Section Three
Blood Toxicology

3.3 Screening of Blood for Commonly Encountered Drugs
3.3.2 Extraction of Strongly Basic Drug Compounds

3.3.2.1 BACKGROUND

This method outlines a preliminary screen of whole blood specimens for a variety of commonly encountered strongly basic drugs. The extract can be analyzed with a gas chromatograph equipped with a nitrogen-phosphorus detector (GC-NPD) and/or a mass selective detector (GC-MSD). The GC-NPD provides a presumptive identification of drug compounds in blood based upon their relative retention times whereas the GC-MSD will provide a qualitative identification. The resulting data is utilized to base the selection of the confirmatory analysis method.

3.3.2.2 SCOPE

Drug compounds are extracted from blood by a liquid-liquid extraction process. Positive controls are spiked for a resulting concentration of 200ng/mL or 500ng/mL of drugs of interest. The blood aliquot is made basic with a pH 12 borate buffer and extracted with n-butyl chloride followed by a back extraction. If necessary, the extract may be washed with hexane. After evaporation and reconstitution, the extract is subjected to analysis by dual column GC-NPD and/or GC-MSD. Two internal standards are used to monitor extraction efficiency and chromatographic performance. A limitation of this method is that it does not detect a variety of compounds such as morphine, hydromorphone, carboxy-THC or the cocaine metabolite benzoylecgonine, due to pKa/pH considerations, a lack of nitrogen and/or chromatographic problems. These analytes can by screened for by enzyme immunoassay (refer to analytical method section one).

3.3.2.3 EQUIPMENT AND SUPPLIES

3.3.2.3.1 Tube rocker
3.3.2.3.2 Vortex mixer
3.3.2.3.3 Evaporative concentrator equipped with nitrogen tank.
3.3.2.3.4 Laboratory centrifuge capable of 3400rpm.
3.3.2.3.5 16x100mm Screw-top round bottom tubes
3.3.2.3.6 Screw cap for 16mm O.D. tubes
3.3.2.3.7 Automated Liquid Sampler (ALS) vials
3.3.2.3.8 GC/MS vial microinsert
3.3.2.3.9 GC equipped with Dual NPDs
3.3.2.3.10 GC equipped with a MSD
3.3.2.3.11 Non-polar Capillary Column (GC-NPD and GC-MSD) 100%-Dimethylsiloxane or a 5%-Diphenyl-95%-Dimethyl-siloxane copolymer.

3.3.2.3.12 Mid-Polar Capillary Column (GC-NPD)
50% Phenyl, 50% methyl-polysiloxane copolymer.

3.3.2.4 REAGENTS

Refer to Manual section 5.12 for solution preparation instructions.

3.3.2.4.1 Methanol (Certified ACS Grade)
3.3.2.4.2 Hexane (Certified ACS Grade)
3.3.2.4.3 n-Butyl chloride (Certified ACS Grade)
3.3.2.4.4 0.1N Sulfuric Acid
3.3.2.4.5 2N Sodium Hydroxide
3.3.2.4.6 Borate Buffer (pH 12)

3.3.2.5 REFERENCE MATERIAL

3.3.2.5.1 Positive Control

Positive Control can be prepared with the working solution described below and/or obtained commercially.

3.3.2.5.1.1 **Positive Control Stock Solution**

Obtain 1mg/mL stock drug standard solutions through Cerilliant, Alltech, Sigma or other appropriate vendor.

3.3.2.5.1.2 **Positive Control Working Solution**

Add the designated volume of stock solution to 10mL methanol.

Solution is stable for 6-months when stored at room temperature or 12-months when stored under refrigeration. Re-make solution when deterioration is noted.

Stock Solution (1.0µg/µL)	**Volume (µL)**
Amitriptyline	20
Caffeine	20
Codeine	20
Diphenhydramine	20
Lidocaine	20
Meperidine	20
Methadone	20
Nicotine	20
PCP	20
Trazodone	50
Zolpidem	20

Internal Standard Mix

3.3.2.5.2.1 **Stock Solutions**
1mg/mL Proadifen
1mg/mL Mepivacaine

3.3.2.5.2.2 **Working Internal Standard Solution [20ng/µL]**
Add 200µL Proadifen and Mepivacaine stock solutions to 10mL volumetric ball flask. QS with DI water.
Solution is stable for one month when stored at 4℃.

3.3.2.6 PROCEDURE

3.3.2.6.1 Initial set-up
Label two sets of extraction tubes, ALS vials with microinserts for controls and case samples.

3.3.2.6.2 Sample Preparation
Use the same lot of negative blood used to prepare the negative control to prepare positive controls.

3.3.2.6.2.1 Transfer 2mL casework, negative control and positive control samples to screw top extraction tube.

3.3.2.6.2.2 Add 200µL of internal standard mixture. Vortex.

3.3.2.6.2.3 Allow sample to stand 10 minutes.

3.3.2.6.2.4 Add 2mL borate buffer (pH 12). Vortex.

3.3.2.6.2.5 Pipet 6mL n-butyl chloride into each tube, cap.

3.3.2.6.2.6 Place tube on rocker for 10 minutes.

3.3.2.6.2.7 Centrifuge for 10 minutes at 3400rpm.

3.3.2.6.2.8 Transfer the butyl chloride (top) layer to clean tube.

3.3.2.6.3 Back Extraction

3.3.2.6.3.1 Pipet 3.0mL 0.1N sulfuric acid, cap.

3.3.2.6.3.2 Place tube on rocker for 5 minutes.

3.3.2.6.3.3 Centrifuge for 5 minutes at 3400rpm.

3.3.2.6.3.4 Discard butyl chloride (top) layer.

3.3.2.6.4 Optional Hexane Wash for Dirty/Fatty samples)

3.3.2.6.4.1 Pipet 5.0mL hexane into each tube, cap.

3.3.2.6.4.2 Place tube on rocker for 5 minutes.

3.3.2.6.4.3 Centrifuge for 5 minutes at 3400rpm.

3.3.2.6.4.4 Discard the hexane (top) layer.

3.3.2.6.5 Final Extraction

3.3.2.6.5.1 Add 500µL 2N NaOH.

3.3.2.6.5.2 Add 3mL n-butyl chloride, cap.

3.3.2.6.5.3 Place tube on rocker for 10 minutes.

3.3.2.6.5.4 Centrifuge for 10 minutes @ ≅3200rpm.

3.3.2.6.5.5 Transfer the butyl chloride (top) layer into tapered bottom centrifuge tube.

3.3.2.6.5.6 Add 50 µL 1% HCl in methanol.

3.3.2.6.6 Evaporation and reconstitution

3.3.2.6.6.1 Evaporate under a gentle stream of nitrogen at ≅37ºC.

3.3.2.6.6.2 Add 100uL butyl chloride to the residue, vortex.

3.3.2.6.6.3 Transfer extract to labeled ALS vial with microinsert.

3.3.2.6.7 Preparation for Analysis Run

3.3.2.6.7.1 Into Sequence log table, enter the sample case numbers, blanks and controls.

3.3.2.6.7.2 Load samples, standards, blank and controls into the quadrant rack as noted in the sequence table.

3.3.2.6.8 Analysis Parameters

3.3.2.6.8.1 Inject 2µL sample extract into GC-MSD or GC-NPD.

3.3.2.6.8.2 Refer to instrument METHOD printouts for analysis parameters.

3.3.2.6.8.3 Analysis method printouts must be stored centrally.

3.3.2.6.9 Detection and Identification Criteria

3.3.2.6.9.1 **GC-NPD**

The presence of a particular drug compound may be indicated if the relative retention time (RRT) for the sample versus applicable standard does not differ by more than ±0.2 minutes.

3.3.2.6.9.2 **GC-MSD**

Retention Time

If the drug of interest is included in the mixed drug standards, the presence of a drug compound is indicated if the retention time for the sample versus applicable standard does not differ by more than ±0.2 minutes.

Mass Spectrum

Due to the preliminary nature of this analysis, the presence of a drug compound is indicated if the MS data shows no significant differences in the unknown mass spectral data versus known data.

3.3.2.7 QUALITY ASSURANCE REQUIREMENTS

3.3.2.7.1 General

3.3.2.7.1.1 Blood samples are to be stored under refrigeration after aliquots are removed for analysis.

3.3.2.7.1.2 Refer to toxicology manual section 5.2 for balance calibration and intermediate check requirements.

3.3.2.7.1.3 Refer to toxicology manual section 5.8 for additional GC-MSD quality assurance

requirements.

3.3.2.7.1.4 Refer to toxicology manual section 5.10 for reference material authentication requirements.

3.3.2.8 ANALYSIS DOCUMENTATION

3.3.2.8.1 A packet containing original data for controls will be prepared for each analysis run and stored centrally in the laboratory where the analysis was performed until archiving.

3.3.2.8.2 A copy of controls need not be included in individual case files. When necessary, a copy of control printouts can be prepared from the centrally stored document.

3.3.2.9 REFERENCES

3.3.2.9.1 Strong Bases Extractions - Screening SOP, Courtesy of Dr. Graham Jones, Office of the Chief Medical Examiner, Edmonton, Canada, 2003.

3.3.2.9.2 Jones, G., *Postmortem Toxicology*. pp. 98-102, **in**: Clarke's Analysis of Drugs and Poisons, 3rd Edition, Moffat, A.C, Osselton, M.D. and Widdop, B., eds., Pharmaceutical Press, 2004.

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Revision No.	Issue Date	History
0	11-21-2006	Method obtained from Edmonton Medical Examiners Office. Method verification for GC-MSD only.
1	07-28-2008	Clarified that negative blood used to prepare positive control is the same lot as used for negative control.