–0.9986 in whole blood and from 0.7 to 136 ng/mL with correlation coefficients of 0.9994–0.9996 in urine. The recoveries of aconitine ranged from 92.08% to 116.98% in whole blood and from 78.84% to 99.43% in urine. Intraday and interday coefficients of variation were less than 9.4% and 14.1% in whole blood and less than 2.1% and 9.1% in urine, respectively. The limit of detection was 0.1 ng.

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ACRIVASTINE

CHEMISTRY

Acrivastine is a nonsedating antihistamine. Its chemical name is (*E*)-3-{6-[(*E*)-3-pyrrolidin-1-yl-1-*p*-tolylprop-1-enyl]-2-pyridyl}acrylic acid. Other names include Acrivastin, Acrivastina, BW-825C and Semprex. Its molecular formula is $C_{22}H_{24}N_2O_2$, with a molecular weight of 348.4 and a CAS number of 87848-99-5.

METHODS

Assay 1 Chang et al. [1] reported the analysis of acrivastine and its metabolite in human plasma by gas chromatography–mass spectrometry. A Hewlett-Packard 9870 gas chromatograph equipped with a J&W on-column injector was used. The stationary phase was a J&W Scientific DB5 capillary column (15 m × 0.25 i.d.) and was directly inserted into the ion source. The column temperature was programmed at 225°C from 0 to 4 min and raised to 300°C at the rate of 8°C/min. The injection volume was 1.5 μL.

A VG70S mass spectrometer was operated in selected-ion recording (SIR) electron ionization mode (70 eV). The source temperature was 200°C. Analytes were monitored at m/z 351 for acrivastine, m/z 422 for the metabolite, m/z 429 for deuterated acrivastine (internal standard for acrivastine), and m/z 358 for deuterated metabolite (internal standard for the metabolite), respectively.

Stock solutions of acrivastine, metabolite, and their respective duterated analogs were prepared in water and stored at 4°C. Standards were prepared by serial dilution of the stock solutions with blank human plasma. An aliquot of a plasma sample or standard was mixed with equal volume (0.5 mL) of internal standard containing 200 ng each of deuterated acrivastine and deuterated metabolite, loaded onto a Waters Sep-Pak C_{18} cartridge that was previously conditioned with 5 mL of methanol followed by 10 mL of water, passed through the cartridge, washed with 3 mL of 30% methanol in water, eluted in 2 mL of methanol, evaporated to dryness at 50°C under a stream of nitrogen, washed with 2 × 3 mL of hexane, dried under nitrogen, reconstituted in 400 μL of methanol, vortexed, sonicated, evaporated to dryness under a stream of nitrogen without heating, reacted with 50 μL of a mixture of bis(trimethylsilyl)trifluoroacetamide, acetonitrile, and

dichloromethane (2 : 1 : 0.1) for 15 min at 100°C, and assayed. Under these conditions, retention times of acrivastine, deuterated acrivastine, metabolite, and deuterated metabolite were about 8.35, 8.33, 6.50, and 6.47 min, respectively (estimated from the published chromatogram).

Calibration curves for acrivastine and its metabolite were constructed in the ranges from 0 to 30 ng/mL and 0 to 250 ng/mL. Coefficients of variation were less than 14.6% for acrivastine and 17.8% for its metabolie in the concentration range of 2–250 ng/mL. At 2 ng/mL, the coefficient of variation for the analysis of acrivastine was 34.4%. The limit of quantification was 2 ng/mL.

Assay 2 Torres et al. [2] reported the determination of acrivastine in human urine by differential pulse polarography. A Potentiostat PGSTAT10-Autolab equipped with a Metrohm 663VA polarographic stand and a three-electrode configuration consisting of a saturated KCl/Ag/AgCl reference electrode, a dropping-mercury electrode (DME) as a working electrode, and a platinum wire as an auxiliary electrode were used. Optimal parameters for the system were as follows: initial potential −0.2 V, modulation amplitude −50 mV, scan rate 10 mV/s, scan direction of negative, current range of 0.1–100 μA, buffer concentration 0.3 M, pH 2.5, and purge time 10 min, where the buffer was prepared by mixing 5 mL of 100 g/L potassium chloride, 0.9 mL of 0.8 M sodium hydroxide, and 6.25 mL of acetic acid.

A stock solution of acrivastine chlorhydrate at 348.0 mg/L was prepared in water. An aliquot of 4 mL of blank urine was mixed with stock solutions of acrivastine, 5 mL of the buffer, and 1 mL of 0.8% Triton X-100 solution, diluted to the 25-mL mark with water, mixed well, transferred into a polarographic cell, purged with a stream of pure nitrogen for 10 min, and scanned by the differential pulse polarography from −0.2 to −1.2 V. Under these conditions, two reduction waves with half-wave potentials at $E_{1/2} = -0.60$ V and $E_{1/2} = -0.99$ V were obtained. The peak height (current) of the reduction wave was found to be in a linear relationship with the concentration of acrivastine.

Calibration curves were constructed over the range from 0.35 to 26.1 mg/L. Correlation coefficients were greater than 0.9997. The coefficient of variation of the assay was less than 4.40%. The limits of detection and quantification were 0.11 and 0.35 mg/L, respectively.

A similar assay was reported by Abdine and Belal [3].

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ACYCLOVIR

CHEMISTRY

Acyclovir is a synthetic purine nucleoside analog antiviral agent. Its chemical name is 2-amino-1,9-dihydro-9-[(2-hydroxyethoxy)methyl]-6*H*-purin-6-one. Other names include Aciclovir, Aciclovirum, BW-248U, Virovir, and Zovirax. Its molecular formula is $C_8H_{11}N_5O_3$, with a molecular weight of 225.2 and a CAS number of 59277-89-3. Acyclovir occurs as white crystalline powders. It has solubilities of 1.3 mg/mL in water at 25°C and 0.2 mg/mL in alcohol. Acyclovir has pK_a values of 2.27 and 9.25.

METHODS

Assay 1 Poirier et al. [1] described an HPLC procedure for acyclovir therapeutic monitoring. A Waters system consisting of a model 510 solvent delivery system, model 486 variable-wavelength UV detector, model 717 WISP autosampler, and model 746D data module integrator was employed. Acyclovir peak purity was verified using a Waters model 996 PDA detector. The stationary phase was a Waters Symmetry C_8 column (150 × 4.6 mm, 5 μm) with a precolumn (10 mm) of the same packing material. The mobile phase was 0.1 M acetate/citrate buffer containing 3.7 mM octanesulfonic acid/methanol (92 : 8, v/v) adjusted to pH 3.0 with 14.8 M phosphoric acid. The flow rate was 1 mL/min. UV detection was performed at 250 nm.

Acyclovir working solutions were prepared in distilled water. Acyclovir standards were prepared by spiking blank human plasma with working solutions and stable at −20°C for at least 1 month. Waters Oasis HLB solid-phase extraction cartridges (30 mg, 1 mL) were conditioned with 1 mL of methanol followed by 1 mL of distilled water. A plasma sample or standard (250 μL) was loaded onto the column, washed with 1 mL of water, eluted with 750 μL of mobile phase, and assayed. The injection volume was 100 μL. Under these conditions, the retention time of acyclovir was 5.3 min.

Calibration curves for acyclovir were linear over the concentration range of 62.5–2000 ng/mL with correlation coefficients greater than 0.999. Within-day and between-day coefficients of variation were 6.1% and 10.4%, respectively. A mean absolute recovery of acyclovir was 90.0 ±3.8%. The limit of detection was 10 ng/mL.

This assay was free of interference from vallacyclovir, acetaminophen, amikacin, amitriptyline, amoxicillin, amphotericin B, caffeine, carbamazepine, chloramphenicol, cilastatin, ciprofloxacin, clonazepam, cyclosporine, desipramine, dexchlorpheniramine, digoxin, disopyramide, ethoxuximide, filgrastine, flecainide, fluconasole, furosemide, gentamicin, imipenen, imipramine, itraconazole, lidocaine, metoclopramide, lynestrenol, methotrexate, morphine, *N*-acetylprocainamide, netilmicin, nortriptyline, omeprazole, phenobarbital, phenytoin, pipotiazine, primidone, procainamide, propranolol, quinidine, salicylate, theophylline, sucralfate, tobramycin, trihexyphenidyle, valproic acid, vancomycin, and related endogenous compounds.

Assay 2 Svensson et al. [2] reported an HPLC–fluorescence method for the determination of acyclovir in serum and urine. The liquid chromatograph consisted of a Pharmacia LKB model 2150 pump, a Rheodyne model 7125 injector with a 20-μL loop, and a Shimadzu model RF530 fluorescence detector. The stationary phase was a Beckman Ultrasphere ODS reversed-phase column (75 × 4.6 mm, 3 μm). The mobile phase was a mixture of 30 mM phosphate buffer (pH 2.1) containing 45 mM dodecylsulfate and acetontrile (82 : 18, v/v), which was prepared by dissolving 4.08 g of monobasic potassium phosphate, 1.45 g of sodium dodecylsulfate, and 15 mL of 3.85 mM phosphoric acid in 800 mL of water; this was mixed further with 180 mL of acetonitrile, and filling to 1 L volume with water. The flow rate was 1.5 mL/min. The detector was set at an excitation wavelength of 285 nm and an emission wavelength of 380 nm.

A serum sample (500 μL) was mixed with 500 μL of a saturated sodium chloride solution in water, pushed through a Sep-Pak Light C_{18} cartridge preconditioned with 1 mL of methanol followed by 1 mL of water, washed with 500 μL of a 50% saturated sodium chloride aqueous solution, eluted with 1000 μL of a 3% acetonitrile in 38 mM phosphoric acid, and assayed. For urine, a urine sample (100 μL) was mixed with 900 μL of 50% saturated sodium chloride aqueous solution. The solid-phase extraction procedure was the same as for the serum described above. The injection volume was 20 μL. The runtime was 5 min. Under these conditions, retention times of acyclovir and its metabolite, 9-carboxymethoxymethylguanine, were about 1.6 and 1.4 min, respectively (estimated from the published chromatogram).

A calibration curve for acyclovir was constructed in the range of 0–16.0 μM. The correlation coefficient was better than 0.998. The limits of detection were 0.12 μM (plasma) and 0.60 μM (urine) for acyclovir and 0.26 μM (plasma) and 1.3 μM (urine) for the metabolite, respectively. This assay was free of interference from cyclosporine, azathioprine, prednisolone, sulfametoxazole, trimetoprim, nifedipine, and furosemide. More than 400 serum samples from organ transplant recipients were analyzed using this assay.

Assay 3 Dao et al. [3] reported the simultaneous determination of aciclovir, ganciclovir, and penciclovir in human plasma using HPLC–fluorescence detection. An Agilent 1100 series equipped with a model G1311A quaternary pump, autosampler, column compartment, and a model G1321A fluorescence detector was used. The stationary phase was a Dikma Diamonsil C_{18} analytical column (250 × 4.6 mm, 5 μm particle size). Solvent A was 0.08% trifluoroacetic acid in water (pH 2.30), and solvent B was methanol. The mobile phase was delivered at 1.5 mL/min in a gradient mode at 96% A from 0 to 7 min, at 40% A from 7.01 to 10 min, and at 96% A from 10.01 to 12.50 min. Fluorescence detection was performed at an excitation wavelength of 260 nm and an emission wavelength of 380 nm. The injection volume was 40 μL.

Stock solutions of aciclovir, ganciclovir, and penciclovir at 1 mg/mL were separately prepared in a mixture of methanol and water (50 : 50, vol/vol). Working solutions were prepared by diluting stock solutions with water. Guanosine 5′-monophosphate at 10 μg/mL in 7% perchloric acid in water was used as an internal standard. Standards were prepared by spiking blank human plasma with working solutions. An aliquot of 200 μL of a sample (blank, standard, control, or patient plasma) were mixed with 50 μL of the internal standard, vortexed vigorously for 30 s, and centrifuged at 15,000 rpm at 4°C. The supernatant was transferred into a autosampler vial and assayed. Under these conditions, retention times of aciclovir, ganciclovir, penciclovir, and the internal standard were 6.5, 4.9, 7.7, and 4.0 min, respectively.

Calibration curves for aciclovir were constructed over the range from 20 to 2000 ng/mL. Correlation coefficients were greater than 0.999. The mean recovery of aciclovir from plasma was 93.9%. Intraday and interday coefficients of variation were less than 6.3% and 7.3%, respectively. The limit of quantification was 20 ng/mL. This assay was not affected by endogenous compounds and the following drugs: acetaminophen, adefovir dipivoxil, almitrine, azathioprine, bepridil hydrochloride, carbamazepine, chlorzoxazone, ciprofloxacin, clindamycin sodium phosphate, clonazepam, clozapine, cyclosporine A, dexamethasone sodium phosphate, dextromethorphan hydrobromide, diazepam, digoxin, diltiazem, dimethylbiguanide, doxepin, famciclovir, fenofibrate, fenofibric acid, gentamicin sulfuric acid, glibenclamide, gliclazide, glipizide, 4-hydroxyantipyrinum, hydrochlorothiazide, 9-hydroxyrisperidone, ibuprofen, indometacin, lorcainide, metoclopramide, metronidazole, naproxen sodium, nevirapine, nifedipine, nimodipine, nitrazepam, ofloxacin, phenacetin, phenylpropanolamine, phenytoin sodium, propafenone, pseudoephedrine, raubasine, spironolactone, tamoxifen, topiramate, valaciclovir, and valganciclovir.

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ALBENDAZOLE

CHEMISTRY

Albendazole is a synthetic anthelmintic agent. Its chemical name is methyl 5-propylthio-1*H*-benzimidazol-2-ylcarbamate. Other names include Albendazol, Albenza, SKF-62979, and Zentel. Its molecular formula is $C_{12}H_{15}N_3O_2S$, with a molecular weight of 265.3 and a CAS

number of 54965-21-8. Albendazole occurs as a white to faintly yellowish powder. Albendazole is practically insoluble in water and alcohol, very slightly soluble in ether and dichloromethane, but freely soluble in anhydrous formic acid.

METHOD

Assay 1 Prochazkova et al. [1] described a simple and fast nonaqueous capillary electrophoresis method for therapeutic drug monitoring of albendazole, albendazole sulfoxide, and albendazole sulfone in human plasma. An Applied Biosystems 270AI IT CE system was equipped with a Polymicro Technologies fused-silica capillary column (58.7 cm $\times$ 75 μm i.d.). The temperature was maintained at 35°C. The capillary column was conditioned with 1 M sodium hydroxide for 20 min, 0.1 M sodium hydroxide for 20 min, and then water for 20 min by applying a vaccum of 67.7 kPa at the outlet end. Between runs, the column was rinsed with 0.1 M sodium hydroxide for 3 min, water for 2 min, and then the background electrolyte for 2 min. Samples were introduced by applying a vaccum of 16.9 kPa for 3 s. The voltage of 23 kV was applied (anode at injection end) and the current was 82 μA. UV detection was 280 nm.

Stock solutions of albendazole (50.8 μM), albendazole sulfoxide (34.9 μM), albendazole sulfone (52.0 μM), and fenbendazole (internal standard, 76.6 μM) were prepared in methanol. Standards were prepared by spiking bovine plasma with stock solutions. The background electrolyte was prepared by mixing 5 mL of 0.05 M disodium tetraborate decahydrate in methanol/*N*-methylformamide (NMF) (1 : 3, vol/vol) and 2 mL of 0.1 M sodium hydroxide in methanol/NMF (1 : 3, vol/vol).

Patient sample or standard (0.5 mL) was mixed with 25 μL of the internal standard, 0.5 mL of 0.25 M sodium carbonate buffer (pH 10.3) and 5 mL of dichloromethane, gently shaken for 10 min, and centrifuged at 3000 rpm for 10 min. The organic phase (lower layer) was collected, evaporated to dryness at 40°C under a gentle stream of air, reconstituted in 200 μL of NMF, and assayed. Under these conditions, migration times of albendazole, fenbendazole, albendazole sulfoxide, and albendazole sulfone were about 5.3, 5.5, 6.0, and 6.5 min, respectively (estimated from the published electropherogram).

Calibration curves for albendazole, albendazole sulfoxide, and albendazole sulfone were obtained in the range of 1.0–10 μM. Limits of detection were 8×10^{-7} M for all three compounds. Intraday and interday coefficients of variation were less than 10% and 12%, respectively. This method was used for the analysis of 45 patient samples. Albendazole sulfoxide and albendazole sulfonate were quantitated, while albendazole was not found in patient plasma.

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ALBUTEROL

CHEMISTRY

Albuterol is a direct-acting sympathomimetic. Its chemical name is 2-*tert*-butylamino-1-(4-hydroxy-3-hydroxymethylphenyl)ethanol. Other names include Proventil, Salbutamol, and Ventolin. Its molecular formula is $C_{13}H_{21}NO_3$, with a molecular weight of 239.3 and a CAS number of 18559-94-9. Albuterol is a white crystalline powder. Albuterol is sparingly soluble in water. It is soluble in alcohol. The drug has pK_a values of 9.3 and 10.3. Albuterol should be protected from light.

METHOD

Assay 1 Lee et al. [1] developed an LC-MS/MS method for the simultaneous determination of 12 ß-blockers and $ß_2$-agonists in sewage samples. A Waters 2695 separation module was used. The stationary phase was an Agilent Zorbax SB C_8 column (150 $\times$ 2.1 mm, 3.5 μm particle size) protected by a SB C_8 guard column (12.5 $\times$ 2.1 mm, 5 μm particle size). The column temperature was maintained at 35°C. Mobile phase A was a mixture of water, acetonitrile, and formic acid (94.5 : 5.0 : 0.5, vol/vol/vol), and mobile phase B was a mixture of acetonitrile and formic acid (99.5 : 0.5, vol/vol). The mobile phase was delivered in a gradient mode from 100% A to 75% A in 13 min, held at 75% A for 13 min, and then pumped at 100% A for another 14 min. The flow rate was 0.2 mL/min. The injection volume was 10 μL. The total runtime of an injection was 40 min.

A Micromass Quattro Ultima triple quadrupole mass spectrometer equipped with an electrospray ionization source was operated in the positive-ion mode. The major parameters were set as follows: nebulizer gas (nitrogen) 50 L/h, desolvation gas (nitrogen) 500 L/h, source temperature 120°C, desolvation temperature 350°C, capillary voltage 3.45 kV, cone energy 30 V, and collision energy 15 kV. Albuterol was analyzed in the multiple-reaction monitoring (MRM) mode at ion transitions m/z 240 $\rightarrow$ 148 for quantitation and m/z 240 $\rightarrow$ 166 for confirmation.

Individual stock solutions of acebutolol, alprenolol, atenolol, bisoprolol, clenbuterol, fenoterol, labetalol, metoprolol, nadolol, pindolol, propranolol, sotalol, terbutaline, and timolol at 1000 μg/mL were prepared in acetonitrile or methanol. A stock solution of salbutamol was prepared at 500 μg/mL. These stock solutions were stored at -20°C. Working solutions were prepared by mixing and diluting these stock solutions with mobile phase B. An aliquot of 250 mL of sewage sample was filtered through a 1.2-μm GF/C filter (90 mm i.d.) with a layer of Celite, acidified to pH 3 with 1 M hydrochloric acid, loaded onto a Waters Oasis MCX cartridge (6 mL, 150 mg, 30 μm) at a flow rate of 10–15 mL/min, which was preconditioned with 6 mL of methanol followed by 10 mL of water at pH 3; this aliquot was dried for 10 min under vacuum; washed with 100 mL of water at pH 3 followed by 6 mL of methanol; eluted with 8 mL of a mixture of dichloromethane, 2-propanol, and ammonium hydroxide (78 : 20 : 2, vol/vol/vol); evaporated to

dryness at 40°C under a stream of nitrogen; reconstituted in 1.0 mL of mobile phase B; filtered through a 0.45-μm nylon syringe filter; and assayed. Under these conditions, retention times for acebutolol, alprenolol, atenolol, bisoprolol, labetalol, metoprolol, nadolol, pindolol, propranolol, sotalol, timolol, clenbuterol, fenoterol, salbutamol, and terbutaline were 17.36, 24.32, 8.96, 21.77, 22.11, 18.02, 13.60, 14.15, 23.87, 7.85, 17.02, 17.91, 11.50, 6.97, and 6.53 min, respectively.

Calibration curves for albuterol were constructed over the range from 50 to 500 pg/μL. The mean recovery of the drug from water ranged from 73% to 87%. The limit of detection was 6 ng/L.

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ALCURONIUM CHLORIDE

CHEMISTRY

Alcuronium chloride is a benzylisoquinolinium competitive neuromuscular blocker. Its chemical name is *N*,*N*′-diallylbisnortoxiferinium dichloride. Other names include Alloferin and Ro-4-3816. Its molecular formula is $C_{44}H_{50}Cl_2N_4O_2$, with a molecular weight of 737.8 and a CAS number of 15180-03-7. Alcuronium chloride is a white or slightly grayish-white, crystalline powder. Alcuronium chloride is freely soluble in water and methanol and soluble in alcohol. It is practically insoluble in cyclohexane.

METHOD

Assay 1 Künzer et al. [1] developed an HPLC method for the analysis of alcuronium in human plasma and urine. A Merck–Hitachi HPLC system consisting of a model AS4000 autosampler, model D6000 interface, model L6200 intelligent pump, model T6300 column thermostat, and model L4250 UV–visible detector was used. The stationary phase was a Spherisorb 5CN column (250 × 4 mm). The mobile phase consisted of 46% acetonitrile and 54% of an aqueous solution containing 60 mM sodium sulfate and 5 mM sulfuric acid and was isocratically delivered at 1 mL/min. UV detection was performed at 294 nm.

A stock solution of alcuronium at 0.4 mg/mL was prepared in deionized water. A plasma stock solution of alcuronium at 2 mg/L and a urine stock solution of alcuronium at 100 mg/L were prepared separately. Standards in plasma and urine were prepared by spiking drug-free plasma or urine with the respective stock solution. For plasma sample, an aliquot of 250 μL of plasma was mixed with 400 μL of laudanosine at 0.12 mg/mL in acetonitrile (as internal standard), vortexed for 15 s, rotated for 2 min, and centrifuged at 15,800 rpm for 10 min. The supernatant was collected and assayed. The injection volume was 75 μL. For urine sample, an aliquot of a urine sample was diluted with an equal amount of 50 mM phosphate buffer (pH 7.3), vortexed, and centrifuged at 2400 *g* for 5 min. The supernatant was collected and assayed. The injection volume was 40 μL. Under these conditions, retention times of laudanosine and alcuronium were 3.2 and 5.4 min, respectively.

For plasma, linear calibration curves were constructed over the range from 0.025 to 2.0 mg/L. Correlation coefficients were greater than 0.999. Intraassay and interassay coefficients of variation were less than 2.7% and 2.1%, respectively. For urine, linear calibration curves were constructed over the range from 1.0 to 80 mg/L. Correlation coefficients were greater than 0.999. Intraassay and interassay coefficients of variation were less than 2.4% and 3.6%, respectively. The limit of detection was 0.025 mg/L. This assay was not affected by the following drugs: thiopental, flunitrazepam, midazolam, disoprivan, ketamine, fentanyl, droperidol, cefazolin, and metronidazole.

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ALFENTANIL HYDROCHLORIDE

CHEMISTRY

Alfentanil hydrochloride is a nonopiate narcotic analgesic. Its chemical name is *N*-{1-[2-(4-ethyl-5-oxo-2-tetrazolin-1-yl)ethyl]-4-(methoxymethyl)-4-piperidyl}propionanilide hydrochloride. Other names include Alfenta, Rapifen, and R-39209. Its molecular formula is $C_{21}H_{32}N_6O_3{\cdot}HCl$, with a molecular weight of 453.0 and a CAS number of 69049-06-5. Alfentanil hydrochloride occurs as a white to almost white powder. It is freely soluble in water, alcohol, chloroform, and methanol, but sparingly soluble in acetone.

METHOD

Assay 1 Kintz et al. [1] reported a capillary GC method for the simultaneous screening and quantification of alfentanil, dextromoramide bitartrate, fentanyl, methadone hydrochloride, pentazocine hydrochloride, pethidine hydrochloride, phenoperidine hydrochloride, and phencyclidine hydrochloride in human plasma. A Perkin-Elmer 8500 gas chromatograph was equipped with a nitrogen–phosphorous detector and a Perkin-Elmer Sigma 15 data collector. The separation of drugs was performed on a vitreous silica capillary, bonded phase BP10 (SGE) column (25 m × 0.22 mm). The flow rate of carrier gas (nitrogen) was 6.2 mL/min. The head pressure on the column was maintained at 18 psi. The temperatures for injector and detector were set at 270°C and 280°C, respectively. The column oven temperature was programmed from 200°C to

270°C at 10°C/min and held at 270°C for 23 min. The total runtime of an injection was 30 min.

Stock solutions of drugs (10 mg/L each) were prepared in methanol and stored at 4°C. SKF525A from Smith, Kline and French (UK) was used as an internal standard. A plasma sample (1 mL) was mixed with 20 μL of 4 N sodium hydroxide solution, 1 mL of dibasic potassium phosphate buffer (40%, pH 9.2), 20 μL of the internal standard (10 mg/L), and 4.5 mL of chloroform/isopropranol/*n*-heptane (50 : 17 : 33, vol/vol/vol), and centrifuged. The organic phase was collected, evaporated to dryness at 45°C, reconstituted in 20 μL of methanol, and assayed. The injection volume was 1 μL. Under these conditions, retention times of pethidine, norpethidine, phencyclidine, methadone metabolite, methadone, pentazocine, fentanyl, dextromoramide, and alfentanil were 5.18, 6.06, 6.42, 6.89, 7.59, 8.88, 19.73, 25.47, and 29.57 min, respectively.

A standard curve for alfentanil was constructed by plotting peak area ratios of the drug to the internal standard against the concentrations. The correlation coefficient was greater than 0.99. The extraction recovery of alfentanil in plasma was 83.1%. Within-day and between-day coefficients of variation were less than 4.9% and 7.4%, respectively. The limit of detection was 0.5 μg/L.

This method was free of interference from endogenous plasma materials.

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ALFUZOSIN HYDROCHLORIDE

CHEMISTRY

Alfuzosin is an α-adrenoceptor blocker. Its chemical name is *N*-{3-[4-amino-6,7-dimethoxyquinazolin-2-yl(methyl)amino]propyl}tetrahydro-2-furamide hydrochloride. Other names include SL-77499-10, UroXatral, and Xatral. Its molecular formula is $C_{19}H_{27}N_5O_4 \cdot HCl$, with a molecular weight of 425.9 and a CAS number of 81403-68-1. Alfuzosin hydrochloride occurs as a white or almost white, slightly hygroscopic, crystalline powder. Alfuzosin hydrochloride is freely soluble in water, sparingly soluble in ethanol, and practically insoluble in dichloromethane. Alfuzosin has a pK_a of 8.13.

METHODS

Assay 1 Li et al. [1] reported an HPLC method for the determination of alfuzosin hydrochloride in plasma. A Shimadzu LC10AT liquid chromatograph was equipped with a model RF10AXL fluoresence detector and model SP4270 integrator. The stationary phase was a Luna C_{18} column (250 × 4.6 mm, 5 μm particle size). The mobile phase consisted of acetonitrile and 0.01 M monobasic potassium phosphate buffer (adjusted to pH 2.5 with phosphoric acid) (1 : 3). The flow rate was 1.0 mL/min. Detection was performed at an excitation wavelength of 270 nm and an emission wavelength of 370 nm.

Tosufloxacin tosilate at 0.5 mg/mL in mobile phase was used as an internal standard. Stock solution of alfuzosin hydrochloride 100 μg/mL was prepared by dissolution in 0.01 N hydrochloric acid and then dilution in distilled water. Standards were prepared by spiking blank plasma with stock solution. Plasma samples and standards (0.5 mL each) were mixed with 100 μL of the internal standard, vortexed for 0.5 min, extracted with 3 mL of *tert*-butylmethyl ether, vortexed for 3 min, and centrifuged at 3000 rpm for 5 min. The organic layer (2.5 mL) was collected. The extraction was repeated two more times. The collected supernatant (7.5 mL) was evaporated to dryness at 40°C under a stream of nitrogen, reconstituted with 100 μL of mobile phase, and assayed. The injection volume was 20 μL. Under these conditions, retention times for alfuzosin and the internal standard were 4.4 and 6.7 min, respectively.

A calibration curve for alfuzosin was constructed in the range of 0.78–50 μg/L. The correlation coefficient was 0.9996. The recovery of alfuzosin in plasma ranged from 72.6% to 75.5%. Intraday and interday coefficients of varation were less than 5.7% and 10.3%, respectively. The limit of detection was 0.7 μg/L.

Assay 2 Guinebault et al. [2] described an HPLC method for the determination of alfuzosin in biological fluids. An LDC Constametric IIG chromatographic system was equipped with a model 725 autoinjector with a 500-μL loop, Kontro model SFM23B spectrofluorimetric detector, and a Perkin-Elmer 56 recorder. The stationary phase was a Spherisorb ODS column (150 × 4.6 mm, 5 μm particle size). The mobile phase consisted of acetonitrile and 0.02 M monobasic potassium phosphate (pH 2.5) (3 : 2). The flow rate was 1.0 mL/min. Detection was performed at an excitation wavelength of 334 nm and an emission wavelength of 378 nm.

N-{3-[(4-Amino-6,7-dimethoxy-2-quinazolinyl)amino] propyl}-*N*-methyltetrahydro-2*H*-pyran-2-carboxamide hydrochloride was used as an internal standard. Stock solution of alfuzosin was prepared in 0.01 M hydrochloric acid and diluted in water. A plasma or blood sample (1 mL) was mixed with 10 μL of the internal standard (5 μg/mL in water) and 1 mL of 0.1 M sodium hydroxide solution, extracted with 7 mL of diethyl ether, shaken for 30 min, and centrifuged at 4°C at 1000 *g* for 5 min. The supernatant (6.5 mL) was collected, evaporated to dryness at 37°C under a stream of nitrogen, reconstituted in 870 μL of acetonitrile and 0.02 M monobasic potassium phosphate (pH 2.5) (1 : 9), and assayed. For urine, samples (100 μL) were diluted in water and extracted as described for plasma samples. Under these conditions, retention times for alfuzosin and its internal standard were 4.4 and 5.6 min, respectively.

Calibration curves were constructed in the concentraton ranges from 0.5 to 100 ng/mL with a correlation coefficient of 0.9999 for alfuzosin in plasma and from 0.05 to 10 μg/mL with a correlation coefficient of 0.999 for

alfuzosin in urine. The coefficient of variation ranged from 0.7% at 100 ng/mL to 6.2% at 1 ng/mL for blood samples and from 0.8% at 5 μg/mL to 10% at 0.05 μg/mL for urine samples, respectively.

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ALLOBARBITAL

CHEMISTRY

Allobarbital is a sedative. Its chemical name is 5,5-diallylbarbituric acid. Other names include Allobarbitone, diallylbarbituric acid, and Pabialgin P. Its molecular formula is $C_{10}H_{12}N_2O_3$, with a molecular weight of 208.2 and a CAS number of 52-43-7. It occurs as crystals or leaflets. Allobarbital dissolves 1 in ~300 of water, 1 in 50 of boiling water, 1 in 20 of cold alcohol, and 1 in 20 of ether. It is very soluble in hot alcohol and in acetone.

METHOD

Assay 1 Lehane et al. [1] developed a GC method for a simultaneous measurement of allobarbital, amobarbital, butabarbital, heptabarbital, pentobarbital, phenobarbital, secobarbital, diphenylhydantoin, and primidone. A Perkin-Elmer model 900 gas chromatograph was equipped with a nitrogen detector. An Applied Science Laboratories single glass column (1.83 m × 2 mm, 3% OV101 on Gas-Chrom Q, 100–120 mesh) was used. Gas flow rates were 30 mL/min for helium, 10 mL/min for hydrogen, and 100 mL/min for air. The injection port and detector oven were maintained at 300°C and 285°C, respectively. The column oven temperature was maintained at 175°C for 1 min, increased to 285°C at 24°C/min, and then kept at 285°C for 4 min.

Stock solutions of drugs (1 g/L) were prepared in methanol. Working solutions were prepared by diluting stock solutions with methanol. Standards were prepared by spiking ion-free serum with working solutions. A mixture of chloroform–isopropanol–benzene (94 : 4 : 1, vol/vol/vol) was prepared as an extraction solvent. 5-(*p*-Methylphenyl)-5-phenylhydantoin in extraction solvent (1.5 mg/L) was used as an internal standard. A serum sample or standard (1 mL) was mixed with 0.1 mL of 1.0 M hydrochloric acid and 12 mL of the internal standard solution, and shaken vigorously for 1 min. The organic phase was filtered through a filter paper, evaporated to dryness at 50°C under a stream of nitrogen, reconstituted with 0.8 mL of extraction solvent, transferred into a vial, evaporated to dryness again under nitrogen, reacted with 0.1 M trimethylphenyl ammonium hydroxide in methanol (50 μL), and assayed. The injection volume was 0.7 μL. Under these conditions, the relative retention ratios were 0.18 for allobarbital, 0.24 for butabarbital, 0.29 for amobarbital, 0.32 for pentobarbital, 0.36 for secobarbital, 0.56 for phenobarbital, 0.68 for heptabarbital, 0.70 for primidone, 0.91 for diphenylhydantoin, and 1.00 for the internal standard, respectively.

A standard curve for allobarbital was constructed in the range 5.0–50 mg/L. The correlation coefficient was greater than 0.995.

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ALLOPURINOL

CHEMISTRY

Allopurinol is a xanthine oxidase inhibitor. Its chemical name is 1,5-dihydro-4*H*-pyrazolo[3,4-*d*]-pyrimidin-4-one. Other names include Alopurinol, BW-56-158, Duovitan, and Uricodue. Its molecular formula is $C_5H_4N_4O$, with a molecular weight of 136.1 and a CAS number of 315-30-0. Allopurinol is a fluffy white to off-white powder. It has the following solubilities at 25°C: 0.48 mg/mL in water, 0.30 mg/mL in ethanol, 0.60 mg/mL in chloroform, and 4.6 mg/mL in dimethylsulfoxide. Allopurinol has a pK_a value of 9.4.

METHOD

Assay 1 Tada et al. [1] presented an HPLC method for the simultaneous determination of allopurinol and its metabolite oxypurinol in human serum. A Waters system consisted of a model 600 pump, model 717 Plus autosampler, and model 2487 dual-absorbance detector. The stationary phase was a Waters Resolve C_{18} Radial-Pak column (100 × 8 mm, 10 μm) coupled with a Waters guard column (3.9 × 20 mm) of the same packing material. The mobile phase was 2% (vol/vol) acetonitrile solution containing 100 mM potassium phosphate (pH 4.0) and 0.5 mM tetra-*n*-butylammonium hydrogen sulfate. The flow rate was 2.0 mL/min. UV detection was performed at 260 nm.

Allopurinol stock solution (50 μ/mL) and oxypurinol stock solution (200 μg/mL) were prepared in distilled ion-free water with one drop of 1 M sodium hydroxide. Standards were prepared by spiking blank serum with stock solutions. Sulfanilamide (5 μg/mL) in distilled ion-free water was used as the internal standard. A serum sample (100 μL) was mixed with 50 μL of the internal standard, followed with 100 μL of 10% trichloracetic acid, shaken, and centrifuged at 7500 *g* for 5 min. The supernatant was collected, filtered through a 0.5 μM Millex-LH filter, and assayed. The injection volume was 30 μL. Under these

conditions, retention times for oxypurinol, allopurinol, and sulfanilamide were 3.99, 4.58, and 5.32 min, respectively.

Intraday and interday coefficients of variation for allopurinol were less than 5.1% and 6.6%, respectively. Intraday and interday coefficients of variation for oxypurinol were less than 5.6% and 5.2%, respectively. The recovery ranged from 97.4% to 101% for allopurinol and 93.2% to 98.1% for oxypurinol. The limits of quantification for allopurinol and oxypurinol were 6 and 4.8 ng/mL, respectively. This assay was free of interference from uric acid, hypoxanthine, xanthine, theophylline, theobromide, 1,7-dimethylxanthine, and caffeine.

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ALPRAZOLAM

CHEMISTRY

Alprazolam is a benzodiazepine. Its chemical name is 8-chloro-1-methyl-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine. Other names include Alprax, Niravam, U-31889, and Xanax. Its molecular formula is $C_{17}H_{13}ClN_4$, with a molecular weight of 308.8 and a CAS number of 28981-97-7. Alprazolam is a white to off-white crystalline powder. Alprazolam is insoluble in water, soluble in alcohol, sparingly soluble in acetone, freely soluble in chloroform, and slightly soluble in ethyl acetate.

METHODS

Assay 1 Wei and Chen [1] reported a reversed-phase HPLC method for the determination of alprazolam in human plasma. A Shimadzu LC5A system included a model SPD2A UV detector, model SIL1A injector, and model CR3A integrator. The stationary phase was a Zorbax ODS column (250 × 4.6 mm). The mobile phase consisted of 50 mM potassium phosphate buffer (pH 6.0) and acetonitrile (65 : 35, vol/vol). The flow rate was 1.5 mL/min. Alprazolam was monitored at 220 nm.

Alprazolam stock solution 100 mg/L and the internal standard triazolam 1 mg/L were prepared in methanol and stored at 4°C. Alprzolam working solutions were prepared by diluting the stock solution with methanol. Standards were prepared by spiking the blank human plasma with working solutions. Patient plasma samples or standards (0.5 mL) were mixed with 200 μL of the internal standard, 1 mL of 1 M sodium borate buffer (pH 9.0), vortexed, mixed with 5 mL of ethyl ether, vortexed again for 5 min, and centrifuged at 3000 rpm for 5 min. Supernatants (ethyl ether layer) were collected, evaporated to dryness at 40°C in a water bath under a stream of nitrogen, reconstituted in 50 μL of the mobile phase, and assayed. The injection volume was 25 μL. Under these conditions, retention times of alprazolam and the internal standard were 10.5 and 12.1 min, respectively.

A calibration curve for alprazolam was constructed over the range from 10 to 200 μg/L. The correlation coefficient was 0.9998. Recoveries of alprazolam in plasma ranged from 90.10% to 91.83%. Within-day and between-day coefficients of variation were less than 2.33% and 3.82%, respectively.

There was no interference for the analysis of alprazolam in plasma from diazepam, clonazepam, nitrazepam, or oxazepam.

Assay 2 Wen and Yun [2] described an HPLC method for the simultaneous determination of alprazolam and doxepin in plasma. A Spectra Physics liquid chromatograph consisted of a model SP8800 ternary pump, model 200 variable-wavelength UV detector, model Focus detector, Datajet integrator, and a Rheodyne 7125 injector. The stationary phase was a reversed-phase Spheri-5 RP18 coumn (220 × 4.6 mm, 5 μm particle size). The mobile phase consisted of 0.05 M ammonium acetate buffer containing 1.0% triethylamine (pH 5.0) and methanol (40 : 60). The flow rate was 1.0 mL/min. UV detection was carried out at 254 nm and 0.002 AUFS.

Stock solutions of alprazolam 100 μg/mL and doxepin 1 mg/mL were prepared in methanol and stored at 4°C. Working solutions of drugs were prepared by diluting stock solutions with methanol. Standards were prepard by spiking blank plasma with working solutions. Plasma samples or standards (1 mL) were mixed with 1 mL of borate buffer (adjusted to pH 13 with 4 N sodium hydroxide solution), extracted in 4 mL of ethyl acetate, vortexed for 5 min, and centrifuged at 3000 rpm for 10 min. The organic layer was collected. The extraction was repeated once with 3 mL of ethyl acetate. The combined ethyl acetate solution was evaporated to dryness at 50°C under a stream of nitrogen, reconstituted in 50 μL of methanol, and assayed. The injection volume was 45 μL. Under these conditions, retention times for alprazolam and doxepin were 10.10 and 11.56 min, respectively.

A calibration curve for alprazolam was constructed in the range from 0 to 100 μg/L. The correlation coefficient was 0.9985. Recovery of the drug in plasma was 77.3%. Intraday and interday coefficients of variation were less than 6.2% and 3.7%, respectively. The limit of detection was 1.2 ng.

Assay 3 Sun et al. [3] reported the simultaneous analysis of six benzodiazepines in serum by HPLC. A Waters liquid chromatograph consisting of model 600 quaternary gradient pump, online degasser, column oven, and model 996 photodiode array detector was used. The stationary phase was a Nova-Pak C_{18} column (150 × 3.9 mm, 4 μm particle size). The column temperature was maintained at 30°C. The mobile phase consisted of 0.01 M phosphate buffer (pH 2.15) and acetonitrile (71 : 29, vol/vol) and was isocratically delivered at 1 mL/min. UV detection was performed at 223 nm. The injection volume was 20 μL.

A stock solution of alprazolam, diazepam, nitrazepam, clonazepam, triazolam, and estazolam at 100 mg/L each was prepared in methanol and stored at 4°C. Standards

were prepared by diluting the stock solution with blank human serum. An aliquot of 1 mL of serum or standard was loaded onto an Accubond C_{18} SPE cartridge (100 mg/1 mL) that was preconditioned with 2 mL of methanol followed by 2 mL of water and rinsed sequentially with 2 mL of water and 2 mL of 25% methanol in water. The cartridge was centrifuged at 3000 rpm for 1 min. Drugs were eluted with 4 mL of methanol/ethyl acetate (1 : 1, vol/vol), evaporated to dryness at 40°C, reconstituted with 100 μL of methanol, and assayed. Under these conditions, retention times for nitrazepam, estazolam, clonazepam, alprazolam, triazolam, and diazepam were 5.8, 7.2, 8.1, 9.4, 11.1, and 13.7 min, respectively.

A calibration curve for alprazolam was constructed in the range of 0.01–10.0 mg/L. The correlation coefficient was 0.9996. Recovery of the drug in serum was 83.4%. Intraday and interday coefficients of variation were 5.9% and 4.8%, respectively. Retention times of aminophylline, sodium valproate, primidone, phenobarbital, sodium phenytoin, carbamazepine, and amitriptyline were 1.4, 1.1, 1.7, 2.7, 4.6, 4.8, and 6.9 min, respectively, and did not interfere with the analysis of alprazolam.

Assay 4 Guo et al. [4] reported the simultaneous determination of estazolam, triazolam, and alprazolam in human plasma by RP-HPLC. A Hewlett-Packard Series 1100 liquid chromatograph equipped with a diode-array detector was used. The stationary phase was a Zorbax RP C_{18} column (150 × 4.6 mm, 5 μm particle size). The mobile phase consisted of methanol and 25 mM ammonium acetate aqueous solution (57 : 43) and was isocratically delivered at 0.6 mL/min. UV detection was performed at 230 nm. The injection volume was 10 μL.

Stock solutions of estazolam, triazolam, and alprazolam at 1.0 mg/mL each were prepared in methanol and working solutions were prepared by diluting stock solutions with methanol. A stock solution of carbamazepine at 1.0 mg/mL was prepared in methanol. A working solution of carbamzapine at 5.0 mg/L was prepared by diluting the stock solution with methanol and used as an internal standard. Standards were prepared by spiking blank human plasma with working solutions. An aliquot of 0.5 mL of plasma or standard was mixed with 50 μL of the internal standard solution, vortexed, mixed with 0.25 mL of a mixture of 1.0 M sodium carbonate and 1.0 M sodium bicarbonate (3 : 1), extracted with 5 mL of ethyl ether, vortexed for 1 min, centrifuged at 3000 rpm for 3 min, and allowed to stand still for 2 min. An aliquot of 4.7 mL of the organic layer was separated, evaporated to dryness at 50°C under a stream of air, reconstituted with 50 μL of methanol, and assayed. Under these conditions, retention times for carbamazepine, estazolam, triazolam, and alprazolam were about 7.9, 9.2, 10.5, and 11.3 min, respectively (estimated from the published chromatogram).

Calibration curves for alprazolam were constructed over the range from 20 to 1000 μg/L. Correlation coefficients were 0.9993. Recovery of the drug from plasma ranged from 76.8% to 79.7%. The coefficient of variation of the assay was less than 4.8%. There was no interference with this method from drugs such as nitrazepam, diazepam, oxazepam, phenobarbital, and chlordiazepoxide.

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ALPRENOLOL

CHEMISTRY

Alprenolol is a noncardioselective β-blocker. Its chemical name is 1-(2-allylphenoxy)-3-isopropylaminopropan-2-ol. Other names include Alprenololi and Alprenololum. Its molecular formula is $C_{15}H_{23}NO_2$, with a molecular weight of 249.3 and a CAS number of 13655-52-2. Alprenolol hydrochloride occurs as a white or almost white crystalline powder or colorless crystals. Alprenolol hydrochloride is very soluble in water and freely soluble in alcohol and dichloromethane.

METHOD

Assay 1 Lee et al. [1] developed an LC-MS/MS method for the simultaneous determination of 12 ß-blockers and $ß_2$-agonists in sewage samples. A Waters 2695 separation module was used. The stationary phase was an Agilent Zorbax SB C_8 column (150 × 2.1 mm, 3.5 μm particle size) protected by a SB C_8 guard column (12.5 × 2.1 mm, 5 μm particle size). The column temperature was maintained at 35°C. Mobile phase A was a mixture of water, acetonitrile, and formic acid (94.5 : 5.0 : 0.5, vol/vol/vol), and mobile phase B was a mixture of acetonitrile and formic acid (99.5 : 0.5, vol/vol). The mobile phase was delivered in a gradient mode from 100% A to 75% A in 13 min, held at 75% A for 13 min, and then pumped at 100% A for another 14 min. The flow rate was 0.2 mL/min. The injection volume was 10 μL. The total runtime of an injection was 40 min.

A Micromass Quattro Ultima triple quadrupole mass spectrometer equipped with an electrospray ionization source was operated in the positive-ion mode. The major parameters were set as follows: nebulizer gas (nitrogen) 50 L/h, desolvation gas (nitrogen) 500 L/h, source temperature 120°C, desolvation temperature 350°C, capillary voltage 3.45 kV, cone energy 50 V, and collision energy 17 V. Alprenolol was analyzed in the multiple-reaction monitoring (MRM) mode at ion transitions m/z 250 → 116 for quantitation and m/z 250 → 173 for confirmation.

Individual stock solutions of acebutolol, alprenolol, atenolol, bisoprolol, clenbuterol, fenoterol, labetalol, metoprolol, nadolol, pindolol, propranolol, sotalol, terbutaline, and timolol at 1000 μg/mL were prepared in acetonitrile or methanol. A stock solution of salbutamol was prepared at 500 μg/mL. These stock solutions were stored at −20°C. Working solutions were prepared by mixing and diluting these stock solutions with mobile phase B. An aliquot of 250 mL of sewage sample was filtered through a 1.2-μm GF/C filter (90 mm i.d.) with a layer of Celite; acidified to pH 3 with 1 M hydrochloric acid, loaded onto a Waters Oasis MCX cartridge (6 mL, 150 mg, 30 μm) at a flow rate of 10–15 mL/min, which was preconditioned with 6 mL of methanol followed by 10 mL of water at pH 3; dried for 10 min under vacuum; washed with 100 mL of water at pH 3 followed by 6 mL of methanol; eluted with 8 mL of a mixture of dichloromethane, 2-propanol, and ammonium hydroxide (78 : 20 : 2, vol/vol/vol); evaporated to dryness at 40°C under a stream of nitrogen; reconstituted in 1.0 mL of mobile phase B; filtered through a 0.45-μm nylon syringe filter; and assayed. Under these conditions, retention times for acebutolol, alprenolol, atenolol, bisoprolol, labetalol, metoprolol, nadolol, pindolol, propranolol, sotalol, timolol, clenbuterol, fenoterol, salbutamol, and terbutaline were 17.36, 24.32, 8.96, 21.77, 22.11, 18.02, 13.60, 14.15, 23.87, 7.85, 17.02, 17.91, 11.50, 6.97, and 6.53 min, respectively.

Calibration curves for alprenolol were constructed over the range from 50 to 500 pg/μL. The mean recovery of the drug from water ranged from 82% to 85%. The limit of detection was 7 ng/L.

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AMBROXOL HYDROCHLORIDE

CHEMISTRY

Ambroxol hydrochloride is a mucolytic. Its chemical name is *trans*-4-(2-amino-3,5-dibromobenzylamino)cyclohexanol hydrochloride. Other names include Ambril, Amxol, Mucoxol, and NA-872. Its molecular formula is $C_{13}H_{18}Br_2N_2O{\cdot}HCl$, with a molecular weight of 414.6 and a CAS number of 23828-92-4. Ambroxol hydrochloride occurs as a white or yellowish crystalline powder. Ambroxol hydrochloride is sparingly soluble in water and practically insoluble in dichloromethane. It is soluble in methanol. Ambroxol hydrochloride should be protected from light.

METHOD

Assay 1 Zhang et al. [1] reported the determination of ambroxol hydrochloride in plasma by HPLC. A Shimadzu LC2010AHT liquid chromatograph was used. The stationary phase was a Diamonsil C_{18} column (150 × 4.6 mm, 5 μm particle size). The mobile phase consisted of methanol, acetonitrile, and 0.01 M phosphate buffer (pH 7.0) (45 : 27 : 28) and was delivered at 1.0 mL/min. UV detection was performed at 245 nm. The injection volume was 30 μL.

Stock solutions of ambroxol hydrochloride at 1.069 mg/mL and nicardipine hydrochloride (internal standard) at 1.616 mg/mL were separately prepared in methanol and diluted to nominal concentrations of 50 and 20 μg/mL, respectively. Standards were prepared by spiking blank human plasma with the stock solution. An aliquot of 0.78 mL of a plasma sample or standard was mixed with 20 μL of nicardipine hydrochloride (200 ng/mL) and 200 μL of 1 M sodium hydroxide solution, vortexed for 30 s, extracted with 4.0 mL of *n*-hexane/*n*-heptane (1 : 1) containing 5% isopropanol, vortexed for 1 min, and centrifuged at 3000 rpm for 10 min. An aliquot of 3 mL of the supernatant was collected, mixed with 200 μL of 0.01 M hydrochloric acid, vortexed for 1 min, and centrifuged at 3000 rpm for 10 min. The aqueous layer was collected and assayed. Under these conditions, retention times of ambroxol and nicardipine were 7.4 and 14.0 min, respectively.

Linear calibration curves were constructed over the range from 10 to 480 ng/mL. Correlation coefficients were 0.9999. The recovery of ambroxol from plasma was more than 94%. Intraday and interday coefficients of variation were less than 4.1% and 4.6%, respectively. The limit of quantification was 10 ng/mL.

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AMIKACIN

CHEMISTRY

Amikacin is a semisynthetic aminoglycoside antibiotic. Its chemical name is (*S*)-*O*-3-amino-3-deoxy-α-D-glucopyranosyl-(1 → 6)-*O*-[6-amino-6-deoxy-α-D-glucopyranosyl-(1 → 4)]-N^1-(4-amino-2-hydroxy-1-oxobutyl)-2-deoxy-D-streptamine. Other names include Amicacina and Amikin. Its molecular formula is $C_{22}H_{43}N_5O_{13}$, with a molecular weight of 585.6 and a CAS number of 37517-28-5. Amikacin and amikacin sulfate occur as white crystalline powders. Amikacin is sparingly soluble in water. Amikacin sulfate is freely soluble in water.

METHODS

Assay 1 Oguri and Miki [1] described the determination of amikacin in human plasma by high-performance capillary electrophoresis (HPCE) with fluorescence detection. A Jasco model CE990 HPCE system was equipped with a model FP920 fluorescence detector wth a capillary cell unit, model CD971 UV detector, and model 807IT data

processor and was set in the micellar electrokinetic chromatography (MERK) mode. A fused-silica capillary tube (75 cm × 50 μm) was used. The carrier electrolyte was 40 mM SDS–20 mM phosphate–borate buffer (pH 7). The applied voltage was 30 kV. The absorption was monitored at an excitation wavelength of 414 and an emission wavelength of 482.

Amikacin stock solution (1.0 mg/mL) was prepared in water. Working solutions were prepared by diluting the stock solution with water. Amikacin standards were prepared by spiking blank human plasma with working solutions. A plasma sample or standard (200 μL each) was centrifuged in Millipore Ultrafree C3LCC tubes at 2000 *g* for 20 min at 4°C and ultrafiltrate plasma with a molecular mass less than 5000 was collected. An aliquot (40 μL) of reaction buffer was added to a tube containing 100 μg of derivatizing agent, 1-methoxycarbonylindolizine-3,5-dicarbaldehyde, ultrasonicated for 30 s, mixed well with 20 μL of ultrafiltrate plasma, incubated at room temperature for 15 min in the dark, and assayed. Under these conditions, the retention time of the derivatized amikacin was 17.6 min.

A linear relationship was obtained over the concentraton range 5–200 μg/mL, with a correlation coefficient of 0.998. Coefficients of variation of the assay were less than 4.08%. The limit of detection was 0.5 mg/mL, and the limit of quantification was 5 mg/mL.

Assay 2 Baranowska et al. [2] developed an HPLC method for simultaneous determination of imipenem, paracetamol, dipyrone, vancomycin, amikacin, fluconazole, cefazolin, prednisolone, dexamethasone, furosemide, and ketoprfen in human urine. The Merck–Hitachi liquid chromatographic system consisting of a model L6200A intelligent pump, model L7480 diode-array detector, a model 7360 fluorescence detector, and a Rheodyne injector with a 20-μL loop was utilized. The stationary phase was a Merck LiChroCART Purospher C_{18}e analytical column (125 × 3 mm, 5 μm particle size) protected with a precolumn (4 × 4 mm, 5 μm particle size) of the same packing material. Solvent A was 0.05% trifluoroacetic acid in water; solvent B, methanol; and solvent C, acetonitrile. The mobile phase was delivered in a gradient mode as follows:

Time (min)	%A	%B	%C	Flow Rate (mL/min)
0	92	6	2	0.75
10	50	42	8	0.65
20	25	55	20	0.60
25	92	6	2	0.75

UV detections were performed at 300, 243, 259, 210, 210, 274, 242, 242, 234, and 254 nm for imipenem, paracetamol, dipyrone, vancomycin, fluconazole, cefazolin, prednisolone, dexamethasone, furosemide, and ketoprofen, respectively. Amikacin after derivatization was monitored by a fluorescence detector at an excitation wavelength of 355 nm and an emission wavelength of 415 nm. The runtime of an injection was 25 min. Under these conditions, retention times were 4.0, 4.9, 6.7, 8.1, 9.5, 10.0, 10.9, 13.3, 14.1, 16.0, and 19.0 min for imipenem, paracetamol, dipyrone, vancomycin, amikacin, fluconazole, cefazolin, prednisolone, dexamethasone, furosemide, and ketoprofen in human urine, respectively.

Stock solutions of these drugs at 1 mg/mL were separately prepared in water/methanol (50 : 50, vol/vol) and stored at −18°C. Working solutions containing these drugs were prepared by mixing individual stock solutions and diluting them with water/methanol (90 : 10, vol/vol). Standards were prepared by spiking drug-free human urine with working stock solutions. A derivatization reagent was prepared by dissolving 40 mg of phthaldialdehyde in 0.8 mL methanol followed by mixing with 7.2 mL of a buffer (boric acid/potassium chloride/sodium hydroxide, pH 9.00) and 40 μL of 3-mercaptopropionic acid and was stored at 4°C in the dark no longer than 48 h before use. An aliquot of 50 μL of a urine sample or standard was mixed with 20 μL of the derivatization reagent, shaken for 1 min, incubated at 22°C for 30 min, and assayed. The injection volume was 20 μL.

A calibration curve for amikacin was constructed in the range of 0.5–35 μg/mL. The correlation coefficient was 0.9999. The recovery of amikacin from urine ranged from 96.1% to 102.3%. The coefficient of variation for the assay was less than 4.7%. The accuracy in the relative percentage error was less than 3.9%. Limit of detection and limit of quantification were 0.25 and 0.65 μg/mL, respectively.

Assay 3 Mendu et al. [3] reported an improved application of the enzyme multiple-immunoassay technique (EMIT) for amikacin. A Dade–Behring Dimension RxL Max automated clinical analyzer was used. Calibrators for amikacin were reconstituted according to the manufacturer's instuctions. Reagent 1 (R1) and Reagent 2 (R2) were separately dissolved with 6 mL of distilled water, and EMIT drug assay buffer concentrate was diluted with distilled water (1 : 14, vol/vol). One part of R1 and R2 was then separately mixed with 8 parts of EMIT drug assay buffer. The optical density changes were monitored for a shorter period of time than recommended by the manufacturer.

A linear calibration curve was constructed over the range from 1 to 50 μg/mL. Within-day and between-day coefficients of variation were less than 2.9% and 5.0%, respectively. Results by the Dimension RxL Max EMIT were compared with those by the Abbott TDx FLx FPIA using linear regression analysis and Bland–Altaman plots to assess bias. A relationship between these two methods was obtained: [TDx FLx FPIA] = 1.01 * [Dimension RxL Max] – 0.075 (Syx = 2.056, $r = 0.986$, $n = 71$). The potential cross-reactant, tobramycin, did not cause a difference greater than 10% of the blank.

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AMILORIDE HYDROCHLORIDE

CHEMISTRY

Amiloride is a weak diuretic. Its chemical name is *N*-amidino-3,5-diamino-6-chloropyrazine-2-carboxamide hydrochloride dihydrate. Other names include Amipramizide, Aridil, MK-870, and Moduretic. Its molecular formula is $C_6H_8ClN_7O{\cdot}HCl{\cdot}2H_2O$, with a molecular weight of 302.1 and a CAS number of 17440-83-4. Amiloride hydrochloride occurs as a yellow to greenish-yellow, odorless or practically odorless powder. Amiloride hydrochloride is slightly soluble in water and insoluble in acetone, chloroform, ether, and ethyl acetate. It is freely soluble in dimethyl sulfoxide.

METHOD

Assay 1 Jin et al. [1] reported a solid-phase extraction (SPE)/RP-HPLC screening procedure for bumetanide, spironolactone, amiloride, acetazolamide, hydrochlorothiazide, chlorothiazide, dichlorphenamide, furosemide, pemoline, triamterene, benzthiazide, bendroflumethiazide, ethacrynic acid, probenecid, and canrenone in urine. An HP1090 series system equipped with a diode-array detector was used. The stationary phase was a LiChrosorb RP18 column (200 × 4.6 mm, 5 μm particle size). The column temperature was maintained at 40°C. The mobile phase consisted of monobasic sodium phosphate buffer (pH 3) (A) and acetonitrile (B), where the phosphate buffer was prepared by dissolving 6.9 g of monobasic sodium phosphate and 1.305 g of ethanolamine in 1000 mL of water and adjusted to pH 3 with phosphoric acid. The mobile phase was initially delivered at 1 mL/min at 15% B from 0 to 3 min, increased to 33% B from 3 to 9.5 min, to 40% B from 9.5 to 9.8 min, and to 80% B from 9.8 to 20 min. UV detections were performed at 216, 230, and 275 nm. The injection volume was 10 μL.

Stock solutions of bumetanide, spironolactone, amiloride, acetazolamide, hydrochlorothiazide, chlorothiazide, dichlorphenamide, furosemide, pemoline, triamterene, benzthiazide, bendroflumethiazide, ethacrynic acid, probenecid, and canrenone at 1 mg/mL were individually prepared in methanol and stored at 4°C. Standards were prepared by spiking drug-free urine with stock solutions. An aliquot of 2 mL of urine or standard was centrifuged. The supernatant was loaded onto a laboratory-made XAD-2 (100–200 μm) SPE column that was preconditioned with 5 mL of water, washed with water to remove water-soluble materials, eluted with 4 mL of ethyl acetate–ether (1 : 1), evaporated to dryness, reconstituted with 300 μL of methanol, and assayed. Under these conditions, retention times for amiloride, acetazolamide, hydrochlorothiazide, caffeine, pemoline, triamterene, dichlorphenamide, chlorothiazide, furosemide, benzthiazide, bendroflumethiazide, ethacrynic acid, bumetanide, probenecid, spironolactone, and canrenone were 3.5, 4.2, 5.6, 6.2, 7.0, 7.9, 10.2, 10.7, 13.5, 14.6, 15.4, 15.8, 16.1, 16.4, 17.8, and 18.4 min, respectively.

The mean recovery of amiloride from urine was 79%. The limit of detection was 1.0 μg/mL when the drug was monitored at 216 nm.

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AMIODARONE HYDROCHLORIDE

CHEMISTRY

Amiodarone hydrochloride is a class III antiarrhythmic agent. Its chemical name is 2-butyl-3-benzofuranyl-4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl ketone hydrochloride. Other names include Cordarone, L-3428, Pacerone, and SKF-33134-A. Its molecular formula is $C_{25}H_{29}I_2NO_3{\cdot}HCl$, with a molecular weight of 681.8 and a CAS number of 19774-82-4. Amiodarone hydrochloride is a white to cream crystalline powder. The drug has solubilities of approximately 0.72 mg/mL in water and 12.8 mg/mL in alcohol at 25°C. Amiodarone has a pK_a value of approximately 6.6.

METHODS

Assay 1 Li et al. [1] developed an LC-MS/MS method for simultaneous determination of 10 antiarrhythmic drugs—diltiazem, amiodarone, mexiletine, propranolol, sotalol, verapamil, bisoprolol, metoprolol, atenolol, and carvedilol—in human plasma. A Shimadzu LC20AD liquid chromatographic system was equipped with two pumps, a vacuum degasser, and an autosampler. The stationary phase was a Capcell Pak C_{18} column (50 × 2.0 mm, 5 μm particle size). Solvent A was 0.02% formic acid in acetonitrile and solvent B 0.02%, formic acid in water. The mobile phase was delivered in a gradient mode from 95% B to 50% B in the first 3.5 min, then from 50% B to 5% B in next 0.5 min, and returned to 95% B in 0.5 min followed by 3-min equilibration. The flow rate was 0.3 mL/min. The temperature of autosamper was maintained at 4°C.

An ABI-SCIEX API3000 triple quadrupole tandem mass spectrometer with TurboIonSpray source was coupled to the liquid chromatograph as a detector. It was operated in positive ionization mode. The ionspray voltage was set at 2.5 kV, source temperature at 450°C, collision-activated dissociation at 12, and the collision gas nitrogen. The declustering potential was 38 V and collision energy 95 V. Analytes were monitored in multiple-reaction monitoring (MRM) mode: m/z 646.1 → 58.2, 279.2 → 124.2,

281.2 → 156.2, and 311.2 → 156.2 for amiodarone, sulfisomedine, sulfamethoxydiazine, and sulfadimethoxine, respectively.

Sulfamethoxydiazine, sulfadimethoxine, and sulfisomedine as internal standards 200 ng/mL were prepared in acetonitrile. Stock solutions of drugs were prepared in methanol. Working solutions were prepared by diluting stock solutions in methanol/water (1 : 1) and storing them at −20°C. Standards were prepared by spiking blank human plasma with working solutions. Plasma samples, standards, and controls (100 μL each) were mixed with 200 μL of internal standard, vortexed for 10 s, and centrifuged at 16,000 *g* for 3 min. An aliquot (50 μL) of the supernatant was mixed with 150 μL of water and assayed. The injection volume was 5 μL. Under these conditions, retention times of amiodarone, sulfisomedine, sulfamethoxydiazine, and sulfadimethoxine were 5.1, 2.8, 3.9, and 4.8 min, respectively.

A calibration curve for amiodarone was constructed in the range from 50 to 10,000 ng/mL. The correlation coefficient was 0.9982. The accuracy ranged from 91.3% to 113.2%. The recovery from plasma ranged from 85.6% to 95.8%. Intraday and interday coefficients of variation were less than 10.3% and 5.3%, respectively.

Assay 2 Hua et al. [2] described an LC-MS method for the simultaneous determination of amiodarone and its metabolite in plasma. A Waters Alliance 2690 liquid chromatograph was coupled with a Waters ZQ mass spectrometer. The stationary phase was a Waters XTerra MS C_{18} column (150 × 3.9 mm, 5 μm particle size). The column temperature was maintained at 40°C. The mobile phase comprised 30 mM ammonium acetate and acetonitrile (12 : 88). The flow rate was 0.85 mL/min. Split of the flow rate (5 to 17) to spectrometer was used. Mass spectrometer was operated in selected-ion recording (SIR) mode of electrospray ionization: m/z 646.3 for amiodarone, 618.2 for desethylamiodarone, and 315.4 for chlorimipramine. Other parameters were as follows: capillary energy 3.0 kV, source temperature 110°C, desolvation temperature 180°C, cone energy: 52 V for amiodarone, 56 V for desethylamiodarone, and 30 V for chlorimipramine, and desolvent gas 400 L/h.

Chlorimipramine 30 μg/mL was used as an internal standard. Plasma samples and standards (0.25 mL) were mixed with 10 μL of the internal standard and 0.75 mL of acetonitrile, vortexed for 2 min, ultrasonicated for 5 min, and centrifuged at 9500 rpm for 5 min. Supernatants were collected and assayed. The injection volume was 20 μL. Under these conditions, retention times for amiodarone, desethylamiodarone, and the internal standard were 5.24, 3.12, and 2.35 min, respectively.

Calibration curves for amiodarone and desethylamiodarone were constructed in the range 0.10–3.20 μg/mL. Correlation coefficients were 0.9993 and 0.9996 for amiodarone and desethylamiodarone, respectively. Limits of detection for amiodarone and desethylamiodarone were 0.025 and 0.030 μg/mL and limits of quantitation for amiodarone and desethylamiodarone were 0.080 and 0.095 μg/mL, respectively.

This assay was free of interference from penicillin, ampicillin, captopril, nifedipine, nimodipine, amlodipine besylate, aminophylline, cephradine, ceftazidine, and sulbutamol.

Assay 3 Saita et al. [3] developed an enzyme-linked immunosorbent assay (ELISA) for the quantification of amiodarone in serum. A Labsystems Fluoroskan Ascent fluorescence microplate reader was used. Buffer A was 20 mM phosphate buffer (pH 7.0) containing 0.1 M sodium chloride, 1 mM magnesium chloride, 0.1% bovine serum albumin (BSA), 0.1% sodium azide and buffer B was 60 mM phosphate buffer (pH 7.4) containing 10 mM ethylenediaminetetraacetate, 0.1% BSA, and 0.1% sodium azide. Antiamiodarone IgG antibody and amiodarone-β-D-galactosidase conjugate were prepared in-house.

Wells in microtiter plates were coated by loading 150 μL of antiamiodarone IgG (2.0 μg/mL) in 10 mM Tris-HCl buffer (pH 8.5) containing 10 mM sodium chloride and 10 mM sodium azide, allowed to stand for 1 h at 37°C, washed twice with buffer B, incubated with 200 μL of 10 mM Tris-HCl buffer (pH 8.5) containg 10 mM sodium chloride and 10 mM sodium azide containing 2% BSA at 37°C for 20 min, filled with 50 μL of samples or buffer B as a control followed immediately by 50 μL of amiodarone-β-D-galactosidase conjugate (diluted 1 : 500 in buffer B), incubated overnight at room temperature, and washed thoroughly with buffer B, filled with 125 μL of 0.1 mM 4-methylumbelliferyl-β-D-galactopyranoside in buffer A, incubated at 37°C for 60 min, mixed with 75 μL of 0.5 M glycine–sodium hydroxide buffer (pH 10.3) to terminate the enzyme reaction, and measured at an excitation wavelength of 355 nm and an emission wavelength of 460 nm.

A standard curve for amiodarone was obtained in the range from 3.2 μg/mL to 10 μg/mL. Recoveries ranged from 95.0% to 104.0%. Intraday and interday coefficients of variation were less than 12.5% and 9.6%, respectively. This ELISA assay had 4.4% crossreactivity with tilorone. No crossreactivity was found in procainamide and 2,6-diiode-4-nitrophenol.

The relationship of this ELISA assay with an HPLC method was found to be HPLC = 0.995∗ELISA − 0.048 ($r = 0.993$, $n = 11$).

Assay 4 Saita et al. [3] also described an HPLC method for the analysis of amiodarone in serum. A Shimadzu model LC10AT liquid chromatograph was equipped with a model SPD10AV detector and model CR7A Chromatopack integrator. The stationary phase was a Merck Lichrospher 100 RP-18 endcapped column (125 × 4 mm, 5 μm particle size). The mobile phase was composed of methanol, water, and 28% ammonium hydroxide (90 : 9.8 : 0.2, vol/vol/vol). The flow rate was 1.5 mL/min. UV detection was carried out at 254 nm.

A serum sample (1 mL) was mixed with 30 μL of 1.2 M hydrochloric acid and 100 μL of mexiletine (5 μg/mL) as an internal standard, extracted with 5 mL of diethyl ether, shaken for 10 min, and centrifuged at 2270 *g* for 5 min. The organic layer (4 mL) was collected, evaporated to dryness at 40°C under a stream of nitrogen, reconstituted in 50 μL of methanol, and assayed. The injection volume was 40 μL.

A linear relationship between peak height ratios of amiodarone to the internal standard was obtained in the concentration range 0.5–8.0 μg/mL.

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AMISULPRIDE

CHEMISTRY

Amisulpride is an atypical antipsychotic agent. Its chemical name is 4-amino-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-5-(ethylsulfonyl)-2-methoxybenzamide. Other names include Amiprid, DAN-216, and Solian. Its molecular formula is $C_{17}H_{27}N_3O_4S$, with a molecular weight of 369.5 and a CAS number of 71675-85-9. Amisulpride is a white or almost white crystalline powder. Amisulpride is practically insoluble in water, sparingly soluble in dehydrated alcohol, and freely soluble in dichloromethane.

METHODS

Assay 1 Kratzsch et al. [1] reported a validated assay for the simultaneous determination of 15 neuroleptics and three of their metabolites in plasma, including amisulpride by liquid chromatography/mass spectrometry with atmospheric-pressure chemical ionization. An Agilent Technologies AT1100 Series HPLC system consisted of a binary pump, autosampler, and degasser. The stationary phase was a Merck LiChroCART Superspher 60 RP Select B column (125 × 2 mm) with a LiChroCART 10-2 guard column of the same packing material. Eluent A was 5 mM aqueous ammonium formate adjusted to pH 3 with formic acid and eluent B, acetonitrile. The mobile phase was delivered in a step-gradient mode: 40% B at 0.4 mL/min from 0 to 5.5 min, 90% B at 0.7 mL/min from 5.5 to 8 min, 40% B at 0.65 mL/min from 8 to 9.5 min, and 40% B at 0.4 mL/min from 9.5 to 10 min.

An Agilent Technologies AT1100 atmospheric-pressure chemical ionization electrospray (APCI) LC/MSD system was used with the following parameters: drying gas, nitrogen (7 L/min, 300°C); nebulizer gas, nitrogen (172.5 kPa), capillary voltage 4000 V; vaporizer temperature 400°C; corona current 5.0 μA; fragmenter voltage 100 V; and positive selected-ion monitoring (SIM) mode. The full-scan in the 100- and 200-V traces was made with the following ions (m/z): 342, 313, 370, 427, 411, 327, 380, 264, 343, 340, 376, 420, 401, 298(IS), 438, 332, 435, and 462. Amisulpride was quantitated in the SIM mode at 100 V fragment voltage at m/z 370 in a time window of 0–3.8 min.

Trimipramine-d_3 0.01 mg/mL in methanol was used as the internal standard. A stock solution of amisulpride 1 mg/mL was prepared in methanol. Working solutions were prepared by diluting the stock solution. Standards were prepared by spiking pooled blank human plasma with working solutions. A plasma sample or standard (0.5 mL) was diluted with 2 mL of purified water; mixed with 0.05 mL of the internal standard for 15 s; loaded onto a Separtis Isolute Confirm HCX solid-phase cartridge that was conditioned with 1 mL of methanol followed with 1 mL of water; washed sequentially with 1 mL of purified water, 1 mL of 0.01 M hydrochloric acid, and 2 mL of methanol; dried under vaccum; eluted with 1 mL of methanol–aqueous ammonia (98 : 2, v/v); evaporated to dryness at 56°C under a gentle stream of nitrogen; reconstituted with 50 μL of methanol; and assayed. The injection volume was 2 μL.

The least-squares ($1/C^2$) calibration curve for amisulpride was calculated by plotting the peak area ratios of the target ion of the drug versus that of the internal standard in the range of 0.025 to 0.5 mg/L. The correlation coefficient was 0.998. Within-day and between-day coefficients of variation were 6.8% and 11.1%, respectively. Accuracy in the relative percentage error was less than 11.3%. The recovery ranged from 93.9% to 98.4%. The limits of detection and quantification were 0.005 and 0.025 mg/L, respectively. This assay has been validated for simultaneous determination of amisulpride, bromperidol, clozapine, droperidol, flupenthixol, fluphenazine, haloperidol, melperone, olanzapine, perazine, pimozide, risperidone, sulpiride, zotepine, zuclopenthixol, norclozapine, clozapine *N*-oxide, and 9-hydroxyrisperidone.

Assay 2 Sachse et al. [2] presented an HPLC method with column switching for the determination of amisulpride for drug-monitoring and pharmacokinetic studies. An Agilent 1100 Series system consisting of a binary pump, autosampler, thermostated column compartment containing an electric six-port switching valve coupled to the autosampler, and a variable-wavelength detector was employed. The stationary phase was a LiChrospher CN column (250 × 4.6 mm, 5 μm). The cleanup column was a silica CN column (10 × 4.6 mm, 20 μm, 10 nm pore size). The mobile phase consisted of 50% acetonitrile and 50% 0.008 M dibasic potassium phosphate, adjusted to pH 6.4 with phosphoric acid, and was delivered at 1.5 mL/min. UV detection was performed at 254 nm.

Amisulpride stock solution 1 mg/mL was prepared in methanol. Working solutions were prepared by diluting the stock solution with deionized water. Standards were prepared by spiking blank human plasma with working solutions and were stable for several months at −20°C. A plasma sample or standard was centrifuged at 10,000 *g* for 5 min. The supernatant (100 μL) was injected onto the cleanup column, washed with deionized water containing 8% (vol/vol) acetonitrile at a flow rate of 1.5 mL/min for 5 min, switched to the analytical column, eluted with the mobile phase at 1.5 mL/min for another 5 min, and switched back to the cleanup column. The cleanup column

was replaced after 100 injections. Under these conditions, the retention time of amisulpride was about 13.6 min.

The linear relationship was obtained over the range 10.00–600.00 ng/mL with a correlation coefficient of 0.9998. Interday coefficients of variation were less than 11.3%. Accuracy in the relative percentage error was less than 9.1%. The limit of detection was 5 ng/mL. This assay was free of interference from alprazolam, amitriptyline, carbamazepine, citalopram, clomipramine, desipramine, diazepam, dipiperone, fluoxetine, haloperidol, imipramine, lorazepam, maprotiline, *N*-desmethyl-venlafaxine, nefazodone, nordiazepam, norclozapine, nortriptyline, *O*-desmethyl-venlafazine, oxazepam, paroxetine, risperidone, sertraline, temazepam, venlafaxine, or zolpidem.

Assay 3 Frahnert et al. [3] reported the analysis of amisulpride in human serum by HPLC for therapeutic drug monitoring. A liquid chromatograph consisting of a Bischoff 2200 pump, a Bischoff SDU2003 solvent degasser, a Waters WISP 717 autosampler, and a Shimadzu SPD10AVP UV detector was used. The stationary phase was a Macherey–Nagel Nucleosil 100-5-Protect 1 analytical column (250 × 4.6 mm, 5 μm particle size). The mobile phase consisted of 25 mM monobasic potassium phosphate buffer (pH 7.0) and acetonitrile (60 : 40) and was isocratically delivered at 1 mL/min. UV detection was performed at 230 nm. The injection volume was 100 μL.

A stock solution of amisulpride at 1.0 mg/mL was prepared in methanol. Working solutions were prepared by diluting this stock solution with water. Standards were prepared by spiking blank human serum with working solutions. Melperone at 3000 ng/mL was used as an internal standard. An aliquot of 1 mL of serum sample or standard was centrifuged at 13000 *g* and at 4°C for 10 min. An aliquot of 0.9 mL of the supernatant was separated; mixed with 0.1 mL of internal standard and 2.0 mL of 0.1 M monobasic potassium phosphate buffer (pH 6.0); loaded onto a Varian 3M-Empore extraction disk cartridge (3 mL) that was preconditioned with 1 mL of methanol followed by 1 mL of water; pulled through the cartridge; washed sequentially with 1 mL of water, 1 mL of 1 M acetic acid, 1 mL of *n*-hexane, 2 mL of *n*-hexane–ethyl acetate (1 : 1), and 1 mL of methanol; eluted with 1 mL of 2-propanol–25% ammonium solution–dichloromethane (20 : 2 : 78), evaporated to dryness, reconstituted with 250 μL of acetonitrile and water (3 : 7), and assayed. Under these conditions, retention times for melperone and amisulpride were 8.8 and 6.1 min, respectively.

Calibration curves for amisulpride were constructed over the range from 10 to 1000 ng/mL. Correlation coefficients were 0.9995. Recovery of the drug from serum ranged from 98.9% to 100.6%. Intraassay and interassay coefficients of variation were less than 3.9% and 5.6%, respectively. There was no interference with the assay from the following drugs and their metabolites (retention times in minutes): sulpiride (4.1), *O*-desmethylvenlafaxine (4.8), 9-OH-risperidone (6.6), *m*-chlorophenylpiperazine (8.0), normirtazapine (8.3), zolpidem (10.2), nordoxepin (10.9), diazepam (11.0), benperidol (11.5), normaprotiline (11.5), dibenzepine (11.5), opipramol (11.6), norfluoxetine (13.4), norclozapine (14.4), haloperidol (15.3), norclomipramine (19.2), trifluperidol (20.8), olanzapine (21.0), ziprasidone (26.4), promethazine (28.1), flupenazine (31.0), nefazodone (32.5), chlorprothixene (36.4), thioridazine (43.2), pimozide (44.1), carbamazepine, perazine, zotepine, valproate, zopiclone, buspirone, lorazepam, and biperidene.

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AMITRIPTYLINE HYDROCHLORIDE

CHEMISTRY

Amitriptyline hydrochloride is a tricyclic antidepressant. Its chemical name is 3-(10,11-dihydro-5*H*-dibenzo [*a,d*]cyclohepten-5-ylidene)propyldimetylamine hydrochloride. Other names include Amitrip, Elavil, and Tryptizol. Its molecular formula is $C_{20}H_{23}N{\cdot}HCl$, with a molecular weight of 313.9 and a CAS number of 549-18-8. Amitriptyline hydrochloride occurs as odorless or practically odorless, colorless crystals or white or almost white powder. It is freely soluble in water, alcohol, chloroform, and methylene chloride. Amitriptyline hydrochloride has a pK_a value of 9.4.

METHODS

Assay 1 Bose et al. [1] developed a micellar liquid chromatographic method for the determination of amitriptyline and nortriptyline in serum samples. An Agilent Series 1100 liquid chromatograph was equipped with a quaternary pump, a degasser, an autosampler, a column oven, and UV–visible and electrochemical detectors. The stationary phase was a Kromasil 5 C_{18} column (250 × 4.6 mm, 5 μm particle size). The micellar mobile phase consisted of 6% (vol/vol) pentanol in 0.15 M sodium dodecyl sulfate solution buffered to pH 7 with monobasic sodium phosphate. The flow rate was 1.5 mL/min. Detections were performed at 240 nm on UV–visible detector and at 650 mV on electrochemical detector.

Stock solutions of amitriptyline and nortriptyline (10 μg/mL) were prepared in micellar mobile phase. Standards and controls were prepared by spiking blank serum with stock solutions. Serum samples, controls, and

standards (0.5 mL each) were diluted 1 : 10 in micellar mobile phase, filtered through a 0.45-μm nylon membrane, and assayed. Under these conditions, retention times for amitriptyline and nortriptyline were about 11.9 and 13.1 min, respectively (estimated from the published chromatogram).

A calibration curve for amitriptyline was constructed over the range of 120–250 ng/mL. The correlation coefficient was 0.997. Mean recoveries ranged from 99.8% to 101.6%. Repeatability and intermediate precision were less than 5.1% and 7.3%, respectively. The limit of detection was 0.25 ng/mL.

Assay 2 Gutteck and Rentsch [2] reported therapeutic drug monitoring of amitriptyline, citalopram, clomipramine, desipramine, dibenzepin, doxepin, escitalopram, flupentixol, fluphenazine, fluvoxamine, imipramine, nortriptyline, opipramol, pipamperone, reboxetine, thioridazine, trimipramine, and zuclopenthixol in serum with liquid chromatography–electrospray ionization mass spectrometry. The liquid chromatograph consisted of a Flux Instruments RHEOS 2000 pump and a CTC LC-PAL autosampler. The stationary phase was a Interchim Silica Uptisphere column (125 × 2 mm, 5 μm) with a guard column (8 × 2 mm). Eluent A was a mixture of 50 mM ammonium acetate buffer (pH 4) and acetonitrile (60 : 40, vol/vol) and eluent B, acetonitrile. The mobile phase was delivered at 90% A and 10% B at a flow rate of 300 μL/min. The ThermoQuest AQA quadrupole mass spectrometer was used in the positive ESI mode. Protonated imipramine-d_3 and amitriptyline were detected in the selected-ion monitoring (SIM) mode: m/z 284 and 278, respectively.

Amitriptyline stock solution 1 μg/μL was prepared in water. Working solutions were prepared by diluting the stock solution with water. Standards were prepared by spiking blank serum with working solutions. Imipramine-d_3 10 ng/μL was used as the internal standard. A patient sample or standard (1 mL) was mixed with 0.5 mL of water, 0.4 mL of ethanol, 0.15 mL of 1 M sodium hydroxide solution, and 25 μL of internal standard, extracted with 5 mL of *n*-hexane/dichloromethane (4 : 1, vol/vol) for 5 min, and centrifuged at 1648 *g* for 5 min. The organic layer was collected, evaporated to dryness, reconstituted in a mixture of 120 μL acetonitrile and 180 μL 50 mM ammonium acetate buffer (pH 4), and assayed. Under these conditions, the retention times for imipramine-d_3 and amitriptyline were 3.09 and 3.43 min, respectively.

Calibration curves for amitriptyline were constructed over the range 360–2884 nmol/L. Correlation coefficients were better than 0.999. Within-day and between-day coefficients of variation were 6.7% and 5.7%, respectively. Accuracy ranged from 90.2% to 102%. Recovery was 79%. The limit of quantification was 50 nmol/L. More than 2000 patient samples were analyzed using this method.

Assay 3 Theurillat and Thormann [3] reported the therapeutic drug monitoring of tricyclic antidepressants, amitriptyline, nortriptyline, imipramine, desipramine, and clomipramine in human serum and plasma by HPLC. A Waters liquid chromatograph consisted of a model 510 pump, model 717 Plus autosampler, a Kratos Analytical model Spectraflow 757 UV detector, and an HP model 3396 Series II integrator. The stationary phase was a Waters Nova-Pak C_{18} reversed phase column (150 × 4.6 mm, 4 μm, 60 Å pore size). The mobile phase consisted of 5 mM monobasic potassium phosphate aqueous buffer, acetonitrile, and diethylamine (500 : 500 : 2, vol/vol/vol), adjusted pH to 8 with concentrated phosphoric acid. The flow rate was 0.9 mL/min. The UV detector was set at 242 nm. The runtime was 34 min.

A stock solution containing amitriptyline, nortriptyline, imipramine, desipramine, and clomipramine (200 μg/mL each) was prepared in methanol and stored at −20°C. Working solutions were prepared by diluting the stock solution with methanol. Standards were prepared by spiking bovine plasma with working solutions. Econazole 90 μg/mL in methanol was used as the internal standard. Patient samples, standards, or controls (1 mL each) were mixed with 50 μL of internal standard, 1 mL of 0.1 M sodium tetraborate solution (adjusted to pH 11 with 30% sodium hydroxide solution), and 6 mL of hexane in 10-mL glass tubes, shaken for 10 min, and centrifuged at 3000 rpm for 10 min. The upper hexane layer was collected, evaporated to dryness at 40°C under a gentle stream of air, reconsitituted in 200 μL of methanol, and assayed. The injection volume was 35 μL. Under these conditions, the retention times for amitriptyline, nortriptyline, imipramine, desipramine, clomipramine, and econazole were about 16.4, 6.1, 11.9, 4.8, 20.5, and 26.5 min, respectively (estimated from the published chromatogram).

Calibration curves for amitriptyline were calculated using the peak area ratio of amitriptyline to econazole in the range of 20–400 ng/mL. The correlation coefficients were greater than 0.990. Intraday and interday coefficients of variation were 3.58% and 3.50%, respectively. The limit of detection was about 60 nM. This method was used for therapeutic drug monitoring and clinical toxicology for a 3-year period.

Assay 4 Hackett et al. [4] compared an HPLC method with a fluorescence polarization immunoassay (FPIA) for therapeutic drug monitoring of tricyclic antidepressants—amitriptyline, clomipramine, dothiepin, doxepin, desipramine, imipramine, nortriptyline, and trimipramine. The HPLC analyses were performed using an Ultrasphere C_8 column (250 × 4.6 mm). The mobile phase was 35% acetonitrile in an aqueous solution containing 4 mM sodium octanesulfonate and 0.5 mM *N,N,N,N*-tetramethylethylenediamine, adjusted to pH 2.5 with phosphoric acid. The flow rate was 2 mL/min. UV detection was performed at 230 nm.

A plasma sample (1 mL) was mixed with 100 ng of desmethyldoxepin as an internal standard, alkalized by the addition of 0.2 mL of 1 M sodium hydroxide solution, extracted by vigorously shaking with 10 mL of hexane containing 1% isoamyl alcohol, and centrifuged. The organic layer was collected, extracted with 0.2 mL of 0.05 M hydrochloric acid, vortexed for 1 min, and centrifuged. An aliquot of 40 μL of the acidic phase was injected onto the column. Under these conditions, retention times for desmethyldoxepin and amitriptyline were 7 and 13.7 min,

respectively. Within-run coefficients of variation were less than 8.1% at 25 μg/L and 3.1% at 250 μg/L.

The FPIA analyses were carried out on an Abbott TDx FPIA system according to the manufacturer's specifications. This assay used a single antibody with imipramine as the calibrator drug (100%). Results were adjusted by applying an appropriate range-dependent correction factor from the Abbott *TDx Assays Manual* for individual drug concentrations. The detection range was 20–1000 μg/L.

The regression relationship between the TDx assay value and the HPLC assay value was TDx = 1.32 * HPLC + 55.2 ($n = 53$, $r^2 = 0.69$). It was found that the TDx assay significantly overestimated therapeutic concentrations of amitriptyline.

Assay 5 Tracqui et al. [5] evaluated the reliability of a drug exposure screening test based on qualitative hair analysis for the antidepressant amitriptyine using a GC-MS method. A Perkin-Elmer 8500 gas chromatograph was equipped with a BP5 capillary column (12.5 × 0.22 mm). Injector and transfer-line temperatures were maintained at 300°C and 280°C, respectively. Column temperature was initially set at 60°C for 1 min, increased to 280°C at 30°C/min, and then maintained at 280°C for 5 min. The carrier gas was helium. The flow rate was 3.2 mL/min. The gas chromatograph was coupled with a Perkin-Elmer ITD mass spectrometer. The spectrometer was set in electron impact mode. Ionization energy was set at 70 eV and electron multiplier voltage 1200 V.

The hair sample was decontaminated by agitation in ethanol for 10 min, dried, weighed, dissolved in 1.0 mL of 1 N sodium hydroxide at 100°C for 30 min, neutralized with 1 N hydrochloric acid, buffered at pH 8.5 with 1 mL of saturated sodium carbonate solution, extracted with 5 mL of *n*-heptane/isoamyl alcohol (98.5 : 1.5, vol/vol) and 20 μL of SKF525A (10 mg/L) as an internal standard, shaken for 10 min, and centrifuged at 2800 *g* for 10 min. The organic layer was collected, evaporated to dryness at 45°C, reconstituted in 20 μL of methanol, and assayed. The injection volume was 1 μL.

Sixty psychiatric patient hairs were qualitatively analyzed using this GC-MS method.

Assay 6 Wilson et al. [6] reported the simultaneous determination of secondary amines (nortriptyline, desmethyldoxepin, desipramine, and protriptyline) and tertiary amines (amitriptyline, doxepin, and imipramine) tricyclic antidepressants by GC/MS chemical ionization mass fragmentography. A Finnigan model 3200F gas chromatograph/mass spectrometer was interfaced with a four-channel PROMIM unit and a Rikadenki Series KA four-pen recorder. The stationary phase was an Applied Science Laboratories 3% OV225 on 100/120-mesh Gas-Chrom Q glass column (1.5 m × 2 mm). Methane (ultrapure) was used as both a carrier gas and a reactant gas for chemical ionization at a flow rate of 16 mL/min. The column temperature was set at 220°C for tertiary amines and 240°C for secondary amines, injector temperature at 240°C, oven temperature at 200°C, and transfer-line temperature at 180°C. The analyzer temperature was maintained below 100°C. The PROMIM channels were set at a sensitivity of 10^{-9} A/V (ampere/volt). A 0.05-Hz filter was used at a sample time of 100 ms. Amitriptyline was monitored at m/z 278 and clomipramine (internal standard), at m/z 317 $(M + 2)^+$ isotope peak.

Stock solutions of individual drugs (200 mg/L) were prepared in methanol and stored at −20°C. Standards containing these drugs were prepared by spiking plasma with stock solutions. Clomipramine (2 mg/L), desmethyltrimipramine (300 μg/L), and protriptyline (500 μg/L) were prepared in deionized water and stored at 4°C. Standards, controls, or patient plasmas (2.0 mL) were mixed with 1.0 mL of internal standard solution, 1.0 mL of 0.1 M sodium hydroxide, and then 25 mL of isopropanol/hexane (2 : 98, vol/vol), shaken for 5 min, and centrifuged at 1500 rpm for 15 min. The organic layer was collected, evaporated to dryness at 50°C under a stream of nitrogen, reconstituted in 25 μL of ethyl acetate, vortexed, and assayed. Under these conditions, retention times for amitriptyline and clomipramine were 2.81 and 6.46 min, respectively.

A calibration curve for amitriptyline was constructed using the ratio of the peak height of the drug to that of the internal standard in the concentration range of 5–500 μg/L. Intraday and interday coefficients of variation were less than 11.2% and 5.5%, respectively.

Assay 7 Volin [7] described a GC method for the routine determination of tricyclic antidepressants in human plasma with a nitrogen-specific detector. A Hewlett-Packard model HP5710A gas chromatograph was equipped with a model 18765A nitrogen–phosphorus detector and model HP3390A data processor. The stationary phase was a Supelco coiled glass column (1.8 m × 2 mm i.d.) containing GP 3% SP-2250 on 80/100 mesh Supelcoport. Temperatures for column, detector, and injector were set at 240°C (or 250°C), 300°C, and 250°C, respectively. The flow rate of nitrogen carrier gas was 40 mL/min. The injection volume was 2–5 μL.

Protriptyline at 1.0 mg/mL in methanol was used as internal standard. A plasma sample or standard (3 mL) was mixed with 1.2 mL of saturated sodium carbonate (Na_2CO_3) by shaking vigorously for 2 min, extracted with 10 mL of *n*-hexane/isoamyl alcohol (97 : 3, vol/vol) containing 0.1 μg/mL internal standard by shaking for 15 min, and centrifuged at 1000 *g* for 5 min. A portion (8 mL) of the organic phase was collected, mixed with 1.2 mL of 0.9 M hydrochloric acid, shaken for 15 min, and centrifuged. The aqueous phase was collected, mixed vigorously with 500 mg of anhydrous sodium carbonate for 1 min, extracted with 3 mL of *n*-hexane/isoamyl alcohol without internal standard for 15 min, and centrifuged. The organic phase was collected, mixed vigorously with 250 mg of anhydrous sodium sulfate, and centrifuged. The supernatant was collected, evaporated to dryness at 40°C under a stream of nitrogen, reconstituted in 120 μL of methanol, and assayed. Relative retention times to internal standard for amitriptyline, trimipramine, imipramine, doxepin, nortriptyline, mianserin, iprindole, maprotiline, and clomipramine were 0.72, 0.74, 0.80, 0.83, 0.86, 0.89, 1.04, 1.11, and 1.22, respectively.

A linear calibration curve for amitriptyline was obtained in the concentration range of 25–175 μg/L. The recovery ranged from 86% to 102%. The coefficient of variation was less than 7.8%. There was no interference from perphenazine, nitrazepam, diazepam, levomepromazine, digoxin, atenolol, melperone, chlorpromazine, thioridazine, lithium, flunitrazepam, fluphenazine, chlordiazepoxide, propranolol, insulin, promazine, or lorazepam.

Assay 8 Aymard et al. [8] reported an HPLC method for simultaneous quantification of imipramine, amitriptyline, maprotiline, fluoxetine, clomipramine, and their respective metabolites. The ThermoSeparation liquid chromatograph consisting of a model P1000 solvent delivery pump, model AS3000 autosampler with a 100-μL loop, and a Spectra Focus model photodiode array detector. The stationary phase was a Waters Symmetry C_{18} column (250 × 4.6 mm, 5 μm particle size). The mobile phase consisted of 0.067 M monobasic potassium phosphate buffer (pH 3.0) and acetonitrile (65 : 35, vol/vol) and was delivered isocratically at 1.2 mL/min. UV detections were performed at 226, 254, and 400 nm. The total runtime of an injection was 20 min.

Stock solutions of drugs at 1.0 mg/mL each were prepared in 0.01 M hydrochloric acid and stored at 4°C. Standards were prepared by spiking drug-free human plasma with stock solutions. Clovoxamine was used as an internal standard. An aliquot of 500 μL of a plasma sample or standard in a 15-mL Venoject silicone tube was alkalinized with 250 μL of 2 M sodium carbonate, mixed with 100 μL of 1 μg/mL internal standard, extracted with 10 mL of *n*-hexane, shaken for 30 min, centrifuged at 3000 *g* for 10 min, and placed in a dry ice–acetone bath. The entire organic layer was collected, mixed with 200 μL of 0.03% phosphoric acid, shaken for 10 min, and centrifuged again. The acidic aqueous solution was collected and assayed. The injection volume was 100 μL. Under these conditions, retention times for clovoxamine, imipramine, maprotiline, amitriptyline, fluoxetine, and clomipramine were about 6.7, 9.8, 10.9, 11.5, 15.5, and 18.9 min, respectively.

A calibration curve for amitriptyline was constructed in the concentration range of 10–3000 ng/mL. The correlation coefficient was greater than 0.998. The recovery of amitriptyline from plasma was better than 67%. Within-day and between-day coefficients of variation were 9.0% and 7.9%, respectively. The limit of quantification determined at 226 nm was 5 ng/mL. Levomepromazine interfered with the determination of the drug. There was no interference with this assay from the following drugs and their metabolites (retention times in minutes): desmethylvenlafaxine (2.7), zopiclone (2.8), sulpiride (2.8), viloxazine (2.8), zolpidem (3.3), venlafaxine (3.5), clozapine (4.5), chlordiazepoxide (4.6), mianserine (5.7), doxepine (6.5), amineptine (6.5), loxapine (7.1), haloperidol (7.2), desipramine (8.9), desmethylmaprotiline (9.2), cyamemazine (9.4), carbamazepine (9.5), fluvoxamine (10.0), nortriptyline (10.5), trimipramine (13.1), norfluoxetine (13.3), lorazepam (13.7), chlorpromazine (14.9), clonazepam (15.4), desmethylclomipramine (16.9), clorazepate dipotassium (19.3), flunitrazepam (24.1), diazepam, and valproic acid.

Assay 9 Kollroser and Schober [9] described the simultaneous determination of seven tricyclic antidepressant drugs—amitriptyline, nortriptyline, doxepin, dosulepin, dibenzepin, opipramol, and melitracen—in human plasma using HPLC-MS/MS with an ion-trap detector and direct injection. A TSP liquid chromatographic system consisting of two model P4000 quaternary pumps, a model AS3000 autosampler, a vacuum degasser, and a six-port switching valve was utilized. The stationary phase was a Waters Symmetry C_{18} column (150 × 3.0 mm, 5 μm particle size) protected by a Waters Symmetry C_{18} guard column (20 × 3.9 mm, 5 μm particle size). The mobile phase consisted of acetonitrile (A) and 0.1% formic acid in water (B) and was delivered at 0.6 mL/min in a step-gradient mode at 28% A for 4 min, increased to 70% A in 1 min, maintained at 70% A for 3 min, returned to 28% A in 0.7 min, and kept at 28% A for another 3.3 min.

A Finnigan LCQ ion-trap mass spectrometer equipped with an APCI source was operated in the positive-ion mode: vaporizer temperature 450°C, capillary temperature 200°C, corona discharge intensity 5 μA, and shealth gas flow 60 units. Analytes were quantified using the following ion transitions: m/z 278.2 → 233.1 for amitriptyline, m/z 264.2 → 233.1 for nortriptyline, m/z 296.2 → 251.2 for dibenzepin, m/z 280.2 → 235.1 for doxepin, m/z 296.1 → 225.1 for dosulepin, m/z 364.2 → 171.1 for opipramol, m/z 292.2 → 247.1 for melitracen, and m/z 419.1 → for lofepramine (internal standard).

Stock solutions of all drugs at 1 mg/mL were separately prepared in methanol. Working solutions were prepared with 0.1% formic acid in water. Lofepramine at 10 mg/L in 0.1% formic acid was used as an internal standard. Standards were prepared by spiking drug-free human plasma with working solutions. Each plasma sample, control, or standard was spiked with 10 μL of the internal standard and diluted with an appropriate amount of 0.1% formic acid. An aliquot of 50 μL of this solution was injected onto a Waters Oasis HLB online extraction column (50 × 2.1 mm, 30 μm particle size) with a mobile phase of 0.1% formic acid at a flow rate of 4 mL/min. The valve was directed to the waste from 0 to 0.3 min, automatically switched to the analytical column for separation of drugs from 0.3 to 9.0 min, and then switched back to the waste. Under these conditions, retention times of dibenzepin, opipramol, doxepin, dosulepin, nortriptyline, amitriptyline, melitracen, and lofepramine were 2.06, 2.10, 2.40, 3.21, 3.59, 3.94, 5.82, and 7.36 min, respectively.

Calibration curves for amitriptyline were constructed in the range from 10 to 800 μg/L. Correlation coefficients were greater than 0.997. Intraassay and interassay accuracies were within 93.2% and 109.1%, respectively. Intraassay and interassay coefficients of variation were less than 6.4% and 7.5%. The limits of detection and quantification were 5 and 10 μg/mL, respectively.

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AMLODIPINE BESYLATE

CHEMISTRY

Amlodipine is a calcium-channel blocking agent. Its chemical name is 3-ethyl 5-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methylpyridine-3,5-dicarboxylate monobenzenesulfonate. Other names include Amlocard, Amlodipine Besilate, Amlostin, Norvasc, and UK-48340-11. Its molecular formula is $C_{20}H_{25}ClN_2O_5 \cdot C_6H_6O_3S$, with a molecular weight of 567.1 and a CAS number of 111470-99-6. Amlodipine besylate occurs as a white to pale yellow crystalline powder. It is slightly soluble in water and sparingly soluble in alcohol.

METHODS

Assay 1 Zarghi et al. [1] reported a rapid and sensitive HPLC method for the determination of amlodipine in human plasma. A Knauer system consisted of a model Wellchrom K1001 pump, model K2501 UV detector, model Eurochrom 2000 integrator, and a Rheodyne 7125 injector. The stationary phase was a Nucleosil C_8 column (125 $\times$ 4.6 mm, 4 μm particle size). The mobile phase consisted of 0.01 M sodium dihydrogen phosphate buffer and acetonitrile (63 : 37, vol/vol) adjusted to pH 3.5. The flow rate was 1.5 mL/min. UV detection was performed at 239 nm.

Diltiazem (0.2 μg/mL) in methanol was used as an internal standard. Amlodipine stock solution (100 μg/mL) was prepared in methanol and stored at 4°C. Standards were prepared by spiking blank human plasma with amlodipine stock solution. Standards or plasma samples (1000 μL) were mixed with 50 μL of internal standard, 900 μL of acetonitrile, and 100 μL of saturated sodium chloride solution; vortexed for 30 s; and centrifuged at 6000 rpm for 15 min. The supernatants were collected, evaporated to dryness under a stream of nitrogen, reconstituted in 100 μL of mobile phase, and assayed. The injection volume was 50 μL. Under these conditions, retention times of amlodipine and diltiazem were about 3.6 and 4.9 min, respectively.

Calibration curves for amlodipine in plasma were obtained over the concentration range of 0.5–16 ng/mL. The correlation coefficients were greater than 0.997. The average recovery of amlodipine in plasma was $96.6 \pm 1.5\%$. Intraday and interday accuracies ranged from 98.1% to 99.1%. Intraday and interday coefficients of variation were less than 9.88% and 9.91%, respectively. The limit of detection was 0.2 ng/mL with a coefficient of variation of less than 8%.

Assay 2 Baranda et al. [2] presented the determination of the calcium channel antagonists amlodipine, lercanidipine, nitrendipine, felodipine, and lacidipine in human plasma using HPLC-MS/MS. A liquid chromatographic system equipped with two Shimadzu model LC10AD gradient pumps and a Chromtech HTC-PAL autosampler with a 100-μL syringe was used. The stationary phase was a Phenomenex Luna RP C_{18} analytical column (150 $\times$ 2 mm, 3.0 μm particle size). The column temperature was maintained at 40°C. The mobile phase consisted of 0.1% formic acid with 1 mM ammonium formate, pH 2.7 (A) and acetonitrile/0.1% formic acid with 1 mM ammonium formate (95 : 5, vol/vol) (B) and was delivered in a gradient mode: 0–1 min at 20% B, 1–3 min from 20% B to 40% B, 3–11 min from 40% B to 70% B, 11–12 min from 70% B to 95% B, 12–12.5 min at 95% B, 12.5–13.5 min from 95% B to 20% B, and 13.5–15.5 min at 20% B. The injection volume was 20 μL.

An Applied Biosystmes/SCIEX API365 triple quadrupole mass spectrometer equipped with TurboIonSpray source was operated in the positive-ion mode. Drugs were quantified in the multiple-reaction monitoring (MRM) mode using the ion transitions m/z 409 $\rightarrow$ 238 for amlodipine, m/z 612 $\rightarrow$ 280 for lercanidipine, m/z 361 $\rightarrow$ 329 for nitrendipine, m/z 384 $\rightarrow$ 338 for felodipine, m/z 354 $\rightarrow$ 310 for lacidipine, and m/z 480 $\rightarrow$ 315 for nicardipine (internal standard).

Stock solutions of drugs at 1000 μg/mL were prepared in methanol and stored at 4°C and protected from light. An aliquot of 1 mL of plasma was spiked with drugs and the internal standard (20 ng/mL), diluted with 1 mL of 0.05 M acetate buffer (pH 5), passed through a Varian disposable solid-phase extraction (SPE) Bond Elut C_{18} cartridge (200 mg/3 mL) that was pre-conditioned with 2 mL of methanol followed by 2 mL of 0.05 M acetate buffer, washed with 1 mL of 0.05 M acetate buffer, dried under vacuum for 10 min, eluted with 2 mL of a mixture of acetonitrile and 25% ammonium hydroxide solution

(97 : 3, vol/vol), evaporated to dryness at 60°C under a stream of nitrogen, reconstituted in 100 μL of the mobile phase (A : B, 70 : 30, vol/vol), vortexed, and assayed. Under these conditions, retention times of nicardipine, amlodipine, lercanidipine, nitrendipine, felodipine, and lacidipine were 4.25, 4.35, 6.61, 9.56, 11.31, and 13.03 min, respectively.

Calibration curves for amlodipine were constructed over the range from 1 to 40 ng/mL. Correlation coefficients were greater than 0.9995. The average recovery of amlodipine from plasma was 93%. Intraday and interday coefficients of variation were less than 8.71% and 13.1%, respectively. Limits of detection and quantification were 0.2 and 1.0 ng/mL, respectively.

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AMOBARBITAL

CHEMISTRY

Amobarbital is a hypnotic and a sedative. Its chemical name is 5-ethyl-5-isopentylbarbituric acid. Other names include Amytal and Pentymalum. Its molecular formula is $C_{11}H_{18}N_2O_3$, with a molecular weight of 226.3 and a CAS number of 57-43-2. Amobarbital is a white crystalline powder. Amobarbital is very slightly soluble in water, freely soluble in alcohol, and soluble in dichloromethane.

METHOD

Assay 1 Lehane et al. [1] developed a GC method for a simultaneous measurement of allobarbital, amobarbital, butabarbital, heptabarbital, pentobarbital, phenobarbital, secobarbital, diphenylhydantoin, and primidone. A Perkin-Elmer model 900 gas chromatograph equipped with a nitrogen detector was used. An Applied Science Laboratories single glass column (1.83 m × 2 mm, 3% OV101 on Gas-Chrom Q, 100–120 mesh) was also used. Gas flow rates were 30 mL/min for helium, 10 mL/min for hydrogen, and 100 mL/min for air. The injection port and detector oven were maintained at 300°C and 285°C, respectively. The column oven temperature was maintained at 175°C for 1 min, increased to 285°C at 24°C/min, and then kept at 285°C for 4 min.

Stock solutions of drugs (1 g/L) were prepared in methanol. Working solutions were prepared by diluting stock solutions with methanol. Standards were prepared by spiking ion-free serum with working solutions. A mixture of chloroform–isopropanol–benzene (94 : 4 : 1, vol/vol/vol) was prepared as an extraction solvent. 5-(*p*-Methylphenyl)-5-phenylhydantoin in extraction solvent (1.5 mg/L) was used as an internal standard. A serum sample or standard (1 mL) was mixed with 0.1 mL of 1.0 M hydrochloric acid and 12 mL of the internal standard solution, and shaken vigorously for 1 min. The organic phase was filtered through a filter paper, evaporated to dryness at 50°C under a stream of nitrogen, reconstituted with 0.8 mL of extraction solvent, transferred into a vial, evaporated to dryness again under nitrogen, reacted with 0.1 M trimethylphenyl ammonium hydroxide in methanol (50 μL), and assayed. The injection volume was 0.7 μL. Under these conditions, the relative retention ratios were 0.18 for allobarbital, 0.24 for butabarbital, 0.29 for amobarbital, 0.32 for pentobarbital, 0.36 for secobarbital, 0.56 for phenobarbital, 0.68 for heptabarbital, 0.70 for primidone, 0.91 for diphenylhydantoin, and 1.00 for the internal standard, respectively.

A standard curve for amobarbital was constructed in the range 5.0–50 mg/L. The correlation coefficient was greater than 0.995.

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AMOXAPINE

CHEMISTRY

Amoxapine is a tricyclic antidepressant. Its chemical name is 2-chloro-11-(piperazin-1-yl)dibenz[*b,f*][1,4]oxazepine. Other names include Amoxapin, Asendin, CL-67772, and Defanyl. Its molecular formula is $C_{17}H_{16}ClN_3O$, with a molecular weight of 313.8 and a CAS number of 14028-44-5. Amoxapine is a white to yellowish-white crystalline powder. It is practically insoluble in water, freely soluble in chloroform, sparingly soluble in methanol and in toluene, slightly soluble in acetone, and soluble in tetrahydrofuran. Amoxapine has a pK_a of 7.6.

METHOD

Assay 1 Jourdil et al. [1] reported the simultaneous determination of amoxapine, dothiepin, fluoxetine, fluvoxamine, medifoxamine, mianserin, and viloxazine in plasma using gas chromatography with nitrogen–phosphorus detection. A Varian Star 3400CX system equipped with a nitrogen–phosphorus detector and a split–splitless injector was used. The stationary phase was a Lara-Siral OV1 fused-silica capillary column (25 m × 0.32 mm i.d.) with a film thickness of 0.25 μm. The operation conditions were as follows: injector and detector temperature 280°C, oven temperature 120°C for 1 min followed by 8°C/min to 160°C, 160°C for 8 min followed by 20°C/min to 290°C, and then

290°C for 10 min, carrier gas (helium) 2.3 mL/min, makeup gas 30 mL/min, hydrogen 4.1 mL/min, and air 175 mL/min. The injection volume was 3 μL.

Protriptyline at 10 mg/L in ethanol was used as an internal standard. An aliquot of 1 mL of 20% ammonia solution was placed in a 20-mL glass vial, vortexed gently for 15 s, mixed with 1 mL of plasma and 15 μL of internal standard at 10 mg/L, vortexed for 5 s, extracted with 9 mL of hexane–dichloromethane–isoamyl alcohol (57 : 42 : 1, vol/vol/vol), shaken for 15 min, and centrifuged at 2200 *g* for 10 min. The upper organic phase was collected, evaporated to dryness at room temperature, reconstituted with 50 μL of ethanol, and assayed. Under these conditions, retention times of medifoxamine, viloxazine, fluoxetine, fluvoxamine, mianserin, dothiepin, amoxapine, and protriptyline were abut 10.4, 11.1, 11.6, 12.7, 17.9, 19.3, 20.6, and 18.3 min, respectively.

Linear calibration curves were obtained up to 2000 μg/L. Intraday and interday coefficients of variation were less than 10%. There was no interference from endogenous substances in plasma. The following drugs (retention time in minutes) were evaluated for any interference: caffeine (9.4), norfluoxetine (10.8), meprobamate (12.1), cocaine (17.9), amitriptyline (18.0), dextropropoxyphene (18.0), nortriptyline (18.1), imipramine (18.2), medazepam (18.2), desipramine (18.3), desmethyltrimipramine (18.3), prometazine (18.5), oxazepam (18.8), maprotiline (19.0), codeine (19.2), lorazepam (19.4), northiaden (19.4), clomipramine (19.5), codethyline (19.5), morphine (19.5), diazepam (19.5), tetrazepam (19.5), desmethylclomipramine (19.6), amineptine (20.0), chlordiazepoxide (20.0), clotiazepam (20.0), levomepromazine (20.1), clobazam (20.2), benzoylecgonine (20.2), paroxetine (20.3), midazolam (20.4), flunitrazepam (20.4), bromazepam (20.4), prazepam (20.7), acepromazine (20.7), temazepam (20.8), lormetazepam (21.0), nitrazepam (21.2), zolpidem (21.4), clozapine (21.9), alprazolam (22.2), haloperidol (22.3), estazolam (22.5), zopiclone (23.1), amphetamine (23.9), pholcodine (24.0), pipotiazine (24.2), tetrahydrocannabinol (24.4), amisulpiride (25.2), buprenorphine (26.0), triazolam (26.6), and lysergic acid diethylamide (27.1). Among these drugs, cocaine and amitriptyline interfered with mianserin, codeine with dothiepin, and desipramine with protriptyline.

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AMOXICILLIN

CHEMISTRY

Amoxicillin is an aminopenicillin antibiotic. Its chemical name is [2*S*-[2α,5α,6β(*S**)]]-6-[[amino-(4-hydroxyphenyl) acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid. Other names include Amoxi, Amoxy, Amoxil, Amoxycillin, and Pasetocin. Its molecular formula is $C_{16}H_{19}N_3O_5S$, with a molecular weight of 365.4 and a CAS number of 26787-78-0. Amoxicillin is a white, practically odorless crystalline powder. It has solubilities of 4.0 mg/mL in water, 7.5 mg/mL in methanol, and 3.4 mg/mL in absolute alcohol. It is insoluble in hexane, benzene, ethyl acetate, and acetonitrile. Amoxicillin has pK_a values of 2.63, 7.55, and 9.64 at 23°C.

METHOD

Assay 1 Pullen et al. [1] investigated the population pharmacokinetics and dosing of amoxicillin by determining its concentrations in plasma using HPLC. A liquid chromatographic system consisting of a Hewlett-Packard model 1050 pump, Waters model 717 autosampler, and Waters model 486 UV detector was used. The stationary phase was a Beckman C_8 column (250 × 4.6 mm, 5 μm particle size) protected by a C_8 guard column. The mobile phase was composed of 0.067 M monobasic potassium phosphate buffer (pH 3.5), methanol, and distilled water (450 : 50 : 100) and was isocratically delivered at 2 mL/min. UV detection was performed at 225 nm. The injection volume was 10 μL.

Standards of amoxicillin in plasma were prepared. Sotalol hydrochloride at 100 mg/L was used as an internal standard. An aliquot of 40 μL of plasma or standard was mixed with an equal volume of internal standard, vortexed, mixed with 80 μL of 3% perchloric acid, and centrifuged at 10,500 *g*. An aliquot of 100 μL of the upper layer was mixed with 40 μL of 1.2 M dibasic potassium phosphate solution, and centrifuged again. The supernatant was collected and assayed.

Calibration curves for amoxicillin were constructed. The limit of quantification was 1 mg/L. Concentrations of amoxicillin in plasma for 150 neonates were analyzed using this method.

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AMPHETAMINE

CHEMISTRY

Amphetamine is an indirect-acting sympathomimetic. Its chemical name is (*R,S*)-α-methylphenethylamine. Other names include Adderall and Amfetamine. Its molecular formula is $C_9H_{13}N$, with a molecular weight of 135.2 and a CAS number of 300-62-9. Amphetamine is a white, odorless crystalline powder. It is freely soluble in water, slightly soluble in alcohol, and practically insoluble in ether.

METHODS

Assay 1 Wu et al. [1] described the determination of stimulants—amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxymethamphetamine, and 3,4-methylenedioxyethylamphetamine—in human urine and hair samples by polypyrrole-coated capillary in-tube solid-phase microextraction (SPME) coupled with liguid chromatography–electrospray mass spectrometry. An Agilent 1100 series liquid chromatograph was used. The stationary phase was a Supelcosil LC-CN column (330 × 4.6 mm, 3 μm particle size). The mobile phase consisted of acetonitrile and 50 mM ammonium acetate buffer (15 : 85) and was isocractically delivered at 0.4 mL/min.

An Agilent mass spectrometer equipped with an atmospheric-pressure–electrospray ionization interface was operated in the positive ionization mode. The optimal conditions were as follows: nebulizer gas (nitrogen) 40 psi; drying gas (nitrogen) 12 L/min and 350°C; capillary voltage 1 kV; dwell time 78 ms; and fragmenter voltage 30 V for amphetamine and 3,4-methylenedioxyamphetamine and 50 V for methamphetamine, 3,4-methylenedioxymethamphetamine, and 3,4-methylenedioxyethylamphetamine. Drugs were quantified in selected-ion monitoring (SIM) mode at m/z 136 for amphetamine, m/z 150 for methamphetamine, m/z 180 for 3,4-methylenedioxyamphetamine, m/z 194 for 3,4-methylenedioxymethamphetamine, and m/z 208 for 3,4-methylenedioxyethylamphetamine.

Urine samples were diluted 10 times with water. An aliquot of the diluted urine sample was mixed with 0.2 mL of 0.5 M sodium carbonate buffer (pH 10.0), filled to 1 mL with water, and microextracted. For hair samples, 10 mg of hair were cleaned, dried, cut into small pieces (0.5 cm), submerged in 5 mL of methanol and 5 M hydrochloric acid (20 : 1, vol/vol), sonicated for 1 h, allowed to stand at room temperature overnight, and filtered. The filtrate was evaporated to dryness under a stream of nitrogen, redissolved in 1 mL of 100 mM carbonate buffer (pH 10.0), and microextracted. A laboratory-made polypyrrole (PPY)-coated capillary (60 cm long) was used as the in-tube SPME device, which was conditioned with 2 × 40 μL of methanol followed by 2 × 40 μL of water and placed between the injection needle and the loop of the autosampler. An aliquot of 40 μL of the diluted urine sample or a hair filtrate was drawn from a sample vial into the capillary at 100 μL/min and then ejected back to the sample vial. This draw/eject process was repeated 10 times. After the tip of the injection needle was washed with 2 μL of methanol and the valve was switched to the analytical column from the load postion, extracted drugs were desorbed from the capillary coating with mobile phase and assayed. Under these conditions, retention times of amphetamine, 3,4-methylenedioxyamphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine, and 3,4-methylenedioxyethylamphetamine were about 3.4, 3.7, 4.1, 4.6, and 5.0 min, respectively (estimated from the published chromatogram).

Calibration curves for amphetamine were constructed over the range from 0.1 to 100 ng/mL in water. Correlation coefficients were greater than 0.9993. Within-day and between-day coefficients of variation were 3.3% and 6.4%, respectively. The limit of detection was 23 ng/L. Calibration curves for amphetamine were also constructed over the range from 0.5 to 100 ng/mL in urine. Correlation coefficients were greater than 0.9993. Within-day and between-day coefficients of variation were 5.0% and 5.3%, respectively. The limit of quantitation was 13 ng/mL. Calibration curves for amphetamine were constructed over the range from 1 to 100 ng/mL in hair samples. Correlation coefficients were greater than 0.9994. Within-day and between-day coefficients of variation were 5.4% and 6.7%, respectively. The limit of quantitation was 0.60 ng/10 mg hair.

Assay 2 Stanaszek and Piekoszewski [2] reported the simultaneous determination of amphetamine (AMP), ephedrine (EP), methcathinone (MTC), paramethoxyamphetamine (PMA), methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), methylenedioxyethylamphetmaine (MDEA), and methamphetamine (MA) in human hair by high-performance liquid chromatography–atmospheric-pressure chemical ionization mass spectrometry (HPLC-APCI-MS). A Hewlett-Packard 1100 series liquid chromatograph coupled to a mass spectrometer equipped with an APCI interface was used. The stationary phase was a Merck LiChroCART Purospher 60 RP18e column (125 × 4.0 mm, 5 μm particle size) protected by a RP18e guard column. The column temperature was maintained at 35°C. The mobile phase consisted of 0.1% formic acid in acetonitrile (A) and 0.1% formic acid in water (B) and was delivered at 1 mL/min in a gradient mode as follows: 100% A at 0 min, 60% A at 15 min, 100% A at 15.2 min, and 100% A at 20 min. The total runtime was 20 min. The mass spectrometer was operated in the positive-ion mode under the following optimal operating parametes: fragmentor voltage 50 V, capillary voltage 3.3 kV, corona current 4 μA, drying gas temperature 280°C, vaporizer temperature 320°C, drying gas flow 3 L/min, and nebulizer (nitrogen) pressure 30 psi. Analytes were detected in the selected-ion monitoring (SIM) mode at ions: m/z 166.2 for EP, 164.2 for MTC, 166.2 for PMA,136.2 for AMP, 150.2 for MA, 180.2 for MDA, 194.2 for MDMA, 208.3 for MDEA, 169.2 for EP-d_3, 141.2 for AMP-d_5, 155.2 for MA-d_5, 185.2 for MDA-d_5, 199.2 for MDMA-d_5, and 213.3 for MDEA-d_5.

Stock solutions of AMP, EP, MTC, PMA, MDA, MDMA, and MDEA at 1 mg/mL and MA, AMP-d_5, EP-d_3, MA-d_5, MDA-d_5, MDMA-d_5, and MDEA-d_5 at 0.1 mg/mL were prepared in methanol. Deuterated amphetamines were used as internal standards. Working solutions were prepared by diluting stock solutions with water. Stock and working solutions were stored at −20°C. Standards were prepared by spiking drug-free samples with working solutions.

A 50-mg hair sample was washed successively by sonication in 15 mL of dichloromethane for 5 min, 15 mL of water for 5 min, and 15 mL of methanol for 5 min, dried at room temperature, cut into 2-cm segments, then cut into 1-mM pieces, pulverized, spiked with 25 μL of internal standard, hydrolyzed in 1 mL of 1 M sodium hydroxide, incubated at 70°C for 20 min, cooled down to room

temperature, extracted with 2 mL of 1-chlorobutane for 10 min, and centrifuged at 4000 rpm for 5 min. The organic layer was collected and acidified with 100 μL of 0.025 M hydrochloric acid. The organic solvent was evaporated at 40°C under a stream of nitrogen and assayed. Under these conditions, retention times in minutes were 5.79 for EP, 7.10 for MTC, 7.35 for PMA, 6.53 for AMP, 7.10 for MA, 7.02 for MDA, 7.38 for MDMA, 8.18 for MDEA, 5.77 for EP-d_3, 6.48 for AMP-d_5, 7.08 for MA-d_5, 6.98 for MDA-d_5, 7.42 for MDMA-d_5, and 8.16 for MDEA-d_5.

Calibration curves for amphetamine were constructed in the range from 0.2 to 20.0 ng/mg. Correlation coefficients were 0.999. The recovery of amphetamine from hair ranged from 75.0% to 97.1%. The accuracy ranged from 84.0% to 99.6%. Intraday and interday coefficients of variation were 5.9% and 5.2%, respectively. Limits of detection and quantification were 0.10 and 0.15 ng/mg, respectively.

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AMPHOTERICIN B

CHEMISTRY

Amphotericin B is a polyene antifungal antibiotic. Other names include Abelcet, AmBisome, Amphotec, and Fungizone. Its molecular formula is $C_{47}H_{73}NO_{17}$, with a molecular weight of 924.1 and a CAS number of 1397-89-3. Amphotericin B occurs as a yellow to orange, odorless or practically odorless, powder. Amphotericin B is insoluble in water, dehydrated alcohol, ether, benzene, and toluene. It is soluble in dimethylformamide, dimethylsulfoxide, and propyleneglycol, and slightly soluble in methanol.

METHODS

Assay 1 Liu et al. [1] described the determination of amphotericin B in human plasma and CSF by HPLC. A Waters model 810 liquid chromatograph consisting of a model 510 pump, model 484 variable-wavelength detector and Baseline 810 manager was used. The stationary phase was a μBondapak C_{18} column (300 × 3.9 mm, 10 μm particle size). The column temperature was maintained at 32°C. The mobile phase consisted of 0.05 M disodium EDTA aqueous solution and acetonitrile (1 : 1) and was isocratically delivered at 1.4 mL/min. UV detection was performed at 405 nm and 0.005 AUFS. The injection volume was 80 μL.

Working solutions of amphotericin B were prepared in acetonitrile. Standards were prepared by spiking blank human plasma with working solutions. An aliquot of 0.4 mL of plasma or standard was mixed with 0.8 mL of acetonitrile, vortexed for 30 s, held still for 5 min, and centrifuged at 13,000 rpm for 5 min. The supernatant was collected and assayed. Under these conditions, the retention time of amphotericin B was about 5.2 min (estimated from the published chromatogram).

Calibration curves for amphotericin B were constructed over the range from 0.05 to 2.0 μg/mL. Correlation coefficients were 0.9996. The recovery of amphotericin B from plasma was greater than 87%. Intraday and interday coefficients of variation were less than 6.3% and 7.9%, respectively. The limit of detection was 0.02 μg/mL.

Assay 2 Espada et al. [2] reported an HPLC assay for the analysis of amphotericin B in biological samples. A liquid chromatographic system consisting of a Jasco PU1580 pump, a Gilson 231XL autosampler with a 100-μL loop, and a Jasco UV1575 UV–visible detector was used. The stationary phase was a ThermoHypersil BDS C_{18} column (250 × 4.6 mm, 5 μm particle size). The mobile phase consisted of acetonitrile, acetic acid, and water (52 : 4.3 : 43.7, vol/vol/vol) and was isocratically delivered at 1 mL/min. UV detection was performed at 406 nm. The runtime of a single injection was 15 min.

A stock solution of amphotericin B was prepared in sodium hydroxide solution (pH 11). Working solutions were prepared by diluting the stock solution with methanol/water (2 : 1, vol/vol). Standards were prepared by spiking the blank human plasma with working solutions. An aliquot of plasma or standard was mixed with 2 volumes of methanol, vortexed for 1 min, and centrifuged at 4500 rpm for 10 min. The supernatant was collected, filtered through a 0.45 μm Millex HV1 sterile syringe filter, and assayed. Under these conditions, the retention time of amphotericin B was 11.6 min.

Calibration curves for amphotericin B were constructed in the range from 0.1 to 10 μg/mL. Correlation coefficients were 0.998. The accuracy experessed in the relative percentage error was 1.9%. Intraday and interday coefficients of variation were 2.9% and 4.4%, respectively. Limits of detection and quantification were 0.016 and 0.054 μg/mL, respectively.

Assay 3 Lee et al. [3] described the determination of free and total amphotericin B in human biological matrices by HPLC-MS/MS. A liquid chromatograph equipped with a Waters model 717 autosampler was used. The stationary phase was a Waters Symmetry C_{18} column (150 × 3.0 mm, 5 μm particle size). The mobile phase consisted of methanol, water, and acetic acid (68.6 : 29.4 : 1.96, vol/vol/vol) and was isocratically delivered at 0.5 mL/min. The runtime was 3.5–4.0 min.

A PE SCIEX API3000 triple quadrupole mass spectrometer equipped with the TurboIonSpray was used for free amphotericin B and API 3+ mass spectrometer for total amphotericin B. The operating parameters were as follows: turbo probe temperature 480°C and drying gas (nitrogen) 8 L/min. Analytes were detected in the multiple-reaction

monitoring (MRM) mode at the following ion transitions: m/z 924 → 906 for amphotericin B and m/z 666 → 503 for natamycin.

A stock solution of amphotericin B at 1 mg/mL was prepared in 50% DMSO in methanol. Working solutions were prepared by diluting the stock solution with methanol. A stock solution of natamycin at 500 μg/mL was prepared in 50% DMSO in methanol. A working solution of natamycin at 0.2 μg/mL was prepared by diluting the stock solution with methanol and was used as an internal standard. For plasma samples, an aliquot of 50 μL of plasma was spiked with 50 μL of a working solution and 20 μL of internal standard, mixed with 2 mL of 16% DMSO in methanol and 2 mL of water, and centrifuged. The supernatant was collected and assayed. For plasma ultrafiltrate, 200 μL of ultrafiltrate was mixed with 20 μL of a working solution, 400 μL of methanol, and 1 mL of water, and was centrifuged for 15 min. The supernatant was loaded onto an Isolute C_2 endcapped SPE cartridge (50 mg/mL) that was preconditioned with methanol followed by water; washed with 1.5 mL of 10% methanol; eluted with 2 × 1 mL of methanol; evaporated to dryness under a stream of nitrogen; reconstituted with 130 μL of methanol, 75 μL of water, and 20 μL of internal standard; and assayed. For urine or fecal homogenate in 20% water, 50 μL urine was mixed with 50 μL of blank human plasma, 50 μL of a working solution, and 300 μL of 16% DMSO in methanol, and centrifuged for 15 min. An aliquot of 200 μL of the supernatant was mixed with 1 mL of water and was extracted using a SPE technique as described above. The volume of the internal standard added was 10 μL. Under these conditions, the retention time of amphotericin B was about 2.20 min (estimated from the published chromatogram).

Calibration curves in plasma were constructed in the range from 2 to 150 μg/mL. The overall recovery was 38%. The accuracy expressed in the relative percentage error was 7.2%. The coefficient of variation was 5.8%. Calibration curves in plasma ultrafiltrate were constructed in the range from 0.001 to 0.20 μg/mL. The accuracy expressed in the relative percentage error was 13.0%. The coefficient of variation was 12.6%. Calibration curves in urine were constructed in the range from 0.05 to 30 μg/mL. The accuracy expressed in the relative percentage error was 15.3%. The coefficient of variation was 13.0%. Calibration curves in fecal homogenate were constructed in the range from 0.40 to 40. The accuracy expressed in the relative percentage error was 9.6%. The coefficient of variation was 17.2%. The limit of quantitation was 1 ng/mL for ultrafitrate. For total amphotericin B, limits of quantification were 2, 0.05, and 0.4 μg/mL for plasma, urine, and fecal homogenates, respectively.

Assay 4 Liu et al. [4] described an HPLC method for the determination of amphotericin B in cerebrospinal fluid (CSF). A Hewlett-Packard 1050 system was used. The stationary phase was a Waters Nova-Pak C_{18} column (150 × 3.9 mm, 4 μm particle size). The mobile phase consisted of 0.01 M EDTA (pH 5) and acetonitrile (65 : 35, vol/vol) and was isocratically delivered at 0.5 mL/min. UV detection was performed at 410 nm. The injection volume was 100 μL. The runtime was 10 min.

Stock solutions of amphotericin B and nystatin (internal standard) were prepared in a mixture of DMSO and methanol (1 : 1, vol/vol). Working solutions were prepared by diluting stock solutions with methanol. Standards were prepared by spiking the blank canine CSF with working solutions. An aliquot of 1.0 mL of CSF, control or standard was mixed with 50 μL of internal standard, vortexed, loaded onto a BakerBond C_{18} SPE cartridge that was preconditioned with 3 mL of methanol followed by 3 mL of 0.1 M sodium carbonate buffer (pH 9), washed with 2 mL of the carbonate buffer, allowed to dry for 2 min, eluted with 2 × 0.5 mL of methanol, evaporated to dryness under a stream of nitrogen, reconstituted with 200 μL of methanol, and assayed. Under these conditions, retention times for amphotericin B and nystatin were 7.6 and 8.5 min, respectively.

Calibration curves for amphotericin B were constructed over the range from 1 to 10 ng/mL. Correlation coefficients were greater than 0.99. The accuracy expressed as the relative percentage error was 9.4%. The coefficient of variation of the assay was 8.4%. The limit of detection was 0.5 ng/mL.

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AMPICILLIN

CHEMISTRY

Ampicillin is an aminopenicillin antibiotic. Its chemical name is [2*S*-[2α,5α,6β(S^*)]]-6-[(aminophenylacetyl) amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid. Other names include Ampicin, AY-6108, BRL-1341, NSC-528986, Magnapen, and Principen. Its molecular formula is $C_{16}H_{19}N_3O_4S$, with a molecular weight of 349.4 and a CAS number of 69-53-4. Ampicillin is a white, practically odorless, crystalline powder. It is sparingly soluble in water at room temperature.

METHODS

Assay 1 Ishida et al. [1] reported an HPLC method for the determination of ampicillin in human serum after solid-phase extraction. A Waters HPLC system consisting of

a model 510 pump, model 710 autoinjector, model 481 UV detector, and a column oven was employed. The stationary phase was a GL Science Inertsil ODS3 column (250 × 4.6 mm). The column oven was set at 30°C. The mobile phase consisted of acetonitrile and 10 mM monobasic sodium phosphate (6.5 : 93.5, v/v). The flow rate was 1.0 mL/min. UV detection was performed at 220 nm.

N^2,N^4,N^6-Trimethylmelamine was used as the internal standard. Ampicillin stock solution and the internal standard were prepared in water at 0.5 and 0.2 mg/mL, respectively and stored at 4°C for a week. Ampicillin standards were prepared by spiking blank human serum with stock solution. Each sample or standard (0.5 mL) was mixed with 2 μL of internal standard and 0.5 mL of water, vortexed, loaded onto a 3M Empore SDB-XC disk cartridge (10 mm/6 mL, 24 mg) that was preconditioned with 0.5 mL of methanol followed by 0.5 mL of water twice, washed with 1 mL of water, eluted with 400 μL of acetonitrile, and directly assayed. The injection volume was 10 μL. Under these conditions, retention times of ampicillin and the internal standard were 20.7 and 15.4 min, respectively.

Calibration curves for ampicillin were linear in the concentration range of 0.19–9.41 μg/mL. Correlation coefficients were 0.999. Intraday and interday coefficients of variation were within 13.9% and 14.1%, respectively. Intraday and interday accuracies of assay were in the ranges of 90.4–103.1% and 90.4–102.1%, respectively. The assay was free of interference from lenampicillin, metabolites of lenampicillin, acetoin, or 2.3-butandiol.

Assay 2 Holt et al. [2] described an HPLC assay for the simultaneous determination of chloramphenicol, metronidazole, cefuroxime, cephalexin, ceftazidime, ampicillin, and benzylpenicillin. A liquid chromatographic system comprised a Waters model 510 pump, model 481 variable-wavelength UV detector, and a Rheodyne injector with a 20-μL loop. The stationary phase was a LiChrosorb C_{18} stainless-steel column (250 × 4.6 mm, 10 μm particle size). The mobile phase was 15% methanol in 100 mM sodium phosphate buffer (pH 6.0). The flow rate was 1.7 mL/min. UV detection was carried out at 214 nm.

Proteins in serum samples (100 μL each) were precipitated with 2 volumes of 4% perchloric acid containing tinidazole 25 mg/L as an internal standard and centrifuged. Supernatants were assayed immediately. The injection volume was 20 μL. Under these conditions, retention times of ampicillin and tinidazole were 11.6 and 15.6 min, respectively. Metabolites of ampicillin did not interfere with its analysis.

Linear calibration curves for ampicillin were established in the range of 1–80 mg/L, with correlation coefficients of 0.99. The coefficient of variation of the assay was 1.3%. The interday coefficient of variation was less than 5%. The mean recovery of ampicillin from serum was 108%. The limit of detection was 1.0 mg/L.

Assay 3 Wang et al. [3] presented an HPLC method for the determination of ampicillin in human plasma. A Beckman liquid chromatograph consisting of a model 125 pump and model 166 UV detector was used. The stationary phase was an ODS2 column (200 × 4.6 mm, 5 μm particle size). The mobile phase was 7.5% acetonitrile in 0.068 M monobasic potassium phosphate buffer and was isocratically delivered at 1 mL/min. UV detection was performed at 210 nm and 0.01 AUFS. The injection volume was 20 μL.

A stock solution of ampicillin at 1 mg/mL was prepared in water. Standards were prepared by spiking the blank human plasma with the stock solution. An aliquot of 200 μL of plasma or standard was mixed with 200 μL of perchloric acid, vortexed for 30 s, and centrifuged at 16000 rpm for 5 min. The supernatant was collected and assayed. Under these conditions, the retention time of ampicillin was 8.95 min.

Calibration curves for ampicillin was constructed over the range from 0.3 to 20 μg/mL. Correlation coefficients were 0.9994. The recovery of amipicillin in plasma was 90.3%. Intraday and interday coefficients of variation were 5.1% and 7.6%, respectively. There was no interference with the assay from endogenous substances in plasma.

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AMPRENAVIR

CHEMISTRY

Amprenavir is a synthetic antiretroviral agent. Its chemical name is (3*S*)-tetrahydro-3-furyl{(*S*)-α-[(1*R*)-1-hydroxy-2-(N^1-isobutylsulfanilamido)ethyl]phenethyl}carbamate. Other names include Agenerase and KVX-478. Its molecular formula is $C_{25}H_{35}N_3O_6S$, with a molecular weight of 505.6 and a CAS number of 161814-49-9. Amprenavir is a white to cream-colored solid. It has solubilities of 0.04 mg/mL in water and 86 mg/mL in alcohol. Amprenavir has a pK_a value of 1.97 at 25°C.

METHODS

Assay 1 Aymard et al. [1] developed an HPLC method for simultaneous determination of 12 antiretroviral drugs in human plasma. For the analysis of amprenavir, indinavir, nelfinavir, ritonavir, saquinavir, and efavirenz, a ThermoQuest liquid chromatographic system equipped with an

isocratic P1000 pump, AS3000 autosmpler, PC1000 integrator, UV1000 variable-wavelength UV detector, and a Waters column heater was used. The stationary phase was a Waters Symmetry C_{18} column (250 × 4.6 mm, 5 μm particle size) protected by a Waters Guard-Pak μBondapak C_{18} precolumn. The column temperature was maintained at 37°C. The mobile phase was composed of 0.04 M dibasic sodium phosphate buffer with 4% (vol/vol) 0.25 M octanesulfonic acid and acetonitrile (50 : 50, vol/vol) and was delivered at 1.3 mL/min. UV detection was carried out at 261 nm between 0 and 9 min, at 241 nm between 9 an 20 min, and at 254 nm between 20 and 32 min. The injection volume was 100 μL.

For the determination of abacavir, didanosine, lamivudine, stavudine, zidovudine, delavirdine, and nevirapine, a liquid chromatographic system consisting of three Beckman model 114M pumps, a Waters model WISP 717 Plus autosampler, model 481 variable-wavelength UV detector, a Shimadzu RF551 fluorescence monitor, two Lea Switch I&T switch valves, a Cil Cluzeau Croco-Cil column heater, and a Beckman System Gold 2 integrator was used. The stationary phase was a Waters Symmetry Shield C_{18} column (250 × 4.6 mm, 5 μm particle size) protected by an UpChurch filter (2 μm). The column temperature was maintained at 30°C. The mobile phase consisted of monobasic potassium phosphate buffer with 1% (vol/vol) 0.25 M octanesulfonic acid and acetonitrile. Mobile phase 1 contained 5% (vol/vol) acetonitrile and was delivered at 1 mL/min for 0–12 min, mobile phase 2 contained 20% (vol/vol) acetonitrile and was delivered at 1 mL/min for 12–35 min, and mobile phase 3 contained 70% (vol/vol) acetonitrile and was delivered at 1.2 mL/min for 35–40 min. UV detection was performed at 260 nm. The fluorescence detector for delavirdine was set at an excitation wavelength of 305 nm and emission wavelength of 425 nm, respectively. The injection volume was 150 μL.

Stock solutions of abacavir, didanosine, lamivudine, and stavudine at 1 mg/mL were prepared in water and stored at 4°C. Stock solutions of zidovudine, delavirdine, efavirenz, amprenavir, indinavir, nelfinavir, ritonavir, and saquinavir at 1 mg/mL, and neviapine at 5 mg/mL were prepared in methanol and stored at −20°C. Working solutions of didanosine, stavudine, and zidovudine at 100 μg/mL were prepared by diluting stock solutions with water. Working solutions of saquinavir and efavirenz at 500 μg/mL were prepared by diluting stock solutions with methanol. All working solutions were stored at 4°C. Standards were prepared by spiking drug-free human plasma with working solutions. A plasma sample or standard (1 mL) was loaded onto a J. T. Baker C_{18} extraction column that was preconditioned with 3 mL of methanol followed with 3 mL of distilled water, drawn through the column under pressure, washed with 2 mL of distilled water, dried under vacuum for 1 min, and eluted with 2.6 mL of methanol. The eluate was divided into two aliquots of 1 and 1.6 mL. These two solutions were evaporated to dryness at 40°C under a gentle stream of nitrogen. One residue was reconstituted with 200 μL of water for the analysis of abacavir, didanosine, lamivudine, stavudine, zidovudine, and nevirapine. The other residue was reconstituted with 150 μL of mobile phase for the analysis of amprenavir, indinavir, nelfinavir, ritonavir, saquinavir, and efavirenz. Retention times for indinavir, amprenavir, ritonavir, efavirenz, saquinavir, nelfinavir, lamivudine, didanosine, stavudine, zidovudine, abacavir, and nevirapine were 4.8, 5.6, 12.9, 15.2, 16.8, 29.2, 8.5, 9.6, 11.1, 17.4, 20.9, and 27.9 min, respectively.

A calibration curve for amprenavir was constructed in the concentration range of 100–10,000 ng/mL. The correlation coefficient was greater than 0.998. Within-day and between-day coefficients of variation were less than 3.9% and 5.8%, respectively. The limit of quantification was 25 ng/mL. There was no interference with the analysis of amprenavir from the following coadministered drugs, except for delavirdine and flunitrazepam: acebutolol, acetaminophen, acetylcysteine, acetylsalicylic acid, acyclovir, albendazole, alimemazine, alizapride, amikacin, amiodarone, amphotericin B, ampicillin, bepridil, buprenorphine, butobarbital, caffeine, calcium folinate, captopril, carbamazepine, carbutamide, chloroquine, ciprofloxacin, clindamycin, clofazimine, clofibrate, clonazepam, clonidine, cloxacillin, clozapine, cocaine, codeine, cortisol, cyamemazine, dantrolene, dexamethasone, dextropropoxyphene, diazepam, diclofenac, digoxin, dihydroergotamine, diltiazem, doxycycline, ethambutol, flecainide, fluconazole, fluoxetine, fluvoxamine, foscarvir, furosemide, ganciclovir, gentamicin, glibenclamide, granisetron, halofantrine, haloperidol, imipramine, indomethacin, interferon alfa, isoniazid, itraconazole, josamycin, ketoconazole, levomepromazine, lidocaine, loperamide, loratadine, losartan, mefloquine, meprobamate, methadone, methylprednisolone, metoclopramide, metronidazole, mianserin, moclobemide, morphine, nifedipine, niflumic acid, nitrofurantoin, omeprazole, paroxetine, pentamidine, phenobarbital, phenytoin, piracetam, prazosin, prednisolone, prednisone, primidone, propranolol, quinidine, quinine, ranitidine, ribavirin, rifabutine, rifampicin, roxithromycin, salicylic acid, simvastatin, sulfadiazine, sulfamethoxazole, sulpiride, thalidomide, theophylline, trimethoprim, valproic acid, venlafaxine, vigabatrin, viloxazine, zolpidem, and zopiclone.

More than 500 plasma samples were assayed on each column without significant loss of resolution.

Assay 2 Hirabayashi et al. [2] described an HPLC method for the simultaneous determination of amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, efavirenz, and the active metabolite of nelfinavir (M8) in human plasma. An Applied Biosystems Integral 100Q system consisting of a binary solvent delivery pump, autosampler, and a dual-wavelength UV detector was used. The stationary phase was a Developsil Ph-UG-3 column (150 × 2.0 mm, 3 μm particle size) protected by a Developsil Ph-UG-S guard column (10 × 1.5 mm). The column temperature was maintained at 40°C. Solvent A was a mixture of 34% acetonitrile and 66% 25 mM monobasic sodium phosphate containing 6 mM sodium 1-hexanesulfonate, adjusted to pH 5.1 with the phosphate buffer. Solvent B was a mixture of 64% acetonitrile and 36% 25 mM monobasic sodium phosphate buffer containing 6 mM sodium 1-hexanesulfonate, adjusted to pH 5.3. The mobile

phase was delivered at 200 μL/min in a gradient mode as follows:

Time (min)	%A	%B
0	100	0
14.6	100	0
16.6	70	30
35.5	70	30
35.6	0	100
40.0	0	100
40.1	100	0
46.8	100	0

UV detections were performed at 212 nm in channel 1 and at 266 nm from 0 to 18.3 min and 239 nm from 18.3 to 46.8 min in channel 2. Amprenavir was monitored at 266 nm.

Stock solutions of eight compounds at 1000 μg/mL were individually prepared in methanol. Working solutions containing these eight compounds at 100 μg/mL were prepared by diluting stock solutions with 50% methanol in water. Standards and controls were prepared by spiking drug-free plasma with working solutions. An aliquot of 400 μL of plasma, standard, or control was alkalinized with 400 μL of 0.1 M ammonium hydroxide adjusted to pH 10.5 with phosphoric acid, vortexed briefly, extracted with 1.8 mL of a mixture of ethyl acetate/acetonitrile (9 : 1, vol/vol), vortexed vigorously for 5 min, and centrifuged at 2000 *g* at 4°C for 10 min. An aliquot of 1.5 mL of the organic layer was collected, evaporated to dryness at 40°C under a stream of nitrogen, reconstituted with 150 μL of solvent A, washed with 1 mL of *n*-hexane, vortexed for 10 s, and centrifuged at 2000 *g* for 2 min. The lower phase was collected and assayed. Under these conditions, retention times for indinavir, amprenavir, nelfinavir (M8), efavirenz, saquinavir, ritonavir, lopinavir, and nelfinavir were 14.4, 16.2, 21.8, 23.7, 25.8, 30.0, 31.4, and 33.0 min, respectively.

Calibration curves for amprenavir were constructed over the range from 0.05 to 15 μg/mL. Correlation coefficients were 0.995. The accuracy expressed as the relative percentage error was 6.9%. The average extraction efficiency was 84.8%. Limit of quantification was 0.05 μg/mL. Intraday and interday coefficients of variation were 8.9% and 7.8%, respectively. There was no interference with the assay from the following drugs: abacavir, acetaminophen, acyclovir, amikacin, amoxicillin, amphotericin B, ampicillin, azithromycin, cefaclor, clarithromycin, clindamycin, didanosine, erythromycin, ethambutol, fluconazole, foscarnet, ganciclovir, hydroxyurea, isoniazid, kanamycin, ketoconazole, lamivudine, metronidazole, nevirapine, pentamidine, prednisolone, pyrazinamide, rifampicin, stavudine, sulfamethoxazole, trimethoprim, vancomycin, zalcitabine, zidovudine, and zidovudine glucuronide.

Assay 3 Dickinson et al. [3] described the simultaneous determination of HIV protease inhibitors amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, ritonavir, and saquinavir in human plasma by HPLC-MS/MS. A Surveyor liquid chromatograph consisting of a pump, autosampler, and PDA detector was used. The stationary phase was a HyPURITY C_{18} column (100 × 2.1 mm, 5 μm particle size) protected by a Merck guard column (Si 60, 5 μm particle size). Mobile phase A was a mixture of acetonitrile and 20 mM ammonium formate buffer (50 : 50) and mobile phase B a mixture of acetonitrile and 20 mM ammonium formate buffer (70 : 30). The mobile phase was delivered at 0.4 mL/min in a gradient mode from 100% A to 0% A in 2 min, held at 0% A for 4.5 min, increased to 100% A in 0.5 min, and held at 100% A for 3 min. The injection volume was 10 μL.

A ThermoElectron ion-trap LCQ Deca XP Plus mass spectrometer equipped with an electrospray ionization source was operated in the positive-ion mode. Drugs were detected over a 9-min period, which was divided into four separate segments. The operating conditions were summarized as follows:

	Segment 1	Segment 2	Segment 3	Segment 4
Time (min)	0.00–1.86	1.86–4.09	4.09–5.80	5.80–9.00
Capillary temperature (°C)	360	360	360	360
Sheath gas (N_2)	50	65	50	60
Auxiliary gas (He)	15	10	15	10
Source voltage (kV)	4.5	5	4.5	5
Capillary voltage (V)	27	3	36	31
Source current (μA)	80	80	80	80

Drugs and internal standard were quantified in the multiple-reaction monitoring (MRM) mode at ion transistions m/z 614.40 → 465.20 for indinavir, 506.20 → 245.00 for amprenavir, 705.40 → 335.10 for atazanavir, 721.40 → 267.90 for ritonavir, 671.40 → 433.20 for saquinavir, 629.40 → 447.10 for lopinavir, 568.30 → 331.10 for nelfinavir, and 674.40 → 388.20 for the internal standard.

Stock solutions of drugs at 1 mg/mL were individually prepared in methanol. Standards were prepared by diluting these stock solutions with drug-free plasma. A stock solution of Ro31-9564 at 100 μg/mL was prepared in methanol. The working solution of Ro31-9564 at 1 μg/mL was prepared by diluting the stock solution with methanol–water (50 : 50, vol/vol) and was used as an internal standard. An aliquot of 100 μL of plasma, standard, or control was spiked with 20 μL of internal standard, mixed with 1 mL of acetonitrile, vortexed, left to stand at room temperature for 15 min, vortexed again, and centrifuged at 4°C at 1780 *g* for 10 min. The organic phase was collected, mixed with 300 μL of 20 mM ammonium formate, and assayed. Under these conditions, retention times for indinavir, amprenavir, atazanavir, ritonavir, saquinavir, lopinavir, nelfinavir, and internal standard were 1.10, 1.38, 1.97, 2.32, 2.63, 2.74, 5.03, and 6.14 min, respectively.

Calibration curves for amprenavir were constructed over the range from 56 to 5136 ng/mL. Correlation coefficients were greater than 0.995. The mean accuracy expressed as the relative percentage error was less than 9.9%. Intraday and interday coefficients of variation were less than 5% and 9%, respectively. Limits of detection and quantification were 29 and 56 ng/mL, respectively.

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AMSACRINE

CHEMISTRY

Amsacrine is an antineoplastic agent. Its chemical name is 4′-(acridin-9-ylamino)methanesulfon-*m*-anisidide. Other names include Amsacrina, Amsidine, and NSC-249992. Its molecular formula is $C_{21}H_{19}N_3O_3S$, with a molecular weight of 393.5 and a CAS number of 51264-14-3.

METHODS

Assay 1 Emonds et al. [1] reported the determination of amsacrine in human plasma by gas chromatography. A Becker type 420 gas chromatograph equipped with a nitrogen–phosphorus (NP) detector was used. The stationary phase was a capillary support coated open tubular column (10 m × 0.45 mm i.d.) coated with Dexsil 300 GC. The operating conditions were as follows: injection temperature 400°C, oven temperature 270°C, detection temperature 310°C, carrier gas nitrogen at 5 mL/min, and auxiliary gas helium at 25 mL/min. The injection volume was 5 μL.

Standards were prepared by spiking 0.2 mL of blank plasma with known amounts of amsacrine. An aliquot of 0.2 mL of plasma or standard was mixed with 2 mL of chloroform and centrifuged. The organic phase was collected, evaporated under a stream of nitrogen, reconstituted with 0.25 mL of methanol/water (9 : 1), washed with 3 × 1 mL of hexane, dried under nitrogen, redissolved in 50 μL of dimethylformamide, and assayed. Under these conditions, the retention time of amsacrine was about 3.2 min (estimated from the published chromatogram).

A calibration curve for amsacrine was constructed in the range of 0.125–1.00 μg/mL. The average recovery of the drug from plasma was 85%. The limit of detection was 50 ng/mL.

Assay 2 Jurlina and Paxton [2] described an HPLC assay fo the determination of amsacrine in plasma. A Waters system consisting of a model 6000A pump, model 440 UV detector, and a model U6K injector was used. The stationary phase was a Radial-Pak C_{18} column (100 × 8 mm, 10 μm particle size). The mobile phase consisted of acetonitrile and water (40 : 60) containing 0.01 M triethylamine phosphate (pH 3.80) and was isocratically delivered at 7 mL/min. UV detection was performed at 254 nm. The injection volume was 20–40 μL. The run time was 5.5 min.

A stock solution of 10 mM amsacrine was prepared in methanol. A stock solution of 1.0 mM 4′-(3-methyl-9-acridinylamino)methanesulfonanilide was prepared in methanol, diluted to a nominal concentration of 20 μM with methanol, and used as an internal standard. Standards were prepared by diluting the stock solution of the drug with blank human plasma and stored at −20°C. An aliquot of 100 μL of 2 nM 4′-(3-methyl-9-acridinylamino)methanesulfonanilide was added to a glass culture tube and evaporated to dryness at 35°C under a stream of nitrogen. An aliquot of 0.5 mL of plasma or standard was added to the tube, adjusted to pH 3.0–4.0 with 120 μL of 0.5 M hydrochloric acid, vortexed, mixed with 5 mL of hexane, shaken for 20 min, and centrifuged at 1720 *g* for 10 min. The lower aqueous layer was collected, adjusted to pH 9.0 with 0.5 mL of saturated sodium tetraborate solution, extracted with 6 mL of diethyl ether for 15 min, and centrifuged again for 15 min. The organic layer was separated, evaporated to dryness at 35°C under a gentle steam of nitrogen, reconstituted with 100 μL of methanol, and assayed. Under these conditions, retention times for amsacrine and the internal standard were 3.4 and 4.3 min, respectively.

The calibration curve for amsacrine was constructed over the range of 0.1–10 μM. The correlation coefficient was 0.9998. The average recovery of the drug from plasma ranged from 104% to 115%. Intraday and interday coefficients of variation were 2.7% and 4.0%, respectively. The limit of quantification was 50 nM. There was no interference with the assay from adriamycin, chlorambucil, cytosine arabinoside, 5-fluorouracil, lomustine, melphalan, methotrexate, prednisolone, 6-thioguanine, vincristine, or vinblastine.

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APOMORPHINE HYDROCHLORIDE

CHEMISTRY

Apomorphine hydrochloride is a dopamine receptor agonist. Its chemical name is (6a*R*)-5,6,6a,7-tetrahydro-6-methyl-4*H*-dibenzo[*de,g*]quioline-10,11-diol hydrochloride hemihydrate. Other names include Apokyn, Apomine, and Uprima. Its molecular formula is $C_{17}H_{17}NO_2 \cdot HCl \cdot 1/2H_2O$, with a molecular weight of 312.8 and a CAS number of 41372-20-7. Apomorphine hydrochloride occurs as odorless white or faintly yellow to green-tinged grayish crystals or crystalline powder. It is soluble 1 in 50 of water and 1 in 20 of water at 80°C, and 1 in 50 of alcohol, and very slightly soluble in ether and chloroform.

METHODS

Assay 1 Bolner et al. [1] described an HPLC method with electrochemical detection for the analysis of apomorphine in plasma. A liquid chromatograph consisting of a Gilson model 307 pump and model 234 autosampler with a 100-μL loop was used. The stationary phase was a Macherey–Nagel Nucleosil 100 C_{18} column (150 × 4.6 mm, 5 μm particle size). The mobile phase consisted of 10.0 mM phosphate buffer (pH 3.6) and acetonitrile (80 : 20) and was isocratically delivered at 1.0 mL/min. An ESA coulometric detector equipped with a model 5011 high-sensitivity cell was operated at applying potentials of 0.00 and +0.35 V at the first and second electrodes, respectively. The injection volume was 100 μL.

A stock solution of apomorphine at 1.0 μg/mL was prepared in 0.1 M hydrochloric acid and stored at −80°C. Standards were prepared by diluting the stock solution with blank human plasma. An aliquot of 200 μL of plasma was mixed with 10 μL of 2.0 M Tris buffer (pH 8.6) and 40 mg of alumina of activity-grade Super 1, stirred for 30 min, and centrifuged. The alumina pellet was separated, washed with 2 × 1.0 mL of water, mixed, centrifuged, eluted by adding 200 μL of 0.2 M perchloric acid in acetonitrile, and stirred for 15 min. The acid layer was diluted 1 : 2 with 0.6 M phosphate buffer (pH 3.6) and assayed. Under these conditions, the retention time of apomorphine was about 5.9 min (estimated from the published chromatogram).

A calibration curve for apomorphine was constructed over the range of 5–1000 ng/mL. The correlation coefficient was 0.999. The average recovery of the drug from plasma was greater than 98.7%. Intrarun and interrun coefficients of variation were 3.7% and 5.6%, respectively.

Assay 2 Van der Geest et al. [2] developed an HPLC assay for the determination of *R*-apomorphine and *S*-apomorphine in plasma. A liquid chromatographic system consisting of an Applied Biosystems Spectroflow 400 solvent delivery system, a Millipore WISP 710B autosampler, a Shimadzu Chromatopack CR3A integrator, and a Leiden Antec electrochemical detector was used. The stationary phase was a Diacel Chiralcel OD-R chiral column (200 × 4.6 mm, 10 μm particle size). The mobile phase consisted of acetonitrile and aqueous solution (35 : 65), where the aqueous solution contained 0.1 M monobasic sodium phosphate, 0.1 M sodium perchlorate hydrate, and 10 mg/L EDTA, adjusted to pH 4 with 98% phosphoric acid. The flow rate was 0.9 mL/min. The electrochemical detection was performed at an applied potential of 0.7 V. The injection volume was 50 μL.

N-Propylnorapomorphine was used as an internal standard. Stock solutions of apomorphine and internal standard were separately prepared in 5 mM citrate buffer (pH 4), where the citrate buffer consisted of 2.1 mM sodium citrate and 2.9 mM citric acid, to which 0.1% sodium metabisulfite and 0.01% EDTA were added. All stock solutions were stored at −20°C. Standards were prepared by diluting stock solutions with blank plasma. Tetraoctylammonium bromide (TOABr) at 0.165% was prepared in octane–hexane (1 : 10). Diphenylborinic acid ethanolamine ester (DPBEA) at 0.2% was prepared in water containing 14 mL/L ammonium hydroxide (25%) and 0.5% EDTA. An aliquot of 1 mL of plasma or standard was mixed with 30 μL of internal standard, 0.5 mL of DPBEA buffer (pH 8.45), and 1.5 mL of TOABr; shaken for 2 min; and centrifuged at 5°C for 15 min. The aqueous phase was mixed with 3 mL of octanol and 0.5 mL of 0.05 M phosphoric acid containing 0.1% sodium metabisulfite and 0.01% EDTA, shaken for 2 min, and centrifuged at 5°C for 15 min. The aqueous phase was collected and assayed.

Calibration curves for *R*-apomorphine were constructed over the range of 6–600 ng/mL. Correlation coefficients were greater than 0.995. The accuracy ranged from 94.7% to 106.2%. The recovery ranged from 78.6% to 85.4%. Intraday and interday coefficients of variation were 5.1% and 3.8%, respectively. Limit of detection was 0.3 ng/mL.

Calibration curves for *S*-apomorphine were constructed over the range of 6–600 ng/mL. Correlation coefficients were greater than 0.995. The accuracy ranged from 98.8% to 102.8%. The recovery ranged from 82.3% to 86.1%. Intraday and interday coefficients of variation were 6.6% and 4.1%, respectively. Limit of detection was 0.6 ng/mL.

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ARIPIPRAZOLE

CHEMISTRY

Aripiprazole is an atypical antipsychotic. Other names include Abilify and OPC-14597. Its chemical name is 7-{4-[4-(2,3-dichlorophenyl)-piperazin-1-yl]butoxy}-3,4-dihydroquinolin-2(1*H*)-one. Its molecular formula is

$C_{23}H_{27}Cl_2N_3O_2$, with a molecular weight of 448.4 and a CAS number of 129722-12-9.

METHODS

Assay 1 Liu et al. [1] described the analysis of aripiprazole in plasma by RP-HPLC-UV. An Agilent 1100 liquid chromatograph consisting of a model G1315B diode-array detector, model G1313A autoinjector, and a workstation. The stationary phase was a Diamonsil C_{18} column (150 × 4.6 mm, 5 μm particle size). The column temperature was maintained at 40°C. The mobile phase consisted of 0.03 M ammonium acetate and acetonitrile (34 : 66) and was isocratically delivered at 0.8 mL/min. UV detection was performed at 257 nm and 0.01 AUFS. The injection volume was 15 μL.

A stock solution of aripiprazole at 1 mg/mL was prepared in methanol. Working solutions were prepared by diluting the stock solution with methanol. Diazepam at 10 mg/L in methanol was used as an internal standard. Standards were prepared by spiking blank human plasma with working solutions. An aliquot of 0.8 mL of plasma or standard was spiked with 40 μL of internal standard, shaken, extracted with 3.2 mL of ethyl acetate–dichloromethane (80 : 20), vortexed for 2 min, and centrifuged at 12,000 rpm for 3 min. The supernatant was evaporated to dryness at 56°C under vacuum, reconstituted with 100 μL of methanol, and assayed. Under these conditions, retention times for diazepam and aripiprazole were 5.0 and 9.3 min, respectively.

A calibration curve was constructed over the range from 5.0 to 600.0 μg/L. The correlation coefficient was 0.9995. The recovery of aripiprazole from plasma ranged from 95.7% to 98.5%. Intraday and interday coefficients were less than 5.4%. Limit of quantification was 5.0 μg/L.

Assay 2 Lancelin et al. [2] developed an HPLC method for the simultaneous determination of aripiprazole and dehydroaripiprazole in human plasma. A Waters system consisting of a model 600 controller pump, inline degasser, and a model 2996 photodiode-array detector was used. The stationary phase was a Waters X Bridge C_{18} column (100 × 4.6 mm, 3.5 μm particle size). The mobile phase consisted of acetonitrile and 10 mM ammonium buffer (pH 8.35) (60 : 40, vol/vol) and was isocratically delivered at 1 mL/min. UV detection was performed at 217 nm. The injection volume was 50 μL.

Stock solutions of aripiprazole and dehydroaripiprazole at 200 μg/mL each were prepared in methanol. Chlorohaloperidol at 10 μg/mL in methanol was used as an internal standard. These solutions were stored at 4°C. Standards were prepared by spiking drug-free human plasma with stock solutions. An aliquot of 1 mL of plasma or standard was mixed with 20 μL of internal standard, 500 μL of 2 M sodium carbonate, and 10 mL of heptane–propanol (98 : 2, vol/vol), shaken for 20 min; and centrifuged at 1800 *g* at 4°C for 10 min. The aqueous layer was collected, mixed with 150 μL of 0.05 M phosphoric acid, and assayed. Under these conditions, retention times for chlorohaloperidol, dehydroaripiprazole, and aripiprazole were 4.2, 5, and 6.8 min, respectively.

Calibration curves for aripiprazole and dehydroaripiprazole were constructed over the range of 2–1000 ng/mL. Correlation coefficients were 0.999. Accuracy ranged from 98.5% to 111.5% for aripiprazole and from 98.1% to 113.0% for dehydroaripiprazole. Intraday and interday coefficients of variation were 7.4% and 18.5% for aripiprazole and 7.2% and 17.8% for dehydroaripiprazole, respectively. There was no interference with this method from endogenous materials in plasma and from the following drugs (retention times in minutes): amisulpride (1.2), *O*-desmethylvenlafaxine (1.5), 9-hydroxyrisperidone (1.5), risperidone (1.7), desmethylescitalopram (1.8), norclozapine (1.9), amoxapine (2.3), hydroxyhaloperidol (2.3), paroxetine (2.4), escitalopram (2.4), fluvoxamine (2.6), diazepam (2.6), venlafaxine (2.7), desipramine (2.7), norfluoxetine (2.8), haloperidol (2.9), pipothiazine (2.9), clozapine (3.0), nortriptyline (3.3), fluoxetine (3.7), desmethylclomipramine (3.7), loxapine (4.5), sertraline (7.4), cyamemazine (8.0), levomepromazine (8.2), imipramine (8.5), clomipramine (8.6), or chlorpromazine (9.2). Fluphenazine and amitriptyline had retention times of 4.2 and 6.6 min and interfered with this assay.

Assay 3 Musenga et al. [3] developed a capillary electrophoresis (CE) method for the determination of aripiprazole in human plasma. An Agilent ^{3D}CE system equipped with a diode array detector was used. A Composite Metal Services uncoated fused-silica capillary (33.0 cm × 50 μm i.d.) was employed. The voltage was set at +20 kV, the capillary temperature was 20°C, and samples were injected by pressure at 50 mbar for 13 s at the anodic end. UV detection was performed at 214 nm. The background electrolyte was 50 mM phosphate buffer (pH 2.5). The capillary was conditioned with the background electrolyte for 15 min, and between two runs it was flushed sequentially with 0.1 M hydrochloric acid for 2 min, water for 1 min, 0.1 N sodium hydroxide solution for 1 min, water for 2 min, and the background electrolyte for 5 min.

A stock solution of aripiprazole at 0.1 mg/mL was prepared in methanol. Working solutions were prepared by diluting the stock solution with diluent A, which consisted of 5 mM phosphate buffer (pH 2.5) and acetonitrile (65 : 35, vol/vol). Loxapine at 1.00 mg/mL in methanol was used as an internal standard. Standards were prepared by spiking blank human plasma with working solutions. An aliquot of 500 μL plasma or standard was mixed with 50 μL of internal standard, diluted with 1000 μL of water, loaded onto a Bond Elut cyano cartridge (1 mL/100 mg) that was preconditioned with 5 × 1 mL of methanol followed by 5 × 1 mL of water, washed with 2 × 1 mL of water followed by 1 mL of methanol–water (20 : 80, vol/vol), eluted with 1.5 mL of methanol, evaporated to dryness, reconstituted with 200 μL of dilutent A, and assayed. Under these conditions, migration times for aripiprazole and loxapine were 4.9 and 2.8 min, respectively.

Calibration curves were constructed over the range from 70.0 to 700 ng/mL. Correlation coefficients were greater than 0.9996. The mean recovery ranged within

91.4–99.0%. Intraday and interday coefficients of variation were less than 4.5% and 6.9%, respectively. Limits of detection and quantification were 35.0 and 70.0 ng/mL, respectively. There was no interference with this method from the following drugs (retention time in minutes): olanzapine (2.1), clozapine (2.2), risperidone (3.0), ziprasidone (4.5), duloxetine (3.4), fluoxetine (5.4), citalopram (3.5), and clomipramine (3.4).

Assay 4 Musenga et al. [3] developed an HPLC method for the determination of aripiprazole in human plasma. An Agilent 1100 system equipped with a diode-array detector was used. The stationary phase was a Varian Microsorb C_8 column (150 × 4.6 mm, 5 μm particle size) protected by a C_8 precolumn (4 × 3 mm, 5 μm particle size). The mobile phase consisted of 12.5 mM phosphate buffer (pH 3.5), triethylamine, and acetonitrile (64.81 : 0.19 : 35, vol/vol/vol) and was isocratically delivered at 1.2 mL/min. UV detection was performed at 254 nm. The injection volume was 50 μL.

Plasma samples and standards were processed as described in Assay 3. Retention times for aripiprazole and loxapine were 9.6 and 6.9 min, respectively.

Calibration curves were constructed over the range from 70.0 to 700 ng/mL. Correlation coefficients were greater than 0.9999. The mean recovery ranged from 94.2% to 97.3%. Intraday and interday coefficients of variation were less than 4.9% and 6.1%, respectively. Limits of detection and quantification were 25.0 and 50.0 ng/mL, respectively.

Assay 5 Kirschbaum et al. [5] described the therapeutic monitoring of aripiprazole by HPLC with column switching and UV detection. An Agilent 1100 series system consisting of an autosampler, a column oven, an electric six-port switching valve, and a variable-wavelength UV detector was used. The stationary phase was a MZ-Analysentechnik LiChrospher CN5 column (100 × 4 mm, 5 μm particle size). The mobile phase consisted of acetonitrile and 10 mM dibasic potassium phosphate buffer adjusted to pH 6.4 with phosphoric acid (50 : 50, vol/vol) and was isocratically delivered at 1.2 mL/min. UV detection was performed at 210 nm. The injection volume was 100 μL.

Perphenazine at 61.5 μg/mL was used as an internal standard. An aliquot of 99 μL of plasma was mixed with 1 μL of internal standard, injected onto a MZ-Analysentechnik CN cleanup column (100 × 4 mm, 20 μm paricle size), washed to the waste with 8% acetonitrile in water at 0.8 mL/min for 5 min, and backflushed to the analytical column by switching the valve using the mobile phase at 1.2 mL/min. Under these conditions, retention times for aripiprazole and perphenazine were about 17.6 and 19.2 min, respectively (estimated from the published chromatogram).

A calibration curve was constructed over the range from 50 to 1000 μg/mL. The correlation coefficient was greater than 0.998. The recovery of the drug from plasma ranged from 96.7% to 115.5%. Intraday and interday coefficients of variation were less than 9.1% and 7.0%, respectively. The limit of quantification was 50 μg/mL. The following drugs (retention time in minutes) interfered with the determination of aripiprazole: reboxetine (17.0), pipamperone 917.8), and norclozapine (18.0).

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AROTINOLOL HYDROCHLORIDE

CHEMISTRY

Arotinolol is a noncardioselective β-blocker. Its chemical name is (±)-5-[2-{[3-(*tert*-butylamino)-2-hydroxypropyl] thio}-4-thiazolyl]-2-thiophenecarboxamide hydrochloride. Other names include Almarl and S-596. Its molecular formula is $C_{15}H_{21}N_3O_2S_3 \cdot HCl$, with a molecular weight of 408.0 and a CAS number of 68377-91-3.

METHOD

Assay 1 Moulin et al. [1] developed an HPLC method for the determination of arotinolol and its main metabolite, 5-[2-(3-tertiarybutylamino-2-hydroxypropyl)thio-4-thiazolyl]-2-thiophenecarboxylic acid hydrochloride (AC623), in biological samples. A Gilson liquid chromatographic system consisting of a model 303 pump with a pulsation dampler, model 231 autosampler, model 401 dilutor, a Shimadzu model RF535 fluorescence detector, model SPD6A UV detector, and a model CR4A integrator was used. The stationary phase was a Hypersil ODS column (250 × 4.6 mm, 5 μm particle size) protected by a C_{18} guard column (15 × 4.6 mm, 7 μm particle size). The mobile phase consisted of acetonitrile, methanol, and 0.07 M phosphate buffer (pH 5.6) containing 6×10^{-4} M tetrabutylammonium chloride (150 : 150 : 900) and was isocratically delivered at 1.2 mL/min. UV detection was performed at 310 nm, and fluorescence detection was carried out at an excitation wavelength of 310 nm and an emission wavelength of 395 nm. The injection volume was 100 μL.

Stock solutions of arotinolol and AC623 were prepared in methanol. Working solutions were prepared by diluting stock solutions with water. Standards were prepared by spiking blank human plasma or urine with working

solutions. Alpiropride was used as an internal standard. An aliquot of 1 mL of plasma or standard was spiked with 50 μL of alpiropride at 5 μg/mL, loaded onto a C_{18} BakerBond solid-phase extraction cartridge that was preconditioned with methanol, washed with 3 × 1 mL of water followed by 3 × 1 mL of diethyl ether–*n*-hexane (50 : 50, vol/vol), and eluted with 2 × 1 mL of chloroform–triethylamine (90 : 10, vol/vol). The organic layer was evaporated under vacuum, reconsitituted in 150 μL of mobile phase, and assayed. An aliquot of 100 μL of urine sample was mixed with 250 μL of blank plasma and 100 μL of internal standard and processed as described above for plasma samples. Under these conditions, retention times for arotinolol, AC623, and alpiropride were 10.0, 8.0, and 4.7 min, respectively.

Calibration curves in plasma were constructed over the ranges 0–60 ng/mL for arotinolol and 0–15 ng/mL for AC623. Correlation coefficients were greater than 0.9951. Extraction efficiencies were better than 72% for arotinolol and 57% for AC623. Accuracy expressed as the relative percentage error was less than 10% for both compounds. Intraday and interday coefficients of variation for both compounds were less than 10%. Limits of detection were 0.11 ng/mL for arotinolol and 0.25 ng/mL for AC623.

Calibration curves in urine were constructed over the ranges from 0 to 1000 ng/mL for both compounds. Correlation coefficients were greater than 0.9932. Extraction efficiencies were better than 79% for arotinolol and 66% for AC623. Accuracy expressed as the relative percentage error was less than 10% for both compounds. Intraday and interday coefficients of variation for both compounds were less than 10%. Limits of detection were 11 ng/mL for arotinolol and 2.5 ng/mL for AC623. There was no interference with this assay from endogenous materials in plasma and urine.

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ARTEMISININ

CHEMISTRY

Artemisinin is an antimalarial. Its chemical name is (3*R*,5a*S*,6*R*,8a*S*,9*R*,12*S*,12a*R*)-octahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-*j*]-1,2-benzodioxepin-10(3*H*)-one. Other names include Arteannuin, Artemisinine, Huanghuahaosu, and Qinghaosu. Its molecular formula is $C_{15}H_{22}O_5$, with a molecular weight of 282.3 and a CAS number of 63968-64-9.

METHOD

Assay 1 Gordi et al. [1] described a direct analysis of artemisinin in plasma and saliva using coupled-column high-performance liquid chromatography with restricted-access material (RAM) precolumn. The system consisting of a Shimdazu model LC10AD pump, two ESA model 580 solvent delivery modules, a Triathlon autoinjector with two Rheodyne six-port switching valves, and a Shimadzu model SPD10A UV detector was used. The stationary phase was a Chrompack reversed-phase C_{18} column (100 × 4.6 mm, 3 μm particle size) protected by an R2 guard column. A Merck inline filter was placed in front of the precolumn and after the guard column. UV detection was performed at 289 nm.

Plasma and urine samples were centrifuged at 8000 *g* for 7 min, and supernatants were injected onto the RAM precolumn with a mobile phase consisting of water and acetonitrile (98 : 2, vol/vol). After injection the flow rate of the mobile phase increased gradually from 1.20 to 3.00 mL/min in 2 min. Then, 9 min after injection, the flow rate was returned to 1.20 mL/min. The valve was switched and the retained analytes were backflushed from the RAM precolumn to the analytical column using the separation mobile phase consisting of water and acetonitrile (50 : 50, vol/vol) at 0.75 mL/min. The postcolumn reaction of artemisinin with 0.3 M potassium hydroxide in ethanol–water (9 : 1, vol/vol) occurred in the online Teflon coil immersed in a 70°C water bath. Under these conditions, the retention time of the derivatized artemisinin was about 5 min (estimated from the published chromatogram).

Calibration curves were constructed over the range from 10 to 2000 ng/mL in plasma and in water. The mean accuracy ranged from 98.6% to 103.4% for plasma and from 98.6% to 100.0% for saliva. Intraday and interday coefficients of variation were less than 10.5% and 15.8% for plasma and 11.1% and 11.8% for saliva. Limit of quantification was 2 ng/mL for saliva and 10 ng/mL for plasma. The following drugs did not interfere with the analysis of artemisinin: mefloquine, chloroquine, deacetyl-chloroquine, sulfodoxine, acetylsulfodoxine, pyrimetamine, proquanil, cycloquanil, and 4-chlorophenylbiquanide.

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ARTESUNATE

CHEMISTRY

Artesunate is a derivative of artemisini as an antimalarial. Its chemical name is (3*R*,5a*S*,6R,8a*S*,9*R*,10*S*,12*R*,12a*R*)-decahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano-[4,3-*j*]-1,2-benzodioxepin-10-ol hydrogen succinate. Other names include Artesunato, Cotecxin, and Larinate. Its molecular formula is $C_{19}H_{28}O_8$, with a molecular weight of 384.4 and a CAS number of 83507-69-1.

METHODS

Assay 1 Na-Bangchang et al. [1] described an assay with electrochemical detection for the simultaneous determination of artesunate and dihydroartemisinin in human plasma. A Bioanalytical Systems model BAS2000B was coupled with a Bioanalytical Systems electrochemical detector and a Rheodyne 7125 injector with a 20-μL sample loop. The stationary phase was a Waters Nova-Pak C_{18} column (150 × 3.9 mm, 5 μm particle size). The mobile phase consisted of acetonitrile and 0.1 M acetic acid aqueous solution (pH 4.8) (45 : 55, vol/vol). The temperature of the mobile phase was maintained at 35°C. The flow rate was 1.5 mL/min. The electrochemical detector was operated in the reductive mode at an applied potential of −1.0 V, using a BAS model MF1000 thin-layer dual-glassy-carbon electrode as working electrode and a BAS model MW2021 Ag/AgCl reference electrode at a sensitivity of 50 nA.

Artemisinin was used as an internal standard. Stock solutions of artesunate and dihydroartemisinin at 0.5 μg/μL were prepared in 50% ethanol. Working solutions were prepared by diluting the stock solution with 50% ethanol and stored at −70°C. Calibrators were prepared by spiking blank plasma with working solutions. A plasma sample or calibrator (1 mL) was mixed with 300 ng of internal standard, vortexed for 30 s, extracted twice with 5 mL of dichloromethane–*tert*-methylbutyl ether (8 : 2, vol/vol) by mechanical tumbling for 20 min, and centrifuged at 4°C at 1200 *g* for 15 min. The supernatant was collected, evaporated to dryness at room temperature under a stream of nitrogen, reconstituted with 60 μL of 50% ethanol, stored at 4°C for 16 h for equilibrium of α and β anomers of dihydroartemisinin, and assayed. The injection volume was 20 μL. Under these conditions, retention times of α-dihydroartemisinin, β-dihydroartemisinin, artesunate, and artemisinin were 2.9, 4.2, 4.5, and 6.0 min, respectively.

Calibration curves for artesunate and α-dihydroartemisinin were constructed in the range from 10 to 800 ng/mL. Correlation coefficients were greater than 0.990. Coefficients of variation were less than 10%. Limits of quantification were 5 and 3 ng/mL for artesunate and α-dihydroartemisinin, respectively. This assay was free of any interference from mefloquine, quinine, chloroquine, pyrimethamine, primaquine, artemether, and arteether.

Assay 2 Gu et al. [2] reported an LC-MS/MS method for artesunate and dihydroartemisinin in human plasma. The stationary phase was a Varian Pursuit C_{18} column (150 × 2.0 mm, 5 μm particle size). The mobile phase consisted of acetonitrile (A) and 10 mM ammonium acetate in water (B) and was delivered in a gradient mode at 65% B for 4 min, increased to 95% B in 0.01 min, held at 95% B for 2.99 min, returned to 65% in 0.01 min, and held at 65% B for 2.99 min. The flow rate was 0.2 mL/min.

A Micromass Quattro II mass spectrometer was operated in the positive-ion electrospray ionization mode. Analytes were monitored in the multiple-reaction mode (MRM).

Standards were prepared by spiking blank human plasma with artesunate and dihydroartemisinin. Indomethacin in water was used as an internal standard. The analytes were extracted from plasma with ethyl acetate. The organic phase was dried, reconstituted with acetonitrile and water containing indomethacin (50 : 50, vol/vol), diluted with 50 μL of water, and assayed.

Calibration curves for artesunate were constructed over the range from 2 to 400 ng/mL. Correlation coefficients were greater than 0.998. The recovery ranged from 56.5% to 81.3%. Intraday and interday coefficients of variation were less than 12.9% and 14.1%, respectively. Limit of quantification was 4.3 ng/mL.

Calibration curves for dihydroartemisinin were constructed over the range from 2 to 400 ng/mL. Correlation coefficients were greater than 0.996. The recovery ranged from 76.0% to 121.6%. Intraday and interday coefficients of variation were less than 10.5% and 12.1%, respectively. Limit of quantification was 2.6 ng/mL.

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ASPIRIN

CHEMISTRY

Aspirin is a salicylate NSAID. Its chemical name is *O*-acetylsalicylic acid. Other names include acetylsalicylic acid, salicylic acid acetate, Ascriptin, and Empirin. Its molecular formula is $C_9H_8O_4$, with a molecular weight of 180.2 and a CAS number of 50-78-2. Aspirin occurs as white crystals, commonly tubular or needle-like, or white crystalline powder. Aspirin is soluble 1 in 300 of water, 1 in 5 of alcohol, 1 in 17 of chloroform, and 1 in 10–15 of ether.

METHOD

Assay 1 Ogunbona [1] reported the simultaneous determination of aspirin and its metabolites, salicylic acid, salicyluric acid, and gentisic acid, in human urine using HPLC. A liquid chromatograph consisting of an LDC Consta Metric III pump, Spectromonitor III variable-wavelength UV detector, and a Rheodyne model 7125 injector with a 100-μL loop was used. The stationary phase was a Spherisorb ODS5 column (250 × 4.6 mm). The mobile phase consisted of water, methanol, and acetic acid (71 : 25 : 4), pH 2.5,

and was isocratically delivered at 1.2 mL/min. UV detection was performed at 245 nm.

Standards were prepared by diluting stock solutions of aspirin, salicylic acid, salicyluric acid, and gentisic acid with blank human urine. *o*-Anisic acid at 500 mg/L in acetonitrile was used as an internal standard. An aliquot of 1 mL of urine or standard was mixed with 1 mL of internal standard, diluted to 10 mL with water, mixed well, and assayed. Under these conditions, retention times for gentisic acid, salicyluric acid, *o*-anisic acid, aspirin, and salicylic acid were 5.8, 7.5, 9.8, 11.1, and 20.8 min, respectively.

Calibration curves were constructed over the range from 2.5 to 10 mg/L for aspirin and from 5 to 100 mg/L for salicylic acid, salicyluric acid, and gentisic acid. Correlation coefficients were greater than 0.995 for all compounds. Interassay coefficients of variation were less than 4%. The limit of detection was 1 mg/L.

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ATAZANAVIR SULFATE

CHEMISTRY

Atazanavir sulfate is an HIV-protease inhibitor with antiviral activity against HIV. Its chemical name is dimethyl (3*S*,8*S*,9*S*,12*S*)-9-benzyl-3,12-di-*tert*-butyl-8-hydroxy-4,11-dioxo-6-(*p*-2-pyridylbenzyl)-2,5,6,10,13-pentaazatetradecanedioate sulfate (1 : 1). Other names include BMS-232632 and Reyataz. Its molecular formula is $C_{38}H_{52}N_6O_7{\cdot}H_2SO_4$, with a molecular weight of 802.9 and a CAS number of 229975-97-7.

METHODS

Assay 1 Dickinson et al. [1] described the simultaneous determination of HIV protease inhibitors amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, ritonavir, and saquinavir in human plasma by HPLC-MS/MS. A Surveyor liquid chromatograph consisting of a pump, autosampler, and PDA detector was used. The stationary phase was a HyPURITY C_{18} column (100 × 2.1 mm, 5 μm particle size) protected by a Merck guard column (Si 60, 5 μm particle size). Mobile phase A was a mixture of acetonitrile and 20 mM ammonium formate buffer (50 : 50) and mobile phase B a mixture of acetonitrile and 20 mM ammonium formate buffer (70 : 30). The mobile phase was delivered at 0.4 mL/min in a gradient mode from 100% A to 0% A in 2 min, held at 0% A for 4.5 min, increased to 100% A in 0.5 min, and held at 100% A for 3 min. The injection volume was 10 μL.

A ThermoElectron ion-trap LCQ Deca XP Plus mass spectrometer equipped with an electrospray ionization source was operated in the positive-ion mode. Drugs were detected over a 9-min period, which was divided into four separate segments. The operating conditions were summarized as follows:

	Segment 1	**Segment 2**	**Segment 3**	**Segment 4**
Time (min)	0.00–1.86	1.86–4.09	4.09–5.80	5.80–9.00
Capillary temperature (°C)	360	360	360	360
Sheath gas (N_2)	50	65	50	60
Auxiliary gas (He)	15	10	15	10
Source voltage (kV)	4.5	5	4.5	5
Capillary voltage (V)	27	3	36	31
Source current (μA)	80	80	80	80

Drugs and internal standard were quantified in the multiple-reaction monitoring (MRM) mode at ion transisitions m/z 614.40 → 465.20 for indinavir, 506.20 → 245.00 for amprenavir, 705.40 → 335.10 for atazanavir, 721.40 → 267.90 for ritonavir, 671.40 → 433.20 for saquinavir, 629.40 → 447.10 for lopinavir, 568.30 → 331.10 for nelfinavir, and 674.40 → 388.20 for the internal standard.

Stock solutions of drugs at 1 mg/mL were individually prepared in methanol. Standards were prepared by diluting these stock solutions with drug-free plasma. A stock solution of Ro31-9564 at 100 μg/mL was prepared in methanol. The working solution of Ro31-9564 at 1 μg/mL was prepared by diluting the stock solution with methanol–water (50 : 50, vol/vol) and was used as an internal standard. An aliquot of 100 μL of plasma, standard, or control was spiked with 20 μL of internal standard, mixed with 1 mL of acetonitrile, vortexed, left to stand at room temperature for 15 min, vortexed again, and centrifuged at 4°C at 1780 *g* for 10 min. The organic phase was collected, mixed with 300 μL of 20 mM ammonium formate, and assayed. Under these conditions, retention times for indinavir, amprenavir, atazanavir, ritonavir, saquinavir, lopinavir, nelfinavir, and internal standard were 1.10, 1.38, 1.97, 2.32, 2.63, 2.74, 5.03, and 6.14 min, respectively.

Calibration curves for atazanavir were constructed over the range from 47 to 6239 ng/mL. Correlation coefficients were greater than 0.995. The mean accuracy expressed as the relative percentage error was less than 10.1%. Intraday and interday coefficients of variation were less than 6% and 8%, respectively. Limits of detection and quantification were 4.6 and 47 ng/mL, respectively.

Assay 2 Cateau et al. [2] reported the analysis of atazanavir in human plasma using solid-phase extraction and high-performance liquid chromatography. A liquid chromatograph consisting of a Merck model L6000 pump, a Waters model 717 Plus autosampler, model 2487 UV detector, and a Kromasystem 2000 integrator was used. The stationary phase was a Varian Kromasil C_{18} column (150 × 3 mm, 5 μm particle size) protected with a ChromSep C_{18}

guard column (10 × 2 mm). The mobile phase consisted of acetonitrile and water (38 : 62, vol/vol) and was isocratically delivered at 1.0 mL/min. UV detection was performed at 210 nm. The injection volume was 50 μL.

Stock solutions of atazanavir at 400 μg/mL and prazepam (internal standard) at 100 μg/mL were individually prepared in methanol. Working solutions were prepared by diluting stock solutions with methanol. Standards were prepared by fortifying 1 mL of drug-free human plasma with 100 μL of working solutions. An aliquot of plasma or standard was mixed with 50 μL of internal standard and 100 μL of 5 N acetic acid; vortexed for 30 s; loaded onto a Waters Oasis MCX solid-phase extraction cartridge (1 mL) that was preconditioned with 1 mL of methanol followed by 1 mL of water; washed with 1 mL of a mixture of ammonium hydroxide, methanol, and water (5 : 5 : 90, vol/vol/vol); eluted with 2 × 500 μL of ammonium-methanol (5 : 95, vol/vol); evaporated to dryness under a stream of nitrogen; reconstituted with 100 μL of mobile phase; and assayed. Under these conditions, the retention times for atazanavir and prazepam were 18.5 and 22.0 min, respectively.

Calibration curves were constructed over the range from 0.156 to 10 μg/mL. Correlation coefficients were greater than 0.999. The mean accuracy ranged from 96.2% to 100.4%. Intraday and interday coefficients of variation were less than 8% and 11%, respectively. Limits of detection and quantification were 0.078 and 0.156 μg/mL, respectively. The following drugs did not interfere with the analysis of atazanavir: abacavir, amphotericin B, amprenavir, bromazepam, diazepam, didanosine, efavirenz, ethambutol, fluconazole, flucytosine, fluoxetine, fluvoxamine, indinavir, isoniazid, itraconazole, lamivudine, lopinavir, methadone, nelfinavir metabolite (M8), nelfinavir, nevirapine, nordazepam, oxazepam, pyrazinamide, rifampin, ritonavir, saquinavir, sulfamethoxazole, vancomycin, and zidovudine.

Assay 3 Crommentuyn et al. [3] described simultaneous analysis of atazanavir and tipranavir in human plasma using LC-MS/MS. An Agilent 1100 series system consisting of a pump and a cooled-well-plate autosampler was used. The stationary phase was a Chrompack Inertsil ODS3 column (50 × 2.0 mm, 5 μm particle size) protected by a Chrompack minibore reversed-phase precolumn (10 × 2.0 mm) and a Upchurch Scientific inline filter (0.5 μm). Solvent A was a mixture of 10 mM ammonium acetate buffer (pH 5.0) and methanol (65 : 35, vol/vol), and solvent B was methanol. The mobile phase was delivered at 100% A from 0 to 0.2 min, 15% A from 0.2 to 1.6 min, and at 100% A again from 1.7 to 3.5 min. The flow rate was 0.5 mL/min. The injection volume was 10 μL.

A SCIEX API3000 triple quadrupole mass spectrometer equipped with the electrospray ionization interface was operated in the positive-ion mode. The following operating conditions were set: electrospray voltage 4 kV, nebulizer gas 1.8 mL/min, turbo gas 7.01 mL/min, curtain gas 1.31 mL/min, and collision gas 240×10^{12} molecules/cm^2. Compounds were monitored in the multiple-reaction mode (MRM) at ion transitions m/z 705 → 168 for atazanavir, 603 → 411 for tipranavir, and 676 → 575 for saquinavir-d_5.

Stock solutions of atazanavir at 2 mg/mL and tipranavir at 1 mg/mL were individually prepared in methanol. Working solutions were prepared by diluting stock solutions with methanol. Saquinavir-d_5 at 400 μg/mL was prepared in methanol, diluted to a nominal concentration of 1.5 μg/mL in methanol–acetonitrile (1 : 1, vol/vol), and used as an internal standard. Standards were prepared by diluting working solutions with drug-free human plasma. An aliquot of 100 μL (50 μL for tipranavir) of plasma or standard was mixed with 200 μL (600 μL for tipranavir) of internal standard solution, vortexed for 30 s, and centrifuged at 23,100 *g* for 15 min. An aliquot of 150 μL of the supernatant was transferred to a 96-well plate, diluted with 150 μL of 50 mM ammonium acetate buffer (pH 5), shaken for 20 min, and assayed. Under these conditions, retention times for atazanavir, tipranavir, and saquinavir-d_5 were 3.3, 3.5, and 3.4 min, respectively.

Calibration curves for atazanavir were constructed over the range from 0.05 to 10 μg/mL. Correlation coefficients were greater than 0.997. The accuracy expressed as the relative percentage error was 7.3%. The mean recovery of atazanavir from plasma ranged from 83.9 to 93.6%. Intraday and interday coefficients of variation were less than 2.5% and 3.8%, respectively. The following drugs did not interfere with the analysis of atazanavir: abacavir, caffeine, co-trimoxazole, didanosine, efavirenz, enfuvirtide, fluconazole, folinic acid, ganciclovir, itraconazole, lamivudine, methadone, nevirapine, oxazepam, paracetamol, pyrazinamide, pyrimethamine, ranitidine, rifampin, stavudine, tenofovir, zalcitabine, zidovudine, zidovudine–glucuronide, amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, and nelfinavir metabolite M8.

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ATENOLOL

CHEMISTRY

Atenolol is a β_1-selective adrenergic blocking agent. Its chemical name is 4-[2-hydroxy-3-[(1-methylethyl)amino] propoxy]benzeneacetamide. Other names include Antipressan, Atenil, Atenix, ICI-66082, and Tenormin. Its

molecular formula is $C_{14}H_{22}N_2O_3$, with a molecular weight of 266.3 and a CAS number of 29122-68-7. Atenolol is a white crystalline powder. Atenolol has a solubility of 26.5 mg/mL in water at 37°C. It is freely soluble in methanol, soluble in acetic acid and dimethylsulfoxide, sparingly soluble in 96% ethanol, slightly soluble in isopropanol, and very slightly soluble in acetone and dioxane. It is practically insoluble in acetonitrile, ethyl acetate, and chloroform. Atenolol has a pK_a of 9.6.

METHODS

Assay 1 Li et al. [1] developed an LC-MS/MS method for simultaneous determination of ten antiarrhythmic drugs—diltiazem, amiodarone, mexiletine, propranolol, sotalol, verapamil, bisoprolol, metoprolol, atenolol, and carvedilol—in human plasma. A Shimadzu LC20AD liquid chromatographic system equipped with two pumps, a vacuum degasser, and an autosampler was employed. The stationary phase was a Capcell Pak C_{18} column (50 × 2.0 mm, 5 μm particle size). Solvent A was 0.02% formic acid in acetonitrile and solvent B, 0.02% formic acid in water. The mobile phase was delivered in a gradient mode from 95% B to 50% B in the first 3.5 min, then from 50% B to 5% B in next 0.5 min, and returned to 95% B in 0.5 min followed by 3-min equilibration. The flow rate was 0.3 mL/min. The temperature of autosamper was maintained at 4°C.

An ABI-SCIEX API3000 triple quadrupole tandem mass spectrometer with TurboIonspray source was coupled to the liquid chromatograph as a detector. It was operated in positive ionization mode. The ionspray voltage was set at 2.5 kV, source temperature at 450°C, collision-activated dissociation at 12, and the collision gas nitrogen. The declustering potential was 28 V and collision energy 36 V. Analytes were monitored in reaction-monitoring (MRM) mode: m/z 267.6 → 145.2, 279.2 → 124.2, 281.2 → 156.2, and 311.2 → 156.2 for atenolol, sulfisomedine, sulfamethoxydiazine, and sulfadimethoxine, respectively.

Sulfamethoxydiazine, sulfadimethoxine, and sulfisomedine as internal standards 200 ng/mL were prepared in acetonitrile. Stock solutions of drugs were prepared in methanol. Working solutions were prepared by diluting stock solutions in methanol/water (1 : 1) and stored at −20°C. Standards were prepared by spiking blank human plasma with working solutions. Plasma samples, standards, and controls (100 μL each) were mixed with 200 μL of internal standard, vortexed for 10 s, and centrifuged at 16,000 *g* for 3 min. An aliquot (50 μL) of the supernatant was mixed with 150 μL of water and assayed. The injection volume was 5 μL. Under these conditions, retention times of atenolol, sulfisomedine, sulfamethoxydiazine, and sulfadimethoxine were 2.1, 2.8, 3.9, and 4.8 min, respectively.

A calibration curve for atenolol was constructed in the range 50–10,000 ng/mL. The correlation coefficient was 0.9973. The accuracy ranged from 87.5% to 110.5%. The recovery from plasma ranged from 87.0% to 96.5%. Intraday and interday coefficients of variation were less than 9.8% and 6.9%, respectively.

Assay 2 Leite et al. [2] developed an HPLC method for the quantification of atenolol in plasma with fluorescence detection. A Shimadzu system consisted of a model LC10AD VP pump, model RF10AXL fluorescence detector, and a model CR6A integrator. The stationary phase was a Shimadzu Shim Pak C_{18} column (150 × 4.6 mm, 4 μm) with a Waters Nova-Pak C_{18} precolumn (4 × 4 mm). The mobile phase consisted of 0.05 N phosphate buffer (pH 5.5) and methanol (80 : 20, v/v). The isocratic flow rate was 0.7 mL/min. Atenolol was monitored at an excitation wavelength of 235 nm and an emission wavelength of 290 nm.

Sotalol (1.25 μg/mL) was used as the internal standard. The plasma sample (200 μL) was mixed with 100 μL of the internal standard, precipitated with 400 μL of acetonitrile, homogenized in a shaker for 15 s, and centrifuged at 5°C and at 6000 rpm for 30 min. The supernatant (200 μL) was collected, evaporated at 37°C under a stream of nitrogen, reconstituted with 200 μL of water/acetonitrile (50 : 50, v/v), and assayed. The injection volume was 20 μL. Under these conditions, retention times of sotalol and atenolol were 10.4 and 12.7 min, respectively.

Standard curves for atenolol were linear in the range of 8–2000 ng/mL. Correlation coefficients were better than 0.998. Intraday and interday coefficients of variation were less than 8.93% and 14.06%, respectively. Intraday and interday accuracies in relative percentage error were less than 0.93% and 1.78%, respectively. The limit of detection was 4 ng/mL, and the limit of quantification was 8 ng/mL. This assay was free of interference from endogenous substances.

Assay 3 Khuroo et al. [3] reported the simultaneous determination of atenolol and chlorthalidone in human plasma by LC-MS/MS. The Perkin-Elmer LC200 series liquid chromatograph was used. The stationary phase was a Waters XTerra C_{18} column (150 × 4.6 mm, 5 μm particle size). The mobile phase consisted of acetonitrile and 10 mM ammonium acetate buffer (pH 7.5) (92 : 8, vol/vol) and was isocratically delivered at 1.5 mL/min. The flow rate of the mobile phase into the mass spectrometer was 0.5 mL/min through a split.

The Applied Biosystems SCIEX API3000 mass spectrometer was operated using TurboIonSpray in both positive and negative modes. It ran in the negative-ion mode for 3.0 min to monitor chlorthalidone and then switched over to the positive-ion mode from 3.1 to 5.5 min to monitor atenolol. The optimal parameters were as follows: ionsource voltage 2500 V, curtain gas (nitrogen) 12, nebulizer gas (nitrogen) 10, and ion source temperature 475°C. Analytes were monitored in the multiple-reaction mode (MRM): m/z 267.1 → 145.2, 337.5 → 190.1, 268.2 → 159.1, and 295.8 → 269.0 for atenolol, chlorthalidone, metoprolol (internal standard for atenolol), and hydrochlorothiazide (internal standard for chlorthalidone), respectively.

Stock solutions of atenolol, chlorthalidone, metoprolol, and hydrochlorothiazide were separately prepared in methanol. A working solution of metoprolol (4.0 μg/mL) and hydrochlorothiazide (0.5 μg/mL) was freshly prepared by diluting the stock solution in methanol/water (50 : 50, vol/vol). Standards and controls were prepared by spiking

blank human plasma with working solutions and stored at −20°C. An aliquot of 300 μL of a plasma sample, standard, or control was mixed with 50 μL of the internal standard and 300 μL of carbonate buffer, which was prepared by dissolving 2.5 g of sodium carbonate and 8.5 g of sodium bicarbonate in 500 mL of water, vortexed for 30 s, loaded onto a solid-phase extraction (SPE) cartridge that was preconditioned using 1 mL of methanol followed by 1 mL of water, washed with 2% methanolic water twice, and eluted with 1 mL of 5% ammoniated acetonitrile. The eluent was evaporated to dryness at 50°C under nitrogen, reconstituted in 300 μL of mobile phase, and assayed. The injection volume was 10 μL. Under these conditions, retention times of chlorthalidone, hydrochlorothiazide, atenolol, and metoprolol were 2.15, 2.10, 3.50, and 4.00 min, respectively.

Calibration curves for atenolol were constructed in the concentration range from 10 to 2050 ng/mL. Correlation coefficients were greater than 0.9800. Intraassay and interassay coefficients of variation for atenolol were 9.4% and 7.1%, respectively. The mean recoveries of atenolol and metoprolol from plasma were 78.8% and 71.5%, respectively. The limit of quantification was 10 ng/mL. The analysis of atenolol was not affected by the biological matrix, metabolites, degradation products, or coadministered drugs.

Assay 4 Vieno et al. [4] developed an LC-MS/MS method for the detection of acebutolol, atenolol, metoprolol, sotalol, carbamazepine, ciprofloxacin, ofloxacin, and norfloxacin in drinking water, surface water, and sewage treatment plant water. An Agilent 1100 series system consisting of a binary pump, vacuum degasser, autosampler, and a thermostated column oven was used. The stationary phase was an Agilent Zorbax XDB C_{18} column (50 × 2.1 mm, 5 μm particle size) protected by an Agilent narrowbore guard column (12.5 × 2.1 mm, 5 μm particle size). The column temperature was maintained at 30°C. The mobile phase consisted of acetonitrile and 1% acetic acid in water and was delivered at 250 μL/min in a gradient mode from 3% to 28% acetonitrile in 12 min and to 53% acetonitrile in another 5 min, maintained at 53% acetonitrile for 1 min, and then returned to the initial condition in 1 min. The column was equilibrated at 3% acetonitrile for 8 min.

A Micromass Quattro Micro triple quadrupole mass spectrometer equipped with an electrospray ionization interface was operated in positive mode: desolvation gas 640 L/h, nebulizing gas 30 L/h, collision gas (argon) 2.8×10^{-3} mbar, source temperature 120°C, and desolvation temperature 325°C. Cone voltage (V) and collision energy (eV) were 28 and 20 for acebutolol, 30 and 23 for atenololol, 25 and 15 for metoprolol, 30 and 23 for sotalol, 25 and 15 for alprenolol (internal standard), 29 and 18 for carbamazepine, 35 and 21 for dihydrocarbamazepine (internal standard), 30 and 17 for ciprofloxacin, 28 and 16 for norfloxacin, 29 and 18 for ofloxacin, and 28 and 18 for enrofloxacin (internal standard). Quantification was performed in multiple-reaction monitoring (MRM) mode using ion transitions at m/z 336.8 → 116.0 for acebutolol, m/z 267.0 → 144.9 for atenolol, m/z 267.9 → 190.9 for metoprolol, m/z 254.8 → 132.9 for sotalol, m/z 249.9 → 172.9 for alprenolol, m/z 237.0 → 193.9 for carbamazepine, m/z 239.0 → 193.9 for dihydrocarbamazepine, m/z 331.9 → 287.9 for ciprofloxacin, m/z 319.8 → 275.9 for norfloxacin, m/z 361.8 → 317.9 for ofloxacin, and m/z 359.9 → 315.9 for enrofloxacin, respectively.

Stock solutions of drugs were prepared in methanol, except that antibiotics were prepared in a mixture of methanol and 0.01 M hydrochloric acid (1 : 1, vol/vol) and stored at −18°C. Working solutions were prepared daily by diluting stock solutions with the same solvents. Standards were prepared by spiking noncontaminated groundwater with working solutions and internal standards. A sample (100, 250, 500, and 1000 mL for sewage influent, sewage effluent, surface water, and groundwater, respectively) was adjusted to pH 10.0 with 2 M sodium hydroxide solution, spiked with 500 ng of the internal standards, and filtered through a 0.45-μm Schleicher & Schuell GF6 filter that was previously washed with *n*-hexane, acetone, methanol, and water. It was then loaded onto a Waters Oasis HLB SPE cartridge (3 mL, 60 mg) by means of PTFE tubes at flow rates of 2, 5, 10, and 20 mL/min (sewage influent water, sewage effluent water, surface water, and groundwater, respectively), which was preconditioned sequentially with 2 mL of *n*-hexane, 2 mL of acetone, 10 mL of methanol, and 10 mL of noncontaminated groundwater (pH adjusted to 10.0); pulled through the cartridge; washed with 2 mL of 5% methanol in 2% aqueous ammonium hydroxide; dried with a stream of nitrogen for 30 min; eluted with 4 × 1 mL of methanol; evaporated to near dryness under a stream of nitrogen; reconstituted with 20 μL of methanol and 480 μL of 1% acetic acid; and assayed. Under these conditions, retention times of sotalol, atenolol, norfloxacin, ofloxacin, ciprofloxacin, enrofloxacin, acebutolol, metoprolol, alprenolol, carbamazepine, and dihydrocarbamazepine were 3.3, 4.4, 9.8, 9.8, 10.2, 10.9, 11.1, 11.2, 15.4, 17.6, and 17.8 min, respectively.

Calibration curves for atenolol were constructed in the range from 10.6 to 6000 μg/L. Correlation coefficients were greater than 0.999. Average recoveries of atenolol from groundwater and tapwater, surface water, sewage effluent water, and sewage influent water were 81%, 90%, 101%, and 108%, respectively. Limits of quantification in drinking water, surface water, sewage effluent water, and sewage influent water were 6.5, 11.8, 21, and 49 ng/L, respectively.

This LC-MS/MS method was successfully applied for the determination of atenolol in sewage influent water, sewage effluent water, and their recipient rivers.

Assay 5 Delamoye et al. [5] developed an HPLC method for simultaneous determination of 13 ß-blockers and one metabolite, atenolol, sotalol, diacetolol, carteolol, nadolol, pindolol, acebutolol, metoprolol, celiprolol, oxprenolol, labetalol, propranolol, tertatolol, and betaxolol. A Spectra liquid chromatographic system consisting of a model P1000XR quaternary gradient pump, model AS3000 autoinjector with a 100-μL loop, and model 6000LP photodiode-array detector was used. The stationary phase was a ThermoHypersil Hypurity C_{18} column (250 × 4.6 mm, 5 μm particle size) protected by a C_{18} precolumn (4 × 4.4 mm, 5 μm particle size). The column temperature was maintained at 35°C. UV detection was performed at 220 nm. The injection volume was 80 μL.

Stock solutions of these compounds at 1.0 g/L each were prepared in methanol. Working solutions were prepared by diluting stock solutions with methanol. A stock solution of medroxalol at 50.0 mg/L in methanol was used as an internal standard. These stock and working solutions were stored at – 20°C. An aliquot of 1 mL of plasma, standard, or control was spiked with 20 μL of the internal standard, mixed with 500 μL of 1 M sodium carbonate (pH 9.7), extracted with 7 mL of chloroform–pentanol–diethyl ether (6 : 2 : 1, vol/vol/vol), shaken for 15 min, and centrifuged at 3000 *g* for 5 min. The organic phase was collected, mixed with 250 μL of 0.05 M phosphoric acid (pH 2.1), shaken for 10 min, and centrifuged at 3000 *g* for 5 min. The aqueous phase was collected and assayed. Under these conditions, retention times for atenolol, sotalol, diacetolol, carteolol, nadolol, pindolol, acebutolol, metoprolol, celiprolol, oxprenolol, medroxalol, labetalol, propranolol, tertatolol, and betaxolol were 5.1, 5.6, 7.9, 9.2, 9.9, 10.5, 14.5, 15.4, 18.8, 20.4, 21.2, 21.8, 24.6, 25.1, and 25.9 min, respectively.

Calibration curves for atenolol were constructed over the range from 25 to 1000 ng/mL. The mean correlation coefficient was 0.997. The mean accuracy was 102.1% at 100 ng/mL. The mean recovery ranged from 100% to 123%. Intraday and interday coefficients of variation were 3.11% and 4.70%, respectively. Limits of detection and quantification were 6 and 25 ng/mL, respectively.

Assay 6 Lee et al. [6] developed an LC-MS/MS method for the simultaneous determination of 12 ß-blockers and $ß_2$-agonists in sewage samples. A Waters 2695 separation module was used. The stationary phase was an Agilent Zorbax SB C_8 column (150 × 2.1 mm, 3.5 μm particle size) protected by a SB C_8 guard column (12.5 × 2.1 mm, 5 μm particle size). The column temperature was maintained at 35°C. Mobile phase A was a mixture of water, acetonitrile, and formic acid (94.5 : 5.0 : 0.5, vol/vol/vol), and mobile phase B was a mixture of acetonitrile and formic acid (99.5 : 0.5, vol/vol). The mobile phase was delivered in a gradient mode from 100% A to 75% A in 13 min, held at 75% A for 13 min, and then pumped at 100% A for another 14 min. The flow rate was 0.2 mL/min. The injection volume was 10 μL. The total runtime of an injection was 40 min.

A Micromass Quattro Ultima triple quadrupole mass spectrometer equipped with an electrospray ionization source was operated in the positive-ion mode. The major parameters were set as follows: nebulizer gas (nitrogen) 50 L/h, desolvation gas (nitrogen) 500 L/h, source temperature 120°C, desolvation temperature 350°C, capillary voltage 3.45 kV, cone energy 50 V, and collision energy 25 kV. Atenolol was analyzed in the multiple-reaction monitoring (MRM) mode at ion transitions m/z 267 → 145 for quantitation and m/z 267 → 190 for confirmation.

Individual stock solutions of acebutolol, alprenolol, atenolol, bisoprolol, clenbuterol, fenoterol, labetalol, metoprolol, nadolol, pindolol, propranolol, sotalol, terbutaline, and timolol at 1000 μg/mL were prepared in acetonitrile or methanol. A stock solution of salbutamol was prepared at 500 μg/mL. These stock solutions were stored at −20°C. Working solutions were prepared by mixing and diluting these stock solutions with mobile phase B. An aliquot of 250 mL of sewage sample was filtered through a 1.2-μm GF/C filter (90 mm i.d.) with a layer of Celite; acidified to pH 3 with 1 M hydrochloric acid; loaded onto a Waters Oasis MCX cartridge (6 mL, 150 mg, 30 μm) at a flow rate of 10–15 mL/min that was preconditioned with 6 mL of methanol followed by 10 mL of water at pH 3; dried for 10 min under vacuum; washed with 100 mL of water at pH 3 followed by 6 mL of methanol; eluted with 8 mL of a mixture of dichloromethane, 2-propanol, and ammonium hydroxide (78 : 20 : 2, vol/vol/vol); evaporated to dryness at 40°C under a stream of nitrogen; reconstituted in 1.0 mL of mobile phase B; filtered through a 0.45-μm nylon syringe filter, and assayed. Under these conditions, retention times for acebutolol, alprenolol, atenolol, bisoprolol, labetalol, metoprolol, nadolol, pindolol, propranolol, sotalol, timolol, clenbuterol, fenoterol, salbutamol, and terbutaline were 17.36, 24.32, 8.96, 21.77, 22.11, 18.02, 13.60, 14.15, 23.87, 7.85, 17.02, 17.91, 11.50, 6.97, and 6.53 min, respectively.

Calibration curves for atenolol were constructed over the range from 50 to 500 pg/μL. Mean recovery of the drug from water ranged from 90% to 96%. The limit of detection was 6 ng/L.

Assay 7 Peng et al. [7] reported the determination of atenolol in human plasma by gas chromatography. An HP5890 gas chromatograph equipped with a Hewlett-Packard ^{63}Ni electron-capture detector and an HP3396A integrator was used. The column was an HP column (1.12 m × 0.2 mm × 0.33 μm). The carrier gas was helium. The major parameters were set as follows: injector temperature 200°C, detector temperature 300°C, and column temperature programmed from 140°C to 190°C by 10°C/min. The injection volume was 2 μL.

Standards were prepared by spiking blank human plasma with stock solutions of atenolol. Metoprolol was used as an internal standard. An aliquot of 1 mL of plasma or standard was mixed with 400 ng of metoprolol and 0.1 mL of 0.5 M sodium hydroxide solution, extracted with 5 mL of *n*-butanol and cyclohexane (7 : 3) by shaking for 3 min, and centrifuged. After the second extraction, the combined organic mixture was mixed further with 4 mL of 0.2 M hydrochloric acid and shaken for 3 min. After the second extraction and centrifugation, the aqueous phase was separated, mixed with 0.2 mL of 5 M sodium hydroxide solution, and extracted with 6 mL of diethyl ether twice. The organic phase was evaporated to dryness under nitrogen, reconstituted with 25 μL of ethyl acetate, and assayed. Under these conditions, retention times for atenolol and metoprolol were 10.4 and 8.7 min, respectively.

Calibration curves for atenolol were constructed over the range from 50 to 200 ng/mL. Correlation coefficients were 0.9984. The mean recovery of atenolol from plasma ranged from 93.5% to 98.0%. Within-day and between-day coefficients of variation were 2.38% and 4.04%, respectively. The limit of detection was 5 ng/mL. There was no interference from endogenous substances from plasma.

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AZATHIOPRINE

CHEMISTRY

Azathioprine is an immunosuppressant. Its chemical name is 6-(1-methyl-4-nitroimidazol-5-ylthio)purine. Other names include Azaprin, Azasan, Azathioprin, BW-57322, Imuran, and NSC-39084. Its molecular formula is $C_9H_7N_7O_2S$, with a molecular weight of 277.3 and a CAS number of 446-86-6. Azathioprine is a pale yellow, odorless powder. Azathioprine is insoluble in water, very slightly soluble in ethanol and chloroform, and sparingly soluble in dilute mineral acids. It is soluble in dilute solutions of alkali hydroxides.

METHOD

Assay 1 Yuan et al. [1] reported the simultaneous determination of azathioprine and 6-mercaptopurine in human plasma by HPLC. A Waters LC system consisting of model 510 pumps, model 490E UV detector, model U6K injector, and model P5200 integrator was used. The stationary phase was a Spherisorb C_{18} stainless-steel column (10 μm particle size). The mobile phase consisted of methanol, water, and diethylamine (200 : 800 : 10, vol/vol/vol) containing 1 g/L sodium acetate, adjusted to pH 8.5 with glacial acetic acid. The flow rate was 0.6 mL/min. UV detection was performed at 313 nm and 0.05 AUFS.

A stock solution of azathioprine at 500 μg/mL was prepared in 0.05 M sodium hydroxide solution. A stock solution of 6-mercaptopurine at 100 μg/mL was prepared by dissolving 10 mg of the reference standard in 5 mL of water and 0.2 mL of 1 M sodium hydroxide solution and filling to 100-mL mark with sodium acetate buffer (pH 6). Standards were prepared by spiking blank human plasma with stock solutions. An aliquot of 0.5 mL of plasma or standard was spiked with 15 μL of metronidazole (internal standard) at 40 μg/mL in water, mixed with 0.4 mL of 10% trichloroacetic acid, vortexed, and centrifuged at 4000 rpm for 10 min. The supernatant was separated and assayed. Under these conditions, retention times for 6-mercaptopurine, metronidazole, and azathioprine were about 5.6, 7.0, and 12 min, respectively (estimated from the published chromatogram).

Calibration curves for azathioprine were constructed over the range from 0.1 to 10 μg/mL. Correlation coefficients were 0.9995. The average recovery of azathiprine from plasma was 96.6%. Intraday and interday coefficients of variation were less than 4.1% and 8.6%, respectively. Cyclosporine A, prednisone, methylprednisolone, and 4-oxadocosane-1,2-diol did not interfere with this method.

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AZITHROMYCIN

CHEMISTRY

Azithromycin is an antibacterial. Its chemical name is 9-deoxo-9a-aza-9a-homoerythromycin A dihyrate. Other names include CP-62993, Zithromax, Zitromax, and Zmax. Its molecular formula is $C_{38}H_{72}N_2O_{12}{\cdot}2H_2O$, with a molecular weight of 785.0 and a CAS number of 117772-70-0. Azithromycin is a white or almost white powder. Azithromycin is practically insoluble in water. It is freely soluble in dehydrated alcohol and in dichloromethane.

METHODS

Assay 1 Yuzuak et al. [1] developed an LC-MS/MS method for the analysis of azithromycin in human plasma. A Waters 2695 separation module was used. The stationary phase was a SunFire C_{18} column (50 × 2.1 mm, 3.5 μm particle size). The column temperature was maintained at 30°C. The mobile phase consisted of water, acetonitrile, and methanol (25 : 57 : 18, vol/vol/vol) containing 1.54 g of ammonium acetate and 0.6 mL of glacial acetic acid per liter. The flow rate was 0.2 mL/min. The injection volume was 10 μL.

A Micromass Quattro mass spectrometer equipped with an electrospray ionization interface was operated in the positive-ion mode. The operating conditions were as follows: source block temperature 120°C, desolvation temperature 350°C, desolvation gas flow 450 L/h, cone gas flow 50 L/h, collision energy 43, and gas pressure (argon) 3.8×10^{-3} mbar. Azithromycin and roxithromycin were monitored in the multiple-reaction monitoring (MRM) mode at

ion transitions m/z 749.58 → 591.6 and 837.64 → 158.2, respectively.

Stock solutions of azithromycin and roxithromycin at 1 mg/mL were individually prepared in methanol. Working solutions were prepared by diluting the stock solution with methanol. A solution of roxithromycin at 5 μg/mL in methanol was used as an internal standard. Standards and controls were prepared by diluting working solutions with drug-free human plasma. An aliquot of 0.2 mL of plasm, standard, or control was spiked with 50 μL of the internal standard, mixed with 250 μL of 0.25 M sodium bicarbonate buffer (pH 9.5), vortexed for 10 s, extracted with 3 mL of diethyl ether and dichloromethane (70 : 30, vol/vol), vortexed for 30 s, centrifuged at 4600 rpm at 4°C for 5 min, and stored at −70°C for 10 min. The organic phase was collected, evaporated to dryness at 40°C under a stream of nitrogen, reconstituted in 200 μL of mobile phase, and assayed. Under these conditions, retention times for azithromycin and roxithromycin were 0.9 and 1.1 min, respectively.

Calibration curves were constructed over the range from 2 to 1000 ng/mL. Correlation coefficients ranged from 0.9894 to 0.9937. The recovery of azithromycin from plasma was 82%. Within-batch and between-batch coefficients of variation were less than 18.8% and 14.1%, respectively. The limit of quantification was 2 ng/mL. There was no interference with this method from endogenous substances in plasma.

Assay 2 Ghone et al. [2] described the determination of azithromycin in human plasma by HPLC with electrochemical detection. A liquid chromatograph consisting of Jasco PU980 pumps, Rheodyne 7125 injector with a 100-μL loop, SP4270 integrator, and an Amor Spark amperometric detector was used. The stationary phase was a Waters μBondapak C_{18} column (300 × 3.6 mm, 10 μm particle size). The mobile phase consisted of 100 mM ammonium acetate, acetonitrile, and methanol (30 : 47 : 23, vol/vol/vol), adjusted to pH 6.5 with acetic acid. The flow rate was 1.0 mL/min. The applied cell potential of the electrode was set at +0.7 V. The injection volume was 100 μL.

Stock solutions of azithromycin and erythromycin at 1 mg/mL were individually prepared in methanol and stored at −20°C. Erythromycin at 50 μg/mL in methanol was used as an internal standard. Standards were prepared by spiking blank human plasma with stock solutions. An aliquot of 1 mL of plasma or standard was spiked with 0.1 mL of the internal standard, alkalinized using phosphate buffer (pH 8.5), extracted with 5 mL of dichloromethane by shaking for 30 min, and centrifuged at 2000 rpm for 10 min. The organic layer was collected, evaporated to dryness under a stream of nitrogen, reconstituted with 200 μL of mobile phase, and assayed. Under these conditions, retention times for erythromycin and azithromycin were 5.8 and 7.5 min, respectively.

Calibration curves were constructed over the range from 0.1 to 3 μg/mL. Correlation coefficients were 0.998. The accuracy ranged from 97.2% to 103.6%. The mean recovery of azithromycin from plasma was 74.9%. Intraday and interday coefficients of variation were less than 4.3% and 5.2%, respectively. The limit of detection was 20 ng/mL.

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