Idaho State Police
Forensic Services

Approval for Quality System Controlled Documents

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3.6.2-Liquid-Liquid Extraction Procedure for the Recovery of Acidic and Neutral Drugs from Blood

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Idaho State Police
Forensic Services
Toxicology Discipline

Section Three
Blood Toxicology

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

3.6.2 Liquid-Liquid Extraction Procedure for the Recovery of Acidic and Neutral Drugs from Blood

3.6.2.1 BACKGROUND

This method is a general blood liquid-liquid extraction procedure for a variety of commonly encountered acidic and neutral drugs along with their metabolites. This method prepares an extract for qualitative confirmatory analysis with a gas chromatograph equipped with a mass selective detector (GC/MSD). This extraction yields excellent recovery of most acid neutral drugs and can be accomplished in under one hour. The extraction is designed to yield fewer and lower levels of endogenous compounds that can interfere with drug detection.

3.6.2.2 SCOPE

Drug compounds are extracted from blood by a liquid-liquid extraction process. Blood pH is 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.

3.6.2.3 EQUIPMENT AND SUPPLIES

3.6.2.3.1 Tube rocker (Fisher or equivalent)
3.6.2.3.2 Evaporative concentrator equipped with nitrogen tank.
3.6.2.3.3 Vacuum Manifold/pump
3.6.2.3.4 Laboratory centrifuge capable of 3200 - 3400rpm.
3.6.2.3.5 Fixed and adjustable volume single channel air displacement pipetters, and appropriate tips, capable of accurate and precise dispensing of volumes indicated.
3.6.2.3.6 16X100mm round bottom glass screw-top tubes
3.6.2.3.7 Screw Cap for 16mm O.D. tubes
3.6.2.3.8 GC/MS Automated Liquid Sample (ALS) vials
3.6.2.3.9 GC/MS Vial Microinsert
3.6.2.3.10 Gas Chromatograph equipped with a Mass Selective Detector
3.6.2.3.11 5%-Diphenyl-95%-Dimethyl-siloxane copolymer capillary GC column, 12.5 to 30M.

3.6.2.4 REAGENTS

Refer to Manual section 5.12 for solution preparation instructions.

3.6.2.4.1 Methanol (Certified ACS Grade)
3.6.2.4.2 Hexane (Certified ACS Grade)
3.6.2.4.3 Ethyl acetate (Certified ACS Grade)
3.6.2.4.4 Acetonitrile (Certified ACS Grade)
3.6.2.4.5 2N Sodium Hydroxide
3.6.2.4.6 Saturated Ammonium Chloride

3.6.2.5 QUALITY ASSURANCE MATERIAL

3.6.2.5.1 Positive Control Working Solution

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

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

3.6.2.5.2.2 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

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

3.6.2.5.2 Internal Standard Mix

3.6.2.5.2.1 **Stock Solutions**

1mg/mL Proadifen
1mg/mL Aprobarbital

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

Add 500µL Proadifen and 500µL Aprobarbital stock solutions to 10.0mL volumetric ball flask. QS with methanol.

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

3.6.2.5.3 Negative Control
Negative Whole Blood

3.6.2.6 PROCEDURE

3.6.2.6.1 Initial set-up
Label ALS vials, with microinserts, and two sets of extraction tubes.

3.6.2.6.2 Positive Control

3.6.2.6.2.1 Prepare or use commercially obtained positive control. To prepare add 100µL mixed working control solution to 1mL negative whole blood.

3.6.2.6.2.2 Positive control must be run in duplicate.

3.6.2.6.2 Negative Control
Transfer 1mL negative whole blood to screw top extraction tube.

3.6.2.6.3 Casework Samples

3.6.2.6.3.1 Transfer 1mL casework samples to screw top extraction tube.

3.6.2.6.3.2 Add 200µL of internal standard mixture. Vortex.

3.6.2.6.3.3 Add 1mL saturated ammonium chloride. Vortex.

3.6.2.6.4 Extraction

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

3.6.2.6.4.2 Place tube on rocker for 10 minutes.

3.6.2.6.4.3 Centrifuge for 10 minutes at 3200 - 3400rpm.

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

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

3.6.2.6.5 Evaporation

Evaporate to dryness under a gentle stream of nitrogen at approximately 37°C.

3.6.2.6.6 Hexane Wash

3.6.2.6.6.1 Pipet 500µL hexane into each tube. Vortex.

3.6.2.6.6.2 Place tube on rocker for 5 minutes.

3.6.2.6.6.3 Pipet 50µL Acetonitrile. Vortex briefly.

3.6.2.3.6.4 Centrifuge for 5 minutes at 3200 - 3400rpm

3.6.2.3.6.5 Discard the hexane (top) layer.

3.6.2.6.6.6 Transfer acetonitrile extract to labeled ALS vial with microinsert.

3.6.2.6.7 Preparation for GC-MSD Analysis Run

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

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

3.6.2.6.8 Analysis Parameters

3.6.2.6.8.1 Refer to instrument METHOD printouts for analysis parameters.

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

3.6.2.6.9 GC-MSD Qualitative Detection and Identification Criteria

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

3.6.2.6.9.2 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.

3.6.2.7 QUALITY ASSURANCE REQUIREMENTS

3.6.2.7.1 General

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

3.6.2.7.1.2 Refer to toxicology analytical method 5.2 for balance calibration requirements.

3.6.2.7.1.3 Refer to toxicology analytical method 5.3.1 for GC-MSD maintenance guidelines.

3.6.2.7.1.4 Refer to toxicology analytical methods 5.8 and 5.10 for reference standard authentication and additional GC-MSD quality assurance requirements.

3.6.2.8 ANALYSIS DOCUMENTATION

3.6.2.8.1 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.

3.6.2.8.2 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.

3.6.2.9 REFERENCES

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

3.6.2.9.2 Foerster, E.H., Dempsey, J., and Garriott, J.D., *A Gas Chromatography Screening Procedure for Acid and Neutral Drugs in Blood,* J Anal Tox, **3**:87-91, 1979.

3.6.2.9.3 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.

3.6.2.9.4 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.

3.6.2.9.5 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.

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Revision #	Issue Date	History
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.

Approval

Discipline Leader: _____ **Date:** _____
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