Section Three
Blood Toxicology

3.3 Screening of Blood for Commonly Encountered Drugs
3.3.1 Extraction of Basic and Neutral Drug Compounds

3.3.1.1 BACKGROUND

This method outlines a non-selective screen of whole blood specimens for a variety of commonly encountered basic and neutral drugs. Due to the non-selective nature of the method, endogenous compounds and contaminants may co-extract which can be removed with the optional back extraction. The extract is analyzed with a gas chromatograph equipped with a nitrogen-phosphorus detector (GC-NPD) and/or a mass selective detector (GC-MSD). The GC-NPD may provide a presumptive identification of drug compounds in blood based upon their relative retention times and the GC-MSD data may provide an initial qualitative identification. The resulting data is utilized as a basis for selection of the analytical method used for confirmatory analysis.

3.3.1.2 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 sample is made basic with a pH 9.2 buffer and extracted with n-butyl chloride. An optional back extraction procedure removes most frequently encountered interfering substances. Following evaporation, the reconstituted extract is subjected to analysis by dual column GC-NPD and/or full scan GC-MSD. Two internal standards are used to monitor extraction efficiency and chromatographic performance. A limitation of this method is that it does not provide a screen for 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 manual section one). An advantage of this method is that it is a reliable and relatively simple protocol.

3.3.1.3 EQUIPMENT AND SUPPLIES

3.3.1.3.1 Tube rocker
3.3.1.3.2 Vortex mixer
3.3.1.3.3 Evaporative concentrator equipped with nitrogen tank.
3.3.1.3.4 Laboratory centrifuge capable of 3400rpm.
3.3.1.3.5 16 x 100mm round bottle screw-top tubes
3.3.1.3.6 Screw cap for 16mm O.D. tubes
3.3.1.3.7 Automated Liquid Sampler (ALS) vials
3.3.1.3.8 Microinsert for GC/MS vial
3.3.1.3.9 Gas Chromatograph equipped with Dual Nitrogen Phosphorus Detectors

3.3.1.3.10 Gas Chromatograph equipped with a Mass Selective Detector

3.3.1.3.11 Non-polar Capillary Column (GC-NPD and GC-MSD)
100%-Dimethylsiloxane or a 5%-Diphenyl-95%-Dimethyl-siloxane copolymer, 12.5 to 30M.

3.3.1.3.12 Mid-Polar Capillary Column (GC-NPD)
50% Phenyl, 50% methyl-polysiloxane copolymer, 12.5 to 30M.

3.3.1.4 REAGENTS

Refer to Manual section 5.12 for solution preparation instructions.

3.3.1.4.1 Methanol (Certified ACS Grade)
3.3.1.4.2 n-Butyl chloride (Certified ACS Grade)
3.3.1.4.3 Borate Buffer, pH 9.2
3.3.1.4.4 1% Hydrochloric Acid in Methanol
3.3.1.4.5 0.1N Sulfuric Acid
3.3.1.4.6 2N Sodium Hydroxide

3.3.1.5 REFERENCE MATERIAL

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

3.3.1.5.1.1 **Positive Control Stock Solution**
Obtain 1mg/mL (1µg/µL) stock drug standard solutions through Cerilliant, Alltech, Sigma or other appropriate vendor.

3.3.1.5.1.2 **Positive Control Working Solution (2ng/µL or 5ng/µL)**
Add the designated volume of stock solution to 10mL methanol. Select a minimum of four of the following compounds. Additional compounds may be added to mix provided that they do not co-elute with selected compounds. 20µL of additional compounds should be added unless insufficient response is noted. The amount added should be optimized for each compound.

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

3.3.1.5.2 Internal Standard Mix

3.3.1.5.2.1 **Stock Solutions**
1mg/mL Proadifen
1mg/mL Prazepam

3.3.1.5.2.2 **Working Internal Standard Solution**
Add 200µL Proadifen and 20µL Prazepam stock solutions to 10mL volumetric ball flask. QS with DI water.

Solution is stable for three months when stored at room temperature or 6-months when stored under refrigeration.

3.3.1.5.3 Negative Control
Negative Whole Blood

3.3.1.6 PROCEDURE

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

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

3.3.1.6.2.1 Prepare two positive control samples by adding 100µL mixed working control solution to two 1mL samples of negative whole blood or pipette two samples of commercially obtained whole blood positive control.

3.3.1.6.2.2 When the optional back extraction will be employed for only selected samples, prepare 2 additional positive controls to parallel the back extraction process.

3.3.1.6.2.3 Transfer 1mL casework and negative control samples to screw top extraction tube.

3.3.1.6.2.4 Add 100µL of internal standard mixture. Vortex.

3.3.1.6.2.5 Allow sample to stand 10 minutes.

3.3.1.6.2.6 Add 1mL borate buffer (pH 9.2). Vortex.

3.3.1.6.2.7 Pipet 3mL n-butyl chloride into each tube, cap.

3.3.1.6.2.8 Place tube on rocker for 10 minutes.

3.3.1.6.2.9 Centrifuge for 10 minutes at 3400rpm.

3.3.1.6.2.10 Transfer the n-butyl chloride layer to second tube.

3.3.1.6.2.11 If no clean-up steps are being performed proceed to 3.3.1.6.5.

3.3.1.6.3 Optional Back Extraction

3.3.1.6.3.1 Pipet 3.0mL 0.1N sulfuric acid, cap.

3.3.1.6.3.2 Place tube on rocker for 5 minutes.

3.3.1.6.3.3 Centrifuge for 5 minutes at 3400rpm.

3.3.1.6.3.4 Discard butyl chloride (top) layer.

3.3.1.6.4 Optional Hexane Wash for Dirty/Fatty samples)

3.3.1.6.4.1 Pipet 5.0mL hexane into each tube, cap.

3.3.1.6.4.2 Place tube on rocker for 5 minutes.

3.3.1.6.4.3 Centrifuge for 5 minutes at 3400rpm.

3.3.1.6.4.4 Discard the hexane (top) layer.

3.3.1.6.5 Final Extraction

3.3.1.6.5.1 Add 500µL 2N NaOH.

3.3.1.6.5.2 Add 3mL n-butyl chloride, cap.

3.3.1.6.5.3 Place tube on rocker for 10 minutes.

3.3.1.6.5.4 Centrifuge for 10 minutes at 3400rpm.

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

3.3.1.6.6 Preparation for Evaporation

3.3.1.6.6.1 Add 50µL 1% HCl in methanol.

3.3.1.6.6.2 Evaporate under a gentle stream of nitrogen at ≅40°C.

3.3.1.6.7 Reconstitution

3.3.1.6.7.1 Add 100uL n-butyl chloride to the residue, vortex.

3.3.1.6.7.2 Transfer extract to labeled ALS vial with microinsert.

3.3.1.6.8 Preparation for Analysis Run

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

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

3.3.1.6.9 Analysis Parameters

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

3.3.1.6.9.2 Refer to instrument METHOD printout for additional analysis parameters.

3.3.1.6.9.3 Current analysis method must be stored centrally as a hard or electronic copy.

3.3.1.6.10 Detection and Identification Criteria

3.3.1.6.10.1 **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.

3.3.1.6.10.2 **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.

3.3.1.7 QUALITY ASSURANCE REQUIREMENTS

3.3.1.7.1 General

3.3.1.7.1.1 Blood samples are to be stored under refrigeration before and after aliquots are removed for analysis.

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

3.3.1.7.1.3 Refer to toxicology manual section 5.8 for additional GC-MSD quality assurance requirements.

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

3.3.1.8 ANALYSIS DOCUMENTATION

3.3.1.8.1 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.

3.3.1.8.2 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.

3.3.1.9 REFERENCES

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

3.3.1.9.2 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.

Revision History

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Revision No.	Issue Date	History/Comments
0	11-21-2006	Based on method obtained from Edmonton Medical Examiners Office. Method verification for GC-MSD only.
1	07-28008	Clarified that negative blood used to prepare positive control is the same lot as used for negative control.