1. PROTOCOL FOR THE ANALYSIS OF DRUGS IN BLOOD AND URINE BY LIQUID/LIQUID EXTRACTION

PURPOSE

The following method describes the preliminary screening and confirmation of drugs which lack sufficient recovery by other extraction methods. Examples of drugs include valproic acid, gabapentin, and modafinil in blood, serum, plasma and urine.

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) Sample concentrator with UHP Nitrogen

(4) Vortex mixer / Multi-tube vortex mixer

B. Reagents:

(1) **Saturated ammonium chloride solution**. To 250 ml of water add 50 grams of ammonium chloride. Stir until dissolved. Label reagent. Store at room temperature. Stable for two years.

(2) **3N HCl**. To 250ml of water add 65ml of concentrated hydrochloric acid. Label reagent. Store at room temperature. Stable for two years.

(3) **Ethyl acetate.** Prepare a 100 ml transfer bottle of ACS/HPLC grade ethyl acetate. Label accordingly. Store in glass at room temperature. Stable until consumed.

C. Internal Standard (Store refrigerated in glass. Stable for 2 years.)

(1) **Hexobarbital/Prazepam Internal Standard Solution (10/25 ng/µl)**. Prepare by diluting 250 µl of 1 mg/ml Hexobarbital (Cerilliant H-013) stock standard and 625 µl of a 1 mg/mL Prazepam (Ceriliant 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 *(Screening)***. Prepare on day of use with applicable drugs at a concentration of 1000 ng/ml into 1mL of negative urine or blood.

(2) **Positive Control (*Confirmation*)**. Prepare on day of use with applicable drugs at concentrations of 500 ng/ml, 1000 ng/ml and 5000 ng/ml into 1 mL of negative urine or blood. (Concentrations may vary depending on the drug.)

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

E. Extraction:

(1) Prepare positive control(s) and pipette 100 µl of the negative control as well as case samples into respectively labeled 16x100mm culture tubes.

(2) Add 50 µl of internal standard.

(3) Add 500 µl of saturated ammonium chloride solution.

(4) Add 20 µl of 3N HCl.

(5) Add 2 ml of ethyl acetate.

(6) Vortex until thoroughly mixed; centrifuge for five minutes at 3500 rpm. After tubes have been centrifuged, transfer top organic layer into appropriately labeled auto sampler vials.

(7) Evaporate to near dryness (approximately 10 µl). Reconstitute residue with 70 µl of ethyl acetate, vortex (transfer to vial insert if using screw cap autosampler vials) and cap.

F. Data Acquisition and Analysis:

(1) Make sure the Autotune was performed, rinse vials filled, etc.

(2) Set up a sequence to include positive and negative controls and 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.

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

(c) Acceptable performance of the positive control will be the identification of applicable 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) and blank prior to sample.