1. PROTOCOL FOR THE ANALYSIS OF DRUGS IN URINE BY A GENERAL L/L EXTRACTION METHOD

PURPOSE

This protocol outlines the procedure to be used for the qualitative screening and confirmation of drugs in urine using a general liquid/liquid (L/L) extraction method.

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) Centrifuge

(3) Top load balance

(4) Sample Concentrator with UHP Nitrogen

(5) Rocker

B. Reagents:

(1) **Saturated carbonate/bicarbonate buffer**. To 1 L of H_2O add 70 g $NaHCO_3$. Stir until dissolved. Then add 50 g Na_2CO_3. Stir until dissolved. Label reagent and check pH~9.5. Stable until consumed.

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

(3) **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.

(4) **Hexane: methylene chloride: isopropanol (7:2:1).** Store in glass at room temperature. Stable until consumed.

(5) **Sodium Chloride**. Store at room temperature. Stable until consumed.

C. Internal Standard:

(1) **Prazepam Internal Standard Solution (25 ng/µl).** Prepare by diluting 250 µl of a 1 mg/ml Prazepam (Cerilliant P-906) solution with methanol in a 10 ml volumetric flask. Store refrigerated in glass. (May also prepare larger stock volume by adjusting volumes accordingly to account for an equivalent concentration.) 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 100 µl of the positive control stock standard above to 1 ml of negative urine.

(3) **Positive Controls (*Confirmation*)**. Prepare on day of use with applicable drugs at concentrations of 200 ng/ml, 500 ng/ml and 2000 ng/ml for basic drugs; and 500 ng/ml, 1000 ng/ml and 5000 ng/ml for acidic drugs into 1 ml of negative urine. (Concentrations may vary depending on the drug, ex: gabapentin at 2,500, 5,000 and 10,000 ng/ml)

(4) **Negative Control**. Urine produced in house will be used as negative control.

E. Liquid/Liquid Extraction:

(1) Weigh out 0.5 g (± 0.02 g) Sodium Chloride to appropriately labeled screw top tubes.

(2) Add to each tube

(a) 1 ml urine

(b) 50 µl prazepam internal standard

(c) 1 ml saturated carbonate/bicarbonate buffer

(d) 2 ml 7:2:1 hexane: methylene chloride: isopropanol

(3) Cap tubes. Place tubes on rocker for 10 minutes or vortex for 15 seconds.

(4) Centrifuge for 5 minutes.

(5) Transfer top organic layer to autosampler vial, additionally add 50 µl of 2% glacial acetic acid.

(6) Evaporate to dryness under nitrogen in sample concentrator.

(7) Reconstitute with 100 µl ethyl acetate. Cap and vortex.

F. Data Acquisition and Analysis:

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

(2) Set up a sequence with the negative and positive control(s) at the beginning. Subsequent injections to include solvent blanks between case samples.

(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.

(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 basic drugs and 4 acidic drugs.

(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 ± 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).