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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 in forensic casework. The procedures contained within this manual will be utilized in the analyses of volatiles and drugs in biological samples as applicable.

The toxicology section follows standardized procedures established through method validation to produce legally defensible results for all analyses performed. The methods used meet the standards established by the ANSI National Accreditation Board (ANAB) for forensic laboratories as well as guidelines set forth in the Illinois Administrative Code.

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** *ISP DRE Toxicology Request Form,* or agency DRE evaluation report, 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 to include volatile and drug testing. Coroner's cases are reviewed by the analyst and appropriate testing is determined based on information from the user agency and case history.

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. If the additional service request is a continuation of the toxicology testing analytical scheme for the evidence submitted then no additional approvals are needed. However, if the service request is for testing outside of the toxicology testing analytical scheme (for example, blood drug testing on a case where urine drug testing was already performed) then supervisor or lab director approval is required and must be documented as a case correspondence.

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 DUI drugs or DUI 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.

NOTE: Some blood only cases may have case offenses that do not fall into a DUI category. Consult with management in these situations to determine if blood drug testing is warranted.

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). Urine that screens positive for cannabinoids is confirmed by quantitation in the blood (if available). If negative or no results are confirmed, proceed to step 3.

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

Urine Only 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. If no urine is submitted, refer to Blood Only DUI protocol, outlined above.

2) Screen the urine sample using EMIT. Report any confirmed findings. Urine that screens positive for cannabinoids is confirmed by quantitation in the blood (if available).

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

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, Officer Involved Shootings

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

2) Blood/Urine cases - Perform a full volatile and drug panel 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).

Note: some other case types may be analyzed using the traffic fatality scheme. Possible offenses include child endangerment and cases involving drivers with a commercial driving license. Analysts will consult with their supervisor on these cases and their direction will be recorded using a "Case Correspondence" entry in LIMS.

Drug Facilitated Sexual Assaults

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

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

Note: Cases with 20 mL of urine sample or less will be analyzed for volatiles and all other testing will be deferred. Deferred analyses on DFSA cases may be revisited upon request by the user agency which includes permission to consume sample as needed.

Coroner Cases

1) Perform full volatile and drug panel analysis on blood and urine samples.

2) 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.

ILLINOIS STATE POLICE

TOXICOLOGY
PROCEDURES MANUAL

PROTOCOL: Volatile Analysis

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

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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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. Unknown liquid samples must be diluted and a 25x dilution is the lowest dilution factor to use. (Note #6)
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. Instruments used to dilute the sample will be annotated in LIMS batch results using a task comment in lieu of scanning the assets.
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 a sample is initially diluted to a volume greater than 25 mL and the result is positive for a volatile but below the 0.010 g/dL limit of quantitation, then the sample will be re-run with a lesser dilution.
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
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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 and will be reported qualitatively only.

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 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".
   c. Results must be above 0.010 g/dL on both columns to be accepted qualitatively.

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:

_____
Sean McCarthy, Chairperson
Toxicology Command Advisory Board

Approved by:

_____
Timothy Tripp
Bureau Chief – Toxicology Command Coordinator

ILLINOIS STATE POLICE

TOXICOLOGY PROCEDURES MANUAL

PROTOCOL: Qualitative Drug Screening

METHOD: Enzyme Multiplied Immunoassay Techniques

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

Reviewed by:

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Sean McCarthy, Chairperson
Toxicology Command Advisory Board

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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

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ILLINOIS STATE POLICE

TOXICOLOGY
PROCEDURES MANUAL

PROTOCOL: Qualitative Drug Screening

METHOD: Enzyme Linked Immunosorbent Assay (ELISA)

Reviewed by:

_____
Casey Craven, Chairperson
Toxicology Command Advisory Board

Approved by:

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Timothy Tripp
Bureau Chief – Toxicology Command Coordinator

INTRODUCTION:

The ELISA 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 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).

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.

<u>In-House Reagent Preparation</u>

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).

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 #3). (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.
3. The THC and PCP assay are excluded from having a PN finding and can only be positive or negative

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:

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Casey Craven, Chairperson
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Approved by:

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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.

This extraction is utilized for both the general drug screen in blood, as well as the targeted confirmation of PCP. Cases will be evaluated appropriately per the Toxicology Analytical Scheme and will utilize the positive control and panel list below. Cases that require both a general drug screen and are PCP positive on ELISA will utilize only the general drug screen task. Cases that are only PCP positive on ELISA will utilize only the PCP task.

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**, **200 Proof.** (Aaper Alcohol Co.).
5. **Hexanes**, **Certified A.C.S. Grade.** (Fisher Scientific Co.)
6. **Drug Screen Positive Control Working Standard.** 2.5 µg/mL of PCP; 15 µg/mL of diphenhydramine; 30 µg/mL of zolpidem, propofol and amitriptyline; 120 µg/mL butalbital. Dilute 25 µL of PCP standard; 150 µL of diphenhydramine standard; 300 µL each of zolpidem, propofol and amitriptyline stock standards; and 1.2 mL of butalbital stock standard together to 10 mL with methanol. Alternative stock standards prepared at appropriate concentrations may be used if necessary.

7. **Hexanes:Ethanol (1:1).** Measure equal parts of Hexanes and Ethanol and mix.

MINIMUM STANDARDS & CONTROLS

Refer to Appendix II.

Controls:

1. In-house positive blood control. Spike 1 mL blank blood with 25 uL positive control working standard.

2. Blank blood for negative control.

INSTRUMENTATION

Gas Chromatograph/Mass Spectrometer

Instrument Conditions (Note #3):

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 positive and negative controls and each of the case samples. Place 1.0 mL aliquots of each control and case sample 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 50 µL hexanes: ethanol (1:1) prior to injection.
8. Inject 1 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.

Full Panel Drug Screen (Note # 2)

Drug Name	**Major Ions**			
10-hydroxy Carbamazepine[1] (Oxcarbazepine metabolite)	193	180	210	167
Amitriptyline	58	202	215	189
Bupropion	44	100	111	224
Butalbital	168	41	167	124
Carbamazepine[1]	193	236	165	139
Carbamazepine 10,11-epoxide (Carbamazepine metabolite)	180	252	152	223
Carisoprodol	55	97	158	245
Chlorophenylpiperazine (mCPP)	154	196	111	138
Chlorpheniramine	203	167	58	72
Citalopram	58	238	208	324
Clonidine	229	194	172	124
Cocaethylene	82	196	94	317
Cocaine	82	182	94	303
Cyclobenzaprine	58	215	202	189
Desipramine	234	193	208	266
Desmethylcitalopram	44	238	208	310
Diphenhydramine	58	165	73	152

Doxepin	58	178	165	219
Doxylamine	58	71	167	200
EDDP (Methadone metabolite) - 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine	277	262	220	165
Fluoxetine	44	104	91	309
Hydroxybupropion	44	116	139	224
Hydroxyzine	201	299	165	374
Imipramine	58	234	193	280
Ketamine	180	209	182	152
Lamotrigine	185	255	123	157
Meperidine	71	103	172	247
Meprobamate	83	55	71	144
Methadone	72	165	91	309
Methorphan	271	59	150	214
Methylenedioxyamphetamine (MDA)	44	136	77	179
Methylenedioxymethamphetamine (MDMA)	58	135	77	193
Methylphenidate	84	91	56	172
Mirtazapine	195	43	194	265
Modafinil (Artifact)[2]	167	152	165	115
Nordoxepin	44	165	178	222
Norketamine	166	168	131	195
Norquetiapine	227	210	239	295
Nortriptyline	44	202	189	215
O-Desmethyltramadol	58	249	121	93
O-Desmethylvenlafaxine	58	120	107	165
Olanzapine	242	229	213	312
Oxcarbazepine	180	209	252	152
Paroxetine	44	192	138	329
Phenobarbital	204	117	115	232
Phenytoin	180	104	77	252
Promazine (ISTD)	58	284	198	86
Promethazine	72	180	198	284
Propofol	163	178	91	117
Quetiapine	210	239	144	321
Sertraline	274	159	276	304
Topiramate (Breakdown)	43	324	59	110
Topiramate (Artifact)	245	229	127	171
Tramadol	58	263	77	135

Trazodone	205	70	138	371
Venlafaxine	58	134	91	179
Zolpidem	235	219	307	92

[1] See limitations #3
[2] See note #5

PCP Panel

Drug Name	**Major Ions**			
Phencyclidine (PCP)	200	242	91	84

Reference collection sources include but are not limited to Certified Reference Materials and In-House Reference Collections created using Certified Reference Materials traceable to National or International Standards. Additional sources such as *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) may also be referred to for comparisons.

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. When a drug found on another class-specific panel (see appendix XI: Drug Panel) is detected via the full panel drug screen, the class-specific confirmation procedure must be utilized for the confirming test unless Supervisor or Technical Leader approval is granted.

3. This method can be retention time locked to promazine so pressure and flow rate are subject to change for each instrument.

4. When panel drugs are detected in samples that are not present in the control, a library comparison spectrum will be included in the notes packet.

5. Modafinil artifact elutes at ~21.17 min (HP-1 column) or 22.68 min (HP-5 column) when retention time locked to promazine.

LIMITATIONS

1. This procedure was validated per research projects 2023-06 and 2023-16.

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.

3. Carbamazepine will not be reported in samples where both 10-hydroxy Carbamazepine and Carbamazepine are identified.[3]

REPORT WORDING

Refer to Appendix I.

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3. Garg, U, Johnson, L, et.al. "False-Positive Carbamazepine Results by Gas Chromatography-Mass Spectrometry and VITROS 5600 Following a Massive Oxcarbazepine Ingestion," *Journal of Applied Laboratory Medicine*, Volume 3, Issue 1, July 2018, 135-139.

ILLINOIS STATE POLICE

TOXICOLOGY PROCEDURES MANUAL

PROTOCOL: Qualitative Drug Screening

METHOD: Gas Chromatography-Mass Spectrometry

PROCEDURE: **DRUG SCREEN FOR URINE**

Reviewed by:

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Approved by:

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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.

This extraction is utilized for both the general drug screen in urine, as well as the targeted confirmation of PCP. Cases will be evaluated appropriately per the Toxicology Analytical Scheme and will utilize the positive control and panel list below. Cases that require both a general drug screen and are PCP positive on EMIT will utilize only the general drug screen task. Cases that are only PCP positive on EMIT will utilize only the PCP task.

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)
2. **Blank Urine** for controls.
3. **Stock Standards.** (Cerilliant). 1.0 mg/mL of drug in methanol.
4. **Methanol, HPLC Grade** (Fisher Scientific Co.)
5. **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.
6. **Drug Screen Positive Control Working Standard.** 2 µg/mL of diphenhydramine; 5 µg/mL of methadone, amitriptyline, and phencyclidine; 50 µg/mL butalbital and zolpidem. Dilute 20 µL of each stock standard targeted at 2 µg/mL; 50 µL of each stock standard targeted at 5 µg/mL; and 500 µL of each stock standard targeted at 50 µg/mL together to 10 mL with methanol. Alternative stock standards prepared at appropriate concentrations may be used if necessary
7. **Hexanes**, **Certified A.C.S. Grade.** (Fisher Scientific Co.)
8. **Ethanol**, **200 Proof.** (Aaper Alcohol Co.).
9. **Hexanes:Ethanol (1:1).** Measure equal parts of Hexanes and Ethanol and mix

MINIMUM STANDARDS & CONTROLS

Refer to Appendix II.

Controls:

1. In-house positive urine control. Spike 5 mL blank urine with 75 µL of the positive control working standard

2. Blank urine for negative control.

INSTRUMENTATION

Gas Chromatography/Mass Spectrometry

Instrument Conditions (Note #2):

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.

Full Panel Drug Screen (Note #1)

Drug Name	**Major Ions**			
10-hydroxy Carbamazepine[1] (Oxcarbazepine metabolite)	193	180	210	167
Amitriptyline	58	202	215	189
Bupropion	44	100	111	224
Butalbital	168	41	167	124
Carbamazepine[1]	193	236	165	139
Carbamazepine 10,11-epoxide (Carbamazepine metabolite)	180	252	152	223
Carisoprodol	55	97	158	245
Chlorophenylpiperazine (mCPP)	154	196	111	138
Chlorpheniramine	203	167	58	72
Citalopram	58	238	208	324
Clonidine	229	194	172	124
Cocaethylene	82	196	94	317
Cocaine	82	182	94	303
Cyclobenzaprine	58	215	202	189
Desipramine	234	193	208	266
Desmethylcitalopram	44	238	208	310
Diphenhydramine	58	165	73	152
Doxepin	58	178	165	219
Doxylamine	58	71	167	200
EDDP (Methadone metabolite) - 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine	277	262	220	165
Fentanyl	245	146	189	202
Fluoxetine	44	104	91	309
Guaifenesin	124	109	198	81
Hydroxybupropion	44	116	139	224
Hydroxyzine	201	299	165	374
Imipramine	58	234	193	280
Ketamine	180	209	182	152
Lamotrigine	185	255	123	157
Levetiracetam	126	69	41	170

Meperidine	71	103	172	247
Meprobamate	83	55	71	144
Methadone	72	165	91	309
Methorphan	271	59	150	214
Methylenedioxyamphetamine (MDA)	44	136	77	179
Methylenedioxymethamphetamine (MDMA)	58	135	77	193
Methylphenidate	84	91	56	172
Mirtazapine	195	43	194	265
Modafinil (Artifact)[2]	167	152	165	115
Nordoxepin	44	165	178	222
Norfentanyl	83	159	175	232
Norketamine	166	168	131	195
Norquetiapine	227	210	239	295
Nortriptyline	44	202	189	215
O-Desmethyltramadol	58	249	121	93
O-Desmethylvenlafaxine	58	120	107	165
Olanzapine	242	229	213	312
Oxcarbazepine	180	209	252	152
Paroxetine	44	192	138	329
Phenytoin	180	104	77	252
Promazine (ISTD)	58	284	198	86
Promethazine	72	180	198	284
Propofol	163	178	91	117
Quetiapine	210	239	144	321
Sertraline	274	159	276	304
Topiramate (Breakdown)	43	324	59	110
Topiramate (Artifact)	245	229	127	171
Tramadol	58	263	77	135
Trazodone	205	70	138	371
Venlafaxine	58	134	91	179
Zolpidem	235	219	307	92

[1] See limitations #3
[2] See note #4

PCP Panel

Drug Name	**Major Ions**			
Phencyclidine (PCP)	200	242	91	84

Reference collection sources include but are not limited to Certified Reference Materials and In-House Reference Collections created using Certified Reference Materials traceable to National or International Standards. Additional sources such as *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) may also be referred to for comparisons.

REPORT WORDING

Refer to Appendix I.

NOTES:

1. When a drug found on another class-specific panel (see appendix XI: Drug Panel) is detected via the full panel drug screen, the class-specific confirmation procedure must be utilized for the confirming test unless Supervisor or Technical Leader approval is granted.

2. This method can be retention time locked to promazine, so pressure and flow rate are subject to change for each instrument.

3. When panel drugs are detected in samples that are not present in the control, a library comparison spectrum will be included in the notes packet.

4. Modafinil artifact elutes at ~21.17 min (HP-1 column) or 22.68 min (HP-5 column) when retention time locked to promazine.

LIMITATIONS

1. This procedure was validated per research projects 2023-05 and 2023-17.

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

3. Carbamazepine will not be reported in samples where both 10-hydroxy Carbamazepine and Carbamazepine are identified.[5]

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.

5. Garg, U, Johnson, L, et.al. "False-Positive Carbamazepine Results by Gas Chromatography-Mass Spectrometry and VITROS 5600 Following a Massive Oxcarbazepine Ingestion," *Journal of Applied Laboratory Medicine*, Volume 3, Issue 1, July 2018, 135-139.

ILLINOIS STATE POLICE

TOXICOLOGY PROCEDURES MANUAL

PROTOCOL: Qualitative Analysis

METHOD: Gas Chromatography/Mass Spectrometry

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

Reviewed by:

_____

Casey Craven, 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: 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).** (Campbell Science).
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** (Cerilliant) 1 mg/mL standard.
7. **GHB-D6** (Cerilliant) 1 mg/mL. Internal standard.
8. **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. 10 µL of GHB standard (1 mg/mL) added to 1 mL DI water.

2. DI 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, negative control, and each case sample.

Sample preparation:

1. Place 1 mL of the positive control, negative control, and case 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 #1):

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 Certified Reference Materials and In-House Reference Collections created using Certified Reference Materials traceable to National or International Standards. Additional sources such as *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) may also be referred to for comparisons.

Notes:

1. This procedure will be used as a second test for the GHB Quantitative Screen for Urine when indicated.

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-Butyrolactone (GBL), p.33.

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. **Temazepam Glucuronide Stock Standard**. (Cerilliant) 100 µg/mL solution.
6. **Positive Control Working Standard**. 32 µg/mL of alpha-hydroxyalprazolam, lorazepam, diazepam, nordiazepam, oxazepam, temazepam glucuronide, midazolam, and alpha-hydroxymidazolam; 64 µg/mL of alprazolam, clonazepam and flualprazolam; 128 µg/mL 7-aminoclonazepam. Dilute 320 µL of each non-glucuronide stock standard targeted at 32 µg/mL; 640 µL of each stock standard targeted at 64 µg/mL; 1.28 mL of 7-aminoclonazepam; and 3.2 mL temazepam glucuronide together to 10 mL with methanol, or appropriate alternatives at appropriate concentrations.
7. **Sodium Acetate Trihydrate, Certified ACS Grade** (Fisher Scientific).
8. **Glacial Acetic Acid** (Fisher Scientific).
9. **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.
10. **n-Butyl Chloride, Certified ACS Grade** (Fisher Scientific).
11. **Prazepam Stock Internal Standard.** (Cerilliant). Prazepam in methanol, 1.0 mg/mL.
12. **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.
13. **Methanol, Certified A.C.S. Grade.** (Fisher Scientific Co.).

MINIMUM STANDARDS & CONTROLS

Refer to Appendix II

Controls:

1. In-house positive urine control. Spike 4 mL blank urine with 75 µL of positive control working standard.

2. Blank urine for negative control.

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 derivatize)	**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 Certified Reference Materials and In-House Reference Collections created using Certified Reference Materials traceable to National or International Standards. Additional sources such as *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) may also be referred to for comparisons.

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)
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.
9. **Stock Standards.** (Cerilliant) 1.0 mg/mL solutions of benzodiazepines in methanol.
10. **Positive Control Working Standard.** 24 µg/mL of alpha-hydroxyalprazolam, lorazepam, diazepam, nordiazepam, oxazepam, temazepam, midazolam, clonazepam, and alpha-hydroxymidazolam; 48 µg/mL of alprazolam, 7-aminoclonazepam, and flualprazolam. Dilute 240 µL of each stock standard targeted at 24 µg/mL and 480 µL of each stock standard targeted at 48 µg/mL together to 10 mL with methanol, or appropriate alternatives at appropriate concentrations.

MINIMUM STANDARDS & CONTROLS

Refer to Appendix II.

Controls:

1. In-house positive blood control. Spike 2 mL blank blood with 25 µL of positive control working standard.

2. Blank blood for negative control.

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 the negative control, positive control, and cases.

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 1-2 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 Certified Reference Materials and In-House Reference Collections created using Certified Reference Materials traceable to National or International Standards. Additional sources such as *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) may also be referred to for comparisons.

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.

4. Bovine and porcine blood sources showed evidence of significant endogenous interference during method validation/verification. It is recommended that controls are not prepared using these matrices.

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.

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. **Stock Standards**. (Cerilliant). 1.0 mg/mL of benzoylecgonine, cocaine, and cocaethylene in methanol.
3. **Positive Control Working Standard**. 30 µg/mL of cocaine, cocaethylene and benzoylecgonine. Dilute 300 µL of each stock standard together to 10 mL with methanol, or appropriate alternatives at appropriate concentrations.
4. **Solid Phase Extraction Tubes** (PHENOMENEX Strata-X-Drug B)
5. **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).
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 4ºC in glass.
7. **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.
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**. 40 µg/mL in methanol. Add 400 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. In-house positive urine control (450 ng/mL). Spike 5 mL blank urine with 75 µL of positive control working standard.

2. Blank urine for negative control.

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 negative control, positive control and each of thecases.

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.

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 Certified Reference Materials and In-House Reference Collections created using Certified Reference Materials traceable to National or International Standards. Additional sources such as *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) may also be referred to for comparisons.

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*

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; minimum of 1.0 mL required.

Reagents:

1. **Blank Blood**.
2. **Cocaine, Cocaetheylene and Benzoylecgonine Stock Standards**. (Cerilliant). 1.0 mg/mL of cocaine, cocaethylene and benzoylecgonine in methanol.
3. **Positive Control Working Standard**. 30 µg/mL of cocaine, cocaethylene and benzoylecgonine. Dilute 300 µL of each stock standard together to 10 mL with methanol, or appropriate alternatives at appropriate concentrations.
4. **Solid Phase Extraction Tubes** (PHENOMENEX Strata-X-Drug B)
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**. 40 µg/mL in methanol. Add 400 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 blood control (300 ng/mL). Spike 1 mL blank blood with 10 µL of positive control working standard.

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 eachcase.

Treat all tubes as follows:

1. Sample Pretreatment
   To 1 mL of blood add 15 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 2 µL on to the GC/MS.

Interpretation of Results:

Drug Name (* denotes drug does not derivatize)	**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 Certified Reference Materials and In-House Reference Collections created using Certified Reference Materials traceable to National or International Standards. Additional sources such as *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) may also be referred to for comparisons.

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.

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). 1 mg/mL in methanol. (Cerilliant.)
2. **Working THC-COOH Solution – 1.0 µg/mL.** Dilute 50 µL of standard (1 mg/mL THC-COOH) to 50 mL with methanol in a volumetric flask. Store in a **silanized** container and 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 Positive Control, 50 ng/mL**. Add 0.25 mL of working THC-COOH solution to 4.75 mL blank urine.

2. **Blank urine 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:	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 case 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 Certified Reference Materials and In-House Reference Collections created using Certified Reference Materials traceable to National or International Standards. Additional sources such as *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) may also be referred to for comparisons.

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. The 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:

_____

Casey Craven, 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)
2. **Bis(trimethylsilyl)trifluoroacetamide/1% Trimethylchlorosilane (BSTFA with 1% TMCS).** (Campbell Science or equivalent).
3. **Nalorphine Stock Internal Standard.** (Millipore Sigma). Nalorphine in methanol made by diluting 5 mg of Nalorphine powder in 5 mL of methanol for a final concentration of 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 #3. **Warning: Prepare in hood. Do not breathe fumes.**
10. **Methanol, HPLC Grade**. (Fisher Scientific).
11. **Stock Standards.** (Cerilliant). Solutions of the appropriate opiates in methanol, 1.0 mg/mL as needed. (Codeine, Morphine, etc.)
12. **Positive Control Working Standard.** 12 µg/mL of morphine, codeine, 6-monoacetylmorphine and dihydrocodeine; 24 µg/mL of oxycodone and oxymorphone; and 72 µg/mL hydrocodone. Dilute 120 µL of morphine, codeine, 6-monoacetylmorphine and dihydrocodeine; 240 µL of oxycodone and oxymorphone; and 720 µL hydrocodone of stock standard together to 10 mL with methanol. Alternative stock standards prepared at appropriate concentrations may be used if necessary.

MINIMUM STANDARDS & CONTROLS

Refer to Appendix II

Controls:

1. In-house positive blood control. Spike 2 mL of blank blood with 25 µL of positive control working standard.
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:	160°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 each control 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.
Evaporate to dryness under N_2 using water bath at 40°C.

Derivatization (Note #1)

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.

Results:

Drug Name (Note #2)	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 Certified Reference Materials and In-House Reference Collections created using Certified Reference Materials traceable to National or International Standards. Additional sources such as *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) may also be referred to for comparisons.

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 these products.

2. When panel opiates are detected in samples that are not present in the control or are identified using derivative forms not detected in the control, a library comparison spectrum will be included in the notes packet.

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.

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.

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.* 1991, 15, 293-298.

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.

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

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

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

ILLINOIS STATE POLICE

TOXICOLOGY PROCEDURES MANUAL

PROTOCOL: Confirmations

METHOD: Opiates

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

Reviewed by:

_____

Casey Craven, 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)
2. **β-Glucuronidase (Abalone**). (Campbell Science), approximately 1 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. **Positive Control Working Standard.** 60 µg/mL of codeine, dihydrocodeine, oxycodone, and hydrocodone; 80 µg/mL hydromorphone and oxymorphone; and

120 µg/mL morphine glucuronide. Dilute 600 µL of codeine, dihydrocodeine, oxycodone, and hydrocodone; 800 µL of hydromorphone and oxymorphone; and 1.2 mL morphine glucuronide stock standards together to 10 mL with methanol. Alternative stock standards prepared at appropriate concentrations may be used if necessary.

8. **Nalorphine Stock Internal Standard.** (Millipore Sigma). Nalorphine in methanol made by diluting 5 mg of Nalorphine powder in 5 mL of methanol for a final concentration of 1.0 mg/mL.
9. **Nalorphine Working ISTD.** 100 µg/mL nalorphine. Dilute 1 mL of stock to 10 mL with methanol.

MINIMUM STANDARDS & CONTROLS

Refer to Appendix II

Controls:

1. In-house positive urine control. Spike 5 mL of blank urine with 75 µL of positive control working standard.

2. Blank urine for negative control.

INSTRUMENTATION

Gas Chromatograph/Mass Spectrometer

Instrument Conditions:

Column:	HP-5MS Ultra Inert or HP-1MS 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 samples and positive and negative controls.
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° 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 (Note #2)	**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 Certified Reference Materials and In-House Reference Collections created using Certified Reference Materials traceable to National or International Standards. Additional sources such as *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) may also be referred to for comparisons.

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.
2. When panel opiates are detected in samples that are not present in the control or are identified using derivative forms not detected in the control, a library comparison spectrum will be included in the notes packet.

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.

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 *SMA Confirmation for Urine.*

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: 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.** (PHENOMENEX Strata-X-Drug B)
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. **Positive Control Working Standard**. 30 µg/mL of amphetamine, methamphetamine, MDA, MDMA. Dilute 300 µL of each stock standard together to 10 mL with methanol, or appropriate alternatives at appropriate concentrations.
14. **N-Propylamphetamine Standard**. 1 mg/mL N-propylamphetamine. 5 mg of N-propylamphetamine hydrochloride dissolved in 5 mL of methanol.
15. **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 positive blood control (300 ng/mL). Spike 1 mL blank blood with 10 µL of positive control working standard.

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 Certified Reference Materials and In-House Reference Collections created using Certified Reference Materials traceable to National or International Standards. Additional sources such as *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) may also be referred to for comparisons.

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:

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Casey Craven, 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 *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, 1 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)
3. **Hexanes, HPLC Grade.** (Fisher Scientific Co.).
4. **Heptafluorobutyric anhydride (HFBA).** (Sigma-Aldrich).
5. **Ethyl Acetate.** (Fisher Scientific).
6. **Stock Standards**. (Cerilliant) 1.0 mg/mL solutions of SMAs in methanol.
7. **Positive Control Working Standard**. 30 µg/mL of amphetamine, methamphetamine, MDA, MDMA. Dilute 300 µL of each stock standard together to 10 mL with methanol. Alternative stock standards prepared at appropriate concentrations may be used if necessary
8. **N-Propylamphetamine Standard.** 1 mg/mL N-propylamphetamine. 5 mg of N-propylamphetamine hydrochloride dissolved in 5 mL of methanol.
9. **N-Propylamphetamine Working ISTD.** 50 µg/mL N-propylamphetamine. Dilute 500 µL of 1 mg/mL N-propylamphetamine standard to 10 mL with methanol.
10. **Ethanol**, **200 Proof.** (Aaper Alcohol Co.).
11. **Hexanes:Ethanol (1:1).** Measure equal parts of Hexanes and Ethanol and mix

MINIMUM STANDARDS & CONTROLS

Refer to Appendix II.

Controls:

1. In-house positive urine control (900 ng/mL). Spike 1 mL blank urine with 30 µL of positive control working standard.

2. Blank urine for negative control (Note #1).

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,
Scan Range:	40 - 420 m/z

PROCEDURE OR ANALYSIS

1. Briefly shake extraction tube.
2. Add 1 mL urine and 50 µL 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 100 µL of hexane:ethanol (1:1) and inject 1 µL on GC/MS with a blank solvent injection between each sample (Note #1).

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.

Notes:

1. A solvent or extracted blank (no internal standard) must be run prior to every case sample as an additional quality control measure to monitor carryover from highly concentrated samples. If no carryover is detected in the blank, the results in the following case sample can be reported. If carryover is detected in the blank, then data from the following case sample must be rejected and reanalyzed. Re-injection on a different instrument may be suitable for reanalysis if all carryover parameters are met.
2. Every case should have 4 components: Positive Control, Negative Control, Case Blank, and Case Sample images. The following format should be used for SMA Confirmation in Urine instrument sequences:

QC UR POS SMA (Scanned from Worklist)
QC UR NEG SMA (Scanned from Worklist)
Scanned Case # BLANK (Blank typed manually)
Scanned Case #
Scanned Case #2 BLANK (Blank typed manually)
Scanned Case #2
*more lines as needed

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.

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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: Triple Quadrupole Mass Spectrometry

PROCEDURE: **THC QUANTITATION FOR BLOOD**

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 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 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. **Δ^9-THC, Δ^9-THC-COOH Control Intermediate Working Standard.** THC 10.0 µg/mL in methanol, and THC-COOH 20.0 µg/mL in methanol. Add 500 µL of THC and 1000 µL THC-COOH stock solutions to a 50 mL volumetric flask and q.s. to 50 mL with methanol.
7. **Deuterated Δ^9-THC Stock Internal Standard** (Cerilliant). Δ^9-THC D_3 in methanol, 100 µg/mL.
8. **Deuterated Δ^9-THC-COOH Stock Internal Standard** (Cerilliant). Δ^9-THC-COOH D_3 in methanol, 100 µg/mL.
9. **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.
10. **Acetonitrile, HPLC Grade.** (Fisher Scientific).
11. **Hexanes, HPLC Grade.** (Fisher Scientific).
12. **Ethyl Acetate.** (Fisher Scientific).
13. **Extraction Solvent.** 90% Hexanes/10% Ethyl Acetate.
14. **Acetic Acid, 10%.** Add 30 mL glacial acetic acid to 270 mL DH_2O.
15. **Formic Acid, 0.1% in DH_2O.** (Fisher Scientific)
16. **Formic Acid, 0.1% in Methanol.** (Fisher Scientific)
17. **Methanol, HPLC Grade.** (Fisher Scientific)
18. **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).
19. **Δ^8-THC Stock Standard** (Cerilliant). 1.0 mg/mL.
20. **11-nor-9-carboxy-Δ^8-THC Stock Standard.** (Cayman Chemical or Cerilliant). 1.0 mg/mL.

21. **Δ^8-THC Combined Intermediate Standard.** 10 ug/mL. Add 500 µL of Δ^8-THC Stock Standard and 500 µL of 11-nor-9-carboxy-Δ^8-THC Stock Standard to a 50 mL volumetric flask and q.s. to 50 mL with methanol.
22. **Δ^8 Working Injection Standard,** 10ng/mL. Add 100 µL of Δ^8-THC combined intermediate standard and 2 mL of deuterated Δ^9-THC, Δ^9-THC-COOH working internal standard to a 100 mL volumetric flask and q.s. with mobile phase.
22. **Reconstitution Solvent** (starting 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, 5 ng/mL of Δ^9-THC and 10 ng/mL Δ^9-THC-COOH or other appropriate control. Prepare by adding 125 µL of the Control Intermediate Working Standard (#6 above) into 250 mL of blank blood. Mix at least 1 hour using a stir bar. NOTICE: Drug standards used to prepare the Control 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.

At least 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

Instrument:	Agilent Ultivo LC/TQ (Ultivo)
Autosampler temp:	10 °C
LC Column:	Phenomenex Kinetex PS C18 100Å, 2.1x50mm, 2.6µm
LC Guard Column:	InfinityLab Poroshell 120 Stablebond C18, 2.1x5mm, 2.7µm
Flow:	0.600 mL/minute
Mobile Phase:	Methanol with 0.1% formic acid : Water with 0.1% formic acid
Gradient:	60:40 at 0.00 minutes 70:30 at 1.00 minutes 70:30 at 2.50 minutes 80:20 at 4.00 minutes 80:20 at 5.00 minutes 100:0 at 5.01 minutes 100:0 at 6.5 minutes 60:40 at 6.51 minutes

	End runtime 9 minutes
Column Compartment:	50°C
Ionization:	Electrospray - Positive Mode

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 reconstitution solvent.
12. Inject 10 µL of the System Suitability Standard, the Δ^8 Working Injection Standard, and all extracted samples into the LC/QQQ.

Evaluation in Mass Hunter and Calculations (**Note #5, #6, #7**):

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. Relative 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 relative retention time is within 2% of the system suitability standard.
    c. Calculated concentration is 2.0 ng/mL or greater.
    d. Δ^8-THC and 11-nor-9-carboxy-Δ^8-THC injection standards relative retention times must be outside 2% of the system suitability standard.

2. Quantitative
    a. R-squared value for the curve must be $\geq$ 0.99. (Not required for Δ^9-THC-COOH)
    b. No less than five calibrators will be used for Δ^9-THC curve evaluation. A point for Δ^9-THC may be dropped and must be recorded in the worklist notes notating who dropped the point, when, and why by utilizing the appropriate comment in the "task comments for all tasks" task option (**Note #8**). The following are examples for why a calibration point may be dropped:

i. Failure to meet identification criteria listed in quality control section 1 (a-d)
ii. Poor chromatography
iii. Mis-spike of the calibrator
iv. Notated error during extraction (E.g. dropping a tube)
v. Dropping of the lowest calibrator will lead to an adjustment of the limit of detection for that run to the next calibrator
vi. Dropping of the highest calibrator will lead to an adjustment of the upper limit of quantitation to the next calibrator (Note #10)

c. 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 ±3σ confidence limit (**Note #9**).
d. Duplicate quantitative results must be within 20% of the mean of the two replicates.

3. Qualitative
   a. Four calibrators can be used for Δ^9-THC-COOH identification. Points may be dropped if any criteria listed in Quality Control 2.b. are met and must be recorded in the worklist notes notating who dropped the point, when, and why by utilizing the appropriate comment in the "task comments for all tasks" task option (**Note #8**).
   b. R-squared value is not strictly evaluated for Δ^9-THC-COOH. However, calibration should demonstrate positive linearity across the range.
   c. The Δ^9-THC-COOH of the control is only evaluated qualitatively (quality control #1 a-d).

4. Case approach
   a. Report Δ^9-THC and/or Δ^9-THC-COOH if either/both meets all identification criteria and are greater than or equal to the 2.0 ng/mL LOD for both replicates.
   b. If Δ^9-THC does not meet identification criteria but Δ^9-THC-COOH does, report Δ^9-THC-COOH. Work on the positive cannabinoid immunoassay is now complete per the toxicology analytical scheme.
   c. If neither Δ^9-THC or Δ^9-THC-COOH meet identification criteria (or they are below the LOD) in either replicate, work on the positive cannabinoid immunoassay is complete per the toxicology analytical scheme. **Note #5**
   d. If RRT of either Δ^8-THC or Δ^8-THC-COOH are matched with a peak within RRT identification criteria, add a task note "indication of Δ^8-THC or Δ^8-THC-COOH". They cannot be reported, but the notes packet will reflect the indication since they can cause a positive screen from immunoassay. The task note can be added whether Δ^9-THC or Δ^9-THC-COOH are reported or not. Work on the positive cannabinoid immunoassay is now complete per the toxicology analytical scheme.

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 of Δ^9-THC are below 2.0 ng/ml, the result is reported as "No Delta-9-Tetrahydrocannabinol (THC) detected".
   b. If one (1) of the two (2) analyzed replicates of Δ^9-THC is quantitatively below 2.0 ng/mL, the result is reported as "No Delta-9-Tetrahydrocannabinol (THC) detected".
   c. If Δ^9-THC-COOH does not meet identification criteria or is below the 2.0 ng/mL LOD, the result does not appear on the report.
   d. If Δ^9-THC-COOH meets identification criteria and is above the 2.0 ng/mL LOD, it will be reported in the blood as "Delta-9 Carboxy THC (THC metabolite) 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.
8. A calibrator may not be dropped purely to make a QC value better fit.
9. A fresh spiked control can be used if a pooled control is unavailable or trending outside of the ±3σ confidence limit. The fresh spiked control should be spiked from a working standard not used for the calibration curve and be within ±20% of the target value.
10. If the highest calibrator was dropped for failing to meet any criteria listed in section quantitative 2 b and one or both of the replicates are above the next highest calibrator, both the worksheet and the report will require manual entry of "Delta-9 Tetrahydrocannabinol (THC) greater than XX.X ng/mL", where "XX.X" is the quantitative value of the replicate below the next highest calibrator or the next highest calibrator if both replicates are above that value.

REPORT WORDING

Refer to Appendix I.

LIMITATIONS

1. This procedure was validated per research projects 2020-11, 2021-06, and 2021-03.

2. Samples remain stable for 7 days in the chilled autosampler. Any samples older than 7 days or not stored in the chilled autosampler must be re-extracted.

REFERENCES

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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:

_____
Casey Craven, 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 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. Dilute to 1000 mL with distilled water. Mix. Store at room temperature in glass or plastic.
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** (20 mg/L). Dilute 1 mL of GHB-D_6 stock solution into a 50 mL volumetric flask with methanol.
9. **GHB Stock Standard** (Cerilliant), GHB in methanol, 1.0 mg/mL.
10. **Deionized Water.** (DH_2O)
11. **Mobile Phase.** Formic Acid, 0.1% in DH_2O. (Fisher Scientific)
12. **Mobile Phase.** Formic Acid, 0.1% in Methanol. (Fisher Scientific)
13. **Reconstitution 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 40 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 4 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, 2 mg/L – 25 mg/L (preparation below).

Preparation:

Label 10 mL screw-capped tubes for each calibrator and control. The control and calibrators are prepared in 10 mL quantities. Performing a single extraction consumes 200 µL of these calibrators and control. Prepare the calibrators and control as follows using certified 10 mL volumetric flasks:

Tube	Water	GHB Stock Standard	Final Concentration (mg/L)
1	10 mL	20 µL	2.0
2	10 mL	50 µL	5.0
3	10 mL	100 µL	10.0
4	10 mL	150 µL	15.0
5	10 mL	200 µL	20.0
6	10 mL	250 µL	25.0
7 (Control)	10 mL	100 µL of alternate stock	10.0

INSTRUMENTATION

Liquid Chromatograph/Mass Spectrometer

Instrument Conditions:

Instrument:	Agilent Ultivo LC/TQ
Autosampler Temp:	10°C
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)

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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	200 µL Control	50 µL
9 and 10	200 µL of Case Sample	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 reconstitution mobile phase (95:5) prior to injection.
7. Inject 5 µL into the LC/MS.

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 and apply method from existing file" and apply the appropriate processing method to the batch (example: GHB Quant).
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. Relative 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. A sample report with the system suitability used to update the ion ratios and retention times will be included in the case file for all cases analyzed.
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. 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.

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 relative retention time is within 2% of the system suitability standard.
    c. The calculated concentration is 2.0 mg/L or greater.

2. Quantitative
    a. R-squared value for the curve must be ≥ 0.99.
    b. No less than five calibrators will be used for GHB curve evaluation. A point may be dropped and must be recorded in the worklist notes notating who dropped the point, when, and why by utilizing the appropriate comment in the "task comment for all tasks" task option. The following are examples for why a calibration point may be dropped.
        i. Failure to meet identification criteria listed in quality control section 1 (a-c)
        ii. Poor chromatography
        iii. Mis-spike of the calibrator
        iv. Notated error during extraction (e.g. dropping a tube)
        v. Dropping of the lowest calibrator will lead to an adjustment of the limit of detection for that run to the next calibrator.
        vi. Dropping of the highest calibrator will lead to an adjustment of the upper limit of quantitation to the next calibrator (note #6)
    c. 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 control value must be within ±20% of the controls target value.
    d. Duplicate quantitative results must be within 20% of the mean of the two replicates.
3. Reporting
    a. In batch results after uploading all reports and quantitative values that meet criteria in quality control #1 and #2, review the "GHB Stats" tab to review the reported range for each case.

b. When the upper limit for measurement uncertainty of a GHB result is greater than or equal to 10.0 mg/L, then a second test must be performed to qualitatively confirm the results. The second test should be procedure TX-IID-4 "GHB Confirmation for Urine Using BSTFA" utilizing a GC/MS.
c. When the upper limit for measurement 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.
d. When the lower limit for measurement uncertainty of a GHB result is less than 10.0 mg/L but within the calibration range, a statement will be automatically added to the report notating that "Urinary GHB concentrations less than 10 mg/L cannot be distinguished from endogenous concentrations."
e. If GHB can not be reported due to the sample being unsuitable for analysis (limitations #4), add "The sample condition is unsuitable for GHB analysis using the approved ISP procedure" as a task comment in batch 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 or fail identification criteria in quality control #1, then GHB will be considered not detected and no additional 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 be listed as "not reported" in the worksheet. Calculated values will be included in the notes packet on the worksheet, but no additional report wording will be necessary outside of the report statement listed in quality control section 3d.
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 utilizing procedure TX-IID-4 "GHB Confirmation for Urine Using BSTFA", then the average of the duplicate analyses will be reported as the calculated concentration. The GHB result in the worklist associated with the TX-IID-4 batch must have a status of "FINAL" for all quantitative results to appear on the report.
    a. A measurement uncertainty statement will be added to the report remarks when the above criteria is met.
    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 will still be included on the worksheet.

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.
6. If the highest calibrator was dropped for failing to meet any criteria listed in section quantitative 2 b and one or both of the replicates are above the next highest calibrator, both the worksheet and report will require manual entry of “Gamma-Hydroxybutyric Acid (GHB) greater than XX.X mg/L”, where “XX.X” is the quantitative value of the replicate below the next higher calibrator or the next highest calibrator if both replicates are above that value.

LIMITATIONS

1. This procedure was validated per research project 2020-15.
2. Extracted samples remain stable for 7 days in the chilled autosampler. Any samples older than 7 days or not stored in the chilled autosampler must be re-extracted.
3. Un-extracted calibrators or controls have not been evaluated for long term stability. If a calibration curve or control do not pass the stock calibrators and controls should be re-made.
4. Some samples may not be suitable for analysis. If the internal standard fails identification criteria for a case sample in one or both replicates, that case should be marked “reject” in batch results and the extraction will be repeated. If the internal standard fails for a case sample on a second extraction attempt then the procedure will not be repeated and the wording in quality control 3e above will be utilized on the worksheet. All required paperwork will still be uploaded for both extractions.

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ILLINOIS STATE POLICE

TOXICOLOGY PROCEDURES MANUAL

APPENDIX I: REPORT WORDING

Reviewed by:

_____
Casey Craven, Chairperson
Toxicology Command Advisory Board

Approved by:

_____
Timothy Tripp
Bureau Chief - Toxicology Command Coordinator

APPENDIX I

REPORT WORDING

This appendix contains the toxicology section's guidelines for report wording. In general, report wording is populated using the data entry fields within LIMS. However, format modifications may be necessary to present the evidence submitted, testing performed, or analytical findings accurately.

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

What, if any, drugs and/or volatiles are detected?

If quantitations are performed, what are the concentrations of these drugs and/or volatiles, including a measurement uncertainty statement as needed?

TOXICOLOGY REPORTING STATEMENTS

I. REPORT FINDINGS

A. POSITIVE IDENTIFICATION
1. Qualitative: (substance) detected
2. Quantitative: (substance) _____ wt/vol (g/dL, ug/L, mg/L, ng/mL etc.).
3. Below quantitation limit (excluding GHB): No (substance) detected
4. Both replicates above quantitation limit: (substance) greater than (higher quantitation limit)
5. One replicate above quantitation limit and one below: (substance) greater than (replicate concentration on the curve)

B. NEGATIVE IDENTIFICATION
1. "No (substance/class) detected"
2. "No panel drugs detected"

C. PRELIMINARY TESTING
1. Preliminary screening results were positive for the following classes: (list classes that were positive).

II. REPORT REMARKS

A. VOLATILE REPORT

1. Volatile analysis of this case is limited to the following: ethanol, methanol, acetone, and isopropanol.
2. Analysis has been limited to volatiles only. If additional testing is required, a service request for drug testing can be submitted using LIMS Prelog.

B. DRUG REPORT

1. Drug analysis has been limited to the following classes: Amphetamine, Benzodiazepine, Cocaine, Opiate, Phencyclidine (PCP), and Cannabinoid. If additional testing is required, a service request must be submitted using LIMS Prelog.
2. Drug analysis includes the full drug panel and the following classes: Amphetamine, Benzodiazepine, Cocaine, Opiate, Phencyclidine (PCP), and Cannabinoid.

C. CSA REPORT

1. Urine ethanol concentrations do not directly reflect blood ethanol concentrations.
2. Urinary GHB concentration less than 10.0 mg/L cannot be distinguished from endogenous concentrations.
3. Additional analysis was performed to screen for Gamma-Hydroxybutyric Acid (GHB) and Lorazepam.

D. PRELIMINARY TESTING

1. Testing was limited to preliminary screening results due to (example qualifiers listed below). No confirmatory analysis was performed. If confirmatory drug testing is required, please contact the laboratory.
2. Testing was limited to preliminary screening results due to (example qualifiers listed below). Confirmatory analysis is pending and will be issued on a separate report.
    a. Insufficient sample amount
    b. Condition of sample not suitable for analysis (e.g. decomposition)
    c. Instrument needed for analysis out of service

E. SERUM CONVERSION

Per Illinois Administrative Code Title 20 Part 1286.40, the blood serum ethanol concentration divided by 1.18 results in an equivalent whole blood ethanol concentration of 0.XXX g/dL ± 0.XXX g/dL at the 99.73% confidence interval.

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

The estimated uncertainty for the (analysis performed) of item XXX is ___ (unit) ± ___ (unit) at the 99.73% confidence interval.

Example: The estimated uncertainty for the ethanol analysis of item 1A1 is 0.079 g/dL ± 0.007 g/dL at the 99.73% confidence interval.

G. ANALYSIS FEE STATEMENT
The following statement will be added to reports with offense codes – DUI alcohol, DUI drugs, DUI drugs and alcohol, and Aggravated DUI:

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.

H. ISO/IEC 17025:2017 ACCREDITATION STATEMENT
Any analysis conducted is accredited under the *ISO/IEC 17025:2017 - Testing Laboratory* accreditation issued by ANSI National Accreditation Board (ANAB). Refer to certificate #FT-0240 and associated Scope of Accreditation. This report contains the conclusions, opinions and/or interpretations of the analyst(s) whose signature(s) appears on the report as authorization of the results. All testing was performed at the location listed in the header of this document, unless otherwise indicated in the Notes Packet. The "Notes Packet" appendix of this report, available in Prelog, contains detailed information on the method(s) used, date(s) of testing, location(s) of testing and environmental conditions associated with this analysis, if applicable. The results relate only to the items submitted and tested, unless otherwise noted. All evidence submitted to the laboratory will be returned upon completion of all service requests, unless otherwise indicated in the body of the report.

I. ACCOUNTABILITY STATEMENT
I have personally completed this report. Under penalties of perjury, I certify I have examined all of the information provided for this document related to the analysis conducted for this report and, to the best of my knowledge, it is true, correct, and complete.

J. ADDITIONAL REPORT REMARKS
1. The findings for (analysis performed) will be issued on a separate report.
2. The findings for (analysis performed) are pending and will be the subject of a separate report.
3. Analysis consisted of (analysis performed) testing only.
4. Limited testing was performed due to insufficient sample.
5. Sample was consumed over the course of analysis. Permission to consume granted by authorized entity.

6. Analysis has been limited to volatiles only due to insufficient sample. Should additional targeted testing be required, please contact the laboratory for more information on available services.

NOTE: Should the analytical scheme and approved procedures yield a testing result for which currently approved wordings are not appropriate, an appropriate report wording approved by the Technical Leader and/or Command may be utilized.

ILLINOIS STATE POLICE

TOXICOLOGY
PROCEDURES MANUAL

APPENDIX II: MINIMUM STANDARDS & CONTROLS

Reviewed by:

_____
Casey Craven, Chairperson
Toxicology Command Advisory Board

Approved by:

_____
Timothy Tripp
Bureau Chief – Toxicology Command Coordinator

APPENDIX II

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.

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) Positive Identification: 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) Negative Identification : 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. Criteria for qualitative identification.
    a. When quantitative results [2b(2)] are not suitable for use 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 will be reported as "detected".

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 the column types and instrument conditions, such as the detector and inlet temperatures. 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 instrument 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 screening 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. Positive identification of a drug may only be made using a procedure who's associated panel includes that targeted drug.

B. TESTS AND CONTROLS

A set of controls as defined in the respective procedures will be run with each set of samples.

1. Gas Chromatography (GC) and Liquid Chromatography (LC)
    a. GC Identification Criteria:
        (1) Positive Identification: 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) Negative Identification: 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) Positive Identification: Retention time for the analyte of interest must be within criteria as set by its associated procedure.

(2) Negative Identification: Retention time for the analyte of interest is not within criteria as set by its associated procedure.

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 column type and instrument conditions such as temperature gradients, injection volume, and scan range. The instrument and method parameters have been uploaded to LAM.

2. Mass Spectrometry
   a. Positive Identification 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, and available reference collections. The scan range used during data acquisition will be the scan range used for validation. 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. Negative Identification 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”.
      (1) An exception to this is when the panel has been limited by case approach such as PCP or criminal sexual assault cases

that are negative for the Benzodiazepine assay on EMIT and are screened for Lorazepam. In these instances, the Benzodiazepine assay will be notated in batch results as "No Lorazepam" and on the printed spectral image as "No Lorazepam detected" or "No Lorazepam 2TBDMS detected". The PCP assay will be notated in batch results as "No Phencyclidine" and on the printed spectral image as "No Phencyclidine detected".

(2) If data analysis is limited for any other reason than those listed above, there must be an explicit case note that notates what the search was limited to.

d. An image of all panel drugs evaluated for comparison that are listed on the worksheet will be included in the case file (including positively identified and possible panel 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. Naming conventions on positive and possible spectral images must be in accordance to what is found in its associated procedure (for example Nordiazepam would be notated as Nordiazepam TBDMS on the spectral image).

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

3. 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.

4. 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.

(1) Technical leader approval is required for any deviations

from the procedure (e.g., changes in sample or solvent volumes) and will 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 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 batch results and included in the notes packet. (e.g. GCMS system, pipette…etc.)

5. Reagents: Stock Chemicals, Purchased Reagents, and Prepared Reagents (See QM-14 for definitions)

a. LAM will be used to track these assets as they are received, prepared, placed in use, and consumed. Reagent locations will be tracked until the asset is consumed; at which time it will be relocated to the "Disposed Asset" location.

(1) Expired reagents that do not pass "Reauthentication" will also be relocated to the "Disposed Asset" location.

b. Reagents are tested concurrently with casework using quality controls for their reliability per QM-14

c. Stock Chemicals and Purchased Reagents will be added under Work With – Chemical/Purchased Reagent items.

(1) When adding the asset, required fields will be entered under the "Chemical Info" tab, including but not limited to: Asset Type, Asset Name, Lot number, Quantity, Units and Date Received.

(2) When adding the asset, date received and received by will be entered under the "Lab Status Info" tab.

(3) If an expiration date is assigned by the manufacturer, it will be entered under the "Lab Status Info" tab.

(4) If an expiration date is not assigned by the manufacturer, it will be entered as "12/31/9999".

(5) A "re-test" date is not considered an expiration date

(6) For immunoassay kits, a certificate of analysis will be uploaded to each replicate asset in LAM.

(7) Stock Chemicals and Purchased Reagents with expiration dates assigned by the producer cannot be "Reauthenticated" (see QM-14).

d. "Prepared Reagents" will be added under the Work With – Prepared

Reagent items.

(1) When adding the asset, required fields will be entered in the "Identification" section under the "Reagent Info" tab, including but not limited to: Assigned Section, Asset Type, Asset Name, Lot Number, Quantity Prepared, and Units.

(a) Lot number for in house reagents will be the date prepared and should follow the format MMDDYY. Additional criteria may be used if multiple of the same asset are prepared on the same day.

(2) When adding the asset, required fields will be entered in the "Preparation" sections under the "Reagent Info" tab, including but not limited to: Prepared by, Date Prepared, and Expiration Date.

(3) All assets (including equipment, chemicals, and standards) used to prepare the reagent will be scanned and recorded in the "Component" tab. This includes volumes and concentrations of chemicals or standards, as well as balances, volumetric glassware, and pipette(s) used in the preparation.

(4) When the reagent is reauthenticated, a "Tox Asset Reauthentication" template must be added under "Lab Status Info".

(a) "Prepared Reagents" with an expiration date assigned by the laboratory can be "Reauthenticated" (see QM-14).

(b) Utilize this template to record "Reauthentication Result".

(c) Expiration dates can be extended up to one year from the reauthentication date per QM-14.

6. Reference Materials: Purchased (Acquired from Producer) and Internal (see QM-14)

a. LAM will be used to track these assets as they are received, prepared, placed in use, and consumed. Reference material locations will be tracked until the asset is consumed, at which time it will be relocated to the "Disposed Asset" location. Expired reference materials for quantitative use will be disposed of or notated that they are "for Qualitative use only".

b. Purchased reference materials (non-matrix), which are acquired from a producer will be added under Work With – Drug Standard items.

(1) When adding the asset, required fields will be entered under the "Drug Standard Info" tab, including but not limited to: Assigned Section, Drug Type, Standard Name, Lot Number, Quantity Received, Units, Manufacturer, and Date Received.

(2) A certificate of analysis will be uploaded to each drug standard asset.
(3) Reference materials (i.e. drug standards or stock standards) used in a procedure for an analyte reported quantitatively must be obtained from accredited reference material producers.
(4) All drug standards that are not sourced as a certified reference material will be verified by mass spectrometry and documented in LAM by uploading an image of the spectra to the images tab for that drug standard entry.

c. Purchased Reference Materials (Matrix and Mass Spec Tuning Solutions) which are acquired from a producer will be added under Work With – Chemical/Purchased Reagent items.
(1) When adding the asset, required fields will be entered under the "Chemical Info" tab, including but not limited to: Asset Type, Asset Name, and Lot Number.
(2) When adding the asset, date received and received by will be entered under the "Lab Status Info" tab.
(3) If an expiration date is assigned by the manufacturer, it will be entered under the "Lab Status Info" tab. Note: Matrix reference materials (i.e. blood, urine) do not have expiration dates.
(4) If an expiration date is not assigned by the manufacturer it will be entered as "12/31/9999".
(5) A "re-test date" is not considered an expiration date.

d. Internal Reference Materials (prepared in the laboratory/"In-House") will be added under Work With – Prepared Reagent items.
(1) When adding the asset, required fields will be entered in the "Identification" section under the "Reagent Info" tab, including but not limited to: Assigned Section. Asset Type, Asset Name, Lot Number, Quantity prepared, and Units.
(a) Lot number for internal reference materials will be the date prepared and should follow the format MMDDYY. Additional criteria may be used if multiple of the same asset are prepared on the same day.
(2) When adding the asset, required fields will be entered in the "Preparation" section under the "Reagent Info" tab, including but not limited to: Prepared By, Date Prepared, and Expiration Date.
(3) All equipment used to prepare the internal reference material will be scanned and recorded in the "Component" tab. This includes volume and concentrations of chemicals or certified reference materials, as well as balances,

volumetric glassware, and pipette(s) used in the preparation.

(4) Preparation of "Internal Reference Materials" will be verified by a second analyst. Equipment used in the preparation will be reviewed in the "Components" tab. Observation of internal controls will be documented in the "Toxicology Reference Material Record".

(5) All "working drug standards" prepared for quantitations and ELISA calibrators will be stored in the freezer until one of the analytes expires per the manufacturer or up to one year from the date of preparation; whichever is earlier.

(a) Internal standards and System Suitability Standards are considered qualitative and do not expire. Note: Any preparations containing water will be stored in the refrigerator (GHB, LCMSMS system suitability, etc.).

(6) All controls and immunoassay calibrators prepared "In-house" (Internal) must be quality checked before being put into service. The newly prepared control will be analyzed using the appropriate procedure to determine whether it meets acceptance criteria. The QC date and the analyst who performed the quality control are recorded in the "Toxicology Reference Material Record". Additionally, the data results will be attached in the "images" section for the LAM record.

e. Drug standards will not be used past the manufacturer's expiration date for quantitative purposes but may be used qualitatively if the standard meets original defined criteria (i.e. analyte detected).

f. When a reference material is placed in-use, a "Toxicology Reference Material" record must be added.

(1) Record will include: Type, Procedural Use, and Procedure Used for Preparation.

(2) If the reference material is utilized as a control the record will include: Preparation reviewed by, QC Date, QC by, QC Result.

g. All controlled drugs 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) Documentation of the annual inventory will include a document with the 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.

C. QUANTITATIONS

1. Routine quantitations will be performed using suitable methods in the Toxicology Procedures Manual.
2. Technical leader approval is required for any deviations from the procedures outlined in the Toxicology Procedures Manual (e.g., changes in sample or solvent volumes) and should be noted in the notes packet.
3. Appropriate controls and a negative will be analyzed with all quantitations. The use of a fresh spiked control requires prior approval from the Toxicology Technical Leader. Consult each procedure for acceptable controls.
4. QC results will be recorded in the batch results worklist or the notes packet.
5. An estimation of measurement uncertainty (MU) will be determined following guidelines provided in Appendix VI and Appendix VII.
6. A quantitation result must be rejected if the measured value of a spiked control is greater than ±20% of the control's target value unless other criteria is outlined in the respective procedure.
7. When establishing a mean for a pooled control chart, the control will be rejected if the established mean is greater than ±20% of the control's target value.
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. There are three types of controls available to an analyst. Any of these controls are acceptable.

Purchased - Purchased 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.

Internal Reference Controls (Pooled) - 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 also 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.

Internal Reference Controls (Fresh spiked) - When purchased controls and pooled internal reference 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 and must be made on a different day or by another analyst. 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.

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, controls, working standards, and ethanol dilutions. 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
      (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.
   b. 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 stock 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.
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.

a. Each worklist that requires documents to be uploaded in a LAM entry must have a secondary analyst verify that the required documents have been uploaded. A worklist comment will be added stating "LAM documentation upload verified by XX" where XX are the initials of the secondary analyst. This includes opening the file to verify it matches the files name (e.g. the entry for "MS8 012225 tune" is actually the tune printout and not another document.

4. Instrument software may need firmware updates occasionally. A tune and test mix after the update will demonstrate that the instrument is still functioning appropriately, unless more function checks are required based on the scope of the update as deemed necessary by the Technical Leader. Software updates will be recorded in LAM with the appropriate function check records attached.

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. 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 and attached in batch results.

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.
      (2) If the instrument stops running for any reason overnight, a new tune will be performed before any samples are injected. The system suitability, curve, negative, and QC sample will be reinjected alongside any cases that did not inject or require re-injection.
         a. If samples can still be run and do not require re-extraction, the data will not be considered rejected.
         b. A new worklist may be beneficial depending on how many samples did not run on the initial injection date.
   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.

3. Software
   a. Instrument software may need firmware updates occasionally. A tune and system suitability run after the update will demonstrate that the instrument is still functioning appropriately, unless more function checks are required based on the scope of the updates as deemed necessary by the Technical Leader. Software updates will be recorded in LAM with the appropriate function check records attached.

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.
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. Scan each assay kit, reagent, and control used into the worklist in batch results.

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. 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. Scan all assay kits, controls, pipettes and reagents used into the worklist in

batch results.

5. Certificate of analysis sheets will be uploaded to LAM when available.

K. 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.

L. 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”.

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 Appendix IX.

B. If controls or data is rejected, the following information will be recorded in the notes packet

1. The reason for rejection
2. The identity of the person rejecting
3. The date of rejection

Analyst may add any supporting documentation as deemed relevant to their testimony. Examples include the TIC, a sequence log for the rejected batch, and/or extracted ion chromatograms.

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C. 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.

D. 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. The sequence log for LC/TQ instruments must be the worklist report.

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, ethanol controls) and semi-quantitative materials (ie. immunoassay cutoffs/controls/system controls, 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. Purchased standards will be treated as quantitative until their listed expiration date, whereas they may be reauthenticated for use as a qualitative material only.

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.

1. Reference collection sources include but are not limited to Certified Reference Materials and In-House Reference Collections created using Certified Reference Materials traceable to National or International Standards. Additional sources such as *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) may also be referred to for comparisons.

2. Spectral comparisons should be made to a traceable certified reference material. This includes analytes in the positive control traceable to national or international standards or the in-house reference collection comprised of certified reference material standard spectra.

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:

_____
Sean McCarthy, Chairperson
Toxicology Command Advisory Board

Approved by:

_____
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

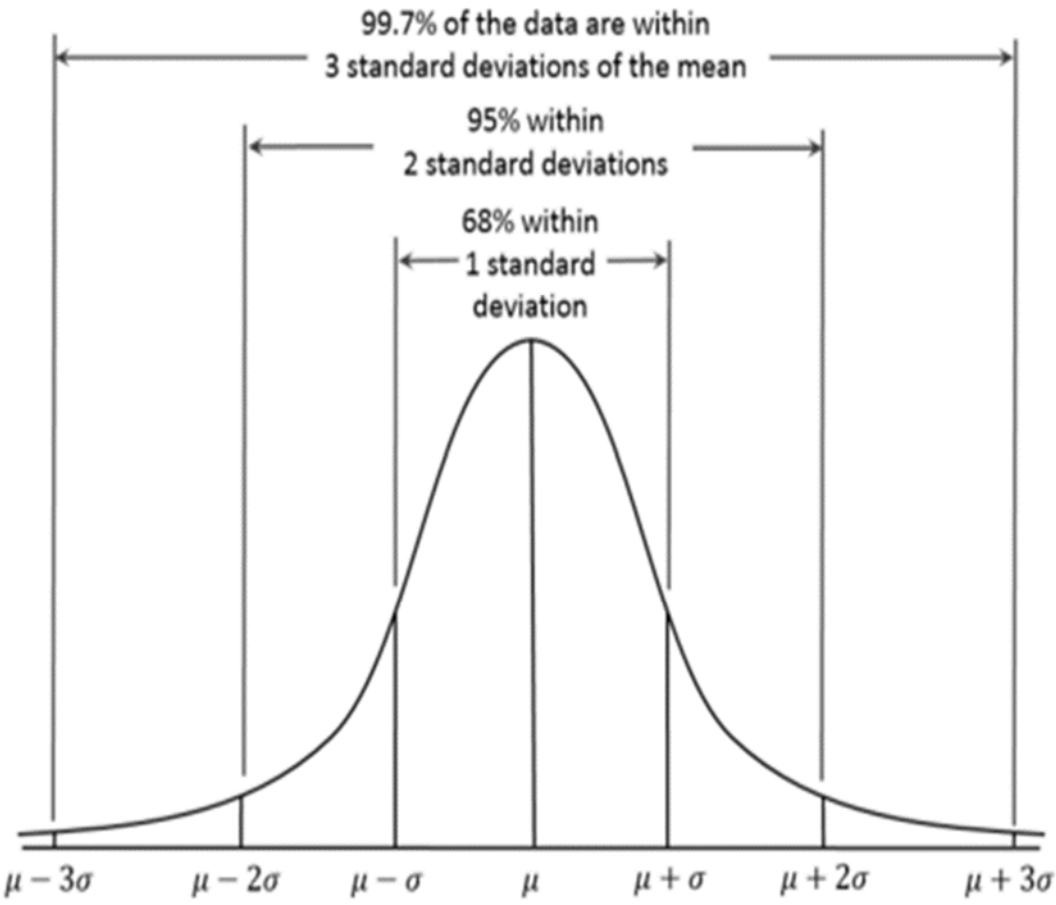
After all factors that contribute to the measurement uncertainly in a process have been added to the UB, the reported uncertainty for the method needs to be calculated.

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

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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).

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ILLINOIS STATE POLICE

TOXICOLOGY
PROCEDURES MANUAL

APPENDIX VIII: RETROGRADE EXTRAPOLATION

Reviewed by:

_____
Casey Craven, Chairperson
Toxicology Command Advisory Board

Approved by:

_____
Timothy Tripp
Bureau Chief – Toxicology Command Coordinator

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 incident occurred and when a blood specimen was obtained. Retrograde extrapolation is routinely used in pharmacology, toxicology, and clinical medicine.

Alcohol metabolizes via several pathways, the primary being through the liver involving the enzymes alcohol dehydrogenase and aldehyde dehydrogenase. In a post-absorptive state, the metabolism is linear until it reaches a sufficiently low enough concentration for the metabolic enzymes to no longer be a limiting factor. An elimination rate range of 0.010 g/dL/hr to 0.025 g/dL/hr encompasses the majority of the population regardless of age, sex, ethnicity, and drinking experience. This linearity may not apply at concentrations below 0.020 g/dL.

In contrast to metabolism, the absorption of alcohol is a complex process which is not linear and can be highly variable. Absorption rates can be impacted by factors such as the presence of food in the stomach, the type and volume of beverage consumed, other drugs consumed, and the condition of the gastrointestinal tract. Studies support that it can take up to 2 hours to reach the post absorptive phase following the last drink.

Laboratory personnel may provide retrograde extrapolation opinions when the attorney of record makes an appropriate request to include the time at which the last drink was consumed. If drinking history details are unknown it is not reasonable to assume the subject is post-absorptive and the request will be denied.

These opinions rely on case history information provided to laboratory personnel through the requesting attorney or law enforcement records. This information in combination with the measured blood alcohol concentration are crucial to preparing a valid opinion. Therefore, when preparing the opinion, the information relied upon will be clearly communicated including but not limited to the following assumptions:

- The measured whole blood ethanol concentration is greater than 0.020 g/dL
- The individual is post-absorptive at the time in question
- The elimination rate of the individual is in the normal range of 0.010-0.025 g/dL/hr

If the drinking history information supports that the subject ceased drinking 2 hours or more prior to the incident, it is reasonable to assume the subject is post absorptive and the following statement will be used as an assumption:

- The individual is considered post absorptive at the time in question. Drinking history information indicated that the individual ceased alcohol consumption 2 hours or more prior to the time of incident in question.

If drinking history information supports that the subject ceased drinking 30 minutes or more prior to the time of incident in question, it is reasonable that the subject can be post absorptive. However, additional clarifications will be added to address the potential impact on the opinion by using the following statement as an assumption.

- The individual is considered post absorptive at the time in question. Drinking history indicated that the individual ceased alcohol consumption at least 30 minutes prior to the time of the incident in question. Research has shown that an individual can reach the post absorptive phase 30 minutes up to 2 hours once alcohol consumption has ceased. However, alcohol absorption can be impacted by various factors including liquid(s) and food(s) in the stomach, other drug(s) consumed, and gastrointestinal health.

Should any information provided by the attorney or case record conflict with the required assumptions the request should be denied.
Performing alcohol calculations is a forensic service request which includes work documentation and peer review. Should information used in the retrograde extrapolation change it may be appropriate to discuss the impact a change would have on the calculations. However, performing calculations during live testimony is discouraged due to inherent risks.

The template on the following pages may be utilized when creating a response to a requested Retrograde Extrapolation. Bracketed sections contain descriptions only and will be replaced with the appropriate case information, as provided by the requesting attorney or generated by the responding analyst during drafting of the response. Additional assumptions and or clarifications may be added when necessary. Current Illinois State Police letterhead with the appropriate header and footer should be used.

A copy of this template will be made available as a fillable document to all analysts.

Completed retrograde extrapolations will be reviewed and initialed by a second analyst before being submitted to the requesting attorney. The proofed letter will be uploaded to the case in LIMS.

[DATE RESPONSE WAS DRAFTED]

[NAME OF REQUESTING ATTORNEY]
[TITLE OF REQUESTING ATTORNEY]
[NAME OF COURTHOUSE]
[STREET ADDRESS OF COURTHOUSE]
[CITY], Illinois [ZIP CODE]

RE: People v. [NAME OF CASE SUBJECT]

Dear [NAME AND TITLE OF REQUESTING ATTORNEY]:

I have reviewed the information provided regarding the following case.
Subject: [SUBJECT'S NAME]
Laboratory Case Number: [LABORATORY CASE NUMBER]
Agency Case Number: [AGENCY CASE NUMBER])

Based on the information provided, and the following listed assumptions, I have calculated an estimation of the subject's blood alcohol concentration at the time of the incident.

Provided Information:

1. The time of the incident was approximately [DATE AND TIME OF INCIDENT].
2. The time of the DUI blood collection was approximately [DATE AND TIME OF BLOOD DRAW].

Assumptions (APP-VIII Toxicology Procedures Manual):

1. The measured whole blood ethanol concentration is greater than 0.020 g/dL
2. The elimination rate of the individual is in the normal range of 0.010-0.025 g/dL/hr

Option 1 for item 3.

3. The individual is considered post absorptive at the time in question. Drinking history information indicated that the individual ceased alcohol consumption 2 hours or more prior to the time of incident in question.

Option 2 for item 3.

3. The individual is considered post absorptive at the time in question. Drinking history indicated that the individual ceased alcohol consumption at least 30 minutes prior to the time of the incident in question. Research has shown that an individual can reach the post absorptive phase 30 minutes up to 2 hours once alcohol consumption has ceased. However, alcohol absorption can be impacted by various factors including liquid(s) and food(s) in the stomach, other drug(s) consumed, and gastrointestinal health.

Based on the above assumptions, the estimated blood alcohol concentration at the time of the incident is calculated as follows:

Calculations:

A. The DUI whole blood alcohol concentration is [REPORTED CONCENTRATION] g/dL, as measured by Headspace Gas Chromatography at [NAME OF LAB].

B. The time difference between the time of the incident, [TIME OF INCIDENT] on [DATE OF INCIDENT], and the DUI blood collection, [TIME OF BLOOD DRAW] on [DATE OF BLOOD DRAW] is approximately [CALCULATE DIFFERENCE IN HOURS ROUNDED TO TWO DECIMAL POINTS] hours.

C. The amount of alcohol eliminated between the time of the incident and the time of the DUI blood collection can be estimated by multiplying the time difference by the metabolic rate as follows:

0.010 g/dL/hr x [TIME CALCULATED ABOVE] hours = [CALCULATED VALUE TRUNCATED TO 3 DECIMAL POINTS] g/dL eliminated

0.025 g/dL/hr x [TIME CALCULATED ABOVE] hours = [CALCULATED VALUE ROUNDED UP TO 3 DECIMAL POINTS] g/dL eliminated

D. The estimated concentration of alcohol in the subject's blood at the time of the incident can be calculated by adding the amount of alcohol eliminated to the measured whole blood alcohol concentration.

[REPORTED CONCENTRATION] g/dL measured concentration + [LOWER CALCULATED VALUE FROM C.] g/dL eliminated = [CALCULATED VALUE] g/dL

[REPORTED CONCENTRATION] g/dL measured concentration + [HIGHER CALCULATED VALUE FROM C.] g/dL eliminated = [CALCULATED VALUE] g/dL

Conclusion:

Therefore, it is my opinion that the estimated blood alcohol concentration for the subject at [TIME AND DATE OF INCIDENT], was between [LOWER CALCULATED VALUE FROM D] g/dL and [HIGHER CALCULATED VALUE FROM D] g/dL.

This opinion is based on data published in scientific literature, the information provided, the assumptions and calculations outlined above, and the Illinois State Police Division of Forensic Services Toxicology Standard Operating Procedures Manual.

Should any of the information change or contradict the assumptions declared to prepare this opinion as the case progresses; the forensic scientist should be consulted to address the potential impact upon the opinion.

Please contact me if I may be of any further assistance.

Sincerely,

[NAME OF ANALYST, WITH SIGNATURE ABOVE]
Forensic Scientist
Illinois State Police

REFERENCES

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ILLINOIS STATE POLICE

TOXICOLOGY PROCEDURES MANUAL

APPENDIX IX: ABBREVIATIONS

Reviewed by:

_____
Casey Craven, Chairperson
Toxicology Command Advisory Board

Approved by:

_____
Timothy Tripp
Bureau Chief – Toxicology Command Coordinator

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
ACN	Acetonitrile
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 extraction
BZE	Benzoylecgonine
BZO	Benzodiazepine
CAL	Calibration or Calibrator
CALMS	Computer Aided Laboratory Management System
CANNAB	Cannabinoid(s)
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**
CONF	**Confirmation**
CONFIRM	**Confirmation**
CONT	**Control**
CPD	**Chicago Police Department**
CRM	**Certified Reference Material**
CSA	**Criminal Sexual Assault**
CT	**Chicago Toxicology**
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**
dH_2O or DI	**Deionized (water)**
DIL	**Dilution**
DEP	**Deputy**
DUI	**Driving Under the Influence**
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 Spectrometry**
GC/NPD	**Gas Chromatography with Nitrogen-Phosphorous Detector**
GHB	**Gamma-hydroxybutyric Acid**
GLUC	**Glucuronidase**
HC	**High Control**
HFB	**Heptafluorobutylated Derivative**
HFBA	**Heptafluorobutyric anhydride**
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	**Metabolites(S)**
MILLS	**Instrumental Date 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 Immunoassay 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**
SCRN	**(Drug) Screen**
SDT	**Subpoena Duces Tecum**
SFSL	**Springfield Forensic Science Laboratory**
Sgt	**Sergeant**
SMA	**Sympathomimetic Amine**
S/N	**Signal-to-noise ratio**
SNRI	**Selective norepinephrine reuptake inhibitor**
SO	**Sheriff's Office (or Department)**
SOFT	**Society of Forensic Toxicologists**
SOP	**Standard Operating Procedure**
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**
ST	**Springfield Toxicology**
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.**
TAT	**Turn around time**
TBDMS	**Tertiary Butyl Dimethyl Silyl Derivative**
TC	**Training Coordinator**
Temp	**Temperature**
THC	**tetrahydrocannabinol or THC-COOH GC\MS method**
TIAFT	**The International Association of Forensic 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**
YET	**Yellow evidence tape**

$\bar{x}$	**Mean (Average)**
α	**Alpha**
β	**Beta**

ILLINOIS STATE POLICE

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APPENDIX X: DATA SUITABILITY FOR COMPARISON

Reviewed by:

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Casey Craven, Chairperson
Toxicology Command Advisory Board

Approved by:

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Timothy Tripp
Bureau Chief - Toxicology Command Coordinator

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. This evaluation considers whether applicable parameters such as detector response, chromatographic peak shape, and resolution are sufficient to ensure reliable comparisons. Once deemed suitable, the data can be further evaluated to determine whether it supports a positive identification of a panel drug. This document defines the key terms used in assessing the suitability of analytical data and outlines how various data outcomes should be interpreted.

I. Terms & Definitions

A. Suitable Data: Analytical data that meets criteria for detector response, chromatographic peak shape and resolution and therefore is considered suitable for comparison to a reference.
B. Unsuitable Data: Analytical data that does not meet criteria for detector response, chromatographic peak shape and resolution and therefore is considered not suitable for comparison to a reference.
C. Positive Identification: Suitable data that upon comparison to a reference has produced a response which meets applicable identification criteria.
D. Negative Identification: Suitable data that upon comparison to a reference has not produced a response which meets applicable identification criteria
E. Possible Data: Suitable data that upon comparison suggest the presence of a drug but does not meet full identification criteria.
F. Interference: Non-targeted substances (i.e. matrix component, other drugs, metabolites, internal standard, impurities) which may impact the ability to detect, identify, or quantitate a targeted panel drug.
G. Rejected Data: Analytical results that are deemed invalid and excluded from interpretation or reporting due to a failure in quality control measures, such as unacceptable performance of calibration, controls, internal standard, or blanks.

II. Criteria for Assessing Data Suitability

A. Data that is suitable for comparison for GC-FID, LC-MS, LC-TQ meets the following requirements when applicable:
    1. Detector Response
        i. When using a technique that produces a detector response, the targeted response should be greater than the baseline to be considered a peak suitable for comparison.
    2. Chromatographic Peak Shape
        i. Acceptable peaks are typically Gaussian, but slight asymmetry and tailing is normal with gradient methods.
        ii. The peak of a single analyte should produce a single apex to be used for comparison.

3. Resolution
    i. In chromatographic techniques, resolution shall be sufficient to show separation of distinct analytes.
    ii. In mass spectrometry techniques resolution shall be sufficient to differentiate mass assignments.

B. When assessing data suitability, concentration disparities may be considered.
    1. Concentration disparities may affect some characteristics of data to include but not limited to chromatographic peak shape, retention time, mass spectral ion ratios etc.

III. Positive Identification and Negative Identification

A. Criteria for positive and negative identification can be found in TX-APP-II or applicable procedures.
    1. Positive identification is the result of suitable data comparisons to a reference standard which meets identification criteria of a panel drug.
    2. Negative Identification is the result of suitable data comparison to reference standard which does not meet identification criteria of a panel drug.

IV. Identification with Interference and Possible Identification

A. Identification with Interference
    1. In some instances, a known or unknown component (e.g. endogenous matrix components, other drugs or metabolites) can interfere with suitable data comparisons.
        i. Some techniques (i.e. mass spectrometry) utilize data processing tools that can minimize interference through spectral averaging and background subtraction.
    2. Interferences should be minimized when possible; however, an identification that meets method specific acceptance criteria can still be made when an interference is not fully eliminated.
    3. The forensic scientist authoring the report is responsible for notating interferences on spectral images or in case notes when deemed applicable.

B. Possible Identification
    1. A possible identification does not fully meet positive identification criteria upon comparison (i.e low signal or concentration, matrix effects).
    2. A possible result requires a positive confirmatory test to report panel drug.

V. Rejected Data

A. Instances where quality control samples including calibrators, controls, or blanks fail and invalidate the batch or individual sample will be documented as rejected data.
    1. The decision to reject data will be made by either the forensic scientist authoring the report or if different, the analyst performing the extraction.

2. Dropping calibration points as allowed by procedure is not considered rejected data.
3. Instances where data is not processed and can be reanalyzed will not be considered rejected data.
    i. For example, instrument malfunctions, power outages, sequence reruns.
    ii. Should sample be re-aliquoted, notation is required.

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APPENDIX XI: Drug Panel

Reviewed by:

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Casey Craven, Chairperson
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Approved by:

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Timothy Tripp
Bureau Chief – Toxicology Command Coordinator

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, 2]
Methylenedioxymethamphetamine (MDMA)[1,2]

Benzodiazepine Panel
7-Aminoclonazepam[1,2]
alpha-Hydroxyalprazolam[2]
alpha-Hydroxymidazolam[2]
Alprazolam
Clonazepam[1,2]
Diazepam
Flualprazolam
Lorazepam[1,2,7]
Midazolam[2]
Nordiazepam
Oxazepam[1]
Temazepam
Cannabinoids Panel
Delta-9 Carboxy THC (THC metabolite)
Delta-9 Tetrahydrocannabinol (THC)[1,2]
Cocaine Panel
Benzoylecgonine (Cocaine metabolite)
Cocaethylene (Cocaine metabolite)[2]
Cocaine[1,2]
Opiate Panel
6-Monoacetylmorphine (Heroin metabolite)[1]
Codeine[1]
Dihydrocodeine[2]
Hydrocodone
Hydromorphone[1,2]
Morphine
Oxycodone [1,2]
Oxymorphone[2]
Phencyclidine (PCP) Panel
Phencyclidine (PCP)

[1] Preliminary drug screening tests in urine 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.

[2] Preliminary drug screening tests in blood 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.

Report Remark
Drug analysis includes the Full Drug Panel.[3] **Or** Drug analysis includes the **Full Drug Panel** and the following classes: **Amphetamine**, **Benzodiazepine**, **Cocaine**, **Opiate**, **Phencyclidine (PCP)**, and **Cannabinoid**.
Full Drug Panel
10-hydroxy Carbamazepine - (Oxcarbazepine metabolite)
Amitriptyline
Bupropion
Butalbital
Carbamazepine
Carbamazepine 10,11-epoxide - (Carbamazepine metabolite)
Carisoprodol
Chlorophenylpiperazine (mCPP)
Chlorpheniramine
Citalopram
Clonidine
Cocaethylene
Cocaine
Cyclobenzaprine
Desipramine
Desmethylcitalopram
Diphenhydramine
Doxepin
Doxylamine
EDDP (Methadone metabolite) – 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
Fentanyl[5]
Fluoxetine
Guaifenesin[5]
Hydroxybupropion
Hydroxyzine
Imipramine
Ketamine
Lamotrigine
Levetiracetam[5]
Meperidine
Meprobamate

Methadone
Methorphan
Methylenedioxyamphetamine (MDA)
Methylenedioxymethamphetamine (MDMA)
Methylphenidate
Mirtazapine
Modafinil
Nordoxepin
Norfentanyl[5]
Norketamine
Norquetiapine
Nortriptyline
O-Desmethyltramadol
O-Desmethylvenlafaxine
Olanzapine
Oxcarbazepine
Paroxetine
Phenobarbital[4]
Phenytoin
Promethazine
Propofol
Quetiapine
Sertraline
Topiramate
Tramadol
Trazodone
Venlafaxine
Zolpidem

[3] This report remark is utilized when only a drug screen is performed.
[4] Analysis limited to full drug panel testing in blood sample types.
[5] Analysis limited to full drug panel testing in urine sample types.

Report Remark
Note: Additional analysis was performed to screen for **Gamma-Hydroxybutyric Acid (GHB)**.
GHB Panel[6,7]
Gamma-Hydroxybutyric Acid (GHB)

[6] Analysis limited to urine sample types
[7] Analysis routinely performed on all criminal sexual assault (CSA) investigations which include urine sample submissions.

ILLINOIS STATE POLICE

TOXICOLOGY
PROCEDURES MANUAL

APPENDIX XII: Validation and Verification of Procedures

Reviewed by:

_____
Sean McCarthy, Chairperson
Toxicology Command Advisory Board

Approved by:

_____
Timothy Tripp
Bureau Chief – Toxicology Command Coordinator

APPENDIX XII

Validation and Verification of Procedures

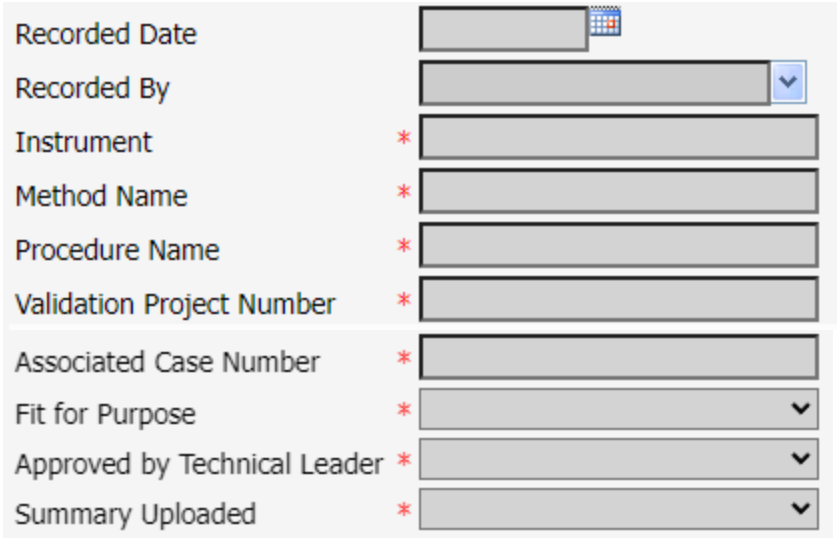
Appendix XII contains the procedures for the verification, and subsequent documentation, of validated procedures in Toxicology.

Novel procedures will be appropriately validated before being added to the Toxicology Procedures Manual and approved for use in casework. Validation protocols will adhere to RES 1 and RES 2 in the Command Directives. Validation addendums may be used to evaluate minor procedural modifications that would not impact all of the parameters evaluated in the initial validation. Addendums will include all supporting data for the affected validation parameters as determined by the technical leader and a summary report determining if the procedure is "fit for purpose" with those modifications.

A verification demonstrating that the validated procedure is fit for purpose must be performed on all instruments that were not directly used in validation experiments. An outline of the verification process is detailed as follows:

I. A verification plan
   a. Utilizes the results of the completed validation as a starting point
   b. Covers one or more parameters assessed in the validation
      i. This can be accomplished over fewer experiments and with fewer blank matrix sources than the initial validation
   c. Approved by the Technical Leader
   d. Summarized in the verification report

II. A verification report
   a. Summarizes results
      i. Specifically notate any results that differ from that of the initial validation
      ii. Concludes if experiments demonstrate the procedure/instrument is or is not fit for purpose
   b. Documented in LAM
      i. Under "Lab"
         1. Forensic Science Center at Chicago – Toxicology
         2. Springfield Toxicology Laboratory
      ii. Under "Section"
         1. Toxicology
      iii. Verification entries will be recorded in a log template under "Other Asset">"Asset Type">"Tox Verifications"> where the "Asset Name" will be "YYYY Verifications"
      iv. Utilize the "Tox Method Verification" template in the "Lab Status Info" tab under the appropriate "YYYY Verification" asset type.
      v. Complete all required fields
      vi. Upload Verification "Summary" Report in "Images"



III. Collected data

a. Experiments should be completed using all instruments that require verification.
    i. A lead analyst may collect and analyze all data however incorporating additional analysts into the process will be preferred.
b. Data from experiments will be archived in LIMS using a mock casefile assigned to the Toxicology Laboratory for quality assurance
    i. Each verification will be given a separate assignment in LIMS within the mock casefile
        1. "Lab Code", "Section", and "Assigned Analyst" (Lead) should be populated
        2. The "Comments" field should be used to describe the verification for quick identification
        3. The Assignment's "Image vault/Paperclip" will be used to archive all verification data as well as any notes or electronic files as needed
        4. Compressed (zipped) folders may be used to compile data into a single upload
    ii. Mock case number and assignment number will be documented in the "Tox Method Verification" record in LAM