Idaho State Police
Forensic Services
Toxicology Section

Section Three
Blood Toxicology

3.6 Liquid-Liquid Extraction Methods for GC/MSD Confirmation

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

3.6.2.1 BACKGROUND

This method is a general blood extraction procedure for a variety of commonly encountered acid drugs of abuse. This method prepares an extract that will be subject to confirmatory analysis by gas chromatography/mass spectrometry (GC\MS).

3.6.2.2 PRINCIPLE

The method is based upon the principle of liquid/liquid extraction. Acidic compounds can be extracted from blood samples under acidic conditions with an organic solvent. The sample is extracted with n-butyl chloride. Following centrifugation, the organic layer is transferred to a new extraction tube and 0.45N sodium hydroxide is added to back extract acidic analytes. The pH is then adjusted to ≤6 with concentrated HCl to convert analytes back to a non-ionic form for a final extraction with n-butyl chloride. The final extract is reconstituted with 1:1 hexane/ethanol for confirmation on the GC/MS using SIM and/or full scan monitoring. The sample is extracted with n-butyl chloride. For sample clean-up, the sample is then back extracted. The final extract is reconstituted with 1:1 hexane/ethanol for confirmation on the GC/MS using SIM and/or full scan monitoring.

3.6.2.3 EQUIPMENT AND SUPPLIES

3.6.2.3.1 Drybath (Fisher or equivalent)

3.6.2.3.2 Evaporative concentrator (Zymark TurboVap or equivalent) equipped with nitrogen tank.

3.6.2.3.3 Glassware

13x100mm Screw top tubes (Fisher 14-959-35C or equivalent)

Screw cap for tubes (Fisher 14-930-15E or equivalent).

16X144mm tapered tip centrifuge tubes (Fisher 05-538-41C or equivalent)

Snap Caps (Fisher 05-538-41N or equivalent)

GC/MS Automated Liquid Sampler (ALS) vials (HP 5182-0865 or equivalent)
GC/MS vial microinsert (HP 5183-2088 or equivalent)

3.6.2.3.4 pH paper (Fisher 09-876-17 or equivalent)

3.6.2.3.5 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)

3.6.2.4 REAGENTS

Refer to Manual section 3.8 for solution preparation

3.6.2.4.1 Methanol (Fisher A412-4 or equivalent)
3.6.2.4.2 Deionized/Distilled (DI) Water
3.6.2.4.3 n-Butyl chloride (Fisher B416-1 or equivalent)
3.6.2.4.4 Concentrated Hydrochloric Acid (Fisher A144-500)
3.6.2.4.5 Hexane (Fisher H292-4 or equivalent)
3.6.2.4.6 Ethanol (Fisher A995-4or equivalent)
3.6.2.4.7 Hexane/Ethanol 1:1
3.6.2.4.8 0.45N Sodium Hydroxide

3.6.2.5 STANDARDS

3.6.2.5.1 Stock Standard Solution

3.6.2.5.1.1 1.0mg/mL Drug standard (obtain as necessary from Cerilliant, Alltech, Sigma or equivalent vendor).

3.6.2.5.2 Working Standard Solution (5000ng/mL)

3.4.5.5.2.1 Add 50µL Stock Solution to 10mL Methanol.
Solution is stable for 12 months when stored at 4°C.

3.6.2.6 CONTROLS

3.6.2.6.1 Liquid Whole Blood Positive Control (Utak 98818 or equivalent)

3.6.2.6.2 Liquid Whole Blood (Utak 44600-WB (F) or equivalent) spiked with working standard solution at 50, 100 and/or 500ng/ml (other levels may be used as needed). To 2mL of negative blood add working standard solution as indicated below.

Desired ng/mL	µL Working Standard Solution
50	20
100	40
500	200

3.6.2.6.3 Liquid Whole Blood Negative Control (Utak 44600-WB (F) or equivalent)

3.6.2.7 PROCEDURE

3.6.2.7.1 Initial set-up

Label test tubes, and GC/MS vials with microinserts for the negative control (NC), positive control (PC), and appropriate laboratory numbers.

3.6.2.7.2 Sample Preparation

- Transfer 1mL sample, negative control and positive control to screw-top extraction tube.

3.6.2.7.3 Initial Extraction

- Pipet 10mL n-butyl chloride into each tube, cap and extract for ≥3 minutes.
- Centrifuge for ≥5 minutes/ Transfer the butyl chloride (top) layer to a second tube.

The following are clean-up steps. If the sample is clean, proceed to 3.6.2.7.6

3.6.2.7.4 Back Extraction

- Pipet 2.0mL of 0.45N sodium hydroxide, cap and extract for ≥3 minutes.
- Centrifuge for ≥5 minutes.
- Discard butyl chloride (top) layer.

3.6.2.7.5 Final Extraction

- Add concentrated HCl until the pH is acidic (≤6).
- Pipet 10mL butyl chloride into extraction tube, cap and extract for ≥5 minutes.
- Centrifuge for ≥ 5 minutes.
- Transfer the butyl chloride (top) layer into centrifuge tube.

3.6.2.7.6 Evaporation and reconstitution

- Evaporate under a gentle stream of nitrogen at ≤37°C.
- Add 100uL of 1:1 hexane/ethanol to the residue.

- Vortex.
- Transfer extract to labeled GC/MSD ALS vial with microinsert.

3.6.2.8 GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS) ANALYSIS

3.6.2.8.1 Analysis Parameters

3.6.2.8.1.1 Inject 1 µL into GC/MS using the ALS.

3.6.2.8.1.2 Analyze sample extract(s) in full scan acquisition or SIM monitoring the appropriate ions.

3.6.2.8.1.3 Refer to attached GC/MSD method printout for current analysis parameters.

3.6.2.8.2 Detection and Identification Criteria

3.6.2.8.2.1 The presence of a drug compound can be established if there are no significant differences in the retention time and mass spectra for the sample versus standards.

- Acceptable retention time window is +/- 2%.

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