Idaho State Police
Forensic Services
Toxicology Section

Section Three
Blood Toxicology

3.6 Liquid-Liquid Extraction Methods for GC/MSD Confirmation

3.6.1 Liquid-Liquid Extraction Procedure for the Recovery of Neutral and Basic Drugs from Blood.

3.6.1.1 BACKGROUND

This method is a general blood extraction procedure for a variety of commonly encountered neutral and basic drugs of abuse. This method prepares and extract which will be subject to confirmatory analysis by gas chromatography/mass spectrometry (GC\MS). This method does not efficiently extract some compounds (morphine and hydromorphone) due to pH considerations.

3.6.1.2 PRINCIPLE

The method is based upon the principle of liquid/liquid extraction. The sample pH is adjusted with a pH 9.5 saturated borate buffer and extracted with n-butyl chloride. Following centrifugation, the organic layer is transferred to a new extraction tube and 1N sulfuric acid is added to back extract basic and neutral analytes. The aqueous layer is washed with the non-polar solvent, hexanes. After the wash the pH is adjusted to greater than 9 with 10N NaOH to convert analytes back to a non-ionic form for a final extraction with n-butyl chloride. The final extract is either reconstituted with 1:1 hexane/ethanol or derivatized for confirmation on the GC/MS using SIM and/or full scan monitoring.

3.6.1.3 EQUIPMENT AND SUPPLIES

3.6.1.3.1 Drybath (Fisher or equivalent)

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

3.6.1.3.3 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)

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

3.6.1.3.5 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)

3.6.1.4 REAGENTS

Refer to Manual section 3.8 for solution preparation

3.6.1.4.1 Methanol (Fisher A412-4 or equivalent)
3.6.1.4.2 Deionized/Distilled (DI) Water
3.6.1.4.3 Hexane (Fisher H292-4 or equivalent)
3.6.1.4.4 n-Butyl chloride (Fisher B416-1 or equivalent)
3.6.1.4.5 Ethanol (Fisher A995-4 or equivalent)
3.6.1.4.6 Hexane/Ethanol 1:1
3.6.1.4.7 1% Hydrochloric Acid in Methanol
3.6.1.4.8 1N Sulfuric Acid
3.6.1.4.9 10N Sodium Hydroxide
3.6.1.4.10 Saturated Borate Buffer (pH 9.5)
3.6.1.4.11 Silylation Reagent Options

- MSFTA (Pierce 48910 or equivalent)
- MSFTA + 1% TMCS (Pierce 48915 or equivalent)
- BSTFA (Pierce 38830 or equivalent)
- BSTFA + 1% TMCS (Pierce 38831 or equivalent)

3.6.1.5 STANDARDS

3.6.1.5.1 Stock Standard Solution

3.6.1.5.1.1 1.0mg/mL Drug standards (obtain as necessary from Cerilliant, Alltech, Sigma or equivalent vendor).

3.6.1.5.2 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.

3.6.1.6 CONTROLS

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

3.6.1.6.2 Liquid Whole Blood (Utak 44600-WB (F) or equivalent) spiked with working standard solution at 50, 100 and/or 300ng/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
300	120

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

3.6.1.7 PROCEDURE

3.6.1.7.1 Initial set-up

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

3.6.1.7.2 Sample Preparation

- Transfer 2mL sample, negative control and positive control to screw-top extraction tube.
- Pipet 2.0mL pH 9.5 saturated borate buffer to each sample and vortex.

3.6.1.7.3 Initial Extraction

- Pipet 10mL n-butyl chloride into each tube, cap and extract for 10 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 3.6.1.7.7

3.6.1.7.4 Back Extraction

- Pipet 2.0mL of 1N sulfuric acid, cap and extract for 5 minutes.
- Centrifuge for ≥5 minutes and discard butyl chloride (top) layer.

3.6.1.7.5 Hexane Wash

- Pipet 5.0mL hexane into each tube, cap and extract for 5 minutes.

- Centrifuge for approx. 5 minutes and discard the hexane (top) layer.

3.6.1.7.6 Final Extraction

- Add 10N NaOH (approx. 6-8 drops) until the pH is basic (> 9).
- 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.

3.6.1.7.7 Evaporation and reconstitution

- Add 2-5 drops of 1% HCl in methanol.
- Evaporate under a gentle stream of nitrogen at 37°C to near dryness.
- Finish drying under nitrogen at room temperature. As each sample dries, *immediately* add 50uL of 1:1 hexane/ethanol to the residue
- Vortex.
- Transfer extract to labeled GC/MSD ALS vial with microinsert.

3.6.1.7.8 Derivatization (when appropriate)

- In fume hood add 50µL silylating agent to the reconstituted extract.
- Cap tubes.
- Vortex.
- Heat tube for 15 minutes in 90°C dry bath.
- Remove from heat and allow to cool.
- Transfer derivative to labeled GC/MSD ALS vial with microinsert.

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

3.6.1.8.1 Analysis Parameters

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

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

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

3.6.1.8.2 Detection and Identification Criteria

3.6.1.8.2.1 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%.

3.6.1.9 REFERENCES

3.6.1.9.1 Foerster, E., Hatchett, D., and Garriott, J. A Rapid, Comprehensive Screening Procedure for Basic Drugs in Blood of Tissues by Gas Chromatography. J. Anal. Toxicol. 2:50-55, 1978.

Idaho State Police
Forensic Services
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Section Three
Blood Toxicology

3.6 Liquid-Liquid Extraction Methods for GC/MSD Confirmation
3.6.1 Liquid-Liquid Extraction Procedure for the Recovery of Neutral and Basic Drugs from Blood.

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

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Section Five
Quality Assurance

5.8 Evaluation of Quality Assurance Measures

5.8.1 SCOPE

This SOP deals with the evaluation of quality assurance measures used to promote confidence in results obtained from toxicology methods.

5.8.2 EVALUATION CRITERIA FOR QUALITATIVE METHODS

5.8.2.1 Analytical Standards

New analytical standards must be authenticated prior to official use. Authentication requires comparison of instrumental data obtained through the analysis of the standard with data from a peer reviewed scientific journal, reference standard compendium, instrumental data and/or library searches.

5.8.2.2 Non-extracted Standards (NES)

Standards should be compared to previously analyzed standards along with literature sources. No significant differences should be apparent.

5.8.2.3 Extracted Positive Controls (EPC)

Positive controls should exhibit proper retention time and mass spectral characteristics for compounds of interest based upon NES.

5.8.2.4 Extracted Negative Control (ENC)

To verify a lack of contamination in the extraction procedure, the negative control should be examined to satisfy the analyst that it does not contain the compound(s) of interest or interfering substances. This should be confirmed by extracting appropriate ions in the apposite retention time window.

5.8.2.5 Solvent Blanks

To verify a lack of carryover between samples analyzed on a gas chromatograph equipped with a mass selective detector (GC-MSD), a solvent blank should be run between samples that are analyzed in selective ion monitoring mode (SIM) or know to have a potential for carry-over. Non-SIM blanks should be run at the discretion of individual analysts. The absence of carryover must be verified prior to the discontinuation of blanks between samples.

If the solvent blank contains a significant amount of the compound(s) of interest, the GC-MSD analysis should be repeated.

5.8.3 DISTRIBUTION OF QUALITY DATA

5.8.3.1 Documentation of analytical standard authentication and originals of casework quality controls (NES, EPC, ENC) will be stored centrally in the file designated for urine toxicology quality data in the laboratory where the analysis was performed. Copies of all quality assurance samples need not be placed in each case file.

5.8.3.2 Copies of analytical standards used to substantiate the identification of each drug compound must be included in each case file if not otherwise indicated in individual SOPs.

Idaho State Police
Forensic Services
Toxicology Section

History Page

Toxicology Procedural Manual
Section Five
Quality Assurance
5.8 Evaluation of Quality Assurance Measures

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