1. PROTOCOL FOR SOLID PHASE EXTRACTIONS OF ACID/BASIC DRUGS IN BLOOD

PURPOSE

The Toxicology section is routinely asked to analyze evidence for the presence of other basic and acidic drugs that are not included in the preliminary screen. This protocol outlines the procedure to follow when performing this type of analysis for the qualitative screening and confirmation of drugs in blood, serum or plasma.

PLAN

A. Equipment:

(1) GC/MS with a 5% diphenylpolysiloxane, 95% dimethylpolysiloxane, (or 50% diphenylpolysiloxane, 50% dimethylpolysiloxane) 15/30 meter, 0.25 micron film thickness column

(2) Positive Pressure Manifold

(3) SPE Column – Silica gel - Co polymeric bonded phase with a hydrophobic cation exchange (CSDAU203)

(4) Sample concentrator with UHP Nitrogen

(5) Centrifuge

(6) Vortex mixer / Multi-tube vortex mixer

B. Reagents:

(1) **100 mM Phosphate buffer solution**. Dissolve 1.70 grams of Na_2HPO_4 and 12.14 g $NaH_2PO_4 \cdot H_2O$ in 800 mL of deionized water. Dilute to 1000 mL with deionized water. Mix well. pH should be 5.5-6.0. If necessary, adjust with 100 mM monobasic sodium phosphate (lowers pH) or 100 mM dibasic sodium phosphate (raises pH). Store refrigerated. Stable for six months.

(2) **Deionized Water** (DI Water) Label. Stable until consumed.

(3) **Methanol.** Prepare a transfer bottle of ACS/HPLC grade methanol. Label accordingly. Store in glass at room temperature. Stable until consumed.

(4) **100 mM Hydrochloric Acid (HCL)**. To 400 mL of deionized water, add 8.4 mL concentrated HCl. Dilute to 1L with deionized water. Mix well. Stable for 2 years.

(5) **78:20:2 methylene chloride: isopropanol: ammonium hydroxide Elution Solvent**. Prepare fresh daily. Add ammonium hydroxide to isopropanol, followed by methylene chloride (i.e. per 10 mL of elution solvent add approximately 200 µL ammonium hydroxide). Mix thoroughly, elution solvent should have a turbid appearance when thoroughly mixed.

(6) **2% glacial acetic acid in methanol**. To 100 mL of methanol add 2.0 mL glacial acetic acid. Store in glass at room temperature. Stable for 2 years.

(7) **Ethyl Acetate**. Prepare a transfer bottle of ACS/HPLC grade ethyl acetate. Label accordingly. Store in glass at room temperature. Stable until consumed.

(8) **Hexane**. Prepare a transfer bottle of ACS/HPLC grade hexane. Label accordingly. Store in glass at room temperature. Stable until consumed.

(9) **Hexane/Ethyl acetate (50/50).** Prepare fresh daily.

C. Internal Standards:

(1) **Hexobarbital/Prazepam Internal Standard Solution (10 ng/µL)**. Prepare by diluting 250 µL of a 1 mg/mL Hexobarbital (Cerilliant H-013) stock standard and 250 µL of a 1 mg/mL Prazepam (Cerilliant P-906) stock standard with methanol in a 25 mL volumetric flask. Store refrigerated in glass. Stable for 2 years.

D. Quality Controls: (Store Refrigerated)

(1) **Positive Control Stock Standard**. Prepare 10 mL of a Stock Standard containing: 10 ng/µL each of methamphetamine, bupropion, meperidine, methadone, amitriptyline, nortriptyline, oxycodone and trazodone; 4.0 ng/µL zolpidem; 0.4 ng/µL fentanyl; 50 ng/µL each of butalbital, meprobamate, carisoprodol, metaxalone, phenytoin; and 500 ng/µL gabapentin; dilute to volume with methanol. Stable for 2 years.

(2) **Positive Control (*Screening*)**. Prepare on day of use by adding 20 µL of the positive control stock standard above to 1 mLof negative blood.

(3) **Positive Controls (*Confirmation*)**. Prepare on day of use with applicable drugs at concentrations of 25, 100 and 500 ng/mL for basic drugs; and 250, 1000 and 5000 ng/mL for acidic drugs into 1 mL of negative blood. (Concentrations may vary depending on the drug, ex: gabapentin at 2,500, 5,000 and 10,000 ng/mL)

(4) **Negative Control**. Blank blood prepared in house consisting of 50% saline, 50% packed red blood cells, and 5 g sodium fluoride/1 g potassium oxalate (per 500 mL prepared blood) will be used as negative control.

E. Solid Phase Extraction (SPE)

(1) ***Sample Preparation***
Prepare in appropriately labeled culture tubes as follows:

(a) 1 mL* of negative control, positive control(s), case samples. Add 50 µL* of Hexobarbital/Prazepam internal standard.

*In case samples where a limited amount of sample is received use the same fraction of internal standard as the sample, as an example ½ mL of blood and 25 µL of internal standard.

(b) Add 1.5 mL DI water and vortex until thoroughly mixed.

(c) Add 1 mL of 100 mM phosphate buffer and vortex until thoroughly mixed.

(d) Centrifuge for 5 minutes at 3500 rpm.

(2) ***Column Conditioning***
Pass through the column sequentially the following reagents at <1.0 mL/min, or gravity only:

(a) 2 mL of methanol

(b) 2 mL of deionized water

(c) 1 mL of 100 mM phosphate buffer

Take care to prevent sorbent from drying out.

(3) ***Sample Application***
Apply sample to column, being careful to not allow the sediment, if present, which will be in the base of the centrifuge tube to pass. Flow rate should be 1-2 mL/minute.

(4) ***Column Rinse***
Pass through the column sequentially the following reagents, at 1-2 mL/min:

(a) 3 mL of deionized water

(b) 1 mL of 100mM HCl

(c) Dry the column for 15 minutes under full pressure.

(d) 1 mL of hexane

(5) ***Elute Acidic and Neutral drugs***

(a) Elute into autosampler vial with approximately 1.5 mL of 50:50 hexane/ethyl acetate under gravity or <1.0 mL/minute. Check no water is present, decant eluate into appropriately labeled autosampler vial if needed. *NOTE:* elute to waste for samples where acid/neutral drugs are not being confirmed

(b) Evaporate to dryness under nitrogen

(c) Reconstitute with 100 µL of ethyl acetate, vortex (transfer to vial inserts if using screw cap vials) and cap

(6) ***Wash Column***

(a) 2 mL of methanol at 1-2 mL/minute

(b) Dry column under full pressure for (≥15 inches Hg) for 10 minutes

(7) ***Elute Basic Drugs***

(a) Elute into autosampler vial by gravity using 1.5 mL of 78:20:2 methylene chloride: isopropanol: ammonium hydroxide elution solvent.

(b) Add 25 µL of 2% glacial acetic acid to eluate

(c) Evaporate to dryness under nitrogen

(d) Reconstitute with 100 µL of ethyl acetate, vortex (transfer to vial inserts if using screw cap vials) and cap

F. Data Acquisition and Analysis:

(1) Perform Autotune, fill rinse vials, etc.

(2) Set up a sequence with the negative and positive control(s); for a screening batch place controls at the beginning, for a confirmation batch controls can be placed throughout the sequence. Subsequent injections to include solvent blanks prior to case samples. Load samples onto autosampler according to sequence and have it verified by another analyst before or after analysis but prior to unloading.

(3) Analyze using the appropriate method on GC/MS.

G. Results and acceptability:

(1) ***Screening*** In order for the analysis to qualify as a preliminary screen for the presence of a drug, the following criteria should be met:

(a) The sample exhibits a published base peak and at least two prominent secondary ions that are consistent with the mass spectrum of a drug in an approved library (AAFS, Cayman Spectral Library, NIST08, PCLTOX, SWGDRUG).

(b) The drug is absent in the negative quality control.

(c) Acceptable performance of the positive control will be the identification of at least 8 drugs in the base, 4 drugs in the acid.

(2) ***Confirmation*** A drug previously identified by a preliminary screen may be reported qualitatively provided the following criteria are met:

(a) The mass spectrum of the sample exhibits a published base peak and at least two prominent secondary ions that are consistent with the corresponding known standard of that drug in the positive control.

(b) The abundance of the drug in the sample is greater than or equal to the abundance of the corresponding drug in the lowest acceptable positive control.

(c) The retention time, or relative retention time (drug/internal standard) of the drug in the sample is within $\pm$ 5% of the corresponding drug in the positive quality control sample (or in exceptional circumstances a positive unextracted quality control sample can be used for the above comparisons to a known standard).

(d) The drug is absent in the negative quality control sample (<10% abundance of lowest acceptable positive control) and the solvent blank prior to the sample.