Section Three
Blood Toxicology

3.3 Screening of Blood for Commonly Encountered Drugs
3.3.3 Extraction of Acidic and Neutral Drug Compounds

3.3.3.1 BACKGROUND

This analytical method outlines a non-selective screen of whole blood specimens for commonly encountered acidic and neutral drugs. These include a wide variety of pharmaceuticals. The extract can be analyzed with a gas chromatograph equipped with a nitrogen-phosphorus detector (GC-NPD) or a mass selective detector (GC-MSD). The GC-MSD provides a presumptive identification of drug compounds in blood based on retention time and mass spectral data. The resulting data is utilized to base the selection of the confirmatory analysis method.

3.3.3.2 SCOPE

Drug compounds are extracted from blood by a liquid-liquid extraction process. Blood pH is first adjusted with saturated ammonium chloride followed by extraction with ethyl acetate. After evaporation and a hexane wash, the final extract is subjected to analysis by GC-MSD. Two internal standards are used to monitor extraction efficiency and chromatographic performance.

3.3.3.3 EQUIPMENT AND SUPPLIES

3.3.3.3.1 Tube rocker
3.3.3.3.2 Vortex mixer
3.3.3.3.3 Evaporative concentrator equipped with nitrogen tank.
3.3.3.3.4 Laboratory centrifuge capable of 3400rpm.
3.3.3.3.5 Fixed and adjustable volume single channel air displacement pipetters, and appropriate tips, capable of accurate and precise dispensing of volumes indicated.
3.3.3.3.6 16 x 100mm screw-top centrifuge tubes
3.3.3.3.7 Screw Cap for 16mm O.D. tubes
3.3.3.3.8 GC/MS Automated Liquid Sample (ALS) vials
3.3.3.3.9 GC/MS Vial Microinsert
3.3.3.3.10 Gas Chromatograph equipped with a Mass Selective Detector
3.3.3.3.11 100%-Dimethylsiloxane or a 5%-Diphenyl-95%-Dimethyl-siloxane copolymer, 12.5 to 30M.

3.3.3.4 REAGENTS

Refer to Manual section 5.12 for solution preparation instructions.

3.3.3.4.1 Methanol (Certified ACS grade)
3.3.3.4.2 Hexane (Certified ACS grade)

3.3.3.4.3 Ethyl acetate (Certified ACS grade)
3.3.3.4.4 Acetonitrile (Certified ACS grade)
3.3.3.4.5 2N Sodium Hydroxide
3.3.3.4.6 Saturated Ammonium Chloride

3.3.3.5 REFERENCE MATERIAL

3.3.3.5.1 Positive Control

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

3.3.3.5.1.1 **Positive Control Stock Solution**

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

3.3.3.5.1.2 **Positive Control Working Solution**

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

Stock Solution	Volume (µL)
Acetaminophen	20
Butalbital	20
Carbamazepine	20
Carisoprodol	20
Meprobamate	20
Phenobarbital	20
Secobarbital	20

Solution is stable for 6-months when stored at room temperature.

3.3.3.5.2 Internal Standard Mix

3.3.3.5.2.1 **Stock Solutions**

1mg/mL Proadifen
1mg/mL Aprobarbital

3.3.3.5.2.2 **Working Internal Standard Solution [50ng/µL]**

Add 500µL Proadifen and 500µL Aprobarbital stock solutions to 10mL volumetric ball flask. QS with methanol.

Solution is stable for 3 months when stored at room temperature.

3.3.3.5.3 Negative Control
Negative Whole Blood

3.3.3.6 PROCEDURE

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

3.3.3.6.2 Sample Preparation

3.3.3.6.2.1 Prepare two 1mL positive control samples. Use the same lot of negative blood as used to prepare the negative control.

Option 1: Add 100µL mixed working control solution to 1mL negative whole blood.

Option 2: Pipette commercially obtained whole blood positive control.

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

3.3.3.6.2.3 Add 200µL of internal standard mixture. Vortex.

3.3.3.6.2.4 Add 1mL saturated ammonium chloride. Vortex.

3.3.3.6.3 Extraction

3.3.3.6.3.1 Pipet 4mL ethyl acetate into each tube, cap.

3.3.3.6.3.2 Place tube on rocker for 10 minutes.

3.3.3.6.3.3 Centrifuge for 10 minutes at 3400rpm.

3.3.3.6.3.4 Transfer the ethyl acetate (top) layer to second tube.

3.3.3.6.3.5 If necessary, this is potential overnight stopping point. Tubes must be capped and refrigerated.

3.3.3.6.4 Evaporation
Evaporate to dryness under a gentle stream of nitrogen at ≅37ºC.

3.3.3.6.5 Hexane Wash

3.3.3.6.5.1 Pipet 500µL hexane into each tube. Vortex.

3.3.3.6.5.2 Place tube on rocker for 5 minutes.

3.3.3.6.5.3 Pipet 50µL Acetonitrile. Vortex.

3.3.3.6.5.4 Centrifuge for 5 minutes at 3400rpm

3.3.3.6.5.5 Discard the hexane (top) layer.

3.3.3.6.5.6 Transfer acetonitrile extract to labeled ALS vial with microinsert.

3.3.3.6.6 Preparation for Analysis Run

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

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

3.3.3.6.7 Analysis Parameters

3.3.3.6.7.1 Refer to instrument METHOD printouts for analysis parameters.

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

3.3.3.6.8 Detection and Identification Criteria

3.3.3.6.8.1 **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.

3.3.3.6.8.2 **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.

3.3.3.7 QUALITY ASSURANCE REQUIREMENTS

3.3.3.7.1 General

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

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

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

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

3.3.3.8 ANALYSIS DOCUMENTATION

3.3.3.8.1 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.

3.3.3.8.2 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.

3.3.3.9 REFERENCES

3.3.3.9.1 Procedure for Acid/Neutral Drug Analysis, Courtesy of Jim Hutchison, Montana Department of Justice, Forensic Services Division, 2005.

3.3.3.9.2 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	Procedure obtained from Montana Department of Justice, Forensic Science Division.
1	07-28-2008	Clarified that negative blood used to prepare positive control is the same lot as used for negative control.