Section Three
Blood Toxicology

3.6 Qualitative Liquid-Liquid Extraction Methods for GC/MSD Confirmation

3.6.7 Liquid-Liquid Extraction Procedure for the Recovery of pKa ≥9 Drug Compounds.

3.6.7.1 BACKGROUND

This method is a general blood extraction procedure for a variety of commonly encountered basic drugs that exhibit a pKa of ≅ ≥9 along with their metabolites. This method prepares an extract for confirmatory analysis with a gas chromatograph equipped with a mass selective detector (GC\MSD). With the addition of appropriate internal standard(s), this same extraction method may be used for quantitative analysis. Refer to analytical method 3.9.2 for requirements.

3.6.7.2 PRINCIPLE

The method is based upon the principle of liquid/liquid extraction. The sample pH is adjusted with a pH 12 saturated borate buffer and extracted with n-butyl chloride. Following an optional back extraction, the extract is evaporated and reconstituted with methanol. Two internal standards are used to monitor extraction efficiency and chromatographic performance. Gas chromatography in conjunction with full scan mass spectrometry is used to confirm the presence of analytes of interest.

3.6.7.3 EQUIPMENT AND SUPPLIES

3.6.7.3.1 Evaporative concentrator equipped with nitrogen tank.
3.6.7.3.2 16 x 100mm round bottom glass screw-top tubes
3.6.7.3.3 Screw Cap for 16mm O.D. tubes
3.6.7.3.4 GC/MS Automated Liquid Sampler (ALS) vials
3.6.7.3.5 GC/MS vial microinsert
3.6.7.3.6 Gas chromatograph equipped with a mass selective detector (HP 6890/5973 or equivalent) and a nonpolar capillary column with a phase composition capable of efficiently separating amines, alkaloids, drugs compounds and other analytes encountered in toxicological specimens (e.g. 100%-dimethylpolysiloxane or 95%-dimethyl-polysiloxane with 5% diphenyl)

3.6.7.4 REAGENTS

Refer to Manual section 5.12 for solution preparation instructions.

3.6.7.4.1 Methanol (Certified ACS Grade)
3.6.7.4.2 n-Butyl chloride (Certified ACS Grade)
3.6.7.4.3 pH 12 Borate Buffer
3.6.7.4.4 100mM HCl
3.6.7.4.5 1% HCl in Methanol

3.6.7.5 QUALITY ASSURANCE MATERIAL

3.6.7.5.1 Positive Control

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

3.6.7.5.1.1 **Positive Control Stock Solution**

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

3.6.7.5.1.2 **Positive Control Working Solution**

Add the designated volume of stock solution to 10mL methanol. A minimum of the following compounds must be used.

Stock Solution (1.0mg/mL)	Volume (µL)
Amitriptyline	20
Caffeine	20
Codeine	20
Diphenhydramine	20
Lidocaine	20
Meperidine	20
Methadone	20
Methamphetamine	20
Nicotine	20
PCP	20
Trazodone	50

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

3.6.7.5.2 Internal Standard

3.6.7.5.2.1 **Stock Solutions**

1 mg/mL Benzphetamine
1mg/mL Papaverine

3.6.7.5.2.2 **Working Internal Standard Solution [10ng/µL]**

Add 100µL Benzphetamine and Papaverine stock solutions to 10mL volumetric ball flask. QS with methanol.

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

3.6.7.5.3 Negative Control
Negative Whole Blood

3.6.7.6 PROCEDURE

3.6.7.6.1 Initial set-up
For each control and case sample. label two sets of extraction tubes and an ALS vial with microinserts,

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

3.6.7.6.2.1 Prepare two positive control samples by adding 200µL mixed working control solution to 2mL negative whole blood (Utak 44600-WB (F) or equivalent) or pipette two samples of commercially obtained whole blood positive control.

3.6.7.6.2.2 When the optional back extraction is used, prepare 2 additional positive controls to parallel the back extraction process.

3.6.7.6.2.3 Transfer 2mL casework and negative control samples to screw top extraction tube.

3.6.7.6.2.4 Add 20µL of internal standard mixture. Vortex.

3.6.7.6.2.5 Allow sample to stand 10 minutes.

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

3.6.7.6.3 Extraction

3.6.7.6.3.1 Pipet 4mL n-butyl chloride into each tube, cap.

3.6.7.6.3.2 Place tube on rocker for a minimum of 10 minutes.

3.6.7.6.3.3 Centrifuge 10 minutes at 3400 rpm.

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

3.6.7.6.3.5 Add 50µL 1% HCl in Methanol.

3.6.7.6.3.6 Evaporate to dryness under a gentle stream of nitrogen at approximately 37ºC.

If no clean-up proceed to 3.6.7.6.5.

3.6.7.6.4 Optional Sample Clean-up

3.6.7.6.4.1 Reconstitute with 50ul of 100mM HCl.

3.6.7.6.4.2 Add 1ml of n-Butyl Chloride. Vortex.

3.6.7.6.4.3 Rock for 5 minutes.

3.6.7.6.4.4 Centrifuge for 5 minutes at 3400 rpm.

3.6.7.6.4.5 Discard upper n-Butyl Chloride layer.

3.6.7.6.4.6 Add 2ml of pH 12 borate solution. Vortex

3.6.7.6.4.7 Add 4 ml of n-Butyl Chloride.

3.6.7.6.4.8 Rock for 5 minutes.

3.6.7.6.4.9 Centrifuge for 5 minutes at 3400 rpm.

3.6.7.6.4.10 Transfer upper n-Butyl Chloride layer into screw-top tube.

3.6.7.6.4.11 Evaporate to dryness under a gentle stream of nitrogen at approximately 37ºC.

3.6.7.6.5 Reconstitution

3.6.7.6.5.1 Add 50uL Methanol to the residue, vortex.

3.6.7.6.5.2 Transfer extract to labeled ALS vial with microinsert.

3.6.7.6.6 Preparation for Analysis Run

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

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

3.6.7.6.7 Analysis Parameters

3.6.7.6.7.1 Refer to instrument METHOD printouts for analysis parameters.

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

3.6.7.6.8 GC-MSD Qualitative Detection and Identification Criteria

3.6.7.6.8.1 For the identification of compounds not included in positive control, analyze appropriate non-extracted reference standards.

3.6.7.6.8.2 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 and there are no significant differences in the mass spectral data.

3.6.7.7 QUALITY ASSURANCE REQUIREMENTS

3.6.7.7.1 General

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

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

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

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

3.6.7.8 ANALYSIS DOCUMENTATION

3.6.7.8.1 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.

3.6.7.8.2 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.

3.6.7.9 REFERENCES

3.6.7.9.1 Procedure for High pKa Drug Analysis, Courtesy of Jim Hutchison, Montana Department of Justice, Forensic Services Division, 2005.

3.6.7.9.2 Procedure for Back Extraction, Courtesy of Jim Hutchison, Montana Department of Justice, Forensic Services Division, 2006.

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

3.6.7.9.4 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.

3.6.7.9.5 Hearn, W.L. and Walls, H.C. Strategies for Postmortem Toxicology Investigation. pp. 937-939. *in*: Drug Abuse Handbook, S.B. Karch, ed., CRC Press, Boca Raton, FL, 1998.

Revision History

Section Three
Blood Toxicology

3.6 Qualitative Liquid-Liquid Extraction Methods for GC/MSD Confirmation

3.6.7 Liquid-Liquid Extraction Procedure for the Recovery of pKa ≥9 Drug Compounds.

Revision #	Issue Date	History/Comments
1	04-25-2002	Original Issue in SOP format
2	05-27-2003	Updated, Clarifications
3	11-21-2006	Addition of internal standard, positive control requirements specified, extraction process restructured.
4	07-28-2008	Clarified that negative blood used to prepare positive control is the same lot as used for negative control.