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TOXICOLOGY
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FOREWORD

The body of knowledge which comprises forensic science is a compilation of procedures adapted from other disciplines that encompass many of the physical and natural sciences. During the history of forensic science, a multitude of scientists have greatly contributed to the protocols, methods and procedures that have become a routine part of analysis. Every effort has been made in this manual to give proper recognition to the authors of specific procedures; however, in some instances, the original source of forensic procedures has been lost in antiquity. For others, the general procedures belong to the public domain and are recorded in many basic references concerning forensic science. In addition, many of the procedures described in this manual have been adapted from standard laboratory practices, and the citation of thousands of references which deserve credit for aiding in the development of these procedures is neither practical nor possible. To all those scientists who have contributed to the knowledge of forensic science contained herein, we do extend collective recognition and gratitude.

Procedures manuals which offer reliable information that is then combined with corresponding training manuals serve as the foundation for effective quality management of analyses. Extensive effort has been made to ensure that the routine procedures described herein will produce accurate and valid analytical results.

May 31, 2016

INTRODUCTION

The *Toxicology Procedures Manual* contains procedures that are approved and deemed fit for their intended use. The procedures contained within this manual will be utilized in the analyses of volatiles and drugs in biological samples as applicable.

Instruments and their operating parameters will vary with time and from laboratory to laboratory. The instrument operating conditions listed in this manual are included to serve as a starting point to help the toxicologist adapt the procedures to their own instrumentation.

Toxicologists should review **Minimum Standards and Controls, Safety Guidelines, and the Toxicology Analytical Schemes (included in this Introduction)** prior to conducting any analytical procedures.

TOXICOLOGY ANALYTICAL SCHEMES

The following are **general** analytical schemes to be utilized for toxicology cases:

DUI This analytical scheme utilizes three levels of testing. Testing is halted and an ISP laboratory report will be issued once alcohol or drugs are detected that either: violate the per se element of the law or may be considered forensically significant to a DUI investigation.

Drug Recognition Expert (DRE) Evaluations. A **signed** *DRE Toxicology Request Form* must be submitted before any additional tests outside of the Toxicology Analytical Scheme will be performed. Blood samples will **only** be tested for volatiles. Drug testing will be limited to urine samples, with the exception of urine that screens positive for cannabinoids where blood was also a submitted item. In these instances a blood THC quantitation will be performed.

Death Investigation. These cases are too diverse to have a specific analytical scheme however all cases follow a general analytical protocol. Coroner's cases are reviewed by the analyst and appropriate testing is determined based on information from the user agency and case history.

Note: When a limited amount of urine sample is available and may prevent complete analysis, the analyst will contact the submitting officer/assigned state's attorney for permission to consume and specific direction on what analyses to perform. This direction will be recorded using a "Case Correspondence" entry in LIMS.

Deferred analyses on DUI cases can be revisited upon request by the user agency. A second toxicology service request must be submitted through LIMS Pre-Log. A supplemental report will be issued for any additional analysis performed.

Blood Only DUI's

1) Test the blood sample for Ethanol. Report finding and do not perform any additional analysis for volatile only service requests. When the lower limit for measurement uncertainty of an Ethanol result is less than 0.080 g/dL and the case offense indicates drugs or volatiles and drugs proceed to step 2.

2) The blood sample will be screened using ELISA. If positive(s) are confirmed, do not perform any additional analysis and report finding(s). If negative or no drugs are confirmed proceed to step 3.

3) Perform a full drug panel analysis and report any confirmed findings.

Blood/Urine DUI's

1) Test the blood sample for Ethanol. When the lower limit for measurement uncertainty of an Ethanol result is greater than or equal to 0.080 g/dL, report finding and do not perform any additional analysis. The urine is not analyzed. If the lower limit for measurement uncertainty of the Ethanol result is less than 0.080 g/dL proceed to step 2.

2) Screen the urine sample using EMIT. If positive(s) are confirmed, stop additional analysis and report finding(s). If negative or no results are confirmed, proceed to step 3.

3) Perform a full drug panel analysis and report any confirmed findings. Urine that screens positive for cannabinoids is confirmed by quantitation in the blood (if available).

Urine DUI's

1) Screen the urine sample using EMIT. If positive(s) are confirmed, stop additional analysis and report finding(s). If negative or no drugs are confirmed proceed to step 2.

2) Perform a full drug panel analysis and report any confirmed findings.

DRE DUI

1) If submitted, test the blood sample for Ethanol.

2) Screen the urine sample using EMIT. Report any confirmed findings.

3) Perform a full drug panel analysis in urine and report any confirmed findings. Urine that screens positive for cannabinoids is confirmed by quantitation in the blood (if available).

4) When a limited amount of urine sample is available and may prevent complete analysis, the analyst will contact the DRE officer for specific direction on what analyses to perform. This direction will be recorded using a "Case Correspondence" entry in LIMS.

Traffic Fatality, Reckless Homicides

1) Blood only cases - Perform a full volatile and drug analysis.

2) Blood/Urine cases - Perform a full volatile and drug analysis. Volatiles analysis performed on the blood sample. Drug analysis performed on the urine sample. Urine that screens positive for cannabinoids is confirmed by quantitation in the blood (if available).

Drug Facilitated Sexual Assaults

1) If submitted, blood is **not** analyzed.

2) Perform a full volatile and drug analysis on the urine sample. The urine sample will also be tested for GHB and lorazepam.

Coroner Cases

Perform full volatile and drug analysis on blood and urine samples. When the volatile analysis is positive, perform a second volatile analysis on vitreous humor. In the absence of vitreous humor, urine or an alternate sample will be analyzed when available.

April 1, 2022

ILLINOIS STATE POLICE

TOXICOLOGY
PROCEDURES MANUAL

PROTOCOL: Volatile Analysis

METHOD: Headspace Gas Chromatography With Flame Ionization Detector

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Reviewed by:

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Larry Shelton, Chairperson
Toxicology Command Advisory Board

Approved by:

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Shannon George
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Volatile Analysis

When biological samples are submitted to the laboratory to be analyzed for volatiles, they will be handled in accordance with Section, Laboratory, and Command guidelines.

The procedures used for analysis should meet standards and controls guidelines outlined in Appendix II in this manual.

The primary responsibility of the analyst is to identify volatiles that may be present in a specimen using acceptable scientific procedures. The methods outlined in this manual represent only those most commonly used procedures for the identification and/or quantitation of volatiles. Other appropriate methods, if necessary, may be used if documented in the case file. In the final analysis, all conclusions reached must reflect a reasonable degree of scientific certainty.

ILLINOIS STATE POLICE

TOXICOLOGY PROCEDURES MANUAL

PROTOCOL: Volatile Analysis

METHOD: Headspace Gas Chromatography With Flame Ionization Detector

PROCEDURE: **DETERMINATION OF ALCOHOL CONTENT IN LIQUIDS**

Reviewed by:

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Sean McCarthy, Chairperson
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Approved by:

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Timothy Tripp
Bureau Chief – Toxicology Command Coordinator

INTRODUCTION

Law enforcement agencies may request that the alcohol (ethanol) content of a liquid be determined. The liquid may be commercially produced or "homemade brew". In either case, Illinois Compiled Statutes defines an "alcoholic liquor" as a liquid or solid capable of being consumed as a beverage by a human being that contains greater than 0.5% ethanol by volume.

The analysis of ethanol is performed by headspace gas chromatography. This technique is based on various gas laws which dictate the phenomena that exists when a volatile liquid in solution comes in contact with a closed air space, "headspace," forming an equilibrium between phases. At a given temperature, the concentration of the volatile in the "headspace" is directly proportional to its concentration in the liquid. This method affords a means of separation and produces extremely clean samples for chromatographic analysis.

Other Related Procedures:

See *Combined Quantitation of Ethanol & Other Volatiles*

SAFETY CONSIDERATIONS

Caution should be taken when opening any container of suspected "homemade brew." The contents may still be fermenting and pressure may have built up inside the container.

PREPARATIONS

Specimen: Suspected Liquid; 1.0 mL required. Prepare as follows:

If the suspected alcoholic liquid appears to be actively fermenting or there is a possibility that fermentation can occur, the fermentation should be stopped before the analysis is performed. Addition of 0.10 grams of Sodium Metabisulfite per 30 mL of liquid should be added to stop fermentation of the liquid. This should be done upon receipt of the sample.

If the suspected alcoholic liquid contains solid material, centrifuge a portion for analysis. If the liquid is carbonated, filter several milliliters through filter paper and collect the decarbonated liquid for analysis.

Pipet 1.0 mL of the liquid into a 25 mL volumetric flask and dilute to the mark with distilled water. Note #1.

Reagents:

See *Combined Quantitation of Ethanol & Other Volatiles*

INSTRUMENTATION

See *Combined Quantitation of Ethanol & Other Volatiles* for Instrument Conditions and Calibration.

MINIMUM STANDARDS & CONTROLS

Refer to Appendix II.

Controls:

1. In house aqueous ethanol control.
2. In house ethanol whole blood control.
3. Negative control: dH_2O.

Reagents:

1. **Ethanol**. Ethyl alcohol, pure – 200 proof (Sigma-Aldrich).
2. **n-Propanol**. A.C.S. Certified. (Fisher Scientific Co.).
3. **Sodium Metabisulfite** (Acros Chemical).

PROCEDURE OR ANALYSIS

1. Retrieve case samples from a secure refrigerator and place the exhibits to be tested on a rotator. Allow sample containers to mix and reach room temperature.
2. Label two auto-sampler vials for each case specimen and one for each control and blank included on the "batch" worklist. (Notes #2, #3, and #4)
3. Dilute and dispense controls and case samples into the appropriately labeled auto-sampler vials using the diluter/dispenser. **Ensure the dispenser tip is touching the inner wall of the auto-sampler vial when dispensing the sample. Continue this action until all specimens and controls are pipetted, rinsing the diluter a minimum of one (1) times with distilled water after each aliquot.**
4. Place a rubber stopper on each vial as it is prepared. Seal with crimp caps.
5. Input all QC and case samples into the instrument sequence by scanning the corresponding barcode from the LIMS batch worklist **in order**. Then place vials onto the auto-sampler carousel appropriately. (Note #5)
6. Begin gas chromatography analysis.
7. "Import" quantitative results and chromatograms to the "batch" worklist in LIMS.

Calculations:

1. Concentrations in weight percent (g/dL) are automatically calculated by the instrument from the calibration curve using linear regression. Volume percent of ethanol is calculated in LIMS.
2. A reported volume percent of ethanol is calculated in LIMS. Samples are analyzed in duplicate and the average concentration is determined using both sample tests from the quantitative column (default is column A or by selecting column B). The average value is divided by the density of ethanol (0.789) and then multiplied by a dilution factor as needed (for example 25 or 50). The calculated value is truncated to 1 decimal place for report "Findings"
3. Determine if replicate concentrations are within ±5% of their average concentration or within ±0.004 g/dL, whichever is greater. Samples outside the tolerated spread will be reanalyzed. For example, if the reportable value is 0.100 g/dL, the two replicates range of ±5% is 0.095 and 0.105 g/dL. Results averaging below 0.080 g/dL will default to the ±0.004 g/dL spread for acceptability. If both sample replicates are within the calculated spread the result can be reported.
4. The limit of quantitation listed in the procedure, *Combined Quantitation of Ethanol and Other Volatiles*, is 0.010 g/dL. This will be the lowest concentration LIMS will use to calculate a % Ethanol by Volume in an unknown liquid (of a diluted x25 sample). 0.010 g/dL samples calculate to 0.3% which is just below the legal cutoff of 0.5% Ethanol by Volume.
5. Refer to App VII when case results are between 0.3% and 1.0% for guidelines on determining an estimation of Measurement Uncertainty.

Notes:

1. Linearity for acetone, ethanol, isopropanol, and methanol has been established up to 0.400 g/dL. Should a sample diluted to 25 mL surpass this upper limit, an additional dilution should be performed and re-analyzed (i.e. times 50 mL dilution). Recommended dilutions are to 100 mL for suspected hard liquor, 50 mL for suspected wine and mixed drinks, and 25 to 50 mL for suspected beer.
2. Samples are prepared in duplicate.
3. At a minimum, a control will be pipetted at the beginning and end of every 20 case vials.
4. For the determination of ethanol concentration, a set of controls includes a whole blood, a positive aqueous, and a negative control. If a whole blood control or positive aqueous control is unavailable at the laboratory, see Note 7. The number of set of controls to use is based on the number of vials being analyzed. For 1-20 vials, use one set; for 21-40 vials, use two sets, for 41-60 vials, use three sets; and so forth.
5. At a minimum, controls are analyzed at the beginning and end of each sample set to bracket the case samples. The negative control must run directly after the in-house aqueous ethanol control.

6. If no volatiles are detected after analyzing a diluted sample, the sample will be reanalyzed without being diluted.
7. Certified reference material (CRM) standards may be used in place of in-house controls if they are unavailable at the laboratory. To accept a calibration or quantitative result for ethanol the measured value for ethanol must be within 5% of the expected CRM value. To accept a calibration or quantitative result for acetone, isopropanol, and methanol the measured value must be within 10% of the expected CRM value.

REPORT WORDING

Refer to Appendix I.

REFERENCES

1. Toxicology Procedures Manual – TX-IA-5 "Combined Quantitation of Ethanol & Other Volatiles." September 1, 2016.

ILLINOIS STATE POLICE

TOXICOLOGY PROCEDURES MANUAL

PROTOCOL: Volatile Analysis

METHOD: Headspace Gas Chromatography with Flame Ionization Detector

PROCEDURE: **COMBINED QUANTITATION OF ETHANOL & OTHER VOLATILES**

Reviewed by:

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Sean McCarthy, Chairperson
Toxicology Command Advisory Board

Approved by:

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Timothy Tripp
Bureau Chief – Toxicology Command Coordinator

INTRODUCTION

The group of substances known as volatiles includes the following compounds: methanol, ethanol, acetone, and isopropanol. Ethanol is primarily a social drug, while isopropanol and methanol are occasionally observed in accidental and intentional intoxications. Acetone is a major metabolite of isopropanol but may also be present in the blood during diabetic or fasting ketoacidosis.

The analysis of ethanol and other volatiles is performed by headspace gas chromatography. This technique is based on various gas laws which demonstrate that at a given temperature the concentration of ethanol or volatiles in the gas phase, "headspace", is directly proportional to the concentration in the liquid solution. Testing is performed by diluting an aliquot of a biological fluid with an internal standard in a glass vial; sealing it and then placing it on the instrument where it is heated before testing. A needle injects a portion of the headspace vapor onto a dual column gas chromatograph (GC) equipped with dual flame ionization detectors (FID). Volatiles are identified by their retention time and concentrations are calculated with software that uses a weighted linear regression (1/x) of a calibration curve. This method affords a means of separation and produces extremely clean samples for chromatographic analysis.

SAFETY CONSIDERATIONS

Warning: Potential Biohazard. Adhere to all blood borne pathogen guidelines.

PREPARATIONS

Specimen: Blood, serum, plasma, urine, and vitreous humor. A minimum of 250 µL is required.

Range: Blood concentrations, g/dL

Volatile	Normal	Toxic	Lethal
Acetone	N/A	0.020 - 0.030	0.055
Ethanol	N/A	0.080 - 0.100	0.350
Isopropanol	N/A	>0.040	>0.150
Methanol	N/A	0.020	>0.089

Reagents:

1. **Ethanol.** 200 proof. Sigma-Aldrich
2. **Mixed Volatile Standards**. NIST traceable standards. Multicomponent Alcohol Calibration Kit (Cerilliant). Multicomponent kit ampules currently contain acetone, ethanol, isopropanol, and methanol at concentrations of 100, 250, 500, 1000, 2000 and 4000 µg/L.
3. **n-Propanol.** A.C.S. Certified. (Fisher Scientific Co.).
4. **Working Internal Standard.** 0.04 g/dL n-propanol. Add 1 mL of n-propanol into a two liter volumetric flask and dilute to volume with distilled water. Mix well before use.

MINIMUM STANDARDS & CONTROLS

Refer to Appendix II.

Controls:

1. In House Blood Ethanol Control. Ethanol 80 mg/dL.
2. In House Aqueous Ethanol Control. Ethanol 320 mg/dL.
3. Negative Control: dH_2O.

Over the course of 2 calibrations, 50 aliquots of a new whole blood control or in-house aqueous control must be analyzed to establish historical data in LIMS. The established mean and standard deviation will be used to evaluate the QCs and therefore case result acceptability. Historical data is instrument specific.

INSTRUMENTATION

Instrument:	Agilent 8890 Gas Chromatograph Agilent 7697A Headspace Sampler
Column 1:	DB-ALC1 30 m x 320 µm x 1.8 µm film.
Column 2:	DB-ALC2 30 m x 320 µm x 1.2 µm film.
Injector:	150ºC.
Front Detector:	200ºC.
Back Detector:	200ºC.
Temperature Program:	35ºC Isothermal; Hold for 6.0 minutes.

Microlab Diluter/Dispenser	Method (Micro)
Sample Volume	100 µL
Working Internal Standard	400 µL

Calibration

The headspace method for volatile analysis is calibrated using aliquots of five mixed volatile standard solutions. The calculations are based on response factors derived from the analysis of these standards with an internal standard, n-propanol. An instrument calibration should be performed when any of the following occur:

1. A new working internal standard is prepared.
2. Control results indicate a calibration is needed.
3. Thirty-one (31) days have elapsed since the previous calibration.
4. Maintenance is performed on the headspace GC/FID or columns.

To calibrate:

1. Pipet one aliquot of each mixed volatile standard into a headspace vial. Vials should include: 100, 500, 1000, 2000, and 4000 µg/L. (See item #2 in the reagent section). **Note: Ensure dispenser tip is touching the inner wall of the auto-sampler vial when dispensing the standards. Continue this action until all calibrators and controls are pipetted, rinsing the diluter a minimum of one (1) time with distilled water after each aliquot.**
2. Analyze each standard using "calibration mode".
3. Once the calibration sequence is complete save the method.
4. Pipet in-house blood control, in-house aqueous control, and 250 ug/L volatile calibration standard. Analyze in sample mode. (Note #10)
5. Check that control results fall within 2 standard deviations of their established mean.
6. Check that the 250 ug/L calibration standard results are within ±10%. Volatiles outside 10% can be reported qualitatively through the duration of the calibration.
7. Print the calibration data. Record the lot numbers and expiration dates of each calibration standard on a coversheet. Initial and date the calibration coversheet. Ensure the calibration data is reviewed and initialed by a second analyst.
8. Upon passing the review, in LAM, "Add" an entry to the Instrument's Calibration History then upload the calibration chromatograms and coversheet using the "Images" button that corresponds with the date analyzed.

PROCEDURE OR ANALYSIS

1. Retrieve case specimens from refrigerator and place the exhibits to be tested on a rotator. Allow sample tubes to mix and reach room temperature. (Note #1)
2. Label two auto-sampler vials for each case specimen and one for each control and blank included on the "batch" worklist. (Notes #2, #3, and #4)
3. Dilute and dispense controls and case specimens into the appropriately labeled auto-sampler vials using the diluter/dispenser. **Ensure dispenser tip is touching the inner wall of the auto-sampler vial when dispensing the sample. Continue this action until all specimens and controls are pipetted, rinsing the diluter a minimum of one (1) times with distilled water after each aliquot.**
4. Place a rubber stopper on each vial as it is prepared. Seal with crimp caps.
5. Input all QC and case samples into the instrument sequence by scanning the corresponding barcode from the LIMS batch worklist **in order**. Then place vials onto the auto-sampler carousel appropriately.
6. Begin gas chromatography analysis.
7. "Import" quantitative results and chromatograms to the "batch" worklist in LIMS.

Quality Control:

This section discusses the preparation of in-house volatile controls, how to prepare quality control charts in LIMS, control acceptance criteria, calculations and serum conversions, and additional notes to assist with consistent analysis.

Reagents:

Ethanol. Ethyl alcohol, pure – 200 proof (Sigma-Aldrich).

Preparation of In-house Controls:

High range aqueous ethanol control (approximately 0.320 g/dL). Pipet 1090 µL of ethanol into a 250 mL volumetric flask (or equivalent ratio) and dilute to volume with distilled water.

Decision point blood ethanol control (approximately 0.080 g/dL). Pipet 272 µL of ethanol into a 250 mL volumetric flask (or equivalent ratio) and dilute to volume with blank blood.

Dispense the control into appropriate vials for refrigerated storage for aqueous or frozen storage for blood. Prepare enough vials to last at least one year. Obtain statistical data using the normal quality control charting procedure.

Preparation of Quality Control Chart:

A quality control report is created to evaluate control results of a unique asset in LIMS. These reports are instrument specific so that system performance can be monitored. The control chart is prepared using the following procedure:

1. Perform at least fifty (50) test quantitations of a new control utilizing at least 2 calibrations.
2. "Import" control data and chromatogram images into LIMS. Ensure the instrument and lot# are selected and notate the worklist number associated in the comments section.
3. Use the dropdown "Custom Procedures" menu and choose "Set EtOH Validation" and click the "gear" button.
4. Status will be set to "Validation" for all ETHANOL results in the worklist. Close the worklist.
5. The LIMS "Ethanol Control Chart" custom report calculates the mean concentration ($\bar{x}=\Sigma x \div n$), standard deviation ($SD=\sqrt{(\Sigma((x-\bar{x})^2)) \div (n-1)}$), and coefficient of variation ($CV= SD \div \bar{x}$). The coefficient of variation should be less than 3%.

6. LIMS will auto calculate the Z score of a QC result imported into a batch worklist. The Z score value will be used to determine if a control and ultimately a group of sample results are accepted or rejected as outlined below.
7. Ethanol control reports are created and stored in LIMS.

Evaluation:

a. Each set of controls will bracket case samples as instructed in the volatile procedures. Only the quantitation column will be considered when evaluating control results.

b. If one control is outside 3 standard deviations (SD) all positive samples bracketed within the control set must be repeated.

c. If both controls are outside 2 SD all positive samples bracketed within the control set must be repeated.

d. When the control chart demonstrates a control is trending outside of 2 SD, the technical leader must be notified. The technical leader will determine appropriate corrective action if warranted.

e. Negative case samples can still be reported qualitatively as "No volatiles detected" even if ethanol controls are rejected quantitatively. Note: control volatiles and internal standard must be detected qualitatively.

Quality Control of Acetone, Isopropanol, and Methanol:

Because volatiles other than ethanol are infrequently probative to a case; these volatiles will not be tracked in a control chart. In the event a mixed volatile result is relevant to a case the acceptable tolerance set by the control manufacturer will be followed.

Calculations:

1. Concentrations are automatically calculated by the instrument from the calibration curve using weighted linear regression (1/x).
2. The calibration curve is generated by plotting the concentration of the calibration standard on the x-axis and the ratio of the peak area of the volatile by that of the internal standard on the y-axis.
3. Reported concentrations are calculated by LIMS. Samples are analyzed in duplicate and the average concentration is determined using both sample tests from the quantitative column (default is column A or by selecting column B). The average value truncated to three decimal places is used for report "Findings".

4. Determine if replicate concentrations are within ±5% of their average concentration or within ± 0.0040% (w/v), whichever is greater. Samples outside the tolerated spread will be reanalyzed. For example, if the reportable value is 0.100 g/dL, the two replicates range of ±5% is 0.095 and 0.105 g/dL. Results averaging below 0.080 g/dL will default to the ±0.004 g/dL spread for acceptability. If both sample replicates are within the calculated spread the result can be reported.

Serum/Plasma Correction Factor (Note #8)

Estimated blood ethanol concentrations are calculated as follows:

$$\text{Plasma or Serum Concentration} = \text{Estimated blood ethanol (g/dL)} \times \frac{}{1.18}$$

Notes:

1. Serum and plasma tubes that contain significant clots should be centrifuged prior to analysis.
2. Specimens are prepared in duplicate.
3. For the determination of ethanol concentration, a set of controls includes a whole blood, a positive aqueous, and a negative control. If a whole blood control or positive aqueous control is unavailable at the laboratory, see Note 10. When volatiles other than ethanol are significant to the case, a volatile serum toxicology control will be used in combination with a negative control. The number of sets of controls to use is based on the number of vials being analyzed. For 1-20 vials, use one set; for 21-40 vials, use two sets; for 41-60 vials, use three sets; and so forth.
4. At a minimum, controls are pipetted and analyzed at the beginning and end of each sample set (20 case vials) to bracket the case samples. The negative control must run directly after the in-house aqueous ethanol control or volatile serum toxicology control.
5. Linearity for acetone, ethanol, isopropanol, and methanol has been established up to 0.400 g/dL. This procedure is not validated for dilutions. Report wording will indicate "greater than 0.400 g/dL" when both measured concentrations are above the linear range. If one (1) of the two (2) replicates is above 0.400g/dL, the final result is reported as "Greater than X g/dL", where "X" is the quantitative value of the replicate below 0.400g/dL. (See note #7 for coroner cases).
6. Limit of detection and quantitation is 0.010 g/dL.
   a. If one (1) of the two (2) analyzed replicates is quantitatively below 0.010 g/dL, the result is not reported.
   b. If both of the analyzed replicates are quantitatively below 0.010 g/dL, the result is reported as "No volatiles detected".
7. When the analysis of a coroner case results in an ethanol concentration greater than 0.400 g/dL an alternate sample will be tested if available.

8. When specimens other than whole blood are analyzed, the analytical result will be reported. An estimated blood ethanol concentration will be reported in the "Remarks" section of the report for serum or plasma. Refer to App I Report Wording.
9. Use prepared value of standard for calibration programming.
10. Certified reference material (CRM) standards may be used in place of in-house controls if the in-house controls are unavailable at the laboratory. To accept a calibration or quantitative result for ethanol the measured value for ethanol must be within 5% of the expected CRM value. To accept a calibration or quantitative result for acetone, isopropanol, and methanol the measured value must be within 10% of the expected CRM value.

REPORT WORDING

Refer to Appendix I.

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ILLINOIS STATE POLICE

TOXICOLOGY
PROCEDURES MANUAL

PROTOCOL: Qualitative Drug Screening

Reviewed by:

_____
Larry Shelton, Chairperson
Toxicology Command Advisory Board

Approved by:

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Shannon George
Toxicology Program Manager

Introduction to Qualitative Drug Screening

When biological samples and other types of evidence are submitted to the laboratory to be analyzed for drugs, they will be handled in accordance with Section, Laboratory, and Command guidelines. The procedures used for analysis will be at the discretion of the analyst as long as they meet standards and controls guidelines found in Appendix II of this manual.

The primary responsibility of the analyst is to identify drugs that may be present in a specimen using acceptable scientific procedures. The methods outlined in this manual represent only those most commonly used. Other appropriate methods, if necessary, may be used if documented in the case file. In the final analysis, all conclusions reached must reflect a reasonable degree of scientific certainty.

This section covers screening procedures, including color tests, immunoassays, thin-layer chromatography and general screening extracts.

ILLINOIS STATE POLICE

TOXICOLOGY PROCEDURES MANUAL

PROTOCOL: Qualitative Drug Screening

METHOD: Enzyme Multiplied Immunoassay Techniques

PROCEDURE: **ENZYME MULTIPLIED IMMUNOASSAY TECHNIQUE V- TWIN**

Reviewed by:

_____
Sean McCarthy, Chairperson
Toxicology Command Advisory Board

Approved by:

_____
Timothy Tripp
Bureau Chief – Toxicology Command Coordinator

INTRODUCTION

The EMIT assay is a homogeneous enzyme immunoassay technique used for the analysis of specific compounds in biological fluids. In the performance of an EMIT assay, urine is mixed with two (2) reagents. Reagent 1 contains antibodies to a particular drug, the coenzyme nicotinamide adenine dinucleotide (NAD), and substrate for the enzyme glucose-6-phosphate dehydrogenase (G6PDH); Reagent 2 contains a drug derivative labeled with enzyme G6PDH. Reagent 1 is added to the sample first, and the antibody binds to the drug it recognizes. Reagent 2 is added second, and the enzyme labeled drug combines with any remaining antibody binding sites; this binding decreases the enzyme activity. If the drug is present in the sample, some enzyme remains unbound and therefore is still active in the reaction mixture. This residual enzyme activity relates directly to the concentration of the drug in the sample. The active enzyme converts NAD to NADH, resulting in an absorbance change that is measured spectrophotometrically.

SAFETY CONSIDERATIONS

Warning: Potential Biohazard. Adhere to all blood borne pathogen guidelines.

Warning: HCl. Corrosive. Use extreme care when handling concentrated acid. Avoid breathing fumes. Always add small amount of acid to water with stirring.

Warning: NaOH. Corrosive to all tissues. Wear goggles and gloves.

PREPARATIONS

Specimen: Urine (~0.5 mL) is the specimen of choice. Serum may be used without further treatment. Other specimens can be used but may require treatment to obtain satisfactory results.

Reagents:

Purchased reagents are available from Siemens.

1. **Calibrators**. Level 1 is used to calibrate the opiate and amphetamine assays. Level 2 is used to calibrate benzoylecgonine at 150 ng/mL. Level 3 is used for all other assays. Check expiration dates of all calibrators used.
2. **Reagents 1 & 2 for drug assays.** The EMIT II Plus reagents are prepared by the manufacturer and require no preparation prior to use. Check expiration dates of all reagents used. In rare circumstances it may be necessary to adjust the pH of a given sample.
3. **HCl, 0.1 N.** (Fisher Scientific). Add 4.2 mL of concentrated HCl to 250 mL of distilled water in 500 mL volumetric flask and q.s. to 500 mL with distilled water with stirring. **Warning: Always add acid to water with stirring.**
4. **NaOH, 0.1 N**. (Fisher Scientific) Dissolve 0.4 g NaOH in 10 mL of distilled water. **Warning: Caustic material. Wear gloves and face shield.**

In-house preparation:

1. **EMIT Positive Control**. 500 mL of blank urine spiked with the following:

Drug Standard (1 mg/mL stock)	Volume (in µL)
Methamphetamine	450
Oxazepam	300
11-Nor-9-Carboxy-Δ9-THC	75
Benzoylecgonine	225
Morphine	450
PCP	35

MINIMUM STANDARDS & CONTROLS

Refer to Appendix II.

Calibrators/Controls:

1. Siemens Level 5 calibrator/control.
2. Siemens Level 3 calibrator/control.
3. Siemens Level 2 calibrator/control.
4. Siemens Level 1 calibrator/control.
5. Siemens Level 0 calibrator/control
6. Blank urine.

INSTRUMENTATION

V-Twin Immunoassay System

Instrument Set-Up

The following steps must be performed prior to analysis:
Review the rotor blank printout. There should be no errors indicated.
Make sure there is sufficient water/system solution.
Empty waste bottles.
Check expiration dates on calibrators, controls, and reagents.
Perform a "System Fill" on the instrument.
Check syringes for leaks or bubbles during the system fill.
Optional: under "Evaluate Samples," clear the results buffer.

Calibration

Calibration is performed daily when in use.

From the main menu screen, select the "Request Samples" button. Near the bottom left, there is a drop-down menu under "Sample Type." Select "Calibrate" and then indicate the assays you wish to calibrate by clicking on the appropriate boxes.

Note: the instrument is set up to do a single point calibration at the cut-off level. If multiple assays are selected for calibration, the software will automatically order the appropriate calibrators.

Controls

At a minimum, controls should be run immediately at the beginning and end of individual runs if more than 10 samples are run. (Analysts may use the same controls if running back-to-back in that the first analyst's ending controls may be used as the next analyst's starting controls. If 10 samples or less are to be analyzed then only one set of controls is needed at the beginning of the run.)

After selecting to calibrate, the "Control" option should be selected from the drop-down menu. Select a control and choose the assays to run them on. Repeat for each control. Other runs of the positive control (or other commercial control) and blank should be entered into the work list as "samples" under the following step.

Samples

Under the “Sample Type” drop-down menu, select “Sample.” Note: this is the default selection. Enter Level 0, Level 5, QC, and case samples by scanning the corresponding barcode from the LIMS batch worklist into the “Patient Name” field (Note: “Sample ID” cannot be blank however LIMS uses “Patient Name” to import the analytical data).. Choose the assays you wish to run on that sample by selecting the appropriate box(es).

Running the Samples

From the main menu, select the “Sample Handling” button. You will see a graphic display of the sample carousel. All slots should be gray, indicating they are available for loading. If not, select the “Confirm Unload” button to clear the tray.

The list of requested calibrators, controls, and samples should be on the right side of the screen. As you click on each item, a slot on the carousel will be selected by the software. The spot will also change colors on the display. If you wish to select a different spot, right-click the sample ID and a dialogue box will display.

Once all of the calibrators, controls and samples have been placed on the carousel, press the “Start Measurement” button to begin analysis.

Exporting/Importing Sample Data

Results can be exported to a data file once the instrument transitions into “stand-by” mode. Select “Sample Handling” > “Results Handling” (archived data may need to be loaded).

Select all items included on the batch worklist using the checkboxes for samples and tests. Click “Export” to create the data file. Save/Transfer to a thumb-drive to import via LIMS.

In LIMS, load the EMIT worklist using “Instrument” > “Batch Results”. Click “Import” and select the data file saved from the “export” data step. Press “Ok”.

PROCEDURE OR ANALYSIS

See instructions contained within *“Instrumentation”* above.

Interpretation of Results:

When the rate of absorbance for one or both measurements of an unknown sample is greater than the low calibrator (“Cutoff”), the sample will be considered positive (POS) for that assay. (In rare instances when duplicate measurements are not consistent, re-analysis may be warranted)

For cannabinoids (THC) and phencyclidine (PCP) assays, when one or both absorbance rate measurements are greater than or equal to the low calibrator (“Cutoff”), the sample will be designated positive (POS). If both measurements are below the low calibrator, this test will be designated negative (NEG).

For all other assays, when one or both sample absorbance readings meet or exceed the “Cutoff Mid” value (defined as the value midway between the negative and low calibrator and calculated by LIMS), analysts will designate the result as positive-negative (PN). (PN) designations will trigger the corresponding confirmation ”task” similar to a positive (POS) result. Measurements below the “Cutoff Mid” will be designated as negative (NEG) for this test.

Measurements elevated above the negative calibrator but below the “Cutoff Mid” value may indicate low levels of a drug or drugs within the assay class. However, falsely elevated results may also be an indication of cross reactivity from unrelated drugs or chemicals. False positives can occur on one or more of the assays when high concentrations of certain drugs are present. Putrification products from postmortem cases and high levels of lactate and/or LDH often found in the urine from insulin dependent diabetes can also result in false positives. Analyst can reference the package insert included with each assay which lists drugs that may cross react. The manufacturer’s separation guidelines may change with reagent lot numbers.

False negatives can result from the adulteration of urine specimens with salt, soap, acid, bleaches and glutaraldehyde (found in "UrinAid"). A specimen with a rate of absorbance that is considerably below the negative calibrator may have been adulterated.

LIMITATIONS

1. This procedure was validated per research projects 2019-08 and 2019-10.

REPORT WORDING

Refer to Appendix I.

REFERENCES

1. *Dade-Behring V-Twin User's Manual*

2. "Other Postmortem Problems," *Forensic Drug Abuse Advisor*. Vol. 8(2) 1996.

3. George, S. and Brainwaite, R.A. "The effect of glutaraldehyde adulteration of urine specimens on Syva EMIT II Drugs-of-Abuse Assay." *J. Anal. Toxicol.* 1996, 20, 195-196.

ILLINOIS STATE POLICE

TOXICOLOGY
PROCEDURES MANUAL

PROTOCOL: Qualitative Drug Screening

METHOD: Enzyme Linked Immunosorbent Assay (ELISA)

Reviewed by:

Sean McCarthy, Chairperson
Toxicology Command Advisory Board

Approved by:

Timothy Tripp
Bureau Chief – Toxicology Command Coordinator

INTRODUCTION:

The ELISA assay is an end-point heterogenous competitive enzyme immunoassay technique used for the analysis of specific compounds in biological materials. During the course of the analysis drugs contained within blood or other fluids compete with drugs conjugated to enzyme (horseradish peroxidase) for binding sites within reaction wells. The reaction wells are coated with antibodies for specific drugs and/or metabolites. After a period of incubation, any unbound drug (or drug/enzyme conjugate) is washed from the reaction well. After washing, an enzyme's substrate (tetramethylbenzimide) is introduced into the reaction well and allowed to incubate. A negative sample will contain more of the drug/enzyme conjugate, since the complex did not have to compete for binding sites with native drug. Because there is more of the enzyme bound to the reaction well, there will be more enzyme activity. For this assay, enzyme activity is proportional to the absorbance of the mixture at specific wavelengths that correspond to the oxidized substrate. Higher concentrations of native drug in the sample will result in lower enzyme activity and color change.

SAFETY CONSIDERATIONS:

Warning: Potential Biohazard. Adhere to all blood borne pathogen guidelines.

Warning: Moving parts. Robotic instruments for ELISA contain motorized moving parts that can present potential for mechanical injury. Stay clear of moving parts.

PREPARATIONS:

Specimens: 400 µL of blood is diluted with 1600 µL manufacturer supplied enzyme immunoassay (EIA) buffer (Note #1). The instrument and reagents are capable of analyzing various biological fluids and tissue extracts. Urine may be analyzed without prior dilution. Follow reagent vendor instruction for sample preparation where applicable.

Instrumentation: Verify that there is an adequate supply of reaction wells, reagents, buffers, substrate and other commodities to complete the entire analysis.

Reagents: All necessary reagents are available from Neogen Corporation. Alternate sources of reaction plates, reagents and consumables may be used if properly validated.

Calibrators and Controls: Negative and "cut-off" calibrators are available from Neogen Corporation. Use of vendor supplied calibrators is recommended for troubleshooting. The laboratory may prepare controls in house at appropriate concentrations.

In-House Reagent Preparation

Prepare the following as needed (Note #2). Mix thoroughly. After preparation dispense 400 µL into appropriate vials and store in a freezer. Each vial must be mixed with 1600 µL of EIA Buffer before use.

1. **Cut-off Calibrator (100 µg/L Amphetamine, 50 µg/L Benzoylecgonine, 50 µg/L Morphine, 100 µg/L Oxazepam, 20 µg/L PCP, and 2 µg/L Fentanyl).**
   Add the following to a 500 mL flask and dilute to volume using a blank blood matrix:
   a. Using a 1 mg/mL standard solution:
      50 µL Amphetamine, 25 µL Benzoylecgonine, 25 µL Morphine,
      50 µL Oxazepam, and 10 µL PCP.
   b. Using a 10 µg/mL standard solution:
      100 µL Fentanyl.

2. **Positive Control (300 µg/L Amphetamine, 150 µg/L Benzoylecgonine, 150 µg/L Morphine, 300 µg/L Oxazepam, 60 µg/L PCP, and 6 µg/L Fentanyl).** Add the following to a 500 mL flask and dilute to volume using a blank blood matrix:
   a. Using a 1 mg/mL standard solution:
      150 µL Amphetamine, 75 µL Benzoylecgonine, 75 µL Morphine, 150 µL Oxazepam, and 30 µL PCP.
   b. Using a 10 µg/mL standard solution:
      300 µL Fentanyl.

3. **Negative Control.** Use a blank blood matrix.

4. **THC-COOH Cut-Off Calibrator (5 µg/L THC-COOH).** Add the following to a 250 mL flask and dilute to volume using a blank blood matrix:
   a. Using a 10 µg/mL standard solution:
      125 µL THC-COOH.

5. **THC-COOH Positive Control (15 µg/L THC-COOH).** Add the following to a 250 mL flask and dilute to volume using a blank blood matrix:
   a. Using a 10 µg/mL standard solution:
      375 µL THC-COOH.

6. **Negative THC-COOH Control.** Use a blank blood matrix.

MINIMUM STANDARDS & CONTROLS:

Refer to Appendix II.

INSTRUMENTATION:

Dynex DSX Automated System or equivalent. Follow all manufactures guidelines for operations and maintenance.

PROCEDURE OR ANALYSIS

Following the manufacturer's instructions, samples are analyzed alongside calibrators and controls. Blood samples must be pre-diluted with EIA buffer 4:1 (ie. 400 µL sample with 1600 µL EIA buffer) prior to placement on the instrument (Note #1). Follow reagent manufacturer guidelines for other sample types and matrices where applicable.

As programmed by the manufacturer, the instrument places an aliquot of sample into reaction wells coated with antibodies. Next, drug conjugate is added to compete for the antibody sites. The wells are then washed with water to remove any unbound fraction. Substrate is added and the bound conjugate enzyme oxidizes the substrate. Finally, an acid is added that stops the reaction and converts the oxidized substrate into a yellow product which absorbs visible light at 450 nm. At the completion of testing, the instrument measures the amount of color change within each reaction well.

Interpretation of Results:

Color change is inversely proportional to the amount of assay specific drug(s) within the sample. The negative control should normally have the most amount of color change. Highly concentrated samples will have lesser amounts of color change. Analysts should use critical judgment and compare the absorbance measurements of unknown samples against the measurements of the "cut-off" and negative calibrators. Samples with absorbance at or near the negative calibrator must be considered negative (NEG). Samples with absorbance lower than the "cut-off" must be considered positive (POS). Absorbance levels in between the "cut-off" and the negative calibrator must be critically evaluated. Analysts may designate the result as positive-negative (PN) in LIMS (Note: THC is excluded and can only be positive or negative). (PN) designations will trigger the corresponding confirmation "task". Analysts should consider cross reactivity and non-linear enzyme kinetics when making analytical decision based on ELISA results.

Notes:

1. Cases with limited sample can be diluted using comparable amounts, keeping a 1:4 ratio of blood to EIA buffer (ex: 200 µL blood and 800 µL EIA buffer).
2. Cutoff calibrators and positive controls may be prepared in different volumes, based on laboratory needs, but will be made to the listed concentrations above.

LIMITATIONS

1. This procedure was validated per research projects 2019-09 and 2019-11.

REPORT WORDING:

Refer to Appendix I.

REFERENCES:

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3. Neogen Amphetamine Ultra kit package insert, Document D130819. Neogen Corporation 3/24/10.

4. Spiehler, V.R., Collison, I.B., Sedgwick, P.R., Perex, S.L., Le, S.D., Farnin, D.A., "Validation of an automated microplate enzyme immunoassay for screening of postmortem blood for drugs of abuse." Journal of Analytical Toxicology, 1998; 22(7): 573-9.

ILLINOIS STATE POLICE

TOXICOLOGY PROCEDURES MANUAL

PROTOCOL: Qualitative Drug Screening

METHOD: Gas Chromatography-Mass Spectrometry

PROCEDURE: **DRUG SCREEN FOR BLOOD**

Reviewed by:

_____

Sean McCarthy, Chairperson
Toxicology Command Advisory Board

Approved by:

_____

Timothy Tripp
Bureau Chief – Toxicology Command Coordinator

INTRODUCTION

A sample of whole blood is mixed with an internal standard and an alkaline buffer, and the mixture is extracted with n-butyl acetate. The extract is concentrated, and a portion is injected into a gas chromatograph equipped with a mass spectrometer for drug identification.

Other Related Procedures:

See *Codeine Quantitation*

SAFETY CONSIDERATIONS

Warning: Potential Biohazard. Adhere to all blood borne pathogen guidelines.

Warning: NaOH. Corrosive to all tissues. Wear goggles and gloves.

Warning: n-Butyl Acetate. Flammable. May cause irritation of mucous membranes, skin and eyes. Avoid breathing fumes.

Warning: Hexanes. Fire and explosion hazard. May cause irritation of mucous membranes, skin and eyes.

PREPARATIONS

Specimen: Blood; 1.0 mL required.

Reagents:

1. **Stock Standards.** (Cerilliant). 1.0 mg/mL of drugs in methanol.
2. **Bicarbonate Buffer (pH 11.0) containing 1 mg/L promazine working ISTD.** Add 4.2 g of reagent grade sodium bicarbonate (Fisher Scientific Co.), 2.3 g of sodium hydroxide (Fisher Scientific Co.) and 500 µL of promazine stock standard to a 500 mL volumetric flask and q.s. with distilled water. Check pH and adjust if necessary. **Warning: NaOH is caustic material. Wear gloves and face mask.**
3. **n-Butyl Acetate.** (Fisher Scientific Co.) **Warning: Avoid breathing fumes.**
4. **Ethanol**, **Punctilious 200 Proof.** (Aaper Alcohol Co.).
5. **Hexanes**, **Certified A.C.S. Grade.** (Fisher Scientific Co.)

MINIMUM STANDARDS & CONTROLS

Refer to Appendix II.

Controls:

1. In-house control containing the following drugs at 1000 µg/L in blood: Caffeine, Cocaine, Nordiazepam, Alprazolam, or other appropriate control. Several hundred milliliters of this control should be made at one time. It can be placed in small vials and stored in the freezer.

2. Blank blood.

INSTRUMENTATION

Gas Chromatograph/Mass Spectrometer

Instrument Conditions:

Column:	HP-1MS Ultra Inert or HP-5MS Ultra Inert; 30 m x 0.25 mm x 0.25 µm
Injector:	250°C
Detector:	280°C
Temperature Program:	60°C for 1 minute; Ramp 18°C/minute to 190°C, hold for 0.5 minutes; Ramp 5°C/minute to 200°C, hold for 0.5 min; Ramp 5°C/minute to 220°C, hold for 0.5 minutes; Ramp 5°C/minute to 230°C, hold for 1 minute; Ramp 5°C/minute to 260°C; Ramp 25°C/minute to 320°C, hold for 5 minutes.
Scan Range:	40 - 500 m/z

PROCEDURE OR ANALYSIS

Label 13 x 100 mm disposable screw-capped culture tubes for the control, the blank, and each of the unknowns. Place 1.0 mL aliquots of control, blank, and unknown blood samples in the appropriate tubes.

Treat all tubes as follows:

1. Add 1 mL of bicarbonate buffer with ISTD to each tube and mix.
2. Add 1 mL of n-butyl acetate to each tube.
3. Cap the tubes and rotate for 10 minutes.
4. Centrifuge the tubes at 2000 RPM for 10 minutes.
5. Transfer the organic layer in each tube to a test tube. Note #1.
6. Evaporate to dryness under N_2 in a 40°C water bath. (Use caution not to over dry the sample, particularly if amphetamines are suspected.)
7. Reconstitute the residue in hexanes:ethanol (1:1) prior to injection.
8. Inject 1-2 µL into the GC/MS. Note #2.

Interpretation:

A drug will be identified by its mass spectrum. Retention time may also be used to support identification.

Drug Name	**Major Ions**		
Amitriptyline	58	202	215
Bupropion	44	100	111
Butalbital	168	41	167
Carbamazepine	193	236	165
Carisoprodol	55	97	158
Chlorpheniramine	203	167	58
Chlorophenylpiperazine (mCPP)	154	196	111
Citalopram	58	238	208
Clonidine	229	194	172
Cocaine	82	182	94
Cocaethylene	82	196	94
Cyclobenzaprine	58	215	202
Desipramine	234	193	208
Desmethylcitalopram	44	238	208
Diphenhydramine	58	165	73
Doxepin	58	178	165
Doxylamine	58	71	167
Fentanyl	245	146	189
Fluoxetine	44	104	91
Gabapentin Breakdown	81	153	110
Hydroxybupropion	44	116	139
Hydroxyzine	201	299	165
Imipramine	58	234	193
Ketamine	180	209	182
Lamotrigine	185	255	123
Meperidine	71	103	172
Meprobamate	83	55	71
Methadone	72	165	91
Methorphan	271	59	150
MDA	44	136	77
MDMA	58	135	77
Methylphenidate	84	91	56
Mirtazapine	195	43	194
Modafinil artifact	167	152	165
Nordoxepin	44	165	178

Norketamine	166	168	131
Norquetiapine	227	210	239
Nortriptyline	44	202	189
O-Desmethyltramadol	58	249	121
O-Desmethylvenlafaxine	58	120	107
Olanzapine	242	229	213
Oxcarbazepine	180	209	252
Paroxetine	44	192	138
Phenobarbital	204	117	115
Phencyclidine (PCP)	200	242	91
Phenytoin	180	104	77
Promazine (ISTD)	58	284	198
Promethazine	72	180	198
Propofol	163	178	91
Quetiapine	210	239	144
Sertraline	274	159	276
Topiramate Breakdown	43	324	59
Tramadol	58	263	77
Trazodone	205	70	138
Valproic Acid	73	102	115
Venlafaxine	58	134	91
Zolpidem	235	219	307

Several sources are available for identifying the mass spectrum. These include, but are not limited to, *Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites*, and *Instrumental Data for Drug Analysis*, and in-house reference collection created using reference materials traceable to national or international standards.

Notes:

1. Any serum or impurity from the lower aqueous layer may cause interference with the drug or internal standard. It is better to leave a little of the extraction solvent in the tube rather than risk transferring any of the aqueous layer.
2. All drugs present in control must be detected to accept a run.

LIMITATIONS

1. This procedure was validated per research project 2023-06.

2. Stability of extracted samples has not been evaluated. Always inject samples with the concurrently extracted controls, to properly evaluate extraction viability. When possible, samples should be injected the same day they are extracted.

REPORT WORDING

Refer to Appendix I.

REFERENCES

1. Cox, R.A.; Crafasi, J.A.; Dickey, R.E.; Ketzler, S.C.; Pshak, G.L., *Journal of Analytical Toxicology.* 1989, 13, 224.

2. Pfleger, K., Maurer, H.H. and Weber, A. *Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites.* 1st and 2nd eds.; Wiley-VCH:New York 1985, 1992, and 2000 (Part 4).

ILLINOIS STATE POLICE

TOXICOLOGY PROCEDURES MANUAL

PROTOCOL: Qualitative Drug Screening

METHOD: Gas Chromatography-Mass Spectrometry

PROCEDURE: **DRUG SCREEN FOR URINE**

Reviewed by:

_____
Sean McCarthy, Chairperson
Toxicology Command Advisory Board

Approved by:

_____
Timothy Tripp
Bureau Chief – Toxicology Command Coordinator

INTRODUCTION

Commercially prepared extraction tubes simplify the extraction step when preparing a urine specimen for gas chromatography/mass spectrometry analysis and are used for the extraction of basic and neutral drugs (analgesics, stimulants and tranquilizers) from urine. The urine is added to the extraction tubes. The tubes are rotated and then centrifuged. The solvent layer is transferred to a concentrator tube and evaporated. The sample is reconstituted and injected on to the GC/MS.

SAFETY CONSIDERATIONS

Warning: Potential Biohazard; Adhere to all blood borne pathogen guidelines

Warning: Follow all standard laboratory safety guidelines when handling commercially prepared extraction tubes.

PREPARATIONS

Specimen: Urine; 5 mL.

Materials:

1. **De-tox A extraction tubes** (Dyna-Tek Industries) or another appropriate extraction tube.
2. **Blank Urine** for controls.
3. **Stock Standards.** (Cerilliant). 1.0 mg/mL of drug in methanol.
4. **Promazine Working Internal Standard.** 50 µg/mL in methanol. Add 500 uL of promazine stock standard to 10 mL volumetric flask and q.s. to 10 mL with methanol.

MINIMUM STANDARDS & CONTROLS

Refer to Appendix II.

Controls:

1. 2 µg/mL of lidocaine, 3 µg/mL of diazepam and 20 µg/mL of phentermine in urine, or other appropriate control. Several hundred milliliters of this control should be made at one time. It can be placed in small vials and stored in the freezer.
2. Blank urine.

INSTRUMENTATION

Gas Chromatography/Mass Spectrometry

Instrument Conditions:

Column:	HP-1MS Ultra Inert or HP-5MS Ultra Inert; 30 m x 0.25 mm x 0.25 µm
Injector:	250°C
Detector:	280°C
Temperature Program:	60°C for 1 minute; Ramp 18°C/minute to 190°C, hold for 0.5 minutes; Ramp 5°C/minute to 200°C, hold for 0.5 min; Ramp 5°C/minute to 220°C, hold for 0.5 minutes; Ramp 5°C/minute to 230°C, hold for 1 minute; Ramp 5°C/minute to 260°C; Ramp 25°C/minute to 320°C, hold for 5 minutes.
Scan Range:	40 - 500 m/z

PROCEDURE OR ANALYSIS

1. Briefly shake extraction tube. Add urine to the 5 mL arrow, 25 µL of Promazine Working Internal Standard cap and mix by inversion for five minutes. Centrifuge for two to five minutes.
2. Transfer solvent layer to a test tube or concentrator tube. Evaporate under N_2 in a 40°C water bath.
3. Reconstitute the residue with 50 µL of hexane:ethanol (1:1) prior to injection.
4. Inject 1 µL onto the GC/MS.

Interpretation:

A drug will be identified by its mass spectrum. Retention time may also be used to support identification.

Drug Name	**Major Ions**		
Amitriptyline	58	202	215
Bupropion	44	100	111
Butalbital	168	41	167
Carbamazepine	193	236	165
Carisoprodol	55	97	158
Chlorpheniramine	203	167	58
Chlorophenylpiperazine (mCPP)	154	196	111
Citalopram	58	238	208
Clonidine	229	194	172
Cocaine	82	182	94
Cocaethylene	82	196	94
Cyclobenzaprine	58	215	202
Desipramine	234	193	208
Desmethylcitalopram	44	238	208

Diphenhydramine	58	165	73
Doxepin	58	178	165
Doxylamine	58	71	167
Fentanyl	245	146	189
Fluoxetine	44	104	91
Gabapentin Breakdown	81	153	110
Guaiphenesin	124	109	198
Hydroxybupropion	44	116	139
Hydroxyzine	201	299	165
Imipramine	58	234	193
Ketamine	180	209	182
Lamotrigine	185	255	123
Levetiracetam	126	69	41
Meperidine	71	103	172
Meprobamate	83	55	71
Methadone	72	165	91
Methorphan	271	59	150
MDA	44	136	77
MDMA	58	135	77
Methylphenidate	84	91	56
Mirtazapine	195	43	194
Nordoxepin	44	165	178
Norfentanyl	83	159	175
Norketamine	166	168	131
Norquetiapine	227	210	239
Nortriptyline	44	202	189
O-Desmethyltramadol	58	249	121
O-Desmethylvenlafaxine	58	120	107
Olanzapine	242	229	213
Oxcarbazepine	180	209	252
Paroxetine	44	192	138
Phencyclidine (PCP)	200	242	91
Phenytoin	180	104	77
Promazine (ISTD)	58	284	198
Promethazine	72	180	198
Propofol	163	178	91
Quetiapine	210	239	144
Sertraline	274	159	276
Topiramate Breakdown	43	324	59
Tramadol	58	263	77
Trazodone	205	70	138

Valproic Acid	73	102	115
Venlafaxine	58	134	91
Zolpidem	235	219	307

Several sources are available for identifying the mass spectrum. These include, but are not limited to, *Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites*, and *Instrumental Data for Drug Analysis*, and in-house reference collection created using reference materials traceable to national or international standards.

REPORT WORDING

Refer to Appendix I.

LIMITATIONS

1. This procedure was validated per research project 2023-05.

2. Stability of extracted samples has not been evaluated. Always inject samples with the concurrently extracted controls, to properly evaluate extraction viability.

REFERENCES

1. Mills, T. and Roberson, J.C. *Instrumental Data for Drug Analysis,* 1st and 2nd eds., Elsevier: New York, 1982 and 1987.

2. Pfleger, K., Maurer, H.H. and Weber, A. *Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites*, 1st and 2nd eds.; Wiley-VCH: New York, 1985, 1992, and 2000 (Part 4).

3. *Toxi-Lab Instruction Manual*: Analytical Systems, Division of Marion Laboratories, Inc. Laguna Hills, CA, 1983.

4. "*Toxi-Lab" Drug Identification Procedures*: Department of Laboratory Medicine, University of Washington Hospitals, Harborview Medical Center, Harborview, Washington, November 1984.

ILLINOIS STATE POLICE

TOXICOLOGY PROCEDURES MANUAL

PROTOCOL: Qualitative Analysis

METHOD: Gas Chromatography/Mass Spectrometry

PROCEDURE: **GHB CONFIRMATION FOR URINE USING BSTFA**

Reviewed by:

_____

Sean McCarthy, Chairperson
Toxicology Command Advisory Board

Approved by:

_____

Timothy Tripp
Bureau Chief – Toxicology Command Coordinator

INTRODUCTION:

Gamma-Hydroxybutyrate (or Gamma-Hydroxybutyric Acid-GHB) is an endogenous metabolite of GABA (Gamma-amino Butyric Acid). GHB plays a role as a central neurotransmitter and neuromodulator. GHB has been employed clinically since 1960 as an anesthetic and hypnotic agent. Illicit use of GHB often involves oral doses of one teaspoon (approximately 2.5 grams).

The effects of GHB include drowsiness, euphoria, dizziness, nausea, visual disturbances and unconsciousness. These effects usually manifest within 15 minutes after administration.

Although GHB is an endogenous compound, drowsiness and sleep only occur at levels significantly higher than is found endogenously. Deep sleep will occur at levels greater than 250 mg/L, with light to moderate sleep occurring at levels between 50 mg/L and 250 mg/L in the blood.

GHB is extracted using solid phase extraction technique. The extract is evaporated to dryness and derivatized using BSTFA to improve the chromatography.

Urinary GHB concentrations less than 10 mg/L cannot be distinguished from endogenous levels.

SAFETY CONSIDERATIONS:

Warning: Potential Biohazard. Adhere to all blood borne pathogen guidelines.

Warning: Dichloromethane. Vapors are heavier than air and will collect in low areas. When exposed to flames, can form HCl gas. Eye, skin and mucous membrane irritant. Use in hood.

Warning: Sodium Phosphate, Dibasic. May cause eye, skin and mucous membrane irritation.

Warning: Sodium Phosphate, Monobasic. May cause eye, skin and mucous membrane irritation.

Warning: BSTFA. Explosion may occur under fire conditions. Eye, skin and mucous membrane irritant.

Warning: Ammonium hydroxide. Corrosive. Avoid breathing fumes.

PREPARATIONS:

Specimen: One mL of urine.

Reagents

1. **Methanol,** certified A.C.S. grade. (Fisher Scientific).
2. **Bis(trimethylsilyl)trifluoroacetamide/1% trimethylchlorosilane (BSTFA with 1% TMCS).** (Sigma Chemical Company).
3. **Phosphate Buffer, 100mM (pH = 6.0).** Dissolve 1.70 g Na_2HPO_4 (Fisher Scientific) and 12.14 g $NaH_2PO_4 \cdot H_2O$ (Fisher Scientific) in 800 mL distilled water. Dilute to 1000 mL using distilled water. Mix. Adjust pH to 6.0 ± 0.1 with 100 mM monobasic sodium phosphate (lowers pH) or 100 mM dibasic sodium phosphate (raises pH).
4. **Ammonium Hydroxide,** (Fisher Scientific Co.).
5. **Extraction Solvent**, 12 mL Methanol with 200 µL NH_4OH. Make fresh daily.
6. **GHB-D6** (Cerilliant) 1 mg/mL. Internal standard.
7. **Solid Phase Extraction Tubes**. (PHENOMENEX Strata-X-Drug B)

MINIMUM STANDARDS & CONTROLS:

Refer to Appendix II

Control:

1. Positive control spiked at 10 mg/L in distilled water. Note #1.

2. Water (for negative control).

INSTRUMENTATION:

Instrument Conditions:

Column:	HP-1MS Ultra Inert or HP-5MS Ultra Inert 30 m x 0.25 mm x 0.25 µm
Injector:	250°C
Detector:	280°C
Temperature Program:	70ºC hold 0 min.; Ramp 10°C/min. to 200°C; hold for 0 min.; Ramp 30°C/min. to 300°C; hold for 0 min.
Scan Range:	50-550 m/z

PROCEDURE:

Label solid phase extraction tubes for the positive control, blank, and each case sample.

Sample preparation:

1. Place 1 mL of the positive control, negative control, and unknown sample in appropriately labeled test tubes.
2. Add 10 µL of 1 mg/ml GHB-D6 to each sample.
3. Add 1 mL of phosphate buffer 100mM (pH 6.0) to each sample. Vortex and centrifuge.

Conditioning of Columns (under vacuum)

3 mL Methanol
3 mL Distilled/Deionized water
1 mL Phosphate buffer 100 mM (pH 6.0)
Avoid column drying.

Apply Sample

4. Place samples on the column and allow the sample to drip through with no vacuum.
5. Slightly dry columns. Apply vacuum to column for approximately 15 seconds.
6. Place appropriately labeled 16x100mm test tubes into vacuum manifold for sample collection.
7. Add 3 mL of extraction solvent (12 mL Methanol with 200 µL NH_4OH made fresh daily) to each column and allow to drip through without vacuum.
8. Evaporate the solvent to dryness.
9. Add 100 µL BSTFA to the dried sample. Cap the tubes.
10. Heat the sample for 10 minutes at 65°C.
11. Inject 2 µL of sample on the GC/MS.

Interpretation of Results (Note #2):

Drug Name	**Major Ions**		
GHB 2TMS	147	233	204
GHB-D6 2TMS (ISTD)	147	239	206

Reference collection sources include but are not limited to: *Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites* by Pfleger, Maurer, and Weber; Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG); American Academy of Forensic Sciences (AAFS); National Institute of Standards and Technology (NIST); and an in house reference collection created using reference materials traceable to national or international standards.

Notes:

1. Batches of this controls may be prepared ahead of time and stored frozen until needed.

2. If the sample is positive, a second aliquot of urine will be analyzed quantitatively. This aliquot will be injected on a different GC/MS method.

REPORT WORDING:

Refer to Appendix I of Toxicology Manual.

LIMITATIONS

1. This procedure was validated per research project 2023-18.

2. GHB was not evaluated for its individual affinity to the derivatizing agent. This limitation may impact samples with additional components present in high concentrations that are also susceptible to reactions with BSTFA.

3. Stability of extracted samples has not been evaluated. Always inject samples with the concurrently extracted controls, to properly evaluate extraction viability. When possible, samples should be injected the same day they are derivatized.

REFERENCES:

1. Baselt, Randall C. *Disposition of Toxic Drugs and Chemicals in Man*, Eighth ed.; Biomedical Publications, Foster City, CA: 2008.

2. Couper, Fiona J., and Logan, Barry, *Determination of Gamma-Hydroxybutyrate (GHB) in Biological Specimens by Gas Chromatography-Mass Spectrometry,* Journal of Analytical Toxicology, Vol. 24, January/February 2000, pp. 1-7.

3. LeBeau, Marc A., Montgomery, Madeline A., Morris-Kukoski, Cynthia, Schaff, Jason E. And Deakin, Anna "*A Comprehensive Study in the Variations in Urinary Concentrations of Endogenous Gamma-Hydroxybutyrate (GHB).*" Journal of Analytical Toxicology, Volume 30, March 2006.

4. United Chemical Technologies, A Solid Phase Method for Gamma-Hydroxybutyrate (GHB) in Urine without Conversion to Gamma-Butrylactone (GBL), p.33.

ILLINOIS STATE POLICE

TOXICOLOGY
PROCEDURES MANUAL

PROTOCOL: Confirmations

Reviewed by:

_____
Larry Shelton, Chairperson
Toxicology Command Advisory Board

Approved by:

_____
Shannon George
Toxicology Program Manager

ILLINOIS STATE POLICE

TOXICOLOGY PROCEDURES MANUAL

PROTOCOL: Confirmations

METHOD: Benzodiazepines

PROCEDURE: **BENZODIAZEPINE CONFIRMATION FOR URINE**

Reviewed by:

_____
Sean McCarthy, Chairperson
Toxicology Command Advisory Board

Approved by:

_____
Timothy Tripp
Bureau Chief – Toxicology Command Coordinator

INTRODUCTION

The benzodiazepines are a class of drugs effective as antianxiety agents, muscle relaxants, sedative hypnotics and anticonvulsants. They are the most frequently prescribed class of psychotropic drugs in the world today. Because benzodiazepines are eliminated from the body largely as glucuronide conjugates and are polar, often thermally unstable compounds, their detection in urine can be difficult. In addition to this, some benzodiazepines are administered in very low doses.

In order to increase their detectability, benzodiazepines are freed from their glucuronide conjugates by performing an enzyme hydrolysis. They are then extracted, concentrated, and derivatized with MtBSTFA (some benzodiazepines, such as diazepam and alprazolam will not derivatize). The MtBSTFA derivatizes hydroxyl and carboxyl groups and primary and secondary amines. The tert-Butyldimethylsilyl derivatives are stable (less susceptible to hydrolysis) and can be analyzed by gas chromatography/mass spectrometry.

SAFETY CONSIDERATIONS

Warning: Potential Biohazard. Adhere to all blood borne pathogen guidelines.

Warning: β-Glucuronidase (Abalone). Avoid skin contact and inhalation.

Warning: MtBSTFA + 1% t-BDMCS [N-Methyl-N-(t-butyldimethylsilyl) trifluoroacetamine +1% t-Butyl-Dimethylchlorosilane)]. Flammable and corrosive. Eye, skin and mucous membrane irritant. Use in hood.

Warning: n-Butyl Chloride. Dangerous fire/explosion hazard when exposed to flames. Vapors are heavier than air and will collect in low areas. Eye, skin and mucous membrane irritant. Use in hood to avoid breathing fumes. Wear gloves.

Warning: Sodium Acetate. May form combustible dust concentrations in air. Causes irritation.

Warning: Glacial Acetic Acid. A moderate fire hazard when exposed to heat or flames; also a skin and eye irritant. Always add small amount of acid to water with stirring.

PREPARATIONS

Specimen: Urine; 4 mL required.

Reagents:

1. **β-Glucuronidase (Abalone**). (Campbell Science). Approximately1 million units/g solid (concentration may vary by lot).
2. **β-Glucuronidase Working Solution**, 25,000 units/mL. Add 1 bottle of β-glucuronidase to 40 mL distilled water to result in final concentration of 25,000 units/mL.
3. **MtBSTFA+1%t-BDMCS (N-Methyl-N-(t-butyldimethylsilyl) trifluoro acetamine + 1% t-Butyl-Dimethylchlorosilane**). (Campbell Science or equivalent).
4. **Stock Standards**. (Cerilliant) 1.0 mg/mL solutions of benzodiazepines in methanol.
5. **Working Standards**. 100 µg/mL of appropriate drug standard. Dilute 500 µL of stock to 5 mL with methanol.
6. **Sodium Acetate Trihydrate, Certified ACS Grade** (Fisher Scientific).
7. **Glacial Acetic Acid** (Fisher Scientific).
8. **Acetate Buffer, 100mM (pH 4.5)**. Dissolve 5.86 g of sodium acetate trihydrate in 90 mL of distilled water. Add 3.24 mL of glacial acetic acid. Dilute to 1000 mL with distilled water. Mix. Store at 25°C in glass or plastic.
9. **n-Butyl Chloride, Certified ACS Grade** (Fisher Scientific).
10. **Prazepam Stock Internal Standard.** (Cerilliant). Prazepam in methanol, 1.0 mg/mL.
11. **Prazepam Working Internal Standard** 40 µg/mL in methanol. Add 400 µL of prazepam stock standard to a 10 mL volumetric flask and q.s. to 10 mL with methanol.
12. **Methanol, Certified A.C.S. Grade.** (Fisher Scientific Co.).

MINIMUM STANDARDS & CONTROLS

Refer to Appendix II

Controls:

1. Appropriate benzodiazepines at 1 µg/mL each. Spike 4 mL of blank urine with 40 µL of benzodiazepine working standard. Batches of this control may be stored frozen until needed.

2. Blank urine.

INSTRUMENTATION

Agilent Gas Chromatograph/Mass Spectrometer

Instrument Conditions:

Column:	HP-5MS Ultra Inert, 30 m x 0.25 mm x.25 µm film
Injector:	250°C
Detector:	280°C
Temperature Program:	200°C for 0 minutes; Ramp 20°C/min. to 280°C; Hold for 15 minutes.
Scan range:	50-600 m/z

Column:	HP-1MS Ultra Inert, 30 m x 0.25 mm x.25 µm film
Injector:	250°C
Detector:	280°C
Temperature Program:	150°C for 2 minutes; Ramp 12.5°C/min. to 250°C; Hold for 5 minutes. Ramp 7.5°C/min to 300°C, Hold for 5.333 minutes
Scan range:	50-600 m/z

PROCEDURE OR ANALYSIS

Hydrolysis

1. Into appropriately labeled 10 mL screw-capped tubes, pipet 4 mL of case urine, blank urine and control urine.
2. Add 50 µL Prazepam Working ISTD to each tube
3. Add 1 mL of 100mM acetate buffer to each tube and vortex.
4. Add 400 µL of β-glucuronidase working solution to each tube. Cap and vortex to mix.
5. Incubate 4 hours at 60°C or overnight at 37°C.
6. Remove tubes from heating block and allow to cool.

Extraction

7. Add 5 mL of n-butyl chloride to each tube. Rotate 10 minutes. Centrifuge at 2000 rpm for 5 minutes.
8. Transfer solvent (top) layer to clean screw capped tubes.
9. Evaporate to dryness under nitrogen in a 40°C-50°C water bath.

Derivatization

10. To the residue, add 50 µL MtBSTFA, cap and vortex to mix.
11. Heat at 70°C for 30 minutes. Allow tubes to cool.
12. Inject 1 µL of solution directly into GC/MS.

Interpretation of Results:

Drug Name (* denotes drug does not derivitize)	**Major Ions**		
7-Aminoclonazepam TBDMS	342	344	399
alpha-Hydroxyalprazolam TBDMS	381	383	423
alpha-Hydroxymidazolam TBDMS	398	440	324
Alprazolam*	308	279	204
Clonazepam TBDMS	372	374	326
Diazepam*	256	283	221
Flualprazolam*	222	297	326
Lorazepam 2TBDMS	73	491	513
Midazolam*	310	325	163
Nordiazepam TBDMS	327	329	383
Oxazepam 2TBDMS	73	457	513
Temazepam TBDMS	357	255	283
Prazepam*	269	295	324

Reference collection sources include but are not limited to: *Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites* by Pfleger, Maurer, and Weber; Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG); American Academy of Forensic Sciences (AAFS); National Institute of Standards and Technology (NIST); and an in house reference collection created using reference materials traceable to national or international standards.

REPORT WORDING

Refer to Appendix I

LIMITATIONS

1. This procedure was validated per research project 2023-13.

2. Panel drugs were not evaluated for their individual affinity to the derivatizing agent. This may impact cases with multiple panel drugs present in high concentrations.

3. The glucuronide form of each panel drug was not evaluated for its individual hydrolysis effectiveness. This may impact cases with concentrations near the laboratory-defined limit of detection.

4. Stability of extracted samples has not been evaluated. Always inject samples with

the concurrently extracted controls, to properly evaluate extraction viability. When possible, samples should be injected the same day they are derivatized.

REFERENCES

1. West, R.E. and Ritz, D.P. "GC/MS Analysis of Five Common Benzodiazepine Metabolites in Urine as tert-Butyl-dimethylsilyl Derivatives." *Journal of Analytical Toxicology*, 17: 114-116, 1993.

ILLINOIS STATE POLICE

TOXICOLOGY
PROCEDURES MANUAL

PROTOCOL: Confirmations

METHOD: Benzodiazepines

PROCEDURE: **BENZODIAZEPINE CONFIRMATION FOR BLOOD**

Reviewed by:

_____
Sean McCarthy, Chairperson
Toxicology Command Advisory Board

Approved by:

_____
Timothy Tripp
Bureau Chief - Toxicology Command Coordinator

INTRODUCTION

The benzodiazepines are a class of drugs effective as anti-anxiety agents, muscle relaxants, sedative hypnotics and anticonvulsants. They are the most frequently prescribed class of psychotropic drugs in the world today. There exist a large number of drugs belonging to the benzodiazepine family. Some benzodiazepines are administered in very low doses such as alprazolam and clonazepam. Blood or plasma concentrations associated with prescribed use range from sub-nanogram per mL to near-microgram per mL pose a challenge both qualitatively and quantitatively.

Other Related Procedures:

See *Benzodiazepine Confirmation for Urine*

SAFETY CONSIDERATIONS

Warning: Potential Biohazard. Adhere to all blood borne pathogen guidelines.

Warning: Sodium Acetate. May form combustible dust concentrations in air. Causes irritation.

Warning: MtBSTFA + 1% t-Butyl-Dimethylchlorsilane. Flammable and corrosive. Eye, skin and mucous membrane irritant. Use in the hood.

PREPARATIONS

Specimen: Whole blood, 2.0 mL required.

Reagents:

1. **Prazepam Stock Internal Standard.** (USP). Prazepam in methanol, 1.0 mg/mL.
2. **Prazepam Working Internal Standard** 40 µg/mL in methanol. Add 400 µL of prazepam stock standard to a 10 mL volumetric flask and q.s. to 10 mL with methanol.
3. **Methanol, Certified A.C.S. Grade.** (Fisher Scientific Co.).
4. **MtBSTFA + 1% t-BDMCS (N-Methyl-N-(t-butyldimethylsily) trifluoroacetamine + 1% tButyl-Dimethylchlorsilane).**

5. **Carbonate Buffer Solution, pH 9.0.** Combine 20 grams each of sodium carbonate (Mallinckrodt) and sodium bicarbonate (Fisher Scientific Co.). Dissolve in 500 mL of distilled water, adjust pH to 9.0 then bring volume to 1000 mL.
6. **Ethyl Acetate**. (Fisher Scientific).
7. **Solid Phase Extraction Tubes.** (PHENOMENEX Strata-X-Drug B; or appropriate cartridge.)
8. **Sodium Acetate Buffer, 100mM (pH 4.5)**. Dissolve 5.86 g of sodium acetate trihydrate in 90 mL of distilled water. Add 3.24 mL of glacial acetic acid. Dilute to 1000 mL with distilled water. Mix. Store at 25°C in glass or plastic.

MINIMUM STANDARDS & CONTROLS

Refer to Appendix II.

Controls:

1. Appropriate benzodiazepines at appropriate level. Batches of this control may be stored frozen until needed.

2. Blank blood.

INSTRUMENTATION

Gas Chromatography/Mass Spectrometry

Instrument Conditions:

Column:	HP-5MS Ultra Inert, 30 m x 0.25 mm x.25 µm film
Injector:	250°C
Detector:	280°C
Temperature Program:	200°C for 0 minutes; Ramp 20°C/min. to 280°C; Hold for 15 minutes.
Scan range:	50-600 m/z

PROCEDURE

Label disposable 16 x 100mm screw-capped tubes for blank, control, and unknowns.

Treat tubes as follows:

1. To 2 mL of blood add 15 µL ISTD and 4 mL of 100 mM Sodium Acetate Buffer (pH 4.5). Vortex for 15 seconds then centrifuge at 3000 rpm for 10 minutes.
2. Label a SPE column for each sample.
3. Column Conditioning.
   2 mL Ethyl Acetate
   2 mL Methanol
   Dry columns for 15 seconds
4. Sample Loading
   Aspirate sample through column at 10-20 mL/min.
5. Wash Column
   1 mL Carbonate buffer pH 9.0
   1 mL DH_2O
   Dry columns
6. Elution
   Place labeled disposable screw-capped tubes into rack, and position under proper SPE columns.
   Elute with 2 mL Ethyl Acetate: NH_4OH (98:2)
   Note: Decant into a new vial leaving water droplets behind.
7. Evaporate the eluate to dryness.
8. To the dry residue add 50 µL MtBSTFA, cap, and vortex to mix.
9. Heat at 70°C for 30 minutes. Allow tubes to cool.
10. Inject 4 µL directly into the GC/MS.

Interpretation of Results:

Drug Name (* denotes drug does not derivitize)	**Major Ions**		
7-Aminoclonazepam TBDMS	342	344	399
alpha-Hydroxyalprazolam TBDMS	381	383	423
alpha-Hydroxymidazolam TBDMS	398	440	324
Alprazolam*	308	279	204
Clonazepam TBDMS	372	374	326
Diazepam*	256	283	221
Flualprazolam*	222	297	326
Lorazepam 2TBDMS	73	491	513
Midazolam*	310	325	163
Nordiazepam TBDMS	327	329	383
Oxazepam 2TBDMS	73	457	513
Temazepam TBDMS	357	255	283
Prazepam (ISTD)*	324	269	295

Reference collection sources include but are not limited to: *Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites* by Pfleger, Maurer, and Weber; Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG); American Academy of Forensic Sciences (AAFS); National Institute of Standards and Technology (NIST); and an in house reference collection created using reference materials traceable to national or international standards.

Notes:

1. Any serum or impurity from the lower aqueous layer may cause interference with the drug or internal standard. It is better to leave a little of the extraction solvent in the tube rather than risk transferring any of the serum layer.

REPORT WORDING

Refer to Appendix I.

LIMITATIONS

1. This procedure was validated per Research Project 2023-15.

2. Panel drugs were not evaluated for their individual affinity to the derivatizing agent. This may impact cases with multiple panel drugs present in high concentrations.

3. Stability of extracted samples has not been evaluated. Always inject samples with the concurrently extracted controls, to properly evaluate extraction viability. When possible, samples should be injected the same day they are derivatized.

REFERENCES

1. Baselt, Randall C.; *Disposition of Toxic Drugs and Chemicals in Man*, Eighth ed.; Biomedical Publications: Foster City, CA: 2008.

2. SPEware Trace Applications Manual. *Benzodiazepines from Whole Blood.* SPEware Corporation, San Pedro, CA.

3. Winek, Charles L., Wahba Wagdy W., Jr. Winek Charles L., Winek-Balzer, Tracey "Drug and Chemical Blood-Level Data 2001." Forensic Science International, 122, 107-123, 2001.

ILLINOIS STATE POLICE

TOXICOLOGY PROCEDURES MANUAL

PROTOCOL: Confirmations

METHOD: Benzoylecgonine

PROCEDURE: **BENZOYLECGONINE CONFIRMATION FOR URINE USING MtBSTFA**

Reviewed by:

_____

Sean McCarthy, Chairperson
Toxicology Command Advisory Board

Approved by:

_____

Timothy Tripp
Bureau Chief – Toxicology Command Coordinator

INTRODUCTION

Cocaine is the most potent of the naturally occurring central nervous system stimulants. It has been widely utilized as a local anesthetic and increasingly by drug abusers for its stimulant properties. Cocaine can be administered topically, by nasal insulation, by intravenous injection, or as the free base by smoking. Cocaine is eliminated in the urine as unchanged drug and as its primary metabolite, benzoylecgonine.

Benzoylecgonine is extracted using a solid phase extraction technique. The extract is evaporated to dryness and the benzoylecgonine is derivatized using MtBSTFA (N-Methyl-N- (t-Butyldimethylsilyl) to improve its chromatography. The derivatized extracts are injected directly on to the gas chromatograph/mass spectrometer. Because solid phase extraction eliminates the series of cleanup steps that are required using the liquid:liquid extraction, this procedure is faster without affecting the recovery of the drugs.

Other Related Procedures:

See *Cocaine/Benzoylecgonine Quantitation II*

SAFETY CONSIDERATIONS

Warning: Potential Biohazard. Adhere to all blood borne pathogen guidelines.

Warning: Sodium Phosphate, Dibasic. May cause eye, skin and mucous membrane irritation.

Warning: Sodium Phosphate, Monobasic. May cause eye, skin and mucous membrane irritation.

Warning: Concentrated HCl. Corrosive. Use extreme care when handling concentrated acid. Always add small amount of acid to water with stirring. Avoid breathing fumes.

Warning: Dichloromethane. Vapors are heavier than air and will collect in low areas. When exposed to flames, can form HCl gas. Eye, skin and mucous membrane irritant. Use in hood.

Warning: Ammonium hydroxide. Corrosive. Avoid breathing fumes.

Warning: MtBSTFA + 1% t-BDMCS (N-Methyl-N- (t-Butyldimethylsilyl) Trifluoroacetamide + 1 % t-Butyl-Dimethylchlorosilane). Flammable and corrosive. Eye, skin and mucous membrane irritant. Use in hood.

PREPARATIONS

Specimen: Urine; 5 mL required.

Reagents:

1. **Blank Urine** for controls.
2. **Benzoylecgonine Stock Standards**. (Cerilliant). 1.0 mg/mL of benzoylecgonine in methanol.
3. **Solid Phase Extraction Tubes** (TECAN Trace-J, PHENOMENEX Strata-X-Drug B; or appropriate cartridge.)
4. **Phosphate Buffer, 100 mM (pH = 6.0)**. Dissolve 1.70 g Na_2HPO_4 (Fisher Scientific) and 12.14 g $NaH_2PO_4 \bullet H_2O$ (Fisher Scientific) in 800 mL distilled water. Dilute to 1000 mL using distilled water. Mix. Adjust pH to 6.0 ± 0.1 with 100 mM monobasic sodium phosphate (lowers pH) or 100 mM dibasic sodium phosphate (raises pH).
5. **Sodium Phosphate, Dibasic, l00 mM.** Dissolve 2.84 g Na_2HPO_4 in 160 mL distilled water. Dilute to 200 mL with distilled water. Mix. Store at 4ºC in glass.
6. **Sodium Phosphate, Monobasic, 100 mM.** Dissolve 2.76g $NaH_2PO_4 \bullet H_2O$ in 160 mL distilled water. Dilute to 200 mL with distilled water. Mix. Store at 4°C in glass.
7. **Methanol**. (Fisher Scientific).
8. Distilled Water.
9. **Hydrochloric Acid, 0.1 M**. (Fisher Scientific). Add 4.2 mL concentrated HCl to 400 mL distilled water. Dilute to 500 mL. Warning: Prepare in hood. Handle HCl with care. Always add acid to water with stirring.
10. **Elution Solvent**. Dichloromethane (Fisher Scientific)/Isopropanol (Fisher Scientific)/Ammonium hydroxide (Mallinckrodt) (78/20/2). Mix 390 mL of dichloromethane with 100 mL of isopropanol. Store in an Oxford pipettor. Before each use add 200 µL of ammonium hydroxide to each 10 mL of dichloromethane/ isopropanol. Mix thoroughly. Warning: Prepare in hood. Do not breathe fumes.
11. **MTBSTFA + 1% T-BDMCS**. (N-Methyl-N- (t-Butyldimethylsilyl) Trifluoroacetamide + 1% t-Butyl-Dimethylchlorosilane.
12. **Methapyrilene Stock Standard**. (Cayman) Methapyrilene hydrochloride. Dissolve 5 mg in 5 mL methanol to make a 1.0 mg/mL solution.
13. **Methapyrilene Working Internal Standard**. 45 µg/mL in methanol. Add 450 µL of methapyrilene stock standard to 10 mL volumetric flask and q.s. to 10 mL with methanol.

MINIMUM STANDARDS & CONTROLS

Refer to Appendix II

Controls:

1. 3 µg/mL benzoylecgonine or appropriate control.
2. Blank urine.

INSTRUMENTATION

Gas Chromatograph with Mass Spectrometer

Instrument Conditions:

Column:	HP-1MS Ultra Inert or HP-5MS Ultra Inert 30 m x 0.25 mm x 0.25 µm.
Injector:	250°C
Detector:	280°C
Temperature Program:	120°C for 1 min Ramp 20°C/min to 300°C Hold for 6 min.
SCAN range:	40 to 500 m/z
Purge valve	ON at 2 minutes.

PROCEDURE OR ANALYSIS

Label 16 x 100 test tubes for a blank, positive control and each of the unknowns.

Treat all tubes as follows:

Sample Pretreatment

1. To 5 mL of urine, add 50 µL working internal standard. Vortex.
2. Add 5 mL of 100 mM phosphate buffer (pH 6.0). Vortex, then centrifuge at 2000 rpm for 10 minutes.
3. Label a SPE column for each sample. Extract as follows:
4. Sample Loading
   Aspirate sample through column at 1-2 mL/min. Note: When decanting supernatant from the tube, use care not to disturb the pellet at the bottom of the tube.
5. Wash Column
   2 mL distilled water
   2 mL 0.1 M HC1
   3 mL methanol. Dry for 5 minutes.
6. Elution
   Place labeled 16 x 100 mm disposable screw-capped tubes into rack, and position under proper SPE columns.
   Elute with 3 mL elution solvent at 1-2 mL/min. Note #1
7. Derivatization

Evaporate the eluate to dryness.
To each tube add 50 µL of MtBSTFA (use under hood). Vortex, cap and heat in a 60°C heating block for 10 minutes. Note #2.

8. Analyze
Transfer the MtBSTFA to properly labeled autosampler vials with micro-inserts and inject 2 µL directly on to the GC/MS. DO NOT EVAPORATE BEFORE INJECTING.

Interpretation of Results:

Drug Name (* denotes drug does not derivitize)	**Major Ions**		
Benzoylecgonine TBDMS (Cocaine metabolite)	82	282	403
Cocaethylene*	82	196	317
Cocaine*	82	182	303
Methapyrilene*	58	97	261

Reference collection sources include but are not limited to: *Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites* by Pfleger, Maurer, and Weber; Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG); American Academy of Forensic Sciences (AAFS); National Institute of Standards and Technology (NIST); and an in house reference collection created using reference materials traceable to national or international standards.

Notes:

1. Add ammonium hydroxide to elution solvent prior to use.
2. After elution, the elution solvent may be evaporated to dryness in the 12x75 culture tubes. Add 50 µL of MtBSTFA to each tube and vortex. Using glass micro-pipettes, transfer the MtBSTFA to labeled autosampler vials with micro-inserts, apply a crimp cap to seal. Place each vial into a 13 x 100 mm culture tube. Using the heating block, heat the vials for 10 minutes at 60°C.

REPORT WORDING

Refer to Appendix I

LIMITATIONS

1. This procedure was validated per research project 2023-12.

2. Benzoylecgonine was not evaluated for its individual affinity to the derivatizing agent. This limitation may impact samples with additional components present in high concentrations that are also susceptible to reactions with MtBSTFA.

3. Stability of extracted samples has not been evaluated. Always inject samples with the concurrently extracted controls, to properly evaluate extraction viability. When possible, samples should be injected the same day they are derivatized.

REFERENCES

1. Baselt, Randall C. “Disposition of Toxic Drugs and Chemicals in Man.” Biomedical Publications, 2nd Edition, 1982, pp. 193-198.

2. Tietz, N. W. “Clinical Guide to Laboratory Tests, Second Edition.” W. B. Saunders Company, Philadelphia: 1990.

3. Worldwide Monitoring. “Clean Screen Extraction Columns Application Manual.” United Chemical Technologies, Bristol, PA: 1995.

ILLINOIS STATE POLICE

TOXICOLOGY
PROCEDURES MANUAL

PROTOCOL: Confirmations

METHOD: Gas Chromatography/Mass Spectrometry

PROCEDURE: **BENZOYLECGONINE CONFIRMATION FOR BLOOD USING MtBSTFA**

Reviewed by:

_____
Sean McCarthy, Chairperson
Toxicology Command Advisory Board

Approved by:

_____
Timothy Tripp
Bureau Chief – Toxicology Command Coordinator

INTRODUCTION

Cocaine is the most potent of the naturally occurring central nervous system stimulants. It has been widely utilized as a local anesthetic and increasingly by drug abusers for its stimulant properties. Cocaine can be administered topically, by nasal insulation, by intravenous injection, or as the free base by smoking. Cocaine is eliminated in the urine as unchanged drug, as its primary metabolite benzoylecgonine, as ecgonine methyl ester and as ecgonine. The combined use of ethanol and cocaine causes the production of *cocaethylene*.

Cocaine, cocaethylene, and benzoylecgonine are extracted together with an internal standard using solid phase extraction technique. The extract is evaporated to dryness and the benzoylecgonine is derivatized using MtBSTFA (N-Methyl-N-(t-Butyldimethylsilyl) to improve its chromatography. These derivatized extracts are injected directly on to the gas chromatograph/mass spectrometer.

Other Related Procedures:

See *Benzoylecgonine Confirmation in urine and Cocaine/Benzoylecgonine Quantitation II.*

SAFETY CONSIDERATIONS

Warning: Potential Biohazard. Adhere to all blood borne pathogen guidelines.

Warning: Sodium Phosphate, Dibasic. May cause eye, skin and mucous membrane irritation.

Warning: Sodium Phosphate, Monobasic. May cause eye, skin and mucous membrane irritation.

Warning: Concentrated HCl. Corrosive. Use extreme care when handling concentrated acid. Always add small amount of acid to water with stirring. Avoid breathing fumes.

Warning: Dichloromethane. Vapors are heavier than air and will collect in low areas. When exposed to flames, can form HCl gas. Eye, skin and mucous membrane irritant. Use in hood.

Warning: Ammonium hydroxide. Corrosive. Avoid breathing fumes.

Warning: MtBSTFA + 1% t-BDMCS (N-Methyl-N-(t-Butyldimethylsilyl) Trifluoroacetamide + 1% t-Butyl-Dimethylchlorosilane). Flammable and corrosive. Eye, skin and mucous membrane irritant. Use in hood.

PREPARATIONS

Specimen: Whole blood, serum, or plasma; minimum of 1.0 mL required.

Reagents:

1. **Blank Blood** for standard curve.
2. **Cocaine, Cocaetheylene and Benzoylecgonine Stock Standards**. (Cerilliant). 1.0 mg/mL of cocaine, cocaethylene and benzoylecgonine in methanol.
3. **Cocaine, Cocaethylene and Benzoylecgonine Working Standard**. 10 µg/mL of Cocaine, cocaethylene and benzoylecgonine in methanol. Dilute 100 µL of each stock to 10 mL with methanol.
4. **Solid Phase Extraction Tubes** (PHENOMENEX Strata-X-Drug B; or appropriate cartridge.)
5. **Phosphate Buffer, 100 mM (pH = 6.0)**. Dissolve 1.70 g Na_2HPO_4 (Fisher Scientific) and 12.14 g $NaH_2PO_4 \cdot H_2O$ (Fisher Scientific) in 800 mL distilled water. Dilute to 1000 mL using distilled water. Mix. Adjust pH to 6.0 ± 0.1 with 100 mM monobasic sodium phosphate (lowers pH) or 100 mM dibasic sodium phosphate (raises pH).
6. **Sodium Phosphate, Dibasic, l00 mM**. Dissolve 2.84 g Na_2HPO_4 in 160 mL distilled water. Dilute to 200 mL with distilled water. Mix. Store at 4°C in glass.
7. **Sodium Phosphate, Monobasic, 100 mM**. Dissolve 2.76 g $NaH_2PO_4 \bullet H_2O$ in 160 mL distilled water. Dilute to 200 mL with distilled water. Mix. Store at 4°C in glass.
8. **Methanol.** (Fisher Scientific).
9. **Distilled Water.**
10. **Hydrochloric Acid**, 0.1 M. (Fisher Scientific). Add 4.2 mL concentrated HCl to 400 mL distilled water. Dilute to 500 mL. Warning: Prepare in hood. Handle HCl with care. Always add acid to water with stirring.
11. **Elution Solvent.** Dichloromethane (Fisher Scientific)/Isopropanol (Fisher Scientific)/Ammonium hydroxide (Mallinckrodt) (78/20/2). Mix 390 mL of dichloromethane with 100 mL of isopropanol. Store in an Oxford pipettor. Before each use add 200 µL of ammonium hydroxide to each 10 mL of dichloromethane/ isopropanol. Mix thoroughly. Warning: Prepare in hood. Do not breathe fumes.
12. **MTBSTFA + 1% T-BDMCS** (N-Methyl-N-(t-Butyldimethylsilyl) Trifluoroacetamide + 1% t-Butyl-Dimethylchlorosilane.
13. **Methapyrilene Stock Standard**. (Cayman) Methapyrilene hydrochloride. Dissolve 5 mg in 5 mL methanol to make a 1.0 mg/mL solution.
14. **Methapyrilene Working Internal Standard**. 10 µg/mL in methanol. Add 10 µL of methapyrilene stock standard to 10 mL volumetric flask and q.s. to 10 mL with methanol.

MINIMUM STANDARDS & CONTROLS

Refer to Appendix II

Control:

1. In-house positive control prepared with benzoylecgonine, cocaethylene, and cocaine at 500 ng/mL or other appropriate control. (Note #2)

2. Blank blood for negative control.

INSTRUMENTATION

Gas Chromatograph with Mass Spectrometer

Instrument Conditions:

Column:	HP-5MS Ultra Inert 30 m x 0.25 mm x 0.25 µm
Injector:	250°C
Detector:	280°C
Temperature Program:	120°C for 1 min Ramp 20°C/min to 300°C Hold for 6 min.
SCAN range:	40 to 500 m/z
Purge valve	ON at 2 minutes.

PROCEDURE OR ANALYSIS

Label 16 x I 00 culture tubes for positive and negative controls and each unknown.

Treat all tubes as follows:

1. Sample Pretreatment
   To 1 mL of blood add 50 µL of working internal standard.
   Add 5 mL of 100 mM phosphate buffer (pH 6.0).
   Vortex. Centrifuge for 10 minutes.
   Label a SPE column for each sample.
2. Column Conditioning.
   1 mL phosphate buffer (avoid sorbent drying)
3. Sample Loading
   Aspirate sample through column at 1-2 mL/min. Note: When decanting supernatant from the tube, use care not to disturb the pellet at the bottom of the tube.
4. Wash Column
   2 mL distilled water
   2 mL 0.1 M HC1
   3 mL methanol
   Dry for 5 minutes.
5. Elution
   Place labeled 16 x 125 mm disposable screw-capped tubes into rack, and position under proper SPE columns.

Elute with 3 mL elution solvent at 1-2 mL/min. Transfer elution solvent to 13x100 mm screw-capped tubes. (Note #1)

6. Derivatization

Evaporate the eluate to dryness.
To each tube add 50 µL of MtBSTFA (use under hood).
Vortex, cap and heat in a 60°C heating block for 10 minutes.
If using an autosampler, transfer the MtBSTFA to properly labeled autosampler vials with micro-inserts and inject on to the GC/MS.
DO NOT EVAPORATE BEFORE INJECTING.

Interpretation of Results:

Drug Name (* denotes drug does not derivitize)	**Major Ions**		
Benzoylecgonine TBDMS (Cocaine metabolite)	82	282	403
Cocaethylene*	82	196	317
Cocaine*	82	182	303
Methapyrilene*	58	97	261

Reference collection sources include but are not limited to: *Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites* by Pfleger, Maurer, and Weber; Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG); American Academy of Forensic Sciences (AAFS); National Institute of Standards and Technology (NIST); and an in house reference collection created using reference materials traceable to national or international standards.

Notes:

1. After elution, the elution solvent may be evaporated to dryness in the 12x75 culture tubes. Add 50 µL of MtBSTFA to each tube and vortex. Using glass micropipettes, transfer the MtBSTFA to labeled autosampler vials with micro-inserts, apply a crimp cap to seal. Place each vial into a 13 x 100 mm culture tube. Using the heating block, heat the tubes for 10 minutes at 60ºC.

2. Batches of this control may be stored frozen until needed. Spike blank blood with 25 µL of each 1mg/mL standard q.s. to 50 mL.

REPORT WORDING

Refer to Appendix I

LIMITATIONS

1. This procedure was validated per research project 2023-11.

2. Benzoylecgonine was not evaluated for its individual affinity to the derivatizing agent. This limitation may impact samples with additional components present in high concentrations that are also susceptible to reactions with MtBSTFA.

3. Stability of extracted samples has not been evaluated. Always inject samples with the concurrently extracted controls, to properly evaluate extraction viability. When possible, samples should be injected the same day they are derivatized.

REFERENCES

1. Baselt, Randall C. Disposition of Toxic Drugs and Chemicals in Man, Biomedical Publications, Eighth Edition, 2008.

2. *SPEware Trace Applications Manual*, updated 11-2006.

3. Tietz, N. W. Clinical Guide to Laboratory Tests, Second Edition, W. B. Saunders Company, Philadelphia, 1990.

4. Winek, C. L., Wahba, W. W., Winek, Jr., C. L., Winek Balzer, T., "Drug and Chemical Blood-Level Data 2001," *Forensic Science International* 122 (2001) 107-123.

5. Worldwide Monitoring. Clean Screen® Extraction Columns Application Manual, United Chemical Technologies, Bristol, PA: 1995.

ILLINOIS STATE POLICE

TOXICOLOGY PROCEDURES MANUAL

PROTOCOL: Confirmations

METHOD: Cannabinoids

PROCEDURE: **CANNABINOIDS CONFIRMATION FOR URINE**

Reviewed by:

_____
Sean McCarthy, Chairperson
Toxicology Command Advisory Board

Approved by:

_____
Timothy Tripp
Bureau Chief – Toxicology Command Coordinator

INTRODUCTION

Cannabis sativa, in the form of marijuana or hashish, is perhaps the most widely used psychoactive drug in the world. It is abused for its euphoric and hallucinogenic properties. Tetrahydrocannabinol (THC) is the most active principle constituent of marijuana (Cannabis sativa) and is administered either orally or by smoking. THC is also used as a drug to reduce intraocular pressure in glaucoma patients and to relieve nausea and suffering in terminal cancer patients.

Cannabinoids are readily metabolized in mammals. In particular, THC, the primary psychoactive agent, is oxidized first to 11-Hydroxy-THC, which is further oxidized to the carboxy derivative (11-Nor-Delta 9-Tetrahydrocannabinol-9-Carboxylic acid or THC Acid). All three drugs, THC, 11-Hydroxy-THC and THC Acid are excreted in the urine with the first two found in trace quantities and the latter predominating. Eighty percent of the THC acid in urine is present in the form of the glucuronide conjugate.

Urine is hydrolyzed and extracted. The carboxylic and hydroxyl groups of THC acid are ethylated resulting in the diethyl derivative of THC acid. An extract of this derivative is then subjected to analysis by gas chromatography-mass spectrometry (GC/MS). This procedure is used as confirmation of a positive EMIT screen for cannabinoids.

SAFETY CONSIDERATIONS

Warning:	Potential Biohazard. Adhere to all blood borne pathogen guidelines.
Warning:	Potassium Hydroxide. Corrosive. May cause severe burns.
Warning:	HCl. Heat will be generated and a water bath is recommended. Always add acid to water with stirring. Prepare in hood. Wear gloves and mask.
Warning:	Ethyl Acetate. Dangerous fire hazard when exposed to heat or flame. Use in hood.
Warning:	Hexanes. Dangerous fire hazard when exposed to heat or flame. Use in hood.
Warning:	Bis(trimethylsilyl)trifluoroacetamide/1% Trimethylchlorosilane (BSTFA with 1% TMCS). Explosion may occur under fire conditions. Eye, skin, and mucous membrane irritant.

PREPARATIONS

Specimen: Urine; minimum of 5 mL required.

Reagents:

1. **11-Nor-Delta 9-Tetrahydrocannabinol-9-Carboxylic Acid.** (THC carboxylic acid). 100 µg/mL in methanol. (Cerilliant.)
2. **Working THC-COOH Solution – 1.0 µg/mL.** Dilute 0.5 mL of standard (100 µg/mL THC-COOH) to 50 mL with methanol in a **silanized** volumetric flask. Refrigerate.
3. **Cannabinol Internal Standard Solution.** 10 µg/mL. Dilute 1.0 mg/mL certified standard of Cannabinol to 100 mL with MeOH. Refrigerate.
4. **Potassium Hydroxide, 11.8 N. (Cannabinoid Hydrolysis Reagent).** (Mallinckrodt). Dissolve 33.1 g of Potassium Hydroxide in 50 mL of distilled H_2O. **Warning: Caustic. Wear mask and gloves.**
5. **50% HCl.** (Fisher Scientific Co.)**.** Slowly add 50 mL of acid to 50 mL of water with stirring. Heat will be generated and a water bath is recommended. **Warning: Always add acid to water with stirring. Prepare in hood. Wear gloves and mask.**
6. **Ethyl Acetate. (**Fisher Scientific Co.).
7. **Hexanes, HPLC Grade.** (Fisher Scientific Co.)**.**
8. **Extraction Solvent,** Hexanes/Ethyl Acetate 7:1 (V/V). **Warning: Prepare in hood. Wear gloves and face mask.**
9. **Bis(trimethylsilyl)trifluoroacetamide/1% Trimethylchlorosilane (BSTFA with 1% TMCS).** (Sigma-Aldrich).

MINIMUM STANDARDS & CONTROLS

Refer to Appendix II.

Controls:

1. **Fresh Spiked Control, 50 ng/mL**. Add 0.25 mL of working THC-COOH solution to 4.75 mL blank urine.

2. **Blank urine**.

INSTRUMENTATION

Gas Chromatograph/Mass Spectrometer

Instrument Conditions:

Column:	HP-1MS Ultra Inert or HP-5MS Ultra Inert 30 m x 0.25 mm x 0.25 µm
Injector:	250°C
Detector:	280°C
Temperature Program:	180°C for 0 min. Ramp 20°C/min. to 280°C Hold for 10 min.
Scan:	200-510 m/z

PROCEDURE OR ANALYSIS

Extraction

1. Label clean 16 x 125 mm screw-capped tubes for each unknown and the positive and negative controls.
2. Add 5.0 mL of appropriate sample to each tube.
3. Add 20 µL of cannabinol internal standard solution to each tube and vortex briefly to mix.
4. Add 200 µL of Cannabinoid Hydrolysis Reagent (11.8 N KOH) to each tube. Cap and vortex to mix. Heat at 60°C for 15 minutes.
5. Cool tubes to room temperature and add 500 µL of 50% HCl to each. Vortex to mix. Check that pH < 2.0.
6. Add 5.0 mL of hexanes/ethyl acetate extraction solvent to each tube, cap, and rotate for 15 minutes.
7. Centrifuge at 2000 rpm for 5 minutes.
8. Transfer organic layer to 16 x 100 mm screw-capped tubes and evaporate to dryness under N_2 using water bath at 40°C.

Derivatization:

9. To the residue, add 50 µL of BSTFA. Cap and vortex to mix. Note #2
10. Heat tubes at 65ºC for 30 minutes. Do Not Evaporate.
11. Inject 2 µL onto the GC/MS.

Interpretation of Results:

Drug Name	Major Ions		
Delta-9-Carboxy-THC 2TMS	371	473	488
Cannabinol (ISTD) TMS	367	382	310

Reference collection sources include but are not limited to: *Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites* by Pfleger, Maurer, and Weber; Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG); American Academy of Forensic Sciences (AAFS); National Institute of Standards and Technology (NIST); and an in house reference collection created using reference materials traceable to national or international standards.

Notes:

1. Any impurities from the lower aqueous layer may cause interference with the drug. It is better to leave a little of the extraction solvent in the tube rather than risk transferring any of the aqueous layer.

2. After elution, the elution solvent may be evaporated to dryness in the 12x75 culture tubes. Add 50 µL of BSTFA to each tube and vortex. Using glass micro-pipettes, transfer the BSTFA to labeled autosampler vials with micro-inserts, apply a crimp cap to seal. Place each vial into a 13 x 100 mm culture tube. Using the heating block, heat the vials for 30 minutes at 65°C.

REPORT WORDING

Refer to Appendix I.

LIMITATIONS

1. This procedure was validated per Research Project 2023-14.

2. Panel drugs were not evaluated for their individual affinity to the derivatizing agent. This may impact cases with multiple panel drugs present in high concentrations.

3. Stability of extracted samples has not been evaluated. Always inject samples with the concurrently extracted controls, to properly evaluate extraction viability. When possible, samples should be injected the same day they are derivatized.

REFERENCES

1. Baker, T.S.; Harry, J.V.; Russell, J.W.; Myers, R.L. "Rapid Method for the GC/MS Confirmation of 11-Nor-9-Carboxy-9-Tetrahydrocannabinol in Urine." *Journal of Analytical Toxicology.* 1984 8, 255-259.

2. Bursey, J.T.; Cook, C.E.; Foltz, R.L.; Irving, J.; Leeb, B; Willette, R.E. "Evaluation of Immunoassays for Cannabinoids in Urine." *Journal of Analytical Toxicology.* 1984 8, 192-196.

3. Callahan, L.S., Childs, P.S., Lewellyn, L.J., McCurdy, H. H. "Evaluation of the Ion Trap Detector for the Detection of 11-nor-delta-9- THC-9-carboxylic acid in Urine After Extraction by Bonded-Phase Adsorption." *Journal of Analytical Toxicology.* 1986, 10, 175-177.

4. Kiser, R.E.; Law, D.; Patterson, J.E., III; Ramsden, H.E.: "Solid Phase Extraction of Delta-9-Carboxy-THC from Urine." *Clin. Chem.*, 1986 32(6), 1115.

5. *Toxi-Lab Cannabinoid (THC) Screen Instruction Manual*: Analytical Systems, Division of Marion Laboratories, Inc., 1983.

ILLINOIS STATE POLICE

TOXICOLOGY PROCEDURES MANUAL

PROTOCOL: Confirmations

METHOD: Opiates

PROCEDURE: **OPIATE CONFIRMATION FOR BLOOD USING BSTFA**

Reviewed by:

_____
Sean McCarthy, Chairperson
Toxicology Command Advisory Board

Approved by:

_____
Timothy Tripp
Bureau Chief – Toxicology Command Coordinator

INTRODUCTION

The presence of opiates such as morphine, codeine, hydromorphone, hydrocodone, dihydrocodeine, oxycodone, oxymorphone and 6-monoacetylmorphine can be confirmed in blood using solid phase extraction and derivatizing with BSTFA to produce the trimethylsilyl (TMS) derivatives. Using this derivatization method, 6-monoacetylmorphine, the specific metabolite of heroin, can be detected when present in a sample. The derivatized sample is analyzed by GC/MS.

Other Related Procedures:

See *Opiate Confirmation for Urine.*

SAFETY CONSIDERATIONS

Warning: Potential Biohazard. Adhere to all blood borne pathogen guidelines.

Warning: BSTFA. Explosion may occur under fire conditions. Eye, skin and mucous membrane irritant.

Warning: Sodium Acetate. May form combustible dust concentrations in air. Causes irritation.

Warning: Glacial Acetic Acid is a moderate fire hazard when exposed to heat or flames; also a skin and eye irritant. Always add small amount of acid to water with stirring.

Warning: Dichloromethane. Vapors are heavier than air and will collect in low areas. When exposed to flames, can form HCl gas. Eye, skin and mucous membrane irritant. Use in hood.

Warning: Ammonium hydroxide. Corrosive. Avoid breathing fumes.

Warning: Sodium Phosphate, Dibasic. May cause eye, skin and mucous membrane irritation.

Warning: Sodium Phosphate, Monobasic. May cause eye, skin and mucous membrane irritation.

PREPARATIONS

Specimen: Blood, 2 mL required

Reagents:

1. **Solid Phase Extraction Tubes.** (PHENOMENEX Strata-X-Drug B; or appropriate cartridge.)

2. **Bis(trimethylsilyl)trifluoroacetamide/1% Trimethylchlorosilane (BSTFA with 1% TMCS).** (Campbell Science or equivalent).
3. **Nalorphine Stock Internal Standard.** (Sigma-Aldrich). Nalorphine in methanol 1.0 mg/mL.
4. **Nalorphine Working ISTD.** 100 µg/mL nalorphine. Dilute 1.0 mL of stock to 10 mL with methanol.
5. **Phosphate Buffer, 100 mM (pH = 6).** Dissolve 1.70 g Na_2HPO_4 (Fisher Scientific) and 12.14 g $NaH_2PO_4 \cdot H_2O$ (Fisher Scientific) in 800 mL distilled H_2O. Dilute to 1000 mL using distilled H_2O. Mix. Adjust pH to 6.0 ± 0.1 with 100 mM monobasic sodium phosphate (lowers pH) or 100 mM dibasic sodium phosphate (raises pH).
6. **Sodium Phosphate, Dibasic, 100mM:** Dissolve 2.84 g Na_2HPO_4 in 160 mL DI H_2O. Dilute to 200 mL using DI H_2O. Mix. Storage: 5ºC in glass.
7. **Sodium Phosphate, Monobasic, 100 mM:** Dissolve 2.76 g $NaH_2PO_4 \cdot H_2O$ in 160 mL DI H_2O. Dilute to 200 mL with DI H_2O. Mix. Storage: 5ºC in glass.
8. **Acetate Buffer, 100 mM.** (pH 4.5) Dissolve 5.86 g of sodium acetate trihydrate in 90 mL of distilled water. Add 3.24 mL of glacial acetic acid. Dilute to 1000 mL with distilled water. Mix. Storage: 25°C in glass or plastic.
9. **Elution Solvent.** Dichloromethane (Fisher Scientific)/Isopropanol (Fisher Scientific)/Ammonium hydroxide (Mallinckrodt) (78/20/2). Mix 390 mL of dichloromethane with 100 mL of isopropanol. Store in an Oxford pipettor. Before each use, add 200 µL of ammonium hydroxide (Fisher Scientific) to each 10 mL of dichloromethane/isopropanol. Mix thoroughly. Note #2. **Warning: Prepare in hood. Do not breathe fumes.**
10. **Methanol**. (Fisher Scientific).
11. **Stock Standards.** (Cerilliant). Solutions of the appropriate opiates in methanol, 1.0 mg/mL as needed. (Codeine, Morphine, etc.)

MINIMUM STANDARDS & CONTROLS

Refer to Appendix II

Controls:

1. In-house control spiked with morphine, 75 µg/L and codeine, 250 µg/L or other appropriate control.
2. Blank blood for negative control.

INSTRUMENTATION

Gas Chromatograph/Mass Spectrometer

Instrument Conditions:

Column: HP-5MS Ultra Inert

	30 m x 0.25 mm x 0.25 µm
Injector:	250°C
Detector:	280°C
Temperature Program:	150°C for 1 min.; Ramp 10°C/min to 280°C; Hold for 5 min.
Scan Range:	80 - 525 m/z

PROCEDURE OR ANALYSIS

Sample Pretreatment

1. Place 2 mL of blood from control, blank and case in appropriately labeled 16 x 100 screw-capped test tubes. Add 20 µL of ISTD.
2. Add 8 mL of 100mM phosphate buffer (pH 6.0). Mix/vortex. Centrifuge for 10 minutes.
3. Label a SPE column for each sample.

Extract as follows:

4. Sample Loading
   Decant supernatant from the tube using care not to disturb the pellet at the bottom of the tube.
   Aspirate sample through column at approximately 1-2 mL/minute.

5. Wash column.
   2 mL distilled water
   2 mL 100 mM acetate buffer
   3 mL methanol
   Dry column for 5 minutes.

6. Elution
   Place labeled 13 x 100 disposable screw-capped tubes into rack, and position under the proper SPE columns.
   Elute with 3 mL elution solvent at 1 to 2 mL/minute. Note #2
   Evaporate to dryness under N_2 using water bath at 40°C.

Derivatization

7. To the residue in each tube, add 50 µL of BSTFA. Cap and vortex to mix.
8. Incubate at 65°C for 30 minutes. **Do Not Evaporate.**
9. Inject 1 µL into the GC/MS.

Interpretation of Results:

Drug Name (Note #1)	Major Ions		
6-Monoacetylmorphine TMS	399	340	287
Codeine TMS	371	178	234
Codeine (underivatized)	299	162	229
Dihydrocodeine TMS	373	315	236
Hydrocodone enol TMS	371	234	356
Hydrocodone (underivatized)	299	242	185
Hydromorphone TMS	357	300	342
Hydromorphone enol 2TMS	429	414	234
Morphine 2TMS	429	414	236
Oxycodone TMS	387	229	372
Oxycodone enol 2TMS	459	444	368
Oxycodone (underivatized)	315	230	201
Oxymorphone TMS	373	288	259
Nalorphine 2TMS (ISTD)	455	414	440

Reference collection sources include but are not limited to: *Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites* by Pfleger, Maurer, and Weber; Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG); American Academy of Forensic Sciences (AAFS); National Institute of Standards and Technology (NIST); and an in house reference collection created using reference materials traceable to national or international standards.

Notes:

1. Some panel opiates may appear as the drug underivatized, or as one or two derivatized products. Detection of these drugs only require positive identification for 1 of these products.
2. Add ammonium hydroxide to elution solvent prior to use.

REPORT WORDING

Refer to Appendix I

LIMITATIONS

1. This procedure was validated per research project 2023-09.

2. Panel drugs were not evaluated for their individual affinity to the derivatizing agent. This may impact cases with multiple panel drugs present in high concentrations.

3. Stability of extracted samples has not been evaluated. Always inject samples with the concurrently extracted controls, to properly evaluate extraction viability. When possible, samples should be injected the same day they are derivatized.

4. Interference between opiates that elute at close retention times may occur. Samples should be analyzed on the HP-5MS UI columns.

REFERENCES

1. Chen, B. H., Taylor, E. H. and Pappas, A. A. "Comparison of Derivatives for Determination of Codeine and Morphine by Gas Chromatography/Mass Spectrometry." *Journal of Analytical Toxicology.* 1990, 14, 12-14.

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3. Mule, S. J. and Casella, G. A. "Rendering the 'Poppy-Seed Defense" Defenseless: Identification of 6-Monoacetylmorphine in Urine by Gas Chromatography/Mass Spectrometry." *Clin. Chem.* 1988, 34/7, 1427-1430.

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ILLINOIS STATE POLICE

TOXICOLOGY PROCEDURES MANUAL

PROTOCOL: Confirmations

METHOD: Opiates

PROCEDURE: **OPIATE CONFIRMATION FOR URINE USING BSTFA**

Reviewed by:

_____

Sean McCarthy, Chairperson
Toxicology Command Advisory Board

Approved by:

_____

Timothy Tripp
Bureau Chief – Toxicology Command Coordinator

INTRODUCTION

The presence of opiates such as morphine, codeine, hydromorphone, hydrocodone, dihydrocodeine, oxycodone, oxymorphone and 6-monoacetylmorphine can be confirmed in urine by first performing an enzyme hydrolysis to free the drugs from their glucuronide conjugates. The sample is then extracted, concentrated and finally derivatized with BSTFA to produce the trimethylsilyl (TMS) derivatives. Using this derivatization method, 6-monoacetylmorphine, the specific metabolite of heroin, can be detected when present in a sample. The derivatized sample is analyzed by GC/MS.

Other Related Procedures:

See *Opiate Confirmation for Blood*

SAFETY CONSIDERATIONS

Warning: Potential Biohazard. Adhere to all blood borne pathogen guidelines.

Warning: Follow all standard laboratory safety guidelines when handling commercially prepared extraction tubes.

Warning: β-Glucuronidase (Abalone). Avoid skin contact and inhalation.

Warning: BSTFA. Explosion may occur under fire conditions. Eye, skin and mucous membrane irritant.

PREPARATIONS

Specimen: Urine, 5 mL required.

Reagents:

1. **De-Tox A Extraction Tubes** (Dyna-Tek Industries) or another appropriate extraction tube.
2. **β-Glucuronidase (Abalone**). (Campbell Science), approximately1 million units/g solid (concentration may vary by lot).
3. **β-Glucuronidase Working Solution**, 25,000 units/mL. Add appropriate amount of β- glucuronidase to 40 mL distilled water to result in final concentration of 25,000 units/mL. **Warning: Avoid contact and inhalation**.
4. **Bis(trimethylsilyl)trifluoroacetamide/1% Trimethylchlorosilane (BSTFA with 1% TMCS).** (Campbell Science).
5. **Morphine-3β-d-Glucuronide Stock Standard.** (Cerilliant). Morphine-3β-d-glucuronide in WATER, 1.0 mg/mL.
6. **Stock Standards.** (Cerilliant). Solutions of the appropriate opiates in methanol, 1.0 mg/mL as needed. (Codeine, hydromorphone, etc.)

7. **Working Standards.** 100 µg/mL of appropriate drug standard. Dilute 500 µL of stock to 5 mL with methanol (WATER for morphine glucuronide).
8. **Nalorphine Stock Internal Standard.** (Cerilliant). Nalorphine in methanol 1.0 mg/mL.
9. **Nalorphine Working ISTD.** 90 µg/mL nalorphine. Dilute 900 µL of stock to 10 mL with methanol.

MINIMUM STANDARDS & CONTROLS

Refer to Appendix II

Controls:

1. Morphine glucuronide and codeine, 1.0 µg/mL each. Spike 5 mL of blank urine with 50 µL of working standards.
2. Other opiate controls may be formulated as needed by spiking 5 mL of blank urine or frozen opiate urine control with 50 µL of working standards to produce concentrations of 1.0 µg/mL.
3. Blank urine.

INSTRUMENTATION

Gas Chromatograph/Mass Spectrometer

Instrument Conditions:

Column:	HP-5MS Ultra Inert 30 m x 0.25mm x 0.25 µm
Injector:	250°C
Detector:	280°C
Temperature Program:	150°C for 0.5 min.; Ramp 20°C/min to 280°C; Hold for 5 min.
Scan Range:	80 - 525 m/z

PROCEDURE OR ANALYSIS

Hydrolysis

1. Into appropriately labeled 16 x 100 mm screw-capped tubes, pipet 5 mL of case urine sample, blank urine and control urine.
2. Add 50 µL of working ISTD to each tube.
3. To each tube add 400 µL of ß-glucuronidase working solution. Cap and vortex to mix.
4. Incubate 4 hours at 60°C,
5. Remove tubes from heating block and allow to cool to room temperature.+

Extraction

6. Pour each sample into labeled extraction tubes. Rotate for at least 10 minutes, then centrifuge at 2000 rpm for 10 minutes.
7. Transfer the solvent (top) layer to 16 x 100 mm screw-capped tubes and evaporate to dryness under nitrogen in a 40-50^{o} C water bath.

Derivatization (Note #1)

8. To the residue in each tube add 50 µL of BSTFA. Cap and vortex to mix.
9. Incubate at 65°C for 30 minutes. **Do Not Evaporate.**
10. Inject 1µL into the GC/MS.

Results:

Drug Name	**Major Ions**		
6-Monoacetylmorphine TMS	399	340	287
Codeine TMS	371	178	234
Codeine (underived)	299	162	229
Dihydrocodeine TMS	373	315	236
Hydrocodone enol TMS	371	234	356
Hydrocodone (underived)	299	242	185
Hydromorphone TMS	357	300	342
Hydromorphone enol 2TMS	429	414	234
Morphine 2TMS	429	414	236
Oxycodone TMS	387	229	372
Oxycodone enol 2TMS	459	444	368
Oxycodone (underived)	315	230	201
Oxymorphone TMS	373	288	259
Nalorphine 2TMS (ISTD)	455	414	440

Reference collection sources include but are not limited to: *Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites* by Pfleger, Maurer, and Weber; Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG); American Academy of Forensic Sciences (AAFS); National Institute of Standards and Technology (NIST); and an in house reference collection created using reference materials traceable to national or international standards.

Notes:

1. Some panel opiates may appear as the drug underivatized, or as one or two derivatized products. Detection of these drugs only require positive identification for one of the products.

REPORT WORDING

Refer to Appendix I

LIMITATIONS

1. This procedure was validated per research project 2023-10.

2. Panel drugs were not evaluated for their individual affinity to the derivatizing agent. This may impact cases with multiple panel drugs present in high concentrations.

3. The glucuronide form of each panel drug was not evaluated for its individual hydrolysis effectiveness. This may impact cases with concentrations near the laboratory-defined limit of detection

4. Stability of extracted samples has not been evaluated. Always inject samples with the concurrently extracted controls, to properly evaluate extraction viability. When possible, samples should be injected the same day they are derivatized.

5. Interference between opiates that elute at close retention times may occur. Samples should be analyzed on the HP5MS UI columns first, if possible.

REFERENCES

1. Chen, B. H., Taylor, E. H. and Pappas, A. A.: "Comparison of Derivatives for Determination of Codeine and Morphine by Gas Chromatography/Mass Spectrometry." Journal of Analytical Toxicology, 14: 12-14, (1990).

2. Grinstead, G. F.: "A Closer Look at Acetyl and Pentafluoropropionyl Derivatives for Quantitative Analysis of Morphine and Codeine by Gas Chromatography/Mass Spectrometry." Journal of Analytical Toxicology, 15: 293-298, (1991).

3. Mule, S. J. and Casella, G. A.: "Rendering the "Poppy-Seed Defense" Defenseless: Identification of 6-Monoacetylmorphine in Urine by Gas Chromatography/Mass Spectrometry." Clinical Chemistry, 34/7 1427-1430, (1988).

4. Romberg, R. W. and Brown V. E.: "Extraction of 6-Monoacetylmorphine from Urine." Journal of Analytical Toxicology, 14: 58-59, (1990).

ILLINOIS STATE POLICE

TOXICOLOGY
PROCEDURES MANUAL

PROTOCOL: Confirmations

METHOD: SMAs

PROCEDURE: **SMA CONFIRMATION FOR BLOOD**

Reviewed by:

_____
Sean McCarthy, Chairperson
Toxicology Command Advisory Board

Approved by:

_____
Timothy Tripp
Bureau Chief – Toxicology Command Coordinator

INTRODUCTION

Sympathomimetic amines (SMAs), such as amphetamine and methamphetamine can be detected in blood by using enzyme linked immunosorbent assay (ELISA). Confirming the identity of these drugs in the blood after initial detection by ELISA can be difficult due to their relative volatility and lack of well differentiated mass spectra.

The SMA's are extracted from the blood using solid phase extraction techniques and can be derivatized with heptafluorobutyric anhydride (HFBA). This derivatizing reagent reacts with both primary and secondary amines to form an amide. The derivatization of the sympathomimetic amines enhances their chromatography and produces more distinctive mass spectra. The extract is analyzed by GC/MS.

Other Related Procedures:

See *Amphetamine/Methamphetamine Quantitation, and SMA Confirmation for Urine.*

SAFETY CONSIDERATIONS

Warning: Potential Biohazard. Adhere to all blood borne pathogen guidelines.

Warning: Heptafluorobutyic anhydride (HFBA). Will cause burns to skin and eyes. Will cause eye, skin and mucous membrane irritation. Use in hood.

Warning: Glacial Acetic Acid is a moderate fire hazard when exposed to heat or flames; also a skin and eye irritant. Always add small amount of acid to water with stirring.

Warning: Sodium Phosphate, Dibasic. May cause eye, skin and mucous membrane irritation.

Warning: Sodium Phosphate, Monobasic. May cause eye, skin and mucous membrane irritation.

Warning: Dichloromethane. Vapors are heavier than air and will collect in low areas. When exposed to flames, can form HCl gas. Eye, skin and mucous membrane irritant. Use in hood.

Warning: Ammonium hydroxide. Corrosive. Avoid breathing fumes.

Warning: Concentrated HCl. Corrosive. Use extreme care when handling concentrated acid. Always add small amount of acid to water with stirring. Avoid breathing fumes.

PREPARATIONS

Specimen: Blood, 1 mL required.

Reagents:

1. **Solid Phase Extraction Tubes.** (TECAN Trace-J, PHENOMENEX Strata-X-Drug B; or appropriate cartridge.)
2. **Phosphate Buffer, 100 mM (pH = 6.0).** Dissolve 1.70 g Na_2HPO_4 (Fisher Scientific) and 12.14 g $NaH_2PO_4 \cdot H_2O$ (Fisher Scientific) in 800 mL distilled H_2O. Dilute to 1000 mL using distilled H_2O. Mix. Adjust pH to 6.0 ± 0.1 with 100 mM monobasic sodium phosphate (lowers pH) or 100 mM dibasic sodium phosphate (raises pH).
3. **Sodium Phosphate, Dibasic, 100mM.** Dissolve 2.84 g Na_2HPO_4 in 160 mL DI H_2O. Dilute to 200 mL using DI H_2O. Mix. Storage: 5ºC in glass.
4. **Sodium Phosphate, Monobasic, 100 mM.** Dissolve 2.76 g $NaH_2PO_4 \cdot H_2O$ in 160 mL DI H_2O. Dilute to 200 mL with DI H_2O. Mix. Storage: 5ºC in glass.
5. **Acetic Acid, 1.0 M** (Mallinckrodt)**.** To 400 mL DI H_2O add 28.6 mL glacial acetic acid. Dilute to 500 mL with DI H_2O. Mix. Storage: 25°C in glass or plastic. **Warning: Always add acid to water with stirring. Prepare in hood**.
6. **Elution Solvent.** Dichloromethane (Fisher Scientific)/Isopropanol (Fisher Scientific)/Ammonium hydroxide (Fisher Scientific) (78/20/2). Mix 390 mL of dichloromethane with 100 mL of isopropanol. Store in an Oxford pipettor. Before each use, add 200 µL of ammonium hydroxide (Fisher Scientific) to each 10 mL of dichloromethane/isopropanol. Mix thoroughly. **Warning: Prepare in hood. Do not breathe fumes.**
7. **Methanol**. (Fisher Scientific Co.).
8. **1% HCl in Methanol.** Add one mL concentrated HCl (Fisher Scientific) to 99 mL of Methanol. **Warning: Prepare in hood. Handle HCl with care.**
9. **Hexanes, HPLC Grade.** (Fischer Scientific Co.)
10. **Heptafluorobutyric anhydride (HFBA).** (Sigma-Aldrich).
11. **Ethyl Acetate.** (Fisher Scientific).
12. **Stock Standards**. (Cerilliant) 1.0 mg/mL solutions of SMAs in methanol.
13. **N-Propylamphetamine Standard**. 1 mg/mL N-propylamphetamine. 5 mg of N-propylamphetamine hydrochloride dissolved in 5 mL of methanol.
14. **N-Propylamphetamine Working ISTD**. 50 µg/mL n-propylamphetamine. Dilute 500 µL of 1 mg/mL N-propylamphetamine standard to 10 mL with methanol.

MINIMUM STANDARDS & CONTROLS

Refer to Appendix II

Controls:

1. Positive control prepared with appropriate sympathomimetic amines at 1-2 µg/mL.
2. Blank blood for negative control.

INSTRUMENTATION

Gas Chromatograph/Mass Spectrometer

Instrument Conditions:

Column:	HP-1MS Ultra Inert or HP-5MS Ultra Inert, 30 m x 0.25 mm x 0.25 µm
Injector:	200°C
Detector:	280°C
Temperature Program:	70°C for 1 min. Ramp 10°C/min to 180°C, Then ramp 30°C/min to 300°C, Hold for 10 min.
Scan Range:	40 - 420 m/z

PROCEDURE OR ANALYSIS

Sample Pretreatment

1. Place 1 mL of blood from each control and case in appropriately labeled 16 x 100 screw-capped test tubes.
2. Add 10 µL of N-propylamphetamine Working ISTD solution to each tube and vortex.
3. Add 3 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Centrifuge for 10 minutes.
4. Label a SPE column for each sample.
5. Sample Loading
   Decant supernatant from the tube using care not to disturb the pellet at the bottom of the tube.
   Aspirate sample through column at approximately 1-2 mL/minute.
6. Wash column.
   2 mL distilled water
   2 mL 1.0 M acetic acid
   3 mL methanol
   Dry column for 5 minutes.
7. Elution
   Place labeled 13 x 100 disposable screw-capped tubes into rack, and position under the proper SPE columns.
   Elute with 3 mL elution solvent at approximately 1-2 mL/minute. Note #1.

Derivatization Using HFBA

8. Add 200 µL of 1% HCl in methanol to each tube of eluate; vortex.
9. Evaporate to dryness under N_2 at 37ºC.
10. To the dry residue, add 500 µL of ethyl acetate; vortex. Add 50 µL of HFBA, cap

and vortex. Incubate for 10 minutes at 70ºC. Warning: Use HFBA under the hood.

11. Allow the tubes to cool to room temperature. Evaporate to dryness.
12. Reconstitute with 50 µL of hexane:ethanol (1:1) and inject 1 µL on GC/MS.

Results:

Drug Name	**Major Ions**		
Amphetamine HFB	240	118	91
Methamphetamine HFB	254	210	118
Methylenedioxyamphetamine (MDA) HFB	135	162	375
Methylenedioxymethamphetamine (MDMA) HFB	254	162	389
N-Propylamphetamine (ISTD) HFB	240	282	91

Reference collection sources include but are not limited to: *Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites* by Pfleger, Maurer, and Weber; Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG); American Academy of Forensic Sciences (AAFS); National Institute of Standards and Technology (NIST); and an in house reference collection created using reference materials traceable to national or international standards.

Notes:

1. Add ammonium hydroxide to elution solvent prior to use.

REPORT WORDING

Refer to Appendix I

LIMITATIONS

1. This procedure was validated per research project 2023-07.

2. Panel drugs were not evaluated for their individual affinity to the derivatizing agent. This may impact cases with multiple panel drugs present in high concentrations.

3. Stability of extracted samples has not been evaluated. Always inject samples with the concurrently extracted controls, to properly evaluate extraction viability. When possible, samples should be injected the same day they are derivatized.

REFERENCES

1. Baselt, R.C. *Disposition of Toxic Drugs and Chemicals in Man*, Eighth ed.; Biomedical Publications: Foster City, CA: 2008.

2. Forensic Drug Abuse Advisor; 14/10; Nov/Dec. 2002.

3. Forensic Drug Abuse Advisor; 15/3; March 2003.

4. Taylor, R.W.; Le, S.D.; Philip, S.; and Jain, N.C. "Simultaneous Determination of Amphetamine and Methamphetamine Using Solid Phase Extraction and Gas Chromatography/Nitrogen Phosphorous Detection or Gas Chromatography/Mass Spectrometry." *Journal of Analytical Toxicology.* 1989, 13, 293-295.

5. Tietz, N.W. "*Clinical Guide to Laboratory Tests."* 2nd ed.; W.B. Saunders; Philadelphia: 1990.

6. *Worldwide Monitoring Clean Screen® Extraction Columns Applications Manual*: United Chemical Technologies, Bristol, PA: 1995.

ILLINOIS STATE POLICE

TOXICOLOGY
PROCEDURES MANUAL

PROTOCOL: Confirmations

METHOD: SMAs

PROCEDURE: **SMA CONFIRMATION FOR URINE**

Reviewed by:

_____
Sean McCarthy, Chairperson
Toxicology Command Advisory Board

Approved by:

_____
Timothy Tripp
Bureau Chief – Toxicology Command Coordinator

INTRODUCTION

Sympathomimetic amines (SMAs), such as amphetamine and methamphetamine are readily detected in urine using enzyme multiplied immunoassay techniques (EMIT). Identifying which of these drugs is present after detection by EMIT, however, is more difficult. Their relative volatility and lack of a well differentiated mass spectrum makes the confirmatory analysis of these drugs challenging.

The SMA's are extracted from urine using liquid:liquid techniques and can be derivatized with heptafluorobutyric anhydride (HFBA). This derivatizing reagent reacts with both primary and secondary amines to form an amide. The derivatization of the sympathomimetic amines enhances the chromatography of the drugs and produces more distinctive mass spectra. The extract is then analyzed by GC/MS.

Other Related Procedures:

See *Amphetamine/Methamphetamine Quantitation, and SMA Confirmation for Blood.*

SAFETY CONSIDERATIONS

Warning: Potential Biohazard. Adhere to all blood borne pathogen guidelines.

Warning: Heptafluorobutyric anhydride (HFBA). Will cause burns to skin and eyes. Will cause eye, skin and mucous membrane irritation. Use in hood.

Warning: Concentrated HCl. Corrosive. Use extreme care when handling concentrated acid. Always add small amount of acid to water with stirring. Avoid breathing fumes.

PREPARATIONS

Specimen: Urine, 3 mL.

Reagents:

1. **1% HCl in Methanol.** Add 1 mL concentrated HCl (Fisher Scientific) to 99 mL of Methanol. **Warning: Prepare in hood. Handle HCl with care.**
2. **De-Tox A extraction tubes** (Dyna-Tek Industries) or another appropriate extraction tube.
3. **Hexanes, HPLC Grade.** (Fischer Scientific Co.).
4. **Heptafluorobutyric anhydride (HFBA).** (Sigma-Aldrich).
5. **Ethyl Acetate.** (Fisher Scientific).
6. **N-Propylamphetamine Standard.** 1 mg/mL N-propylamphetamine. 5 mg of N-propylamphetamine hydrochloride dissolved in 5 mL of methanol.
7. **N-Propylamphetamine Working ISTD.** 50 µg/mL N-propylamphetamine. Dilute 500 µL of 1 mg/mL N-propylamphetamine standard to 10 mL with methanol.

MINIMUM STANDARDS & CONTROLS

Refer to Appendix II.

Controls:

1. In-house frozen urine control. Drugs may include 4 µg/mL amphetamine, methamphetamine, MDA, MDMA, or appropriate alternatives.
2. Blank urine.

INSTRUMENTATION

Gas Chromatograph/Mass Spectrometer

Instrument Conditions:

Column:	HP-1MS Ultra Inert or HP-5MS Ultra Inert, 30 m x 0.25 mm x 0.25 µm
Injector:	250°C
Detector:	280°C
Temperature Program:	70°C for 1 min. Ramp 10°C/min to 180°C, Then ramp 30°C/min to 300°C, Hold for 10 min.
Scan Range:	40 - 420 m/z

PROCEDURE OR ANALYSIS

1. Briefly shake extraction tube.
2. Add 3 mL urine and 50 uL of working internal standard.
3. Cap and mix by inversion for 5 minutes.
4. Centrifuge at 2000 rpm for 5 minutes.
5. Transfer solvent layer to appropriately labeled screw top test tubes.
6. Add 200 µL of 1% HCl in methanol to each tube of transferred organic and vortex.
7. Evaporate to dryness under N_2 at 37ºC.
8. To the dry residue, add 500 µL of ethyl acetate and vortex. Add 50 µL of HFBA, cap and vortex. Incubate for 10 minutes at 70ºC. Warning: Use HFBA under the hood.
9. Allow the tubes to cool to room temperature. Evaporate to dryness.
10. Reconstitute with 50 µL of hexane:ethanol (1:1) and inject 1 µL on GC/MS with a blank solvent injection between each sample.

Results:

Drug Name	Major Ions		
Amphetamine HFB	240	118	91
Methamphetamine HFB	254	210	118
Methylenedioxyamphetamine (MDA) HFB	135	162	375
Methylenedioxymethamphetamine (MDMA) HFB	254	162	389
N-Propylamphetamine (ISTD) HFB	240	282	91

Reference collections sources include but are not limited to: *Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites* by Pfleger, Maurer, and Weber; Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG); American Academy of Forensic Sciences (AAFS); National Institute of Standards and Technology (NIST); and an in house reference collection created using reference materials traceable to national or international standards.

REPORT WORDING

Refer to Appendix I

LIMITATIONS

1. This procedure was validated per research project 2023-08.

2. Panel drugs were not evaluated for their individual affinity to the derivatizing agent. This may impact cases with multiple panel drugs present in high concentrations.

3. Stability of extracted samples has not been evaluated. Always inject samples with the concurrently extracted controls, to properly evaluate extraction viability. When possible, samples should be injected the same day they are derivatized.

4. Carryover may occur at high concentrations. To account for this possibility, a blank solvent injection will be analyzed between unknown samples. An unknown sample immediately following a blank injection that has evidence of carryover will be rejected and reanalyzed.

REFERENCES

1. Baselt, R.C. *Disposition of Toxic Drugs and Chemicals in Man*, Eighth ed.; Biomedical Publications: Foster City, CA: 2008.

2. Forensic Drug Abuse Advisor; 14/10; Nov/Dec. 2002.

3. Forensic Drug Abuse Advisor; 15/3; March 2003.

4. Taylor, R.W.; Le, S.D.; Philip, S.; and Jain, N.C. "Simultaneous Determination of Amphetamine and Methamphetamine Using Solid Phase Extraction and Gas Chromatography/Nitrogen Phosphorous Detection or Gas Chromatography/Mass Spectrometry." *Journal of Analytical Toxicology.* 1989, 13, 293-295.

5. Tietz, N.W. "*Clinical Guide to Laboratory Tests."* 2nd ed.; W.B. Saunders; Philadelphia: 1990.

6. *Worldwide Monitoring Clean Screen®* *Extraction Columns Applications Manual*: United Chemical Technologies, Bristol, PA: 1995.

ILLINOIS STATE POLICE

TOXICOLOGY
PROCEDURES MANUAL

PROTOCOL: Quantitations

Reviewed by:

_____
Larry Shelton, Chairperson
Toxicology Command Advisory Board

Approved by:

_____
Shannon George
Toxicology Program Manager

ILLINOIS STATE POLICE

TOXICOLOGY PROCEDURES MANUAL

PROTOCOL: Quantitations

METHOD: Gas Chromatography/Mass Spectrometry

PROCEDURE: **AMPHETAMINE/METHAMPHETAMINE QUANTITATION**

Reviewed by:

_____

Sean McCarthy, Chairperson
Toxicology Command Advisory Board

Approved by:

_____

Shannon George
Toxicology Program Manager

INTRODUCTION

Methamphetamine and its metabolite, amphetamine, are sympathomimetic phenethylamine derivatives with prominent central stimulant activity. These drugs are frequently abused for their stimulant effects and may be self-administered either orally or by intravenous injection in amounts of up to 2000 mg daily by tolerant addicts.

Methamphetamine and amphetamine are extracted from an alkaline sample (serum/blood/plasma) after the addition of deuterated amphetamine and methamphetamine as internal standards. The sample is extracted and evaporated to dryness. The concentrate is then derivatized using heptafluorobutyrylimidazole (HFBI).

HFBI reacts with both primary and secondary amines to form an amide. The sample is quantitated by gas chromatography/mass spectrometry using SIM analysis.

Other Related Procedures:

See *D,L-Enantiomers of Methamphetamine/Amphetamine Differentiation, SMA Confirmation for Blood, and SMA Confirmation for Urine*

SAFETY CONSIDERATIONS

Warning: Potential Biohazard. Adhere to all blood borne pathogen guidelines.

Warning: Heptafluorobutyrylimidazole (HFBI). Will cause burns to skin and eyes. Will cause eye, skin and mucous membrane irritation. Use in hood.

Warning: Glacial Acetic Acid is a moderate fire hazard when exposed to heat or flames; also a skin and eye irritant. Always add small amount of acid to water with stirring.

Warning: Sodium Phosphate, Dibasic. May cause eye, skin and mucous membrane irritation.

Warning: Sodium Phosphate, Monobasic. May cause eye, skin and mucous membrane irritation.

Warning: Dichloromethane. Vapors are heavier than air and will collect in low areas. When exposed to flames, can form HCl gas. Eye, skin and mucous membrane irritant. Use in hood.

Warning: Ammonium hydroxide. Corrosive. Avoid breathing fumes.

Warning: Concentrated HCl. Corrosive. Use extreme care when handling concentrated acid. Always add small amount of acid to water with stirring. Avoid breathing fumes.

PREPARATIONS

Specimen: Blood, serum or plasma, minimum of 1 mL required per replicate.

Range: Blood concentrations, µg/L

Drug	Therapeutic	Toxic	Lethal
Amphetamine	30-110	> 500	> 1000
Methamphetamine	10-50	600-5000	> 10000

Amphetamine blood/plasma concentration ratio: 0.6 - 1.0 (concentration dependent)

Reagents:

1. **Blank Blood** for standard curve.
2. **Stock Standards.** (Sigma-Aldrich) Methamphetamine and Amphetamine in methanol, 1 mg/mL.
3. **Working Standards.** Methamphetamine and Amphetamine, 10 µg/mL. Add 100 µL of Methamphetamine stock solution and/or 100 µL of Amphetamine stock to a 10 mL volumetric flask and q.s. to 10 mL with methanol.
4. **Stock Internal Standard.** (Cerilliant) Methamphetamine-d5 (100 µg/mL in methanol) and Amphetamine-d5 (100 µg/mL in methanol).
5. **Working Internal Standard.** 10 µg/mL solution of Methamphetamine-d5 and Amphetamine-d5. Put 1.0 mL each of stock Methamphetamine-d5 and Amphetamine-d5 in 10 mL volumetric flask and q.s. to 10 mL with methanol. Store ISTD in refrigerator.
6. **Solid Phase Extraction Tubes** (ZSDAU020 or ZCDAU020, United Chemical Technologies, Inc.; TB-0506, SPE Ware; or appropriate cartridge.)
7. **Heptafluorobutyrylimidazole (HFBI),** (Sigma-Aldrich). (Store in freezer).
8. **Phosphate Buffer, 100mM (pH = 6).** Dissolve 1.70 g Na_2HPO_4 (Fisher Scientific) and 12.14 g $NaH_2PO_4 \cdot H_2O$ (Fisher Scientific) in 800 mL distilled H_2O. Dilute to 1000 mL using distilled H_2O. Mix. Adjust pH to 6.0 ± 0.1 with 100 mM monobasic sodium phosphate (lowers pH) or 100 mM dibasic sodium phosphate (raises pH).
9. **Sodium Phosphate, Dibasic, 100mM:** Dissolve 2.84 g Na_2HPO_4 in 160 mL DI H_2O. Dilute to 200 mL using DI H_2O. Mix. Storage: 5°C in glass.
10. **Sodium Phosphate, Monobasic, 100 mM**: Dissolve 2.76 g $NaH_2PO_4 \cdot H_2O$ in 160 mL DI H_2O. Dilute to 200 mL with DI H_2O. Mix. Storage: 5°C in glass.
11. **1.0 M Acetic Acid** (Mallinckrodt). To 400 mL DI H_2O add 28.6 mL glacial acetic acid. Dilute to 500 mL with DI H_2O. Mix. Storage: 25°C in glass or plastic. **Warning: Always add acid to water with stirring. Prepare in hood**.

12. **Elution Solvent.** Dichloromethane (Fisher Scientific)/Isopropanol (Fisher Scientific)/ Ammonium hydroxide (Mallinckrodt) (78/20/2). Mix 390 mL of dichloromethane with 100 mL of isopropanol. Store in an Oxford pipettor. Before each use add 200 µL of ammonium hydroxide to each 10 mL of dichloromethane/isopropanol. Mix thoroughly. **Warning:** Prepare in hood. Do not breathe fumes.
13. **1% HCl in Methanol.** Add one mL concentrated HCl (Fisher Scientific) to 99 mL of Methanol. **Warning: Prepare in hood. Handle HCl with care.**
14. **Hexanes, HPLC Grade.** (Fisher Scientific Co.).

MINIMUM STANDARDS & CONTROLS

Refer to Appendix II

Controls:

UTAK whole blood

1. Drugs of Abuse Level 1 #98818, 100 µg/L d-Amphetamine and d-Methamphetamine

OR

2. Drugs of Abuse Level 2 #98819, 500 µg/L d-Amphetamine and d-Methamphetamine

INSTRUMENTATION

Gas Chromatograph/Mass Spectrometer

Instrument Conditions:

Column:	DB-5 30m x 0.25mm x .25 µm
Injector:	200°C
Detector:	280°C
Temperature Program:	110°C for 0.5 min., ramp 10°C/min to 140°C; then 30°C/min to 280°C, hold 4 min.
SIM Ions:	91, 96, 118, 123, 240, 244, 254, 258 using the d5 internal standards.

PROCEDURE OR ANALYSIS

Label five 16 x 125 mm disposable screw-capped culture tubes for standards, one for a blank, one for the control, and one for each unknown. Prepare the tubes as follows:

Tube	Blank Blood	Working Standard	Internal Standard	Final Concentration
1	1.0 mL	5 µL	30 µL	50 µg/L
2	1.0 mL	10 µL	30 µL	100 µg/L
3	1.0 mL	20 µL	30 µL	200 µg/L
4	1.0 mL	30 µL	30 µL	300 µg/L
5	1.0 mL	40 µL	30 µL	400 µg/L
6	1.0 mL	50 µL	30 µL	500 µg/L
7	1.0 mL	---	30 µL	Blank

Tube	Control	Unknown Blood	Internal Standard	Final Concentration
8	1.0 mL	---	30 µL	Control Value
9	---	1.0 mL	30 µL	Unknown

Treat all tubes as follows:

1. Sample Pretreatment.
   To 1 mL of blood add 5 mL of 100 mM phosphate buffer (pH 6.0).
   Mix/vortex. Centrifuge for 10 minutes. Note #4.
   Label a SPE column for each sample. (It may be more convenient to extract 6 or 7 samples at a time instead of an entire large batch.)

2. Column Conditioning. (Note #1)
   3 mL Methanol.
   3 mL distilled water.
   1 mL 100 mM phosphate buffer (pH 6.0).

3. Sample Loading.
   Aspirate sample through column at 1-2 mL/min. Note: When decanting supernatant from the tube, use care not to disturb the pellet at the bottom of the tube.

4. Wash Column.
   3 mL distilled water.
   1 mL 1.0 M acetic acid.
   3 mL methanol.
   Dry column for 5 minutes.

5. Elution.
   Place labeled 13 x 100 screw-cap tubes into rack and position under proper SPE columns.

Elute with 3 mL elution solvent at 1 to 2 mL/minute. **Note: Add NH_4OH to elution solvent daily.**

Derivitization.

6. Add 200 µL of 1% HCl in methanol to each tube of eluate; vortex.
7. Evaporate to dryness under a gentle stream of nitrogen at 37°C.
8. To the dry residue, add 500 µL of hexane (**OR** ethyl acetate), vortex .Note #5.
9. Add 50 µL of HFBI, cap and vortex. Incubate for 10 minutes at 70°C. **Warning: Use HFBI under the hood.** Note #3 and #4.
10. Allow tubes to cool to room temperature.
11. Add 2 mL hexane (**OR** ethyl acetate) and 1 mL of distilled water to each tube. Shake well to mix and to dissolve all HFBI residue from inside the tubes. Centrifuge for 5 minutes.
12. Carefully transfer the hexane layer to a clean 12 x 75 culture tube.
13. Evaporate to dryness.
14. Reconstitute with hexane:ethanol (1:1) and inject on the GC/MS. Note #5

Alternative Derivatization Using HFBA

6. Add 200 µL of 1% HCl in methanol to each tube of eluate; vortex.
7. Evaporate to dryness under N2 at 37ºC.
8. To the dry residue, add 500 µL of ethyl acetate; vortex.
9. Add 50 µL of HFBA, cap and vortex.
10. Incubate for 10 minutes at 70ºC. Warning: Use HFBA under the hood.
11. Allow the tubes to cool to room temperature. Evaporate to dryness.
12. Reconstitute with hexane:ethanol (1:1) and inject on GC/MS. Note #6

Calculations:

1. For each sample run, integrate the peak area for Amphetamine (ion 240), Methamphetamine (ion 254), and internal standard (ions 244 for amphetamine-d5 and 258 for methamphetamine-d5).
2. Calculate the ratios for each standard. Divide the peak area of the Amphetamine and Methamphetamine by the internal standard peak area. (Peak height ratios may also be used.)
3. Prepare a standard curve using the ratios for the five standards. Plot the respective concentrations (µg/L) on the X-Axis and the peak area ratios on the Y-Axis. Use linear regression to determine correlation coefficient (r), slope, and y-intercept.
4. Calculate the area ratios for the control and unknown samples. If the unknown ratio is higher than the highest standard, see Note #2.
5. Using linear regression, determine the concentrations for the control and unknowns.

Notes:

1. The column conditioning step can be omitted when using a polymer column. When using a silica based column it is important that the sorbent not be allowed to dry.
2. If the unknown sample ratio is higher than that of the highest standard, it must be run diluted so that it will fall within the standard curve. When high levels are suspected initially, it may be useful to prepare a dilution at the start of the analysis.
3. Ethyl acetate may be substituted for hexane in the derivatization step and the rinse step. Ethyl acetate appears to be the better solvent when only methamphetamine-d5 and is used as an internal standard. When amphetamine-d5 is also present in the internal standard, it does not appear to matter. Use only hexane:ethanol for reconstitution.
4. After the addition and mixing of 100 mM phosphate buffer, samples may be sonicated for 15 minutes using an ultrasonic bath.
5. SMA-HFB derivatives are not stable for long periods of time. Ideally, samples should be analyzed the same day derivatization is performed.

REPORT WORDING

Refer to Appendix I

REFERENCES

1. Baselt, Randall C. *Disposition of Toxic Drugs and Chemicals in Man*, Eighth ed.; Biomedical Publications: Foster City, CA: 2008.

2. *SPEware Trace Applications Manual*, updated 11-2006.

3. Taylor, R. W., et al. "Simultaneous Determination of Amphetamine and Methamphetamine Using Solid Phase Extraction and GC/NPD or GC/MS." *Journal of Analytical Toxicology,* 1989, 13, 293-295.

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5. Winek, C.L., Wahba, W. W., Winek, Jr., C.L. Winek Balzer, T., "Drug and Chemical Blood-Level Data 2001," *Forensic Science International* 122 (2001) 107-123.

6. *Worldwide Monitoring Clean Screen®Extraction Columns Applications Manual*: United Chemical Technologies, Bristol, PA: 1995.

ILLINOIS STATE POLICE

TOXICOLOGY PROCEDURES MANUAL

PROTOCOL: Quantitations

METHOD: Gas Chromatography/Mass Spectrometry

PROCEDURE: **COCAINE/BENZOYLECGONINE QUANTITATION II**

Reviewed by:

_____

Larry Shelton, Chairperson
Toxicology Command Advisory Board

Approved by:

_____

Shannon George
Toxicology Program Manager

INTRODUCTION

Cocaine is the most potent of the naturally occurring central nervous system stimulants. It has been widely utilized as a local anesthetic and increasingly by drug abusers for its stimulant properties. Cocaine can be administered topically, by nasal insulation, by intravenous injection, or as the free base by smoking. Cocaine is eliminated in the urine as unchanged drug, as its primary metabolite benzoylecgonine, as ecgonine methyl ester and as ecgonine.

Cocaine and benzoylecgonine are extracted together with a deuterated cocaine/ benzoylecgonine internal standard using solid phase extraction technique. The extract is evaporated to dryness and the benzoylecgonine is derivatized using MtBSTFA (N-Methyl-N-(t-Butyldimethylsilyl) to improve its chromatography. These derivatized extracts are injected directly on to the gas chromatograph/mass spectrometer.

Other Related Procedures:

See *Benzoylecgonine Confirmation.*

SAFETY CONSIDERATIONS

Warning: Potential Biohazard. Adhere to all blood borne pathogen guidelines.

Warning: Sodium Phosphate, Dibasic. May cause eye, skin and mucous membrane irritation.

Warning: Sodium Phosphate, Monobasic. May cause eye, skin and mucous membrane irritation.

Warning: Concentrated HCl. Corrosive. Use extreme care when handling concentrated acid. Always add small amount of acid to water with stirring. Avoid breathing fumes.

Warning: Dichloromethane. Vapors are heavier than air and will collect in low areas. When exposed to flames, can form HCl gas. Eye, skin and mucous membrane irritant. Use in hood.

Warning: Ammonium hydroxide. Corrosive. Avoid breathing fumes.

Warning: MtBSTFA + 1% t-BDMCS (N-Methyl-N-(t-Butyldimethylsilyl) Trifluoroacetamide + 1% t-Butyl-Dimethylchlorosilane). Flammable and corrosive. Eye, skin and mucous membrane irritant. Use in hood.

PREPARATIONS

Specimen: Whole blood, serum, or plasma; minimum of 1.0 mL required.
Range: Blood concentrations, µg/L

Drug	Therapeutic	Toxic	Lethal
Cocaine	50-930	900	1000-20000
Benzoylecgonine	N/A	N/A	N/A

Cocaine blood/plasma concentration ration: 1

Reagents:

1. **Blank Blood** for standard curve.
2. **Cocaine and Benzoylecgonine Stock Standards**. (Sigma-Aldrich). 1.0 mg/mL of cocaine and benzoylecgonine in methanol.
3. **Cocaine and Benzoylecgonine Working Standard**. 10 µg/mL of cocaine and benzoylecgonine in methanol. Dilute 100 µL of cocaine stock and 100 µL of benzoylecgonine stock to 10 mL with methanol.
4. **Dueterated Cocaine and Benzoylecgonine Stock Internal Standard.** (Cerilliant) 100 µg/mL cocaine-d3 in methanol. 100 µg/mL benzoylecgonine-d3 in methanol.
5. **Dueterated Cocaine and Benzoylecgonine Working Internal Standard**. (Cerilliant) 20 µg/mL cocaine-d3 and 20 µg/mL benzoylecgonine-d3 in methanol. Dilute 1.0 mL cocaine-d3 and 1.0 mL benzoylecgonine-d3 to 5.0 mL with methanol.
6. **Solid Phase Extraction Tubes** (ZSDAU020 or ZCDAU020, United Chemical Technologies, Inc.; TB-0506, SPE Ware; or appropriate cartridge.)
7. **Phosphate Buffer, 100 mM (pH = 6.0)**. Dissolve 1.70 g Na_2HPO_4 (Fisher Scientific) and 12.14 g $NaH_2PO_4 \cdot H_2O$ (Fisher Scientific) in 800 mL distilled water. Dilute to 1000 mL using distilled water. Mix. Adjust pH to 6.0 ± 0.1 with 100 mM monobasic sodium phosphate (lowers pH) or 100 mM dibasic sodium phosphate (raises pH).
8. **Sodium Phosphate, Dibasic, l00 mM**. Dissolve 2.84 g Na_2HPO_4 in 160 mL distilled water. Dilute to 200 mL with distilled water. Mix. Store at 4°C in glass.
9. **Sodium Phosphate, Monobasic, 100 mM**. Dissolve 2.76 g $NaH_2PO_4 \cdot H_2O$ in 160 mL distilled water. Dilute to 200 mL with distilled water. Mix. Store at 4°C in glass.
10. **Methanol.** (Fisher Scientific).
11. **Distilled Water.**
12. **Hydrochloric Acid**, 0.1 M. (Fisher Scientific). Add 4.2 mL concentrated HCl to 400 mL distilled water. Dilute to 500 mL. Warning: Prepare in hood. Handle HCl with care. Always add acid to water with stirring.
13. **Elution Solvent.** Dichloromethane (Fisher Scientific)/Isopropanol (Fisher Scientific)/Ammonium hydroxide (Mallinckrodt) (78/20/2). Mix 390 mL of

dichloromethane with 100 mL of isopropanol. Store in an Oxford pipettor. Before each use add 200 µL of ammonium hydroxide to each 10 mL of dichloromethane/ isopropanol. Mix thoroughly. Warning: Prepare in hood. Do not breathe fumes.

14. **MTBSTFA + 1% T-BDMCS** (N-Methyl-N-(t-Butyldimethylsilyl) Trifluoroacetamide + 1% t-Butyl-Dimethylchlorosilane.

MINIMUM STANDARDS & CONTROLS

Refer to Appendix II

Control:

UTAK whole blood (See Note #6)

1. Drugs of Abuse Level 1 #98818, 100 µg/L Cocaine and Benzoylecgonine.

OR

2. Drugs of Abuse Level 2 #98819, 500 µg/L Cocaine and Benzoylecgonine.

INSTRUMENTATION

Gas Chromatograph with Mass Spectrometer

Instrument Conditions:

GC parameters:

Column:	HP-5 ms, 30 m x 0.25 mm x 0.25 µm
Injector:	250°C
Detector:	280°C
Temperature Program:	150°C for 0.5 min, ramp 25°C/min to 280°C; hold for 6 min.
Purge valve:	ON at 0.8 minutes
Constant Flow:	ON: Reference temperature 150°C

MSD parameters:

Electron multiplier:	100 above autotune
Solvent delay:	6 minutes
Acquistion:	SIM mode
SIM ions:	82, 85, 182, 185, 303, 306, 282, 285, 403, 406. Note# 3.
SIM Dwell time:	50

PROCEDURE OR ANALYSIS

Label 16 x I 00 culture tubes for a blank, standards, controls and each unknown. Prepare the tubes as follows:

Tube	Blank Blood	Working Standard	Internal Standard	Final Concentration
1	1.0 mL	5 µL	30 µL	50 µg/L
2	1.0 mL	10 µL	30 µL	100 µg/L
3	1.0 mL	30 µL	30 µL	300 µg/L
4	1.0 mL	50 µL	30 µL	500 µg/L
5	1.0 mL	80 µL	30 µL	800 µg/L
6	1.0 mL	100 µL	30 µL	1000 µg/L
7	1.0 mL	---	30 µL	Blank

Tube	Control	Unknown Blood	Internal Standard	Final Concentration
8	1.0 mL	---	30 µL	Control Value
9	---	1.0 mL	30 µL	Unknown

Treat all tubes as follows:

1. Sample Pretreatment
   To 1 mL of blood add 5 mL of 100 mM phosphate buffer (pH 6.0).
   Vortex. Centrifuge for 10 minutes. Note #5.
   Label a SPE column for each sample.
2. Column Conditioning. (Note #1)
   3 mL methanol
   3 mL distilled water
   1 mL phosphate buffer (avoid sorbent drying)
3. Sample Loading
   Aspirate sample through column at 1-2 mL/min. Note: When decanting supernatant from the tube, use care not to disturb the pellet at the bottom of the tube.
4. Wash Column
   2 mL distilled water
   2 mL 0.1 M HC1
   3 mL methanol
   Dry for 5 minutes.
5. Elution
   Place labeled 16 x 125 mm disposable screw-capped tubes into rack, and position under proper SPE columns.
   Elute with 3 mL elution solvent at 1-2 mL/min. Transfer elution solvent to 13x100 mm screw-capped tubes. Note #4.
6. Derivatization
   Evaporate the eluate to dryness.
   To each tube add 50 µL of MtBSTFA (use under hood). Vortex, cap and heat in a 60°C heating block for 10 minutes. (Note #4)

If using an autosampler, transfer the MtBSTFA to properly labeled autosampler vials with micro-inserts and inject on to the GC/MS. OR If injecting samples manually, inject MtBSTFA directly on to the GC/MS. **DO NOT EVAPORATE BEFORE INJECTING**.

Calculations:

1. For each sample run, integrate the peak area of the following ions: 303, 306, 403 and 406 for cocaine, cocaine-d3, benzoylecgonine (tBDMS derivative) and benzoylecgonine-d3 (tBDMS derivative) respectively.
2. Calculate the peak area ratios for cocaine and benzoylecgonine (tBDMS derivative) in each standard.
3. Prepare two (2) standard curves -- one for cocaine and one for benzoylecgonine (tBDMS derivative) -- using the ratios for the five standards. Plot the respective concentrations (µg/L) on the X-Axis and the peak area or height ratios on the Y-Axis for each curve. Using linear regression, determine correlation coefficient (r), slope, and y-intercept.
4. Calculate the area ratios for the controls and unknown samples for cocaine and for benzoylecgonine (tBDMS derivative). If unknown ratio is higher than the highest standard, see Note #2.
5. Using linear regression, determine the concentrations for the controls and unknowns for both cocaine and benzoylecgonine.

Notes:

1. The column conditioning step can be omitted when using a polymer column. When using a silica based column it is important that the sorbent not be allowed to dry.
2. If the unknown sample ratio is higher than that of the highest standard, it must be diluted before extracting so that it will fall within the standard curve. When high levels are suspected initially, it may be useful to prepare a dilution at the start of the analysis.
3. Drugs and their associated ions Cocaine 82, 182, 303; Cocaine-d3 85, 185, 306; Benzoylecgonine (TBDMS derivative) 82, 282, 403; Benzoylecgonine-d3 (tBDMS derivative) 85, 285, 406.
4. After elution, the elution solvent may be evaporated to dryness in the 12x75 culture tubes. Add 50 µL of MtBSTFA to each tube and vortex. Using glass micropipettes, transfer the MtBSTFA to labeled autosampler vials with micro-inserts, apply a crimp cap to seal. Place each vial into a 13 x 100 mm culture tube. Using the heating block, heat the tubes for 10 minutes at 60°C.
5. After the addition and mixing of 100 mM phosphate buffer, samples may be sonicated for 15 minutes using an ultrasonic bath.
6. The Utak control should be made fresh. Even though Utak controls have a 30 day expiration date it has been demonstrated that cocaine/benzoylecgonine levels may not be stable.

REPORT WORDING

Refer to Appendix I

REFERENCES

1. Baselt, Randall C. Disposition of Toxic Drugs and Chemicals in Man, Biomedical Publications, Eighth Edition, 2008.

2. *SPEware Trace Applications Manual*, updated 11-2006.

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5. Worldwide Monitoring. Clean Screen® Extraction Columns Application Manual, United Chemical Technologies, Bristol, PA: 1995.

ILLINOIS STATE POLICE

TOXICOLOGY
PROCEDURES MANUAL

PROTOCOL: Quantitations

METHOD: Gas Chromatography/Mass Spectrometry

PROCEDURE: **LORAZEPAM QUANTITATION**

Reviewed by:

_____
Sean McCarthy, Chairperson
Toxicology Command Advisory Board

Approved by:

_____
Shannon George
Toxicology Program Manager

INTRODUCTION

Lorazepam, the active ingredient in Ativan, is a benzodiazepine administered as an antianxiety agent. Lorazepam is extracted together with an internal standard from alkalinized sample into an organic solvent. The extract is evaporated to dryness, the residue is dissolved in a small amount of MtBSTFA, a derivatizing reagent, and analyzed by Gas Chromatography/Mass Spectrometry using SIM analysis.

Other Related Procedures:

See *Benzodiazepine Confirmation for Urine*

SAFETY CONSIDERATIONS

Warning: Potential Biohazard. Adhere to all blood borne pathogen guidelines.

Warning: Sodium Hydroxide. Corrosive to all tissues. Wear goggles and gloves.

Warning: Acetone. Dangerous fire hazard when exposed to heat or flame. Use in hood.

Warning: Hexanes. Dangerous fire hazard when exposed to heat or flame. Use in hood.

Warning: Anhydrous Ethyl Ether. Highly flammable. Tends to form explosive peroxides. Keep away from heat, sparks and flame. Highly volatile. Harmful if swallowed, inhaled or absorbed through skin. Use in hood.

Warning: MtBSTFA + 1% t-Butyl-Dimethylchlorsilane. Flammable and corrosive. Eye, skin and mucous membrane irritant. Use in the hood.

PREPARATIONS

Specimen: Whole blood, serum, or plasma, minimum of 4.0 mL required. (2 mL per sample replicate)

Range: Blood concentrations in µg/L

Therapeutic	Toxic	Lethal
10-240	500-2000	700-12000

Reagents:

1. **Blank Blood** for standard curve.
2. **Lorazepam Stock Standard**. (Alltech). Lorazepam, 1.0 mg/mL.

3. **Lorazepam Working Standard.** 4 µg/mL in methanol. Add 40 µL of stock standard to a 10 mL volumetric flask and q.s. to 10 mL with methanol.
4. **Lorazepam-d4 Stock Standard.** (Cerilliant). Lorazepam-d4. 100 µg/mL in acetonitrile.
5. **Lorazepam-d4 Working Standard.** 4 µg/mL in methanol. Add 250 µL of stock standard to a 10 mL volumetric flask and q.s. to 10 mL with methanol.
6. **Prazepam Stock Internal Standard.** (Alltech). Prazepam in methanol, 1.0 mg/mL.
7. **Prazepam Working Internal Standard** 4 µg/mL in methanol. Add 40 µL of prazepam stock standard to a 10 mL volumetric flask and q.s. to 10 mL with methanol.
8. **Methanol, Certified ACS Grade.** (Fisher Scientific).
9. **NaOH, 0.1N.** (Fisher Scientific) Dissolve 0.4 gram sodium hydroxide in 100 mL distilled water. **Warning: Caustic material. Wear gloves.**
10. **Acetone**, **Certified ACS Grade**. (Fisher Scientific).
11. **Hexanes**, **HPLC Grade.** (Fisher Scientific).
12. **Anhydrous Ethyl Ether**, **Certified ACS Grade**. (J.T. Baker). **Warning: Extremely flammable.**
13. **Extraction Solvent.** 10% Acetone/40% Hexanes/50% Ethyl Ether. **Warning: Prepare in hood. Avoid breathing fumes.**
14. **MtBSTFA + 1% t-BDMCS (N-Methyl-N-(t-butyldimethylsily) trifluoroacetamine + 1% tButyl-Dimethylchlorsilane).**

MINIMUM STANDARDS & CONTROLS

Refer to Appendix II

Control:

Custom UTAK whole blood or appropriate control.

INSTRUMENTATION

Gas Chromatography/Mass Spectrometry

Instrument Conditions:

Column:	DB-5 30 m x 0.25 mm x 25 µm
Injector:	250°C
Detector:	280°C
Temperature Program:	120°C for 0.5 min., ramp 35°C/min to 280°C hold for 7.50 min
SIM Ions:	Lorazepam 491, 493, 513, 515
	Prazepam 269, 295, 324
	Lorezapam-D4 495, 497, 517, 519

PROCEDURE

Label disposable screw-capped culture tubes for standards, blank, control, and each unknown. Prepare the tubes as follows:

Tube	Blank Blood	Working Standard	Internal Standard	Final Concentration
1	2.0 mL	10 µL	40 µL	20 µg/L
2	2.0 mL	30 µL	40 µL	60 µg/L
3	2.0 mL	50 µL	40 µL	100 µg/L
4	2.0 mL	150 µL	40 µL	300 µg/L
5	2.0 mL	250 µL	40 µL	500 µg/L
6	2.0 mL	---	40 µL	Blank

Tube	Control	Unknown Blood	Internal Standard	Final Concentration
7	2.0 mL	---	40 µL	100 µg/L
8	---	2.0 mL	40 µL	Unknown

Treat all tubes as follows:

1. Add 1.0 mL of 0.1 N NaOH to each tube and mix.
2. Add 8 mL of extraction solvent to each tube.
3. Cap tubes and rotate for 10 minutes.
4. Centrifuge at 2000 RPM for 10 minutes.
5. Transfer the organic layer to new test tubes (screw cappped). Note #1.
6. Evaporate to dryness using N_2 and a 40°C water bath.
7. To the dry residue add 50 µL MtBSTFA, cap, and vortex to mix.
8. Heat at 70°C for 30 minutes. Allow tubes to cool.
9. Inject 2 µL directly into the GC/MS.

Calculations:

1. For each sample run, integrate the area (or height) of the lorazepam ion (513) and lorezepam-d4 internal standard ion (519) peaks or prazepam.
2. Calculate the peak area (or height) ratios for each standard. Divide the area of the lorazepam by that of the internal standard.
3. Prepare a standard curve using the peak area ratios for the five standards. Plot the respective concentrations (µg/L) on the X-Axis and the peak area ratios on the Y-Axis. Using linear regression, determine correlation coefficient (r), slope, and y-axis.
4. Calculate the peak area ratios for the control and unknown samples. Note #2.

5. Using the linear regression, determine the concentrations for the control and unknowns.

Notes:

1. Any serum or impurity from the lower aqueous layer may cause interference with the drug or internal standard. It is better to leave a little of the extraction solvent in the tube rather than risk transferring any of the serum layer.
2. If the unknown sample ratio is higher than that of the highest standard, it must be run diluted so that it will fall within the standard curve. When high levels are suspected initially, it may be useful to prepare a dilution at the start of the analysis.

REPORT WORDING

Refer to Appendix I

REFERENCES

1. Baselt, Randall C.; *Disposition of Toxic Drugs and Chemicals in Man*, Eighth ed.; Biomedical Publications: Foster City, CA: 2008.

2. Winek, C.L.; Wahba, W. W.; Winek Jr., C. L.; Winek Balzer, T. "Drug and Chemical Blood-Level Data 2001." Forensic Science International 122 (2001), 107-123.

ILLINOIS STATE POLICE

TOXICOLOGY
PROCEDURES MANUAL

PROTOCOL: Quantitations

METHOD: Gas Chromatography/Mass Spectrometry

PROCEDURE: **DIPHENHYDRAMINE QUANTITATION II**

Reviewed by:

_____
Larry Shelton, Chairperson
Toxicology Command Advisory Board

Approved by:

_____
Shannon George
Toxicology Program Manager

INTRODUCTION

Diphenhydramine was one of the first effective antihistamines. It is available over-the-counter for the relief of allergies and motion sickness. Because it is also a central nervous system depressant, it is used as a sedative. Overdoses can result in death.

A sample of blood is combined with an internal standard of deuterated diphenhydramine and alkalinized. It is extracted using hexanes. The extract is concentrated by evaporation and then analyzed by Gas Chromatography/Mass Spectrometry.

SAFETY CONSIDERATIONS

Warning: Potential Biohazard. Adhere to all blood borne pathogen guidelines.

Warning: Sodium Hydroxide. Corrosive to all tissues. Wear goggles and gloves.

Warning: Hexanes. Dangerous fire hazard when exposed to heat or flame. Use in hood.

PREPARATIONS

Specimen: Whole blood, serum, or plasma; minimum of 1 mL required.

Ranges: Blood concentrations in µg/L

Therapeutic	Toxic	Lethal
25-112	5000	>8000

Reagents:

1. **Blank Blood** for standard curve.
2. **Diphenhydramine Stock Standard**. (Sigma-Aldrich). Diphenhydramine in methanol, 1.0 mg/mL.
3. **Diphenhydramine Working Standard**. Diphenhydramine in methanol, 10 µg/mL. Add 100 µL of diphenhydramine stock solution to a 10 mL volumetric flask and q.s. to 10 mL with methanol.
4. **Deuterated Diphenhydramine Stock Standard**. (Cerilliant). Diphenhydramine-d3 in methanol, 100 µg/mL.
5. **Deuterated Diphenhydramine Working Standard**. Diphenhydramine-d3 in methanol, 10 µg/mL. Add 1.0 mL of Diphenhydramine-d3 stock standard to a 10 mL volumetric flask and q.s. to 10 mL with methanol.
6. **Hexanes, HPLC Grade**. (Fisher Scientific Co.).
7. **Sodium Hydroxide Solution, 0.1 N**. (Fisher Scientific Co.) Add 2.0 g of sodium hydroxide to a 500 mL volumetric flask and q.s. to 500 mL with distilled water. Warning: Caustic material. Wear gloves.

MINIMUM STANDARDS & CONTROLS

Refer to Appendix II.

Control:

Blood control containing 250 µg/L diphenhydramine.

INSTRUMENTATION

Gas Chromatograph with Mass Spectrometer

Instrument Conditions:

Column:	HP-5 ms, 30 m x 0.25 mm x 0.25 µm
Injector:	250°C
Detector:	280°C
Temperature Program:	160°C for 0 min.; ramp 10°C /min. to 260°C; hold for 2 min.
Purge ON time:	2.00 min.
Solvent delay:	4.00 min.
Acquisition:	SIM mode
SIM ions:	58, 61, 73, 76, 152, 165

PROCEDURE OR ANALYSIS

Label 16 x 125 mm disposable screw-capped culture tubes for each standard, blank, control, and unknown. Prepare the tubes as follows:

Tube	Blank Blood	Working Standard	Internal Standard	Final Concentration
1	1.0 mL	5 µL	25 µL	50 µg/L
2	1.0 mL	10 µL	25 µL	100 µg/L
3	1.0 mL	20 µL	25 µL	200 µg/L
4	1.0 mL	30 µL	25 µL	300 µg/L
5	1.0 mL	50 µL	25 µL	500 µg/L
6	1.0 mL	---	25 µL	Blank

Tube	Control	Unknown Blood	Internal Standard	Final Concentration
7	1.0 mL (Note #3)	---	25 µL	250 µg/L
8	---	1.0 mL	25 µL	Unknown

Treat all tubes as follows:

1. Add 250 µL of 0.1 N sodium hydroxide solution to each tube and mix.
2. Add 8 mL of hexanes to each tube.
3. Cap the tubes and shake for 15 minutes.
4. Centrifuge the tubes at 2000 RPM for 5 minutes.
5. Transfer the organic layer in each tube to a small test tube, using caution not to transfer any of the lower aqueous layer. Note #1.
6. Evaporate to dryness under a stream of N_2 in a 40°C water bath.
7. Dissolve the sample in hexanes:ethanol (1:1) prior to injection.
8. Inject 1-2 µL into the GC/MS.

Calculations:

1. For each sample run, integrate the peak area (or height) of the following ions: 58 for diphenhydramine and 61 for diphenhydramine-d3 internal standard.
2. Calculate the ratios for each standard. Divide the peak area (or height) of the diphenhydramine by the diphenhydramine-d3 peak area (or height).
3. Prepare a standard curve using the ratios for the five standards. Plot the respective concentrations (µg/L) on the X-axis and the peak ratios on the Y-axis. Using linear regression, determine correlation coefficient (r), slope, and y-intercept.
4. Calculate the area (or height) ratios for the control and unknown samples. (Note #2)
5. Using linear regression, determine the concentrations for the control and unknowns.

Notes:

1. Any serum or impurity from the lower aqueous layer may cause interference with the drug or internal standard. It is better to leave a little of the extraction solvent in the tube rather than risk transferring any of the aqueous layer.
2. If the unknown sample ratio is higher than that of the highest standard, it must be run diluted so that it will fall within the sample curve. When high levels are suspected initially, it may be useful to prepare a dilution at the start of the analysis.
3. If a fresh spiked control must be used, the working standard for preparing the control must be made from a separate stock solution. Prepare at the same concentration of the Diphenhydramine Working Standard used for the curve. Spike 25 µL of control working standard into 1.0 mL blank blood for final concentration of 250 µg/L.

REPORT WORDING

Refer to Appendix I.

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ILLINOIS STATE POLICE

TOXICOLOGY
PROCEDURES MANUAL

PROTOCOL: Quantitations

METHOD: Gas Chromatography/Mass Spectrometry

PROCEDURE: **GHB QUANTITATION IN URINE**

Reviewed by:

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Sean McCarthy, Chairperson
Toxicology Command Advisory Board

Approved by:

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Shannon George
Toxicology Program Manager

INTRODUCTION

Gamma-Hydroxybutyrate (or Gamma-Hydroxybutyric Acid-GHB) is an endogenous metabolite of GABA (Gamma-amino Butyric Acid). GHB plays a role as a central neurotransmitter and neuromodulator. GHB has been employed clinically since 1960 as an anesthetic and hypnotic agent. Illicit use of GHB often involves oral doses of one teaspoon (approximately 2.5 grams). The effects of GHB include drowsiness, euphoria, dizziness, nausea, visual disturbances and unconsciousness. These effects usually manifest within 15 minutes after administration.

Although GHB is an endogenous compound, drowsiness and sleep only occur at levels significantly higher than is found endogenously. Peak urine concentrations, in abusive situations, have been found to be between 1000 mg/L and 2000 mg/L. GHB levels in urine are considered to be above endogenous when at or above 10 mg/L.

GHB is extracted using solid phase extraction technique. The extract is evaporated to dryness and derivatized using BSTFA to improve the chromatography.

SAFETY CONSIDERATIONS

Warning: Potential Biohazard. Adhere to all blood borne pathogen guidelines.

Warning: Sodium Phosphate, Dibasic. May cause eye, skin and mucous membrane irritation.

Warning: Sodium Phosphate, Monobasic. May cause eye, skin and mucous membrane irritation.

Warning: BSTFA. Explosion may occur under fire conditions. Eye, skin and mucous membrane irritant.

Warning: Ammonium hydroxide. Corrosive. Avoid breathing fumes.

PREPARATIONS

Specimen: One mL of urine or appropriate dilution if 1.0 mL is not available.

Curve and control will be spiked in water.

Reagents

1. **Methanol, Certified A.C.S. Grade.** (Fisher Scientific).
2. **Bis (trimethylsilyl) trifluoroacetamide/1% Trimethylchlorosilane (BSTFA with 1% TMCS).** (Sigma Chemical Company).

3. **Phosphate Buffer, 100mM (pH = 6.0).** Dissolve 1.70 g Na_2HPO4 (Fisher Scientific) and 12.14 g $NaH_2PO_4 \cdot H_2O$ (Fisher Scientific) in 800 mL distilled water. Dilute to 1000 mL using distilled water. Mix. Adjust pH to 6.0 ± 0.1 with 100 mM monobasic sodium phosphate (lowers pH) or 100 mM dibasic sodium phosphate (raises pH).
4. **Ammonium Hydroxide.** (Fisher Scientific Co.).
5. **Extraction Solvent**, 12 mL Methanol with 200 µL NH_4OH. Make fresh daily.
6. **GHB-D6 Stock Standard** (Cerilliant), Internal standard. GHB-D6 in methanol, 1.0 mg/mL.
7. **GHB Stock Standard** (Cerilliant/Lipomed), GHB in methanol, 1.0 mg/mL.
8. **Distilled/Deionized Water.**

MINIMUM STANDARDS & CONTROLS

Refer to Appendix II

Control:

Frozen Control GHB, 20 mg/L in water. Prepare by putting 1.0 mL of 1.0 mg/mL stock GHB into 50 mL volumetric flask and q.s. to 50 mL with dH_2O.

INSTRUMENTATION

Solid Phase Extraction columns ZSGHB020 (United Chemical Technologies, Inc. Part # ZSGHB020) or Trace B TB-0506, or appropriate cartridge

Gas Chromatograph/Mass Spectrometer .

Instrument Conditions:

Column:	DB-5, 30 m x 0.25 mm I.D. x 0.25 µm film thickness
Injector:	250°C
Detector:	280°C
Temperature Program:	60ºC hold for one minute; ramp 8°C/min. to 140°C hold for 0 min.; ramp 35°C/min. to 300°C hold for four minutes.
SIM ions:	73, 117, 120, 147, 204, 206, 233, 239

PROCEDURE

Label 16 x 125 mm disposable screw-capped culture tubes for each standard, blank, control, and unknown. Prepare the tubes as follows:

Tube	Blank Water	Working Standard	Internal Standard	Final Concentration
1	1.0 mL	2 µL	10 µL	2 mg/L
2	1.0 mL	5 µL	10 µL	5 mg/L
3	1.0 mL	10 µL	10 µL	10 mg/L
4	1.0 mL	15 µL	10 µL	15 mg/L
5	1.0 mL	25 µL	10 µL	25 mg/L
6	1.0 mL	50 µL	10 µL	50 mg/L
7	1.0 mL	---	10 µL	Blank
8	1.0 mL	10 µL (Note #1)	10 µL	20 mg/L (Control)

Tube	Unknown Urine	Internal Standard	Final Concentration
9	1.0 mL (Note #2)	10 µL	Unknown

Treat all tubes as follows:

1. Sample Pretreatment
   Add 1 mL of phosphate buffer 100 mM (pH 6.0) to each sample. Vortex and centrifuge at 2000 RPM for 15 minutes.
   Label ZSGHB020 solid phase extraction tubes (UCT) or appropriate tube for each sample.
2. Column Conditioning.
   3 mL Methanol
   3 mL Distilled/Deionized water
   1 mL Phosphate buffer 100 mM (pH 6.0)
   NOTE: Avoid column drying.
3. Sample Loading.
   Place samples on the column and allow the sample to drip through with no vacuum.
   Slightly dry columns. Apply vacuum to column for approximately 15 seconds.
   Place appropriately labeled 16x100mm test tubes into vacuum manifold for sample collection.
4. Elution
   Add 3 mL of extraction solvent (12 mL Methanol with 200 µL NH_4OH made fresh daily) to each column and allow to drip through without vacuum.
5. Derivatization.
   Evaporate the solvent to dryness.
   Add 100 µl BSTFA to the dried sample. Cap the tubes.
   Heat the sample for 5 minutes at 70°C.
   Inject 2 µL of sample on the GC/MS.

Calculations:

1. For each sample run, integrate the peak area for GHB (ion 233), and internal standard, GHB-D6 (ion 239).
2. Calculate the ratios for each standard. Divide the peak area of the GHB by the internal standard peak area. (Peak height ratios may also be used.)
3. Prepare a standard curve using the ratios for the six standards. Plot the respective concentrations (mg/L) on the X-Axis and the peak area ratios on the Y-Axis. Use linear regression to determine correlation coefficient (r), slope, and y-intercept.
4. Using linear regression, determine the concentrations for the control and unknowns.

Notes:

1. The control must be made from a separate stock solution.
2. If abuse is suspected, an additional sample of the unknown urine should be analyzed after it is diluted at a 1:10 and 1:100 ratio.
3. If the case is positive, a second extraction must be performed (quantitative or qualitative) to confirm the results using a different GC/MS column.

REPORT WORDING

Refer to Appendix I

1. If the GHB concentration is below 10 mg/L standard, then report "GHB less than 10 mg/L." Include the following statement in the report conclusion: "Urinary GHB concentrations which are less than 10 mg/L cannot be distinguished from endogenous GHB concentrations."
2. If the GHB concentration is equal to or greater than 10 mg\L, report "GHB detected."
3. If the GHB concentration exceeds the quantitation limits, then report "GHB detected."

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ILLINOIS STATE POLICE

TOXICOLOGY
PROCEDURES MANUAL

PROTOCOL: Quantitations

METHOD: Triple Quadrupole Mass Spectrometry

PROCEDURE: **THC QUANTITATION FOR BLOOD**

Reviewed by:

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Sean McCarthy, Chairperson
Toxicology Command Advisory Board

Approved by:

_____
Shannon George
Toxicology Program Manager

INTRODUCTION:

Cannabis sativa, in the form of marijuana or hashish, is perhaps the most widely used illicit psychoactive drug in the world. It is abused for its euphoric and hallucinogenic properties. The most active principle constituent of marijuana (cannabis sativa) is Δ^9-Tetrahydrocannabinol (Δ^9-THC), which is most commonly administered either orally or by smoking. THC is also used as a drug to reduce intraocular pressure in glaucoma patients and to relieve nausea and suffering in terminal cancer patients.

Cannabinoids are readily metabolized in mammals. In particular, Δ^9-THC, the primary psychoactive agent, is metabolized to 11-Nor-9-carboxy-Δ^9-Tetrahydrocannabinol (Δ9-THC-COOH). Both constituents, Δ^9-THC and Δ^9-THC-COOH, are present in the blood in varying concentrations. The statutory limit for Δ^9-THC in blood is 5 ng/mL.

Large macromolecules are precipitated out of blood using an acetonitrile crash. Δ^9-THC and Δ^9-THC-COOH, along with their deuterated internal standards, are extracted from an acidified sample into an organic solvent. This extract is evaporated to dryness, reconstituted in a small amount of mobile phase, and subjected to analysis by liquid chromatography-triple quadrupole mass spectrometry (LC/QQQ) using Multiple Reaction Monitoring (MRM).

SAFETY CONSIDERATIONS:

Warning: Potential Biohazard. Adhere to all blood borne pathogen guidelines.

Warning: Acetonitrile. Dangerous when exposed to heat or flame. Vapors are heavier than air and may travel a considerable distance to source of ignition and flashback. Toxic Chemical Asphyxiant. Avoid breathing vapors. May irritate skin.

Warning: Hexanes. Fire and explosion hazard. May cause irritation of mucous membranes, skin, and eyes.

Warning: Ethyl Acetate. Dangerous fire hazard when exposed to heat or flame. Vapor-air mixtures are readily explosive. Irritates mucous membranes, skin and eyes. Use in hood.

Warning: Glacial Acetic Acid is a moderate fire hazard when exposed to heat or flames; also a skin and eye irritant. Always add small amount of acid to water with stirring.

PREPARATIONS:

Specimen: Whole blood, serum, or plasma; minimum of 1.0 mL required per replicate.

Reagents

1. **Blank Blood** for standard curve.
2. **Δ^9-THC Stock Standard** (Cerilliant). THC in methanol, 1.0 mg/mL.
3. **Δ^9-THC-COOH Stock Standard** (Cerilliant). THC-COOH in methanol, 1.0 mg/mL.
4. **Δ^9-THC, Δ^9-THC-COOH Intermediate Working Standard.** THC, THC-COOH, in methanol, 10.0 µg/mL. Add 500 µL each of THC and THC-COOH stock solutions to a 50 mL volumetric flask and q.s. to 50 mL with methanol.
5. **Δ^9-THC, Δ^9-THC-COOH Working Standard.** THC, THC-COOH in methanol, 0.5 µg/mL. Add 2.5 mL of the THC, THC-COOH intermediate working standard to a 50 mL volumetric flask and q.s. to 50 mL with methanol.
6. **Deuterated Δ^9-THC Stock Internal Standard** (Cerilliant). Δ^9-THC D_3 in methanol, 100 µg/mL.
7. **Deuterated Δ^9-THC-COOH Stock Internal Standard** (Cerilliant). Δ^9-THC-COOH D_3 in methanol, 100 µg/mL.
8. **Deuterated Δ^9-THC, Δ^9-THC-COOH Working Internal Standard.** Δ^9-THC D_3, Δ^9-THC-COOH D_3 in methanol, 0.5 µg/mL. Add 250 µL of Δ^9-THC D_3 and Δ^9-THC-COOH D_3 stock solutions to a 50 mL volumetric flask and q.s. to 50 mL with methanol.
9. **Acetonitrile, HPLC Grade.** (Fisher Scientific).
10. **Hexanes, HPLC Grade.** (Fisher Scientific).
11. **Ethyl Acetate.** (Fisher Scientific).
12. **Extraction Solvent.** 90% Hexanes/10% Ethyl Acetate.
13. **Acetic Acid, 10%.** Add 30 mL glacial acetic acid to 270 mL DH_2O.
14. **Formic Acid, 0.1% in DH_2O.** (Fisher Scientific)
15. **Formic Acid, 0.1% in Methanol.** (Fisher Scientific)
16. **Methanol, HPLC Grade.** (Fisher Scientific)
17. **System Suitability Standard,** 10 ng/mL**.** Add 5 mL of Δ^9-THC, Δ^9-THC-COOH Working Standard and 5 mL of Deuterated Δ^9-THC, Δ^9-THC-COOH Working Internal Standard to a 250 mL volumetric flask and q.s. to 250 mL with mobile phase (60:40 Methanol/DH_2O with 0.1% formic acid).
18. **Δ^8-THC Stock Standard** (Cerilliant). 1.0 mg/mL.
19. **Δ^8-THC Intermediate Standard,** 10 ug/mL. Add 500 uL of stock solution to a 50 mL volumetric flask and q.s. to 50 mL with mobile phase.
20. **Δ^8-THC Working Injection Standard,** 10 ng/mL. Add 100 uL of Δ^8-THC Intermediate Working Standard to a 100 mL volumetric flask and q.s. with mobile phase.
21. **11-nor-9-carboxy-Δ^8-THC Stock Standard.** (Cayman Chemical). 1.0 mg/mL.
22. **11-nor-9-carboxy-Δ^8-THC Intermediate Standard.** 10 ug/mL. Add 500 uL of stock solution to a 50 mL volumetric flask and q.s. to 50 mL with mobile phase.
23. **11-nor-9-carboxy-Δ^8-THC Working Injection Standard,** 10ng/mL. Add 100 uL of 11-nor-9-carboxy-Δ^8-THC Intermediate Standard to a 100 mL volumetric flask and q.s. with mobile phase.
24. **Mobile Phase.** 60% Methanol with 0.1% formic acid/40% DH_2O with 0.1% formic acid.

MINIMUM STANDARDS & CONTROLS:

Refer to Appendix II
Control:

Pooled whole blood control, 10 ng/mL of Δ^9-THC, Δ^9-THC-COOH or other appropriate control. Prepare by adding 100 µL of an intermediate working standard (#5 above) into 100 mL of blank blood. Mix at least 1 hour using a stir bar. NOTICE: Drug standards used to prepare the intermediate working standard should be from a different manufacturer or Lot#. When alternate standards are unavailable, an intermediate working standard prepared separately can be used.

19 aliquots of a new control must be analyzed to establish historical data in LIMS. The established mean and standard deviation will be used to evaluate an extracted QC and therefore case result acceptability. Historical data is instrument specific.

INSTRUMENTATION:

Liquid Chromatography/Triple-Quadrupole Mass Spectrometry

PROCEDURE:

Label 16 x 100 mm disposable culture tubes for each standard, blank, control, and each of the unknowns (in duplicate). Prepare the tubes as follows:

Table 2.

Sample Type	**Level**	**Blank Blood**	**Working Standard**	**D_3 Internal Standard**	**Final Concentration**
Calibrator	1	1.0 mL	4 µL	20 µL	2 ng/mL
Calibrator	2	1.0 mL	8 µL	20 µL	4 ng/mL
Calibrator	3	1.0 mL	16 µL	20 µL	8 ng/mL
Calibrator	4	1.0 mL	24 µL	20 µL	12 ng/mL
Calibrator	5	1.0 mL	32 µL	20 µL	16 ng/mL
Calibrator	6	1.0 mL	40 µL	20 µL	20 ng/mL
Blank	----	1.0 mL	----	20 µL	Blank
Sample Type	**Level**	**Control Blood**	**Unknown Blood**	**D_3 Internal Standard**	**Concentration**
QC	----	1.0 mL	----	20 µL	Control Value
Unknown	----	----	1.0 mL	20 µL	Unknown

Treat all tubes as follows:

1. Vortex each tube for 5 seconds.
2. Precipitate blood with 2 mL cold Acetonitrile added dropwise (full amount added over 20-30 seconds) while vortexing. **Note #2.**
3. Centrifuge samples at 3000 rpm for 10 minutes.
4. Decant supernatant into clean 16 x 100 mm disposable screw-capped test tubes. **Note #3.**
5. Add 800 µL of 10% Acetic Acid. Vortex.
6. Add 8 mL of Extraction Solvent (9:1 Hexanes/Ethyl Acetate).
7. Cap and rotate 30 minutes.
8. Centrifuge samples at 3000 rpm for 10 minutes.
9. Transfer the organic layer to new 16 x 100 mm disposable test tubes. **Note #4**.
10. Evaporate to dryness in a 40°C water bath under N_2.
11. To the dry residue, reconstitute with 100 µL of mobile phase.
12. **Inject the System Suitability Standard, Δ^8-THC Working Injection Standard, and 11-nor-9-carboxy-Δ^8-THC Working Injection Standard, and all extracted samples into the LC/QQQ.**

Evaluation in Mass Hunter and Calculations (**Note #5, #6**):

1. Create a "New Batch" in Mass Hunter for the quant sequence.
2. In the "Method" tab select "open method from existing file" and apply the appropriate processing method to the batch (example: THC QUANT FINAL). Mass Hunter will display the method tables on screen. Select "Exit" and Confirm "Yes" to apply this method to the batch.
3. Select the appropriate system suitability tube (typically the second injection). An arrow will be displayed next to the sample name when selected.
4. Within the "Method" tab, select "Update Retention Time" and select all compounds.
5. Return to the "Home" tab. Use "Analyze Batch" to select "Integrate Batch".
6. Once integration is complete. Return to the "Method" tab and use "Update Qualifier Ions" and select all compounds.
7. Return to the "Home" tab and click "Analyze Batch" to then select "Analyze Batch".
8. Retention times, product ion ratios, and concentrations are now processed by the instrument software using an unweighted calibration curve applying linear regression.
9. Review the system suitability tube to verify product ion ratio and retention times have been updated.
10. The calibration curve is generated by plotting the concentration of the calibration standard on the x-axis vs. the peak height ratio (drug to internal standard) on the y-axis.
11. Case samples will be analyzed in duplicate. Results will be averaged in LIMS using values to two decimal places. Reported value will be truncated to one decimal place.

Quality Control:

1. The identification of Δ^9-THC or Δ^9-THC-COOH by this procedure is dependent on meeting the following criteria:
    a. Product ion ratios must be within 20% of the system suitability standard.
    b. The retention time is within 0.05 minutes of the system suitability standard.
    c. The compound is within 0.05 minutes of the analogous internal standard from the same injection.
    d. Calculated concentration is 2.0 ng/mL or greater.
    e. Δ^8-THC and 11-nor-9-carboxy-Δ^8-THC injection standards retention times must be outside 0.05 minutes of the system suitability standard.

2. Quantitative
    a. R-squared value for the curve must be ≥ 0.99.
    b. Acceptability of the results for the entire analytical run is dependent upon the agreement of the results from the QC materials within established ranges as determined through QC charting. The QC must fall within $\pm 3\sigma$ confidence limit.
    c. Duplicate quantitative results must be within 20% of the mean of the two replicates.

Notes:

1. Instrument conditions may differ based on validation research studies.
2. Store acetonitrile used for precipitation in the freezer. It is important to add the acetonitrile slowly to the sample; a visual change in the specimen should be evident.
3. Pour supernatant slowly when decanting so as not to disturb the precipitate.
4. Any impurity from the lower aqueous layer may cause interference with the drug or internal standard. It is better to leave a little of the extraction solvent in the tube, rather than risk transferring any of the aqueous layer.
5. Limits of detection and quantitation are 2 ng/mL.
    a. If both of the analyzed replicates are below 2.0 ng/ml, the result is reported as "delta9-Tetrahydrocannabinol not detected".
    b. If one (1) of the two (2) analyzed replicates is quantitatively below 2.0 ng/mL, the result is reported as "delta9-Tetrahydrocannabinol not detected".
6. This procedure is not validated for dilutions. If both sample results are above 20 ng/mL, it is administratively reported as "Greater than 20 ng/mL".
7. If only one (1) of the two (2) replicates is above 20 ng/mL, the final result is reported as "Greater than X ng/mL", where "X" is the quantitative value of the replicate below 20 ng/mL.

REPORT WORDING

Refer to Appendix I.

REFERENCES

1. "Standard Practices for Method Validation in Forensic Toxicology", AAFS Standards Board, LLC., ASB Standard 036, First Edition, 2019.

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ILLINOIS STATE POLICE

TOXICOLOGY
PROCEDURES MANUAL

PROTOCOL: Quantitations

METHOD: Triple Quadrupole Mass Spectrometry

PROCEDURE: **GHB QUANTITATIVE SCREEN FOR URINE**

Reviewed by:

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Sean McCarthy, Chairperson
Toxicology Command Advisory Board

Approved by:

_____
Shannon George
Toxicology Program Manager

INTRODUCTION

Gamma-Hydroxybutyrate (or Gamma-Hydroxybutyric Acid-GHB) is an endogenous metabolite of GABA (Gamma-amino Butyric Acid). GHB plays a role as a central neurotransmitter and neuromodulator. GHB has been employed clinically since 1960 as an anesthetic and hypnotic agent. Illicit use of GHB often involves oral doses of one teaspoon (approximately 2.5 grams).

The effects of GHB include drowsiness, euphoria, dizziness, nausea, visual disturbances and unconsciousness. These effects usually manifest within 15 minutes after administration.

Although GHB is an endogenous compound, drowsiness and sleep only occur at levels significantly higher than what is found endogenously. Peak urine concentrations, in abusive situations, have been found to be between 1000 mg/L and 2000 mg/L. Deep sleep will occur at levels greater than 250 mg/L, with light to moderate sleep occurring at levels between 50 mg/L and 250 mg/L. GHB levels in urine are considered to be above endogenous when at or above 10 mg/L.

GHB is extracted using a liquid-liquid extraction technique. The extract is evaporated to dryness and reconstituted with HPLC mobile phase.

SAFETY CONSIDERATIONS

Warning: Potential Biohazard. Adhere to all blood borne pathogen guidelines.

Warning: Ethyl Acetate. Dangerous fire hazard when exposed to heat or flame. Vapor-air mixtures are readily explosive. Irritates mucous membranes, skin and eyes. Use in hood.

Warning: Sodium Acetate. May form combustible dust concentrations in air. Causes irritation.

Warning: Glacial Acetic Acid. A moderate fire hazard when exposed to heat or flames; also a skin and eye irritant. Always add small amount of acid to water with stirring.

Warning: Formic Acid. Flammable liquid and vapor. Harmful if swallowed. Causes severe skin burns and eye damage. Toxic if inhaled. May cause respiratory irritation.

Warning: Methanol. Highly flammable liquid and vapor. Toxic if swallowed, in contact with skin or if inhaled.

PREPARATIONS

Specimen: 400 µL of urine. (200 µL per replicate)

Curve and control will be spiked in water.

Reagents

1. **Methanol, HPLC Grade.** (Fisher Scientific).
2. **Ethyl Acetate, Certified A.C.S. Grade.** (Fisher Scientific).
3. **Sodium Acetate Trihydrate, Certified ACS Grade.** (Fisher Scientific).
4. **Glacial Acetic Acid.** (Fisher Scientific).
5. **Acetate Buffer, 100mM (pH 4.5).** Dissolve 5.86 g of sodium acetate trihydrate in 90 mL of distilled water. Add 3.24 mL of glacial acetic acid.
6. **Formic Acid.** (Fisher Scientific Co.).
7. **GHB-D_6 Stock Internal Standard** (Cerilliant). GHB-D_6 in methanol, 1.0 mg/mL.
8. **GHB-D_6 Working Internal Standard** (100 mg/L). Dilute 1.0 mL of GHB-D_6 stock solution into a 10 mL volumetric flask.
9. **GHB Stock Standard** (Cerilliant), GHB in methanol, 1.0 mg/mL.
10. **Deionized Water.** (DH_2O)
11. **Formic Acid, 0.1% in DH_2O**. (Fisher Scientific)
12. **Formic Acid, 0.1% in Methanol.** (Fisher Scientific)
13. **Mobile Phase.** 95% DH_2O with 0.1% formic acid / 5% Methanol with 0.1% formic acid.
14. **System Suitability Standard.** Add 100 µL of GHB stock standard and 200 µL of GHB-D_6 stock standard to a 10 mL volumetric flask and q.s. to 10 mL with mobile phase. The resulting concentration of GHB is 10 mg/L and GHB-D_6 is 20 mg/L.

MINIMUM STANDARDS & CONTROLS

Refer to Appendix II

Calibrators and Controls:

1. Water (for negative control).
2. Spiked control containing 10 mg/L Gamma-Hydroxybutyrate in DH_2O (preparation below).
3. Six calibrators for a six-point curve, 2mg/L – 25 mg/L (preparation below).

Preparation:

Label 2.0 mL screw-capped sterile cryogenic vials for each calibrator and control. The control and calibrators are prepared in 2.0 mL quantities. Performing a single extraction consumes 200 µL of these calibrators and control. The remainder of the prepared calibrator and control can be used for subsequent testing for up to 30 days. Prepare the calibrators and control as follows:

Tube	Water	GHB Stock Standard	Final Concentration (mg/L)
1	2.0	4 µL	2.0
2	2.0	10 µL	5.0
3	2.0	20 µL	10.0
4	2.0	30 µL	15.0
5	2.0	40 µL	20.0
6	2.0	50 µL	25.0
7 (Control)	2.0	20 µL of alternate stock	10.0

INSTRUMENTATION

Liquid Chromatograph/Mass Spectrometer

Instrument Conditions:

Instrument:	Agilent Ultivo LC/TQ
LC Column:	InfinityLab Poroshell 120 Phenyl-Hexyl, 100 mm x 3 mm, 2.7 µm
LC Guard Column:	InfinityLab Poroshell 120 Phenyl-Hexyl, 5 mm x 3 mm, 2.7 µm
Flow:	0.500 mL/min
Mobile Phase:	Water : Methanol with 0.1% formic acid
Gradient:	95:5 at 0 minutes, hold for 0.5 minutes
	50:50 at 3 minutes
	95:5 at 3.01 minutes
	95:5 at 5 minutes
Column Section:	35°C
Ionization:	Electrospray – Positive Mode
MRM ions:	Parent ions: 105.1 (GHB), 111.1 (GHB-D_6)
	Product ions: 87.1, 45.2 (GHB) and 93.1, 49.2 (GHB-D_6)

Note: Exact instrument conditions may differ based on validation research studies.

PROCEDURE:

Label 16 x 100 mm disposable culture tubes for each standard, blank, control, and unknown. Prepare the tubes as follows:

Tube	Sample Aliquot	D6-GHB Working ISTD
1	200 µL of 2.0 mg/L calibrator	50 µL
2	200 µL of 5.0 mg/L calibrator	50 µL
3	200 µL of 10.0 mg/L calibrator	50 µL
4	200 µL of 15.0 mg/L calibrator	50 µL
5	200 µL of 20.0 mg/L calibrator	50 µL
6	200 µL of 25.0 mg/L calibrator	50 µL
7	200 µL Blank Water	50 µL
8 and 9	200 µL Control (in duplicate)	50 µL
10 and 11	200 µL of Case Sample (in duplicate)	50 µL

Sample Extraction:

1. Place 1 mL of acetate buffer into each labeled test tube. Vortex briefly to mix.
2. Add 2 mL of ethyl acetate to each tube. Vortex briefly.
3. Centrifuge the tubes at 3000 RPM for 5 minutes
4. Transfer the organic layer in each tube to a clean test tube, using caution not to transfer any of the lower aqueous layer.
5. Evaporate to dryness under a stream of N_2 in a 40°C water bath,
6. Dissolve the residue in 100 µL of mobile phase (95:5) prior to injection.
7. Inject 15 µL into the LC/MS.
    a. Inject each control 3 separate times, for a total of 6 results.

Evaluation in Mass Hunter and Calculations:

1. Open sequence and create a "New Batch" in Mass Hunter Quantitative Analysis.
2. In the "Method" tab select "Open method from existing file" and apply the appropriate processing method to the batch (example: GHB Quant). Mass Hunter will display the method tables on screen. Select "Exit" and confirm "Yes" to apply this method to the batch.
3. Select the appropriate system suitability tube (typically the second injection). An arrow will be displayed next to the sample name when selected.
4. Within the "Method" tab, select "Update Retention Time" and select all compounds.
5. Return to the "Home" tab. Use "Analyze Batch" to select "Integrate Batch"
6. Once integration is complete, return to the "Method" tab and use "Update Qualifier Ions" and select all compounds.
7. Return to the "Home" tab and click "Analyze Batch" to then select "Analyze Batch".

8. Retention times, product ion ratios, and concentrations are now processed by the instrument software using an unweighted calibration curve applying linear regression.
9. Review the system suitability tube to verify product ion ratio and retention times have been updated.
10. Review peak integration of all samples.
11. The calibration curve is generated by plotting the concentration of the calibration standard on the x-axis vs. the peak height ratio (drug to internal standard) on the y-axis.
12. Case samples will be analyzed in duplicate. If both results are within the calibration range, results will be averaged using values to two decimal places. The reported value will be truncated to one decimal place.
13. Analysts will complete a LIMS measurement uncertainty worksheet to determine the upper and lower limits of uncertainty for the reported value.
    a. Sample values calculated as greater than 25 mg/L can be used for the MU estimate.
14. When the upper limit for measurement of uncertainty of a GHB result is less than 10.0 mg/L, then the quantitative amount will be considered indistinguishable from endogenous levels and a second test will not be needed. A task comment notating that "Urinary GHB concentrations less than 10 mg/L cannot be distinguished from endogenous concentrations" will be added in batch results.

Quality Control

1. The identification of GHB and GHB-D_6 by this procedure is dependent on meeting the following criteria:
    a. The ion ratio for the product ions of both GHB and GHB-D_6 must be within 20% of the ion ratios of the concurrently run suitability standard.
    b. The retention time is within 0.05 minutes of the contemporaneously run suitability standard.

2. Quantitative
    a. R-squared value for the curve must be ≥ 0.99.
    b. Acceptability of the results for the entire analytical run is dependent upon the QC values / results agreeing with all standards as determined through Appendices II and VI. The average of all control values must be within ±20% of the control's target value.

3. Reporting
    a. When the upper limit for measurement of uncertainty of a GHB result is greater than or equal to 10.0 mg/L, then a second test must be performed to qualitatively verify results.

REPORT WORDING

Refer to Appendix I.

Notes:

1. Limits of detection and quantitation are 2 mg/L. If any of the sample duplicates are less than 2 mg/L, then GHB will be considered not detected and no further report wording will be necessary.
2. If the calculated GHB concentration is within the calibration range, but the upper limit for measurement of uncertainty is less than 10 mg/L, then GHB will be considered indistinguishable from endogenous and will not be reported. Calculated values will be included in the notes packet, but no additional report wording will be necessary.
3. If the calculated GHB concentration is within the calibration range, the upper limit for measurement of uncertainty is greater than 10 mg/L, and qualitative criteria have been met, then the average of the duplicate analyses will be reported as the calculated concentration after GHB has been qualitatively confirmed in a separate extraction.
    a. A measurement uncertainty statement will be added to the report remarks when the above criteria is met and GHB is quantitatively reported.
    b. A measurement uncertainty statement will not be added to report remarks when a quantitative finding is reported as "greater than XX.X." or when the upper limit for measurement of uncertainty is below 10 mg/L. The measurement uncertainty document will be still included in the notes packet.
    c. If the uncertainty range includes 10 mg/L, the following statement will be added to the report remarks: "Urinary GHB concentrations less than 10 mg/L cannot be distinguished from endogenous concentrations."
4. This procedure is not validated for dilutions. If both duplicate values are greater than 25 mg/L, then the reported GHB value will be "Gamma-Hydroxybutyric Acid (GHB) greater than 25 mg/L."
5. If only one (1) of the two (2) replicates is above 25 mg/L, the final result is reported as "Gamma-Hydroxybutyric Acid (GHB) greater than XX.X mg/L", where "XX.X" is the quantitative value of the replicate below 25 mg/L.

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ILLINOIS STATE POLICE

TOXICOLOGY PROCEDURES MANUAL

APPENDIX I: REPORT WORDING

Reviewed by:

_____
Sean McCarthy, Chairperson
Toxicology Command Advisory Board

Approved by:

_____
Shannon George
Toxicology Program Manager

APPENDIX I

REPORT WORDING

This appendix contains the toxicology section's guidelines for report wording. Each analyst is responsible for following these guidelines; however, individual case variations may require format modification in order to accurately reflect the evidence submitted.

A toxicology report represents a summary of the analytical findings and may not include all of the information contained in the case notes. The analyst's report typically answers two essential questions:

1. What, if any, drugs and/or volatiles are present?

2. What are the concentrations of these drugs and/or volatiles?

TOXICOLOGY REPORTING FOR DRUGS/VOLATILES

	CRITERIA	RECOMMENDED REPORT FORMAT
1.	Positive Identification	
	a. Qualitative	(substance) detected.
	b. Quantitative	(substance) _____ wt/vol (g/dL, ug/L, mg/l, etc.); Carbon Monoxide _____ % saturation.
	c. Below quantitation limit	(substance) less than (lowest quantitation limit). Note: results below limit of detection are reported as not detected.
2.	Preliminary testing Note: preliminary testing may be reported if it is of investigative value (e.g., coroners' cases)	Preliminary testing indicates the possible presence of (substance / class) (optional qualifiers listed below)
3.	Negative	No (substance / class) detected.
4.	No Analysis Performed	Not analyzed for volatiles/drugs. (optional qualifiers listed below)
5.	Optional Qualifiers	
	a. Preliminary testing	Not confirmed due to _____. * Insufficient sample amount * Condition of sample (e.g., decomposition) However, this could not be confirmed.
	b. No analysis performed	Sample unsuitable for analysis due to _____. * sample amount

* sample condition
* sample type
or
(type of analysis) analysis not offered by this laboratory.

c. Drug report

Drug analysis was limited to the following classes: Amphetamine, Benzodiazepine, Cocaine, Opiate, Phencyclidine (PCP), and Cannabinoid. If additional drug testing is required, a service request can be submitted using LIMS pre-log.

Drug analysis includes, full drug panel and the following classes: Amphetamine, Benzodiazepine, Cocaine, Cannabinoid, Opiate, and Phencyclidine (PCP).

d. Volatile report

Volatile analysis of this case is limited to the following: ethanol, methanol, acetone, and isopropanol.

e. Urine Ethanol Result

Urine ethanol concentrations do not directly reflect blood ethanol concentrations.

6. Report Status

a. No further analysis will be performed unless specifically requested by contacting the (Name of Laboratory) at (Phone Number).
b. Drug analysis report to follow.
c. Volatile report previously issued.
d. Drug quantitation report to follow.
e. Qualitative drug report previously issued.
f. Note: Analysis has been limited to volatiles only. Should additional testing be required, please contact the (Name of the Laboratory) at (Phone Number).
g. GHB test results will be issued on a separate report.

7. Conclusion Section

A toxicology report may include a narrative section that contains a serum to whole blood calculation, interpretations as to physiological effect of a reported drug, clarification of a reanalysis or amended report, and/or other pertinent information about the case when needed.

Example: Note: Estimated blood ethanol concentration, calculated from the serum ethanol concentration, is 0.100 g/dL.

8. Measurement Uncertainty Statement

The following statement will be added to express measurement uncertainty as defined in Appendix VII (TX-App-VII).

NOTE: The estimated uncertainty for ethanol analysis is ± X.X% at the 99.73% confidence interval, resulting in an ethanol range of XXX to XXX g/dL (or % by volume).

Examples: 1) For an ethanol result of 0.080 g/dL with a calculated uncertainty of 2.2%, the conclusion added to the report is:

NOTE: The estimated uncertainty for ethanol analysis is ±2.2% at the 99.73% confidence interval, resulting in an ethanol range of 0.078 to 0.082 g/dL.

2) For an open liquor ethanol result of 0.5% by volume with a calculated uncertainty of 2.4%, the conclusion added to the report is:

NOTE: The estimated uncertainty for ethanol analysis is ±2.4% at the 99.73% confidence interval, resulting in an ethanol range of 0.4 to 0.6% by volume.

9. Analysis fee statement.

Reports on offense codes - DUI alcohol, DUI drugs, DUI drugs and alcohol, and Aggravated DUI contain the following statement:

> Section 5-9-1.9 of the Unified Code of Corrections (730ILCS) authorizes a criminal laboratory analysis fee of $150.00 to be imposed for persons adjudged guilty of an offense in violation of Section 11-501 of the Illinois Vehicle Code.

ILLINOIS STATE POLICE

TOXICOLOGY
PROCEDURES MANUAL

APPENDIX II: MINIMUM STANDARDS & CONTROLS

Reviewed by:

_____
Sean McCarthy, Chairperson
Toxicology Command Advisory Board

Approved by:

_____
Timothy Tripp
Bureau Chief – Toxicology Command Coordinator

MINIMUM STANDARDS AND CONTROLS

Appendix II contains toxicology minimum standards and controls. They represent a minimum standard which has been agreed upon. All analysts have a responsibility to follow these minima; however, it is the primary responsibility of each analyst to perform proper scientific procedures rather than simply fulfill these standards.

This appendix also contains information on preparing ethanol controls and control charts. Lastly, guidelines for the quality control and drug quantitations, including how to choose an appropriate control, are also found in this appendix.

MINIMUM TOXICOLOGY STANDARDS AND CONTROLS

I. VOLATILES/BLOOD ALCOHOL ANALYSIS

A. MINIMUM STANDARDS OF ANALYSIS

The headspace gas chromatography procedure used for volatiles/blood alcohol analysis is considered a primary test for these substances. It can, by itself, be used as the basis for a conclusive finding. The procedure yields both qualitative and quantitative information and is the minimum test to be performed.

1. Volatiles/Blood Alcohol analysis will be performed as outlined in the Toxicology Procedures Manual.
2. Criteria for positive identification and quantitation.
   a. Identification:
      (1) Acceptance: Retention time for the analyte of interest must be within ±0.030 mins of the retention time of a reference standard using the same instrument and method.
      (2) Rejection: Retention time for the analyte of interest is not within ±0.030 mins of the retention time of a reference standard using the same instrument and method.
   b. Quantitation:
      (1) Duplicate quantitative levels must agree within the acceptable range listed in the procedure.
      (2) Duplicate samples that have been reanalyzed but do not agree within acceptable range limits of the procedure may be reported after consulting the Technical Leader.
      (3) Volatiles detected but quantitatively less than the lowest standard used for calibration, 0.010 g/dL, will be reported as "No volatiles detected.".
      (4) Volatiles detected in blood greater than 0.010 g/dL should be quantitated.
      (5) Class A glassware will be used for the dilution of suspected alcoholic liquids.
3. Criteria for qualitative identification.

a. When quantitative results [2b(2)] are not acceptable and reanalysis is prevented either by sample condition or insufficient amount of sample, only qualitative results will be reported.
b. When quantitative value is of limited investigative value (e.g. acetone in a urine sample, controlled drugs identified) the volatile may be reported as "detected" in lieu of repeating analyses with a mixed volatile control. Further quantitative testing will be deferred.

4. No Volatiles Detected
Samples analyzed according to the procedures manual will be reported as "No volatiles detected" when the acceptance criteria for acetone, ethanol, isopropanol, and methanol is not met.

5. No Analysis Performed
a. Sample amount inappropriate for analysis.
b. Sample condition inappropriate for analysis.
c. Sample type inappropriate for analysis.
d. Analysis requested not offered by laboratory**.**

B. CONTROLS

1. A set of controls as defined in the respective volatile procedure will be run with each set of samples. Control acceptance/rejection criteria can be reviewed in the "Quality Control" section of the procedure.
2. Quality control data will be recorded in ethanol worklists in LIMS.
3. The case file will include a reference to the method used for analysis. The method is included in the validation and maintained in LAM.
4. Calibration data will be maintained in LAM. The current calibration date will be included on the worksheet.
5. A batch worklist will document all QC and samples analyzed during an instruments sequence or run.
6. QC chromatograms will be included in the notes packet.

II. DRUG ANALYSIS

A. MINIMUM STANDARDS OF ANALYSIS

1. Qualitative drug analysis will be performed using analytical techniques outlined in the Toxicology Procedures manual.
2. Each positive result should be confirmed by analyzing a separate aliquot. A different analytical technique (e.g. extraction, instrument method, or column) should be used whenever possible.
3. No Drugs Detected
Samples will be reported as "No drugs detected" when they are analyzed according to the screening procedures outlined in the Toxicology Procedures Manual and do not meet the acceptance criteria for the identification of any panel drugs (see "Appendix XI: Drug Panel").

4. No Analysis Performed
   a. Sample amount inappropriate for analysis.
   b. Sample condition inappropriate for analysis.
   c. Sample type inappropriate for analysis.
   d. Analysis requested not offered by laboratory.

B. TESTS AND CONTROLS

1. Routine Formulations and Analysis Procedures
   Analysts do not need to record how tests are performed or the formulation of reagents within the notes packet if this information is available in the Toxicology Procedures Manual.
2. Gas Chromatography (GC) and Liquid Chromatography (LC)
   a. GC identification criteria:
      (1) Acceptance: Retention time for the analyte of interest must be within ±0.300 mins of the retention time of a reference standard using the same instrument/method, when available.
      (2) Rejection: Retention time for the analyte of interest is not within ±0.300 mins of the retention time of a reference standard using the same instrument/method, when available.
   b. LC identification criteria:
      (1) Acceptance: Retention time for the analyte of interest must be within ±0.050 mins of the retention time of a reference standard using the same instrument and method.
      (2) Rejection: Retention time for the analyte of interest is not within ±0.050 mins of the retention time of a reference standard using the same instrument and method.
   c. An image of the sample analysis will be included in the notes packet.
   d. Comparisons will be made to reference materials.
   e. The case file will include the instrument conditions and column type used for analysis. The instrument and method parameters have been uploaded to LAM.
3. Mass Spectrometry
   a. Acceptance Criteria
      Positive identifications of drugs in unknown samples are determined by visual comparison of fragmentation patterns. Full scan mass spectral identification is not rigidly defined; rigid criteria may lead to misidentification or under-identification. The analyst should base identification on a number of factors: unique ions, ion abundance, S/N and available reference collections. Full scan mass spectral identifications use the following:
      (1) Spectra should contain the base peak and all major ions unique to the analyte with a fragmentation pattern consistent to a reference library or certified reference standard spectrum.
      (2) Consideration of the following factors when making an

identification:

(a) Missing or additional ions may indicate low signal concentrations, co-eluting components, and background noise.

(b) Analyte concentration can shift base peaks to a secondary ion in high abundance.

(c) Spectral averaging and background subtraction can be utilized.

(d) All identifications will be peer reviewed.

b. Rejection Criteria

If any of the following occur, an identification will not be made:

(1) Spectrum does not contain the base peak.

(2) The fragmentation pattern is not consistent with a reference library or certified reference standard spectrum.

c. Analysts are responsible for confirming the presence, or absence, of each panel drug within a target extraction (see Appendix XI for current drug panel). Extractions fully analyzed and resulting in no positive identifications of any panel drugs will be notated as "no panel drugs detected".

d. An image of all reported drugs, and an image of the specific reference used for comparison, will be included in the notes packet. Any additional images an analyst determines to be relevant can be included in the notes packet.

e. Reference collection sources include but are not limited to *Mass Spectral and GC data of Drugs, Poisons, Pesticides, Pollutants, and their Metabolites* by Pfleger, Maurer, and Weber, Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG), American Academy of Forensic Sciences (AAFS), National Institute of Standards and Technology (NIST), and an In House Reference Collection.

f. All reference collection sources used for comparison will be documented in the notes packet.

g. The case file will include the instrument and method parameters used for analysis. The instrument and method parameters have been uploaded to LAM.

4. Ultraviolet, Visible, and Fluorescence Spectrophotometry

a. An image of sample spectra will be included in the notes packet.

b. Solvent and scan conditions will be documented in the procedure or notes packet.

5. Immunoassays

a. EMIT and ELISA

(1) LIMS will maintain batch results data including: calibration, quality control and sample data.

(2) At a minimum, worksheets will import sample data and calibration cut-offs. Each assay will indicate if the sample

result is positive (POS), negative (NEG), or positive/negative (PN).

(3) Immunoassay QC results are evaluated by the instrument and verified by the analyst. The measured absorbance values of the positive QC and the cutoff QC should be more positive than the measured values for the negative QC. The measured absorbance values for the positive QC should also be more positive than the measured values for the cutoff QC. Interpretation of absorbance values for EMIT and ELISA can be found in their respective procedures.

6. Extractions

a. Extractions performed as part of a procedure outlined in the Toxicology Procedures Manual do not have to be recorded on the assignment worksheet. However, any deviations from the procedure (e.g., changes in sample or solvent volumes) should be noted.

b. Sample aliquots are only prepared by the analyst with custody of the test item(s) utilized. The date aliquots are prepared will be annotated in the notes packet if different than the extraction date.

c. Once aliquoted, removed sample is considered to be a work product. Work product does not require chain of custody and will be discarded upon completion of analysis unless otherwise noted in the case notes.

(1) The analyst who extracts the aliquot and the date of that extraction will be annotated in the notes packet.

d. Appropriate QC samples will be extracted with unknown samples. An image of QC sample results will be included in the notes packet.

e. Specific instrumentation utilized during analysis will be recorded in the notes packet. (e.g. GCMS system, pipette…etc.)

f. Extracted samples are intended for internal use only. Should circumstances arise where analysis needs to be performed at another laboratory, work initially completed will be reported and all items/sub-items will be transferred following appropriate procedures.

7. Chemicals, Solvents, Reagents and In-House Controls

LAM will be updated as reagents are received, prepared and consumed, as determined by their asset type. When a reagent is completely consumed the item will be relocated to the "Disposed Assets" location using the appropriate barcode. Additional protocols can be found in the Command Quality Manual (QM-14).

a. In-house Prepared Reagents and Controls

(1) Lot/Batch number, Asset Type, Asset Name, the name of the analyst who prepared the reagent, the date it was prepared, and the expiration date will be recorded in the Reagent Info section of the LAM.

(2) Additional preparation notes will be included, when applicable, to notate reauthentication and any other

information pertinent to preparing the reagent.

(3) The volumes and concentrations of all chemicals and standards used to create the reagent or control, as well as the glassware and pipettes implemented, will be scanned and recorded in the Component section in the LAM, when possible.

(4) Preparation of in-house controls will be observed and verified by a second analyst, whose initials will be recorded in the preparation notes in the Reagent Info section of the LAM.

(5) In-house prepared reagents and controls must be quality checked before being put into service. This can be done by utilizing the newly prepared reagent in its associated procedure on an additional QC sample. Those results will be compared to the existing QC sample results to ensure that they meet all acceptance criteria. The QC date and the analyst who performed the quality control are recorded in the Lab Status Info section in the LAM. For in-house prepared controls, the QC results will be printed and attached in the images section for the LAM entry of the lot being tested.

b. Chemical/Purchased Reagents

(1) Asset type, asset name, and lot number will be recorded in the Chemical Info section in the LAM.

(2) Date received, expiration date, date first opened/in-use and the analyst who first used it will be recorded in the Lab Status Info section in the LAM.

(3) Form, quantity, units and storage fields may be recorded in the Chemical Info section in the LAM.

8. Drug Standards

a. A drug's name, manufacturer, lot number, expiration date, and date received will be recorded in LAM. LAM will be updated as drugs are received, prepared and consumed.

b. Drug standards may be utilized qualitatively past their manufacture's expiration date, once they have been appropriately reauthenticated per QM-14. Reauthentication may be performed at any time following expiration but must be completed before it is used for casework.

(1) Reauthentication will be documented in LAM.

(2) Reauthenticated qualitative standards will have a new expiration date set for 5 years.

(3) Drug standards will not be reauthenticated for quantitative purposes but may be reauthenticated for qualitative use.

c. All controlled drug standards will be inventoried once a year.

(1) Inventory records of drug standards will include the name of the standard, lot number, date received, and the manufacturer.

(2) If a quantitative standard is stored, the expiration date will also be tracked.

(3) Documentation of the annual inventory will include a document with a physical (or digital equivalent) signature certifying the audit, the number of standards listed in the inventory and the number of standards found during the physical inventory.

d. All drug standards that are not certified reference materials will be verified by mass spectrometry before use, when possible, and an in-house reference collection will be maintained in LAM.

e. All drug standards or stock standards used for quantitative procedures must be obtained from accredited reference material producers per QM-14.

f. All working standards prepared for drug quantitations may be stored in the freezer for a period of up to one year. Working standards containing the analyte of interest will not be used past the manufacturer's expiration date. Internal standards are an exception to this rule and can be re-authenticated per QM-14.

g. All primary and secondary standards will be stored and tracked separately. All secondary standards will have an indication that they must be used for qualitative purposes only.

h. Purchased or frozen controls are preferred. If a fresh spiked control is used, it must be made from a second stock standard that is prepared on a different day or by another analyst.

9. Co-Oximeter

a. Positive and negative controls will be run with each set of samples.

b. Control result will be documented in the case file.

C. QUANTITATIONS

1. Routine quantitations will be performed using suitable methods in the Toxicology Procedures Manual.
2. Any deviations from the procedures outlined in the Toxicology Procedures Manual (e.g., changes in sample or solvent volumes) should be noted in the notes packet.
3. Appropriate controls and a blank will be analyzed with all quantitations. Commercially purchased controls are preferred. Control preference will be in the following order: commercially purchased, prepared batch frozen, and then fresh spiked. If a fresh spiked control is used, it must be made from a second stock standard that is prepared on a different day or by another analyst, when purchased or frozen controls are not available. The use of a fresh spiked control requires prior approval from the Toxicology Technical Leader. Control types and target values will be indicated in the case file.
4. QC results will be recorded in the batch results worklist or the notes packet.

5. Class A glassware will be used to prepare working drug standards and controls.
6. An estimation of measurement uncertainty (MU) will be determined following guidelines provided in Appendix VI and Appendix VII.
7. A quantitation result must be rejected if the measured value of the control is greater than ±20% of the control's target value. If one or more controls in the repeatability analysis for measurement uncertainty is outside ±20%, the average value of the six control measurements will be used to determine acceptability. (Note: Quantitation acceptance criteria for THC, Ethanol and other volatiles can be reviewed in this document or in the respective procedure)
8. Undiluted unknown sample replicate quantitative values must be within 20% of their calculated mean value.

Quality Control of Drug Quantitation

All confirmations and quantitations require a positive and negative control. This section discusses the types of positive controls available and their use. All assets used for quality control and or drug quantitations are tracked using the LAM.

Control Samples

Drug calibration standards contain drugs spiked into a blank sample at exact concentrations and are used to calibrate an instrument during quantitation. Controls are used to verify the accuracy of the instrument's calibration. Control results determine whether a quantitation is accepted or rejected. For acceptance criteria guidelines review section II.C.7. There are three types of controls available to an analyst. Any of these controls are acceptable.

Commercially Prepared - Commercially prepared controls are purchased from a company who has prepared biological samples at known drug concentrations. Generally, the company assays the samples in order to provide an acceptable range for result. The laboratory should evaluate statistical mean values for these controls using established procedures. These values will be used to determine measurement uncertainty.

In-house Batch-Prepared Controls - Not all controls needed by the section's procedures can be purchased in an appropriate concentration or matrix. Therefore, In-house batch controls containing one or more drugs can be prepared in large quantities and frozen for individual use. To prepare this type of control:

1. Check the specific procedure in this manual to determine the proper concentration at which to spike the control.
2. A large amount of the control should be made so that a statistical mean concentration can be determined.
3. The amount of drug required to reach the desired concentration in blank matrix should be determined. For example: To prepare one liter of a 250 µg/L control add

250 µg of drug (250 µL of a 1 mg/mL stock solution).
A crucial part of preparing this type of control is thoroughly mixing the matrix after adding the drug. A minimum of 30 minutes is recommended on a stir plate with a stir bar. Once it has been mixed, it can be aliquoted into smaller containers. Usually, 1.5 mL portions are suitable for most extraction procedures.

4. A standard curve should not be accepted or rejected on the basis of an in-house control that does not have a target value established. A fresh spiked control must be analyzed until a target value has been established. A minimum of two values is required to establish a target.

If the drug standard used to make the control is not pure, the drug concentrations determined will be artificially low. This may not be detected if the same drug standard is used to generate the standard curve for quantitation. When possible, a standard drug from two separate sources or lots should be used for preparing batch controls and calibration standards. This also applies to fresh spiked controls.

Fresh spiked controls - When commercially prepared controls and in-house batched controls are not available for an analysis, a fresh spiked control can be used. It should be spiked at the concentration recommended in the procedure. The fresh spiked control should be made from a different traceable stock solution than the stock solution used to prepare calibration standards. From this second stock solution, a working solution is made and used to prepare the controls. Any mistakes made in the weighing or dilution of the drug standard could easily be duplicated in both the standard and the control. Also, impure drug standards would not be discovered using this procedure. A variation of this type of control is to prepare working standard per the procedure and store it in the refrigerator or freezer for use as a long term control. Every time the quantitation is performed, this working standard could be used to spike and act as the control. This type of control allows multiple control values to be obtained and a mean concentration to be generated. It can also detect dilution errors.

Case Samples

It is recognized that for a variety of reasons, analytical results will occasionally be outliers; that is, analytical values which deviate significantly and spuriously from the true value. Outlier results of controls, blanks or calibrators will be obvious; however outlier results of case specimen may not be identified if only a single extract is analyzed. For this reason, two aliquots of a case sample will be quantitated. A dilution of a case sample may serve as one of the aliquots. Note: diluted and undiluted values should not be averaged.

III. INSTRUMENT CALIBRATIONS AND/OR FUNCTION CHECKS

Note: Records of maintenance and calibrations and/or function checks will be kept in LAM for each instrument in the toxicology section. These records provide a history of the instrument and are useful for troubleshooting and for verification of preventive and corrective maintenance.

A. MAINTENANCE LOGS

All repairs including parts replacement and scheduled preventative maintenance will be documented in an instrument maintenance log in LAM

B. CALIBRATION LOGS OR FILES

LAM or instrument batch results will contain the results of all required calibrations and/or calibration checks. Entries will note the date performed and recording analyst. Images that capture instrument conditions, results, or demonstrate calibration will be attached in LIMS as needed.

C. CLASS A GLASSWARE

Class A volumetric glassware will be used for in-house preparation of calibrators and controls. All Class A glassware certificates of accuracy are stored in LAM. Recalibration shall recur at least once every 10 years by an appropriate accredited calibration service provider. Alternatively, glassware can be replaced after 10 years.

Volumetric glassware will be stored and maintained to protect integrity. However, visual checks will be performed prior to use to inspect for damage. Damaged volumetrics will be discarded.

A certificate of accuracy can be issued to an asset with a unique identification number such as 100 mL volumetric flask (#1234). A certificate can also be issued to an entire lot of items such as 1 mL pipets (10 pipets from lot# 5678).
If a unique asset is used for precise measurement of a sample, the asset will be documented in the notes packet using its identification number. Drug standard assets will document unique identification numbers with Class A glassware used for preparation in LAM. Class A glassware with lot# certifications but are not individually unique do not need to be documented.

D. MASS SPECTROMETERS

1. Tune daily before use. Sequence samples that run overnight and into the next day will be accepted using the previous day's tune. New samples will not be added that following day until the instrument has been tuned.
   a. Agilent (Older GCs such as 7890 or comparable)
      (1) A targeted tune is utilized. Note: tune file name used is set in instrument method.
      (2) The tune utilizes Perfluorotributylamine (PFTBA) as a reference sample. The following criteria are used to accept the tune:
      (3) Mass assignments of 69, 219, and 502 are ±0.2 amu.

(4) The relative abundance of mass 69 is 100%, mass 219 falls between 35% and 75%, and mass 502 falls between 2% and 10%.

(5) The isotopic ratio of mass 70 to 69 falls in the 0.5-1.5 range, the ratio of mass 220 to 219 falls in the 3.0-5.5 range, and the ratio of mass 503 to 502 falls in the 6.5-13.5 range.

b. Shimadzu

(1) The tune utilizes Perfluorotributylamine (PFTBA) as a reference sample. Mass assignments of 69, 219, and 502 are evaluated using the following criteria to accept the tune:

(2) Check that the FWHM (full width at half maximum) values are in the range of 0.5 to 0.7.

(3) Check that the detector voltage does not exceed 2 kV.

(4) Check that the base peak values are 18 or 69.

(5) Check that the relative intensity ratio for the m/z 502 is at least 2%.

c. Agilent (Newer GCs 8890+)

(1) Autotune may be utilized. Note: tune file name used is set in instrument method.

(2) The autotune utilizes Perfluorotributylamine (PFTBA) as a reference sample. The following criteria are used to accept the autotune:

(3) Mass assignments of 69, 219, and 502 and isotope assignments of 70, 220, 503 are ±0.2 amu.

(4) The isotopic ratio of mass 70 to 69 falls in the 0.5-1.5 range, the ratio of mass 220 to 219 falls in the 3.0-5.5 range, and the ratio of mass 503 to 502 falls in the 6.5-13.5 range.

d. Additional parameters to consider when determining if maintenance may be warranted:

(1) Peak shape should be symmetrical.

(2) Peak widths (Pw50) should be between 0.45 - 0.65.

(3) Ion abundances and number of background peaks should stay consistent from day to day.

(4) Relative abundance of 502 to 69 should be >3% for autotunes.

(5) EM voltage should be below 3000.

If more than one of the above parameters (1) through (5) are not being met and are not consistent with the previously analyzed tune, maintenance should be considered.

2. Run a performance test mix daily before use.

a. Helium Carrier Gas – The test mix solution is prepared with 10 ug/mL PCP and 40 ug/mL Nordiazepam and stored in a freezer to prolong stability.

(1) PCP or Nordiazepam will be the largest peak in the total ion chromatogram.

If parameter (1) is not met, corrective action will be taken to minimize extraneous peaks. Corrective action may include: changing the liner or gold seal, trimming the column, cleaning the ion source, or installing a new column.

b. Hydrogen Carrier Gas – The test mix solution is prepared with 10 ug/mL Ketamine and 40 ug/mL Nordiazepam and stored in the freezer to prolong stability.

(1) Ketamine or Nordiazepam will be the largest peak in the total ion chromatogram.

(2) Nordiazepam (242)/Ketamine (180) extracted ion ratio cut-off will be set to 0.20.

If parameters (1) and (2) are not met, corrective action will be taken to minimize extraneous peaks and improve the ion ratio. Corrective action may include: changing the liner or gold seal, trimming the column, cleaning the ion source, or installing a new column.

3. An image of the daily tune, test mix, and sequence log will be uploaded to the corresponding instrument in LAM. Image names will contain an identifier of the instrument used (if the site has multiple instruments of the same type), the date of analysis, and a description of the image. Required format: MS1 MMDDYY ImageName.

E. GAS CHROMATOGRAPHS

1. Headspace Volatile Analysis
   a. Calibration will be performed using certified reference materials as outlined in the Toxicology Procedures Manual.
   b. Calibration data will be maintained in LAM.
2. Drug Analysis
   a. Quantitative analysis: A working or an extracted drug standard will be analyzed to check instrument performance and maintained in the notes packet. (Function checks may be a working standard, working solution, an extracted standard or a standard curve. A hard copy does not need to be placed in a log book since it is in the notes packet.)
   b. Qualitative analysis: The control sample will be used to check instrument performance.
      (1) Positive QC samples must have all expected drugs meet identification criteria to be considered detected.
      (2) Negative QC samples must have no panel drugs included in the positive QC that meet identification criteria.

F. LIQUID CHROMATOGRAPH/TANDEM MASS SPECTROMETER (LC/TQ)

1. Liquid Chromatograph
   a. Check for any errors displayed by the instrument. Clear and address any indicated. Any maintenance performed will be documented in

LAM.

b. An appropriate system suitability control will be used to check instrument performance and to determine the expected retention time and ion ratios for any analyte of interest. Images of this data will be preserved in LAM.

c. Allow binary pump to run at starting mobile phase levels until pressure stabilizes. Pressure at equivalent mobile phase ratios should be comparable between runs. Increased pressure may be indicative of additional maintenance needs.

2. Mass Spectrometer

a. Autotune once every 31 days, or as needed.

b. Perform check tune prior to each use.

(1) Instrument autotune function includes a check tune. Autotuning prior to use is sufficient.

c. Images of the passing autotune/check tune will be preserved in LAM.

d. The tune utilizes ESI Tuning Mix. Parameters are automatically evaluated by the instrument as part of a tune. An overall result of "passed", highlighted in green in the header of the tune printout, will indicate that the instrument is performing within its acceptable range.

(1) An overall result of "out of tolerance", highlighted in yellow in the header of the tune printout, will indicate that an autotune should be performed, or that additional maintenance is required. An overall result of "failed", highlighted in red in the header of the tune printout, or an error indicating that the tune is unable to run to completion, will indicate that additional maintenance is required.

G. BALANCES

1. Perform weight check daily when using the microbalance for weighing quantitative drug standards.

a. Microbalances with a maximum weight capacity less than 10 g will utilize the following weights for their daily checks: 100 mg, 1 g, and 2 g.

2. All other balances should be checked monthly, or before use if used less frequently than monthly, using three appropriate certified weights per QM-11.

3. Weight checks will be documented in LAM.

4. The weight check must be ±1% of the certified weight.

5. Weight sets will be certified by an ISO certified vendor per QM-11. Certification documentation will be maintained in LAM.

H. EMIT SYSTEM

1. Perform function checks with each use as outlined in the Toxicology Procedures Manual and record in LAM.
2. Auto-maintenance/Blank rotor printouts will be attached in LAM and included in the notes packet.
3. Check that all reagents and controls are not past their expiration date. Ensure that lot numbers and expiration dates are updated in the EMIT computer when new items are placed in service.

I. QUANTITATIVE PIPETTES

1. Quantitative pipettes will be calibrated on-site annually.
   a. Whenever necessary for a pipette, ensure the calibration vendor is provided the necessary guidelines or commodity to perform the appropriate calibration service. For example, pipettes that can dispense across a range of volumes need to be calibrated so the calibration range spans the volumes utilized in casework.
   b. Should a pipette be found to be out of calibration, it will be taken out of service, documented in LAM, and its impact on completed casework will be evaluated in accordance with QM-11 and the Quality Manual.
2. Calibration documentation will be recorded in LAM.

J. CO-OXIMETER

1. The instrument must be performance checked whenever any of the following occur:
   a. Controls are out of range.
   b. Maintenance is performed on the instrument.
2. Performance checks will be recorded in LAM.

K. ELISA

1. Check that all instrument self-check tests pass on startup and attach documentation in LAM.
2. Performance check the instrument by running a wash and aspirate worklist. Ensure the plate wells are filled and emptied appropriately and record in LAM.
3. Check that reagents are not past their expiration date. Verify assay lot #'s and record in LAM.
4. Update the reagent kit worksheet if any lot numbers or expiration dates have changed. Attach this worksheet to "worklist documents".
5. Certificate of analysis sheets will be uploaded to LAM when available.

L. REFRIGERATION UNITS

1. It is good scientific practice that samples be kept refrigerated for long-term

storage.

2. Refrigerators and freezers have laboratory-set ranges of acceptability that are continuously monitored by an outside vendor. Staff will be automatically notified by an on-site alarm.
    a. Refrigerators are kept between 2°C and 8°C
    b. Freezers are kept between -25°C and -10°C
    c. Analysts are to ensure the above temperature ranges are sufficient for any newly purchased reagents with temperature control requirements. Any differences will be discussed with the supervisor.
3. Refrigerators and freezers will be performance checked monthly.
    a. The temperature of the refrigeration unit is recorded using a thermometer that is traceable to national or international standards. If necessary, adjust the temperature dial and recheck. Record these results in the LAM.
    b. If the temperature cannot be maintained within the specified tolerance window listed above for a period of 24 hours, all contents will be relocated to an appropriate working refrigeration unit and the out of range unit will be serviced.

M. THERMOMETERS
1. New thermometers are entered in LAM and their certificates are attached.
2. Thermometers may be used until the expiration date on their certificate
    a. This date will be documented in the Lab Status Info tab in LAM.

IV. WORKSHEETS/BATCH WORKLISTS/SEQUENCE LOGS

A. An assignment worksheet shall document all tests performed and their results. At a minimum the worksheet for a toxicology assignment will contain the following:

1. Items tested.
2. Tasks performed and the date of analysis.
    a. If the extraction date and the injection date are not the same date, then the injection date will be used in batch results in LIMS as the "run date" or annotated as a comment in batch results. The extraction date will still be included in the notes packet.
    b. Sequences analyzed overnight may use the date that analysis began as the "run date".
    c. Rejected analyses will be documented in the notes packet. This will include documentation of who rejected the data, when it was rejected, and the reason for the rejection.
        (1) For rejection of batch analyzed data due to a failed control, the batch will be reanalyzed. The batch worksheet will serve as the documentation of any failed batches.

(2) For rejection of a single sample, the rejected data and its associated controls will be included in the notes packet. The sample will be reanalyzed, and the new accepted data and its associated controls will be included in the notes packet.

(3) Should data resulting from analysis for any other situation arise, the rejected data will be maintained in LIMS following the same requirements as above.

3. The drugs/volatiles reported including qualitative and quantitative results as appropriate.

The notes packet can include additional images or documentation not noted on the worksheet so long as the documentation is not used to support "Findings" on the report. Examples include: unknown mass spectra or mass spectra not included in the toxicology drug panel.

In general, there is no reason to write everything on a worksheet in long hand if abbreviations can be found in the Toxicology Procedures Manual.

B. Batch worklists are case samples and QCs grouped together to complete an analytical task. Batch worklist numbers can be used to determine all samples in the sequential analysis. These worklists are saved in LIMS.

C. Sequence logs are files generated by an instrument that detail the order in which a group of samples were analyzed.

GC/MS, LC/TQ, EMIT, and ELISA sequence logs will be uploaded to the corresponding instrument in LAM. "Images" should be attached to the daily tune/test mix log entry for GC/MS and the ultivo general entry for LC/TQ.

V. REFERENCE MATERIALS

Guidelines for the expiration or re-authentication of reference materials are established in QM-14. Reference materials commonly used in toxicology have been categorized into three groups: purchased reference materials, internal reference materials, and reference collections. Reference materials not listed within these groups should refer to QM-14 or consult with the toxicology program manager for clarification if needed.

Quantitative materials (ie. drug/volatile standards, prepared working quant standards/controls) and semi-quantitative materials (ie. immunoassay cutoffs, LC/MS tuning solution) must adhere to manufacturer's expiration date or expire 1 year from the date the material is placed in use, whichever is earlier.

Qualitative materials (ie. confirmation positive/negative controls, internal standards, test mix solutions) and PFTBA do not have expiration dates as they are routinely re-authenticated concurrently with casework.

A. Purchased Reference Materials – to include:

1. Drug standards
2. Volatile standards
3. Whole blood volatile controls
4. Whole blood drug controls
5. Mixed volatile control
6. PFTBA
7. LC/MS tuning solution
8. EMIT system controls

B. Internal Reference Materials – reference materials prepared in-house to include:

1. Working drug standards
2. Urine confirmation controls
3. Blood confirmation controls
4. Aqueous ethanol controls
5. Whole blood ethanol controls
6. Blank urine
7. Blank blood
8. EMIT positive control
9. ELISA cutoffs
10. ELISA positive controls
11. Test mix and system suitability solutions

C. Reference Collections – databases containing reference comparison information used for substance identification. Reference collections include:

1. Mass spectral database libraries (i.e. Pfleger, Mills, AAFS, NIST, SWDRUG)
2. In-house spectral library. May include drugs, internal standards and derivatives traceable to reference materials.

VI. DEVELOPMENT OF NEW PROCEDURES

A. Procedures proposed for inclusion in the Toxicology Procedures Manual must be scientifically acceptable and appropriately referenced. Procedures are eligible for incorporation into the Toxicology Procedures Manual after they have been validated in accordance with Command Directive RES 1.

1. Additional standards in the field of toxicology, such as "ASB Standard 036: Standard Practices for Method Validation in Forensic Toxicology" may be utilized as a reference for creating validation plans and outlining experimentation. Validation acceptance criteria will be determined administratively in-house consistent with ISP policies, as the laboratory may not choose to strictly adhere to these standards in their entirety.

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TOXICOLOGY PROCEDURES MANUAL

APPENDIX III: SAFETY PROCEDURES

Reviewed by:

_____

Sean McCarthy, Chairperson
Toxicology Command Advisory Board

Approved by:

_____

Shannon George
Toxicology Program Manager

APPENDIX III

SAFETY PROCEDURES

Introduction:

The first reference for information on safety related issues is the facility safety manual. No one document can contain all of the available safety information; therefore, this manual is intended to provide a means for organizing and referencing safety information from a variety of sources.

The Command safety guidelines are contained in the Command Safety Manual. These, as well as the guidelines contained in the facility manuals, shall be observed by all analysts.

The potential hazards and appropriate safety precautions for any laboratory procedure must be considered prior to attempting any laboratory activity. Prior to conducting any laboratory procedure, it is the analyst's responsibility to review the safety information. The analyst shall assume all samples are hazardous.

The Safety Committee:
The safety committee is outlined in the Command Safety Manual.

Safety Inspections:
The laboratory inspection process is used to maintain safety standards. It can be used to identify problems and potential problems and determine whether established goals are being met. It can also be used to increase employee awareness of safety in the laboratory. The inspections information is given in the Command Safety Manual (appendix 1.1).

Accidents and Emergency Procedures:
All accidents shall be reported according to the procedures outlined in the Command Safety Manual and the facility manuals. The safety committee chairperson shall keep a log of all accidents with personal injury.

Analysts should be familiar with the procedures to be followed for emergency situations, including fire evacuation, tornado warning, and bomb threats.

Hazards:
The Illinois Toxic Substances Disclosure to Employees Act requires that an employer provide information concerning toxic substances. See the Material Safety Data Sheets maintained at your facility.

Radiation safety procedures are in the facility safety manual.
For labeling and storage of chemicals, consult the facility manual. Additional information is contained in the Command Safety Manual.

For disposal procedures, consult the Command Safety Manual and facility manual.

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TOXICOLOGY PROCEDURES MANUAL

APPENDIX VII: ESTIMATION OF MEASUREMENT UNCERTAINTY

Reviewed by:

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Toxicology Command Advisory Board

Approved by:

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Timothy Tripp
Bureau Chief – Toxicology Command Coordinator

APPENDIX VII

ESTIMATION OF MEASUREMENT UNCERTAINTY

Scope:

An estimation of Measurement Uncertainty (MU) will be determined for all cases where a quantitative result is reported. Calculations are performed by LIMS and documented on the assignment's worksheet. Measurement Uncertainty will be applied to laboratory reports when any quantitative results are reported.

Definitions:

Measurement Uncertainty (MU): An estimate of the range values with which a measurand is likely to be found.

Measurand: The quantitative expression of that which is being determined.

Expression of Uncertainty: The mean measured value ± uncertainty at a given confidence level.

Confidence Level: A probability statement that a particular measurand lies within the quoted range of uncertainty.

Standard Uncertainty: The estimated standard deviation.

Combined Standard Uncertainty: A result of the combination of the standard uncertainty components.

Expanded Uncertainty: An expression obtained by multiplying the combined uncertainty by a coverage factor.

Random (Type A) Uncertainties: Type A uncertainties result from measurement values being scattered in a random fashion due to the laws of chance, and thus a normal or Gaussian shape distribution. Type A uncertainty is best determined by historical data from a large number of repeated measurements. This estimation may be called measurement repeatability or reproducibility and reflects the combined effects of many contributing factors. When a new procedure lacks historical data, repeatability data may be used from the validation

Systematic (Type B) Uncertainties: Type B uncertainties result from the inherent biases in measurement systems. These uncertainties may be reduced by optimizing the method or measurement systems but can never be completely eliminated. Examples of systematic uncertainties include weighing a powdered standard with an analytical balance or preparing a calibrator or control using a volumetric flask. Control data included in Type A uncertainties includes some Type B uncertainties such as instruments, maintenance, and analysts. Furthermore,

the use of an internal standard for quantitative analysis minimizes other sources of uncertainty including instrumental factors such as the injection and detector fluctuations.

Uncertainty Budget (UB): A table that itemizes all components contributing to the measurement uncertainty for a process. It shall include both Type A and Type B uncertainties. All contributing factors will be expressed as a percentage to avoid the need to convert components to the same units.

ESTIMATING MEASUREMENT UNCERTAINTY

Estimation of MU starts with an assessment of all factors that contribute to the uncertainty of a measurand. These components can be categorized into various groups, including but not limited to those included in quality assurance (QA) activities, analyst technique, method development and validation, and quality control (QC) data. Factors that are negligible in practice will be excluded from the Uncertainty Budget (UB). Uncertainty Budgets will be uploaded to LAM and maintained by the Technical Leader, Training Coordinator, or laboratory designee.

Guidelines for Calculating MU:

Overall MU considering the contributions listed below is calculated per the frequency in QM-18 (or as deemed necessary upon the incorporation of new equipment, staff, or calibrations) and entered into the uncertainty budget to update the MU used for reporting.

Uncertainty Contribution from Process Reproducibility

Process reproducibility involves replicate analysis of a control by multiple analysts across all sites and incorporating all major equipment. This measurement captures the effects of the internal standard, environmental conditions, the instrument method, matrix effects, sample stability, and human factors.

1) Determine the mean (x) and standard deviation (σ) of a minimum of 60 historical control measurements that have been collected using all instrumentation and represents all staff members and environmental conditions statewide.
   a) When a new procedure lacks historical data, repeatability data may be used from the validation
2) Calculate the relative standard deviation (RSD) for the data. This is done by dividing the standard deviation by the mean and expressing it as a percentage. This percent RSD is entered into the budget table for the appropriate method.

Uncertainty Contributions from Calibrated Materials and Equipment

In additional to MU using historical data there are other factors that need to be considered for the Uncertainty Budget. What is included depends on what is used in the quantitative method.

1) Items that contribute to MU will have a certificate of analysis (COA) that states the measurement uncertainty.

a) This could include manufacturer provided COAs or COAs that are obtained as the result of calibration by an ISO 17025 certified vendor.
2) Examples include volumetric glassware, certified reference materials, fixed pipettes, repeater pipettes, and pipettor-dilutors.
3) If not already a percent RSD, convert by dividing the standard deviation by the mean and expressing it as a percentage.
4) When evaluating what %MU to include in the Uncertainty Budget for an asset, all COAs for that asset statewide will be evaluated and the highest %MU will be recorded.

Uncertainty Contribution from Duplicate Analysis

Another factor to consider in the Uncertainty Budget is data reproducibility from multiple samplings. All quantitative procedures require duplicate measurements for case samples.

1) When historical case data is available, it will be assessed to determine the standard deviation of the percent difference from the mean for each replicate sample.
2) When historical data is unavailable, such as an initial validation or a significant change in the analytical procedure, the range that the duplicate measurements can fall while still being acceptable will be entered into the budget.
   a) This range is to be stated in the appropriate procedure in the procedure manual.
   b) If the acceptable range is changed prior to the availability of historical data, the UB will need to be updated.

Uncertainty Budget Calculations

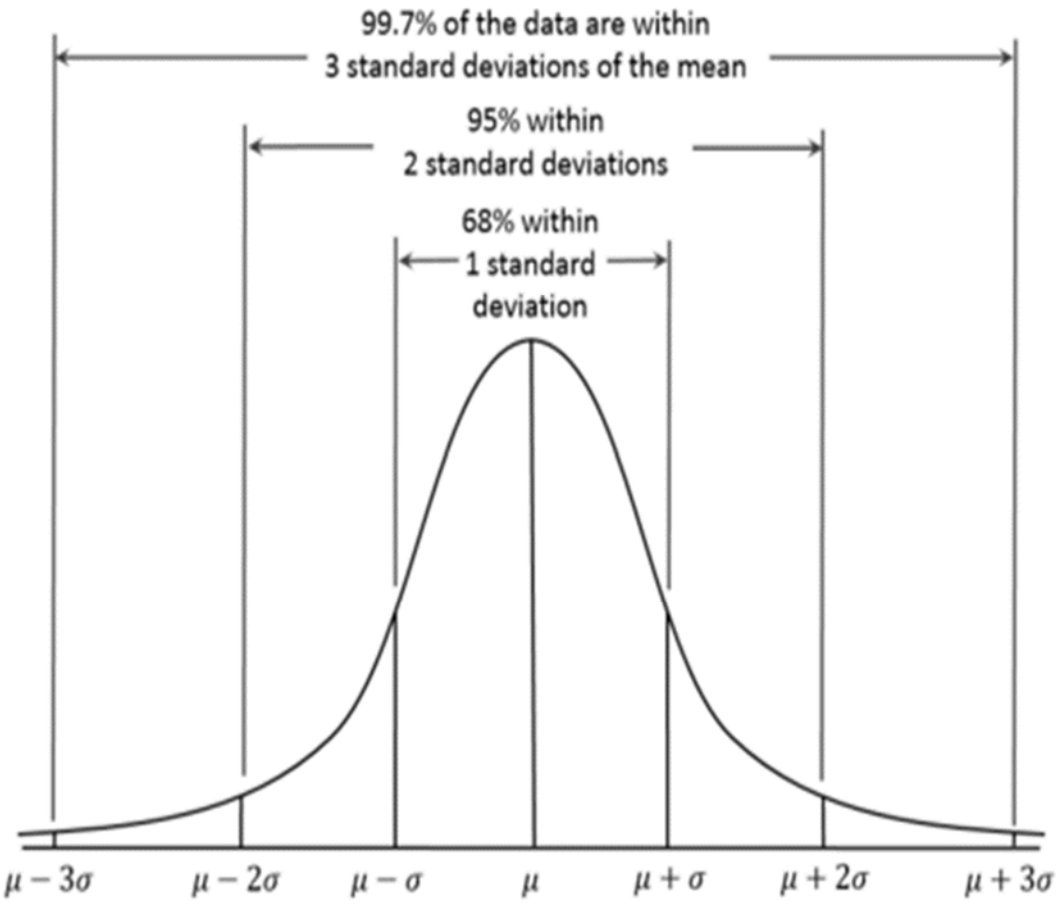
After all factors that contribute to the measurement uncertainly in a process have been added to the UB, the reported uncertainty for the method needs to be calculated.

1) For each uncertainty component in the UB, divide the percentage contribution by a divisor to get the standard uncertainty ($U_{line\ item\#}$). For any line item that is considering case or control data the divisor will be $\sqrt{2}$ since case samples are run in duplicate for all quantitative methods. For all other line items the divisor will be the coverage factor listed on the certificate (usually k = 2).

2) The combined standard uncertainty needs to be calculated using the root sum squares technique. This is done with the following equation

   a. $U_{combined} = \sqrt{(U_{line\ item\ 1}^2 + U_{line\ item\ 2}^2 + U_{line\ item\ N}^2)}$

3) The combined uncertainty is then multiplied by a coverage factor (k). For a confidence interval of 95.45%, k is equal to 2 and for a confidence interval of 99.73%, k is equal to 3. This is the reported uncertainty value. All quantitative procedures will use a 99.73% reported uncertainty unless specifically stated in the procedure.



4) Since the uncertainty of measurement is an estimate, the % uncertainty will not exceed two significant digits and will be rounded up to overestimate the uncertainty.

5) The reported uncertainty percentage value is applied (±) to the appropriate analytical result. When applied to the case result, the lower end will be truncated and the high end will be rounded up to maintain the unknown result's value significance.

When to re-evaluate the Uncertainty Budget:

Over time, factors that contribute to the Uncertainty Budget can change. Below is outlined how changes will be addressed and when it will be deemed necessary to recalculate the measurement uncertainty for a process. All evaluations and recalculations will be documented in LAM.

1) The interval defined in QM-18 has passed, requiring a new calculation of the MU.
   a) The most recent historical data statewide will be evaluated for process uncertainty and duplicate measurements, and the budget table will be updated.
   b) All values in the budget table will be updated, and any new contributions will be included.

2) Addition of new staff or new instrumentation
   a) A minimum of 20 data points will be collected by the new staff member or on the new instrumentation. The data points will be added to the current historical data and the reproducibility will be recalculated and evaluated against the current budget table.
      i) If there is no change or a decrease to the reproducibility contribution, then it will be deemed still fit for purpose and no changes will be made
      ii) If there is an increase to the reproducibility contribution, then the measurement uncertainty will be recalculated.
         (1) If there is no change to the expanded uncertainty, the current calculation remains fit for purpose.
         (2) If there is an increase to the expanded measurement uncertainty, the value will be updated, a new budget table uploaded, and notification will be made to the section.

3) Recalibration of an asset type occurs resulting in a new COA
   a) All COAs for that asset statewide will be evaluated for their percent uncertainty against the current budget table
      i) If there is no change or a decrease to the equipment contribution, then it will be deemed still fit for purpose and no changes will be made
      ii) If there is an increase to the equipment contribution, then the measurement uncertainty will be recalculated.
         (1) If there is no change to the expanded uncertainty, the current calculation remains fit for purpose.
         (2) If there is an increase to the expanded measurement uncertainty, the value will be updated, a new budget table uploaded, and notification will be made to the section.

Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results

Table B.1 – Value of $t_p(v)$ from the t-distribution for degrees of freedom v that defines an interval $-t_p(v)$ to $+t_p(v)$ that encompasses the fraction p of the distribution

Degrees of freedom	**Fraction *p* in percent**					
v	**68.27 [a]**	**90**	**95**	**95.45 [a]**	**99**	**99.73 [a]**
1	1.84	6.31	12.71	13.97	63.66	235.80
2	1.32	2.92	4.30	4.53	9.92	19.21
3	1.20	2.35	3.18	3.31	5.84	9.22
4	1.14	2.13	2.78	2.87	4.60	6.62
5	1.11	2.02	2.57	2.65	4.03	5.51
6	1.09	1.94	2.45	2.52	3.71	4.90
7	1.08	1.89	2.36	2.43	3.50	4.53
8	1.07	1.86	2.31	2.37	3.36	4.28
9	1.06	1.83	2.26	2.32	3.25	4.09

10	1.05	1.81	2.23	2.28	3.17	3.96
11	1.05	1.80	2.20	2.25	3.11	3.85
12	1.04	1.78	2.18	2.23	3.05	3.76
13	1.04	1.77	2.16	2.21	3.01	3.69
14	1.04	1.76	2.14	2.20	2.98	3.64
15	1.03	1.75	2.13	2.18	2.95	3.59
16	1.03	1.75	2.12	2.17	2.92	3.54
17	1.03	1.74	2.11	2.16	2.90	3.51
18	1.03	1.73	2.10	2.15	2.88	3.48
19	1.03	1.73	2.09	2.14	2.86	3.45
20	1.03	1.72	2.09	2.13	2.85	3.42
25	1.02	1.71	2.06	2.11	2.79	3.33
30	1.02	1.70	2.04	2.09	2.75	3.27
35	1.01	1.70	2.03	2.07	2.72	3.23
40	1.01	1.68	2.02	2.06	2.70	3.20
45	1.01	1.68	2.01	2.06	2.69	3.18
50	1.01	1.68	2.01	2.05	2.68	3.16
100	1.005	1.660	1.984	2.025	2.626	3.077
∞	1.000	1.645	1.960	2.000	2.576	3.000

[a]For a quantity z described by a normal distribution with expectation μ_z and standard deviation σ, the interval $\mu_z \pm k\sigma$ encompasses p = 68.27, 95.45, and 99.73 percent of the distribution for k = 1, 2, and 3, respectively. [3,4]

REPORT WORDING

Refer to Appendix I.

REFERENCES

1. American Society of Crime Laboratory Directors / Laboratory Accreditation Board International, ASCLD/LAB Policy on Measurement Uncertainty. (July 1, 2012)

2. Joint Committee for Guides in Metrology (JCGM), *Evaluation of measurement data - Guide to the expression of uncertainty in measurement* (GUM) (GUM 1995 with minor corrections). (Sevres, France: International Bureau of Weights and Measures [BIPM]-JCGM 100], September 2008).

   Even though the electronic version of the 2008 edition of the GUM is available free of charge on the BIPM's website, copyright of that document is shared jointly by the JCGM member organizations (BIPM, IEC, IFCC, ILAC, ISO, IUPAC, IUPAP and OIML).

3. JCGM, International Vocabulary of Metrology – Basic and General Concepts and Associated Terms (VIM), 3rd ed. (Sevres, France: BIPM-JCGM 200, 2008).

Even though the electronic version of the 3rd edition of the VIM is available free of charge on the BIPM's website, copyright of that document is shared jointly by the JCGM member organizations (BIPM, IEC, IFCC, ILAC, ISO, IUPAC, and OIML).

4. United States Department of Commerce Technology Administration, National Institute of Standards and Technology (NIST), *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*, NIST Technical Note 1297, 1994 ed. (Barry N. Taylor and Chris E. Kuyatt).

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TOXICOLOGY
PROCEDURES MANUAL

APPENDIX VIII: RETROGRADE EXTRAPOLATION

Reviewed by:

_____
Larry Shelton, Chairperson
Toxicology Command Advisory Board

Approved by:

_____
Shannon George
Toxicology Program Manager

APPENDIX VIII

RETROGRADE EXTRAPOLATION

Retrograde Extrapolation is a scientific analysis used to determine a person's Blood Alcohol Concentration (BAC) at a previous time, for example, at the time of driving. It is a mathematical process, based on sound scientific principles. This calculation may be necessary due to a lapse in time between when an accident occurred and when a breath, blood, or urine specimen was obtained. Retrograde extrapolation is routinely used in pharmacology, toxicology, and clinical medicine.

When performing retrograde extrapolation the following assumptions are required:

- The measured whole blood ethanol level is greater than 0.020 g/dL
- The individual is at or past peak absorption at the time in question
- The metabolism of the individual is in the normal range of 0.010-0.020 g/dL/hr

Retrograde extrapolation will be performed when an appropriate request is made by the attorney.

REFERENCES

1. Bostic, Nicholas J. "Alcohol-Related Offenses: Retrograde Extrapolation after Wager," Michigan Bar Journal, 2000, Jun: 79(6).

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ILLINOIS STATE POLICE

TOXICOLOGY PROCEDURES MANUAL

APPENDIX IX: ABBREVIATIONS

Reviewed by:

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APPENDIX IX

ABBREVIATIONS

The following is a list of abbreviations utilized by Forensic Scientists in the Toxicology Section:

Abbreviation	Meaning
6-MAM	6-MONOACETYLMORPHINE
-A or A	ARTIFACT
AAFS	AMERICAN ACADEMY OF FORENSIC SCIENCES MASS SPECTRUM LIBRARY
AGG	Aggravated
ALD	Assistant Laboratory Director
AMPHET	AMPHETAMINE/SMA
AMT	Amount
AQ	Aqueous
ASA	ASSISTANT STATE'S ATTORNEY
AVG	Average
BARB	BARBITURATE
Baselt	Book "Disposition of Toxic Drugs and Chemicals in Man" by Randall C. Baselt (cite edition)
BD	Break Down
BE	Benzoylecgonine
BENZO	BENZODIAZEPINE
BLD	BLOOD
BSTFA	Bis(trimethylsilyl)trifluoroacetamide or it's derivative
BTWN	Between
B/U	Blood/Urine
BUAC	BUTYL ACETATE EXTRACT
BZE	BENZOYLECGONINE
BZO	BENZODIAZEPINE
CAL	CALIBRATION OR CALIBRATOR
CALMS	Computer Aided Laboratory Management System
CAYMAN	Cayman Spectral Library
CCSAO	Cook County State's Attorney's Office
CERT MAIL	CERTIFIED MAIL
CHI	Chicago

CLARKE	**REFERENCE BOOK "Clarke's Analysis of Drugs and Poisons" by Moffat, A., et.al.**
Co.	**County**
COC	**COCAINE**
COMBO	**Referring to the "Combined Drug Confirmation for Urine using BSTFA" procedure**
CONF	**CONFIRMATION**
CONFIRM	**CONFIRMATION**
CONT	**CONTROL**
CPD	**Chicago Police Department**
CSA	**Criminal Sexual Assault**
CTRL	**CONTROL**
D or d	**Deuterated**
D(#) or DIST	**District (related to the ISP District Number)**
DDRUG	**"Mass Spectra of Designer Drug" Library**
DERIV	**DERIVATIVE**
Det	**Detective**
DETOX	**Urine drug screen using commercially prepared extraction tubes**
DFS	**Division of Forensic Services**
DIL	**DILUTION**
DEP	**Deputy**
DUI	**Driving Under the Infuence**
ELISA	**Enzyme Linked Immunosorbent Assay**
EMIT	**ENZYME MULTIPLIED IMMUNOASSAY TECHNIQUE**
ET	**Evidence Technician**
ETOH	**ETHANOL**
EVID	**EVIDENCE**
EVT	**EVIDENCE TAPE**
EXP	**EXPIRES**
EXT	**EXTRACT(ED)**
FA	**Formic Acid**
FD	**Fire Department**
FID	**FLAME IONIZATION DETECTOR**
FOM	**Facility Operations Manual**
FP	**Forest Preserve**
FS	**Forensic Scientist**

FSC-C	**Forensic Science Center at Chicago**
FSL	**Forensic Science Laboratory**
GBH	**Great Bodily Harm**
GC/FID	**GAS CHROMATOGRAPHY WITH FLAME IONIZATION DETECTOR**
GC/MS	**GAS CHROMATOGRAPHY WITH MASS SPECTROSCOPY**
GC/NPD	**GAS CHROMATOGRAPHY WITH NITROGEN-PHOSPHOROUS DETECTOR**
GHB	**GAMMA-HYDROXYBUTYRIC ACID**
GLUC	**Glucuronidase**
HC	**High Control**
HFB	**HEPTAFLUOROBUTYRL DERIVATIVE**
HI	**High, often referring to a high control**
HP	**Hewlett-Packard**
HPLC	**HIGH PERFORMANCE LIQUID CHROMATOGRAPHY**
HS	**HEAD SPACE**
HTS	**Heights**
IDNR	**Illinois Department of Natural Resources**
Incub.	**Incubation**
IND	**Indication**
INJ	**INJECTED**
INS or I/S	**Insufficient sample**
IPA	**ISOPROPANOL**
ISP	**Illinois State Police**
ISTD	**INTERNAL STANDARD**
JAT	**JOURNAL OF ANALYTICAL TOXICOLOGY**
JFSL	**Joliet Forensic Science Laboratory**
LAM	**Laboratory Asset Manager**
LC\MS	**Liquid Chromatography\Mass Spectrometry**
LC/TQ or LC/QQQ	**Liquid Chromatography/Triple Quadrupole Mass Spectrometry**
LD	**Laboratory Director**
LIMS	**Laboratory Information Management System**
LIQ	**LIQUID or Liquor**
LOD	**Limit of detection**
LOQ	**Limit of Quantitation**

LSD	**Lysergic acid diethylamide**
Lt	**Lieutenant**
-M or M	**METABOLITE**
-M/A or M/A	**METABOLITE/ARTIFACT**
mCPP	**m-CHLOROPHENYLPIPERAZINE**
MDA	**METHYLENEDIOXYAMPHETAMINE**
MDMA	**METHYLENEDIOXYMETHAMPHETAMINE**
MEOH	**METHANOL**
MET	**METABOLITE(S)**
METAB	**METABOLITE(S)**
MILLS	**INSTRUMENTAL DATA FOR DRUG ANALYSIS BY MILLS, ET AL**
MP	**Mobile Phase**
(MV)	**Volatiles including but not limited to ethanol, acetone, isopropanol, and methanol**
MS	**Mass spectrometer**
MSGT	**Master Sergeant**
MtBSTFA	**N-Methyl-N-(t-Butyldimethylsilyl) Trifluoroacetamide or it's derivative**
MU	**Measurement uncertainty**
NA or N/A	**NOT APPLICABLE**
NDD	**NO DRUGS DETECTED**
NED	**No Expiration Date**
NEG	**NEGATIVE**
NIST	**National Institute of Standards and Technology. Often used to reference a spectral library.**
NP(NR)	**Not Pursued (Not reported)**
NSAID	**Non-steroidal anti-inflammatory drug**
NVD	**NO VOLATILES DETECTED**
Ofc	**Officer**
-OH	**Hydroxy**
OP	**OPIATE**
OPI	**OPIATE**
PCP	**PHENCYCLIDINE**
PD	**POLICE DEPARTMENT**
Pfleger	**"Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites" by Maurer, Pfleger, and Weber**
Pg	**Page**

PM	**Procedure's Manual**
PN	**Positive/Negative for EMIT results**
POS	**POSITIVE**
POSS	**POSSIBLE**
PPA	**PHENYLPROPANOLAMINE**
PROF	**PROFICIENCY**
-Q	**Quantitation**
QC	**Quality Control**
QNS	**QUANTITY NOT SUFFICIENT**
QRC	**Quality Review Coordinator**
q.s.	**Quantum satis (Latin) meaning the amount which is enough**
QUAL	**Qualitative**
QUANT	**QUANTITATION**
RCVD	**Received**
RE	**REGARDING**
RECON	**RECONSTITUTE**
REF	**REFERENCE**
REG MAIL	**REGISTERED MAIL**
RFSL	**Rockford Forensic Science Laboratory**
RRT	**RELATIVE RETENTION TIME**
RT	**RETENTION TIME**
SA	**State's Attorney**
SAO	**STATE'S ATTORNEY'S OFFICE**
SDT	**Subpoena Duces Tecum**
SFSL	**Springfield Forensic Science Laboratory**
Sgt	**SERGEANT**
SMA	**SYMPATHOMIMETIC AMINE**
S/N	**Signal-to-noise ratio**
SNRI	**Selective norephinepherine reuptake inhibitor**
SO	**SHERIFF'S OFFICE (or Department)**
SOFT	**Society of Forensic Toxicologists**
SOS	**Secretary of State**
SP?	**Spelling of word prior to abbreviation has not been confirmed and may be incorrect**
SPE	**SOLID PHASE EXTRACTION**
SPEC	**SPECTRUM or Spectrometer**

SSRI	**Selective serotonin reuptake inhibitor**
STD	**STANDARD**
s/w	**Spoke with**
SWGDRUG	**Scientific Working Group for the Analysis of Seized Drugs Mass Spectral Library**
SYNCANN	**United States Army Criminal Investigation Laboratory (USACIL) Synthetic Cannabinoids Mass Spectral Library**
TAS	**Statewide Case Flow Toxicology Analytical Scheme as listed in the Introduction to the Toxicology Procedure Manual.**
TBDMS	**TERTIARY BUTYL DIMETHYL SILYL DERIVATIVE**
TC	**Training Coordinator**
Temp	**Temperature**
THC	**TETRAHYDROCANNABINOL or THC-COOH GC\MS method**
TIAFT	**The International Association of Foresic Toxicologists**
TIC	**Total Ion Chromatogram**
TL	**Technical Leader**
TMIX	**Test mix**
TMS	**TRIMETHYLSILYL DERIVATIVE**
TOX	**Toxicology**
Tpr	**Trooper**
UNDERIV	**UNDERIVATIZED**
UNK	**UNKNOWN**
UR	**URINE**
URN	**URINE**
UV/VIS	**ULTRAVIOLET/VISIBLE**
VM	**VOICEMAIL**
VOL	**VOLATILE**
w/	**with**
WBC	**WHOLE BLOOD CONTROL**
WKSTD	**WORKING STANDARD**
WL	**Worklist**
$\bar{x}$	**Mean (Average)**
α	**Alpha**
β	**Beta**

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APPENDIX X: DATA SUITABILITY FOR COMPARISON

Reviewed by:

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APPENDIX X

DATA SUITABILITY FOR COMPARISON

Before the data of an unknown sample analysis can be compared to a reference standard, it must first be evaluated for suitability. Occasionally, this includes an assessment of limitations inherent to any particular analytical technique given the presence of a specific substance (e.g. isomer identification by GC-MS). The following suitability assessments shall be considered prior to any comparison between a sample and a reference standard.

ASSESSING THE SUITABILITY FOR COMPARISON OF SAMPLE DATA TO A REFERENCE STANDARD

I. For Instrumental Techniques (GC-FID, GC-MS, LC-TQ)

A. Detector Response

1. When using a technique that produces a detector response, the response should be greater than the noise level to be considered a peak suitable for comparison. The short-term noise levels (those directly adjacent to the signal response) should be used for determining response suitability.

B. Chromatographic Peak Shape

1. Acceptable peaks are typically Gaussian, but slight asymmetry and tailing is normal with temperature-gradient methods.

2. The peak of a single analyte should produce a single apex to be used for comparison.

3. In the event that a chromatographic peak is not Gaussian-shaped, the analyst shall evaluate the level of fronting or tailing to determine the significance of the irregularity. Even in circumstances of asymmetry, a single apex should be present for a single sample component to be integrated when retention time is referenced during analysis. In the event that a single apex is not discernible when retention time is to be considered, mitigation steps shall be taken to generate acceptable peak shapes for use in comparison to a reference standard.

C. Resolution

1. Resolution shall be sufficient to show separation of distinct analytes in chromatographic techniques. Resolution shall also be sufficient to differentiate mass assignments (GC-MS). Analysts must assess the extent to which any

shouldering or overlap may affect identification of a specific analyte to the exclusion of interfering analytes or impurities, and note any manipulations of data employed to produce a clear result from a composite of overlapping substances.

D. Concentration Disparities

1. Characteristics that may be affected by concentration disparities may include chromatographic peak shape, retention time, mass spectral ion ratios, etc. Acceptance criteria is defined in Minimum Standards and Controls.

MINIMUM STANDARDS AND CONTROLS FOR TESTING TECHNIQUES

Minimum standards and controls for each individual procedure or testing technique can be found in the relevant section of the procedures manual.

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APPENDIX XI: Drug Panel

Reviewed by:

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APPENDIX XI

Drug Panel

Appendix XI contains the toxicology Drug Panel list. This list represents the approved reportable drugs analyzed within the toxicology section and will be included on every Toxicology Laboratory Worksheet generated within LIMS.

The drug panel will be reevaluated annually to address the ever-changing trends in drugs of abuse. Recommendations for changes can be submitted to the Command Advisory Board for consideration. Changes to this list will be reflected in this appendix, in the individual class confirmation procedures in the Toxicology Procedures Manual, and in the printed list within LIMS included as a part of the Toxicology Laboratory Worksheet.

This document has been prepared as a guide to understanding toxicology case approach. This guide also serves as a complete list of the drugs and volatiles tested for within each respective panel.

1. Reference the toxicology laboratory report and locate the **Report Remarks**.
2. Match all of the **Report Remarks** to the respective section below (in red).
3. Review each **Drug Class Panel** included on the report to determine which drugs were screened for during testing.
4. Evaluate superscripts for additional details as needed.

Report Remark
Volatile analysis of this case is limited to the following: Ethanol, Methanol, Acetone, and Isopropanol.
Volatile Panel
Acetone
Ethanol
Isopropanol
Methanol

Report Remark
Drug analysis has been limited to the following classes: **Amphetamine**, **Benzodiazepine**, **Cocaine**, **Opiate**, **Phencyclidine (PCP)**, and **Cannabinoid**. If additional drug testing is required, a service request must be submitted using LIMS pre-log.
Amphetamine Panel
Amphetamine
Methamphetamine
Methylenedioxyamphetamine (MDA)[1]
Methylenedioxymethamphetamine (MDMA)[1]

Benzodiazepine Panel
7-Aminoclonazepam
alpha-Hydroxyalprazolam
alpha-Hydroxymidazolam
Alprazolam
Clonazepam
Diazepam
Flualprazolam
Lorazepam[1]
Midazolam
Nordiazepam
Oxazepam
Temazepam
Cannabinoids Panel
Delta-9 Carboxy THC (THC metabolite)
Delta-9 Tetrahydrocannabinol (THC)
Cocaine Panel
Benzoylecgonine (Cocaine metabolite)
Cocaethylene (Cocaine metabolite)
Cocaine
Opiate Panel
6-Monoacetylmorphine (Heroin metabolite)
Codeine
Dihydrocodeine
Hydrocodone
Hydromorphone
Morphine
Oxycodone
Oxymorphone
Phencyclidine (PCP) Panel
Phencyclidine (PCP)

[1] Preliminary screening tests can result in a negative result at concentrations detected in a confirmation test. If this drug is not detected but material to the case please contact the laboratory to discuss if additional analysis is warranted.
Not used

Report Remark
Drug analysis includes, **Full Drug Panel** and the following classes: **Amphetamine**, **Benzodiazepine**, **Cocaine**, **Opiate**, **Phencyclidine (PCP)**, and **Cannabinoid**.
Full Drug Panel
Amitriptyline
Bupropion
Butalbital
Carbamazepine
Carisoprodol
Chlorophenylpiperazine (mCPP)
Chlorpheniramine
Citalopram
Clonidine
Cocaethylene
Cocaine
Cyclobenzaprine
Desipramine
Desmethylcitalopram
Diphenhydramine
Doxepin
Doxylamine
Fentanyl
Fluoxetine
Gabapentin
Guaiphenesin
Hydroxybupropion
Hydroxyzine
Imipramine
Ketamine
Lamotrigine
Levetiracetam
Meperidine
Meprobamate
Methadone
Methorphan
Methylenedioxyamphetamine (MDA)
Methylenedioxymethamphetamine (MDMA)
Methylphenidate

Mirtazapine
Modafinil
Nordoxepin
Norfentanyl
Norketamine
Norquetiapine
Nortriptyline
O-Desmethyltramadol
O-Desmethylvenlafaxine
Olanzapine
Oxcarbazepine
Paroxetine
Phenobarbital
Phenytoin
Promethazine
Propofol
Quetiapine
Sertraline
Topiramate
Tramadol
Trazodone
Valproic Acid
Venlafaxine
Zolpidem

Report Remark
Note: Additional analysis was performed to screen for **Gamma-Hydroxybutyric Acid (GHB)**.
GHB Panel[5/6]
Gamma-Hydroxybutyric Acid (GHB)

[5] Analysis limited to urine sample types

[6] Analysis routinely performed on all criminal sexual assault (CSA) investigations which include urine sample submissions.