Idaho State Police
Forensic Services
Toxicology Section

Section Three
Blood Toxicology

3.2 Specimen Volume Requirements for Blood Toxicology

3.2.1 VOLUME REQUIREMENTS

3.2.1.1 Screening Techniques

3.2.1.1.1 Enzyme Immunoassay (EIA)
Minimum volume required for analysis: 1.0mL

3.2.1.1.2 Gas Chromatograph equipped with a Nitrogen Phosphorus Detector (GC-NPD)
Minimum volume required for analysis: 2.0mL

3.2.1.2 Confirmatory Techniques

3.2.1.2.1 Gas Chromatography equipped with a Mass Selective Detector (GC/MSD)
Minimum volume required for analysis is dependent upon the number and class of drug compounds indicated by screening techniques and the concentration of the compounds present. A rule of thumb is 2mL per constituent.

Idaho State Police
Forensic Services
Toxicology Section

Section Three
Blood Toxicology

3.2 Specimen Volume Requirements for Blood Toxicology

Revision #	Issue Date	History
1	04-16-02	Original Issue in SOP format

Approval

Technical Leader: _____ **Date:** _____
S. C. Williamson

Issuance

QC Manager: _____ **Date:** _____
Rick D. Groff

Idaho State Police
Forensic Services
Toxicology Section

Section Three
Blood Toxicology

3.3 Analysis of Blood for Common Drugs of Abuse by Gas Chromatography Equipped with Dual Nitrogen Phosphorus Detectors

3.3.1 BACKGROUND

The presence of phosphorus and/or nitrogen in the structure of most drug compounds facilitates their detection using a gas chromatograph equipped with a nitrogen-phosphorus detector (GC-NPD). The GC-NPD allows for the presumptive identification of drug compounds in blood based upon their relative retention times without extensive extraction procedures.

3.3.2 PRINCIPLE

This method outlines a sensitive method to screen blood specimens for a variety of commonly encountered basic and neutral drugs of abuse. Drug compounds are extracted from blood by a liquid-liquid extraction process, the blood is made basic and extracted with n-butyl chloride. The extract is subjected to analysis by dual column gas chromatography (GC). The nitrogen phosphorus detector (NPD) utilizes an alkali metal salt, such as rubidium or cesium, positioned above the detector's flame. The NPD is thus configured to selectively ionize compounds containing nitrogen and phosphorus.[1] A limitation of this method is that it does not detect morphine, hydromorphone, carboxy-THC or the cocaine metabolite benzoylecgonine, due to pH considerations, the lack of nitrogen and chromatographic problems.. These analytes can by screened for by enzyme immunoassay (refer to manual section one).

3.3.3 EQUIPMENT

3.3.3.1 Drybath (Fisher or equivalent)

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

3.3.3.3 Vacuum Manifold/pump

3.3.3.4 Laboratory centrifuge

3.3.3.5 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)
Automated Liquid Sampler (ALS) vials (HP 5182-0865 or equivalent)
GC/MS vial microinsert (HP 5183-2088 or equivalent)

3.3.3.6 Gas Chromatograph equipped with Dual Nitrogen Phosphorus Detectors (Agilent/HP 6890 or equivalent)

3.3.3.7 Non-polar Capillary Column
100%-Dimethylsiloxane or a 5%-Diphenyl-95%-Dimethyl-siloxane copolymer, 12.5 to 30M.

3.3.3.8 Mid-Polar Capillary Column
50% Phenyl, 50% methyl-polysiloxane copolymer, 12.5 to 30M.

3.3.4 REAGENTS

Refer to Manual section 3.8 for solution preparation instructions.

3.3.4.1 Methanol (Fisher A412-4 or equivalent)
3.3.4.2 Hexane (Fisher H292-4 or equivalent)
3.3.4.3 n-Butyl chloride (Fisher B416-1 or equivalent)
3.3.4.4 Ethanol (Fisher A-995-4 or equivalent)
3.3.4.5 Hexane/Ethanol 1:1
3.3.4.6 1% Hydrochloric Acid in Methanol
3.3.4.7 1N Sulfuric Acid
3.3.4.8 10N Sodium Hydroxide
3.3.4.9 Saturated Borate Buffer (pH 9.5)

3.3.5 STANDARDS

3.3.5.1 Stock Standard Solution

3.3.5.1.1 1.0mg/mL Drug standard (obtain as necessary from Cerilliant, Alltech, Sigma or equivalent vendor).*
*** Standards requiring acetonitrile as a solvent may not be used in this method.**

3.3.5.2 Working Standard Solution (5000ng/mL)

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

3.3.5.3 Internal Standard Stock Solution (1.0mg/mL)

3.3.5.3.1 Add 10mg iprindole to 10mL methanol. Solution is stable for 12 months when stored at 4°C.

3.3.5.4 Internal Standard Working Solution (5000ng/mL)

3.3.5.4.1 Add 50µL stock solution to 10mL methanol. Solution is stable for 12 months when stored at 4°C.

3.3.5.5 Performance Standard Solution(5000ng/mL)

3.3.5.5.1 Add 50µL of the following stock solutions to 10mL methanol:

Stock (1.0mg/mL)	Potential Vendors
Methamphetamine	Cerilliant M-009, Alltech 010013
PCP	Cerilliant P-007, Alltech 017263
Cocaine	Cerilliant C-008, Alltech 018003
Diazepam	Cerilliant D-907, Alltech 017213

Solution is stable for 12 months when stored at 4°C.

3.3.6 CONTROLS

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

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

3.3.6.3 Performance Standard

3.3.6.3.1 Add 100µL of the Performance Standard Solution to 2.0mL Liquid Whole Blood Negative Control. (Utak 44600-WB (F) or equivalent.

3.3.7 PROCEDURE

3.3.7.1 Initial set-up

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

3.3.7.2 Sample Preparation

- Transfer 2mL sample, negative control and positive control to screw-top extraction tube.
- To each sample add:
  - 100µL of 5ng/µL iprindole internal standard (500ng)
  - 2.0mL saturated borate buffer (pH 9.5)
  - Vortex.

3.3.7.3 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 3.3.7.7

3.3.7.4 Back Extraction

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

3.3.7.5 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.
- Verify that the pH of the aqueous phase is ≤6.

3.3.7.6 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.

3.3.7.7 Evaporation and reconstitution

- 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.

3.3.8 GAS CHROMATOGRAPHY/NITROGEN PHOSPHORUS DETECTOR (GC/NPD) ANALYSIS

3.3.8.1 Analysis Parameters

3.3.8.1.1 Inject 1to 2 µL into GC/NPD using the ALS.

3.3.8.1.2 Run a hexane/ethanol (1:1) blank between each sample.

3.3.8.1.3 Refer to attached GC/NPD method printout for current analysis parameters.

3.3.8.2 Detection and Identification Criteria

3.3.8.2.1 The presence of a drug compound may be indicated if the following criteria is met.

- There are no significant differences in the relative retention time (RRT) for the sample versus standards.

3.3.9 REFERENCES

3.3.9.1 Stafford, D.T., *Chromatography*, pp. 112, **in**: Principles of Forensic Toxicology, B. Levine, ed., AACC, 1999.

3.3.9.2 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
Toxicology Section

Section Three
Blood Toxicology

3.3 Analysis of Blood for Common Drugs of Abuse by Gas Chromatography Equipped with Dual Nitrogen Phosphorus Detectors

Revision #	Issue Date	History
1	04-25-02	Original Issue in SOP format

Approval

Technical Leader: _____ **Date:** _____
S. C. Williamson

Issuance

QC Manager: _____ **Date:** _____
Rick D. Groff

Idaho State Police
Forensic Services
Toxicology Section

Section Three
Blood Toxicology

3.4 Manual Solid Phase Extraction (SPE) Methods

3.4.1 Extraction of Basic and Neutral Drugs in Blood Employing the United Chemical Technologies (UCT) 200 mg CLEAN SCREEN® DAU Extraction Column

3.4.1.1 PRINCIPLE

This procedure outlines the use of the UCT 200mg CLEAN SCREEN® DAU column for the extraction of basic and neutral drugs from blood. The CLEAN SCREEN® DAU column utilizes a copolymeric sorbent which combines a cationic exchanger and a hydrophobic functionality (reverse phase) to interact effectively, physically and chemically, with analytes of interest and minimally with interfering substances in the blood sample.

The anionic sorbent cation exchanger binds to cations. Additional retention mechanisms include hydrophobic interactions and polar adsorption. The nonpolar aspect of the column serves to extract nonpolar compounds from a polar sample matrix. For the extraction of basic and neutral drugs, the blood sample is diluted and centrifuged, adjusted to pH 6 with a phosphate buffer, and loaded onto a pre-conditioned SPE column. The blood pH is adjusted to maximize the ionic character of analytes. The conditioning creates an environment which allows for optimal interaction between the sorbent and the analytes of interest. The column is subsequently washed with water and a weak aqueous buffer, to selectively remove matrix components and interfering substances from the column. Next, the column is dried to remove traces of aqueous and organic solvents. When the column is dry the analytes of interest are recovered from the column with a basic organic solvent mixture. Following the elution from the SPE column the extract is either reconstituted with ethyl acetate or derivatized for confirmation on the GC/MSD.

3.4.1.2 EQUIPMENT AND SUPPLIES

3.4.1.2.1 200mg CLEAN SCREEN® extraction column (ZSDAU020 or ZCDAU020 or equivalent)

3.4.1.2.2 Evaporative concentrator (Zymark TurboVap® LV, Pierce Reacti-Vap™/Reacti-Therm™ or equivalent) equipped with nitrogen tank.

3.4.1.2.3 Vacuum Manifold/pump

3.4.1.2.4 Dry bath

3.4.1.2.5 Glassware

16X100mm disposable culture tubes (Fisher 14-961-29 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 Sample (ALS) vials (HP 5182-0865 or equivalent)

GC/MS Vial Microinsert (HP 5183-2088 or equivalent)

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

3.4.1.2.7 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 GHB and its analogs in toxicological specimens (e.g. 100%-dimethylpolysiloxane or 95%-dimethyl-polysiloxane with 5%-diphenyl)

3.4.1.3 REAGENTS

Refer to manual section 3.8 for solution preparation instructions.

3.4.1.3.1 Deionized/distilled (DI) water

3.4.1.3.2 Methanol (Fisher A412-4 or equivalent)

3.4.1.3.3 Methylene Chloride (Fisher D37-4 or equivalent)

3.4.1.3.4 Ethyl Acetate (Fisher E145-4 or equivalent)

3.4.1.3.5 Isopropanol (Fisher A416-1 or equivalent)

3.4.1.3.6 Ammonium Hydroxide (Fisher A669-500 or equivalent)

3.4.1.3.7 100mM Phosphate Buffer (pH 6.0)

3.4.1.3.8 100mM Hydrochloric Acid (HCl) or 100mM Acetic Acid

3.4.1.3.9 100mM Monobasic sodium phosphate

3.4.1.3.10 100mM Dibasic sodium phosphate

3.4.1.3.11 Elution Solvent

Mix 78mL Methylene Chloride, 20mL Isopropanol and 2mL Ammonium Hydroxide. ***Make fresh.***

3.4.1.3.12 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)
- TFAA (Pierce 67363 or equivalent)
- HFAA (Pierce 63164 or equivalent)

3.4.1.4 CONTROLS

3.4.1.4.1 Liquid Whole Blood Positive Control (Utak 98818 or equivalent).

3.4.1.4.2 Liquid Whole Blood Negative Control (Utak 44600-WB (F) or equivalent) spiked with drugs of interest (more than one level may be used where appropriate).

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

3.4.1.5 PROCEDURE

3.4.1.5.1 Initial set-up

Label 200mg CLEAN SCREEN® Extraction Column, test tubes, and GC/MS vials with microinserts for the negative control (NC), positive control (PC), and appropriate laboratory numbers.

3.4.1.5.2 Sample Preparation

- Transfer 2mL sample, negative control and positive control to screw-top extraction tube.
- Add 8mL DI water, vortex, let stand for 5 minutes.
- Centrifuge for 10 minutes.
- Transfer liquid to second tube.
- Add 4mL 100mM phosphate buffer. (pH 6.0)
- Sample pH should be 6.0 ±0.5. Adjust as necessary with 100mM Monobasic sodium phosphate or 100mM Dibasic sodium phosphate

3.4.1.5.3 Sample Extraction

- Insert labeled 200mg CLEAN SCREEN® Extraction column in the vacuum manifold.
- Add 3mL of methanol to the column and aspirate and aspirate at ≤ 3 in. Hg to prevent sorbent drying.
- Add 3mL of deionized water to the column and aspirate at ≤ 3 in. Hg.
- Add 1mL of 100mM phosphate buffer (pH 6.0) and aspirate at ≤ 3 in. Hg.
- Load sample into column at 1 to 2mL/minute.
- Wash column with the following and aspirate at ≤ 3 in. Hg:
  - 2mL of deionized water
  - 2mL 100mM HCl or 100mM acetic acid
  - 3mL methanol
- Increase vacuum to ≥ 10 in. Hg (≥34 kPa) and dry extraction disc for ≥ 5 minutes.
- Open vacuum manifold, wipe collection tips, and insert the collection rack containing the labeled tapered tip centrifuge tubes.

- Add 6mL elution solvent to the column and aspirate at < 3 in. Hg (<10 kPa) or by gravity.
- Evaporate solvent to dryness, under a gentle stream of nitrogen, in evaporator at ≤ 40°C.

3.4.1.5.4 Sample Reconstitution

- Add 50µL ethyl acetate.
- Vortex.
- Transfer extract to labeled GC/MSD ALS vial with microinsert.

3.4.1.5.5 Derivatization Option One (when appropriate)

- In fume hood add the following:
  - 50µL ethyl acetate.
  - 50µL silylating agent.
- Cap tubes.
- Vortex.
- Heat tube for 20 minutes in 70°C dry bath.
- Remove from heat and allow to cool.
- Transfer derivative to labeled GC/MSD ALS vial with microinsert.

3.4.1.5.6 Derivatization Option Two (when appropriate)

- In fume hood add 50 ul TFAA or HFAA.
- Cap tubes.
- Vortex
- Heat tube for 20 minutes in 70°C dry bath.
- Remove from heat and allow to cool.
- Evaporate samples to dryness with nitrogen at room temperature.
- Add 50ul ethyl acetate.
- Vortex
- Transfer derivative to labeled GC/MSD ALS vial with micro-insert and cap.

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

3.4.1.6.1 Analysis Parameters

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

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

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

3.4.1.6.2 Detection and Identification Criteria

3.4.1.6.2.1 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%.

3.4.1.7 REFERENCES

3.4.1.7.1 UCT CLEAN SCREEN® Extraction Columns Application Manual

3.4.1.7.2 Platoff, G.E., Gere, J.A. Solid Phase Extraction of Abuse Drugs from Urine, For. Sci. Review, 3 (2):117-132; 1991.

Idaho State Police
Forensic Services
Toxicology Section

Section Three
Blood Toxicology

3.4 Manual Solid Phase Extraction (SPE) Methods

3.4.1 Extraction of Basic and Neutral Drugs in Blood Employing the United Chemical Technologies (UCT) 200 mg CLEAN SCREEN® DAU Extraction Column

Revision #	Issue Date	History
1	04-16-02	Original Issue in SOP format

Approval

Technical Leader: _____ **Date:** _____
S. C. Williamson

Issuance

QC Manager: _____ **Date:** _____
Rick D. Groff

Idaho State Police
Forensic Services
Toxicology Section

Section Three
Blood Toxicology

3.4 Manual Solid Phase Extraction (SPE) Methods

3.4.2 Extraction of Benzodiazepines from Blood Employing the United Chemical Technologies (UCT) 200 mg CLEAN SCREEN® DAU Extraction Column

3.4.2.1 BACKGROUND

Refer to manual section 2.4.3.

3.4.2.2 PRINCIPLE

This procedure outlines the use of the 200mg UCT CLEAN SCREEN® DAU Column for the extraction of Benzodiazepines from blood. The CLEAN SCREEN® DAU column utilizes a copolymeric sorbent which combines a cationic exchanger and a hydrophobic functionality (reverse phase) to interact effectively, physically and chemically, with analytes of interest and minimally with interfering substances in the blood sample.

The benzodiazepines are retained on the SPE by hydrophobic interactions and polar adsorption. The nonpolar aspect of the column serves to extract nonpolar compounds from a polar sample matrix.[2] For the extraction of benzodiazepines, the blood sample is diluted and centrifuged, adjusted to pH 6 with a phosphate buffer, and loaded onto a pre-conditioned SPE column. The conditioning creates an environment which allows for optimal interaction between the sorbent and the analytes of interest. The analyte is retained by hydrophobic interaction of the functional groups present on both the analyte and the sorbent. The column is subsequently washed to selectively remove matrix components and interfering substances from the column. Next, the column is dried to remove traces of aqueous and organic solvents. When the column is dry, the analytes of interest are recovered from the column with a basic organic solvent. Following the elution from the SPE column the extract is derivatized for confirmation on the GC/MSD.

3.4.2.3 EQUIPMENT AND SUPPLIES

3.4.2.3.1 200mg CLEAN SCREEN® Extraction Column (ZSDAU020 or ZCDAU020 or equivalent)

3.4.2.3.2 Drybath (Fisher or equivalent)

3.4.2.3.3 Evaporative concentrator (Zymark TurboVap® LV, Pierce Reacti-Vap™/Reacti-Therm™ or equivalent) equipped with nitrogen tank.

3.4.2.3.4 Vacuum Manifold/pump

3.4.2.3.5 Glassware

13x100mm Screw Cap Tubes (Fisher 14-959-35C or equivalent)

Screw Cap for 13X100 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 Sample (ALS) vials (HP 5182-0865 or equivalent)

GC/MS Vial Microinsert (HP 5183-2088 or equivalent)

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

3.4.2.3.7 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 GHB and its analogs in toxicological specimens (e.g. 100%-dimethylpolysiloxane or 95%-dimethyl-polysiloxane with 5%-diphenyl)

3.4.2.4 STANDARDS

3.4.2.4.1 Stock Standard Solutions

Stock (1.0mg/mL)	Potential Vendors*
Diazepam	Cerilliant D-907 Alltech 017213
Desmethyldiazepam	Cerilliant N-905 Alltech 013453
Lorazepam	Cerilliant L-901 Alltech 013583
Oxazepam	Cerilliant O-902 Alltech 013703
Prazepam	Cerilliant P-906 Alltech 013913
Flurazepam	Cerilliant F-003 Alltech 017953
Triazolam	Cerilliant T-910 Alltech 014283
Desalkylflurazepam	Cerilliant D-915 Alltech 6013423
Chlordiazepoxide	Cerilliant C-022 Alltech 01719
Alprazolam	Cerilliant A-903 Alltech 014273

*or equivalent.

3.4.2.4.2 Working Standard Solution (5000ng/mL)

3.4.2.4.2.1 Add 50µL Stock Solution to 10mL Methanol.

Solution is stable for 12 months when stored at 4°C.

3.4.2.5 CONTROLS

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

3.4.2.5.2 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

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

3.4.2.6 REAGENTS

Refer to manual section 3.8 for solution preparation instructions.

3.4.2.6.1 Deionized/distilled (DI) water

3.4.2.6.2 Methanol (Fisher A412-4 or equivalent)

3.4.2.6.3 Methylene Chloride (Fisher D37-4 or equivalent)

3.4.2.6.4 Ethyl Acetate (Fisher E145-4 or equivalent)

3.4.2.6.5 Isopropanol (Fisher A416-1 or equivalent)

3.4.2.6.6 Ammonium Hydroxide (Fisher A669-500 or equivalent)

3.4.2.6.7 100mM Phosphate Buffer (pH 6.0)

3.4.2.6.8 100mM Hydrochloric Acid (HCl) or 100mM Acetic Acid

3.4.2.6.9 100mM Monobasic sodium phosphate

3.4.2.6.10 100mM Dibasic sodium phosphate

3.4.2.6.11 Elution Solvent

Mix 78mL Methylene Chloride, 20mL Isopropanol and 2mL Ammonium Hydroxide. ***Make fresh.***

3.4.2.6.12 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)

3.4.2.7 PROCEDURE

3.4.2.7.1 Initial set-up

Label 200mg CLEAN SCREEN® Extraction Column, test tubes, and GC/MS vials with microinserts for the negative control (NC), positive control (PC), and appropriate laboratory numbers.

3.4.2.7.2 Sample Preparation

- Transfer 2mL sample, negative control and positive control to screw-top extraction tube.
- Add 8mL DI water, vortex, let stand for 5 minutes.
- Centrifuge for 10 minutes.
- Transfer liquid to second tube.
- Add 4mL 100mM phosphate buffer.
- Sample pH should be 6.0 ±0.5. Adjust as necessary with 100mM Monobasic sodium phosphate or 100mM Dibasic sodium phosphate.

3.4.2.7.3 Sample Extraction

- Insert labeled 200mg CLEAN SCREEN® Extraction column in the vacuum manifold.
- Add 3mL of methanol to the column and aspirate and aspirate at ≤ 3 in. Hg to prevent sorbent drying.
- Add 3mL of deionized water to the column and aspirate at ≤ 3 in. Hg.
- Add 1mL of 100mM phosphate buffer (pH 6.0) and aspirate at ≤ 3 in. Hg.
- Load sample into column at 1 to 2mL/minute.
- Wash column with the following and aspirate at ≤ 3 in. Hg:
  - 2mL of deionized water
  - 2mL 100mM HCl or 100mM acetic acid
  - 3mL methanol
- Increase vacuum to ≥10 in. Hg (≥34 kPa) and dry extraction disc for ≥ 5 minutes.
- Open vacuum manifold, wipe collection tips, and insert the collection rack containing the labeled tapered tip centrifuge tubes.
- Add 6mL elution solvent to the column and aspirate at < 3 in. Hg (<10 kPa).
- Evaporate solvent to dryness, under a gentle stream of nitrogen, in evaporator at ≤ 40°C.

3.4.2.7.4 Derivatization

- In fume hood add the following:
  - 50µL ethyl acetate.
  - 50µL silylating agent.
- 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.

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

3.4.2.8.1 Analysis Parameters

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

3.4.2.8.1.2 Analyze sample extract(s) in SIM. Refer to table below.

3.4.2.8.1.3 Refer to attached GC/MS method printout for current analysis parameters.

3.4.2.8.2 Detection and Identification Criteria

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

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

Compound	Ions
Desalkylflurazepam	245*, 247, 341*, 342, 343, 344, 345, 346, 347, 348, 359*, 360, 361, 362, 363.
Desmethyldiazepam	227*, 327*, 328, 329, 341*, 342, 343, 344, 345.
Lorazepam-TMS	347*, 349, 429*, 430, 431*, 432
Diazepam	165, 177, 221*, 255, 256*, 257, 258, 283*, 284, 285, 286.
Oxazepam-TMS	147, 313*, 340, 401, 415*, 429*, 430, 431
Prazepam	241, 242, 243, 267, 268, 269*, 270, 271, 295*, 296, 297, 298, 323, 324*, 326, 327
Flurazepam	245*, 315*, 318, 387*, 388, 389, 390.
Triazolam	238*, 239, 279, 313*, 314, 315,342* 343, 344, 345.
Alprazolam	204, 273*, 279*, 307, 308*.
Chlordiazepoxide	163, 165, 205, 220*, 247*, 282*, 283, 284

*Minimum ions to monitor.

3.4.2.9 REFERENCES

3.4.2.9.1 UCT CLEAN SCREEN® Extraction Columns Application Manual

3.4.2.9.2 Platoff, G.E., Gere, J.A. Solid Phase Extraction of Abuse Drugs from Urine, For. Sci. Review, 3 (2):117-132; 1991.

Idaho State Police
Forensic Services
Toxicology Section

Section Three
Blood Toxicology

3.4 Manual Solid Phase Extraction (SPE) Methods
3.4.2 Extraction of Benzodiazepines from Blood Employing the United Chemical Technologies (UCT) 200 mg CLEAN SCREEN® DAU Extraction Column

Revision #	Issue Date	History
1	04-25-02	Original Issue in SOP format

Approval

Technical Leader: _____ **Date:** _____
S. C. Williamson

Issuance

QC Manager: _____ **Date:** _____
Rick D. Groff

Idaho State Police
Forensic Services
Toxicology Section

Section Three
Blood Toxicology

3.4 Manual Solid Phase Extraction (SPE) Methods

3.4.3 Extraction of Cocaine and Benzoylecgonine from Blood Employing the United Chemical Technologies (UCT) 200 mg CLEAN SCREEN® DAU Extraction Column

3.4.3.1 BACKGROUND

Cocaine is a naturally occurring alkaloid derived from leaves of the South American shrub, Erythroxylon coca. Cocaine can also be produced synthetically. Cocaine is one of the most potent stimulants to the central nervous system due to its mechanism of action, which involves blocking reuptake of stimulatory neurotransmitters. Cocaine is used licitly as a local anesthetic in ophthalmology. The positive effects of cocaine include an increased mental awareness and alertness, a sense of clarity and feelings of elation. The fictional detective Sherlock Holmes used cocaine for its transcendently stimulating and mind clarifying properties to the displeasure of Doctor Watson. As with all drugs, the effects of cocaine depend on the dosage, the form in which it is taken and the route of administration. Other significant factors include the setting or circumstances in which the drug is used and the expectations of the user. Side effects can include pupillary dilation, restlessness, dizziness, dyskinesia, tremor, dysphoria, and paranoia. Additional major side effects of cocaine are a consequence of discontinued use. If the user does not readminister the drug, they may experience increased anxiety, agitation, restlessness and the disturbance of normal sleep patterns, which leads to fatigue. Due to these effects following cocaine use, an individual's ability to operate a motor vehicle is impaired both during and following cocaine use. Routes of administration include snorting, injection and smoking. The metabolism of cocaine and its metabolites involves hydrolysis, transesterification and n-demethylation.

3.4.3.2 PRINCIPLE

This procedure outlines the use of the 200mg CLEAN SCREEN® DAU SPE column for the extraction of cocaine and benzoylecgonine from blood. The CLEAN SCREEN® DAU column utilizes a copolymeric sorbent which combines a cationic exchanger and a hydrophobic functionality (reverse phase) to interact effectively, physically and chemically, with analytes of interest and minimally with interfering substances in the blood sample.

The anionic sorbent for the cation exchanger binds to cations. Additional retention mechanisms include hydrophobic interactions and polar adsorption. The nonpolar aspect of the column serves to extract nonpolar compounds from a polar sample matrix. The cation exchanger component of the phase is effective for compounds which are present in the urine sample in a cationic form bonding ionically to the sorbent.

For the extraction of cocaine and benzoylecgonine, the blood sample is diluted and centrifuged, adjusted to pH 6 with a phosphate buffer, and loaded onto a pre-conditioned SPE column. The blood pH is adjusted to maximize the ionic character of the analyte. The conditioning creates an environment which allows for optimal interaction between the sorbent and the analytes of interest. The analyte is retained by ionic interaction of the amine functional groups present on the drug and the anionic sulfonic acid on the sorbent. The column is subsequently washed with water and a weak aqueous buffer, to selectively remove matrix components and interfering substances from the column. The wash also disrupts the hydrophobic and adsorption interactions but not the ionically bound material. Next, the column is dried to remove traces of aqueous and organic solvents. When the column is dry the analytes of interest are recovered from the column with a basic organic solvent mixture. Following the elution from the SPE column the extract is derivatized for confirmation on the GC/MSD.

3.4.3.3 EQUIPMENT AND SUPPLIES

3.4.3.3.1 200mg CLEAN SCREEN® Extraction Column (ZSDAU020 or ZCDAU020 or equivalent)

3.4.3.3.2 Drybath (Fisher or equivalent)

3.4.3.3.3 Evaporative concentrator (Zymark TurboVap® LV, Pierce Reacti-Vap™/Reacti-Therm™ or equivalent) equipped with nitrogen tank.

3.4.3.3.4 Vacuum Manifold/pump

3.4.3.3.5 Glassware

13x100mm Screw Cap Tubes (Fisher 14-959-35C or equivalent)

Screw Cap for 13X100 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 Sample (ALS) vials (HP 5182-0865 or equivalent)

GC/MS Vial Microinsert (HP 5183-2088 or equivalent)

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

3.4.3.3.7 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).

3.4.3.4 REAGENTS

Refer to manual section 3.8 for solution preparation instructions.

3.4.3.4.1 Deionized/distilled (DI) water
3.4.3.4.2 Methanol (Fisher A412-4 or equivalent)
3.4.3.4.3 Methylene Chloride (Fisher D37-4 or equivalent)
3.4.3.4.4 Ethyl Acetate (Fisher E145-4 or equivalent)
3.4.3.4.5 Isopropanol (Fisher A416-1 or equivalent)
3.4.3.4.6 Ammonium Hydroxide (Fisher A669-500 or equivalent)
3.4.3.4.7 100mM Phosphate Buffer (pH 6.0)
3.4.3.4.8 100mM Hydrochloric Acid (HCl) or 100mM Acetic Acid
3.4.3.4.9 100mM Monobasic sodium phosphate
3.4.3.4.10 100mM Dibasic sodium phosphate
3.4.3.4.11 Elution Solvent
Mix 78mL Methylene Chloride, 20mL Isopropanol and 2mL Ammonium Hydroxide. ***Make fresh.***
3.4.3.4.12 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)

3.4.3.5 STANDARDS

3.4.3.5.1 Stock Standard Solution

3.4.3.5.1.1 1.0mg/mL Benzoylecgonine (Cerilliant B-004, Alltech 018203 or equivalent)

3.4.3.5.1.2 1.0mg/mL Cocaine (Cerilliant C-008, Alltech 018003 or equivalent)

3.4.3.5.2 Working Standard Solution (5000ng/mL)

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

3.4.3.6 CONTROLS

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

3.4.3.6.2 Liquid Whole Blood Negative Control (Utak 44600-WB (F) or equivalent) spiked with cocaine and benzoylecgonine at the 50, 100 and/or 300 ng/ml level(s) (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

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

3.4.3.7 PROCEDURE

3.4.3.7.1 Initial set-up

Label 200mg CLEAN SCREEN® Extraction Column, test tubes, and GC/MS vials with microinserts for the negative control (NC), positive control (PC), and appropriate laboratory numbers.

3.4.3.7.2 Sample Preparation

- Transfer 2mL sample, negative control and positive control to screw-top extraction tube.
- Add 8mL DI water, vortex, let stand for 5 minutes.
- Centrifuge for 10 minutes.
- Transfer liquid to second tube
- Add 4mL 100mM phosphate buffer (pH 6.0)
- Sample pH should be 6.0 ±0.5. Adjust as necessary with 100mM Monobasic sodium phosphate or 100mM Dibasic sodium phosphate

3.4.3.7.3 Sample Extraction

- Insert labeled 200mg CLEAN SCREEN® Extraction column in the vacuum manifold.
- Add 3mL of methanol to the column and aspirate and aspirate at ≤ 3 in. Hg to prevent sorbent drying.
- Add 3mL of deionized water to the column and aspirate at ≤ 3 in. Hg.
- Add 1mL of 100mM phosphate buffer (pH 6.0) and aspirate at ≤ 3 in. Hg.

- Load sample on to column at 1 to 2mL/minute.
- Wash column with the following and aspirate at ≤ 3 in. Hg:
  - 2mL of deionized water
  - 2mL 100mM HCl or 100mM acetic acid
  - 3mL methanol
- Increase vacuum to ≥ 10 in. Hg (≥34 kPa) and dry extraction disc for ≥ 5 minutes.
- Open vacuum manifold, wipe collection tips, and insert the collection rack containing the labeled tapered tip centrifuge tubes.
- Add 6mL elution solvent to the column and aspirate at < 3 in. Hg (<10 kPa).
- Evaporate solvent to dryness, under a gentle stream of nitrogen, in evaporator at ≤ 40°C.

3.4.3.7.4 Derivatization

- In fume hood add the following:
  - 50µL ethyl acetate.
  - 50µL silylating agent.
- 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.

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

3.4.3.8.1 Analysis Parameters

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

3.4.3.8.1.2 Analyze sample extract(s) in SIM. Refer to table below.

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

3.4.3.8.2 Detection and Identification Criteria

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

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

Compound	Ions
Cocaine	*82, *182, 83, 77, 94, 96, 105, 198, 272, *303
Benzoylecgonine-TMS	*82, *240, 105, 94, 73, 241, 256, 346, *361

**Minimum ions to monitor*

3.4.3.9 REFERENCES

3.4.3.9.1 UCT CLEAN SCREEN® Extraction Columns Application Manual

3.4.3.9.2 Platoff, G.E., Gere, J.A. Solid Phase Extraction of Abuse Drugs from Urine, For. Sci. Review, 3 (2):117-132; 1991.

Idaho State Police
Forensic Services
Toxicology Section

Section Three
Blood Toxicology

3.4 Manual Solid Phase Extraction (SPE) Methods
3.4.3 Extraction of Cocaine and Benzoylecgonine from Blood Employing the United Chemical Technologies (UCT) 200 mg CLEAN SCREEN® DAU Extraction Column

Revision #	Issue Date	History
1	04-16-02	Original Issue in SOP format

Approval

Technical Leader: _____ **Date:** _____
S. C. Williamson

Issuance

QC Manager: _____ **Date:** _____
Rick D. Groff

Idaho State Police
Forensic Services
Toxicology Section

Section Three
Blood Toxicology

3.4 Manual Solid Phase Extraction (SPE) Methods

3.4.4 Extraction of Free (Unbound) Opiates in Blood Employing the United Chemical Technologies (UCT) 200 mg CLEAN SCREEN® DAU Extraction Column

3.4.4.1 BACKGROUND

Morphine and codeine are natural derivatives of the opium poppy, *Papaver somiferum*. Opium contains several alkaloids including morphine, codeine and papaverine. These natural products lead to the development of numerous synthetic analgesics. Narcotic analgesics are divided into 3 classes, the phenanthrenes (morphine, codeine, oxycodone, pentazocine), phenylpiperidines (meperidine, fentanyl), and the phenylheptanes (methadone, propoxyphene). As illustrated in the chart below, the effects of opiate class drugs are dependent upon interactions with specific receptor sites within the central nervous system (CNS). In addition to analgesia and cough suppression, effects of opiate use include euphoria, respiratory depression, sedation, reduced GI motility/constipation, hypothermia, dysphoria, miosis, bradycardia, nausea, and physical tolerance and dependence.

Compound	Trade Name	Receptor/ Action	Metabolites	Therapeutic uses
Buprenorphine	Buprenex®	μ agonist, κ antagonist	Norbuprenorphine	moderate-severe pain
Butorphanol	Stadol®, Stadol NS®	κ agonist, μ antagonist	3-hydroxybutorphanol, norbutorphanol	moderate-severe pain
Codeine	Tylenol 3®	μ agonist, δ agonist	morphine, norcodeine	mild-moderate
Dihydrocodeine	Paracodin®	μ agonist	dihydromorphine, nordihydrocodeine	mild-moderate
Fentanyl	Sublimaze®	μ agonist	despropionylfentanyl, norfentanyl, hydroxyfentanyl, hydroxynorfentanyl	moderate-severe
Heroin	NA in US	μ agonist	6-acetylmorphine, morphine, normorphine	----
Hydrocodone	Hycodan®, Vicodin®, Codone®, Lortab®	μ agonist	hydromorphone, norhydrocodone, dihydrocodeine hydromorphol	moderate-severe
Hydromorphone	Dilaudid®	μ agonist	hydromorphol	moderate-severe
Levorphanol	levo-dromoran®	μ agonist, κ agonist	norlevorphanol	moderate-severe

Compound	Trade Name	Receptor/ Action	Metabolites	Therapeutic uses
Meperidine	Demerol®	μ agonist	normeperidine	moderate-severe
Methadone	Dolophine®, Methadose®	μ agonist	methadol, normethadol, EDDP, EMDP	Detoxification
Morphine	MS-IR Roxanol	μ agonist, κ agonist, δ agonist	normorphine	moderate-severe
Nalbuphine	Nubain®	κ agonist, σ agonist, μ antagonist	nornalbuphine	moderate-severe
Oxycodone	Percolone®, Roxicodone®, Oxycontin®, Oxy®	μ agonist	oxymorphone, noroxycodone	moderate-severe
Oxymorphone	Numorphan®	μ agonist	6-oxymorphol	moderate-severe
Pentazocine	Talwin®	μ agonist, κ agonist, σ agonist	cis- and trans-hydroxypentazocine, trans-carboxypentazocine	moderate-severe
Propoxyphene	Darvon®, Darvocet®	μ agonist	norpropoxyphene,	mild-moderate
Tramadol	Ultram®	μ agonist	nortramadol, O-desmethyltramadol, N-desmethyltramadol	moderate

3.4.4.2 **PRINCIPLE**

This procedure outlines the use of the UCT 200 mg CLEAN SCREEN® extraction column for the extraction of Opiates from blood. The CLEAN SCREEN® DAU column utilizes a copolymeric sorbent which combines a cationic exchanger and a hydrophobic functionality (reverse phase) to interact effectively, physically and chemically, with analytes of interest and minimally with interfering substances in the blood sample. The cation exchanger utilizes an anionic sorbent (-) to bind to cations. Additional retention mechanisms include hydrophobic interactions and polar adsorption.

For the extraction of opiate class drugs, the blood sample is diluted and centrifuged, adjusted to pH 6 with a phosphate buffer, and loaded onto a pre-conditioned SPE column. The blood pH is adjusted to maximize the ionic character of the analyte. The conditioning creates an environment which allows for optimal interaction between the sorbent and the analytes of interest. The analyte is retained by ionic interaction of the amine functional groups present on the drug and the anionic sulfonic acid exchanger on the sorbent. The column is subsequently washed with water and a weak aqueous buffer, to selectively remove matrix components and interfering substances from the column. The wash also disrupts the hydrophobic and adsorption interactions but not the ionically bound material. Next, the column is dried to remove traces of aqueous and

organic solvents. When the column is dry the analytes of interest are recovered from the column with a basic organic solvent mixture. Following the elution from the SPE column the extract is derivatized for confirmation on the GC/MSD.

3.4.4.3 EQUIPMENT AND SUPPLIES

3.4.4.3.1 200mg CLEAN SCREEN® Extraction Column (ZSDAU020 or ZCDAU020 or equivalent)

3.4.4.3.2 Drybath (Fisher or equivalent)

3.4.4.3.3 Evaporative concentrator (Zymark TurboVap® LV, Pierce Reacti-Vap™/Reacti-Therm™ or equivalent) equipped with nitrogen tank.

3.4.4.3.4 Vacuum Manifold/pump

3.4.4.3.5 Glassware

13x100mm Screw Cap Tubes (Fisher 14-959-35C or equivalent)

Screw Cap for 13X100 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 Sample (ALS) vials (HP 5182-0865 or equivalent)

GC/MS Vial Microinsert (HP 5183-2088 or equivalent)

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

3.4.4.3.7 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 GHB and its analogs in toxicological specimens (e.g. 100%-dimethylpolysiloxane or 95%-dimethyl-polysiloxane with 5%-diphenyl)

3.4.4.4 REAGENTS

Refer to manual section 3.8 for solution preparation instructions.

3.4.4.4.1 Deionized/distilled (DI) water

3.4.4.4.2 Methanol (Fisher A412-4 or equivalent)

3.4.4.4.3 Methylene Chloride (Fisher D37-4 or equivalent)

3.4.4.4.4 Ethyl Acetate (Fisher E145-4 or equivalent)

3.4.4.4.5 Isopropanol (Fisher A416-1 or equivalent)

3.4.4.4.6 Ammonium Hydroxide (Fisher A669-500 or equivalent)

3.4.4.4.7 100mM Phosphate Buffer (pH 6.0)

3.4.4.4.8 100mM Acetate Buffer (pH 4.5)

3.4.4.4.9 100mM Monobasic sodium phosphate

3.4.4.4.10 100mM Dibasic sodium phosphate

3.4.4.4.11 Elution Solvent
Mix 78mL Methylene Chloride, 20mL Isopropanol and 2mL Ammonium Hydroxide. ***Make fresh.***

3.4.4.4.12 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)

3.4.4.5 STANDARDS

3.4.4.5.1 Stock Standard Solution

3.4.4.5.1.1 1.0mg/mL Codeine (Cerilliant C-006 or equivalent)

3.4.4.5.1.2 1.0mg/mL Morphine (Cerilliant M-005, or equivalent)

3.4.4.5.1.3 1.0mg/mL 6-acetylmorphine (Cerilliant A-009, or equivalent)

3.4.4.5.1.4 Additional opiate class standards as required.

3.4.4.5.2 Working Standard Solution (5000ng/mL)

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

3.4.4.6 CONTROLS

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

3.4.4.6.2 Liquid Whole Blood (Utak 44600-WB (F) or equivalent) spiked with codeine, morphine and/or 6-acetylmorphine at 50, 100 and/or 300ng/ml (other levels and standards may be used as needed). To 2mL of negative blood add working standard solution as indicated below.

Desired ng/mL	µL Volume Working Standard Solution
50	20
100	40
300	120

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

3.4.4.7 PROCEDURE

3.4.4.7.1 Initial set-up

Label 200mg CLEAN SCREEN® Extraction Column, test tubes, and GC/MS vials with microinserts for the negative control (NC), positive control (PC), and appropriate laboratory numbers.

3.4.4.7.2 Sample Preparation

- Transfer 2mL sample, negative control and positive control to screw-top extraction tube.
- Add 8mL DI water, vortex, let stand for 5 minutes.
- Centrifuge for 10 minutes.
- Transfer liquid to second tube.
- Add 4mL 100mM phosphate buffer (pH 6.0).
- Sample pH should be 6.0 ±0.5. Adjust as necessary with 100mM monobasic sodium phosphate or 100mM dibasic sodium phosphate.

3.4.4.7.3 Sample Extraction

- Insert labeled 200mg CLEAN SCREEN® Extraction column in the vacuum manifold.
- Add 3mL of methanol to the column and aspirate and aspirate at ≤ 3 in. Hg to prevent sorbent drying.
- Add 3mL of deionized water to the column and aspirate at ≤ 3 in. Hg.
- Add 2mL of 100mM phosphate buffer (pH 6.0) and aspirate at ≤ 3 in. Hg.
- Load sample on to column at 1 to 2mL/minute.
- Wash column with the following and aspirate at ≤ 3 in. Hg:
  - 2mL of deionized water
  - 2mL 100mM acetate buffer (pH 4.5)
  - 3mL methanol
- Increase vacuum to ≥ 10 in. Hg (≥34 kPa) and dry extraction disc for ≥ 5 minutes.
- Open vacuum manifold, wipe collection tips, and insert the collection rack containing the labeled tapered tip centrifuge tubes.
- Add 6mL elution solvent to the column and aspirate at < 3 in. Hg (<10 kPa).
- Evaporate solvent to dryness, under a gentle stream of nitrogen, in evaporator at ≤ 40°C.

3.4.4.7.4 Derivatization

- In fume hood add the following:

- 50µL ethyl acetate.
- 50µL silylating agent.
- 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.

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

3.4.4.8.1 Analysis Parameters

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

3.4.4.8.1.2 Analyze sample extract(s) in SIM. Refer to table below and laboratory.

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

3.4.4.8.2 Detection and Identification Criteria

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

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

Compound	Ions
Morphine-2TMS	196, 234, 236*, 287, 371, 401, 414*, 429*
6-Acetylmorphine-TMS	73, 204, 287*, 324, 340*, 342, 399*, 400
Codeine-TMS	178*, 196, 229, 234*, 371*, 372
Hydrocodone-TMS	371*, 73, 234*, 313, 314, 356*, 242, 243, 299, 185, 214
Hydromorphone-TMS	357*, 300*, 73, 59, 342*, 243, 272, 301, 358
Oxycodone-TMS	73, 242, 297, 312*, 368, 444*, 459*, 460

** minimum ions to monitor.*

3.4.4.9 REFERENCES

3.4.4.9.1 UCT CLEAN SCREEN® Extraction Columns Application Manual

3.4.4.9.2 Platoff, G.E., Gere, J.A. Solid Phase Extraction of Abuse Drugs from Urine, For. Sci. Review, 3 (2):117-132; 1991.

Idaho State Police
Forensic Services
Toxicology Section

Section Three
Blood Toxicology

3.4 Manual Solid Phase Extraction (SPE) Methods
3.4.4 Extraction of Opiates from Blood Employing the United Chemical Technologies (UCT) 200mg CLEAN SCREEN® DAU Extraction Column

Revision #	Issue Date	History
1	04-25-02	Original Issue in SOP format

Approval

Technical Leader: _____ **Date:** _____
S. C. Williamson

Issuance

QC Manager: _____ **Date:** _____
Rick D. Groff

Idaho State Police
Forensic Services
Toxicology Section

Section Three
Blood Toxicology

3.4 Manual Solid Phase Extraction (SPE) Methods

3.4.5 Extraction of Hydrocodone from Blood Employing the United Chemical Technologies (UCT) 200 mg CLEAN SCREEN® DAU Extraction Column

3.4.5.1 BACKGROUND

Hydrocodone is a semi synthetic narcotic analgesic prepared by the catalytic rearrangement of codeine. Therapeutic uses of hydrocodone include treatment of moderate pain (analgesic) and cough relief (antitussive). Trade names include Hycodan®, Vicodin®, Codone®, and Lortab®. Preparations containing hydrocodone often are in combination with acetaminophen.

Hydrocodone is metabolized in the liver by O-demethylation, N-dealkylation and reduction of its 6-keto group to produce the active metabolites, hydromorphone, norhydrocodone and hydrocodol, respectively. In addition to analgesia and cough suppression, effects may include euphoria, respiratory depression, sedation, reduced GI motility/constipation, hypothermia, dysphoria, miosis, bradycardia, nausea, and physical tolerance and dependence.

3.4.5.2 PRINCIPLE

This procedure outlines the use of the UCT 200mg CLEAN SCREEN® extraction column for the extraction of hydrocodone from blood. The CLEAN SCREEN® DAU column utilizes a copolymeric sorbent which combines a cationic exchanger and a hydrophobic functionality (reverse phase) to interact effectively, physically and chemically, with analytes of interest and minimally with interfering substances in the blood sample. The cation exchanger utilizes an anionic sorbent to bind to cations. Additional retention mechanisms include hydrophobic interactions and polar adsorption.

For the extraction of hydrocodone, the blood sample is diluted and centrifuged, adjusted to pH 6 with a phosphate buffer, and loaded onto a pre-conditioned SPE column. The blood pH is adjusted to maximize the ionic character of the analyte. The conditioning creates an environment which allows for optimal interaction between the sorbent and the analytes of interest. The analyte is retained by ionic interaction of the amine

functional groups present on the drug and the anionic sulfonic acid exchanger on the sorbent. The column is subsequently washed with water and a weak aqueous buffer, to selectively remove matrix components and interfering substances from the column. The wash also disrupts the hydrophobic and adsorption interactions but not the ionically bound material. Next, the column is dried to remove traces of aqueous and organic solvents. When the column is dry the analytes of interest are recovered from the column with a basic organic solvent mixture. Following the elution from the SPE column the extract is prepared for confirmation on the GC/MSD.

3.4.5.3 EQUIPMENT AND SUPPLIES

3.4.5.3.1 200mg CLEAN SCREEN® Extraction Column (ZSDAU020 or ZCDAU020 or equivalent)

3.4.5.3.2 Drybath (Fisher or equivalent)

3.4.5.3.3 Evaporative concentrator (Zymark TurboVap® LV, Pierce Reacti-Vap™/Reacti-Therm™ or equivalent) equipped with nitrogen tank.

3.4.5.3.4 Vacuum Manifold/pump

3.4.5.3.5 Glassware

13x100mm Screw Cap Tubes (Fisher 14-959-35C or equivalent)

Screw Cap for 13X100 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 Sample (ALS) vials (HP 5182-0865 or equivalent)

GC/MS Vial Microinsert (HP 5183-2088 or equivalent)

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

3.4.5.3.7 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 GHB and its analogs in toxicological specimens (e.g. 100%-dimethylpolysiloxane or 95%-dimethyl-polysiloxane with 5%-diphenyl).

3.4.5.4 REAGENTS

Refer to manual section 3.8 for solution preparation instructions.

3.4.5.4.1 Deionized/distilled (DI) water

3.4.5.4.2 Methanol (Fisher A412-4 or equivalent)

3.4.5.4.3 Methylene Chloride (Fisher D37-4 or equivalent

3.4.5.4.4 Isopropanol (Fisher A416-1 or equivalent)

3.4.5.4.5 Hexane (Fisher H292-4 or equivalent)
3.4.5.4.6 Ethanol (Fisher A407-4 or equivalent)
3.4.5.4.7 Ammonium Hydroxide (Fisher A669-500 or equivalent)
3.4.5.4.8 100mM Phosphate Buffer (pH 6.0)
3.4.5.4.9 100mM Acetate Buffer (pH 4.5)
3.4.5.4.10 100mM Monobasic sodium phosphate
3.4.5.4.11 100mM Dibasic sodium phosphate
3.4.5.4.12 Elution Solvent
Mix 78mL Methylene Chloride, 20mL Isopropanol and 2mL Ammonium Hydroxide. ***Make fresh.***
3.4.5.4.13 Reconstituting Solvent
Mix equal parts of Hexane and Ethanol.

3.4.5.5 STANDARDS

3.4.5.5.1 Stock Standard Solution
3.4.5.5.1.1 1.0mg/mL Hydrocodone (Cerilliant H-003 or equivalent)
3.4.5.5.1.2 1.0mg/mL Hydromorphone (Cerilliant H-004, Alltech 013553 or equivalent)
3.4.5.5.1.3 1.0mg/mL Oxycodone (Cerilliant O-008, Alltech 013543 or equivalent)

3.4.5.5.2 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.

3.4.5.6 CONTROLS

3.4.5.6.1 Liquid Whole Blood Positive Control (Utak 98818 or equivalent)
3.4.5.6.2 Liquid Whole Blood (Utak 44600-WB (F) or equivalent) spiked with hydrocodone, hydromorphone and/or oxycodone at 5, 50 and/or 100ng/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
5	2
50	20
100	40

3.4.5.7 PROCEDURE

3.4.5.7.1 Initial set-up

Label 200mg CLEAN SCREEN® Extraction Column, test tubes, and GC/MS vials with microinserts for the negative control (NC), positive control (PC), and appropriate laboratory numbers.

3.4.5.7.2 Sample Preparation

- Transfer 2mL sample, negative control and positive control to screw-top extraction tube.
- Add 8mL DI water, vortex, let stand for 5 minutes.
- Centrifuge for 10 minutes.
- Transfer liquid to second tube.
- Add 4mL 100mM phosphate buffer (pH 6.0)
- Sample pH should be 6.0 ±0.5. Adjust as necessary with 100mM monobasic sodium phosphate or 100mM dibasic sodium phosphate.

3.4.5.7.3 Sample Extraction

- Insert labeled 200mg CLEAN SCREEN® Extraction column in the vacuum manifold.
- Add 3mL of methanol to the column and aspirate at ≤ 3 in. Hg to prevent sorbent drying.
- Add 3mL of deionized water to the column and aspirate at ≤ 3 in. Hg.
- Add 2mL of 100mM phosphate buffer (pH 6.0) and aspirate at ≤ 3 in. Hg.
- Load sample on to column at 1 to 2mL/minute.
- Wash column with the following and aspirate at ≤ 3 in. Hg:
  - 2mL of deionized water
  - 2mL 100mM acetate buffer (pH 4.5)
  - 3mL methanol
- Increase vacuum to ≥ 10 in. Hg (≥34 kPa) and dry extraction disc for ≥ 5 minutes.
- Open vacuum manifold, wipe collection tips, and insert the collection rack containing the labeled tapered tip centrifuge tubes.
- Add 6mL elution solvent to the column and aspirate at < 3 in. Hg (<10 kPa).
- Evaporate solvent to dryness, under a gentle stream of nitrogen, in evaporator at ≤ 40°C.

3.4.5.7.4 Reconstitution

- Add 50µL ethyl acetate.

- Cap tube and vortex.
- Transfer extract to labeled GC/MSD ALS vial with microinsert.

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

3.4.5.8.1 Analysis Parameters

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

3.4.5.8.1.2 Analyze sample extract(s) in SIM. Refer to table below.

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

Compound	Ions
Hydrocodone (underivatized)	185*, 199, 214, 228, 242*, 299*.
Hydromorphone	73, 243, 272, 286, 300*, 314, 342*, 357*
Oxycodone	70, 115, 140, 201, 230*, 258*, 315*

*Minimum ions to monitor.

3.4.5.8.2 Detection and Identification Criteria

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

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

3.4.5.9 REFERENCES

3.4.5.9.1 UCT CLEAN SCREEN® Extraction Columns Application Manual

3.4.5.9.2 Platoff, G.E., Gere, J.A. Solid Phase Extraction of Abuse Drugs from Urine, For. Sci. Review, 3 (2):117-132; 1991.

3.4.5.9.3 Hutchison TA & Shahan DR (Eds): DRUGDEX® System. MICROMEDEX, Inc., Greenwood Village, Colorado, 12/01 Edition.

3.4.5.9.4 Baselt RC, Disposition of Toxic Drugs and Chemicals in Man 5th ed., Chemical Toxicology Institute, 2000.

Idaho State Police
Forensic Services
Toxicology Section

Section Three
Blood Toxicology

3.4 Manual Solid Phase Extraction (SPE) Methods
3.4.5 Extraction of Hydrocodone from Blood Employing the United Chemical Technologies (UCT) 200mg CLEAN SCREEN® DAU Extraction Column

Revision #	Issue Date	History
1	04-16-02	Original Issue in SOP format

Approval

Technical Leader: _____ **Date:** _____
S. C. Williamson

Issuance

QC Manager: _____ **Date:** _____
Rick D. Groff

Idaho State Police
Forensic Services
Toxicology Section

Section Three
Blood Toxicology

3.4 Manual Solid Phase Extraction (SPE) Methods

3.4.6 Extraction of Propoxyphene and Norpropoxyphene from Blood Employing the United Chemical Technologies (UCT) 200 mg CLEAN SCREEN® DAU Extraction Column

3.4.6.1 BACKGROUND

Propoxyphene is a narcotic agonist analgesic compound that is structurally similar to methadone. It is used for mild-to-moderate pain including cancer pain, backache, arthritis, headache, and orthopedic pain. It is an effective analgesic with a potency approximately two-thirds that of Codeine. Propoxyphene has been shown to have a high potential for abuse and the chronic use may produce psychic and physical dependence. When used in combination with alcohol and/or other CNS depressants an additive affect is observed. Trade names include Darvon® (hydrochloride salt) and Darvocet-N® (napsylate salt).

Propoxyphene undergoes N-demethylation in the liver to form the active metabolite Norpropoxyphene. Norpropoxyphene has substantially less central-nervous-system depressant effect than propoxyphene, but a greater local anesthetic effect and longer half-life. Potential adverse effects include dizziness, drowsiness, blurred vision, nausea and vomiting.

3.4.6.2 PRINCIPLE

This procedure outlines the use of the UCT 200mg CLEAN SCREEN® extraction column for the extraction of propoxyphene and norpropoxyphene from blood. The CLEAN SCREEN® DAU column utilizes a copolymeric sorbent which combines a cationic exchanger and a hydrophobic functionality (reverse phase) to interact effectively, physically and chemically, with analytes of interest and minimally with interfering substances in the blood sample. The cation exchanger utilizes an anionic (sulfonic acid) sorbent to bind to cations. Additional retention mechanisms include hydrophobic interactions and polar adsorption.

For the extraction of propoxyphene and norpropoxyphene, the blood sample is diluted and centrifuged, adjusted to pH 6 with a phosphate buffer, and loaded onto a pre-conditioned SPE column. The blood pH is adjusted to maximize the ionic character of the analyte. The conditioning

creates an environment which allows for optimal interaction between the sorbent and the analytes of interest. The column is subsequently washed with water and a weak aqueous buffer, to selectively remove matrix components and interfering substances from the column. The wash also disrupts the hydrophobic and adsorption interactions but not the ionically bound material. Next, the column is dried to remove traces of aqueous and organic solvents. When the column is dry the analytes of interest are recovered from the column with a basic organic solvent mixture. Following the elution from the SPE column the extract is prepared for confirmation on the GC/MSD.

3.4.6.3 EQUIPMENT AND SUPPLIES

3.4.6.3.1 200mg CLEAN SCREEN® Extraction Column (ZSDAU020 or ZCDAU020 or equivalent)

3.4.6.3.2 Drybath (Fisher or equivalent)

3.4.6.3.3 Evaporative concentrator (Zymark TurboVap® LV, Pierce Reacti-Vap™/Reacti-Therm™ or equivalent) equipped with nitrogen tank.

3.4.6.3.4 Vacuum Manifold/pump

3.4.6.3.5 Glassware

13x100mm Screw Cap Tubes (Fisher 14-959-35C or equivalent)

Screw Cap for 13X100 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 Sample (ALS) vials (HP 5182-0865 or equivalent)

GC/MS Vial Microinsert (HP 5183-2088 or equivalent)

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

3.4.6.3.7 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 GHB and its analogs in toxicological specimens (e.g. 100%-dimethylpolysiloxane or 95%-dimethyl-polysiloxane with 5%-diphenyl)

3.4.6.4 REAGENTS

Refer to manual section 3.8 for solution preparation instructions.

3.4.6.4.1 Deionized/distilled (DI) water

3.4.6.4.2 Methanol (Fisher A412-4 or equivalent)

3.4.6.4.3 Methylene Chloride (Fisher D37-4 or equivalent

3.4.6.4.4 Isopropanol (Fisher A416-1 or equivalent)

3.4.6.4.5 Hexane (Fisher H292-4 or equivalent)
3.4.6.4.6 Ethanol (Fisher A407-4 or equivalent)
3.4.6.4.7 Ammonium Hydroxide (Fisher A669-500 or equivalent)
3.4.6.4.8 100mM Phosphate Buffer (pH 6.0)
3.4.6.4.9 100mM Acetate Buffer (pH 4.5)
3.4.6.4.10 100mM Monobasic sodium phosphate
3.4.6.4.11 100mM Dibasic sodium phosphate
3.4.6.4.12 Elution Solvent
Mix 78mL Methylene Chloride, 20mL Isopropanol and 2mL Ammonium Hydroxide. ***Make fresh.***
3.4.6.4.13 Ethyl Acetate (Fisher E145-4 or equivalent)

3.4.6.5 STANDARDS

3.4.6.5.1 Stock Standard Solution
3.4.6.5.1.1 1.0mg/mL Propoxyphene (Cerilliant P-011 or equivalent)
3.4.6.5.1.2 1.0mg/mL Norpropoxyphene (Cerilliant N-913 or equivalent)

3.4.6.5.2 Working Standard Solution (5000ng/mL)
3.4.6.5.2.1 Add 50µL Stock Solution to 10mL Methanol.
Solution is stable for 12 months when stored at 4°C.

3.4.6.6 CONTROLS

3.4.6.6.1 Liquid Whole Blood Positive Control (Utak 98818 or equivalent)
3.4.6.6.2 Liquid Whole Blood (Utak 44600-WB (F) or equivalent) spiked with propoxyphene and norpropoxyphene at 5, 100 and/or 300ng/ml level(s) (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

3.4.6.7 PROCEDURE

3.4.6.7.1 Initial set-up

Label 200mg CLEAN SCREEN® Extraction Column, test tubes, and GC/MS vials with microinserts for the negative control (NC), positive control (PC), and appropriate laboratory numbers.

3.4.6.7.2 Sample Preparation

- Transfer 2mL sample, negative control and positive control to screw-top extraction tube.
- Add 8mL DI water, vortex, let stand for 5 minutes.
- Centrifuge for 10 minutes.
- Transfer liquid to second tube.
- Add 4mL 100mM phosphate buffer (pH 6.0)
- Sample pH should be 6.0 ±0.5. Adjust as necessary with 100mM monobasic sodium phosphate or 100mM dibasic sodium phosphate.

3.4.6.7.3 Sample Extraction

- Insert labeled 200mg CLEAN SCREEN® Extraction column in the vacuum manifold.
- Add 3mL of methanol to the column and aspirate and aspirate at ≤ 3 in. Hg to prevent sorbent drying.
- Add 3mL of deionized water to the column and aspirate at ≤ 3 in. Hg.
- Add 2mL of 100mM phosphate buffer (pH 6.0) and aspirate at ≤ 3 in. Hg.
- Load sample on to column at 1 to 2mL/minute.
- Wash column with the following and aspirate at ≤ 3 in. Hg:
- 2mL of deionized water
- 2mL 100mM acetate buffer (pH 4.5)
- 3mL methanol
- Increase vacuum to ≥ 10 in. Hg (≥34 kPa) and dry extraction disc for ≥ 5 minutes.
- Open vacuum manifold, wipe collection tips, and insert the collection rack containing the labeled tapered tip centrifuge tubes.
- Add 6mL elution solvent to the column and aspirate at < 3 in. Hg (<10 kPa).
- Evaporate solvent to dryness, under a gentle stream of nitrogen, in evaporator at ≤ 40°C.

3.4.6.7.4 Reconstitution

- Add 50µL ethyl acetate

- Cap tube and vortex.
- Transfer extract to labeled GC/MSD ALS vial with microinsert.

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

3.4.6.8.1 Analysis Parameters

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

3.4.6.8.1.2 Analyze sample extract(s) in scan or SIM. SIM ions are listed below.

Compound	Ions
Propoxyphene	58*, 91*, 115*, 130, 208, 178, 193, 266
Norpropoxyphene	44*, 220*, 57, 129, 100*, 205, 178, , 307

**Minimum ions to monitor.*

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

3.4.6.8.2 Detection and Identification Criteria

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

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

3.4.6.9 REFERENCES

3.4.6.9.1 UCT CLEAN SCREEN® Extraction Columns Application Manual

3.4.6.9.2 Platoff, G.E., Gere, J.A. Solid Phase Extraction of Abuse Drugs from Urine, For. Sci. Review, 3 (2):117-132; 1991.

3.4.6.9.3 King W & Wang RI: Propoxyphene (Drug Evaluation). In: Hutchison TA & Shahan DR (Eds): DRUGDEX® System. MICROMEDEX, Inc., Greenwood Village, Colorado (Edition expires 12/2001).

3.4.6.9.4 Baselt RC, Disposition of Toxic Drugs and Chemicals in Man 5th ed., Chemical Toxicology Institute, 2000.

Idaho State Police
Forensic Services
Toxicology Section

Section Three
Blood Toxicology

3.4 Manual Solid Phase Extraction (SPE) Methods

3.4.6 Extraction of Propoxyphene and Norpropoxyphene from Blood Employing the United Chemical Technologies (UCT) 200 mg CLEAN SCREEN® DAU Extraction Column

Revision # Issue Date History

1	04-16-02	Original Issue in SOP format

Approval

Technical Leader: _____ **Date:** _____
S. C. Williamson

Issuance

QC Manager: _____ **Date:** _____
Rick D. Groff

Idaho State Police
Forensic Services
Toxicology Section

Section Three
Blood Toxicology

3.4 Manual Solid Phase Extraction (SPE) Methods

3.4.7 Extraction of THC and Carboxy-THC from Blood Employing the United Chemical Technologies (UCT) 200 mg CLEAN SCREEN® THC Extraction Column

3.4.7.1 BACKGROUND

Refer to section 2.4.4.

3.4.7.2 PRINCIPLE

This procedure outlines the use of the 200mg UCT CLEAN SCREEN® THC Column for the extraction from blood of the cannabinoids Δ^9-tetra-hydrocannabinol (THC) and 11-nor-Δ^9-THC-9-COOH (Carboxy-THC). The CLEAN SCREEN® THC column utilizes a copolymeric sorbent which combines a cationic exchanger and a hydrophobic functionality (reverse phase) to interact effectively, physically and chemically, with analytes of interest and minimally with interfering substances in the blood sample. The cation exchanger utilizes an anionic sorbent to bind to cations. Additional retention mechanisms include hydrophobic interactions and polar adsorption.

For the extraction of cannabinoids, the blood sample is diluted and centrifuged, adjusted to pH 4.5 with an acetate buffer, and loaded onto a pre-conditioned SPE column. The conditioning creates an environment which allows for optimal interaction between the sorbent and the analytes of interest. The column is subsequently washed to selectively remove matrix components and interfering substances from the column. Next, the column is dried to remove traces of aqueous and organic solvents. When the column is dry, the analytes of interest are recovered from the column with an organic solvent. Following the elution from the SPE column the extract is derivatized for confirmation on the GC/MSD.

3.4.7.3 EQUIPMENT AND SUPPLIES

3.4.7.3.1 200mg CLEAN SCREEN® THC Extraction Column (ZSTHC020 or equivalent)

3.4.7.3.2 Drybath (Fisher or equivalent)

3.4.7.3.3 Evaporative concentrator (Zymark TurboVap® LV, Pierce Reacti-Vap™/Reacti-Therm™ or equivalent) equipped with nitrogen tank.

3.4.7.3.4 Vacuum Manifold/pump

3.4.7.3.5 Glassware

13x100mm Screw Cap Tubes (Fisher 14-959-35C or equivalent)

Screw Cap for 13X100 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 Sample (ALS) vials (HP 5182-0865 or equivalent)

GC/MS Vial Microinsert (HP 5183-2088 or equivalent)

3.4.7.3.6 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 GHB and its analogs in toxicological specimens (e.g. 100%-dimethylpolysiloxane or 95%-dimethyl-polysiloxane with 5%-diphenyl)

3.4.7.4 REAGENTS

Refer to manual section 3.8 for solution preparation instructions.

3.4.7.4.1 Deionized/distilled (DI) water

3.4.7.4.2 Methanol (Fisher A412-4 or equivalent)

3.4.7.4.3 Hexane (Fisher H292-4 or equivalent)

3.4.7.4.4 Ethyl Acetate (Fisher E145-4 or equivalent)

3.4.7.4.5 Acetonitrile (Fisher A21-1 or equivalent)

3.4.7.4.6 100mM Acetate Buffer (pH 4.5)

3.4.7.4.7 100mM HCl

3.4.7.4.8 Hexane/Ethyl Acetate 85:15

3.4.7.4.9 HCl/Acetonitrile 70:30

3.4.7.4.10 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)

3.4.7.5 STANDARDS

3.4.7.5.1 Stock Standard Solution

3.4.7.5.1.1 100µg/mL (+) 11-nor-9-carboxy-Δ^9-THC (Cerilliant T-006, Alltech 01468 or equivalent).

3.4.7.5.1.2 1mg/mL Δ^9-THC (Cerilliant T-005 or equivalent.

3.4.7.5.2 Working Standard Solution (1000ng/mL)

3.4.7.5.2.1 Add 100µL c-THC Stock Solution and 10uL Δ^9-THC Stock Solution to 10mL Methanol. *Solution is stable for 12 months when stored at 4 °C.*

3.4.7.6 CONTROLS

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

3.4.7.6.2 Liquid Whole Blood (Utak 44600-WB (F) or equivalent) spiked with c-THC and Δ^9-THC at 10, 30 and/or 60ng/ml level(s) (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
10	20
30	60
60	120

3.4.7.7 PROCEDURE

3.4.7.7.1 Initial set-up

Label 200mg CLEAN SCREEN® Extraction Column, test tubes, and GC/MS vials with microinserts for the negative control (NC), positive control (PC), and appropriate laboratory numbers.

3.4.7.7.2 Sample Preparation

- Transfer 2mL sample, negative control and positive control to screw-top extraction tube.
- Add 2mL acetonitrile, vortex, let stand for 5 minutes.
- Centrifuge for 10 minutes.
- Transfer liquid to second tube.
- Add 6mL 100mM acetate buffer (pH 4.5)
- Vortex to mix.
- Centrifuge an addition 5 minutes to remove blood fragments or foam.

3.4.7.7.3 Sample Extraction

- Insert labeled 200mg CLEAN SCREEN® Extraction column in the vacuum manifold.
- Add 3mL Hexane/Ethyl acetate 85:15 and aspirate at ≤ 3 in. Hg to prevent sorbent drying.
- Add 3mL of methanol to the column and aspirate at ≤ 3 in. Hg.
- Add 3mL of deionized water to the column and aspirate at ≤ 3 in. Hg.
- Add 1mL of 100mM HCl and aspirate at ≤3 in. Hg.
- Load sample onto column and allow to gravity flow or apply minimal vacuum.
- Wash column with the following and aspirate at ≤ 3 in. Hg:
  - 2mL of deionized water
  - 2mL 100mM HCl/Acetonitrile [70:30]
- Increase vacuum to ≥10 in. Hg (≥34 kPa) and dry extraction disc for ≥ 5 minutes.
- Open vacuum manifold, wipe collection tips, and insert the collection rack containing the labeled tapered tip centrifuge tubes.
- Add 200uL hexane (do not allow to dry use no flow!!).
- Add 6mL hexane/ethyl acetate (85:15) elution solvent to the column and collect eluate with gravity flow or apply minimal vacuum.
- Slowly evaporate solvent to dryness, under a gentle stream of nitrogen, in evaporator at ≤ 40°C.

3.4.7.7.4 Derivatization

- In fume hood add the following:
  - 25µL ethyl acetate.
  - 25µL silylating agent.
- 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.

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

3.4.7.8.1 Analysis Parameters

3.4.7.8.1.1 Inject 1µL to 2µL into GC/MS using the ALS.

3.4.7.8.1.2 Analyze sample extract(s) in SIM. Refer to table below.

Compound	Ions
THC	303, 315, 386
Carboxy-THC	371, 473, 488

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

3.4.7.8.2 Detection and Identification Criteria

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

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

3.4.7.9 REFERENCES

3.4.7.9.1 UCT CLEAN SCREEN® Extraction Columns Application Manual

Idaho State Police
Forensic Services
Toxicology Section

Section Three
Blood Toxicology

3.4 Manual Solid Phase Extraction (SPE) Methods
3.4.7 Extraction of THC and Carboxy-THC from Blood Employing the United Chemical Technologies (UCT) 200 mg CLEAN SCREEN® THC Extraction Column

Revision #	Issue Date	History
1	04-16-02	Original Issue in SOP format

Approval

Technical Leader: _____ **Date:** _____
S. C. Williamson

Issuance

QC Manager: _____ **Date:** _____
Rick D. Groff

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 along with their metabolites. This method prepares an extract which will be subjected to confirmatory analysis with a gas chromatograph with a mass selective detector (GC\MSD). 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 Derivatizing Reagent Options

- BSTFA (Pierce 38830 or equivalent)
- BSTFA + 1% TMCS (Pierce 38831 or equivalent)
- TFAA (Pierce 67363 or equivalent)

3.6.1.5 STANDARDS

3.6.1.5.1 Stock Standard Solution

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)

Add 50µL Stock Solution to 10mL Methanol.

Solution is stable for 12 months when stored at 4 °C.

3.6.1.6 QUALITATIVE CONTROLS

3.6.1.6.1 **Positive Control**

Options for a whole blood positive control include the following:

- Utak 98818 or equivalent
- Utak 44600-WB (F) or equivalent spiked with working standard solution of compounds of interest at 50, 100

and/or 300ng/mL. 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

Other levels and/or unextracted standards may be used as required.

3.6.1.6.2 **Negative Control**
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 control(s) and appropriate laboratory numbers.

3.6.1.7.2 Sample Preparation
- Transfer 2mL sample and control(s) to screw-top extraction tube.
- Pipette 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/MSD using the ALS.

3.6.1.8.1.2 Analyze sample extract(s) in full scan acquisition or SIM, monitoring a minimum of three ions (target and two qualifier ions). Appropriate ions may be selected through the examination of reference standard mass spectra analyzed in full scan monitoring and/or toxicology literature sources. The selected ions should be prominent and characteristic of the compound. Higher mass ions are typically more diagnostic. The molecular ion should be included when feasible.

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

3.6.1.8.2 Criteria to Designate a Positive Result

3.6.1.8.2.1 **Full Scan Acquisition**

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

- Acceptable retention time window is +/- 2% of the standard's retention time.

3.6.1.8.2.2 **Qualitative Selected Ion Monitoring (SIM)**

The presence of a drug compound can be established if there are no significant differences in the retention time and the ion ratios for casework sample versus reference standard.

- Acceptable retention time window is +/- 2% of the standard's retention time.
- Ratios between a minimum of three monitored ions should agree within ±20% of the standard's ratios.

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.

3.6.1.9.2 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.

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.

Revision #	Issue Date	History
1	04-25-02	Original Issue in SOP format
2	05-27-03	Updated, Clarifications

Approval

Technical Leader: _____ Date: _____
S. C. Williamson

Issuance

QC Manager: _____ Date: _____
Rick D. Groff

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 and Neutral Drugs from Blood.

3.6.2.1 BACKGROUND

This method is a general blood extraction procedure for a variety of commonly encountered acidic and neutral drugs along with their metabolites. This method prepares an extract for confirmatory analysis with gas chromatograph equipped with a mass selective detector (GC/MSD).

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 first 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. If sample is clean, this back extraction step may be omitted. 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/MSD 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

3.6.2.4.9 Iprindole (Wyeth-Alerst S-455-A-2 or equivalent)

3.6.2.5 STANDARDS

3.6.2.5.1 Stock Standard Solution

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

3.6.2.5.2 Working Standard Solution (5000ng/mL)

Add 50µL Stock Solution to 10mL Methanol.

Solution is stable for 12 months when stored at 4 °C.

3.6.2.6 QUALITATIVE CONTROLS

3.6.2.6.1 **Positive Control**

Options for a whole blood positive control include the following:

- Utak 98818 or equivalent
- Utak 44600-WB (F) or equivalent spiked with working standard solution of compounds of interest at 50, 100 and/or 300ng/mL. 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

Other levels and/or unextracted standards may be used as needed.

3.6.2.6.2 **Negative Control**
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 a minimum of three ions (target and two qualifier ions). Appropriate ions may be selected through the examination of reference standard mass spectra analyzed in full scan monitoring and/or toxicology literature sources. The selected ions should be prominent and characteristic of the compound. Higher mass ions are typically more diagnostic. The molecular ion should be included when feasible.

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

3.6.2.8.2 Detection and Identification Criteria

3.6.2.8.2.1 **Full Scan Acquisition**

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

- Acceptable retention time window is +/- 2% of the standard's retention time.

3.6.2.8.2.2 **Ion ratios - Qualitative Selective Ion Monitoring (SIM)**

The presence of a drug compound can be established if there are no significant differences in the retention time and the ion ratios for casework sample versus reference standard.

- Acceptable retention time window is +/- 2% of the standard's retention time.
- Ratios between a minimum of three monitored ions should agree within ±20% of the standard's ratios.

3.6.2.9 REFERENCES

3.6.2.9.1 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.

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.

Revision #	Issue Date	History
1	04-25-02	Original Issue in SOP format
2	05-27-03	Updated, Clarifications

Approval

Technical Leader: _____ **Date:** _____
S. C. Williamson

Issuance

QC Manager: _____ **Date:** _____
Rick D. Groff

Idaho State Police
Forensic Services
Toxicology Section

Section Three
Blood Toxicology

3.6 Liquid-Liquid Extraction Methods for GC/MSD Confirmation

3.6.3 Liquid-Liquid Extraction and Derivatization Procedure for the Detection of Amphetamine and Methamphetamine in Blood

3.6.3.1 BACKGROUND

Amphetamine and methamphetamine are sympathomimetic drugs that mimic the actions of naturally occurring stimulatory neurotransmitters. Although still prescribed for the treatment of attention deficit disorder (ADD), narcolepsy, and obesity, these compounds have a high potential for abuse. Methamphetamine is produced clandestinely often through the reduction of ephedrine/pseudoephedrine. Psychological side effects may include agitation, nervousness, restlessness, paranoia and an increased sense of power. Physiological effects may include mydriasis, loss of appetite, insomnia, increased blood pressure and heart rate. The manifestation of adverse affects is dependent on the time since drug administration. The drugs may be introduced into the system through inhalation (smoking and snorting) or intravenous injection.

3.6.3.2 PRINCIPLE

The method is based upon the principle of liquid/liquid extraction. At a basic pH, sympathomimetic amines are unionized and thus extract into organic solvents. In this procedure, the sample pH is adjusted with a pH 9.5 saturated borate buffer and extracted with n-butyl chloride. The evaporated extract is derivatized for confirmation on the GC/MS using SIM and/or full scan monitoring.

3.6.3.3 EQUIPMENT AND SUPPLIES

3.6.3.3.1 Drybath (Fisher or equivalent)

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

3.6.3.3.3 Vacuum Manifold/pump

3.6.3.3.4 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)

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

3.6.3.3.6 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)

3.6.3.4 REAGENTS

Refer to Manual section 3.8 for solution preparation

3.6.3.4.1 n-Butyl chloride (Fisher B416-1 or equivalent)

3.6.3.4.2 Ethyl Acetate (Fisher E145-4 or equivalent)

3.6.3.4.3 1% Hydrochloric Acid in Methanol

3.6.3.4.4 Saturated Borate Buffer (pH 9.5)

3.6.3.4.5 Derivatizing Agents - Select from the following:

- Trifluoroacetic Acid Anhydride (TFAA) (Pierce 67363)
- Heptafluorobutyric Acid Anhydride (HFAA) (Pierce 63164 or equivalent)

3.6.3.5 STANDARDS

3.6.3.5.1 Stock Standard Solutions

Stock (1.0mg/mL)	Potential Vendors*
Methamphetamine	Cerilliant M-009, Alltech 010013
Amphetamine	Cerilliant A-007, Alltech 010023
MDMA	Cerilliant M-013, Alltech 014093
MDA	Cerilliant M-012, Alltech 014603
Phenylpropanolamine	Cerilliant P-038, Alltech 6017803
Phentermine	Cerilliant P-023, Alltech 017833
Ephedrine	Cerilliant E-024, Alltech 017403
Pseudoephedrine	Cerilliant P-035, Alltech 6013213

*or equivalent.

3.6.3.5.2 Working Standard Solution (5000ng/mL)

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

3.6.3.6 CONTROLS

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

3.6.3.6.2 Liquid Whole Blood (Utak 44600-WB (F) or equivalent) spiked with amphetamine, and/or methamphetamine at 50, 100 and/or 300ng/ml (other levels and standards 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

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

3.6.3.7 PROCEDURE

3.6.3.7.1 Initial set-up

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

3.6.3.7.2 Sample Preparation

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

3.6.3.7.3 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.

3.6.3.7.4 Evaporation

- Add 2-5 drops 1% HCl in methanol.
- Evaporate under a gentle stream of nitrogen at ≤37°C to *near* dryness.

3.6.3.7.5 Derivatization

- Finish drying under nitrogen at room temperature.

- As each sample dries, *immediately* add 50uL of TFAA or HFAA derivatizing agent to the residue.
- Cap and vortex.
- Heat samples at 70°C for 20 minutes.
- Evaporate samples to dryness with nitrogen at room temperature.
- Pipet 50ul ethyl acetate to each sample
- Vortex.
- Transfer extract to labeled GC/MSD ALS vial with microinsert.

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

3.6.3.8.1 Analysis Parameters

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

3.6.3.8.1.2 Analyze sample extract(s) in full scan acquisition or SIM monitoring the appropriate ions (refer to table below).

Compound	Ions
Amphetamine-TFAA	65, 91*, 92, 117, 118*, 140*.
Methamphetamine-TFAA	65, 91*, 110*, 118*, 154*.
Pseudoephedrine-TFAA	69, 91*, 110*, 118*, 154*
Phentermine-TFAA	65, 91*, 114, 118, 132*, 154*
MDMA-TFAA	77, 110, 135, 154*, 162*, 289*

* *minimum ions to monitor.*

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

3.6.3.8.2 Detection and Identification Criteria

3.6.3.8.2.1 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%.

Idaho State Police
Forensic Services
Toxicology Section

Section Three
Blood Toxicology

3.6 Liquid-Liquid Extraction Methods for GC/MSD Confirmation
3.6.3 Liquid-Liquid Extraction and Derivatization Procedure for the Detection of Amphetamine and Methamphetamine in Blood

Revision #	Issue Date	History
1	04-25-02	Original Issue in SOP format

Approval

Technical Leader: _____ **Date:** _____
S. C. Williamson

Issuance

QC Manager: _____ **Date:** _____
Rick D. Groff

Idaho State Police
Forensic Services
Toxicology Section

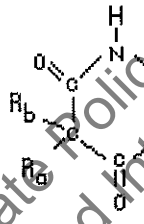
Section Three
Blood Toxicology

3.6 Liquid-Liquid Extraction Methods for GC/MSD Confirmation

3.6.4 Liquid-Liquid Extraction Procedure for the Detection of Barbiturate Class Drugs in Blood

3.6.4.1 BACKGROUND

Barbiturate class compounds are nonselective central nervous system depressants which are used therapeutically for their sedative-hypnotic and anticonvulsant properties. They can are also used in migraine therapy and the reduction of cerebral edema secondary to head injury. The strength and duration of action varies with R-group substitution (see chart below).



DRUG	R_a	R_b	Y	Z	Trade Name®	Duration	Therapeutic Uses
Amobarbital	ethyl	isopentyl	O	H	Amytal	intermediate	sedation, anticonvulsant (convulsive)
Aprobarbital	propylene	isopropyl	O	H	Alurate	short-intermediate	sedation, insomnia
Butobarbital	ethyl	sec-butyl	O	H	Butisol Sodium	intermediate	sedation, pre-operation sedation, insomnia
Butalbital	allyl	isobutyl	O	H	Fiorinal	short-intermediate	dental pain, tension headache
Mephobarbital	ethyl	phenyl	O	H	Mebaral		anticonvulsant (generalized tonic-clonic seizures and absence seizures) - additional methyl group replaces the hydrogen on the nitrogen
Pentobarbital	ethyl	1-methylbutyl	O	H	Nembutal Sodium	short	anesthesia adjunctive, insomnia, epilepsy
Phenobarbital	ethyl	phenyl	O	H	Luminal	long	sedative for relief of anxiety, tension, epilepsy, hypnotic for insomnia
Primidone	ethyl	phenyl	H	H	Mysoline		anticonvulsant (Good for all types of seizers except for absent)
Secobarbital	allyl	1-methylbutyl	O	H	Seconal	Short	insomnia, pre-op sedation, epilepsy

The ultrashort-acting barbiturates are very potent and are used as anesthesia during surgical procedures whereas the short and intermediate acting barbiturates are typically used as sedative-hypnotics. The use of barbiturates has declined, because they produce significant CNS-depression and thus impairing affects. This has lead with the development of other compounds such as the benzodiazepines.

3.6.4.2 PRINCIPLE

Barbiturates are weakly acidic compounds and therefore 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.

3.6.4.3 EQUIPMENT AND SUPPLIES

3.6.4.3.1 Drybath (Fisher or equivalent)

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

3.6.4.3.3 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)

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

3.6.4.3.5 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)

3.6.4.4 REAGENTS

Refer to manual section 3.8 per solution preparation instructions.

3.6.4.4.1 n-Butyl chloride (Fisher B416-1 or equivalent)

3.6.4.4.3 Concentrated Hydrochloric Acid (Fisher A144-500)
3.6.4.4.2 Hexane (Fisher H292-4 or equivalent)
3.6.4.4.4 200 proof Ethanol (Acros 45, 984-4 or equivalent)
3.6.4.4.5 Hexane/Ethanol 1:1
3.6.4.4.6 0.45N Sodium Hydroxide

3.6.4.5 STANDARDS

3.6.4.5.1 Stock Standard Solutions

Stock (1.0mg/mL)	Potential Vendors*
Butalbital	Cerilliant B-006
Butabarbital	Cerilliant B-024
Pentobarbital	Cerilliant P-010
Phenobarbital	Cerilliant P-008
Secobarbital	Cerilliant S-002
Amobarbital	Cerilliant A-020

**or equivalent.*

3.6.4.5.2 Working Standard Solution (5000ng/mL)

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

3.6.4.6 CONTROLS

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

3.6.4.6.2 Liquid Whole Blood (Utak 44600-WB (F) or equivalent) spiked with butalbital, butabarbital, pentobarbital, phenobarbital, and/or secobarbital at 50, 300 and/or 500ng/ml (other levels and standards 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
300	120
500	200

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

3.6.4.7 PROCEDURE

3.6.4.7.1 Initial set-up

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

3.6.4.7.2 Sample Preparation

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

3.6.4.7.3 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 3.6.4.7.6

3.6.4.7.4 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.

3.6.4.7.5 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.

3.6.4.7.6 Evaporation and reconstitution

- Evaporate under a gentle stream of nitrogen at ≤37°C.
- Add 100uL of 1:1 hexane/ethanol.
- Vortex.
- Transfer extract to labeled GC/MSD ALS vial with microinsert.

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

3.6.4.8.1 Analysis Parameters

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

3.6.4.8.1.2 Analyze sample extract(s) in full scan acquisition or SIM monitoring the appropriate ions (refer to chart below).

Compound	Ions
Amobarbital	98, 141*, 142, 156*, 157, 183, 197*.
Aprobarbital	97*, 124*, 153, 167*, 168, 169, 195
Butalbital	124, 141*, 153, 167*, 168*, 209
Butobarbital	41*, 55, 98, 141*, 142, 155, 156*.
Mephobarbital	103, 115, 117*, 118, 146*, 218*, 246
Pentobarbital	156*, 141*, 157, 98, 197*, 69
Phenobarbital	204*, 117*, 232*, 174, 103, 115,161, 146, 217.
Primidone	77, 91, 103, 117, 146, 161, 190,
Secobarbital	97, 124, 153, 167*, 168*, 169, 170,195*, 209

**minimum ions to monitor*

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

3.6.4.8.2 Detection and Identification Criteria

3.6.4.8.2.1 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%.

3.6.4.9 REFERENCES

3.6.4.9.1 Hutchison TA & Shahan DR (Eds): DRUGDEX® System. MICROMEDEX, Inc., Greenwood Village, Colorado, 12/01 Edition.

3.6.4.9.2 Baselt RC, Disposition of Toxic Drugs and Chemicals in Man. 5th ed., Chemical Toxicology Institute, 2000.

3.6.4.9.3 Baselt RC, Drug Effects on Psychomotor Performance, Biomedical Publications, 2001.

3.6.4.9.4 Barry Levine (Eds): Principles of Forensic Toxicology, American Association for Clinical Chemistry, Inc, 1999.

3.6.4.9.5 Moffat, AC, Jackson JV, Moss MS et al. (Eds): Clark's Isolation and Identification of Drugs, 2nd ed., The Pharmaceutical Press, 1986.

Idaho State Police
Forensic Services
Toxicology Section

Section Three
Blood Toxicology

3.6 Liquid-Liquid Extraction Methods for GC/MSD Confirmation
3.6.4 Liquid-Liquid Extraction Procedure for the Detection of Barbiturate Class Drugs in Blood

Revision #	Issue Date	History
1	04-25-02	Original Issue in SOP format

Approval

Technical Leader: _____ **Date:** _____
S. C. Williamson

Issuance

QC Manager: _____ **Date:** _____
Rick D. Groff

Idaho State Police
Forensic Services
Toxicology Section

Section Three
Blood Toxicology

3.6 Liquid-Liquid Extraction Methods for GC/MSD Confirmation

3.6.5 Liquid-Liquid Extraction and Derivatization Procedure for the Detection of Carboxy-THC in Blood

3.6.5.1 BACKGROUND

Refer to section 2.4.4.

3.6.5.2 PRINCIPLE

This method utilizes a protein precipitation and liquid/liquid extraction to separate and identify 11-nor-Δ^9-THC-9-COOH (Carboxy-THC). The sample is treated with acetonitrile to precipitate out the proteins. Following centrifugation, the sample pH is adjusted to ≤6 with concentrated HCl to convert analytes back to a non-ionic form for a final extraction with hexane/ethyl acetate. The evaporated extract is derivatized for confirmation on the GC/MS using SIM and/or full scan monitoring.

3.6.5.3 EQUIPMENT AND SUPPLIES

3.6.5.3.1 Drybath (Fisher or equivalent)

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

3.6.5.3.3 Vacuum Manifold/pump

3.6.5.3.4 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)

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

3.6.5.3.6 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)

3.6.5.4 REAGENTS

Refer to manual section 3.8 per solution preparation instructions.

3.6.5.4.1 Acetonitrile (Fisher A21-1 or equivalent)

3.6.5.4.2 Hexane (Fisher H292-4 or equivalent)

3.6.5.4.3 Ethyl Acetate (Fisher E145-4 or equivalent)

3.6.5.4.4 Concentrated HCl

3.6.5.4.5 Extraction Solvent

Mix 9 parts Hexane with 1 part Ethyl Acetate.

3.6.5.4.6 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)

3.6.5.5 STANDARDS

3.6.5.5.1 Stock Standard Solution

3.6.5.5.1.1 100µg/mL (+) 11-nor-9-carboxy-Δ^9-THC (Cerilliant T-006, Alltech 01468 or equivalent

3.6.5.5.2 Working Standard Solution (1000ng/mL)

3.6.5.5.2.1 Add 100µL Stock Solution to 10mL Methanol.

Solution is stable for 6 months when stored at 4°C.

3.6.5.6 CONTROLS

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

3.6.5.6.2 Liquid Whole Blood (Utak 44600-WB (F) or equivalent) spiked with c-THC at 10, 30 and/or 60ng/ml level(s) (other levels may be used as needed). To 2mL of negative blood add working standard solution as indicated below. Prepare standard and allow equilibrate for 1 hour prior to extraction.

Desired ng/mL	µL Working Standard Solution
10	20
30	60
60	120

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

3.6.5.7 PROCEDURE

3.6.5.7.1 Initial set-up

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

3.6.5.7.2 Sample Preparation

- Transfer 2mL sample, negative control and positive control to screw-top extraction tube.

3.6.5.7.3 Protein Precipitation

- While vortexing, pipet 4mL acetonitrile into each tube.
- Vortex for an additional 30 seconds.
- Centrifuge for ≥10 minutes.
- Transfer the supernatant to a second tube.

3.6.5.7.4 Extraction

- Reduce the solvent to ~1mL under nitrogen at ≤37°C.
- Add 1mL 1N HCl.
- Add 6mL hexane/ethyl acetate (9:1).
- Cap and extract for 30 minutes.
- Centrifuge for ≥10 minutes.
- Transfer the top layer into centrifuge tube with disposable glass pipet.

3.6.5.7.5 Evaporation and Derivatization

- Evaporate under a gentle stream of nitrogen at ≤37°C.
- In fume hood add the following:
  - 25µL ethyl acetate.
  - 25µL silylating agent.
- 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.

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

3.6.5.8.1 Analysis Parameters

3.6.5.8.1.1 Inject 1µL to 2µL into GC/MS using the ALS.

3.6.5.8.1.2 Analyze sample extract(s) in SIM. Refer to table below.

Compound	Ions
Carboxy-THC	371, 473, 488

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

3.6.5.8.2 Detection and Identification Criteria

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

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

Idaho State Police
Forensic Services
Toxicology Section

Section Three
Blood Toxicology

3.6 Liquid-Liquid Extraction Methods for GC/MSD Confirmation
3.6.5 Liquid-Liquid Extraction and Derivatization Procedure for the Detection of Carboxy-THC in Blood

Revision #	Issue Date	History
1	04-16-02	Original Issue in SOP format

Approval

Technical Leader: _____ **Date:** _____
S. C. Williamson

Issuance

QC Manager: _____ **Date:** _____
Rick D. Groff

Idaho State Police
Forensic Services
Toxicology Section

Section Three
Blood Toxicology

3.7 Guidelines for the Identification of Compounds in Blood

3.7.1 BACKGROUND

This section describes the criteria that must be met in order to establish the presence of a compound in blood.

3.7.2 IDENTIFICATION GUIDELINES FOR SCREENS

3.7.2.1 OraSure (STC)/PersonalLAB Enzyme Immunoassay (EIA) Screen

3.7.2.1.1 A positive indication for the compound of interest is designated when the concentration of the compound exceeds the administrative cutoff. This cutoff is specific for each drug-of-abuse class or compound.

3.7.2.1.2 This requirement applies only to compounds possessing sufficient cross-reactivity such that a presumptive presence can be correctly evaluated on the basis of EIA.

3.7.2.1.3 The cross-reactivity displayed by individual compounds for a particular assay are indicated in STC Technologies Technical Bulletins. As indicated in Section One {1.1.3.6.1}, the decision to proceed with confirmatory testing is at the discretion of the analyst.

3.7.2.1.4 Refer to manual section one for further information on the OraSure (STC)/PersonalLAB Enzyme Immunoassay (EIA) Screen.

3.7.2.2 Gas Chromatograph/Nitrogen Phosphorus Detector (GC-NPD) Screen

3.7.2.2.1 The presumptive presence of a drug compound can be established if the following criteria are met:

- There are no significant differences in the relative retention time (RRT) for the sample versus standards.
- All standards run should have comparable RRT established for the instrument.
- Acceptable RRT window is +/-5%.

3.7.3 IDENTIFICATION GUIDELINES FOR CONFIRMATORY TESTING

3.7.3.1 Gas Chromatograph - Mass Selective Detector (GC/MSD) Confirmation

3.7.3.1.1 A positive indication for the compound of interest is indicated if the retention time and mass spectral characteristics meet identification criteria.

3.7.3.1.2 Refer to individual SOPs for further information on GC/MSD identification criteria.

Idaho State Police
Forensic Services
Toxicology Section

Section Three
Blood Toxicology

3.7 Guidelines for the Identification of Compounds in Blood

Revision #	Issue Date	History
1	04-25-02	Reformatted in SOP format

Approval

Technical Leader: _____ **Date:** _____
S C Williamson

Issuance

QC Manager: _____ **Date:** _____
Rick D. Groff

Idaho State Police
Forensic Services
Toxicology Section

Section Three
Blood Toxicology

3.8 Solution Preparation

3.8.1 SCOPE

This section describes the proper preparation of solutions and buffers used in the extraction of drug compounds from blood specimens.

3.8.2 EQUIPMENT AND SUPPLIES

3.8.2.1 Glassware
Adequately sized beakers, volumetric flasks, graduated cylinders and volumetric pipettes

3.8.2.2 Laboratory balance

3.8.2.3 pH Meter

3.8.2.4 Stirring hotplate with magnetic stirrer

3.8.2.5 Safety Equipment

- Chemical Fume Hood
- Acid Resistant Apron
- Laboratory Coat
- Safety Goggles
- Laboratory Gloves

3.8.3 CHEMICALS

3.8.3.1 Acids

- Hydrochloric (Fisher A144-500 or equivalent)
- Sulfuric (Fisher A300-500 or equivalent)

3.8.3.2 Salts

- Sodium Acetate Trihydrate(Fisher S209-3 or equivalent)
- Sodium Hydroxide (Fisher S318-3 or equivalent)
- Sodium Phosphate Monobasic (Fisher S369-3 or equivalent)
- Sodium Phosphate Dibasic (Fisher S374-3 or equivalent)

3.8.3.3 Solvents

- Methanol (Fisher A412-4 or equivalent)

3.8.4 PROCEDURES

Note: appropriate safety equipment should be worn during the preparation of solutions to prevent exposure to caustic/corrosive solutions. The order of the addition of chemicals may be crucial to prevent exothermic reactions. Please follow the procedures verbatim.

3.8.4.1 Acetic Acid (HOAc)

3.8.4.1.1 0.1M/100mM Acetic Acid (100mL)

Place approximately 300mL distilled/deionized (DI) water into a 500mL volumetric flask. Add 2.9mL **glacial HOAc**, mix. QS to 500mL.
Solution is stable for six months.

3.8.4.2 Acetate Buffer

3.8.4.2.1 0.1M/100mM Acetate Buffer, pH 4.5 (500mL)

Dissolve 2.93g **sodium acetate trihydrate** in 400mL DI water. Add 1.62mL **glacial acetic acid**, and mix well. QS to 500mL. Adjust to pH 4.5±0.1 with **100mM sodium acetate** or **100mM acetic acid.**
Solution is stable for six months.

3.8.4.3 Hydrochloric Acid (HCl)

3.8.4.3.1 100mM HCl (500mL)

Place approximately 300mL DI water into a 500mL volumetric flask. Add 4.2mL **concentrated hydrochloric acid**, mix. QS to 500mL.
Solution is stable for one year

3.8.4.3.2 1N HCl (500mL)

Place approximately 400mL DI water into a 500mL volumetric flask. Add 42mL **concentrated hydrochloric acid**, mix. QS to 500mL.
Solution is stable for one year.

3.8.4.3.3 1% HCl in Methanol

Place approximately 30mL **MeOH** in a 50mL volumetric flask. Add 0.5mL concentrated **HCl.** QS to 50mL. Store at 5°C
Solution is stable for six months.

3.8.4.4 Sodium Hydroxide (NaOH)

Note: The addition of NaOH to water will generate heat, exercise due caution.

3.8.4.4.1 0.45N NaOH (500mL)

Gradually add 9g **NaOH** in 500mL DI water. (Caution: Exothermic)
Solution is stable for one year.

3.8.4.4.2 **10N NaOH (500mL)**
Place approximately 250mL DI water into a 1000mL beaker. Gradually add 200g **NaOH.** Transfer to 500mL volumetric flask and QS to 500mL. (Caution: Exothermic)
Solution is stable for one year.

3.8.4.5 Saturated Sodium Borate Buffer , pH >9.5 (500mL)
Place 250mL DI water into a 500mL beaker. Stir while adding **sodium borate** until solution is saturated.
Solution is stable for six months.

3.8.4.6 100mM Sodium Phosphate Dibasic (200mL)
Dissolve 2.84g **sodium phosphate dibasic** in ≈160mL DI water. QS to 200mL and mix.
Solution is stable for 1 month. Store in glass container.

3.8.4.7 100mM Sodium Phosphate Monobasic (200mL)
Dissolve 2.76g **sodium phosphate dibasic** in ≈160mL DI water. QS to 200mL and mix.
Solution is stable for 1 month. Store in glass container.

3.8.4.8 Phosphate Buffer
0.1M/100mM Phosphate Buffer, pH 6 (250mL)
Dissolve 0.42g **sodium phosphate dibasic** (Na_2HPO_4) and **3.03 sodium phosphate monobasic** ($NaH_2PO_4 \cdot H_20$) in approximately 200mL DI water in a 250mL volumetric flask. QS to 250mL. Adjust to pH 6.0 ±0.1 with 100mM **monobasic sodium phosphate** (to lower pH) or 100mM **dibasic sodium phosphate** (to raise the pH).
Solution is stable for 1 month. Store in glass container.

3.8.4.9 Sulfuric Acid (H_2SO_4)
1N Sulfuric Acid (H_2SO_4) (500mL)
Place approximately 400mL DI water into a 500mL volumetric flask. Add 13.4mL **concentrated H_2SO_4**, mix. QS to 500mL.
Solution is stable for one year.

3.8.5 REFERENCES

3.8.5.1 Shugar, G.J., Shugar, R.A. and Bauman, L. *Grades of Purity of Chemicals* pp. 145-154, *pH Measurement.* pp. 232-234. *in:* Chemical Technicians' Ready Reference Handbook, McGraw Hill: New York, 1973.

3.8.5.2 Ansys, Inc. SPEC Extraction Methods

3.8.5.3 United Chemical Technologies, Inc. Applications Manual

Idaho State Police
Forensic Services
Toxicology Section

Section Three
Blood Toxicology

3.8 Solution Preparation

Revision #	Issue Date	History
0	04-25-02	Original Issue

Approval

Technical Leader: _____ **Date:** _____
S. C. Williamson

Issuance

QC Manager: _____ **Date:** _____
Rick D. Groff

Idaho State Police
Forensic Services

Approval for Quality System Controlled Documents

Discipline/Name of Document: Toxicology
3.8 -Solution Preparation

Revision Number: 1

Issue Date: 11/21/2006

APPROVED BY: _____
Quality Manager

11/21/06
Date Signed

Idaho State Police
Forensic Services
Toxicology Discipline

Section Three
Blood Toxicology

3.8 Solution Preparation

3.8.1 SCOPE

This section describes the proper preparation of solutions and buffers used in the extraction of drug compounds from blood specimens.

3.8.2 EQUIPMENT AND SUPPLIES

3.8.2.1 Glassware
Adequately sized beakers, volumetric flasks, graduated cylinders and volumetric pipettes
3.8.2.2 Laboratory balance
3.8.2.3 pH Meter
3.8.2.4 Appropriate pH buffer solutions
3.8.2.5 Stirring hotplate with magnetic stirrer
3.8.2.6 Safety Equipment
3.8.2.6.1 Chemical Fume Hood
3.8.2.6.2 Acid Resistant Apron
3.8.2.6.3 Laboratory Coat
3.8.2.6.4 Safety Goggles
3.8.2.6.5 Laboratory Gloves

3.8.3 CHEMICALS

3.8.3.1 Acids

- Acetic, Glacial (Fisher A38-500 or equivalent)
- Hydrochloric (Fisher A144-500 or equivalent)
- Sodium Acetate Trihydrate (Fisher S209-3 or equivalent)
- Sulfuric (Fisher A300-500 or equivalent)

3.8.3.2 Salts

- Ammonium Chloride (Fisher A661-500 or equivalent)
- Potassium Hydroxide (Fisher P250-500 or equivalent)
- Potassium Phosphate Monobasic (Fisher P285-3 or equivalent)
- Potassium Phosphate Dibasic (Fisher P288-3 or equivalent)
- Sodium Acetate Trihydrate(Fisher S209-3 or equivalent)
- Sodium Hydroxide (Fisher S318-3 or equivalent)

- Sodium Phosphate Monobasic (Fisher S369-3 or equivalent)
- Sodium Phosphate Dibasic (Fisher S374-3 or equivalent)
- Sodium Tetraborate Decahydrate (Fisher S249-500 or equivalent)

3.8.3.3 Solvents

- Methanol (Fisher A412-4 or equivalent)

3.8.4 PROCEDURES

Note: appropriate safety equipment should be worn during the preparation of solutions to prevent exposure to caustic/corrosive solutions. The order of the addition of chemicals may be crucial to prevent exothermic reactions. Please follow the procedures verbatim.

3.8.4.1 Acetic Acid (HOAc)

3.8.4.1.1 **0.1M/100mM Acetic Acid (500mL)**

Place approximately 300mL distilled/deionized (DI) water into a 500mL volumetric flask. Add 2.9mL **glacial HOAc**, mix. QS to 500mL.

Solution is stable for six months.

3.8.4.1.2 **1.0M Acetic Acid (500mL)**

Place approximately 300mL distilled/deionized (DI) water into a 500mL volumetric flask. Add 29mL **glacial HOAc**, mix. QS to 500mL.

Solution is stable for six months.

3.8.4.2 Acetate Buffer

3.8.4.2.1 **0.1M/100mM Acetate Buffer, pH 4.5 (500mL)**

Dissolve 2.93g **sodium acetate trihydrate** in 400mL DI water. Add 1.62mL **glacial acetic acid**, and mix well. QS to 500mL. Adjust to pH 4.5±0.1 with **100mM sodium acetate** or **100mM acetic acid.**

Solution is stable for six months.

3.8.4.3 Ammonium Chloride

3.8.4.3.1 **Saturated Ammonium Chloride (500mL)**

Place approximately 300mL DI water in a beaker and heat/stir over low heat. Add **ammonium chloride** until the solution is saturated. QS to 500mL.

Solution is stable for 6-months.

3.8.4.4 Borate Buffer

3.8.4.4.1 **Saturated Borate Buffer , pH >9.5 (500mL)**

Place ≅250mL DI water into a 500mL volumetric flask. Stir while adding **sodium tetraborate (≅60g)** until solution is saturated. QS.

Solution is stable for six months.

3.8.4.4.2 **Borate Buffer, pH 9.2**

Place ≅500mL DI water into a 1000mL beaker. Heat and stir while adding 50g sodium tetraborate ($Na_2B_4O_7 \cdot 10\ H_2O$). Once dissolved, allow to cool. Bring volume up to ≅950mL with DI water. Verify pH and adjust as necessary to pH 9.2 ±0.2 with 1N NaOH/KOH or 1N HCl. Place solution in 1000mL volumetric flask and QS with DI water.

Solution is stable for six months.

3.8.4.4.3 **Borate Buffer, pH 12**

Place ≅500mL DI water into a 1000mL beaker. Heat and stir while adding 50g sodium tetraborate ($Na_2B_4O_7 \cdot 10\ H_2O$). Once dissolved, allow to cool. Bring volume up to ≅ 900mL with DI water. Add 25mL 10N NaOH and stir. Verify pH and adjust as necessary to pH 12 ±0.2 with 10N NaOH or 6N HCl. Place solution in 1000mL volumetric flask and QS with DI water.

Solution is stable for six months.

3.8.4.5 Hydrochloric Acid (HCl)

3.8.4.5.1 **0.1N/100mM HCl (500mL)**

Place approximately 300mL DI water into a 500mL volumetric flask. Add 4.2mL **concentrated hydrochloric acid**, mix. QS to 500mL.

Solution is stable for one year

3.8.4.5.2 **1N HCl (500mL)**

Place approximately 400mL DI water into a 500mL volumetric flask. Add 42mL **concentrated hydrochloric acid**, mix. QS to 500mL.

Solution is stable for one year.

3.8.4.5.3 **1% HCl in Methanol**

Place approximately 30mL **MeOH** in a 50mL volumetric flask. Add 0.5mL concentrated **HCl.** QS to 50mL. Store at 5°C

Solution is stable for six months.

3.8.4.6 Potassium Hydroxide (KOH)

Note: The addition of KOH to water will generate significant heat, exercise due caution.

3.8.4.6.1 **1N/1M Potassium Hydroxide (1000mL)**

Gradually add 56.0g **potassium hydroxide** to approximately 800mL DI water; stir on stir plate to dissolve. QS to 1000mL.

Solution is stable for one year.

1.0M Potassium Hydroxide (100mL)

Dissolve 5.6g **potassium hydroxide** in approximately 80mL DI water in a 100mL volumetric flask. QS to 100mL.

Solution is stable for one year.

3.8.4.6.2 **2N/2M Potassium Hydroxide (1000mL)**

Gradually add 112.0g **potassium hydroxide** to approximately 800mL DI water; stir on stir plate to dissolve. QS to 1000mL.

Solution is stable for one year.

3.8.4.7 Potassium Phosphate Buffers

3.8.4.7.1 **Saturated Potassium Phosphate Buffer (1000mL)**

Place approximately 1000mL DI water in a beaker and heat/stir over low heat. Add **potassium phosphate monobasic** until the solution is saturated. Allow solution to cool. Adjust pH to approximately 1.8 with **concentrated phosphoric acid.**

Solution is stable indefinitely at room temperature.

3.8.4.7.2 **0.1M/100mM Potassium Phosphate Buffer (100mL) - Adjusted to pH 6**

Dissolve 1.36g **potassium phosphate monobasic** in ≈90mL DI water in a 100mL volumetric flask. Adjust to pH 6.0 with 1.0M **potassium hydroxide.** QS to 100mL.

Solution is stable for 1-month.

3.8.4.7.3 **100mM Potassium Phosphate Buffer (1000mL) Adjusted to pH 6 (Varian)**

Weigh 13.6g of potassium phosphate monobasic (KH_2PO_4) into a 1000mL volumetric flask. Add ≅900mL DI water. Stir to dissolve. Adjust pH to

6.0 ±0.1 with 1M KOH while stirring. Bring up to volume with DI water.
Solution is stable for 1 month. Store in glass container.

3.8.4.8 Sodium Hydroxide (NaOH)
Note: The addition of NaOH to water will generate heat, exercise due caution.

3.8.4.8.1 **0.45N NaOH (500mL)**
Gradually add 9g **NaOH** in 500mL DI water. (Caution: Exothermic)
Solution is stable for one year.

3.8.4.8.2 **2N NaOH (1000mL)**
Place approximately 250mL DI water into a 1000mL beaker. Gradually add 80g **NaOH.** Transfer to 500mL volumetric flask and QS to 500mL. (Caution: Exothermic)
Solution is stable for one year.

3.8.4.8.3 **10N NaOH (500mL)**
Place approximately 400mL DI water into a 1000mL beaker. Gradually add 200g **NaOH.** Transfer to 500mL volumetric flask and QS to 500mL. (Caution: Exothermic)
Solution is stable for one year.

3.8.4.9 Sodium Phosphate

3.8.4.9.1 **100mM Sodium Phosphate Dibasic (200mL)**
Dissolve 2.84g **sodium phosphate dibasic** in ≈160mL DI water. QS to 200mL and mix.
Solution is stable for 1 month. Store in glass container.

3.8.4.9.2 **100mM Sodium Phosphate Dibasic (500mL)**
Dissolve 7.1g **sodium phosphate dibasic** in ≈400mL DI water. QS to 500mL and mix.
Solution is stable for 1 month. Store in glass container.

3.8.4.9.3 **100mM Sodium Phosphate Monobasic (200mL)**
Dissolve 2.76g **sodium phosphate dibasic** in ≈160mL DI water. QS to 200mL and mix.
Solution is stable for 1 month. Store in glass container.

3.8.4.9.3 **100mM Sodium Phosphate Monobasic (500mL)**
Dissolve 6.9g **sodium phosphate dibasic** in ≈400mL DI water. QS to 500mL and mix.
Solution is stable for 1 month. Store in glass container.

3.8.4.10 Sodium Phosphate Buffers

3.8.4.10.1 **0.1M/100mM Phosphate Buffer, pH 6 (250mL)**
Dissolve 0.42g **sodium phosphate dibasic** (Na_2HPO_4) and **3.03 sodium phosphate monobasic** ($NaH_2PO_4 \cdot H_20$) in approximately 200mL DI water in 250mL volumetric flask. QS to 250mL. Adjust to pH 6.0±0.1 with 100mM **monobasic sodium phosphate** (lowers pH) or 100mM **dibasic sodium phosphate** (raises pH).
Solution is stable for 1 month. Store in glass container.

3.8.4.11 Sulfuric Acid (H_2SO_4)

3.8.4.11.1 **1N Sulfuric Acid (H_2SO_4) (500mL)**
Place approximately 400mL DI water into a 500mL volumetric flask. Add 14mL **concentrated H_2SO_4**. QS to 500mL, mix.
Solution is stable for one year.

3.8.4.11.2 0.1N/0.05M/50mM Sulfuric Acid (500mL)
Place approximately 300mL DI into a 500mL volumetric flask. Add 50mL of 1N Sulfuric Acid, QS to 500mL, mix.
Solution is stable for one year.

3.8.12 REFERENCES

3.8.12.1 Shugar, G.J., Shugar, R.A. and Bauman, L. *Grades of Purity of Chemicals* pp. 145-154, *pH Measurement*. pp. 232-234. *in:* Chemical Technicians' Ready Reference Handbook, McGraw Hill: New York, 1973.

3.8.12.2 Ansys, Inc. SPEC Extraction Methods

3.8.12.3 United Chemical Technologies, Inc. Applications Manual

Idaho State Police
Forensic Services
Toxicology Discipline

Section Three
Blood Toxicology

3.8 Solution Preparation

Revision #	Issue Date	History
0	04-25-02	Original Issue
1	11-22-06	Updated for new analytical methods

Approval

Discipline Leader: _____ **Date:** _____
Susan C. Williamson

Issuance

QA Manager: _____ **Date:** _____
Alan C. Spanbauer