Idaho State Police Forensic Services

TOXICOLOGY
ANALYTICAL METHODS

Table of Contents

Revision History

Revision #	Description of Changes
1	Original Version: Combination of methods. Changes made during combination: removal of quantitative references in AM #11, Adjustments made to the control and calibration requirements in AM #13 and AM #14, specified required steps for use of calibrated pipette in LC methods.
2	Addition of LCQQQ ToxBox Methods, minor formatting corrections, removal of running blanks from LCQQQ Benzo/Z-drugs and Cannabinoid methods based on evaluation for carry over. Clarification made for LCMS confirmations in AM 19, Added requirements for ToxBox to AM 21. Added tolerances of balance checks to AM #17.
3	Corrected footer in AM #3, addition of AM 28 Multi-Drug Confirmation by LCQQQ, corrected volumes of low control to add in AM #14, addition of required instrument parameters to all extraction AM's, removed requirement for calibrated pipettes in AM 13 and AM 14, removed cannabinol from scope in AM 14 and made cannabidiol optional, removed methadone from scope of AM 1.
4	Removed Lipomed exclusion in AM #14, corrected oxazepam glucuronide spiking amounts in AM #13, clarified wording for cals and controls in AM #7, added additional requirement for after pipettes are calibrated, added requirement to check of authenticated reference materials.
5	Added panel 2 compounds to AM #28, added needle rinse instructions to AM #25-28, changed storage requirements for ISTD in AM #8, changed calibrator numbers in AM #13.
6	Added mitragynine to scope of AM #28, removed confirmation ranges, dropped s/n requirement for secondary ion from 10 to 5, and included note about confirming amitriptyline/maprotiline to AM #28, included serial dilution instructions for working solution in AM #13, removed methadone from AM #1, archived AM #'s 10, 11, 12, 14 and 15, removed serial dilution instructions for preparing controls and calibrators in AM #7, added quantitative ranges and reporting guidelines for 5 compounds in AM #28, added reconstitution guidelines for AM #'s 25, 26, 27, and 28.
7	Removed work instruction flowcharts from several methods, removed AM #22, clarified requirements for blanks in AM 26-28, added requirements for infrequently performed tests, added and removed compounds in AM #25 and AM #28, added

	additional guidelines for confirming compounds quantitatively (AM #28), added additional compounds for quantitative reporting in AM #28.
8	Removed some compounds from AM #25 and AM #28, corrected a couple spelling errors, added in an additional guideline for dropping highest calibrator for samples that are reported quantitatively in AM #28.
9	Included urine in the scope of samples for AM 25, AM 26, AM 27, and AM 28, added instructions for processing urines for AM 25, AM 26, AM 27, and AM 28, updated testing method types and requirements in AM 20, removed the requirement for external controls in AM 25, 26, 27, and 28.
10	Added key ions to search for in AM #2 and AM #8, clarified quality requirements in AM #7, added the addition of HCl to the steps for urine samples in AM #25 and AM #28 (analysts' discretion), included the option for an external control in AM #25, added to list of compounds for quantitative reporting in AM 28, removed AM #4.
11	Removed AM #1 and AM #7, added AM #29 and AM #30 (Q-TOF screening methods), changed AM #6 method from GCMS method to updated LCMS method, added Q-TOF guidelines to AM #24, added wording for not pursuing compounds in AM #25, removed recipes for solutions no longer used in AM #23, lowered c-THC cutoff in AM 27, removed lists of compounds included in scope of testing for AM 25, 28, and 29, and replaced with a statement to refer to a separate document, minor formatting changes.
12	Included the option to add 1% HCl in MeOH to all samples in AM 25, 28, and 29. Changed the minimum number of batches for a compound to be added from 5 to 3, added in additional criteria for confirmation in AM #30, specified limitation for freezing of plates in AM #25-30, dropped THC cutoff for confirmation in AM #27 to 1ng/mL, minor formatting/wording changes.
13	Added in specifications for mobile phases and column for AM #6, updated criteria for evaluating proficiency tests, added manual integration guidelines to AM #24, added sample bracketing requirement and guideline to AM #27 and AM #28, changed reporting guidelines and examples of wording/circumstances regarding reporting of metabolites in AM #20, also added in examples of extenuating circumstances and guidelines for additional testing in AM #20, added requirement for reviewing ToxBox COA's and added in clarification regarding external controls in AM #21.
14	Removed AM #5 and AM #8, removed requirement for storing 1% HCl in MeOH in amber bottle, also removed expiration for 1% HCl in MeOH (AM #23), removed

	unnecessary shaking step in AM #25 and AM #28, added in mobile phases specifications in AM #26, changed amount of blood + base or urine + base to add to SLE to range in AM #28, made note about using fresh formic acid in MeOH in AM #6, added that required urine control can be external or internal in AM #25, AM #26, AM #27, and AM #28, changed reporting for THC in AM #27, changed requirements for what needs to be reviewed on balance and weights certificates in AM #17, removed comment about calling buprenorphine and norbuprenorphine inconclusive in AM #28, other minor formatting/wording changes.
15	Removed fentanyl reporting limitation in AM #28, added requirement for 6 consecutive points to be included in calibration curve in order to report quantitative value in AM #28, made range for THC in AM #27 qualitative from 1-3ng/mL, minor wording changes.
16	Added in Drug Screening Using Randox MultiSTAT method, made some clarifications regarding the purpose and use of QC's for reinjected in AM 27 and 28, added clarifications to AM 28 acceptance criteria, minor formatting/wording changes.
17	Removed duplicate steps in AM #31.
18	Removed AM 9, made clarifications for requirements in AM 25, 26, 27, 28, 29, and 30. Increased expiration date for ISTD working solution in AM 6, minor formatting/wording changes.
19	Added additional option for urine hydrolysis for methods 26, 27, and 30. Removed extra shaking step in methods 26, 27, and 30. Added additional specifications for transporting, storing, and handling reference materials in AM 21, minor formatting/wording changes.
20	Removed AM #3 and changed urine hydrolysis procedure for AM #25, 28, and 29.
21	Added a statement for changing GHB ISTD working solution preparation instructions to allow for making less than 5mL, corrected urine cutoff concentrations in AM 25 and AM 29 and removed comment in AM 28, specified whole blood in AM 31, changed upper end of quantitative range for THC and THC-OH in AM 27.

Toxicology AM #2: General Extraction of Urine for Basic/Neutral or Acidic/Neutral Compounds

1.0 Background/References

1.1 Background

These extractions are extensions of the TOXI-LAB TOXI-A and TOXI-B thin layer chromatography (TLC) drug detection systems. The samples are extracted as with the TLC system, however, instead of concentrating the extract onto a disc, the solvent extract is concentrated and placed into an automated liquid sampler (ALS) vial for analysis by a gas chromatograph equipped with a mass selective detector (GC/MSD). Discussions of TLC and GC/MS theory can be found in most college-level chemistry and/or instrumental texts. In 2013 the TOXI-LAB line was discontinued. An equivalent product, De-Tox Tubes by Dyna-Tek, were evaluated and found to be a suitable replacement.

2.0 Scope

2.1 This procedure describes the extraction of drug compounds from urine. Depending upon the pKa of a drug compound, either De-Tox Tubes A or B (or verified equivalents) are used. Basic and neutral compounds are extracted with an A tube. Addition of urine to the De-Tox A tube results in the sample becoming alkaline and basic and neutral drugs thus extract into a solvent mixture. The B tube is used for acidic and neutral compounds. Urine placed into the De-Tox B tube becomes acidic resulting in acidic and neutral compounds being extracted into a solvent mixture. Either resulting extract is analyzed by full scan GC/MS in EI mode.

3.0 Equipment/Reagents

3.1 Equipment and Supplies

3.1.1 Tube Rocker

3.1.2 Evaporative concentrator and appropriate concentration cups or tubes

3.1.3 Laboratory Centrifuge capable of 3000 rpm

3.1.4 Laboratory oven or water bath

3.1.5 Fixed and/or adjustable volume pipettes, and appropriate tips.

3.1.6 Automated Liquid Sampler (ALS) vials

3.1.7 GC/MS Vial Microinserts

3.1.8 Gas Chromatograph equipped with a mass selective detector and a low bleed (5%-Diphenyl-95%-Dimethylsiloxane copolymer) capillary column.

3.2 Reagents

3.2.1 De-Tox Tubes A and B (or equivalent Toxi Tubes)

3.2.2 b-Glucuronidase Solution

3.2.3 2M Acetate buffer, pH 4.8

3.3 Qualitative Controls

3.3.1 Positive control

3.3.1.1 Tube A positive control may be commercially obtained or prepared in-house. At a minimum, the control must contain at least one phenethylamine at an approximate concentration between 500 and 3000 ng/mL, and one opiate at an approximate concentration between 300 and 3000 ng/mL.

3.1.1.2 Tube B positive control may also be commercially obtained or prepared in-house. At a minimum, the control must contain two barbiturates at an approximate concentration between 300 and 1000 ng/mL.

3.3.2 Negative Urine

3.3.2.1 Negative urine can be commercially obtained, or in-house urine verified to be negative for drugs of interest. Refer to Toxicology AM#19 for additional details.

3.3.3 Morphine-Glucuronide Positive and Negative Controls for Optional Enzymatic Hydrolysis Step

3.3.3.1 A commercially-obtained control or in-house spiked urine containing morphine-glucuronide should be used. The same negative urine must be used to prepare both the positive and negative glucuronide controls for in-house spiking. Morphine-glucuronide should be used for these controls and must be at a minimum concentration of 375 ng/mL. The positive and negative glucuronide controls are used to demonstrate the glucuronidase cleavage was effective.

3.4 Qualitative Non-Extracted Reference Material

3.4.1 Run necessary reference material as indicated by examination of GC/MSD data. Reference material mixes may be used.

3.4.2 Dilute reference material as necessary. A suggested dilution for a 1 mg/mL solution is 1 in 3 parts of appropriate solvent.

4.0 Procedure

4.1 Extraction Procedure

4.1.1 De-Tox Tubes-A Extraction (Basic and Neutral Compounds)

4.1.1.1 Label DE-TOX TUBE A and ALS vials with micro-inserts for negative control, positive control and appropriate laboratory case numbers.

4.1.1.2 Transfer approx. 5 mL of casework, negative and positive control urine to appropriate DE-TOX TUBE A (pH=9). If case sample volume is limited, less than 5 mL may be used.

4.1.1.3 Agitate the DE-TOX tube to break up salts. This assists in reducing the occurrence of emulsions.

4.1.1.4 Rock DE-TOX TUBE A for at least 10 minutes.

4.1.1.5 Centrifuge tube at ~2500-3000 rpm for ~10 minutes.

4.1.1.6 Transfer solvent and evaporate to ~100-300 µL.

4.1.1.7 Transfer solvent to labeled GC/MS ALS vial with micro-insert.

4.1.1.8 OPTIONAL: Analyst may, at their discretion, perform an enzymatic hydrolysis on a sample aliquot prior to the above De-Tox Tube A extraction. If done, this must be done in addition to the regular (non-hydrolyzed) extraction of the sample. Positive and negative morphine glucuronide controls (see section 3.3.3 of this method) should be run in addition to the regular controls required by the method.

4.1.1.8.1 Optional Enzyme Hydrolysis: To 4.5 mL of urine, add 150µL of 2M acetate buffer and vortex. To all but the glucuronidase negative control, add 100 µL of Kura BG-100 β-glucuronidase solution. Cap and vortex gently to mix. Place in a 60°C laboratory oven or water bath for 2 hours. Allow sample to cool before proceeding with steps 4.1.1.2 through 4.1.1.7.

4.1.2 De-Tox Tubes-B Extraction (Acidic and Neutral Compounds)

4.1.2.1 Label DE-TOX TUBES B and ALS vials with microinserts for negative control, positive control and appropriate laboratory numbers.

4.1.2.2 Transfer approx. 4.5 mL of casework, negative and positive control urine to appropriate DE-TOX TUBE B (pH=4.5). If case sample volume is limited, less than 4.5 mL may be used.

4.1.2.3 Rock DE-TOX TUBE B for at least 10 minutes.

4.1.2.4 Centrifuge tube at ~2500-3000 rpm for ~10 minutes.

4.1.2.5 Transfer solvent and evaporate to ~100-300µL.

4.1.2.6 Transfer solvent to labeled GC/MS ALS vial with microinsert.

4.2 Preparation for Analysis Run

4.2.1 Into Sequence log table, enter the sample case numbers, blanks and controls.

4.2.2 Load samples, reference materials, blanks and controls into the quadrant rack as noted in the sequence table.

4.2.3 GC-MSD Analysis Parameters

4.2.3.1 Refer to instrument method for current analysis parameters.

4.2.3.1.1 Instrument Run Parameters

- Inlet at 280, splitless injection, injection volume: 1 µL
- Oven at 80^{0}C, hold 2.5 minutes
- Ramp 25^{0}C
- Final temperature: 300^{0}C, hold at least 7.5 minutes.
- Additional sample runs may be done with adjustments to these parameters, for example if there is a coeluting peak or a late eluting compound.

4.2.3.2 Current analysis method must be stored centrally as a hard or electronic copy. Setting the instrument parameters to store with the data file is also approved.

4.2.4 Detection and Identification Criteria

4.2.4.1 The presence of a drug compound is indicated if the retention time for the sample versus applicable reference material does not differ by more than ±0.2 minutes and there are no significant differences in the mass spectral data.

NOTE: Early eluting drugs, as well as drugs known to have similar retention times and mass spectral fragmentation patterns (e.g. Phentermine and Methamphetamine), may not differ from the retention time of the applicable reference material by more than ±0.1 minutes.

4.2.4.2 Ions that are common to numerous compounds and should be extracted for in the data include: 44, 58, 71, 205, 235, and 245.

4.3 Quality Assurance Requirements

4.3.1 Refer to applicable sections of Toxicology AM #19 and Toxicology AM #21 for additional quality assurance and reference material authentication requirements.

4.4 Analysis Documentation

4.4.1 The printed results for each case sample and accompanying blank will be included with the analysts' notes. Case results are to be recorded in the iLIMS system.

4.4.2 Original data for controls will be prepared for each analysis run and stored centrally in the laboratory where the analysis was performed until archiving, or electronically on a shared drive.

4.4.3 A copy of controls may be stored electronically in a central location and need not be included in individual case files. When necessary, a copy of control printouts can be prepared from the centrally stored document.

Toxicology AM #6: Screening for Gamma-Hydroxybutyrate (GHB) in Urine

1.0 Background/References

1.1 Background:

GHB occurs naturally in minute quantities as a result of the metabolism of the inhibitory neurotransmitter, GABA.[6,8] The drug GHB is a potent tranquilizer that was previously used as an anesthetic and as a treatment for major depressive illnesses, alcohol withdrawal, and narcolepsy.[1-8] Legitimate use is limited due to the side effects associated with the drug. GHB can produce visual disturbances, nausea, vomiting, drowsiness, dizziness, severe respiratory depression, unconsciousness and involuntary muscle spasms.[1,5,6] Overdoses can require emergency medical treatment including intensive care due to the respiratory depression, bradycardia and coma.[5,6,8]

The use of GHB has been increasing since the 1980s and currently is popular among body builders, teenagers and on the club/dance scene. Body builders use the drug due to its alleged role as a growth hormone releasing agent to enhance muscle growth.[5,6,8] GHB has not been proven to possess any anabolic effects.[6] GHB has gained notoriety as a date rape drug due to its ability to produce short-term amnesia and decreased inhibitions.[1-8] The FDA banned the use of GHB in 1990 except for FDA approved physician supervised protocols.[6,8]

A dose of GHB is anywhere from 2.5 to 4.0 grams in approximately 1 teaspoon ("capful") of liquid. The taste of GHB has been described as salty or soapy, the odor is said to be mothball-like.[5,6] Due to the short half-life of GHB (0.3 to 1.0 hours[5, 27] 5 minutes[7]) the person will re-administer every 45 minutes to 1 hour. The onset of effects is 15 to 60 minutes. The effects of the drug will be detectable during a DRE exam for 4 to 6 hours. GHB is classified as a central nervous system depressant. The observed effects include horizontal and vertical nystagmus, lack of convergence, body tremors, and slowed breathing. The person will also exhibit a lowered pulse, blood pressure, and body temperature. In addition, the muscle tone will exhibit flaccidity and the person may be in a trance-like state, the pupils will exhibit a lack of reaction to light.[5] Lower doses will promote an agitated, combative state however their pulse and other vitals will be depressed.[5,6] Combining GHB with alcohol plus a stimulant or marijuana allows the user to remain conscious during use. This allows them to experience the euphoric “buzz” that is the desired effect of its abuse. The desired effect is a state of relaxation and tranquility, a pleasant drowsiness, mild euphoria, hallucinations and a release of inhibitions. Combining GHB with alcohol or other central nervous system depressant will provide an additive depressant effect.[5] GHB is detectable in blood for up to eight hours and in urine for up to 12 hours.[5,6,7] Peak plasma levels are obtained in 20 to 45 minutes.[7] Peak urine GHB concentrations on the order of 1100 µg/mL (1,100,000 ng/mL) are observed within the first four hours after a 100 mg/kg oral dose.[3,7]

GHB is manufactured by reacting butyrolactone with sodium hydroxide in an aqueous solution in the presence of a low molecular weight alcohol (methanol, ethanol).[5,6] The average yield of GHB is 70%. If the yield of the manufacturing process is low, there will be significant amounts of the lactone present in the product. Abuse of this compound will continue due to its relatively simple synthesis and the availability and low cost of starting materials.[5] Users can ingest Gamma butyral lactone (GBL), a degreaser and floor stripper, and it is converted in vivo into GHB. GBL is therefore diverted from legitimate sources to either be taken directly or converted to GHB.

This compound has been referred to by a variety of names as listed in the table below.

Street Names	Marketing Names
"G"	Revitalize
"G" caps	Rejuvenate
Liquid X	Renewtrient
Soap	Revivarant
Easy Lay	Blue Nitro
Georgia Home Boy	Thunder Nectar
G-riffic	Rest-Eze
Grievous Bodily Harm	Energy Drink

There are thousands of documented GHB overdoses with numerous deaths. The danger in GHB use stems from its steep dose-response curve. A small increase in dose can create a dramatic difference in adverse effects. This makes the potential of overdosing with GHB very high. This is compounded by the fact that GHB affects users so differently. A dose that one individual uses could adversely affect another, thus word of mouth is a poor determiner of how much of the substance to use.

1.2 References:

1.2.1 Frommhold, S. Gamma-Hydroxybutyrate (GHB): What's "the Scoop?" in: Toxi-News 16(1), 1997; pp. 3-8.

1.2.2 Ferrare, S.D., Tedeschi, L. Frison, G., et. al, Therapeutic gamma-hydroxybutyric acid monitoring in plasma and urine by gas chromatography-mass spectrometry. J Pharm. Biomed Anal, 1993, 11(6):483-487.

1.2.3 Stephens, B. and Baselt, R.C. Driving Under the Influence of GHB? J Anal Tox, 1994, 18:357-358.

1.2.4 ElSohly, M.A. and Salamone, S.J. Prevalence of Drugs used in Cases of Alleged Sexual Assault J Anal Tox, 1999, 23:141-146.

1.2.4 Chase, D.A., Gamma Hydroxy Butyrate, "GHB", Presentation at IACP DRE Conference, Minnesota, 1999.

1.2.5 Good, P.J., Selected Abuse Substances, Presentation at IACP DRE Conference, Portland, Oregon, 1998.

1.2.6 https://www.dfs.virginia.gov/wp-content/uploads/2020/03/220-D100-Toxicology-Procedures-Manual.pdf

1.2.7 https:// phenomenex.blob.core.windows.net/documents/8f784003-a01d-4e01-a9c6-b565570a23ff.pdf

1.2.6 Determination of Gamma-Hydroxybutyric Acid by GC/MS, Dade County Medical Examiner's Toxicology Lab SOP.

1.2.7 Microgram, Volume XXXI, No. 3, March 1998.

1.2.8 Couper, F.J. and Logan, B.K. Determination of gamma-Hydroxybutyrate (GHB) in Biological Specimens by Gas Chromatograph-Mass Spectrometry, J Anal Tox, 2000, 24:1-7.

1.2.9 SOFT/AAFS Forensic Toxicology Laboratory Guidelines, 1997.

1.2.10 Goldberger, B.A., Huestis, M.A., Wilkins, D.G. Commonly Practiced Quality Control and Quality Assurance Procedures for Gas Chromatography/Mass Spectrometry Analysis in Forensic Urine Drug-Testing Laboratories, Forensic Sci. Rev., 1997, 9(2):59-79.

2.0 Scope

2.1 This method provides an efficient qualitative analysis option for the liquid-liquid extraction of urine samples suspected of containing Gamma-Hydroxybutyrate/ gamma-Hydroxybutyric Acid (GHB). This method may not provide adequate sensitivity for weaker concentrations of GHB. This method should only be used for driving under the influence of drugs (DUID) situations where GHB is suspected or for screening purposes for drug-facilitated sexual assault (DFSA). Cases that indicate a positive result by this method must be outsourced for quantitative confirmation.

3.0 Equipment/Reagents

3.1 Equipment

3.1.1 16X100mm centrifuge tubes or 16X144mm tapered-end centrifuge tubes

3.1.2 Caps for 16mm Tubes

3.1.3 Laboratory Centrifuge capable of 3400 rpm

3.1.4 Fixed and/or adjustable volume pipettes, and appropriate tips, capable of accurate and precise dispensing of volumes indicated.

3.1.5 Automated Liquid Sampler (ALS) or LCMS vials

3.1.6 ALS or LCMS vial inserts

3.1.7 Approved LC/MS/MS and MassHunter or equivalent software

3.1.8 Phenomenex Phenyl Hexyl (4.6 x 50 mm; 2.6µm) or equivalent column

3.2 Reagents

3.2.1 0.1% formic acid in water (LCMS, Optima LCMS or equivalent) (Mobile phase A)

3.2.2 0.1% formic acid in methanol (LCMS, Optima LCMS, or equivalent) (Mobile phase B)

3.3 Reference Material

3.3.1 Stock Solution

- 1 mg/mL GHB
- 100,000 ng/mL GHB-D6

3.3.2 GHB Spiked Urine Positive Controls (One required)

- Use the same lot of negative urine to prepare both the negative and spiked positive controls.

3.3.2.1 Preparation of 200,000 ng/mL Positive Control Working Solution

3.3.2.1.1 Add 200 µL of GHB 1 mg/mL stock solution to 800 µL negative urine. Vortex. This solution will be stored in the freezer and is good for approximately six months.

3.3.2.2 Preparation of 10,000 ng/mL Positive Control

3.3.2.2.1 Add 10 µL of GHB 200,000 ng/mL working solution to 190 µL negative urine. Vortex. This control should be made fresh with each run.

3.3.3 GHB-D6 Internal Standard

3.3.3.1 Preparation of 20,000 ng/mL Internal Standard Working Solution

3.3.2.1.1 Add 1 mL of GHB-D6 100,000 ng/mL stock solution to 4 mL methanol. Vortex. Volumes may be adjusted as long as the final concentration remains the same. This solution will be stored in the freezer and is good for one year. If a case sample has limited volume, the analyst may choose to perform the extraction with the controls prior to extracting the sample.

3.3.4 Negative Control

3.3.4.1 Negative Urine can be commercially obtained, or in-house urine verified to be negative for drugs of interest.

4.0 Procedure

4.1 Extraction Procedure

4.1.1 Initial set-up

4.1.1.1 Label centrifuge tubes for positive control, negative control, and case samples.

4.1.1.2 Label ALS or LCMS vials for positive control, negative control, and case samples. Place insert in all vials.

4.1.2 Extraction Procedure

4.1.2.1 Pipette positive and negative controls (for negative control, 200 µL urine will be added to the appropriate tube). Add 200 µL urine to each centrifuge tube for case samples.

4.1.2.2 Add 100 µL of the GHB-D6 Internal Standard Working Solution to each tube.

4.1.2.3 Add 900 µL of 0.1% formic acid in methanol to each tube. Vortex. NOTE: Using older 0.1% formic acid in methanol for this step can result in poor chromatography. If the data indicates this, the extraction should be repeated using fresh reagent.

4.1.2.4 Centrifuge at ~3400 rpm for 15 minutes.

4.1.2.5 Add 100 µL 0.1% formic acid in water to each vial insert.

4.1.2.6 Transfer 10 µL of sample from each centrifuge tube to the corresponding vial insert (avoid disturbing the pellet at the bottom). Vortex.

4.2 Instrument and Run set up

4.2.1 See Toxicology AM #24 for LCMS instrument maintenance and operation.

4.2.2 Set up the worklist in MassHunter.

4.2.3 Instrument run parameters and the acquisition methods for this Analytical Method will be printed out (paper or electronically) and stored centrally in the lab or in a common file folder electronically.

4.2.5.1 Acquisition Required Settings

4.2.5.1.1 Column Temperature - 30^0C

4.2.5.1.2 Injection volume –1-5 µL

4.2.5.1.3 Mobile Phase Flow rate - 0.4 mL/min

4.2.5.1.3: Binary Pump Gradient Settings (Pump Time Table) (Note: the gradient parameters may be adjusted slightly as long as it is demonstrated that the compounds can still be distinguished from one another).

Time (min)	% Mobile A	% Mobile B
0	60	40
0.5	5	95
5.0	5	95
5.1	60	40
6	60	40

4.3 Evaluation of Results

4.3.1 Batch Review, Criteria for Positive Results, and Reporting

4.3.1.1 Using the positive control, a 1-point calibration curve will be established. The curve will be set to linear, non-weighted and origin set to force.

4.3.1.2 If a sample gives a response that is greater than 10,000 ng/mL, a statement on the report will be included saying that preliminary testing indicated a possible presence of an elevated level of GHB and it is recommended that the sample be sent to a private lab for quantitation. If a sample gives a response between 7,000 and 10,000 ng/mL, an inconclusive statement can be added to the report.

4.3.1.3 The S/N for samples and controls at and over 10,000 ng/mL must be 5 or greater.

4.3.1.4 Case samples and negative controls will generally be considered negative if the calculated concentration is less than 7,000 ng/mL.

4.3.1.5 Samples should have a significant internal standard response. If a sample has little to no internal standard response, the sample should be reinjected or re-extracted.

4.4 Quality Assurance Requirements

4.4.1 Refer to Toxicology AM #19 and AM #21 for additional quality assurance and reference material authentication.

4.5 Analysis Documentation

4.5.1 The printed results for each case sample and accompanying blank will be included with the analysts' notes. Case results will be recorded in the ILIMS system.

4.5.2 The reports for the batch, including calibration curves and controls will be stored centrally in the lab or electronically on a common drive.

4.5.3 The raw data from the run will be stored electronically and will be backed up at least every two months.

Toxicology AM #16: Pipette Calibration and Intermediate Checks

1.0 Background/References

1.1 Background

The initial calibration of piston or plunger operated volumetric apparatus (POVA) is performed by the manufacturer. Upon receipt of a newly obtained pipette or syringe and thereafter periodically, the calibration must be verified to substantiate that the volume delivered is both accurate and precise. This is accomplished by determining the mass of a volume of liquid of known density that has been delivered into a closed vessel.

1.2 References

1.2.1 ASTM Method E-1154-89 (reapproved 2003), Standard Specification for Piston or Plunger Operated Volumetric Apparatus.

1.2.2 Curtis, R.H., Performance Verification of Manual Action Pipets: Part I, Am. Clin. Lab. 12(7):8-9; 1994.

1.2.3 Curtis, R.H., Performance Verification of Manual Action Pipets: Part II, Am. Clin. Lab. 12(9):16-17; 1994.

1.2.4 Byer, B.J., How to Use and Check Pipetting Equipment, Scientific Newsletters, Inc., 1977.

1.2.5 ISO 8655-6:2002, Piston-operated volumetric apparatus - Part 6: Gravimetric method for the determination of measurement error.

2.0 Scope

2.1 Scope

The reliability of the volume delivered by a pipette is dependent upon verification of calibration. This method sets forth the requirements for both intermediate checks and calibration. The intermediate check is performed to maintain confidence in calibration.

3.0 Equipment/Reagents

3.1 Analytical Balance

- Capable of accurately weighing volumes of interest.

3.2 Thermometer

- Subdivisions of ≤0.5 degree
- Capable of reading 20° - 28°C

3.3 Weighing Vessel

3.4 Appropriate disposable pipette tips

3.5 Water

4.0 Procedure

4.1 Calibration

4.1.1 All pipettes that require calibration (this is designated in the analytical method) will be calibrated within 365 days prior to use by a qualified vendor or after repair or service. If the pipette is a variable volume apparatus at least one level from the table below will be checked for the volumes that apparatus spans.

4.1.2 After calibration is complete an analyst will review the calibration certificate and the calibration report provided by the vendor and will be centrally stored. In addition, an intermediate check must be performed before the pipette is used if the calibration was done off-site.

4.1.3 The criteria listed in table 1A must be met when reviewing the calibration certificate.

Table 1A

Volume	Accuracy	Precision
2-15 µL	± %5	± 2%
16-100 µL	± 2%	± 2%
101-1000 µL	± 2%	± 2%
1000-5000 µL	± 2%	± 2%

4.1.4 The analyst will document the review and approval by initialing and dating the certificate. If the tolerances are not met the analyst will make a notation on the certificate and will take that pipette out service for use in methods that require calibrated pipettes.

4.2 Intermediate Checks

4.2.1 All pipettes that require calibration will have an intermediate check performed within 45 days prior to use. The results will be recorded. The pipette will be clearly marked with the date the next intermediate check is due.

4.2.2 Allow water to equilibrate to room temperature.

4.2.3 Determine and record the water temperature on the log sheet.

4.2.4 Place a volume of water in the weighing vessel which completely covers the bottom of the container.

4.2.5 Place the weighing vessel on the balance and tare.

4.2.6 Use designated pipette, to dispense the appropriate volume of water. If the pipette is a variable volume apparatus at least one level from the table below will be checked for the volumes that apparatus spans.

4.2.7 The criteria listed in table 1B must be met when evaluating the results of the intermediate check.

Table 1B

Volume	Accuracy
2-15 µL	+/- 7%
16-100 µL	+/- 5%
101-1000 µL	+/- 5%
1000-5000 µL	+/- 5%

4.2.7 From the Table 2 below, note the conversion factor (Z) for the mean water temperature. The conversion factor is based upon an air pressure of 1013 hPa.

Table 2

Temperature(°C)	Conversion Factor (Z) (µL/mg)
20.0	1.0029
20.5	1.0030
21.0	1.0031
21.5	1.0032
22.0	1.0033
22.5	1.0034
23.0	1.0035
23.5	1.0036
24.0	1.0038
24.5	1.0039
25.0	1.0040
25.5	1.0041
26.0	1.0043
26.5	1.0044
27.0	1.0045
27.5	1.0047
28.0	1.0048

4.2.8 Calculate the Volume Delivered **(*V*)** at the recorded temperature.

$$V = (W) \cdot Z$$

4.2.9 Inaccuracy Calculation

4.2.9.1 Determine inaccuracy by calculating the percent error (E_t) between the expected (V_o) and calculated (V) volume.

$$E_t = V - V_o / V_o \text{ x } 100$$

4.2.9.2 Record the inaccuracy and refer to table 1B above to for acceptable tolerances. Note on the log sheet if the check passes or fails. If the check fails the pipette needs to be taken out of service for quantitative use and repaired, the pipette must be calibrated before being returned to service.

Toxicology AM #17: Balance Calibration and Intermediate Checks

1.0 Background/References

1.1 Background: Refer to manufacturer's manual for balance specific procedures.

1.2 References

1.2.1 ASTM Method E-617-97, Standard Specification for Laboratory Weights and Precision Mass Standards.

2.0 Scope

2.1 The procedure is to ensure accuracy of measurement when an analytical or top-loading balance is used to prepare solutions or reference material for application to toxicology methods.

3.0 Equipment/Reagents

3.1 ANSI/ASTM Type I, Class 1 or Class 2 laboratory weights

3.2 Analytical and/or Top-Loading, Direct-Reading Laboratory Balances

4.0 Procedure

4.1 Intermediate Check Procedure

4.1.1 Inspect balance pan, clean if necessary.

4.1.2 Inspect level bubble, level if necessary.

4.1.3 Tare balance.

4.1.4 Place weight on balance.

4.15 Record weight on balance logsheet.

4.1.6 The allowable deviation from the standard weights will be 0.01 g or 0.1%, whichever is greater.

4.1.7 The verification procedure should be repeated if the value does not fall within the acceptable range. If value is still out of range, contact a service vendor to set up a service call. A note must be placed on the balance to indicate that it is not in range and must not be used.

4.2 Quality Assurance Requirements

4.2.1 Within 45 days prior to use, the balance is to have its calibration checked (intermediate check is acceptable) against an appropriate set of NIST traceable weights. Results are to be recorded for future reference. All balances will be calibrated yearly by a qualified vendor. Calibration certificates will be checked to ensure that the accuracy is within 0.01 g of the expected values, and that other tolerances listed on the certificates pass. The certificate will be checked and initialed before the balance is placed back in use.

4.2.2 Weights used for intermediate checks of calibration will be NIST traceable and will be calibrated every 5 years by a qualified vendor. Upon return to the laboratory and before the weights are placed back into service, the calibration will be verified by weighing them on an analytical balance. The weights must be within 0.005 g of their expected weight. Calibration certificates will be reviewed to ensure that the weights are within 0.005 g of their expected weight, and initialed.

4.2.3 An in-house intermediate check of balances in use will be performed within 45 days prior to use. Results are to be recorded in logbook.

4.2.4 The weights used for the intermediate check should depend on the application of the balance. Three weights must be used to represent the weight range in question. For instance, if the balance is being used to prepare buffer solutions, then perhaps 100 g, 500 g and 1000 g weights would characterize the weight range. If the balance is used to prepare mg/mL reference material then 1 mg, 10 mg and 100 mg weights may be appropriate.

4.2.5 The type of balance employed should be a consideration. For weights less than 100 mg, an analytical balance should be used. For the preparation of a solution involving gram quantities, a top-loading balance should be used.

4.2.6 Do not touch weights with bare hands. Weights should be transferred with forceps or gloves. The weights will be stored and transported in their case.

Toxicology AM #18: Toxicology Proficiency Tests

1.0 Background/References

1.1 Background

As set forth in the Idaho State Police Forensic Services *Procedure and Quality Manual*, proficiency testing is an integral part of a quality program.

2.0 Scope

2.1 This method describes the criteria to be applied to the evaluation of results obtained for proficiency testing for the toxicology discipline.

3.0 Equipment/Reagents

3.1 Not Applicable

4.0 Procedure

4.1 Urine and Blood Analysis Proficiency Testing

4.1.1 Only analytes that are routinely tested for with current ISP-FS analytical methods will be evaluated. If the parent drug or a metabolite of a drug is reported, additional metabolites of that drug are not required to be reported.

4.1.2 The measured value given (or reported grand mean), not the preparation concentration value reported by the provider, will be used for the evaluation of the results.

4.1.3 The provider measured value (or grand mean) and standard deviation will determine the acceptance criteria for quantitatively reported compounds. The measured mean of a value reported by the analyst/laboratory must fall within the acceptance criteria. (below).

4.1.3.1 An analyst/laboratory result that is greater than 2 standard deviations or less of the provider result or a result that falls within the lab's expanded uncertainty from the mean result for that method (whichever is greater) will be scored as passing.

4.1.3.2 An analyst/laboratory result that is greater than 2 standard deviations, but less than 3 standard deviations of the provider result will be flagged as a "Warning" result. A warning result is NOT immediately considered nonconforming work. Items that receive this flag should be investigated and may require documentation in a QAR. A corrective action may or may not be necessary pending the investigation.

4.1.3.3 An analyst/laboratory result that is greater than 3 standard deviations and outside the lab's expanded uncertainty from the mean result will be considered as failing and thus nonconforming work. A QAR and corrective action will be initiated.

4.1.3.4 If the score is not passing or is flagged as a warning and the analyst/discipline lead/lab manager determines there is a legitimate discrepancy, an investigation may be initiated and documented in a QAR; however a corrective action may or may not be necessary pending the investigation.

4.1.4 If the analyst does not correctly identify all target analytes and/or quantitative values do not fall within the acceptable range, the analyst's training will be reviewed, and additional training may be required as deemed appropriate by the Toxicology Discipline Lead. The analyst may be required to complete a competency test prior to resuming casework. The number of samples will be determined by the nature of the discrepancy.

Toxicology AM #19: Quality Assurance Measures

1.0 Background/References

1.1 Background

The quality assurance measures applied towards analysis of toxicological samples promote confidence in results.

1.2 References

1.2.1 Wu Chen, N.B. Cody, J.T., Garriott, J.C., Foltz, R.L., et al., Report of the Ad hoc Committee on Forensic GC/MS: Recommended guidelines for forensic GC/MS procedures in toxicology laboratory associated with offices of medical examiners and/or coroners, J. Foren. Sci, 236 (35): 236-242, 1990.

1.2.2 Goldberger, B.A., Huestis, M.A., Wilkins, D.G., Commonly practiced quality control and quality assurance procedures for gas chromatograph/mass spectrometry analysis in forensic urine drug-testing laboratories, For Sci Review, 9(2): 60-79, 1997.

1.2.3 SOFT/AAFS Forensic Toxicology Laboratory Guidelines, 2002

2.0 Scope

2.1 This analytical method addresses general acceptance requirements for qualitative and quantitative analysis data obtained through analysis by gas chromatography equipped with a mass selective detective (MSD). Requirements for analysis with other instrumentation are addressed in relevant analytical methods.

3.0 Equipment/Reagents

3.1 Refer to specific analytical method for necessary equipment and supplies.

3.2 Refer to appropriate analytical method for required reagents as well as Toxicology AM #23 for Solution Preparation instructions.

4.0 Procedure

4.1 Instrument Quality Assurance GCMS

4.1.1 Instrument Maintenance

NOTE: Refer to manufacturer's hardcopy or electronic instrument manuals for maintenance indicators and instructions.

4.1.2 MSD Tuning

4.1.2.1 A Tune must be run within a week prior to analysis of casework.

4.1.2.2 A Tune Evaluation should be completed, and parameters should be reviewed for acceptance based on the report's predefined criteria, set by the manufacturer. *Note: H_2O and N_2 values should be monitored for possible instrumentation leaks (acceptance parameters are listed in parentheses on the tune evaluation report).*

4.1.3 Instrument Performance Monitoring

4.1.3.1 Instrument performance will be monitored through evaluation of the positive and negative controls run with every extraction batch. The control requirements for an extraction are found in the applicable analytical methods. The instrument will be determined as working properly if the expected responses are obtained for the controls evaluated.

4.1.3.2 Analysts may use the control data to determine if instrument maintenance is needed.

4.1.3.3 Troubleshooting, repairs and maintenance that takes place will be documented.

4.1.4 Data file back up

4.1.4.1 Data files will be retained and backed up to external media at least once every two months.

4.2 Instrument Quality Assurance LC/MS- Refer to Toxicology AM# 24

4.3 Sample Preparation Quality Assurance

4.3.1 Qualitative Analysis

4.3.1.1 Matrix Controls

4.3.1.1.1 Quality controls are to be prepared and analyzed as designated in the appropriate analytical method.

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

4.3.1.1.3 Negative controls should be examined for compound(s) of interest and interfering substances prior to the completion of analysis, the negative control can be included in the batch.

4.3.1.1.4 Commercially obtained reference materials/standards may only be utilized for qualitative purposes after the manufacturer's expiration date. If the reference material is being used to establish a cutoff or evaluation point (for example the controls in AM #3 and AM #6), the reference material/standard may not be expired even though the results of the analysis are reported qualitatively. Reference material used for urine controls in analytical methods #25-30 may be expired since the urines are reported qualitatively only.

4.3.1.1.5 Analytical plates may be utilized for qualitative and/or quantitative analysis after the manufacturer's re-test date *provided* all method control requirements are met AND an additional in-house control **that is not expired** be run. If any of the compounds in the in-house control fails due to the concentration being outside of the acceptable accuracy parameters, the results of the run may be reported qualitatively only.

4.3.1.1.6 It is applicable, for example, to use an expired commercially obtained c-THC control when performing Toxicology AM# 3; this method requires one (non-expired) spiked control be run in addition to the commercially obtained control.

4.3.1.2 GC/MS Solvent Blanks

4.3.1.2.1 An appropriate solvent blank will be run before case sample extracts. If the same sample is injected for more than one instrument method, only one blank needs to be run preceding the sample injections.

4.3.1.2.2 If the solvent blank contains a reportable analyte of interest, the corrected area of the analyte peak in the sample data must be a minimum of 10 times stronger than the corresponding peak in the blank preceding it. Ideally, no contamination should be apparent.

4.3.1.2.3 *Reportable* is defined as a complete fragmentation pattern at the appropriate retention time. Analytes of interest include, but are not limited to, analytes routinely reported.

4.3.1.2.4 If significant contamination is present, as discussed in 4.3.1.2.2, rerun the solvent blank that was used in that analysis and the sample extract in question. If the contamination is still apparent, or the original samples are no longer available, troubleshoot the instrument to determine the source of contamination. In addition, the sample in question must be re-extracted prior to reanalysis on the rectified instrument.

4.3.2 Quantitative Analysis

4.3.2.1 Quality measures are optimized for the analytes in question and are addressed in each individual quantitative analytical method.

4.3.3 Distribution of Quality Data

4.3.3.1 Original data for matrix controls will be stored in a designated central location in the laboratory where the analysis was performed or electronically on a shared network drive.

4.3.3.2 Copies of all quality assurance control data need not be placed in each case file except those required under 4.3.3.3.

4.3.3.3 Copies of analytical reference material used to substantiate the identification of each drug compound confirmed using GC/MS must be included in each case file, if not otherwise indicated in the relevant analytical method.

4.4 Sample Storage

4.4.1 Blood samples should be stored under refrigeration; this includes Combo Collection Kits.

4.4.2 Urine samples should be stored under refrigeration or frozen. If samples are going to be stored longer than two weeks prior to analysis, they should be frozen. Once analysis is complete, samples should be frozen until they are returned to the agency.

Toxicology AM #20: Testing Guidelines and Reporting Criteria

1.0 Background/References

1.1 Background

To best utilize the resources available to support the ISP-FS toxicology discipline, the degree of analysis pursued should be guided by all available information. It may not always be necessary and/or appropriate to confirm all drug compounds present. With urine analysis, when a subject has admitted to use of prescription and/or over-the counter drugs that may impair driving, confirmation of all drugs present may not serve to strengthen pending charges. With drugs-of-abuse, confirming the presence of all drug compounds may not be necessary, depending on the circumstances. For instance, for Probation and Parole cases, prescription pharmaceuticals are most likely not a consideration. This method also covers reporting criteria.

2.0 Scope

2.1 This method addresses requirements to confirm a drug as well as reporting the indication of drugs. It also covers the factors to consider when determining the extent of analysis a toxicology case sample requires. It is intended to provide guidance to analysts; however, the decision to pursue testing remains at the discretion of each analyst. The goal of these considerations is for the efficient utilization of resources in order to provide timely analysis results to user agencies. This method covers reporting criteria to ensure consistent reporting in the lab system and to ensure limitations are properly expressed.

3.0 Equipment/Reagents

3.1 Not applicable

4.0 Procedure

4.1 Assignment of classifications for analytical methods and instrumental techniques.

4.1.1 Class A (indicative/screening test)

- Full scan GCMS mass spectrum comparison to a library (no comparison of retention time or to standard run on instrument)
- GCMS full scan compared to an instrument run standard with acceptable retention time but mass spectral information is too weak.
- LCMS-QQQ targeted screen (analytical methods 6, 25 and 26).
- LC-QTOF targeted screen (analytical methods 29 and 30).
- LCMS-QQQ targeted confirmation below limit of confirmation with defined indicative range in method.

Class B (confirmatory test, or indicative test- provides basic structural information)

- GCMS methods in SIM (selective ion monitoring mode)
- LCMS-QQQ targeted confirmation methods (analytical methods 27, and 28)

Class C (confirmatory test- provides detailed structural information)

- GCMS full scan mass spectrum with comparison of retention time and spectrum to reference material.

4.2 Testing requirements to confirm a drug.

4.2.1 To confirm a drug, positive test results with the following test combinations must be obtained (the testing may be performed by different analysts):

- Class A and Class B
- Class A and Class C
- Two different Class B methods
- Class B and Class C
- Class C with two sampling events (may be same method)

4.2.2 Exceptions/Clarifications:

- A positive response for a targeted compound may be used as positive indication of metabolites, breakdown products, or the parent compound.

  Example 1. A urine sample screens positive for 6-MAM in a targeted screen. The sample is then analyzed on the GCMS and heroin is detected. The heroin can then be reported without running a second GCMS test as the 6-MAM was detected in the screen.

  Example 2. Buprenorphine and norbuprenorphine are detected in a targeted screen but the buprenorphine cannot be evaluated due to any number of circumstances (there is a response for that compound in the negative control, the peaks were cutting off, the retention time had shifted, etc.) if a confirmatory test is run and is positive for buprenorphine, it can be reported since there was an indicator of it in the screen (and the metabolite was present) and it was positive in the confirmation.

4.3 Testing requirements to report a drug was indicated.

4.3.1 To report a positive screening test, it must be clear on the report that the drug was indicated but not confirmed. For example, "preliminary testing indicates the possible presence of (indicated drug/drug class), not confirmed due to (reason)". The indicative testing may be reported when further testing is not pursued, is not possible, or will be pursued at a later date.

4.4 Positive Class A, and Class B, and single sample Class C tests may be reported as indicative.

4.5 Guidelines for method selection and reporting

4.5.1 Reporting confirmed drugs

4.5.1.1 When a drug produces positive tests required for confirmatory testing, it will be reported. It will be reported as Drugs Confirmed: any drugs confirmed will be listed. If no drugs are confirmed it will be reported Drugs Confirmed: None.

4.5.2 Reporting indicative drug results

4.5.2.1 When a sample has indicative screening drug testing results, it is at the analyst's discretion whether or not to report it. Indicative results must be clearly qualified. For example: Inconclusive for zolpidem, as it does not meet ISP Forensic Services toxicology criteria for identification. This is due to mass spectral differences between sample and reference material.

4.5.2.2 If a sample is positive on one indicative test but does not give an indication on a more sensitive valid test, it will not be reported unless otherwise specified. For example, a sample has a positive result for tramadol using AM 25, AM 28 is then run and tramadol meets all the criteria for evaluation but gives a response below the AM 28 limit of confirmation, it will **not** be reported indicatively.

4.5.2.3 When a drug is indicated in a confirmatory test but does not meet the criteria for identification and the analyst chooses to report it, it will be reported as "Inconclusive for (drug), as it does not meet ISP Forensic Services toxicology criteria for identification. This is due to (reason)".

4.6 Method selection

4.6.1 When available, the type of case associated with a toxicology sample should be determined.

4.6.2 The extent of analysis should be based on background information and the type of case.

4.6.3 If no background information is provided, it is at the discretion of the analyst to perform only basic testing.

4.6.4 The typical basic drug testing protocol will start with a targeted screen using LCMS-QQQ, LC-QTOF, or a combination of these tests to cover general drugs of abuse. The toxicology supplemental report will identify the drugs or classes covered in the screen.

4.6.5 The analyst will pursue indicated drugs as appropriate. Not all drugs indicated are required to have confirmation pursued. When LC-QTOF or LCMS-QQQ targeted screening results are positive but confirmatory testing

is not done, the following comment may be added to the report: "Preliminary testing indicates the possible presence of a (drug class or drug); confirmatory testing not pursued due to (reason)". If the parent drug is indicated and confirmed it is at the analyst's discretion to pursue confirmatory testing for metabolites or to report that a metabolite or metabolites was/were indicated.

Example 1. A targeted LCMS-QQQ screen is run and is positive for zolpidem and zopiclone. Zolpidem is confirmed by LCMS-QQQ. Additional confirmatory testing for zopiclone is not required. The analyst would report. Drug(s) confirmed: Zolpidem. Preliminary testing indicates the possible presences of zopiclone; confirmatory testing not pursued due to the presence of other drugs.

Example 2. A targeted LCMS-QQQ screen is run and is positive for tramadol and o-desmethyltramadol. A GCMS confirmation is run and is positive for tramadol but o-desmethyltramadol and n-desmethyltramadol also appear to be in the sample but the laboratory does not have standards to compare the spectra to. The analyst would report Drug(s) confirmed: Tramadol. It is at the analyst's discretion to also report that preliminary testing indicates the possible presence of o-desmethyltramadol and n-desmethyltramadol; confirmatory testing not pursued due to the presence of other drugs.

4.6.6 Drugs that are indicated in general extractions such as a basic GCMS extraction but not confirmed are at the analyst's discretion to report. For example: in a DUI case where the subject presented with symptoms consistent with a CNS stimulant, a targeted drug screen using LCM-QQQ is run and is positive for methamphetamine and amphetamine. A basic extraction with full scan GCMS is then run. Methamphetamine and amphetamine are confirmed, and lidocaine is indicated. The analyst may report out just the amphetamine and methamphetamine with no mention of the lidocaine on the report, or the analyst may report it out as preliminary testing indicated the possible presences of lidocaine confirmatory testing not pursued due to the presence of other drugs.

4.6.7 Tests in addition to the typical screening tests may be run if information is provided that merits running the additional tests. For example, in a suspected drug facilitated sexual assault case, where urine was collected 3-12 hours after the suspected dosing, and no other drugs were detected, a test for GHB may be run. Another example would be a DUI case where the subject presented with symptoms consistent with a CNS depressant and an

alcohol level of 0.04; prescription therapy of butalbital is listed, an acidic extraction with full scan GCMS may be run.

4.6.8 Specific reporting criteria and limitations are listed in the appropriate analytical method.

4.6.9 Reporting listed RX therapy will be at the analyst's discretion but is recommended for cases where it could alleviate confusion of where a drug came from. For example, if oxazepam was detected in urine, the comment could read: Prescription drug therapy is said to include Valium (diazepam), oxazepam is an active metabolite of diazepam.

4.6.10 All examples of reporting criteria are guidelines and can be altered to fit the circumstance, and the exact wording specified does not need to be followed.

4.7 Testing Guidelines: Post-Blood Alcohol or Breath Testing Analysis

4.7.1 When the ethanol concentration is 0.10g/100cc, or greater, further testing for additional drugs, in either blood or urine, should not be pursued unless justified by case-related circumstances. This is in consideration that the legal limit for ethanol is 0.08 grams per 100 cc blood.

4.7.2 Extenuating circumstances may include the following:

- Fatality or injury accidents.
- Death investigations.
- Sexual assaults.

4.7.2.1 In the case of crashes where the subject is the driver and is deceased and further toxicology testing is requested, testing will be performed on samples that have a blood alcohol content of less than 0.20 grams per 100 cc of blood, accident victim "AV" samples that are for statistical purposes only will be run for both alcohol and toxicology, it will be at the analyst's discretion whether or not confirmatory or indicative screening test only are run.

4.7.3 The submitting officer or agency is responsible for providing justification for additional testing. Justification could take the form of a note on the submittal information, memo, e-mail or letter outlining the situation, or a case report.

4.7.4 If the ethanol concentration is 0.10 g/100cc or higher future testing for other impairing drugs will not be pursued if additional testing is not requested. Analysts are encouraged to contact the agency if it is believed that further analysis is recommended. Additional analysis may be prudent if impairment described cannot reasonably be explained by ethanol/other volatiles results.

4.8 Infrequently Used Methods

4.8.1 If a particular type of analysis is not performed by any analyst in the toxicology section at a specific lab within the previous 6-month period, it will be considered an infrequently used method.

4.8.1.1 If any of the analytical methods of the same analysis type have been completed in the previous 6 months, the extraction/analysis without case samples is not required.

4.8.1.2 If it has been longer than 6 months, then prior to the use of the method for case samples the analytical method will be completed using only the calibrators/controls specified in the method. This will ensure that any controls, calibrators, solvents, etc. used in the method are still viable. Once it has been verified that the method is working as intended, it may be used for case samples, a new set of calibrators/controls will be used during the extraction with the case samples. The results from the first extraction will be included in the central file.

4.8.1.3 Methods will be grouped based on instrumentation and extraction techniques used. The analysis types are listed below:

- Methods that utilize LC-QTOF and SLE extraction
- Methods that utilize the GCMS and liquid/liquid extraction methods
- Methods that utilize the LCMS-QQQ and SLE or liquid/liquid extraction

Toxicology AM #21: Authentication of Reference Materials

1.0 Background/References

1.1 Background

The quality assurance measures applied towards analysis promote confidence in results. This analytical method was created so that the shared requirements did not have to be included in every toxicology discipline analytical method.

1.2 References

1.2.1 Wu Chen, N.B. Cody, J.T., Garriott, J.C., Foltz, R.L., et al., Report of the Ad Hoc Committee on Forensic GC/MS: Recommended guidelines for forensic GC/MS procedures in toxicology laboratory associated with offices of medical examiners and/or coroners, J. Foren. Sci, 236 (35): 236-242, 1990.

1.2.2 Goldberger, B.A., Huestis, M.A., Wilkins, D.G., Commonly practiced quality control and quality assurance procedures for gas chromatograph/mass spectrometry analysis in forensic urine drug-testing laboratories, For Sci Review, 9(2): 60-79, 1997.

1.2.3 SOFT/AAFS Forensic Toxicology Laboratory Guidelines, 2002.

2.0 Scope

2.1 This analytical method addresses qualitative and quantitative authentication of reference materials. Reference materials include both standards and controls. Sections 4.1-4.5 do not apply to ToxBox plates. Those are addressed in section 4.6.

3.0 Equipment/Reagents

3.1 Refer to appropriate analytical method for necessary equipment and reagents as well as solution preparation instructions.

4.0 Procedure

4.1 General

4.1.1 Appropriate authentication must be documented for reference materials prior to an analyst reporting a conclusion in casework in which that reference material was used. Authentication data should be stored centrally. If more than one laboratory within the ISP Forensic Services system will use the same lot of a commercial control or reference material, authentication at each individual laboratory is not required, provided personnel in each laboratory have ready access to authentication data.

4.1.2 When a reference material or control contains more than one constituent, only the compound(s) of interest need be authenticated. It should be clearly marked what compounds are authenticated.

4.1.3 Whenever possible, the source of reference material used to prepare matrix controls must differ from that used to prepare a quantitative response curve. If different vendors are not available, a different lot number should be used. As a last resort, if different lot numbers are not available, a second qualified analyst may prepare one of the working solutions.

4.1.4 Unauthenticated reference material must be stored in a designated area or clearly marked that authentication is needed.

4.1.5 It is the responsibility of each analyst to verify that each standard or control used has been properly authenticated. If an analyst uses a previously authenticated reference material, the analyst will initial the authentication data cover sheet indicating that he/she has looked at the authentication paperwork.

4.1.6 Reference materials should be handled carefully and stored at the recommended temperatures provided by the manufacturers. Shipping or transporting laboratory materials from one lab to another is permitted. Care should be taken to make sure that the reference materials stay cold during the shipping or transporting process, and that the reference materials are properly stored at the manufacturer's specifications upon receipt at the other lab.

4.2 Qualitative Reference Material Authentication

4.2.1 Certified reference material may be obtained from ISO Guide 34 or 17034 approved providers that are included on the supply service list. These reference materials may be authenticated by a review of the Certificate of Analysis (COA) associated with the reference material. The COA will be centrally filed (in the laboratory or electronically). The review and approval by the analyst will be noted on the first page of the COA. The analyst will ensure the compound was tested and evaluated as positive for containing the anticipated compound by the provider.

4.2.1.1 Qualitative standards that are not obtained by an approved ISO Guide 34 provider will be authenticated by an instrument that provides structural information (such as GCMS or FTIR) and has been validated and approved for use in the lab. A standard will be considered authenticated when the match (Q) is at least 85%, (or an 850 match factor for NIST Library) as compared to a library search *and* the analyst confirms that the spectra matches with no significant differences. If the spectra does not have a library match of 85% or greater, the spectra may be authenticated by comparing it to a peer reviewed scientific journal, reference standard compendium or a library match that is less than 85%. For these three options, two analysts trained to use the authentication instrumentation must initial the documentation signifying that it is an appropriate match.

4.2.1.2 When comparison to a journal, compendium or other document is not an option, mass spectral interpretation may be used in conjunction with the COA. This would apply in cases where instrumental data for a drug metabolite is not yet published, but a structurally similar compound is available to assist with interpretation. A second trained analyst must also review and initial the printout verifying the interpretation.

4.2.1.3 A coversheet providing the information necessary for authentication will be prepared and placed with the MSD or FTIR data and a copy of the reference spectra. The coversheet must, at a minimum, list the lot number, vendor, date of analysis, analyst name, and mode of authentication.

4.2.2 Commercially obtained reference materials/standards may only be utilized for qualitative purposes after the manufacturer's expiration date. If the reference material is being used to establish a cutoff or evaluation point (for example the controls in AM #3 and AM #6), the reference material/standard may not be expired even though the results of the analysis are reported qualitatively. Reference material used for urine controls in analytical methods #25-30 may be expired since the urines are reported qualitatively only.

4.3 Quantitative Reference Materials Authentication

4.3.1 The qualitative properties of these reference materials will be evaluated using the procedures described in 4.2.

4.3.2 The quantitative values on the COA will be accepted. The COA will be centrally stored for quantitative reference materials. Quantitative reference materials will be marked or stored in a designated location to prevent those that are only authenticated for qualitative use from being inadvertently used in quantitative applications.

4.3.3 The manufacturer of reference materials used for quantitative purposes must either utilize balances calibrated with weights traceable to National Institute of Standards and Technology (NIST) standards *or* be ISO Guide 34 or 17034 certified to produce reference materials. The certificate of analysis or manufacturer's accreditation certificate(s) and scope must be consulted to verify compliance with this requirement.

4.3.4 Quantitative reference materials will be discarded or designated for qualitative use only after they expire.

4.4 Qualitative and Quantitative Matrix Control Authentication

4.4.1 Matrix controls are analyzed in parallel with casework samples to demonstrate that a procedure and/or analytical method performed as intended.

4.4.2 Quantitative Matrix controls also serve to verify the accuracy of a response curve.

4.4.3 Matrix controls may be prepared with authenticated reference materials or obtained through a vendor. The quantitative and qualitative properties of these controls will be based on the certificate of analysis, or the in-house preparation information. In addition, controls used in qualitative analysis may be authenticated following 4.2, if a certificate of analysis is not available.

4.4.4 The qualitative identity and quantitative values of component(s) in a commercially obtained matrix control will be based on the package insert or certificate of analysis. Certificates of Analysis (COA) and package inserts for commercially obtained matrix controls will be stored centrally in the laboratory in which they are used.

4.4.5 To authenticate the qualitative presence of components when the manufacturer does not provide a certificate of analysis or package insert, the analyst will authenticate each compound in the same way a qualitative reference material is authenticated (see 5.10.5.2). If a previous lot of that control has been authenticated with this process, the analyst may simply compare the new lot to the previously authenticated lot.

4.5 Internal Standard Authentication

4.5.1 Internal standards can be used to demonstrate the efficiency of an extraction, that the injection on the instrument worked properly, and for quantitation.

4.5.2 The qualitative identity and quantitative values of component(s) used as internal standards will be based on the package insert or certificate of analysis. Certificate of Analysis (COA) and package inserts for internal standards will be stored centrally in the laboratory in which they are used.

4.5.3 If the Certificate of Analysis is not available for an internal standard that is only used in qualitative analysis, it may be authenticated the same way a qualitative reference material is authenticated (see 4.2).

4.6 Qualitative and Quantitative ToxBox Internal Standard, Reference Material and Control Authentication.

4.6.1 The qualitative identity and quantitative values of component(s) in the ToxBox kits from Cayman Chemical or PinPoint Testing will be based on the certificate of analysis. The Certificates of Analysis (COA) will be stored electronically on a common drive. Before being put into use, the COA for the plate lot(s) shall be reviewed to ensure the plate map, compounds included, and concentrations are correct. It is the responsibility of each analyst to verify that the COA for the plate lot they are using has been reviewed prior to use. After verifying the COA has been reviewed, the analyst should initial the COA.

4.6.2 Reference material used for urine controls in analytical methods #25-30 may be expired since the urines are reported qualitatively only. If a plate is used beyond the re-test date, an expired urine external control can still be used, provided an additional blood external control that is not expired is also included in the run.

Toxicology AM #23: Solution Preparation

1.0 Background/References

1.1 Background: Refer to references

1.2 References

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

1.2.2 Ansys, Inc. SPEC Extraction Methods

1.2.3 United Chemical Technologies, Inc. Applications Manual.

2.0 Scope

2.1 This section describes the proper preparation of solutions and buffers used in ISP Forensic Services toxicology methods for the extraction of drug compounds from blood and urine specimens.

3.0 Equipment/Reagents

3.1 Equipment

3.1.1 Glassware

- Adequately sized beakers, volumetric flasks, graduated cylinders and volumetric pipettes

3.1.2 Laboratory balance

3.1.3 pH Meter and/or Indicator Strips

3.1.4 Appropriate buffer solutions for pH meter

3.1.5 Stirring hotplate

3.1.6 Magnetic stirrers

3.1.7 Safety Equipment

- Chemical Fume Hood
- Acid Resistant Apron
- Laboratory Coat
- Safety Goggles and/or face Shield
- Laboratory Gloves

3.2 Reagents

All chemicals must be ACS Grade or better.

3.2.1 Acids

- Acetic Acid, Glacial
- Hydrochloric Acid
- Phosphoric Acid
- Formic Acid (this may be ACS, LCMS, Optima LCMS grade, or equivalent)

3.2.2 Salts

- Ammonium Chloride
- Potassium Phosphate Monobasic
- Potassium Phosphate Dibasic
- Sodium Acetate Trihydrate
- Sodium Bicarbonate
- Sodium Hydroxide

3.2.3 Solvents

- Methanol
- Water

4.0 Procedure

4.1 Preparation of the following solutions must be recorded on corresponding preparation logs. Solutions may be made in different volumes by adjusting reagent ratios.

Note: Appropriate safety equipment must be worn during the preparation of solutions to minimize exposure to caustic/corrosive solutions. The order of the addition of chemicals may be crucial to minimize exothermic reactions. Refer to appropriate MSDS sheets for more information on handling chemicals.

4.1.1 Ammonium Chloride

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

- Place approximately 300 mL DI water in a beaker and heat/stir over low heat. Add ammonium chloride until the solution is saturated. QS to 500 mL.
- *A positive and negative control will be run with each use. Remake as indicated by control data.*

4.1.2 Ammonium Formate

4.1.2.1 **5 mM Ammonium Formate +0.01% Formic Acid in LCMS Water**

- Add 0.316 grams ammonium formate to 1000 mL of LCMS, LCMS Optima, or equivalent grade water, spike with 100 µL Formic Acid. Mix thoroughly.

4.1.2.2 **10mM Ammonium Formate in LCMS Water**

- Add 0.631 grams ammonium formate to 1000 mL LCMS, LCMS Optima or equivalent grade water, mix thoroughly.

4.1.2.3 **Concentrated Ammonium Formate in LCMS Water**

- A concentrated ammonium formate solution may also be prepared and spiked into LCMS water for mobile phase preparation.

4.1.2.3.1 For instance, make a 2M solution by adding 25.22 grams of ammonium formate to 200 mL of LCMS grade water. For 10 mM mobile phase, spike 5 mL of 2 M ammonium formate into 1000 mL of LCMS grade water.

4.1.3 Ammonium Hydroxide

4.1.3.1 **0.5M Ammonium Hydroxide**

- Add 33.6 mL Stock (28-30%w/w) ammonium hydroxide to 466.4 mL of DI water.

4.1.4 Formic Acid for LC/MS Mobile Phases

4.1.4.1 **0.1% Formic Acid in LCMS Grade Water (1L)**

- Place approximately 600 mL Water (LCMS, Optima LCMS grade, or equivalent) into instrument solvent bottle. Add 1 mL of Formic Acid, mix. QS to approximately 1 L.
- *Make appropriate volume adjustments if needed, or purchase from an approved vendor.*

4.1.4.2 **0.01% Formic Acid in LCMS Grade Methanol (1 L)**

- Spike 100 µL of formic acid into 1 L methanol (LCMS, Optima LCMS grade, or equivalent), prepare in an instrument solvent bottle. Make appropriate volume adjustments if needed, or purchase from an approved vendor (if available).

4.1.4.3 **0.1% Formic Acid in LCMS Grade Methanol (1L)**

- Spike 1 mL of formic acid into 1 L methanol (LCMS, Optima LCMS grade, or equivalent), prepare in an instrument solvent bottle. Make appropriate volume adjustments if needed, or purchase from an approved vendor (if available).

4.1.5 Hydrochloric Acid

4.1.5.1 **0.1M/100mM Hydrochloric Acid (500 mL)**

- Place approximately 300 mL DI water into a 500 mL volumetric flask. Add 4.2 mL **concentrated hydrochloric acid**, mix. QS to 500 mL.
- *A positive and negative control will be run with each use. Remake as indicated by control data.*

4.1.5.2 **1% HCl in Methanol (10 mL)**

- Add approximately 5 mL of methanol to a 10 mL volumetric flask. Pipet 100 µL of *concentrated HCl*, QS and mix. Make appropriate volume adjustments if needed. Remake as needed.

4.1.6 Potassium Hydroxide (KOH)

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

4.1.6.1 **1M/1N Potassium Hydroxide (1N KOH) (100 mL)**

- Dissolve 5.6g potassium hydroxide in approximately 80 mL DI water in a 100 mL volumetric flask. QS to 100 mL.
- *A positive and negative control will be run with each use. Remake as indicated by control data.*

4.1.7 Potassium Phosphate Buffers

4.1.7.1 **Saturated Potassium Phosphate Buffer (1L)**

- Place approximately 1L DI water in a beaker and heat/stir over low heat. Add potassium phosphate monobasic until the solution is saturated. Allow solution to cool. Adjust pH to approximately 1.8 with concentrated phosphoric acid.
- *A positive and negative control will be run with each use. Remake as indicated by control data.*

4.2 Quality Assurance

4.2.1 Refer to Toxicology AM #17 for balance intermediate check and calibration requirements.

Note: Balances properly monitored by drug discipline analysts fulfill quality assurance requirements. Additional check need not be performed.

Toxicology AM #24: LCMS-QQQ and LC-QTOF Instrument Maintenance and Operation

1.0 Background/References

1.1 Background

Recent instrument improvements have led to the accepted use of Liquid Chromatography Mass Spectrometry Mass Spectrometry and Liquid Chromatography Quadrupole Time-of Flight use in the toxicology field. These instruments utilize theory of separation of analytes using liquid chromatography, coupled with identification of analytes based on specified fragmentation and transitional patterns and/or time of flight.

1.2 References

1.2.1 The applicable methods were developed in conjunction with Patrick Friel from Agilent in 2012, and Pinpoint Testing in 2016-2020.

2.0 Scope

2.1 This method provides maintenance and operational instructions for Liquid Chromatograph Mass Spectrometer Mass Spectrometer (LC-QQQ or LC Triple Quad) and Liquid Chromatograph Quadrupole Time-of-Flight (LC-QTOF) instruments used by the Idaho State Police Forensic Services Toxicology Section.

3.0 Equipment/Reagents

3.1 Equipment and Supplies

3.1.1 Agilent 6470 or Equivalent LC/MS/MS system and MassHunter software

3.1.2 Agilent 6575 or Equivalent LC-QTOF system and MassHunter software

3.2 Reagents

3.2.1 LC/MS Low Tuning Solution

3.2.2 Methanol (LCMS, Optima LCMS or equivalent grade)

3.2.3 Needle Rinse (40/40/20: Water/Methanol/Isopropanol or 75% MeOH in water)

3.2.4 Seal Wash Solution (90% Water, 10% isopropanol)

3.2.5 LC-QTOF Reference Mix

3.2.5.1 Add 95 mL of acetonitrile (LCMS, Optima LCMS, or equivalent grade) and 5 mL of water (LCMS, Optima LCMS, or equivalent grade) to a bottle. Add in 4 µL of 5 mM purine and 100 µL of 2.5 mM HP-0921 ((Hexakis (1H, 1H, 3H-tetrafluoropropoxy) Phosphazine)).

3.2.6 LC-QTOF Tuning Mix

3.2.6.1 Add 10 mL of undiluted ESI-L Tune Mix, 85.5 mL acetonitrile (LCMS, Optima LCMS, or equivalent grade), 4.5 mL water (LCMS, Optima LCMS, or equivalent grade), and 3 µL of 0.1 mM HP-0321 to the appropriate bottle.

4.0 Procedure

4.1 Instrument Maintenance

4.1.1 Refer to Manufacturer's Recommendation for Scheduled Preventative Maintenance.

(Note: Preventative Maintenance is not required as long as response and successful tuning is completed)

4.2 Instrument Operation

4.2.1 Instrument Start-up

4.2.1.1 If necessary, clean the Electrospray Ion Source using lint free cloth and LCMS Grade Methanol or IPA, the use of ultrafine grit cleaning cloth may be necessary (*ensure thorough rinse with LCMS grade methanol after this type of cleaning*). A drop in response from one checktune/mass calibration to the next may indicate the need to clean the electrospray ion source.

4.2.1.2 Select the appropriate mobile phase set for the desired method being used. Ensure that the aqueous mobile phase is connected to channel A, and the organic mobile phase is connected to channel B. Reset the solvent levels in the acquisition software.

4.2.1.3 Turn the LC/MS/MS or LC-QTOF on and let it run for approximately 10-15 minutes prior to starting the run. During this time, monitor the pressure curve to ensure it is not higher or lower than is expected for that method.

4.2.1.4 Verify the column and plate configuration are correct for the desired method(s).

4.2.1.5 Select Tune in MassHunter Acquisition and run a checktune (LCMS-QQQ) or mass calibration (LC-QTOF), if necessary. NOTE: for the LC-QTOF, verify that the switcher valve is closed before tuning. Review tune report. Pay attention to abundances and if there are significant drops in abundance. If successful, continue with pre-run instrument start-up. If unsuccessful, or if a significant drop in abundance(s) is seen, perform an autotune (LCMS-QQQ) or system tune and/or transmission tune (LC-QTOF).

4.2.1.5.1 Checktunes and autotunes are run to ensure the instrument is operating optimally and to indicate if any issues are occurring or may occur in the near future (for instance, if abundances are dropping rapidly, it may be an indication that the capillary needs to be replaced). Autotunes should be performed as needed, and checktunes should be performed periodically to be able to identify any potential issues that may be indicated.

4.2.1.5.2 If analysis mode for samples to run is Positive mode, only tunes in Positive mode are necessary.

4.2.2 Analytical Run Set-up

4.2.2.1 In MassHunter Acquisition, load the appropriate acquisition method based on the analysis to be run on the instrument. Allow column temperature and LC pressure to stabilize for about 15 minutes. Verify that the binary pump is functioning optimally by monitoring the tuning (tuning should be +/- 0.2). This value does not affect the validity of the results and does not need to be documented.

4.2.2.2 Open or start a new worklist. Enter the calibrators, blanks, controls and samples as needed. Designate the appropriate acquisition method for the samples.

4.2.2.3 Select Worklist then Worklist Run Parameters, and create a Data Path for this Batch (e.g. 110808BZ).

4.2.2.4 Also in Worklist Run Parameters, select Acquisition Cleanup/Standby, to put the instrument in Standby after the Worklist, or if a Not Ready Timeout occurs.

4.2.2.5 Save the Worklist. Do not overwrite the Master Worklist file.

4.2.2.6 Verify sufficient volume of needle rinse and seal wash solutions (if applicable) are present, refill if needed.

4.2.2.7 Verify the correct plate configuration is selected for your plate position(s).

4.2.2.8 For LC-QTOF runs, verify that the switching valve is open prior to running.

4.2.2.9 Begin the Worklist

4.2.3 Data Analysis

4.2.3.1 Open MassHunter Quantitative Analysis.

4.2.3.2 Create a New Batch.

4.2.3.3 Navigate to the MassHunter/Data directory and open the folder containing the data files for the current Batch. Assign a name to the Batch (e.g. 110808BZ), and select Open.

4.2.3.4 Add Samples to the batch. Any column rinse injections that will not contain meaningful results can be removed from the Add Samples list and or the batch.

4.2.3.5 Select Method/Open/Open and Apply from Existing File or batch.

4.2.3.6 Navigate to the location of the appropriate Quantitative Analysis Data Analysis Method, select it, and select Open.

4.2.3.7 When the method has been opened and applied, the Batch Table appearance will change, but the results will not yet be populated.

4.2.3.8 Select Analyze Batch, or F5, to complete the Batch analysis, and Save the Batch.

4.2.3.9 The Batch Table view will show the Batch Table with results, Compound Information, and the Calibration Curve. Navigation by Compound can be accomplished by using either the arrows or the drop-down menu in the Compound section of the Batch Table.

4.2.3.10 To update the retention times and qualifier ion ratios for the current Batch, go to Method/Edit, or use F10, to enter the Method Editor view of MassHunter Quantitative Analysis. Ratios should be updated using Average Qualifier ratios (LC/MS/MS). Review the retention times and qualifier ion ratios from the calibrators and make updates as appropriate. Save the updated method by selecting Save As, use the appropriate name format including the date.

4.2.3.11 To return to the Batch Table and apply the updated retention times and qualifier ion ratios, select the Exit button, answer Yes, and in the Batch Table select Analyze Batch, or F5.

4.2.4 General Batch Review *(Additional or more restrictive criteria may be included in the designated Analytical method)*

4.2.4.1 The lab criterion for acceptable calibration curve R^2 will be defined in the appropriate analytical method.

4.2.4.2 A **minimum of four calibration points** are required for a valid curve, unless different designation is noted in the applicable analytical method. If the confirmation decision point is removed from the curve, the new administrative cutoff will be the lowest calibrator that meets quality assurance requirements.

4.2.4.3 Outliers are highlighted in the Batch Table with the color codes blue and red, for below or above acceptable limits (respectively).

4.2.4.4 The default criterion for accuracy is that each calibrator result should agree with the target value ± 20%. There are special guidelines for values at or below 10 ng/mL. Those guidelines are laid out in the individual analytical methods.

4.2.4.5 The default criteria for a defining a positive result and reporting criteria are defined in the appropriate analytical methods.

4.2.4.6 Manual integration should not be needed frequently. When it is needed, it is enabled with the Start/End Manual Integration Tool in the Compound Information section of the Batch Table. Manual integration should only be used on the quantifier peak when there is an issue with the shape of the peak (the wrong peak is integrated, part of the peak is being cut off, there is tailing, the shape does not match that of the internal standard, etc.).

4.2.4.7 Manual integration is accomplished by left-clicking and dragging on the black boxes at peak start and end. (*Adjustments to the Retention time windows in the Data Analysis method should be made if excessive manual integration is needed.)*

4.2.4.8 Review the results for each analyte in the Batch. Check for outliers, R^2 values, and check QC values.

4.2.4.9 When Batch review is complete, Save the Batch.

4.2.5 Generating Reports

4.2.5.1 Select Report/Generate and navigate to the report method (Ex – ISP_Summary_07_LCMS_1Qual), select it, then select OK. Alternatively, the generated reports may be saved as electronic files (Ex – pdfs) and stored electronically per any requirements in the ISP-FS Quality Manual.

4.3 Addition of New Compounds to Existing Methods (LC/MS/MS and LC-QTOF)

4.3.1 Compounds that have been seen in numerous requests, have been seen in solid dosage casework, and/or are being reported at a high rate nationally may be considered for addition to the methods.

4.3.2 Determine transitions based on literature review or Optimizer results.

4.3.3 Dilute appropriately (for example ~ 10 µL of a 1 mg/mL solution standard in 1 mL of methanol.)

4.3.4 Prepare Instrument for acquisition as generally performed.

4.3.5 Load or create a new analyte MRM method using the current acquisition parameters for all but the QQQ tab. Set the analyte transitions in the appropriate location within the acquisition method.

4.3.6 Run each diluted analyte vial using the MRM method with the Optimizer or literature transitions.

4.3.7 Review data using Qualitative Data Analysis to determine Retention time of the analyte.

4.3.8 Add transitions into the currently used Acquisition method. Run the compound(s) using the acquisition method to confirm it does not interfere with the other compounds in the existing method.

4.3.9 Request addition of new analyte to the ToxBox plate order (Should only be completed by the person designated to order the plates).

4.3.10 Evaluate the calibration curves of each analyte, if acceptance criteria is met over a minimum of 3 batches, the new analytes may be added to the analytical method and the documentation shall be centrally stored by the discipline lead.

4.3.11 At no point should analytes being verified for addition be included in the analytical notes/documentation for case work. **Note:** A separate data analysis method should be used in the evaluation of new analytes.

4.4 Removing Analyte(s)

4.4.1 Compounds that have not been seen in requests, have not been seen in solid dosage casework, and/or are not being reported at a high rate nationally may be considered for removal from the method(s).

4.4.2 If detection of the analyte has not been seen over the specified time frame, recommendation for removal should be sent to the discipline lead.

4.4.3 The discipline lead shall review all documentation associated with the analyte removal and update the corresponding analytical method.

4.5 Quality Assurance Requirements

4.5.1 Refer to Toxicology AM #19 and AM #21 for additional quality assurance and reference material authentication requirements.

4.6 Analysis Documentation

4.6.1 Case results are to be recorded in the ILIMS system.

4.6.2 Reports for the batch and controls, if printed, will be stored centrally in the lab in which the analysis was performed. Electronic storage is also permitted in lieu of printed copies. A copy of data for controls may be stored electronically in a central location and need not be included in individual case files.

4.6.3 The data from the run will be stored electronically, and if it is on a computer, will be backed up at least every two months.

Toxicology AM #25: Blood and Urine Multi-Drug Screen by LCMS-QQQ

1.0 Background/References

1.1 References

1.1.1 This method was obtained from PinPoint Testing, LLC. PinPoint provided training and consultation on the method validation and implementation from June 2016 to November 2016.

2.0 Scope

2.1 This method is used to achieve rapid and accurate screening of multiple analytes in biological specimens. This method has simple sample preparation that uses isotopically-labeled internal standards.

2.2 The analytes included are listed in a separate document (maintained in Qualtrax) titled "Compounds Included in Analytical Methods".

3.0 Equipment/Reagents

3.1 Equipment and Supplies

3.1.1 Approved LC/MS/MS and MassHunter or equivalent software

3.1.2 Shaker/incubator

3.1.3 Positive Pressure Manifold

3.1.4 SPE dry or other heated nitrogen dryer

3.1.5 Phenomenex Phenyl Hexyl (4.6 x 50 mm; 2.6 µm) or equivalent column

3.1.6 Calibrated pipette for dispensing blood and/or urine samples

3.1.7 Single or Multi-channel pipettes for all other transfers and additions

3.1.8 ToxBox kit with 96 well plate containing internal standards, and controls, 96 well SLE+ plate, and 96 well blank sample preparation plate

3.1.9 Appropriate sized pipette tips

3.1.10 Heat sealing foil covers for 96 well plate

3.1.11 ACT plate cover for use during drying step with SPE dry (if run contains urine samples)

3.1.12 Additional 48 or 96-well plate (if run contains urine samples)

3.2 Reagents

See AM# 23 for Solution Preparation instructions

3.2.1 10 mM Ammonium Formate in Water (LCMS, Optima LCMS grade, or equivalent) (mobile phase A)

3.2.2 0.1% Formic Acid in Methanol (LCMS, Optima LCMS grade, or equivalent) (mobile phase B)

3.2.3 0.5 M Ammonium Hydroxide

3.2.4 Formic Acid (LCMS grade)

3.2.5 Water (LCMS, Optima LCMS grade, or equivalent)

3.2.6 Methanol (LCMS, Optima LCMS grade, or equivalent)

3.2.7 Ammonium Formate (LCMS, Optima LCMS grade, or equivalent)

3.2.8 Ammonium Hydroxide (ACS or higher)

3.2.9 Ethyl Acetate (ACS or higher)

3.2.10 20% Methanol in water (Reconstitution Solvent) (LCMS, Optima LCMS grade, or equivalent)

3.2.11 LC/MS Needle Rinse (40/40/20: Water/Methanol/Isopropanol or 75% MeOH in water)

3.2.12 Beta-glucuronidase (BGTurbo) (if run contains urine samples)

3.2.13 Instant Buffer I (provided with BGTurbo)

3.2.14 1% HCl in MeOH (if run contains urine samples)

3.3 Quality Assurance: Reference Materials and Controls

3.3.1 Internal Controls, and Internal Standards.

3.3.1.1 Internal standards are prepared by the ToxBox plate manufacturer and contained on the 96 well plate. If the run contains urine samples, a positive external or internal urine control must also be run.

3.3.2 A negative control will be run with each extraction. If the run contains urine samples, both a blood and urine negative control must be included. Urine controls are used for the evaluation of urine samples only.

4.0 Procedure

4.1 Extraction Procedure

4.1.1 Allow refrigerated or frozen samples and frozen components to reach ambient temperature.

4.1.2 Using a **calibrated** single channel pipette, add 250 µL of blood/urine into the appropriate wells of the analytical plate. For urine samples/controls, hydrolysis of the samples must occur prior to extracting. For urine hydrolysis: add 40 µl of BGTurbo and 100 µl of Instant Buffer I to the wells containing urine. Place covered plate on shaking incubator at approximately 900 rpm for at least 5 minutes.

4.1.3 Pipette 250 µL of 0.5 M ammonium hydroxide buffer into all wells in use on the analytical (standards) plate.

4.1.4 Place covered plate on shaking incubator at approximately 900 rpm for approximately 15 minutes.

4.1.5 Transfer 200-450 µL of blood+base and urine+base mixture (if applicable) to the corresponding wells of SLE+ plate.

4.1.6 Apply positive pressure for approximately 5 seconds or until no liquid remains on top of sorbent. Wait 5 minutes. (Recommended setting 15-50 PSI)

4.1.7 Add 900 µL of ethyl acetate and allow to flow through for approximately 5 minutes under gravity.

4.1.8 Apply positive pressure for approximately 10-15 seconds. (Recommended setting 12-15 PSI).

4.1.9 Add 900 µL of ethyl acetate and allow to flow through for approximately 5 minutes under gravity.

4.1.10 Apply positive pressure for approximately 10-15 seconds. (Recommended setting 12-15 PSI).

4.1.11 Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approximately 35^{0}C. Optional step: add 50 µl of 1% HCl in MeOH to all wells in the run and place ACT cover on top of plate prior to drying. The samples may also be dried in another type of heated nitrogen dryer (the temperature should be set at approximately 30-40^{0}C). Temperatures for this step are not critical and it is appropriate to use the equipment temperature readings. The temperatures do not need to be monitored or verified with a traceable thermometer. If this option is chosen, the samples will all be transferred to 10 mL glass centrifuge tubes, HCl added, and dried down in the dryer. The reconstitution step (below) will take place in the glass tubes and the samples will then be transferred back to the plate and sealed.

4.1.12 Reconstitute in 100 µL 20% methanol in water and heat seal plate with foil.

4.1.13 Analyze samples or freeze for future analysis. The plate must be injected within 7 days of the extraction date.

4.2 Instrument and Run set up.

4.2.1 See Toxicology AM #24 for instrument maintenance and operation.

4.2.2 Instrument run parameters and the acquisition methods for this analytical method will be printed out (paper or electronically) and stored centrally in the lab or in a common file folder electronically.

4.2.2.1 Acquisition Required Settings

4.2.2.1.1 Column Temperature - 35^{0}C

4.2.2.1.2 Injection volume – 0.5-10 µL

4.2.2.1.3 Mobile Phase Flow rate - 0.5 mL/min

4.2.2.1.4 Binary Pump Gradient Settings (Pump Time Table) (Note: the gradient parameters may be adjusted slightly but adequate separation of isobaric compounds must be demonstrated).

Time (min)	% Mobile A	% Mobile B
0	85	15
4	0	100
5	0	100
5.1	95	5
6	95	5

4.2.3 Worklist Set up should include internal control, negative control(s) and case samples.

4.3 Evaluation of Results

4.3.1 Minimum Criteria:

4.3.1.1 Signal to noise of primary transition greater than 5.

4.3.1.2 Signal to noise of secondary transition greater than 3.

4.3.1.3 In cases that do not meet the criteria above, the analyst may also evaluate peak Symmetry/Resolution and it is at their discretion to determine if the sample exhibits a positive response for that compound. Correct peak shape (as compared with QCs) and the sufficient resolution of potential interferents from the analyte of interest can be considered.

4.3.1.4 To ensure the response is adequate, each compound in the internal control will be evaluated using the criteria listed above. If a compound does not meet these standards, samples cannot be evaluated for that compound. At the analyst's discretion, that compound can be removed from the list of compounds that the sample was evaluated for, or the sample can be reinjected or re-extracted. If a sample needs to be reinjected, the analyst may add more of the reconstitution solvent to facilitate this. The internal standards must give an adequate response in order for the sample to be evaluated. If a sample needs to be reinjected but the mobile phase has run out, new mobile phase may be prepared, and the sample(s) may be reinjected. The negative control must also be reinjected to demonstrate that there was no contamination introduced with the new mobile phases.

4.3.1.5 Using the internal plate control, a 1-point calibration curve will be established. The curve will be set to linear, non-weighted and origin set to force. The concentration will be set to 10 for all compounds. This is not the actual concentration of the compounds, but a number used to compare sample responses.

4.3.1.6 Retention time criterion for peak identification is a ±2% or +/-0.100 min, whichever is greater, retention time window relative to the internal control and/or internal standards around the analyte's retention time. At the analyst's discretion when the analyte peak falls outside the retention time window and the internal standard shifts comparably that sample may be evaluated as positive if the other criteria are met.

4.3.1.7 Samples should have a significant internal standard response. If a sample has little to no internal standard response for a compound, an evaluation for that sample cannot be made. The analyst will need to reinject the sample, remove that drug from the list of drugs that sample was screened for or re-extract the sample.

4.3.1.8 Case samples and negative controls will generally be considered negative if the calculated concentration is less than 5. Samples with a calculated concentration between 2 and 5 may be evaluated as positive at the analyst's discretion. If calculated concentration is less than 10 for methamphetamine (in blood) it may be evaluated as negative. Calculated concentrations for amphetamine, codeine, methamphetamine, morphine, and pseudoephedrine of less than 50 (in urine) may be evaluated as negative.

4.3.1.9 If a compound in a sample meets the minimum requirements but displays characteristics that are inconsistent with the calibrator (i.e. the ratio is significantly different, the retention time is barely within the allotted window, etc.) it is at the discretion of the analyst to evaluate that compound as negative for that sample, provided they detail an explanation in the case notes.

4.3.2 Limitations of Method

4.3.2.1 Samples flagged as positive for Maprotiline and Amitriptyline will be reported as Amitriptyline/Maprotiline.

4.3.2.2 Samples that indicate as positive for Benzoylecgonine will be reported as Benzoylecgonine/Cocaine break-down product (or other wording that indicates that the response may be from a breakdown of cocaine).

4.3.2.3 This method is a preliminary screen. The screen results are an indication of drugs that may be present in a sample. The weight or confidence in screening results cannot be given the same as a confirmatory test. This is not a quantitative method and quantitative results cannot be reported from this method. The quantitative aspects of the method are only for establishing a minimum response threshold. If these results are reported it must be clear on the report that it is an indicator the drug is present in the sample, not a confirmation of the drug in the sample.

4.4 Quality Assurance Requirements

4.4.1 Refer to Toxicology AM #19 and AM #21 for additional quality assurance and reference material authentication requirements.

4.5 Analysis Documentation

4.5.1 The printed results for each case sample will be included with the analyst's notes. Case results are to be recorded in the ILIMS system.

4.5.2 The printout (paper or electronic) for the negative control and internal control will be stored centrally in the lab in which they were performed or stored on a network drive.

4.5.3 The data from the run will be stored electronically, and if it is on a computer, will be backed up at least every two months.

Toxicology AM #26: Blood and Urine THC and Metabolites Screen by LCMS-QQQ

1.0 Background/References

1.1 Background

Δ^9-THC (Figure 1) is the chief psychoactive cannabinoid resulting from exposure to marijuana. Δ^9-THC has a peak blood concentration within 5 to 15 minutes following smoking of a marijuana cigarette.[4,5,6] This blood concentration drops rapidly after cessation of smoking.[4,5] The level may fall to less than 5 ng within 30 to 60 minutes, although longer detection times have been reported.[4,5] Detection of low dose (1.75%) post-smoking Δ^9-THC has been reported to vary from 3 to 12 hours.[5] This detection window was based on a limit of quantitation of 0.5 ng/mL. The number, duration, spacing of puffs, hold time, and inhalation volume all impact the degree of drug exposure and thus bioavailability.[5] Longer detection times have been observed for frequent users. Δ^9-THC is metabolized to an active metabolite, 11-hydroxy-tetrahydrocannabinol (Hydroxy-THC, THC-OH), and an inactive metabolite, 11-nor-9-carboxy-tetrahydrocannabinol (carboxy-THC). The concentration of the Δ^9-THC inactive metabolite, carboxy-THC, gradually increases and may plateau for several hours.[5] There is poor correlation between blood Δ^9-THC and psychoactive effects since the Δ^9-THC concentrations begin to decline prior to the time of peak effects.[4,5,6] The detection window for the active analytes is much shorter than that of carboxy-THC.[7] Cannabinol and cannabidiol are minor cannabinoids also found in marijuana.[8,9] Due to their low abundance, rapid clearance from blood, and similar pharmacokinetic profiles in chronic versus occasional smokers, the presence of cannabidiol and cannabinol in blood has been suggested to be a sufficient but unnecessary marker for recent marijuana use.[8]

Negative behavioral effects reported from exposure to marijuana include altered time perception, lack of concentration, and impaired learning and memory which can lead to impairment of cognitive and performance tasks.[5] Establishing impairment in an individual is based on evaluation of all available information in conjunction with toxicology results.

For additional background, refer to Toxicology AM #3, obsolete analytical method 3.10.1, and the provided references.

1.2 References

1.2.1 This method was obtained from PinPoint Testing, LLC. The method validation and implementation was completed by ISPFS personnel from October 2016 to November 2016.

1.2.2 [(4)]Standard Operating Procedure for Blood SPE THC and Carboxy-THC GC/MSD Assay, Edmonton, Canada Office of the Chief Medical Examiners, 2003.

1.2.3 [(5)] Huestis, M.A., *Cannabis (Marijuana) - Effects on Human Behavior and Performance,* Forensic Science Rev. 14(1/2): 16-60, 2002.

1.2.4 [6]Drummer, O.H., *Cannabis*, pp. 178-212. In*:* The Forensic Pharmacology of Drugs of Abuse, Arnold: London, 2001.

1.2.5 [7]Huestis, M. *Marijuana*. pp. 229-244. In*:* Principles of Forensic Toxicology, Second Edition. Levine, B. ed., AACC, 2003.

1.2.6 [8]Desrosiers, N.A.; Himes, S.K.; Scheidweiler, K.B.; Concheiro-Guisan, M.; Huestis, M.A. *Phase I and II Cannbinoid Disposition in Blood and Plasma of Occasional and Frequent Smokers Following Controlled Smoked Cannabis.* Clinical Chemistry, 60:4, pp. 631-643, 2014.

1.2.7 [9]Nadulski, T., et al. *Simultaneous and Sensitive Analysis of THC, 11-OH0THC, THC-COOH, CBD, and CBN by GC-MS in Plasma after Oral Application of Small Doses of THC and Cannabis Extract*. Journal of Analytical Toxicology, Vol 29, pp. 782-789, November/December 2005.

2.0 Scope

2.1 This method is used to achieve rapid and accurate screening of cannabinoids in biological specimens. This method has simple sample preparation that uses isotopically-labeled internal standards.

2.2 The analytes included are as follows:

Analyte	Administrative Threshold
THC	3 ng/mL
Carboxy-THC	10 ng/mL
THC-OH	3 ng/mL

3.0 Equipment/Reagents

3.1 Equipment and Supplies

3.1.1 Approved LC/MS/MS and MassHunter or equivalent software

3.1.2 Shaking incubator

3.1.3 ToxBox kit with 48 Well plate containing internal standards and controls, 48 well SLE+ plate, and 48 well blank sample collection plate.

3.1.4 Test tube rocker

3.1.5 Calibrated pipette for dispensing blood and/or urine samples

3.1.6 Single or Multi-channel pipettes for all other transfers and additions

3.1.7 Positive Pressure Manifold

3.1.8 SPE sample evaporator concentrator or other heated nitrogen dryer

3.1.9 ACT plate cover for use during drying step with SPE dry (if run contains urine samples)

3.1.10 Additional 48-well plate (if run contains urine samples)

3.1.11 Phenomenex Phenyl Hexyl (4.6 x 50 mm; 2.6 µm) or equivalent column

3.2 Reagents

Refer to Toxicology AM #23 for Solution Preparation instructions.

3.2.1 0.1% Formic Acid in Water (LCMS, Optima LCMS grade, or equivalent)

3.2.2 10 mM Ammonium Formate in Water (LCMS, Optima LCMS grade, or equivalent) (mobile phase A)

3.2.3 0.1% Formic Acid in Methanol (LCMS, Optima LCMS grade, or equivalent) (mobile phase B)

3.2.4 Methyl Tert-Butyl Ether (MTBE) 99.9%

3.2.5 Hexanes (ACS)

3.2.6 Methanol (LCMS, Optima LCMS grade, or equivalent)

3.2.7 LC/MS Needle Rinse (40/40/20: Water/Methanol/Isopropanol or 75% MeOH in water)

3.2.8 1 N KOH (if run includes urine samples and chemical hydrolysis is chosen)

3.2.9 Saturated Phosphate Buffer (if run includes urine samples and chemical hydrolysis is chosen)

3.2.10 Beta-glucuronidase (BGTurbo) (if run contains urine samples and enzymatic hydrolysis is chosen)

3.2.11 Instant Buffer I (provided with BGTurbo) (if run contains urine samples and enzymatic hydrolysis is chosen)

3.3 Quality Assurance: Reference Materials, Controls, and Blanks

3.3.1 Plate controls and internal standards are prepared by the ToxBox Manufacturer and are contained on the 48 well plate. A minimum of one internal positive plate control will be included in each run. A minimum of three calibrators will be run to establish a calculated response value for the case samples. For THC and THC-OH, a 3ng/mL or 5ng/mL calibrator must be included in the run. For C-THC, a 5ng/mL or 10ng/mL calibrator must be included in the run.

3.3.2 A negative control will be run with each extraction. If the run contains urine samples, a negative urine control and internal or external positive urine control must also be included. Urine controls are used for the evaluation of urine samples only.

4.0 Procedure

4.1 Extraction Procedure

4.1.1 Allow refrigerated or frozen samples and frozen components to reach ambient temperature.

4.1.2 Urine samples must be hydrolyzed prior to extraction. Only the urine samples/controls will be hydrolyzed. There are two different options for hydrolysis.

- Chemical hydrolysis: Add 1.5 mL of blank urine to a clean 48-well plate. Add 250 µL of 1 N KOH to the wells containing urine. Place a cover on the plate and shake at 900 rpm for approximately 15 minutes at 40^0C. Proceed to step 4.1.3.

- Enzymatic hydrolysis: using a **calibrated** pipette, add 1mL of urine to the appropriate wells of the analytical plate (case samples, negative, QC samples). To the wells containing urine, add 100ul of BG Turbo and 200ul of BG Turbo buffer then proceed to step 4.1.4.

4.1.3 Using a **calibrated** single channel pipette, add 1.0 mL of blood/urine into the appropriate wells of the analytical plate. If chemical hydrolysis was done, transfer 1.0 mL of hydrolyzed urine from the plate the hydrolysis was performed in to the appropriate wells of the analytical plate.

4.1.4 Add 0.5 mL 0.1% formic acid in LCMS water to all of the wells containing blood (and urine if the enzymatic hydrolysis option was chosen). Add 0.5 mL of saturated phosphate buffer to all of the wells containing urine (if the chemical hydrolysis option was chosen).

4.1.5 Place covered plate on shaking incubator at approximately 900 rpm for approximately 15 minutes.

4.1.6 Transfer 700-800 µL of blood+acid or urine+acid mixture (if applicable) to the corresponding wells of the SLE+ plate.

4.1.7 Apply positive pressure for approximately 10-15 seconds or until no liquid remains on top of sorbent. *(Recommended setting for Positive Pressure manifold for this loading step is between 15-50 PSI)*

4.1.9 Wait 5 minutes for sample to completely absorb.

4.1.9 Add 2.25 mL MTBE and allow to flow for 5 minutes under gravity. *This should be completed in increments, as the sample well accommodates a maximum volume of 1 mL. (Recommended for this step is: three (3) repetitions of adding 750* µL*)*

4.1.10 Apply positive pressure to complete elution- approximately 15 seconds. *(Positive Pressure manifold set between 12-15 PSI)*

4.1.11 Add 2.25 mL hexane and allow to flow for 5 minutes under gravity. *This should be completed in increments, as the sample well accommodates a maximum volume of 1 mL. (Recommended for this step is: three (3) repetitions of adding 750 µL)*

4.1.12 Apply positive pressure to complete elution- approximately 15 seconds. *(Positive Pressure manifold set between 12-15 PSI)*

4.1.13 Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approximately 35^{0}C. Temperatures for this step are not critical and it is appropriate to use the equipment temperature readings. The temperature does not need to be monitored or verified with a traceable thermometer. The samples may also be dried in another type of heated nitrogen dryer (the temperature should be set at approximately 30-40^{0}C). If this option is chosen, the samples will all be transferred to 10 mL glass centrifuge tubes and dried down in the dryer. The reconstitution step (below) will take place in the glass tubes and the samples will then be transferred back to the plate and sealed.

4.1.14 Reconstitute each sample with 100 µL LCMS Grade Methanol. Heat seal plate with foil to prevent evaporation.

4.1.15 Analyze samples or freeze for future analysis. The plate must be injected within 7 days of the extraction date.

4.2 Instrument and Run set up.

4.2.1 ***See Toxicology AM #24 for instrument maintenance and operation.***

4.2.2 Instrument run parameters and the acquisition methods for this Analytical Method will be printed out (paper or electronically) and stored centrally in the lab or in a common file folder electronically.

4.2.2.1 Acquisition Required Settings

4.2.2.1.1 Column Temperature - 35°C

4.2.2.1.2 Injection volume – 0.5-10 µL

4.2.2.1.3 Mobile Phase Flow rate - 0.6 mL/min

4.2.2.1.4 Binary Pump Gradient Settings (Pump Time Table) (Note: the gradient parameters may be adjusted slightly but adequate separation of the compounds must be demonstrated).

Time (min)	% Mobile A	% Mobile B
0	55	45
3.0	5	95
4.5	5	95
4.51	50	50
6	50	50

4.2.3 Worklist Set up should include a positive control, calibrators, negative control, and case samples.

4.3 Evaluation of Results

4.3.1 Minimum Criteria:

4.3.1.1 The lab criterion for acceptable calibration curve R^2 is >0.98

4.3.1.2 A sample or control with a concentration of 3 ng/mL or greater for THC or THC-OH, or 10 ng/mL or greater for carboxy-THC will be considered positive for cannabinoids. Samples and controls with a carboxy-THC response between 5 and 10 ng/mL may be evaluated as positive at the analyst's discretion, samples and controls with a THC response between 1 and 3 ng/ml may be evaluated as positive at the analyst's discretion.

4.3.1.3 Retention time for a control or sample must be within +/- 2% or +/-0.100 min., whichever is greater, of the average retention time of the calibrators. At the analyst's discretion when the analyte peak falls outside the retention time window and the internal standard shifts comparably that control or sample may be evaluated as positive if the other criteria are met.

4.3.1.4 The accuracy for calibrators that are included in the curve must be within +/- 30%).

4.3.1.5 To ensure the response is adequate, each compound in the internal control will be evaluated using the criteria listed above. If a compound does not meet these standards, samples cannot be evaluated for that compound. At the analyst's discretion, that compound can be removed from the list of compounds that the sample was evaluated for, or the sample can be reinjected or re-extracted. If a sample needs to be reinjected, the analyst may add more of the reconstitution solvent to facilitate this. The internal standards must give an adequate response in order for the sample to be evaluated. If a sample needs to be reinjected but the mobile phase has run out, new mobile phase may be prepared, and the sample(s) may be reinjected. The negative control must also be reinjected to demonstrate that there was no contamination introduced with the new mobile phases.

4.3.1.6 Controls and samples should have a significant internal standard response, if a control or sample has little to no internal standard response for a compound, an evaluation for that control or sample cannot be made. If it is a sample, the analyst will need to re-inject or re-extract that sample. If it is a control, it can be re-injected, but if there is no marked improvement, then the control cannot be evaluated as passing.

4.3.1.7 In cases that do not meet the minimum criteria, the analyst may also evaluate peak symmetry/resolution and it is at their discretion to determine if the sample exhibits a positive response for that compound. Correct peak shape (as compared with QCs) and the sufficient resolution of potential interferents from the analyte of interest can be considered.

4.3.1.8 If a compound in a sample meets the minimum requirements but displays characteristics that are inconsistent with the calibrator(s) (for example, the retention time is barely within the allotted window, etc.) it is at the discretion of the analyst to evaluate that compound as negative for that sample, provided they detail an explanation in the case notes.

4.4 Quality Assurance Requirements

4.4.1 Refer to Toxicology AM #19 and AM #21 for additional quality assurance and reference material authentication requirements.

4.5 Analysis Documentation

4.5.1 The printed results for each case sample will be included with the analyst's notes. Case results are to be recorded in the ILIMS system.

4.5.2 The printout (paper or electronic) for the calibrators and controls will be stored centrally in the lab in which they were performed or stored on a network drive.

4.5.3 The data from the run will be stored electronically, and if it is on a computer, will be backed up at least every two months.

4.6 Limitation of method

4.6.1 This method is a preliminary screen; the screen results are an indication that cannabinoids **may** be present in a sample. The weight or confidence in screening results cannot be given the same as a confirmatory test. This is not a quantitative method and quantitative values cannot be inferred or reported from this method. The quantitative aspects of this method are only for establishing a minimum response threshold. If these results are reported it must be clear on the report that it is an indicator the drug is potentially present in the sample, not a confirmation of the drug in the sample.

Toxicology AM #27: Quantitative Analysis of THC and Metabolites in Blood and Urine by LCMS-QQQ

1.0 Background/References

1.1 Background

Δ^9-THC (Figure 1) is the chief psychoactive cannabinoid resulting from exposure to marijuana. Δ^9-THC has a peak blood concentration within 5 to 15 minutes following smoking of a marijuana cigarette.[4,5,6] This blood concentration drops rapidly after cessation of smoking.[4,5] The level may fall to less than 5 ng within 30 to 60 minutes, although longer detection times have been reported.[4,5] Detection of low dose (1.75%) post-smoking Δ^9-THC has been reported to vary from 3 to 12 hours.[5] This detection window was based on a limit of quantitation of 0.5 ng/mL. The number, duration, spacing of puffs, hold time, and inhalation volume all impact the degree of drug exposure and thus bioavailability.[5] Longer detection times have been observed for frequent users. Δ^9-THC is metabolized to an active metabolite, 11-hydroxy-tetrahydrocannabinol (Hydroxy-THC, THC-OH), and an inactive metabolite, 11-nor-9-carboxy-tetrahydrocannabinol (carboxy-THC). The concentration of the Δ^9-THC inactive metabolite, carboxy-THC, gradually increases and may plateau for several hours.[5] There is poor correlation between blood Δ^9-THC and psychoactive effects since the Δ^9-THC concentrations begin to decline prior to the time of peak effects.[4,5,6] The detection window for the active analytes is much shorter than that of carboxy-THC.[7] Negative behavioral effects reported from exposure to marijuana include altered time perception, lack of concentration, and impaired learning and memory which can lead to impairment of cognitive and performance tasks.[5] Establishing impairment in an individual is based on evaluation of all available information in conjunction with toxicology results.

For additional background, refer to Toxicology AM #3 and obsolete analytical methods 3.10.1, as well as provided references.

1.2 References

1.2.1 This method was obtained from PinPoint Testing, LLC. PinPoint provided training and consultation on the method validation and implementation from June 2016 to October 2016.

1.2.2(4) Standard Operating Procedure for Blood SPE THC and Carboxy-THC GC/MSD Assay, Edmonton, Canada Office of the Chief Medical Examiners, 2003.

1.2.3(5) Huestis, M.A., *Cannabis (Marijuana) - Effects on Human Behavior and Performance,* Forensic Science Rev. 14(1/2): 16-60, 2002.

1.2.4 (6)Drummer, O.H., *Cannabis*, pp. 178-212. *in:* The Forensic Pharmacology of Drugs of Abuse, Arnold: London, 2001.

1.2.5(7) Huestis, M. *Marijuana*. pp. 229-244. *in:* Principles of Forensic Toxicology, Second Edition. Levine, B. ed., AACC, 2003.

1.2.6 [8]Desrosiers, N.A.; Himes, S.K.; Scheidweiler, K.B.; Concheiro-Guisan, M.; Huestis, M.A. *Phase I and II Cannbinoid Disposition in Blood and Plasma of Occasional and Frequent Smokers Following Controlled Smoked Cannabis.* Clinical Chemistry, 60:4, pp. 631-643, 2014.

1.2.7 [9]Nadulski, T., et al. *Simultaneous and Sensitive Analysis of THC, 11-OH0THC, THC-COOH, CBD, and CBN by GC-MS in Plasma after Oral Application of Small Doses of THC and Cannabis Extract*. Journal of Analytical Toxicology, Vol 29, pp. 782-789, November/December 2005.

2.0 Scope

2.1 This method is used for the confirmation of Δ^9-THC, 11-nor-Δ^9-THC-9-COOH (Carboxy-THC) and 11-hydroxy-Δ^9-THC (Hydroxy-THC) in blood and urine. The words calibrator and calibration are used to coincide with the terminology in instrument software and manufacturer manuals. The manufacturer's term calibrator refers to what is considered by ISPFS as reference material that has a certified concentration of drug present.

3.0 Equipment/Reagents

3.1 Equipment and Supplies

3.1.1 Approved LC/MS/MS and MassHunter or equivalent software

3.1.2 Shaking incubator

3.1.3 ToxBox kit with 48 Well plate containing internal standards and controls, 48 well SLE+ plate, and 48 well blank sample collection plate.

3.1.4 Test tube rocker

3.1.5 Calibrated pipettes for dispensing blood and/or urine samples.

3.1.6 Pipettes for all other transfers and additions.

3.1.7 Positive Pressure Manifold

3.1.8 SPE sample evaporator concentrator or other heated nitrogen dryer

3.1.9 UCT Selectra DA 100 x 2.1 mm, 3um or equivalent LC Column

3.1.10 ACT plate cover for use during drying step with SPE dry (if run contains urine samples)

3.1.11 Additional 48-well plate (if run contains urine samples)

3.2 Reagents

Refer to Toxicology AM #23 for Solution Preparation instructions.

3.2.1 0.1% Formic Acid in Water (LCMS, Optima LCMS grade, or equivalent) (mobile phase A)

3.2.2 0.1% Formic Acid in Acetonitrile (LCMS, Optima LCMS grade, or equivalent) (mobile phase B)

3.2.3 Methyl Tert-Butyl Ether (MTBE) 99.9%

3.2.4 Hexanes (ACS)

3.2.5 Methanol (LCMS, Optima LCMS grade, or equivalent)

3.2.6 LC/MS Needle Rinse (40/40/20: Water/Methanol/Isopropanol or 75% MeOH in water)

3.2.7 1 N KOH (if run includes urine samples and chemical hydrolysis is chosen)

3.2.8 Saturated Phosphate Buffer (if run includes urine samples and chemical hydrolysis is chosen)

3.2.9 Beta-glucuronidase (BGTurbo) (if run contains urine samples and enzymatic hydrolysis is chosen)

3.2.10 Instant Buffer I (provided with BGTurbo) (if run contains urine samples and enzymatic hydrolysis is chosen)

3.3 Quality Assurance: Reference Materials and Controls

3.3.1 Plate controls and internal standards are prepared by the ToxBox vendor and are contained on the 48 well plate. A minimum of one positive plate control for each drug compound will be included in each run.

3.3.2 A negative control will be run with each extraction. If the run contains urine samples, a negative urine control and positive internal or external urine control will also be included in the run. Urine controls are used for the evaluation of urine samples only.

3.3.3 A non-extracted blank containing internal standard will be run directly before each case sample to rule out carryover. If confirmation criteria (e.g. ion ratios, RT, S/N) are not met, the analyte is not considered present. If confirmation criteria is met, the response of the blank will be considered. The area response of the blank preceding a sample must be at least 10 times less than any compound confirmed in the case sample and must be below the limit of confirmation for any analyte confirmed in the case sample to be considered negative. If a blank does contain a compound, the blank and the sample can be reinjected.

3.3.3.1 To prepare blank, dilute 1-part ISTD (obtained from a blank well in the extraction plate) in 4-5 parts LCMS grade methanol.

3.3.4 Samples must be bracketed by QC samples. It is at the discretion of the analyst to choose which controls to run. If the QC run either directly before the samples or directly after fails to inject or has an injection issue, it may be reinjected. If the QC cannot be used, the run will need to be re-extracted. Reinjected samples (and calibrators) do not need to be bracketed, but a QC should be run after the reinjected sample(s).

4.0 Procedure

4.1 Extraction Procedure

4.1.1 Allow refrigerated or frozen samples and frozen components to reach ambient temperature. Urine samples that have shown high responses in the screening methods may be diluted with negative urine prior to being extracted. The same negative urine used for the dilution should be used for the negative control. It is at the analyst's discretion as to what the dilution should be. Any dilutions made must be documented in the case notes.

4.1.2 Urine samples must be hydrolyzed prior to extraction. Only the urine samples/controls will be hydrolyzed. There are two different options for hydrolysis.

- Chemical hydrolysis: Add 1.5 mL of blank urine to a clean 48-well plate. Add 250 µL of 1 N KOH to the wells containing urine. Place a cover on the plate and shake at 900 rpm for approximately 15 minutes at 40^0C. Proceed to step 4.1.3.
- Enzymatic hydrolysis: using a **calibrated** pipette, add 1mL of urine to the appropriate wells of the analytical plate (case samples, negative, QC samples). To the wells containing urine, add 100ul of BG Turbo and 200ul of BG Turbo buffer then proceed to step 4.1.4.

4.1.3 Using a **calibrated** single channel pipette, add 1.0 mL of blood/urine into the appropriate wells of the analytical plate. If chemical hydrolysis was done, transfer 1.0 mL of hydrolyzed urine from the plate the hydrolysis was performed in to the appropriate wells of the analytical plate.

4.1.4 Add 0.5 mL 0.1% formic acid in LCMS water to all of the wells containing blood (and urine if the enzymatic hydrolysis option was chosen). Add 0.5 mL of saturated phosphate buffer to all of the wells containing urine (if the chemical hydrolysis option was chosen).

4.1.5 Place covered plate on shaking incubator at approximately 900 rpm for approximately 15 minutes.

4.1.6 Transfer 700- 800 µL of blood+acid or urine+acid mixture (if applicable) to the corresponding wells of the SLE+ plate.

4.1.7 Apply positive pressure for approximately 10-15 seconds or until no liquid remains on top of sorbent. *(Recommended setting for Positive Pressure manifold for this loading step is between 15-50 PSI)*

4.1.8 Wait 5 minutes for sample to completely absorb.

4.1.9 Add 2.25 mL MTBE and allow to flow for 5 minutes under gravity. This should be completed in increments, as the sample well accommodates a maximum volume of 1 mL. (Recommended for this step is: three (3) repetitions of adding 750 µL)

4.1.10 Apply positive pressure to complete elution- approximately 10-15 seconds. *(Positive Pressure manifold set between 12-15 PSI)*

4.1.11 Add 2.25 mL hexane and allow to flow for 5 minutes under gravity. This should be completed in increments, as the sample well accommodates a maximum volume of 1 mL. (Recommended for this step is: three (3) repetitions of adding 750 µL)

4.1.12 Apply positive pressure to complete elution- approximately 10-15 seconds. *(Positive Pressure manifold set between 12-15 PSI)*

4.1.13 Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approximately 35^{0}C. The samples may also be dried in another type of heated nitrogen dryer (the temperature should be set at approximately 30-40^{0}C). Temperatures for this step are not critical and it is appropriate to use the equipment temperature readings. The temperature does not need to be monitored or verified with a traceable thermometer. If this option is chosen, the samples will all be transferred to 10 mL glass centrifuge tubes and dried down in the dryer. The reconstitution step (below) will take place in the glass tubes and the samples will then be transferred back to the plate and sealed.

4.1.14 Reconstitute each sample with 100 µL LCMS grade Methanol. Heat-seal plate with foil to prevent evaporation.

4.1.15 Analyze samples or freeze for future analysis. The plate must be injected within 7 days of the extraction date.

4.2 Instrument and Run set up

4.2.1 See Toxicology AM #24 for LCMS instrument maintenance and operation.

4.2.2 Set up the worklist in MassHunter.

4.2.3 A solvent blank is generally run before the calibration curve, then the calibration curve is run, then a negative control.

4.2.4 A non-extracted or extracted solvent blank will be run directly before a case sample. A sample run immediately after a negative control does not require a solvent blank immediately before it.

4.2.5 Instrument run parameters and the acquisition methods for this Analytical Method will be printed out (paper or electronically) and stored centrally in the lab or in a common file folder electronically.

4.2.5.1 Acquisition Required Settings

4.2.5.1.1 Column Temperature - 50^{0}C

4.2.5.1.2 Injection volume –0.5-10 µL

4.2.5.1.3 Mobile Phase Flow rate - 0.6 mL/min

4.2.5.1.4 Binary Pump Gradient Settings (Pump Time Table) (Note: the gradient parameters may be adjusted slightly as long as it is demonstrated that the compounds can still be distinguished from one another).

Time (min)	% Mobile A	% Mobile B
0	40	60
3.5	40	60
3.51	5	95
4.5	5	95
4.51	40	60

4.3 Evaluation of Results

4.3.1 Batch Review

4.3.1.1 The lab criterion for acceptable calibration curve R^2 is >0.98

4.3.1.2 Calibration curves for compounds reported quantitatively (Δ^9-THC and 11-hydroxy-Δ^9-THC (Hydroxy-THC)) shall be set to linear with a 1/x weighting. Calibration curves for qualitatively reported compounds 11-nor-Δ^9-THC-9-COOH (Carboxy-THC) shall be at the analyst's discretion.

4.3.1.3 The limits of detection (LOD) are as follows:

Analyte	LOD
THC	1 ng/mL
Carboxy-THC	5 ng/mL
THC-OH	3 ng/mL

4.3.2 The default criteria for a positive result are:

4.3.2.1 The control or sample must have a concentration no less than the established LOD for that analyte.

4.3.2.2 A minimum of 4 consecutive points must be included in the calibration curve in order to report a compound qualitatively. All the qualitative confirmation criteria must be met for all calibrators included in the curve.

4.3.2.3 The analyte retention time must be within +/- 3% or +/- 0.100 min., whichever is greater, of the average retention time of the calibrators for that analyte. At the analyst's discretion when the analyte peak falls outside the retention time window and the internal standard shifts comparably that control or sample may be evaluated as positive if the other criteria are met.

4.3.2.4 The ion ratios for selected transitions must fall within 20% of the averaged calibrators for each run.

4.3.2.5 The S/N of detected analytes must be greater than 10.

4.3.2.6 If any points are dropped from the approved quantitative range of the curve, the compound will be reported qualitatively. The quantitative range for THC is 3-100ng/mL. Samples less than 3ng/mL but greater than 1ng/mL will be reported qualitatively (if the 1ng/mL calibrator is included in the curve). The uncertainty of measurement budgets for each compound have been established and will be evaluated annually by the discipline lead.

4.3.2.7 All the qualitative confirmation criteria must be met for the calibrators included in the curve. In addition to the criteria for confirmation, the accuracy for all controls (QC's) and calibrators for that compound, that are included in the curve, must be within the appropriate tolerances (+/- 20%). The allowable accuracy for calibrators and controls with a target concentration of 10ng/mL and less is +/- 30%. However, if the accuracy for a 10ng/mL or less calibrator or control is between +/- 20% and +/- 30%, then the compound will be reported qualitatively between that point and the cutoff. If all quantitative criteria are met for the remaining points, the compound will be reported quantitatively. The highest and lowest calibrators may be dropped due to linearity, even if the accuracy parameters are met for those calibrators. If a control or QC falls outside the accuracy range, at the analyst's discretion, the compound may be reported qualitatively.

4.3.2.8 Samples with a higher response than the 100 ng/mL will be reported out as greater than 100 ng/mL. The uncertainty of measurement has been evaluated for THC and THC-OH. The estimated expanded uncertainty will only be reported when it impacts evaluation of a statute, legal requirement, or upon customer request. When the measurement uncertainty is reported it will be on the report in the same units as the measurement and a statement regarding the coverage probability of 99.73% (k=3) will also be on the report. The current expanded uncertainty is published as a protected document is in Qualtrax and is available to all analysts.

4.3.2.9 Manual integration shall not be done on quantifier peaks for the purposes of getting the accuracy to fall within tolerances. Some situations may arise where manual integration is necessary.

4.3.2.10 The negative control will be evaluated as passing if the compound of interest does not meet all of the above listed criteria and the internal standard gives an anticipated response to demonstrate the sample extracted and injected correctly.

4.3.2.11 Samples must be bracketed by QC samples. It is at the discretion of the analyst to choose which controls to run. If the QC run either directly before the samples or directly after fails to inject or has an injection issue, it may be reinjected. If the QC cannot be used, the run will need to be re-extracted. Reinjected case samples do not need to be bracketed, but a QC should be run after the reinjected sample(s). Reinjected QC's are used to evaluate and set limitations for reinject samples only. If sample(s) need to be reinjected but the mobile phase has run out, new mobile phase may be prepared and the sample(s) may be reinjected. The negative control must also be reinjected. The negative control and cases sample(s) must be bracketed by QC samples.

4.3.2.12 Unless a valid reason is noted, all QC's greater than or equal to the cutoff must meet the minimum criteria for confirmation in order for a compound to be evaluated. If a QC does not meet the minimum requirements and a valid reason is not noted, the compound may not be evaluated. An example of a valid reason to not evaluate a QC would be if the response is poor for that compound and/or the QC is below the limit of confirmation for that run. A notation will be made in the case record if a compound is not being evaluated.

4.3.2.13 To ensure the response is adequate, each compound in the internal control will be evaluated using the criteria listed above. If a compound does not meet these standards, samples cannot be evaluated for that compound. At the analyst's discretion, that compound can be removed from the list of compounds that the sample was evaluated for, or the sample can be reinjected or re-extracted. If a sample needs to be reinjected, the analyst may add more of the reconstitution solvent to facilitate this. The internal standards must give an adequate response in order for the sample to be evaluated.

4.3.2.14 Calibrators and QCs should not be reinjected if the only issue with the injection is that the accuracy is outside of the specified parameters.

4.3.2.15 No quantitative values will be reported for urine cases.

4.3.2.16 Any limitations to curve ranges and or evaluation of compounds should be noted.

4.4 Quality Assurance Requirements

4.4.1 Refer to Toxicology AM #19 and AM #21 for additional quality assurance and reference material authentication.

4.5 Analysis Documentation

4.5.1 The printed results for each case sample and accompanying blank will be included with the analysts' notes. Case results will be recorded in the ILIMS system.

4.5.2 The reports for the batch, including calibration curves and controls will be stored centrally in the lab or electronically on a common drive.

4.5.3 The raw data from the run will be stored electronically and will be backed up at least every two months.

4.5.4 Results of the controls will be entered onto the Control Monitoring Chart.

Toxicology AM #28: Blood and Urine Multi-Drug Confirmatory Analysis by LCMS-QQQ

1.0 Background/References

1.1 Background

1.1.1 This method was obtained from PinPoint Testing, LLC. PinPoint provided training and consultation on the method validation and implementation from June 2016 to November 2016.

2.0 Scope

2.1 This method is used to achieve rapid and accurate confirmation of multiple analytes in biological specimens. This method has simple sample preparation that uses isotopically-labeled internal standards.

2.2 The analytes included are listed in a separate document (maintained in Qualtrax) titled "Compounds Included in Analytical Methods".

3.0 Equipment/Reagents

3.1 Equipment and Supplies

3.1.1 Approved LC/MS/MS and MassHunter or equivalent software

3.1.2 Shaker/incubator

3.1.3 Positive Pressure Manifold

3.1.4 SPE dry or other heated nitrogen dryer

3.1.5 Agilent 120 EC-C18 (2.1 x 100-4 um) or equivalent LC column

3.1.6 Calibrated pipettes for dispensing blood and/or urine samples.

3.1.7 Pipettes for all other transfers and additions

3.1.8 Appropriate sized pipette tips

3.1.9 ToxBox kit with 96 well plate containing internal standards, calibrators, and controls, 96 well SLE+ plate and 96 well blank sample preparation plate.

3.1.10 Heat sealing foil covers

3.1.11 ACT plate cover for use during drying step with SPE dry (if run contains urine samples)

3.1.12 Additional 48 or 96-well plate (if run contains urine samples)

3.2 Reagents

3.2.1 5 mM Ammonium Formate + 0.01% Formic Acid in Water (LCMS, Optima LCMS grade or equivalent) (mobile phase A)

3.2.2 0.01% Formic Acid in Methanol (LCMS, Optima LCMS grade, or equivalent) (mobile phase B)

3.2.3 0.5 M Ammonium Hydroxide

3.2.4 Formic Acid (LCMS, Optima LCMS grade, or equivalent)

3.2.5 Water (LCMS, Optima LCMS grade, or equivalent)

3.2.6 Methanol (LCMS, Optima LCMS grade, or equivalent)

3.2.7 Ammonium Formate (LCMS, Optima LCMS grade, or equivalent)

3.2.8 Ammonium Hydroxide (ACS grade or higher)

3.2.9 Ethyl Acetate (ACS grade or higher)

3.2.10 20% Methanol in Water (LCMS, Optima LCMS grade, or equivalent) (Reconstitution Solvent)

3.2.11 LC/MS Needle Rinse (40/40/20: LCMS Water/Methanol/Isopropanol or 75% MeOH in water)

3.2.10 Beta-glucuronidase (BGTurbo) (if run includes urine samples)

3.2.12 Instant Buffer I (provided with BGTurbo)

3.2.13 1% HCl in MeOH (if run includes urine samples)

3.3 Quality Assurance: Reference materials and Controls

3.3.1 Internal Controls, Calibrators, Internal Standards, and Blanks.

3.3.1.1 Internal controls, calibrators, and internal standards are prepared by the ToxBox manufacturer and contained on the 96 well plate. A minimum of one internal positive control that has a concentration near the administrative cut off level for each compound will be run with each extraction.

3.3.2 Negative Control

3.3.2.1 A negative control will be run with each panel extraction. If the run contains urine samples, a negative urine and positive internal or external urine control will also be included in the run. Urine controls are used for the evaluation of urine samples only.

3.3.4 Blanks

3.3.4.1 A non-extracted or extracted blank containing internal standard will be run directly before each case sample to rule out carryover if the sample injected before it contains the same compound. A sample run immediately after a negative control does not require a solvent blank immediately before it. When evaluating the blank, if the confirmation criteria (e.g. ion ratios, RT, S/N) are not met, the analyte is not considered present. If all confirmation criteria is met, the response of the blank will be considered. The area response of the blank preceding a sample must be at least 10 times less than any compound confirmed in the case sample and must be below the limit of confirmation for any analyte confirmed in the case sample to be considered negative. If a blank does contain a compound, the blank and the sample can be reinjected.

3.3.4.2 It is recommended that a blank follow any samples that exhibit a very strong response in the screen.

3.3.4.3 To prepare blank, dilute 1-part ISTD (obtained from a blank well in the extraction plate) in 4-5 parts LCMS grade methanol.

4.0 Procedure

4.1 Extraction Procedure

4.1.1 Allow refrigerated or frozen samples and frozen components to reach ambient temperature. Urine samples that have shown high responses in the screening methods may be diluted with negative urine prior to being extracted. The same negative urine used for the dilution should be used for the negative control. It is at the analyst's discretion as to what the dilution should be. Any dilutions made must be documented in the case notes.

4.1.2 Using a **calibrated** single channel pipette, add 250 µL of blood/urine into the appropriate wells of the analytical plate. For urine samples/controls, hydrolysis of the samples must occur prior to extracting. For urine hydrolysis: add 40 µl of BGTurbo and 100 µl of Instant Buffer I to the wells containing urine. Place covered plate on shaking incubator at approximately 900 rpm for at least 5 minutes.

4.1.3 Add 250 µL of 0.5 M ammonium hydroxide buffer into all wells in use on the analytical (standards) plate.

4.1.4 Place covered plate on shaking incubator at approximately 900 rpm for approximately 15 minutes.

4.1.5 Transfer 200-450 µL of blood+base or urine+base mixture (if applicable) to corresponding wells of SLE+ plate.

4.1.6 Apply positive pressure for approximately 10-15 seconds or until no liquid remains on top of sorbent. Wait 5 minutes. (Positive Pressure manifold setting for this loading step is between 15-50 PSI)

4.1.7 Add 900 µL of ethyl acetate and allow to flow through for approximately 5 minutes under gravity.

4.1.8 Apply positive pressure for approximately 10-15 seconds. *(Positive Pressure manifold setting for this step is between 12-15 PSI).*

4.1.9 Add 900 µL of ethyl acetate and allow to flow through for approximately 5 minutes under gravity.

4.1.10 Apply positive pressure for approximately 10-15 seconds. *(Positive Pressure manifold setting for this step is between 12-15 PSI).*

4.1.11 Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approximately 35^{0}C. Optional step: add 50 µl of 1% HCl in MeOH to all wells in the run and place ACT cover on top of plate prior to drying. The samples may also be dried in another type of heated nitrogen dryer (the temperature should be set at approximately 30-40^{0}C). Temperatures for this step are not critical and it is appropriate to use the equipment temperature readings. The temperatures do not need to be monitored or verified with a traceable thermometer. If this option is chosen, the samples will all be transferred to 10 mL glass centrifuge tubes and dried down in the dryer. The reconstitution step (below) will take place in the glass tubes and the samples will then be transferred back to the plate and sealed.

4.1.12 Reconstitute in 100 µL 20% LCMS grade methanol in LCMS Water and heat seal plate with foil.

4.1.13 Analyze samples or freeze for future analysis. The plate must be injected within 7 days of the extraction date.

4.2 Instrument and Run set up.

4.2.1 See Toxicology AM #24 for instrument maintenance and operation.

4.2.2 Set up the worklist in MassHunter.

4.2.3 A solvent blank is generally run before the calibration curve, then the calibration curve is run, then a negative control. At a minimum, one internal positive control will be run with each run.

4.2.4 Instrument run parameters and the acquisition methods for this Analytical Method will be printed out (paper or electronically) and stored centrally in the lab or in a common file folder electronically.

4.2.4.1 Acquisition Required Settings

4.2.4.1.1 Column Temperature - 55^0C

4.2.4.1.2 Injection volume – 0.5-10 µL

4.2.4.1.3 Mobile Phase Flow rate - 0.5 mL/min

4.2.4.1.4 Binary Pump Gradient Settings (Pump Time Table) (Note: the gradient parameters may be adjusted slightly but adequate separation of the compounds must be demonstrated).

Time (min)	% Mobile A	% Mobile B
0	95	5
2.00	85	15
4.50	50	50
5.50	5	95
7.50	5	95
7.60	95	5
12.00	95	5

4.3 Evaluation of Results

4.3.1 Batch Review

4.3.1.1 The lab criterion for acceptable calibration curve R^2 is >0.98

4.3.1.2 The confirmation limits of detection (LOD) and quantitative ranges (if applicable) are listed in a separate document (maintained in Qualtrax) titled "Compounds Included in Analytical Methods." Urine samples will be reported qualitatively only. The uncertainty of measurement budgets for quantitative compounds have been established and will be evaluated annually by the discipline lead.

4.3.1.2.1 (Blood only) Compounds that have been evaluated and are approved for quantitative reporting will have a quantitative range value included in the document listed in section 2.2 of this method. Samples approved for quantitative reporting with a higher response than the upper limit of the range will be reported out as greater than (upper limit) ng/mL.

4.3.1.2.1.1 The estimated expanded uncertainty will only be reported when it impacts evaluation of a statute, legal requirement, or upon customer request.

4.3.1.2.1.2 When the measurement uncertainty is reported it will be on the report in the same units as the measurement and a statement regarding the coverage probability of 99.73% (k=3) will also be on the report. The current expanded uncertainty is published as a protected document is in Qualtrax and is available to all analysts.

4.3.1.2.2 A minimum of 4 consecutive points must be included in the calibration curve in order to report a compound qualitatively. All the qualitative confirmation criteria must be met for all calibrators included in the curve.

4.3.2 The default criteria for a positive result are:

4.3.2.1 The sample must have a concentration no less than the established confirmation LOD for that analyte.

4.3.2.2 The analyte retention time must be within +/- 5% or 0.100 min., whichever is greater, of the average retention time of the calibrators for that analyte. The retention time for tramadol must be within +/-2% of the average retention time of the calibrators. At the analyst's discretion when the analyte peak falls outside the retention time window and the internal standard shifts comparably that control or sample may be evaluated as positive if the other criteria are met.

4.3.2.3 The ion ratios for transitions must fall within 20% of the averaged calibrators for each run.

4.3.2.4 S/N for each primary transition of detected analyte must be greater than 10. The S/N for the secondary transition(s) must be greater than 5.

4.3.2.5 For any qualitatively reported compound, if a point(s) is dropped at the low end of a calibration curve, the lowest used point that is greater or equal to that compounds LOD listed in 2.2 will be the LOD for that compound for that run.

4.3.2.6 For quantitative reporting: A minimum of 6 consecutive points must be included in the curve. All the qualitative confirmation criteria must be met for the calibrators included in the curve. In addition to the criteria for confirmation, the accuracy for all controls (QC's) and calibrators for that compound, that are included in the curve, must be within the appropriate tolerances (+/- 20%). The allowable accuracy for calibrators and controls with a target concentration of 10ng and less is +/- 30%. However, if the accuracy for a 10ng or less calibrator or control is between +/- 20% and +/- 30%, then the compound will be reported qualitatively between that point and the cutoff. If all quantitative criteria are met for the remaining points, samples with concentrations within that portion of the curve will be reported quantitatively. Any samples exceeding the highest calibrator in the curve will be reported as greater than (upper limit) ng/ml. The highest and lowest calibrators may be dropped due to linearity, even if the accuracy parameters are met for those calibrators. If a QC included in the curve range fails to meet the accuracy requirements, the compound will be reported qualitatively. Refer to 4.3.2.10 for additional QC requirements.

4.3.2.7 Manual integration shall not be done on quantifier peaks for the purposes of getting the accuracy to fall within tolerances. Manual integration may only be done on quantifier peaks if the peak is getting cut off, if background or an adjacent peak is getting integrated, if an incorrect peak is getting integrated, or if background noise is getting integrated and the threshold is increased to eliminate this and the peak falls below that threshold.

4.3.2.8 The negative control will be evaluated as passing if the compound of interest does not meet all of the above listed criteria and the internal standard gives an anticipated response to demonstrate the sample extracted and injected correctly.

4.3.2.9 Samples must be bracketed by QC samples. It is at the discretion of the analyst to choose which controls to run. If the QC run either directly before the samples or directly after fails to inject or has an injection issue, it may be reinjected. If the QC cannot be used, the run will need to be re-extracted. Reinjected case samples do not need to be bracketed, but a QC should be run after the reinjected sample(s). Reinjected QC's are used to evaluate and set limitations for reinject samples only. If sample(s) need to be reinjected but the mobile phase has run out, new mobile phase may be prepared and the sample(s) may be reinjected. The negative control must also be reinjected. The negative controls and cases sample(s) must be bracketed by QC samples.

4.3.2.10 Unless a valid reason is noted, all QC's greater than or equal to the cutoff must meet the minimum criteria for confirmation in order for a compound to be evaluated. If a QC does not meet the minimum requirements and a valid reason is not noted, the compound may not be evaluated, and a notation will be made in the case record. An example of a valid reason to not evaluate a QC would be if the response/chromatography is poor for that compound. If a QC ratio is out of tolerance on the lower end of the curve and it is due to chromatography issues, calibrators may be removed from the curve to exclude that QC.

4.3.2.11 To ensure if an injection is valid the analyst will evaluate the internal standard response. The analyst will do this by looking at the internal standard responses and comparing them to the others in the run. If one is significantly lower, the analyst will determine if injection is valid or if the compound can be evaluated. If all or most of the internal standards in a sample give a low or no response, this indicates that the sample did not inject properly and should be re-injected. The analyst will determine if the response is adequate to evaluate the compound.

4.3.2.12 Calibrators and QCs should not be reinjected if the only issue with the injection is that the accuracy is off.

4.3.2.13 Any limitations to curve ranges and or evaluation of compounds should be noted.

4.3.3 Limitations of Method

4.3.3.1 Samples that indicate as positive for Benzoylecgonine will be reported as Benzoylecgonine/Cocaine break-down product (or other wording that indicates that the response may be from a breakdown of cocaine).

4.4 Quality Assurance Requirements

4.4.1 Refer to Toxicology AM #19 and AM #21 for additional quality assurance and reference material authentication.

4.5 Analysis Documentation

4.5.1 The printed results for each case sample and (when applicable) accompanying blank will be included with the analyst's notes. Case results will be recorded in the ILIMS system.

4.5.2 The reports for the batch, including calibration curves and controls will be stored centrally in the lab or electronically on a common drive. Calibration curves and control data only need to be evaluated and printed for compounds being evaluated for that run.

4.5.3 The raw data from the run will be stored electronically and will be backed up at least every two months.

4.5.4 Results of the controls will be entered onto the Control Monitoring Chart.

Toxicology AM #29: Blood and Urine Multi-Drug Screen by LC-QTOF

1.0 Background/References

1.1 References

1.1.1 This method was obtained from PinPoint Testing, LLC. PinPoint provided training and consultation on the method validation and implementation in 2019-2020.

2.0 Scope

2.1 This method is used to achieve rapid and accurate screening of multiple analytes in biological specimens. This method has simple sample preparation that uses isotopically-labeled internal standards.

2.2 The analytes included are listed in a separate document (maintained in Qualtrax) titled "Compounds Included in Analytical Methods".

3.0 Equipment/Reagents

3.1 Equipment and Supplies

3.1.1 Approved LC-QTOF and MassHunter or equivalent software

3.1.2 Shaker/incubator

3.1.3 Positive Pressure Manifold

3.1.4 SPE dry or other heated nitrogen dryer

3.1.5 Thermo Accucore BiPhenyl (2.1 x 50 mm; 2.6 µm), Phenomenex Phenyl Hexyl (4.6 x 50 mm; 2.6 µm), or equivalent LC column

3.1.6 Calibrated pipette for dispensing blood and/or urine samples

3.1.7 Single or Multi-channel pipettes for all other transfers and additions

3.1.8 Appropriately sized pipette tips

3.1.9 ToxBox kit with 96 well plate containing internal standards, and controls, 96 well SLE+ plate, and 96 well blank sample preparation plate

3.1.10 Heat sealing foil covers for 96 well plate

3.1.11 ACT plate cover for use during drying step with SPE dry (if run contains urine samples)

3.1.12 Additional 48 or 96-well plate (if run contains urine samples)

3.2 Reagents

See AM# 23 for Solution Preparation instructions

3.2.1 10 mM Ammonium Formate in Water (LCMS, Optima LCMS grade, or equivalent) (mobile phase A)

3.2.2 0.1% Formic Acid in Methanol (LCMS, Optima LCMS grade, or equivalent) (mobile phase B)

3.2.3 0.5 M Ammonium Hydroxide

3.2.4 Formic Acid (LCMS grade)

3.2.5 Water (LCMS, Optima LCMS grade, or equivalent)

3.2.6 Methanol (LCMS, Optima LCMS grade, or equivalent)

3.2.7 Ammonium Formate (LCMS, Optima LCMS grade, or equivalent)

3.2.8 Ammonium Hydroxide (ACS or higher)

3.2.9 Ethyl Acetate (ACS or higher)

3.2.10 20% Methanol in water (Reconstitution Solvent) (LCMS, Optima LCMS grade, or equivalent)

3.2.11 LC/MS Needle Rinse (40/40/20: Water/Methanol/Isopropanol or 75% MeOH in water)

3.2.12 Beta-glucuronidase (BGTurbo) (if run contains urine samples)

3.2.13 Instant Buffer I (provided with BGTurbo)

3.2.14 1% HCl in MeOH (if running urine samples)

3.3 Quality Assurance: Reference Materials and Controls

3.3.1 Internal Controls, and Internal Standards.

3.3.1.1 Internal standards are prepared by the ToxBox plate manufacturer and contained on the 96 well plate. If the run contains urine samples, a positive external or internal urine control must also be run.

3.3.2 A negative control will be run with each extraction. If the run contains urine samples, both a blood and urine negative control must be included. Urine controls are used for the evaluation of urine samples only.

4.0 Procedure

4.1 Extraction Procedure

4.1.1 Allow refrigerated or frozen samples and frozen components to reach ambient temperature.

4.1.2 Using a **calibrated** single channel pipette, add 250 µL of blood/urine into the appropriate wells of the analytical plate. For urine samples/controls, hydrolysis of the samples must occur prior to extracting. For urine hydrolysis: add 40 µl of BGTurbo and 100 µl of Instant Buffer I to the wells containing urine. Place covered plate on shaking incubator at approximately 900 rpm for at least 5 minutes.

4.1.3 Pipette 250 µL of 0.5 M ammonium hydroxide buffer into all wells in use on the analytical (standards) plate.

4.1.4 Place covered plate on shaking incubator at approximately 900 rpm for approximately 15 minutes.

4.1.5 Transfer 200-450 µL of blood+base and urine+base mixture (if applicable) to the corresponding wells of SLE+ plate.

4.1.6 Apply positive pressure for approximately 5 seconds or until no liquid remains on top of sorbent. Wait 5 minutes. (Recommended setting 15-50 PSI)

4.1.7 Add 900 µL of ethyl acetate and allow to flow through for approximately 5 minutes under gravity.

4.1.8 Apply positive pressure for approximately 10-15 seconds. (Recommended setting 12-15 PSI).

4.1.9 Add 900 µL of ethyl acetate and allow to flow through for approximately 5 minutes under gravity.

4.1.10 Apply positive pressure for approximately 10-15 seconds. (Recommended setting 12-15 PSI).

4.1.11 Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approximately 35^{0}C. Optional step: if run contains urine samples, add 50 µl of 1% HCl in MeOH to all the wells in the run and place an ACT cover on top of plate prior to drying. The samples may also be dried in another type of heated nitrogen dryer (the temperature should be set at approximately 30-40^{0}C). Temperatures for this step are not critical and it is appropriate to use the equipment temperature readings. The temperatures do not need to be monitored or verified with a traceable thermometer. If this option is chosen, the samples will all be transferred to 10 mL glass centrifuge tubes, HCl added, and dried down in the dryer. The reconstitution step (below) will take place in the glass tubes and the samples will then be transferred back to the plate and sealed.

4.1.12 Reconstitute in 100 µL 20% methanol in water (LCMS grade or better) and heat seal plate with foil.

4.1.13 Analyze samples or freeze for future analysis. The plate must be injected within 7 days of the extraction date.

4.2 Instrument and Run set up.

4.2.1 See Toxicology AM #24 for instrument maintenance and operation.

4.2.2 Instrument run parameters and the acquisition methods for this analytical method will be printed out (paper or electronically) and stored centrally in the lab or in a common file folder electronically.

4.2.2.1 Acquisition Required Settings

4.2.2.1.1 Column Temperature - 35^{0}C

4.2.2.1.2 Injection volume – 0.5-10 µL

4.2.2.1.3 Mobile Phase Flow rate - 0.5 mL/min

4.2.2.1.4 Binary Pump Gradient Settings (Pump Time Table) (Note: the gradient parameters may be adjusted slightly but adequate separation of isobaric compounds must be demonstrated).

Time (min)	% Mobile A	% Mobile B
0	85	15
5.2	10	90
6.5	10	90
6.51	85	15

4.2.3 Worklist Set up should include internal control, negative control(s) and case samples.

4.3 Evaluation of Results

4.3.1 Minimum Criteria:

4.3.1.1 Retention time criterion for peak identification is a ±2% or +/-0.100 min, whichever is greater, retention time window relative to the internal control and/or internal standards around the analyte's retention time. At the analyst's discretion when the analyte peak falls outside the retention time window and the internal standard shifts comparably that sample may be evaluated as positive if the other criteria are met.

4.3.1.2 Mass Accuracy of 0 (+/-10) and/or Mass Abundance Score of 40 or greater

4.3.1.3 To ensure the response is adequate, each compound in the internal control will be evaluated using the criteria listed above. If a compound does not meet these standards, samples cannot be evaluated for that compound. At the analyst's discretion, that compound can be removed from the list of compounds that the sample was evaluated for, or the sample can be reinjected or re-extracted. If a sample needs to be reinjected, the analyst may add more of the reconstitution solvent to facilitate this. The internal standards must give an adequate response in order for the sample to be evaluated.

4.3.1.4 Using the internal plate control, a 1-point calibration curve will be established. The curve will be set to linear, non-weighted and origin set to force. The concentration will be set to 10 for all compounds. This is not the actual concentration of the compounds, but a number used to compare sample responses. In addition to meeting the other criteria, the calculated concentration for a positive sample should be greater than 5. Samples with a calculated concentration between 2 and 5 may be evaluated as positive at the analyst's discretion. If the calculated concentration for amphetamine, methamphetamine, pseudoephedrine, codeine, or morphine is less than 50 (in urine) it/they may be evaluated as negative.

4.3.1.5 In cases that do not meet the minimum criteria, the analyst may also evaluate peak symmetry/resolution and it is at their discretion to determine if the sample exhibits a positive response for that compound. Correct peak shape (as compared with QCs) and the sufficient resolution of potential interferents from the analyte of interest can be considered.

4.3.1.6 If a compound in a sample meets the minimum requirements but displays characteristics that are inconsistent with the calibrator (i.e. the ratio is significantly different, the retention time is barely within the allotted window, etc.) it is at the discretion of the analyst to evaluate that compound as negative for that sample, provided they detail an explanation in the case notes.

4.3.1.7 Samples should have a significant internal standard response. If a sample has little to no internal standard response for a compound, an evaluation for that sample cannot be made. The analyst will need to reinject the sample, remove that drug from the list of drugs that sample was screened for or re-extract the sample. If a sample needs to be reinjected but the mobile phase has run out, new mobile phase may be prepared, and the sample(s) may be reinjected. The negative control must also be reinjected to demonstrate that there was no contamination introduced with the new mobile phases.

4.3.2 Limitations of Method

4.3.2.1 Samples that indicate as positive for Benzoylecgonine will be reported as Benzoylecgonine/Cocaine break-down product (or other wording that indicates that the response may be from a breakdown of cocaine).

4.3.2.2 Samples flagged as positive for amitriptyline will be reported as Amitriptyline/Maprotiline.

4.3.2.3 This method is a preliminary screen. The screen results are an indication of drugs that may be present in a sample. The weight or confidence in screening results cannot be given the same as a confirmatory test. If these results are reported it must be clear on the report that it is an indicator the drug is present in the sample, not a confirmation of the drug in the sample.

4.4 Quality Assurance Requirements

4.4.1 Refer to Toxicology AM #19 and AM #21 for additional quality assurance and reference material authentication requirements.

4.5 Analysis Documentation

4.5.1 A print out showing compound name, retention time, calculated concentration, internal standard retention time and response will be printed out for the case sample and included in the analyst's notes.

4.5.2 The printout (paper or electronic) for the negative control and internal control will be stored centrally in the lab in which they were performed or stored on a network drive.

4.5.3 The data from the run will be stored electronically, and if it is on a computer, will be backed up at least every two months.

Toxicology AM #30: Blood and Urine THC and Metabolites Screen by LC-QTOF

1.0 Background/References

1.1 Background

Δ^9-THC (Figure 1) is the chief psychoactive cannabinoid resulting from exposure to marijuana. Δ^9-THC has a peak blood concentration within 5 to 15 minutes following smoking of a marijuana cigarette.[4,5,6] This blood concentration drops rapidly after cessation of smoking.[4,5] The level may fall to less than 5 ng within 30 to 60 minutes, although longer detection times have been reported.[4,5] Detection of low dose (1.75%) post-smoking Δ^9-THC has been reported to vary from 3 to 12 hours.[5] This detection window was based on a limit of quantitation of 0.5 ng/mL. The number, duration, spacing of puffs, hold time, and inhalation volume all impact the degree of drug exposure and thus bioavailability.[5] Longer detection times have been observed for frequent users. Δ^9-THC is metabolized to an active metabolite, 11-hydroxy-tetrahydrocannabinol (Hydroxy-THC, THC-OH), and an inactive metabolite, 11-nor-9-carboxy-tetrahydrocannabinol (carboxy-THC). The concentration of the Δ^9-THC inactive metabolite, carboxy-THC, gradually increases and may plateau for several hours.[5] There is poor correlation between blood Δ^9-THC and psychoactive effects since the Δ^9-THC concentrations begin to decline prior to the time of peak effects.[4,5,6] The detection window for the active analytes is much shorter than that of carboxy-THC.[7]

Negative behavioral effects reported from exposure to marijuana include altered time perception, lack of concentration, and impaired learning and memory which can lead to impairment of cognitive and performance tasks.[5] Establishing impairment in an individual is based on evaluation of all available information in conjunction with quantitative blood levels.

For additional background, refer to Toxicology AM #3, obsolete analytical method 3.10.1, and the provided references.

1.2 References

1.2.1 This method was obtained from PinPoint Testing, LLC. The method validation and implementation was completed by ISPFS in 2019-2020.

1.2.2 [(4)]Standard Operating Procedure for Blood SPE THC and Carboxy-THC GC/MSD Assay, Edmonton, Canada Office of the Chief Medical Examiners, 2003.

1.2.3 [(5)] Huestis, M.A., *Cannabis (Marijuana) - Effects on Human Behavior and Performance,* Forensic Science Rev. 14(1/2): 16-60, 2002.

1.2.4 [(6)]Drummer, O.H., *Cannabis*, pp. 178-212. In*:* The Forensic Pharmacology of Drugs of Abuse, Arnold: London, 2001.

1.2.5 [(7)]Huestis, M. *Marijuana*. pp. 229-244. In*:* Principles of Forensic Toxicology, Second Edition. Levine, B. ed., AACC, 2003.

1.2.6 [8]Desrosiers, N.A.; Himes, S.K.; Scheidweiler, K.B.; Concheiro-Guisan, M.; Huestis, M.A. *Phase I and II Cannbinoid Disposition in Blood and Plasma of Occasional and Frequent Smokers Following Controlled Smoked Cannabis.* Clinical Chemistry, 60:4, pp. 631-643, 2014.

1.2.7 [9]Nadulski, T., et al. *Simultaneous and Sensitive Analysis of THC, 11-OH0THC, THC-COOH, CBD, and CBN by GC-MS in Plasma after Oral Application of Small Doses of THC and Cannabis Extract*. Journal of Analytical Toxicology, Vol 29, pp. 782-789, November/December 2005.

2.0 Scope

2.1 This method is used to achieve rapid and accurate screening of cannabinoids in biological specimens. This method has simple sample preparation that uses isotopically-labeled internal standards.

2.2 The analytes included are as follows:

Analyte	Administrative Threshold
THC	3 ng/mL
Carboxy-THC	10 ng/mL
THC-OH	3 ng/mL

3.0 Equipment/Reagents

3.1 Equipment and Supplies

3.1.1 Approved LC/MS/MS and MassHunter or equivalent software

3.1.2 Shaking incubator

3.1.3 ToxBox kit with 48 Well plate containing internal standards and controls, 48 well SLE+ plate, and 48 well blank sample collection plate.

3.1.4 Test tube rocker

3.1.5 Calibrated pipette for dispensing blood and/or urine samples

3.1.6 Single or Multi-channel pipettes for all other transfers and additions

3.1.7 Positive Pressure Manifold

3.1.8 SPE sample evaporator concentrator or other heated nitrogen dryer

3.1.9 ACT plate cover for use during drying step with SPE dry (if run contains urine samples)

3.1.10 Additional 48-well plate (if run contains urine samples)

3.1.11 Phenomenex Phenyl Hexyl (4.6 x 50 mm; 2.6 µm) or equivalent column

3.2 Reagents

Refer to Toxicology AM #23 for Solution Preparation instructions.

3.2.1 0.1% Formic Acid in Water (LCMS, Optima LCMS grade, or equivalent)

3.2.2 0.1% Formic Acid in Methanol (LCMS, Optima LCMS grade, or equivalent) (mobile phase B)

3.2.3 Methyl Tert-Butyl Ether (MTBE) 99.9%

3.2.4 Hexanes (ACS)

3.2.5 Methanol (LCMS, Optima LCMS grade, or equivalent)

3.2.6 10 mM Ammonium Formate (LCMS, Optima LCMS grade, or equivalent) (mobile phase A)

3.2.7 LC/MS Needle Rinse (40/40/20: Water/Methanol/Isopropanol or 75% MeOH in water)

3.2.8 1 N KOH (if run includes urine samples and chemical hydrolysis is chosen)

3.2.9 Saturated Phosphate Buffer (if run includes urine samples and chemical hydrolysis is chosen)

3.2.10 Beta-glucuronidase (BGTurbo) (if run contains urine samples and enzymatic hydrolysis is chosen)

3.2.11 Instant Buffer I (provided with BGTurbo) (if run contains urine samples and enzymatic hydrolysis is chosen) 3.3 Quality Assurance: Reference Materials, Controls, and Blanks

3.3.1 Plate controls and internal standards are prepared by the ToxBox Manufacturer and are contained on the 48 well plate. A minimum of one internal positive plate control will be included in each run. A minimum of three calibrators will be run to establish a calculated response value for the case samples (one must be between the nominal value of 3 and 5) control will be included in each run.

3.3.2 A negative control will be run with each extraction. If the run contains urine samples, a negative urine control and external or internal positive urine control must also be included. Urine controls are used for the evaluation of urine samples only.

4.0 Procedure

4.1 Extraction Procedure

4.1.1 Allow refrigerated or frozen samples and frozen components to reach ambient temperature.

4.1.2 Urine samples must be hydrolyzed prior to extraction. Only the urine samples/controls will be hydrolyzed. There are two different options for hydrolysis.

- Chemical hydrolysis: Add 1.5 mL of blank urine to a clean 48-well plate. Add 250 µL of 1 N KOH to the wells containing urine. Place a cover on the plate and shake at 900 rpm for approximately 15 minutes at 40^{0}C. Proceed to step 4.1.3.
- Enzymatic hydrolysis: using a **calibrated** pipette, add 1mL of urine to the appropriate wells of the analytical plate (case samples, negative, QC samples). To the wells containing urine, add 100ul of BG Turbo and 200ul of BG Turbo buffer then proceed to step 4.1.4.

4.1.3 Using a **calibrated** single channel pipette, add 1.0 mL of blood/urine into the appropriate wells of the analytical plate. If chemical hydrolysis was done, transfer 1.0 mL of hydrolyzed urine from the plate the hydrolysis was performed in to the appropriate wells of the analytical plate.

4.1.4 Add 0.5 mL 0.1% formic acid in LCMS water to all of the wells containing blood (and urine if the enzymatic hydrolysis option was chosen). Add 0.5 mL of saturated phosphate buffer to all of the wells containing urine (if the chemical hydrolysis option was chosen).

4.1.5 Place covered plate on shaking incubator at approximately 900 rpm for approximately 15 minutes.

4.1.6 Transfer 700-800 µL of blood+acid or urine+acid mixture (if applicable) to the corresponding wells of the SLE+ plate.

4.1.7 Apply positive pressure for approximately 10-15 seconds or until no liquid remains on top of sorbent. *(Recommended setting for Positive Pressure manifold for this loading step is between 15-50 PSI)*

4.1.8 Wait 5 minutes for sample to completely absorb.

4.1.9 Add 2.25 mL MTBE and allow to flow for 5 minutes under gravity. *This should be completed in increments, as the sample well accommodates a maximum volume of 1 mL. (Recommended for this step is: three (3) repetitions of adding 750* µL*)*

4.1.10 Apply positive pressure to complete elution- approximately 15 seconds. *(Positive Pressure manifold set between 12-15 PSI)*

4.1.11 Add 2.25 mL hexane and allow to flow for 5 minutes under gravity. *This should be completed in increments, as the sample well accommodates a maximum volume of 1 mL. (Recommended for this step is: three (3) repetitions of adding 750 µL)*

4.1.12 Apply positive pressure to complete elution- approximately 15 seconds. *(Positive Pressure manifold set between 12-15 PSI)*

4.1.13 Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approximately 35^0C. Temperatures for this step are not critical and it is appropriate to use the equipment temperature readings. The temperature does not need to be monitored or verified with a traceable thermometer. The samples may also be dried in another type of heated nitrogen dryer (the temperature should be set at approximately $30\text{-}40^0$C). If this option is chosen, the samples will all be transferred to 10 mL glass centrifuge tubes and dried down in the dryer. The reconstitution step (below) will take place in the glass tubes and the samples will then be transferred back to the plate and sealed.

4.1.14 Reconstitute each sample with 100 µL LCMS Grade Methanol. Heat seal plate with foil to prevent evaporation.

4.1.15 Analyze samples or freeze for future analysis. The plate must be injected within 7 days of the extraction date.

4.2 Instrument and Run set up.

4.2.1 ***See Toxicology AM #24 for instrument maintenance and operation.***

4.2.2 Instrument run parameters and the acquisition methods for this Analytical Method will be printed out (paper or electronically) and stored centrally in the lab or in a common file folder electronically.

4.2.2.1 Acquisition Required Settings

4.2.2.1.1 Column Temperature - 35°C

4.2.2.1.2 Injection volume – 0.5-10 µL

4.2.2.1.3 Mobile Phase Flow rate - 0.5 mL/min

4.2.2.1.4 Binary Pump Gradient Settings (Pump Time Table) (Note: the gradient parameters may be adjusted slightly but adequate separation of the compounds must be demonstrated).

Time (min)	% Mobile A	% Mobile B
0	85	15
5.2	10	90
7.0	10	90
7.01	85	15

4.2.3 Worklist Set up should include a positive control, calibrators, negative control, and case samples.

4.3 Evaluation of Results

4.3.1 Minimum Criteria:

4.3.1.1 Retention time criterion for peak identification is a ±2% or +/-0.100 min, whichever is greater, retention time window relative to the internal control and/or internal standards around the analyte's retention time. At the analyst's discretion when the analyte peak falls outside the retention time window and the internal standard shifts comparably that sample may be evaluated as positive if the other criteria are met.

4.3.1.2 A sample or control with a concentration of 3 ng/mL or greater for THC or THC-OH, or 10 ng/mL or greater for carboxy-THC will be considered positive for cannabinoids. Samples and controls with a carboxy-THC response between 5 and 10 ng/mL may be evaluated as positive at the analyst's discretion.

4.3.1.3 Mass Accuracy of 0 (+/-10) and/or Mass Abundance Score of 40 or greater

4.3.1.4 To ensure the response is adequate, each compound in the internal control will be evaluated using the criteria listed above. If a compound does not meet these standards, samples cannot be evaluated for that compound. At the analyst's discretion, that compound can be removed from the list of compounds that the sample was evaluated for, or the sample can be reinjected or re-extracted. If a sample needs to be reinjected, the analyst may add more of the reconstitution solvent to facilitate this. The internal standards must give an adequate response in order for the sample to be evaluated. If a sample needs to be reinjected but the mobile phase has run out, new mobile phase may be prepared, and the sample(s) may be reinjected. The negative control must also be reinjected to demonstrate that there was no contamination introduced with the new mobile phases.

4.3.1.5 The lab criterion for acceptable calibration curve R^2 is >0.98

4.3.1.6 In cases that do not meet the minimum criteria, the analyst may also evaluate peak symmetry/resolution and it is at their discretion to determine if the sample exhibits a positive response for that compound. Correct peak shape (as compared with QCs) and the sufficient resolution of potential interferents from the analyte of interest can be considered.

4.3.1.7 If a compound in a sample meets the minimum requirements but displays characteristics that are inconsistent with the calibrator (i.e. the ratio is significantly different, the retention time is barely within the allotted window, etc.) it is at the discretion of the analyst to evaluate that compound as negative for that sample, provided they detail an explanation in the case notes.

4.3.1.8 Samples should have a significant internal standard response. If a sample has little to no internal standard response for a compound, an evaluation for that sample cannot be made. The analyst will need to reinject the sample, remove that drug from the list of drugs that sample was screened for or re-extract the sample.

4.4 Quality Assurance Requirements

4.4.1 Refer to Toxicology AM #19 and AM #21 for additional quality assurance and reference material authentication requirements.

4.5 Analysis Documentation

4.5.1 The printed results for each case sample will be included with the analyst's notes. Case results are to be recorded in the ILIMS system.

4.5.2 The printout (paper or electronic) for the calibrators and controls will be stored centrally in the lab in which they were performed or stored on a network drive.

4.5.3 The data from the run will be stored electronically, and if it is on a computer, will be backed up at least every two months.

4.6 Limitation of method

4.6.1 This method is a preliminary screen; the screen results are an indication of drugs that may be present in a sample. The weight or confidence in screening results cannot be given the same as a confirmatory test. This is not a quantitative method and quantitative results cannot be reported from this method. The quantitative aspects of this method are only for establishing a minimum response threshold. If these results are reported it must be clear on the report that it is an indicator the drug is present in the sample, not a confirmation of the drug in the sample.

Toxicology AM #31:
Drug Screening Using Randox Evidence MultiSTAT

1.0 Background/References

1.1 Background

The Randox Evidence MultiSTAT analyzer is an automated analyzer that utilizes Biochip Array Technology (BAT). Competitive chemiluminescent immunoassays are used for the Biochip Arrays. A light signal is generated and detected by the instrument using digital imaging technology and then compared to that from a calibration curve to determine if an analyte is present in the sample at or above the cutoff concentration. The results reported are purely qualitative.

1.2 References

1.2.1 Randox Evidence MultiSTAT User Manual (August 2019)

1.2.2 Randox Evidence MultiSTAT Drugs of Abuse Array Blood Product Insert

2.0 Scope

2.1 This Biochip Array Technology is applied for the qualitative screening for drugs in whole blood specimens. The outcome of the assay is intended as only a preliminary analytical test result. The presence of a particular drug compound must be verified through analysis with a confirmatory instrument such as a liquid chromatograph equipped with a mass selective detector.

As indicated in the table below, each assay in use has an established administrative threshold or cutoff. For this reason, a negative result does not indicate that no drug is present, only that the concentration is less than the administrative cutoff. For this reason, there may be situations where confirmation of an analyte may be pursued even if a negative result is indicated for the compound or a class of compounds in question.

2.2 Drug Class/Compounds included

Assay	**Cutoff**
6-MAM	10 ng/mL
AB-CHMINACA	5 ng/mL
AB-PINACA	2 ng/mL
Alpha-PVP	5 ng/mL
Amphetamine	50 ng/mL
Barbiturates	50 ng/mL
Benzodiazepines	20 ng/mL
BZE/Cocaine	25ng/mL
Buprenorphine	2 ng/mL
Cannabinoids	10 ng/mL
Ethylglucuronide (ETG)	500 ng/mL
Fentanyl	1 ng/mL
Methadone	10 ng/mL
Methamphetamine	50 ng/mL
Pregabalin	1000 ng/mL
Opiate	80 ng/mL
Oxycodone	10 ng/mL
Phencyclidine	5 ng/mL
Tramadol	5 ng/mL
Tricyclic Antidepressant (TCA)	60 ng/mL

3.0 Equipment/Reagents

3.1 Equipment

3.1.1 Sample Preparation Supplies

3.1.1.1 Air-displacement pipettes and appropriate tips.

3.1.1.2 Microcentrifuge

3.1.1.3 Microcentrifuge tubes

3.1.2 Randox Evidence MultiSTAT Analyzer

3.2 Reagents

3.2.1 Randox Evidence MultiSTAT Drugs of Abuse Array- Blood (Remember to check expiration date prior to use.)

3.2.1.1 Assay Kits:

- Blood Test Cartridges
- Blood Cutoff
- Blood Positive Control
- Blood Sample Diluent
- Reconstitution Buffer
- Sample Droppers

3.2.2 Processing of New Assay Supplies

3.2.2.1 When a new kit is opened, check the expiration date prior to opening to ensure the kit is not expired, then proceed with the Batch Update.

3.2.2.2 Set Up the Batch Update for the New Kit

3.2.2.2.1 Insert the USB to the USB port located on the bottom right-hand side of the analyzer.

3.2.2.2.2 Select the import data button on the screen.

3.2.2.2.3 Select the batch update then select OK.

3.2.2.2.4 The batch update will now be complete.

3.2.2.2.5 For each assay kit, an initial Batch QC must be run on the analyzer prior to running case samples. This will consist of running the provided Cutoff and positive control material. The Batch QC is good for 30 days. The Batch QC should be repeated at 30-day intervals (if the kit is not used up within the 30 days).

3.2.2.3 Prepare the Cutoff Reagent

3.2.2.3.1 Gently tap the Cutoff vial on the bench to ensure that all material moves to the bottom.

3.2.2.3.2 Carefully lift (do not remove) the rubber stopper, avoiding any loss of material.

3.2.2.3.3 Pipette 1mL of Reconstitution Buffer to the vial and lower the rubber stopper.

3.2.2.3.4 Wait 2 minutes.

3.2.2.3.5 Gently swirl the vial and invert 3 times to ensure that all of the material is dissolved.

3.2.2.3.6 Let sit for at least 30 minutes (out of bright light) before using.

3.2.2.3.7 Remove and discard the rubber stopper then write the date of reconstitution on the bottle or cap.

3.2.2.3.8 Once reconstituted, the cutoff material is stable for 14 days when stored at 2-8 degrees Celsius. The vial should be stored upright.

3.2.2.4 Prepare the Positive Control

3.2.2.4.1 Gently tap the Positive Control vial on the bench to ensure that all material moves to the bottom.

3.2.2.4.2 Carefully lift (do not remove) the rubber stopper, avoiding any loss of material.

3.2.2.4.3 Pipette 1mL of Reconstitution Buffer to the vial and lower the rubber stopper.

3.2.2.4.4 Wait 2 minutes.

3.2.2.4.5 Gently swirl the vial and invert 3 times to ensure that all the material is dissolved.

3.2.2.4.6 Let sit for at least 30 minutes (out of bright light) before using.

3.2.2.4.7 Remove and discard the rubber stopper then write the date of reconstitution on the bottle or cap.

3.2.2.4.8 Once reconstituted, the Positive Control is stable for 14 days when stored at 2-8 degrees Celsius. The vial should be stored upright.

3.2.2.5 Prepare the Blood Case Samples (Whole Blood)

3.2.2.5.1 Place blood tubes on rocker and allow them to reach ambient temperature before being analyzed.

4.0 Procedure

4.1 Remove cartridges (only the ones you will be using that day), Cutoff Reagent, and Positive Control (if applicable) from the box. Let sit for at least 30 minutes before using. Do not open the test cartridge bag until immediately prior to using.

4.2 Prepare Case Sample (Note: samples should be analyzed immediately following preparation)

4.2.1 Pipette 150 µl of sample blood into a labeled microcentrifuge tube containing 450 µl of sample diluent. Close the tube and vortex or use the pipette to draw liquid up and down to mix the sample and the diluent then close the cap.

4.2.2 Centrifuge at 13,000rpm for 10 minutes. This step may be repeated if the sample is particularly thick and does not appear to separate after the initial spin step.

4.3 Perform the initial Batch QC (if this has not yet been done or if the last Batch QC was longer than 30 days prior). The batch QC is done the same as the samples, but it is done using the positive control.

4.4 Eject the drawers and place the tip cartridge into the appropriate spot. Push it in gently until it clicks.

4.5 Remove the cartridge from the bag and pierce the foil covering the left well of the cartridge with a pipette tip.

4.6 Pipette 200 µl of cutoff into the left well.

4.7 Pipette 200 µl of sample (or positive control if doing a Batch QC) into the right well. When removing sample from microcentrifuge tube, make sure to draw the liquid from the top of the sample.

4.8 Insert the cartridge into the drawer in the appropriate spot and close the drawer (the cartridge will click when it is in properly). Close the front cover.

4.9 Follow the prompts on the screen to start the testing. It should take approximately 25 minutes.

4.10 When the testing is complete, discard the tip and test cartridge in a biohazard container. Power down the instrument when not in use or at the end of the day.

4.11 Analysis Documentation

4.11.1 The results (paper or electronic) for each case sample will be included with the analyst's notes. Positive or negative results for each assay will be recorded in the ILIMS system and will appear on the reports.

4.11.2 The positive control results (paper or electronic) for the initial Batch QC (and subsequent Batch QCs if done) will be stored centrally in the lab in which they were performed or stored on a network drive.

4.11.3 The data from the run will be stored electronically, and if it is on a computer, will be backed up at least every two months.

4.12 Limitation of method

4.12.1 In addition to listing the results of the assays (positive or negative), the reports shall have a statement explaining that the results reported are from a preliminary screen; the screen results are merely an indication of drugs that may be present in the sample and the weight or confidence in screening results cannot be given the same as a confirmatory test.