Idaho State Police Forensic Services

TOXICOLOGY
ANALYTICAL METHODS

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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

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	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.

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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 waterbath

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

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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 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.

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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 degrees Celsius, hold 2.5 minutes
- Ramp 25 degrees Celsius
- Final temperature: 300 degrees Celsius, 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.

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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.

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Toxicology AM #3: Qualitative 11-nor-9-THC-D9-COOH (Carboxy-THC) in Urine

1.0 Background/References

1.1 Background

Cannabis sativa use dates back to 2700 B.C.[2,5] Marijuana (MJ) refers to a mixture of the leaves and flowering tops.[3] The smoke from burning cannabis includes 61 different cannabinoids.[2,6] The major active ingredient in marijuana is delta–9-tetrahydrocannabinol (D9-THC). The D9-THC content varies from 2 to 10% with an average of four to five percent. The quality of marijuana is reported to have improved over the last 20 years due to superior cultivation practices. The medicinal effects of MJ include anti-nausea, muscle relaxing, anticonvulsant and reduction of intraocular pressure.[6] Cannabis therefore has found use as an antiemetic to deal with the nausea associated with anticancer chemotherapy and for relief for those suffering from glaucoma. The debate continues on medical use and the complete legalization of the drug.

Several factors come into play when considering the behavioral effects of (D9)-THC. These include the route of administration (smoked or ingested), THC concentration of the plant (dose), the experience of the user, the user's vulnerability to psychoactive effects, and the setting of the use.[5,6] The desirable effects of MJ include an increased sense of well-being, mild euphoria, relaxation and a mild sedative-hypnotic effect.[5,6] Its clinical effects are similar to those of alcohol and the anti-anxiety agents.[5] The side-effects of MJ use include impairment of cognitive functions, alteration of the user's perception of time and distance, reaction time, learning and short-term memory.[2,5,6] MJ has been shown to interfere with a person's ability or willingness to concentrate. Cannabis causes temporal disintegration such that the individual loses the ability to store information in the short term and is easily distracted.[2] Impairment from use is thought to last from 4 to 8-hours with more recent studies reporting 3 to 6 hours. Dr. Huestis reported that most behavioral and physiological effects return to baseline within three to six hours after use with residual effects in specific behaviors for up to 24 hours.

Impairment of coordination and tracking behavior has been reported to persist several hours beyond the perception of the high.[6] Due to the variable period of impairment, the relating of urine Carboxy-THC to the time of use, and thus impairment, requires the development of the scenario surrounding the stop for DUI. The presence of Carboxy-THC in urine only indicates exposure to MJ at some previous, indeterminate time.

The physiological effects may include an increase in heart rate and blood pressure, conjunctival suffusion, vasodilation, dry mouth and throat and a decrease in respiratory rate. The individual may also experience increased hunger (munchies).

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D9-THC is rapidly metabolized to the inactive metabolite, Carboxy-THC.[1,4,5,6] In urine, this major metabolite, Carboxy-THC is pursued due to D9-THC only being present in minute quantities.[6] Carboxy-THC in urine has been conjugated with glucuronic acid to improve excretion. The detection time of Carboxy-THC in urine following marijuana use varies dependent upon various pharmacological factors such as the dose obtained, the route of administration, and the rates of metabolism and excretion.[1] D9-THC is deposited in body fat due to its high lipid solubility. It is slowly released from this storage depot over time.[1] The amount of D9-THC stored in fat is a function of the amount, frequency and potency of drug exposure. The detection time can therefore vary from days to months.

1.2 References

1.2.1 Huestis, M.A., Mitchell, J.M. and Cone, E.J. Detection Times of Marijuana Metabolites in Urine by Immunoassay and GC-MS J. Anal. Tox. 19:443-449, 1995.

1.2.2 Huestis, M. Marijuana. pp. 269-304 in: Principles of Forensic Toxicology, Third Edition. Levine, B. ed., AACC, 2010.

1.2.3 Cannabis in: Clark's Isolation and Identification of Drugs. pp. 423-425, Moffat, A.C. ed., Pharmaceutical Press: London, 1986.

1.2.4 Drug Evaluation and Classification Training Manual, U.S. Dept. of Transportation, 1993.

1.2.5 Julien, R.M. Marijuana: A Unique Sedative-Euphoriant-Psychedelic Drug in: A Primer of Drug Action. pp. 319-349, W.H. Freeman and Company: New York, 1998.

1.2.6 O'Brien, C.P. Drug Addiction and Drug Abuse. pp. 572-573. In: Goodman & Gilman's The Pharmacological Basis of Therapeutics, Ninth edition, Hardman, J.G. ed., McGraw-Hill, 1996.

2.0 Scope

2.1 This method is to qualitatively confirm the presence of a major metabolite of marijuana, Carboxy-THC, in urine specimens.

3.0 Equipment/Reagents

3.1 Equipment and Supplies

3.1.1 Tube Rocker

3.1.2 Laboratory Centrifuge capable of 3500 rpm

3.1.3 Waterbath

3.1.4 Drybath

3.1.5 Evaporative Concentrator equipped with nitrogen tank.

3.1.6 pH Indicator Strips

3.1.7 Glassware

3.1.7.1 16X100mm tubes

3.1.7.2 16X144mm tapered tip centrifuge tubes

3.1.7.3 Caps for 16mm OD tubes

3.1.7.4 GC/MS ALS vials

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3.1.7.5 GC/MS vial microinserts

3.1.8 Gas Chromatograph equipped with a mass selective detector and a nonpolar capillary column (e.g. 100%-dimethylpolysiloxane or 95%-dimethyl-polysiloxane with 5% diphenyl).

3.2 Reagents

Refer to Toxicology AM#23 for solution preparation instructions. Purity of chemicals must be ACS Grade or equivalent.

3.2.1 1N KOH

3.2.2 Saturated Potassium Phosphate Monobasic pH approx. 1.8

3.2.3 87:13 Hexane with Ethyl Acetate (v/v)

3.2.4 Ethyl acetate

3.2.5 Silylating Agent (select from): BSTFA/1% TMCS or MSTFA

3.3 Standards/Reference Material

3.3.1 Stock Standard Solution

- 100 µg/mL (+) 11-nor-9-carboxy-D9-THC

3.3.2 Working Standard Solution (1800 ng/mL)

3.3.2.1 Add 180 µL Stock Solution to 9.82 mL Methanol. Other volumes may be prepared. Document preparation on appropriate log sheet. Solution is stable for 1-year (or at the date of the earliest expiring Stock solution) when stored under refrigeration.

3.4 Qualitative Controls

3.4.1 Positive Controls

3.4.1.1 A minimum of one spiked 60 ng/mL and one commercial Carboxy-THC containing control must be analyzed in each batch of samples.

3.4.1.2 60 ng/mL Carboxy-THC Spiked Control

3.4.1.2.1 Add 3 mL of the same lot of negative urine used to prepare the negative control to extraction tube. Add 100 µL of working standard solution, and vortex.

3.4.1.3 Suitable nominal concentration range for commercial control is 15 ng/mL to 150 ng/mL.

3.4.2 Negative Control

3.4.2.1 Negative urine commercially obtained or in-house urine verified to be negative for drugs of interest.

4.0 Procedure

4.1 Extraction Method

4.1.1 Initial set-up

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4.1.1.1 Label extraction tubes, tapered bottom derivatization tubes and GC/MS vials with microinserts for the negative control, spiked positive control, commercial positive control(s), and casework samples.

4.1.2 Sample Preparation

4.1.2.1 Transfer 3 mL urine specimen, negative urine, spiked positive control and commercial positive control(s) to extraction tubes.

4.1.3 Sample Hydrolysis

4.1.3.1 Add 0.5 mL 1.0N KOH to each extraction tube.

4.1.3.2 Vortex gently to mix.

4.1.3.3 Check resulting pH.

4.1.3.4 pH must be > 12. If pH <12, add additional 0.5 mL of KOH.

4.1.3.5 Place in 40°C water bath for 15 minutes.

4.1.3.6 Allow samples to cool before proceeding with solvent extraction.

4.1.4 Extraction

4.1.4.1 If original pH was > 12:

4.1.4.1.1 Add 1.5 mL Saturated Phosphate Buffer (pH 1.8).

4.1.4.1.2 Add 3 mL Hexane/Ethyl Acetate (87:13).

4.1.4.1.3 Rock for 10 minutes.

4.1.4.2 If original pH was < 12:

4.1.4.2.1 Add 3.0 mL Saturated Phosphate Buffer (pH 1.8).

4.1.4.2.2 Add 4 mL Hexane/Ethyl Acetate (87:13).

4.1.4.2.3 Rock for 10 minutes.

4.1.4.3 Centrifuge tubes at ~3500 rpm for 10 minutes.

4.1.4.4 Transfer upper organic phase from tube into labeled tapered bottom tube.

4.1.4.5 Evaporate solvent to dryness, under a gentle stream of nitrogen, at ~37°C.

4.1.5 Derivatization

4.1.5.1 To dried extract in tapered bottom tubes, add 50 µL ethyl acetate and 50 µL silylating reagent.

4.1.5.2 Cap tubes.

4.1.5.3 Vortex.

4.1.5.4 Heat tube for 15 minutes in 95°C drybath.

4.1.5.5 Remove from heat and allow samples to cool.

4.1.5.6 Transfer derivative to labeled GC/MS ALS vial with microinsert.

4.2 Gas Chromatograph/Mass Spectrometry (GC/MS)

4.2.1 Preparation for Analysis Run

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4.2.1.1 Into Sequence log table, enter information for case samples, controls and pre-sample solvent blanks. A 60 ng/mL spiked positive control should run both early and late in the sequence.

4.2.1.2 Load case samples, controls and solvent blanks into the quadrant rack(s) as noted in the sequence table.

4.2.2 GC-MSD Acquisition Parameters

4.2.2.1 Refer to instrument method for current acquisition parameters.

4.2.2.1.1 Instrument Run Parameters

- Inlet at 280 degrees Celsius, splitless injection, injection volume: 1µL
- Oven at 185 degrees Celsius
- Ramp 30 degrees Celsius
- Final temperature: 285 degrees Celsius, hold 4 minutes

4.2.2.2 Current acquisition method must be stored centrally as a hard or electronic copy.

4.2.2.3 Acquire sample data in SIM (selected ion monitoring) utilizing the ions 371, 473 and 488.

4.2.3 Detection and Identification Criteria

4.2.3.1 Retention Time

4.2.3.1.1 Identification requires a peak within ±0.1 minutes of the retention time established for Carboxy-THC with the in-run control(s).

4.2.3.2 Ion ratios - Selective Ion Monitoring (SIM)

4.2.3.2.1 Carboxy-THC Ion ratio for the early and late 60 ng/mL control must be calculated and averaged. This mean ratio must be compared to ratio obtained from casework and the mean of the 60 ng/mL control samples. Ratio between monitored ions, 371:473 and 371:488, must agree within ±20%.

4.2.3.2.1.1 Incorrect Ratios

4.2.3.2.1.1.1 If the casework or control sample ion ratios do not agree within ~20% due to high concentration of c-THC in the sample, the extract may be diluted, and run again. Alternatively, carboxy-THC in the sample may be confirmed using full scan data, provided that a derivatized reference material is also run in full scan mode. The analyte may be confirmed from full scan data if there are no significant differences in the mass spectral data as compared to the appropriate reference material and the retention time is within ±0.1 minutes of the appropriate reference material.

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4.2.3.2.1.1.2 Assessment of relative strength of case sample to 60 ng/mL control. The response of case samples will be compared to a 60 ng/mL control sample. The analyst will pick either of the responses from the positive control and divide the response of the 371 ion by 5; this will be defined as the approximate minimum response. The approximate minimum response will be documented in the analyst's notes. -The analyst will compare this response to the response for each case sample.

4.2.3.2.1.1.3 If the response for the case sample is less than the approximate minimum response established by the control. Carboxy-THC will generally not be confirmed. If it is below the minimum response, it is at the analyst's discretion whether or not to call the drug. Other factors such as enzyme screen results and the sample response in relation to the baseline must be considered and noted in the analyst's notes.

4.3 Quality Assurance Requirements

4.3.1 Refer to relevant 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 compiled for each analysis run and must be stored centrally in the laboratory where the analysis was performed, or electronically.

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Toxicology AM #5: Qualitative Benzodiazepines and Ancillary Compounds in Urine

1.0 Background/References

1.1 Background

Approximately 20 benzodiazepines are approved for use in the US.[2] Benzodiazepines were first introduced in the 1960s in pursuit of the perfect sedative hypnotic agent, and have replaced barbiturates as the major class of central nervous system (CNS)-depressant drugs.[2] Chlordiazepoxide (Librium) was introduced in 1962 followed by the introduction of Diazepam (Valium) in 1968. There are four main classes of benzodiazepines, the 1,4-benzodiazepines, the triazolobenzodiazepines, the diazolobenzodiazepines, and the 7-nitrobenzodiazepines. Refer to the following chart for a compilation of benzodiazepines currently prescribed in the US or ones that are commonly encountered.

1,4-Benzodiazepines	Trade Name	Major Metabolite(s)
Diazepam	Valium®	Nordiazepam, Oxazepam, Temazepam
Nordiazepam	---	Oxazepam
Oxazepam	Serax®	Glucuronide conjugate
Temazepam	Restoril®	Oxazepam
Clorazepate	Tranxene®	Nordiazepam, Oxazepam
Chlordiazepoxide	Librium®	Demoxepam, Nordiazepam, Oxazepam
Halazepam	Paxipam®	3-Hydroxy-Halazepam, Nordiazepam, Oxazepam
Quazepam	Dormalin®, Doral®	2-Oxoquazepam
Flurazepam	Dalmane®	Desalkylflurazepam
Lorazepam	Ativan®	3-Glucuronide
7-Nitrobenzodiazepines		
Clonazepam	Klonopin®	7-Aminoclonazepam
Flunitrazepam	Rohypnol® Not Prescribed in US	7-Aminoflunitrazepam
Triazolobenzodiazepines		
Alprazolam	Xanax®	α-Hydroxy-alprazolam, 4-Hydroxy-alprazolam
Triazolam	Halcion®	α-Hydroxy-triazolam
Estazolam	ProSom®	---
Diazolobenzodiazepine		
Midazolam	Versed (Parenteral)	α-Hydroxymidazolam

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Benzodiazepines are used primarily as anti-epileptics in the treatment of seizure disorders, as anxiolytics for the short-term relief of anxiety disorders, as sedative-hypnotics for the treatment of sleep disorders, and as muscle relaxants to relieve spasticity. The primary side effects that accompany their use include dose-related extensions of the intended actions, including sedation and sleepiness/drowsiness. In addition, other undesired effects that will influence the outcome of field sobriety tests include ataxia, a blocked ability to coordinate movements, a staggering walk and poor balance, lethargy/apathy, indifference or sluggishness, mental confusion, disorientation, slurred speech and amnesia. Impairment of motor abilities, especially a person's ability to drive an automobile, is common. This impairment is compounded by the drug-induced suppression of ones' ability to assess their own level of physical and mental impairment. Alcohol and other CNS depressants (e.g., barbiturates, antidepressants, etc.) will increase CNS depressant effects, such as sedation and impairment of psychomotor function, in an additive manner.[4-6]

The benzodiazepines are lipid soluble and are absorbed well from the GI tract with good distribution to the brain. They are metabolized primarily in the liver. Their CNS active metabolites extend their duration of action. The benzodiazepines work by enhancing, facilitating or potentiating the action of the inhibitory neurotransmitter GABA. They serve to increase the frequency of GABA-mediated chloride ion channel opening.

Benzodiazepines are metabolized primarily in the liver via several different microsomal enzyme systems.[6] Many products of their metabolism are active. Since many of the active metabolites have been marketed as therapeutic agents, it is difficult to ascertain which drug was ingested solely upon the basis of the results of analysis. Current drug therapy will assist in determining the source of a particular compound. The detection of a particular agent is determined partly by whether its metabolism yields active metabolites. Excretion of benzodiazepines is predominantly in the urine. Depending upon the particular benzodiazepine, the urine may contain parent compounds, N-dealkylation and oxidative (hydroxylation) metabolism products, and/or glucuronide conjugates.

1.1.1 At the analyst’s discretion, the samples may be extracted with or without derivatizing, and there are two options in the method for derivatization.

1.2 References

1.2.1 Valentine, J.L., Middleton, R., Sparks, C. Identification of Urinary Benzodiazepines and their Metabolites: Comparison of Automated HPLC and GC-MS after Immunoassay Screening of Clinical Specimens. J. Anal. Tox. 20:416-424, 1996.

1.2.2 Levine, B. Central Nervous System Depressants. pp. 191-197. In: Principles of Forensic Toxicology. Levine, B. ed., AACC, 1999.

1.2.3 Huang, W. and Moody, D.E. Immunoassay Detection of Benzodiazepines and Benzodiazepine Metabolites in Blood. J. Anal. Tox. 19:333-342, 1995.

1.2.4 Drug Facts and Comparisons Prescription Drug Information Binder, Updated monthly.

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1.2.5 Julien, R.M. A Primer of Drug Action. pp. 95-107, W.H. Freeman and Company: New York, 1998.

1.2.6 Hobbs, W.R., Rall, T.W. and Verdoorn, T.A. Hypnotics and Sedatives. pp. 362-373. In: Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th edition, Hardman, J.G. ed., McGraw-Hill, 1996.

2.0 Scope

2.1 This extraction method is a modification of the method developed by Valentine, et al., for the extraction of benzodiazepines from urine.[1] This method has also been found to be effective in the extraction of opiates and various other drugs such as Zolpidem, Buprenorphine and Trazodone.

3.0 Equipment/Reagents

3.1 Equipment and Supplies

3.1.1 Tube Rocker

3.1.2 Laboratory oven or water bath

3.1.3 Laboratory Centrifuge capable of 3500 rpm

3.1.4 Fixed and adjustable volume single channel air displacement pipettes, and appropriate tips, capable of accurate and precise dispensing of volumes indicated.

3.1.5 Dry bath

3.1.6 Evaporative Concentrator equipped with nitrogen tank.

3.1.7 Threaded-end 16X100 Round Bottom Glass Tubes and/or 16X114 Tapered Bottom Glass Centrifuge Tubes

3.1.8 Screw caps for 16mm O.D. Tubes

3.1.9 pH Indicator Strips

3.1.10 ALS Vials

3.1.11 ALS Vial Microinserts

3.1.12 Gas Chromatograph equipped with a mass selective detector and a nonpolar capillary column with a phase composition capable of efficiently separating amines, alkaloids, drug compounds and other analytes encountered in toxicological specimens (e.g. 100%-dimethylpolysiloxane or 95%-dimethyl-polysiloxane with 5% diphenyl).

3.2 Reagents

Refer to Toxicology AM#23 for solution preparation instructions.

3.2.1 BG100 b-Glucuronidase Solution (Kura Biotec)

3.1.2 2M Acetate buffer, pH 4.8

3.1.3 50mM Sodium Bicarbonate, pH 11

3.1.4 Chloroform/Isopropanol 9:1 (Each Certified ACS Grade)

3.1.5 Ethyl Acetate (Certified ACS Grade)

3.1.6 Silylating agent: MSFTA or BSTFA with 1% TMCS

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3.3 Qualitative Reference Material and Controls

3.3.1 Positive Control

3.3.1.1 Positive Control can be prepared with single or multi-component working solutions and/or obtained commercially.

3.3.2 Non-extracted Reference Material

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

3.3.3 Non-extracted Derivatized Reference Material

3.3.3.1 Prepare derivatized reference material as necessary based on current drug therapy and examination of GC/MSD data.

3.3.3.2 Add ~3-5 µL (1mg/mL) stock reference material to labeled centrifuge tube. Derivatize as described in 4.1.2.2

3.3.4 Internal Standard

3.3.4.1 Stock Solutions

1 mg/mL Prazepam

3.3.4.2 Working Internal Standard Solution [10ng/µL]

3.3.4.2.1 Add 100 µL Prazepam stock solution to 10 mL volumetric ball flask. QS with methanol.

NOTE: Working Solution is stable for 1 year (or at the date of the earliest expiring Stock solution) when stored under refrigeration.

3.3.5 Required Extracted Controls (inclusive of all options for method)

3.3.5.1 Extracted Negative Control

NOTE: Commercially obtained or in-house urine verified to be negative for drugs of interest.

3.3.5.2 Positive Control

Positive Control can be prepared with single or multi-component working solutions and/or obtained commercially. The positive control must have at least one compound in it that is appropriate for demonstrating that each chosen extraction and derivatization (if applicable) is working. The preferred concentration range of this control is 300–600ng/mL. Examples of preparation and dilutions for an in-house control are given at the end of this method in Section 6.0: Comments.

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3.3.5.3 Positive and Negative Glucuronide Controls.

3.3.5.3.1 These controls may be obtained commercially or prepared in-house by spiking negative urine. The same negative urine must be used to prepare both the positive and negative glucuronide controls. Oxazepam glucuronide, lorazepam glucuronide or morphine glucuronide may be used for these controls and must be at a minimum concentration of 375 ng/mL. Examples of preparation are given in Appendix III. Derivatization will be required for the controls prepared in-house, even if there are no case samples requiring derivatization. The positive and negative glucuronide controls are used to demonstrate the glucuronidase was effective (if these samples are run in conjunction with samples that are not derivatized or are derivatized using option 2, one set of glucuronide controls can be used for both.)

4.0 Procedure

4.1 Extraction Procedure

NOTE: This method provides three options for the analyst. The method describes the preparation of an ethyl acetate extract and two options for a derivatized extract. Based on compounds of interest, both extracts need not be prepared and only the corresponding control material must be included.

4.1.1 Non-Derivatized Ethyl Acetate Samples

4.1.1.1 Casework and Control samples

4.1.1.1.1 Transfer 6 mL casework samples and controls to screw top extraction tubes.

4.1.1.2 Internal Standard Addition

4.1.1.2.1 To each prepared sample, add 300 µL of internal standard (10ng/µL working solution) or 3µL of 1 mg/mL stock solution. Vortex to mix.

4.1.1.3 Sample Hydrolysis

4.1.1.3.1 Add 200 µL 2M acetate buffer to each tube, vortex.

4.1.1.3.2 To all but the glucuronidase negative, add 150 µL BG100 b-Glucuronidase Solution. Cap and vortex gently to mix.

4.1.1.3.3 Place all tubes in 60°C laboratory oven or water bath for one hour.

4.1.1.3.4 Allow samples to cool before proceeding with solvent extraction.

4.1.1.4 Extraction

4.1.1.4.1 Add 2 mL 50mM sodium bicarbonate to each sample tube. Vortex.

4.1.1.4.2 Check pH. If necessary, adjust pH to approximately pH 9 with 1N NaOH or KOH.

4.1.1.4.3 Add 4 mL of chloroform/isopropanol {9:1}.

4.1.1.4.4 Rock for approximately 15 minutes.

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4.1.1.4.5 Centrifuge (~3300-3500 for about 10-15 minutes)

4.1.1.4.6 Transfer lower organic phase from tube into labeled tapered bottom tube.

4.1.1.4.7 Evaporate solvent to dryness under a gentle stream of nitrogen at ~37°C. Proceed to 4.1.1.5 if not derivatizing, or to 4.1.2 if derivatizing before running on GC/MS.

4.1.1.5 Reconstitution with Ethyl Acetate (No Derivatization)

4.1.1.5.1 Add 50 µL ethyl acetate. Vortex.

4.1.1.5.2 Transfer extract to labeled ALS vial with micro-insert.

4.1.1.6 Preparation for Analysis Run

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

4.1.1.6.2 Load samples, reference material, blanks and controls into the quadrant rack(s) as noted in the sequence table.

4.1.2 Derivatization of Samples: Option 1

4.1.2.1 Follow Ethyl acetate sample preparation steps included in 4.1.1.2 - 4.1.1.4

4.1.2.2 Derivatization

4.1.2.2.1 To the tapered-bottom tubes add 20 µL ethyl acetate and 30 µL of silylating agent.

4.1.2.2.2 Cap tubes. Vortex.

4.1.2.2.3 Heat tube for 15 minutes in 75°C drybath.

4.1.2.2.4 Remove from heat and allow samples to cool. Transfer derivative to labeled ALS vial with micro-insert.

4.1.2.3 Preparation for Analysis Run

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

4.1.2.3.2 Load samples, reference material, blanks and controls into the quadrant rack(s) as noted in the sequence table.

4.1.3 Derivatization of Samples: Option 2

(Complete ethyl acetate sample Extraction Procedure (4.1.1.2-4.1.1.5), run samples on GC/MS, then complete derivatization).

4.1.3.1 Derivatization

4.1.3.1.1 Once the ethyl acetate extracts have run on the GC/MS, add 20 µL of silylating agent to remaining extract in the autosampler insert and vortex.

4.1.3.1.2 Heat vials for 15 minutes at 75°C.

4.1.3.1.3 Remove from heat and allow to cool.

4.1.3.2 Preparation for Analysis Run

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

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4.1.3.2.2 Load samples, reference material, blanks and controls into the quadrant rack(s) as noted in the sequence table.

4.1.3.3 GC-MSD Analysis Parameters

4.1.3.3.1 Refer to instrument method for current analysis parameters.

4.1.3.3.1.1 Instrument Run Parameters

- Inlet at 280 degrees Celsius, splitless injection, injection volume: 1µL
- Oven at 80 degrees Celsius, hold 2.5 minutes
- Ramp 25 degrees Celsius
- Final temperature: 300 degrees Celsius, hold at least 7.5 minutes.

4.1.3.3.2 Current analysis method must be stored centrally as a hard or electronic copy.

4.1.4 Detection and Identification Criteria

4.1.4.1 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.

4.2 Methods Limitations and Application to Other Analytes

4.2.1 This method is applicable to other compounds, which require an enzymatic hydrolysis to liberate the compound of interest. Both the ethyl acetate extraction and the TMS derivative can be applied toward the identification of these compounds.

4.2.2 This method has proven useful in the identification of opiate class compounds such as codeine, morphine, 6-monoacetylmorphine, hydrocodone, and buprenorphine.

4.2.3 Care should be taken when estazolam is detected, particularly in samples containing alprazolam and/or alpha-hydroxyalprazolam. For samples containing alprazolam and/or alpha-hydroxyalprazolam, estazolam must be detected in both underivatized and derivatized GC/MSD data to be considered reportable. Estazolam shall not be reported if alprazolam and/or alpha-hydroxyalprazolam are detectable in the sample and derivatized estazolam is not detected.

4.3 Quality Assurance Requirements

4.3.1 Refer to Toxicology AM #19 for additional quality assurance and Toxicology AM #21 for 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 LIMS 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 destruction.

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

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5.0 Work Instructions

5.1 Reference Material

5.1.1 Benzodiazepine Positive Control

- 5.1.1.1 Stock Solutions
  - 1 mg/mL single component or 250 µg/mL multi-component benzodiazepine class reference solutions.
- 5.1.1.2 Direct spiking
  - Spike negative urine with 3µL of each single component stock or 12µL of 250 µg/mL stock solution.
- 5.1.1.3 Working Control Solution (10 ng/µL)
  - Add 100 µL each 1 mg/mL or 400 µL 250 µg/mL Stock Solution to ≅ 9 mL MeOH in 10 mL ball flask. Bring up to volume with MeOH. Solution is stable for one-year when stored under refrigeration. (Note: A minimum of two benzodiazepine class compounds must be included in the control solution. One of these compounds must form a TMS derivative.)

5.1.2 Conjugated Controls

5.1.2.1 Commercially obtained conjugated control (example: Bio-Rad C3)

5.1.2.2 Stock Solution

5.1.2.2.1 1 mg/mL Oxazepam Glucuronide or 100 µg/mL Lorazepam Glucuronide or 100 µg/mL Morphine Glucuronide

5.1.2.2.1 Direct spiking

5.1.2.2.1.1 Spike negative urine with 3 µL of 1 mg/mL stock solution or 30 µL of 100 µg/mL stock solution.

5.1.2.2.2 Working Glucuronide Solution (10ng/µL)

5.1.2.2.2.1 Add 100 µL of 1mg/mL Stock Solution (or 1 mL 100 µg/mL Stock Solution) to 10 mL MeOH. Solution is stable for one year when stored under refrigeration.

5.1.3 Internal Standard

5.1.3.1 Stock Solution

5.1.3.1.1 1 mg/mL Prazepam.

5.1.3.1.1 Direct addition

5.1.3.1.1.1 Add 3 µL of 1 mg/mL stock solution to each 6 mL urine sample and control.

5.1.3.1.2 Working Internal Standard Solution

5.1.3.1.2.1 Add 100 µL Stock Solution to ≅ 9 mL MeOH in 10 mL ball flask. Bring up to volume with MeOH. Solution is stable for one year when stored under refrigeration.

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5.1.4 Comments: This method has instructions for the preparation of both an underivatized and a TMS-derivatized extract. The analyst has the option of preparing either or both of these extracts.

6.0 Comments

6.1 Positive Control Sample

Use the lot of negative urine that will be used to prepare negative control.

- Pipette 6 mL of commercially obtained positive control or prepare positive control as described below.
- Use the table below as a guide for spiked positive control preparation. Prepare control for a concentration between 300 to 600 ng/mL.

Spiking Solution Concentration	Amount to Add (µL)	Resulting ng/mL
100 ng/µL (100 µg/mL)	30	500
10 ng/µL	300	500
1 mg/mL	3	500
250 ng/µL (250 µg/mL)	12	500

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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 marihuana 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]

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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

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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.

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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

3.2 Reagents

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3.2.1 0.1% formic acid in methanol (LCMS, Optima LCMS or equivalent)

3.2.2 0.1% formic acid in water (LCMS, Optima LCMS, or equivalent)

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 20,000 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. This solution will be stored in the freezer and is good for approximately six months.

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.

4.1.2.4 Centrifuge at ~3400 rpm for 15 minutes.

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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 that 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.

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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.

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Toxicology AM #8: Basic and Neutrals Drugs in Blood

1.0 Background/References

1.1 Background

This method is a general blood extraction procedure for a variety of commonly encountered neutral and basic drugs along with their metabolites. The method is based upon the principle of liquid/liquid extraction. Positive controls are spiked for a resulting concentration of 200 ng/mL or 500 ng/mL of drugs of interest. The sample pH is adjusted with a pH 9.2 borate buffer and extracted with n-butyl chloride. An optional back extraction procedure removes most frequently encountered interfering substances. Two internal standards are used to monitor extraction efficiency and chromatographic performance. Gas chromatography in conjunction with full scan mass spectrometry is used to confirm the presence of basic and neutral analytes of interest.

1.2 References

1.2.1 Procedure for Basic Drug Analysis, Courtesy of Jim Hutchison, Montana Department of Justice, Forensic Services Division, 2005.

1.2.2 Procedure for Back Extraction, Courtesy of Jim Hutchison, Montana Department of Justice, Forensic Services Division, 2006.

1.2.3 Strong Bases Extractions - Screening SOP, Courtesy of Dr. Graham Jones, Office of the Chief Medical Examiner, Edmonton, Canada, 2003.

1.2.4 Jones, G., Postmortem Toxicology. pp. 98-102, in: Clarke's Analysis of Drugs and Poisons, 3rd Edition, Moffat, A.C, Osselton, M.D. and Widdop, B., eds., Pharmaceutical Press, 2004.

1.2.5 Hearn, W.L. and Walls, H.C., Strategies for Postmortem Toxicology Investigation. pp. 937-939. In: Drug Abuse Handbook, S.B. Karch, ed., CRC Press, Boca Raton, FL, 1998.

2.0 Scope

2.1 This method is a general blood extraction procedure for a variety of commonly encountered neutral and basic drugs, along with their metabolites. This method has also been shown capable of extracting some acidic drugs (e.g. butalbital) if sufficient quantity is present in the sample. This method does not efficiently extract some basic compounds, such as morphine and hydromorphone, due to pKa/pH considerations. The method allows for the analyst to use either methanol or ethyl acetate as a reconstitution solvent. Some benzodiazepines are more efficiently extracted using ethyl acetate than methanol as a reconstitution solvent. In addition, samples reconstituted in ethyl acetate can also be derivatized to increase sensitivity and detection of some compounds. Some drugs are more efficiently extracted using methanol as a reconstitution solvent. It is at the analyst's discretion to determine which solvent to use.

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3.0 Equipment/Reagents

3.1 Equipment and Supplies

3.1.1 Tube Rocker

3.1.2 Vortex Mixer

3.1.3 Evaporative concentrator equipped with nitrogen tank.

3.1.4 Laboratory centrifuge capable of 3400 rpm

3.1.5 Fixed and adjustable single channel pipettes, and appropriate tips

3.1.6 16X100mm round bottom glass screw-top tubes

3.1.7 Screw Cap for 16mm O.D. tubes

3.1.8 GC/MS Automated Liquid Sampler (ALS) vials

3.1.9 GC/MS Vial Microinsert

3.1.10 Gas Chromatograph equipped with a Mass Selective Detector

3.1.11 5%-Diphenyl-95%-Dimethyl-siloxane copolymer capillary GC column, 12.5 to 30M.

3.2 Reagents

Refer to Toxicology AM #23 for Solution Preparation instructions.

3.2.1 Methanol (ACS Grade)

3.2.2 n-Butyl chloride (ACS Grade)

3.2.3 Borate Buffer (pH 9.2)

3.2.4 Deionized/Distilled (DI) Water

3.2.5 1% Hydrochloric Acid in Methanol

3.2.6 100mM Hydrochloric Acid

3.2.7 Ethyl Acetate (ACS Grade)

3.2.8 Silylating Agent (select from):

- BSTFA/1% TMCS
- MSTFA

3.3 Reference Materials

3.3.1 Positive Control

NOTE: Positive Control can be prepared with the working solution described below and/or obtained commercially.

3.3.1.1 Positive Control Stock Solution

3.3.1.1.1 Obtain 1 mg/mL stock drug standard solutions through Cerilliant, Grace, Sigma or other appropriate vendor.

3.3.1.2 Positive Control Working Solution

3.3.1.2.1 Add the designated volume of stock solution to 10 mL methanol. A minimum of four compounds must be used.

NOTE: Solution is stable for 6-months when stored at room temperature or 12-months when stored under refrigeration. Re-make solution if deterioration is noted.

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Stock Solution (1.0 mg/mL)	Volume (µL)
Amitriptyline	20
Caffeine	20
Codeine	20
Diphenhydramine	20
Lidocaine	20
Meperidine	20
Methadone	20
Nicotine	20
PCP	20
Trazodone	50
Methamphetamine	20
Phentermine	20

3.3.2 Internal Standard

3.3.2.1 Stock Solutions

- 1 mg/mL Benzphetamine
- 1 mg/mL Papaverine

3.3.2.2 Working Internal Standard Solution [10 ng/µL]

3.3.2.2.1 Add 100 µL Benzphetamine and Papaverine stock solutions to 10 mL volumetric ball flask. QS with methanol.

NOTE: Solution is stable for three months when stored in the freezer.

3.3.3 Negative Control

- Negative Whole Blood

3.3.4 Reference Standards

3.3.4.1 Stock Standard Solution

3.3.4.1.1 Obtain 1 mg/mL stock drug standard solutions through Cerilliant, Grace, Sigma or other appropriate vendor.

3.3.4.2 Working Drug Standard Solution

3.3.4.2.1 Add the designated volume found in 3.3.1.2.1 of stock solution to 10 mL methanol.

4.0 Procedure

4.1 Extracted Procedure

4.1.1 Initial set-up

4.1.1.1 For each control and case sample, label two screw-top extraction tubes and one ALS vial with micro-insert.

4.1.2 Positive Control Samples

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NOTE: The same lot of negative blood must be used for the preparation of both negative and positive spiked controls.

4.1.2.1 Prepare control sample by adding 200 µL mixed working control solution to 2 mL negative whole blood or pipette a 2 mL sample of commercially-obtained whole blood positive control.

4.1.2.2 When the optional back extraction is used, prepare an additional positive control to parallel the back extraction process.

4.1.2.3 When some samples in a batch are going to be reconstituted with methanol and others with ethyl acetate, a separate positive control must be run for each reconstitution solvent.

4.1.3 Casework Samples

4.1.3.1 Transfer 2 mL casework sample to screw top extraction tube.

4.1.4 Negative Control Sample

4.1.4.1 Transfer 2 mL negative whole blood to extraction tube. If some samples are going to be run with ethyl acetate and some with methanol as a reconstitution solvent, a negative control must be prepared and run for each reconstitution solvent. If the optional back extraction is used, prepare an additional negative control to parallel the back extraction process.

4.1.5 Internal Standard

4.1.5.1 Add 20 µL of internal standard mixture and vortex. If benzodiazepines are of interest, 3 µL of Prazepam (1 mg/mL) may also be spiked into the sample.

NOTE: If the analyst has reason to suspect the sample may contain one or more of these internal standard compounds, it is permissible for the analyst to eliminate one of the internal standard compounds (e.g. papaverine) or replace one of the internal standard compounds with an appropriate alternative (prazepam may be used). Clear notation of the replacement, along with justification, must be included in the analysis notes. If the analyst prefers to use a different internal standard than those listed here, s/he must confer with the toxicology discipline leader in the selection of said internal standard.

4.1.5.2 Allow sample to stand 10 minutes.

4.1.6 Initial Extraction

4.1.6.1 Add 2 mL borate buffer. Vortex.

4.1.6.2 Pipet 4 mL n-butyl chloride into each tube, cap.

4.1.6.3 Place tube on rocker for 10 minutes.

4.1.6.4 Centrifuge for 10 minutes at 3400 rpm.

4.1.6.5 Transfer the n-butyl chloride layer (upper) to second screw-top tube.

4.1.6.6 Add 50 µL 1% HCl in Methanol.

4.1.6.7 Evaporate to dryness under N2 at ≤37°C.

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4.1.6.7.1 If no clean-up proceed to 4.1.8.

4.1.7 Optional Sample Clean-up (Back Extraction Procedure)

4.1.7.1 Reconstitute with 50 µL 100mM HCl.

4.1.7.2 Add 1 mL of n-Butyl Chloride. Vortex.

4.1.7.3 Rock for 5 minutes.

4.1.7.4 Centrifuge for 5 minutes at 3400 rpm.

4.1.7.5 Discard upper n-Butyl Chloride layer.

4.1.7.6 Add 2 mL of pH 9.2 borate solution. Vortex

4.1.7.7 Add 4 mL of n-Butyl Chloride.

4.1.7.8 Rock for 5 minutes.

4.1.7.9 Centrifuge for 5 minutes at 3400 rpm.

4.1.7.10 Transfer upper n-Butyl Chloride layer into screw-top tube.

4.1.7.11 Evaporate to just dryness under N_2 at ≤45°C.

4.1.8 Reconstitution

4.1.8.1 Add 50 µL Methanol or Ethyl Acetate to the residue, vortex.

4.1.8.2 Transfer extract to labeled ALS vial with micro-insert.

4.1.9 Preparation for Analysis Run

4.1.9.1 Into Sequence log table, enter the case samples, blanks and controls.

4.1.9.2 Load samples, standards, blank and controls into the quadrant rack as noted in the sequence table.

4.1.10 Optional Derivatization *(samples that are extracted with ethyl acetate may be derivatized at the analyst's discretion)*

4.1.10.1 After ethyl acetate extraction samples have run on the GC-MSD, add 20 µL silylating agent to the sample. In addition to the case samples, the extracted positive and negative controls must also be derivatized and run.

4.1.10.2 Heat at about 75°C for ~15 minutes.

4.1.10.3 Allow samples to cool; run on GC-MSD.

4.1.11 GC-MSD Acquisition Parameters

4.1.11.1 Refer to instrument method printout for current acquisition parameters.

4.1.11.1.1 Instrument Run Parameters (Short Run)

- Inlet at 250 degrees Celsius, splitless injection, injection volume: 1µL
- Oven at 70 degrees Celsius, hold 2 minutes
- Ramp 35 degrees Celsius to 290 degrees Celsius, hold 3 minutes
- Ramp 40 degrees Celsius to 300 degrees Celsius, hold at least 6 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.

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4.1.11.1.2 Instrument Run Parameters (Longer Run)

- Inlet at 250 degrees Celsius, splitless injection, injection volume: 1μL
- Oven at 90 degrees Celsius, hold 4 minutes
- Ramp 15 degrees Celsius
- Final temperature: 290 degrees Celsius, hold at least 10.667 minutes
- Additional sample runs may be done with adjustments to these parameters, for example if there is a co-eluting peak or a late eluting compound.

4.1.11.2 Current acquisition method must be stored centrally as a hard or electronic copy.

4.1.12 GC-MSD Qualitative Detection and Identification Criteria

4.1.12.1 For the confirmation/identification of compounds, analyze appropriate non-extracted reference materials.

4.1.12.2 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.

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

4.2 Quality Assurance Requirements

4.2.1 General

4.2.1.1 Blood samples are to be stored under refrigeration after aliquots are removed for analysis.

4.2.1.2 Refer to Toxicology AM #17, AM #19 and AM #21 for quality assurance and reference material authentication requirements.

4.3 Analysis Documentation

4.3.1 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 LIMS system.

4.3.2 A packet containing 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 destruction.

4.3.3 A copy of controls need not be included in individual case files. When necessary, a copy of control printouts can be prepared from the centrally stored document.

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Toxicology AM #9: Acidic and Neutral Drugs in Blood

1.0 Background/References

1.1 This method is a general liquid-liquid procedure to extract a variety of commonly encountered acidic and neutral drugs along with their metabolites from blood. Drug compounds are extracted from blood by a liquid-liquid extraction process. Blood pH is adjusted with saturated ammonium chloride followed by extraction with ethyl acetate. After evaporation and a hexane wash, the final extract is subjected to analysis by GC-MSD. Two internal standards are used to monitor extraction efficiency and chromatographic performance. This extraction yields excellent recovery of most acidic and neutral drugs, and can generally be accomplished in under one hour. The extraction is designed to yield fewer and lower levels of endogenous compounds that can interfere with drug detection.

1.2 Reference

1.2.1 Procedure for Acid/Neutral Drug Analysis, Courtesy of Jim Hutchison, Montana Department of Justice, Forensic Services Division, 2005.

1.2.2 Foerster, E.H., Dempsey, J., and Garriott, J.D., A Gas Chromatography Screening Procedure for Acid and Neutral Drugs in Blood, J Anal Tox, 3:87-91, 1979.

1.2.3 Jones, G., Postmortem Toxicology. pp. 98-102, in: Clarke's Analysis of Drugs and Poisons, 3rd Edition, Moffat, A.C, Osselton, M.D. and Widdop, B., eds., Pharmaceutical Press, 2004.

1.2.4 Hearn, W.L. and Walls, H.C. Strategies for Postmortem Toxicology Investigation. pp. 937-939. In: "Drug Abuse Handbook" S.B. Karch, ed., CRC Press, Boca Raton, FL: 1998.

2.0 Scope

2.1 This method is used to extract a variety of commonly encountered acidic and neutral drugs, and their metabolites, from blood. Some basic drug compounds (e.g. diazepam), if in sufficient quantity in the sample, have been successfully extracted with this method.

3.0 Equipment/Reagents

3.1 Equipment and Supplies

3.1.1 Tube rocker

3.1.2 Evaporative concentrator equipped with nitrogen tank.

3.1.3 Vortex mixer

3.1.4 Laboratory centrifuge capable of 3400 rpm.

3.1.5 Fixed and adjustable volume single channel pipettes, and appropriate tips

3.1.6 16X100mm round bottom glass screw-top tubes

3.1.7 Screw Cap for 16mm O.D. tubes

3.1.8 GC/MS Automated Liquid Sample (ALS) vials

3.1.9 GC/MS Vial Microinsert

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3.1.10 Gas Chromatograph equipped with a Mass Selective Detector

3.1.11 5%-Diphenyl-95%-Dimethyl-siloxane copolymer capillary GC column, 12.5 to 30M.

3.2 Reagents

Refer to Toxicology AM #23 for Solution Preparation instructions.

3.2.1 Methanol (Certified ACS Grade)

3.2.2 Hexane (Certified ACS Grade)

3.2.3 Ethyl acetate (Certified ACS Grade)

3.2.4 Acetonitrile (Certified ACS Grade)

3.2.5 2N Sodium Hydroxide

3.2.6 Saturated Ammonium Chloride

3.3 Quality Assurance Material

3.3.1 Positive Control Working Solution

Note: Positive Control can be prepared with the working solution described below and/or obtained commercially.

3.3.1.1 Obtain 1 mg/mL stock drug standard solutions through Cerilliant, Grace, Sigma or other appropriate vendor.

3.3.1.2 Add the designated volume of stock solution to 10 mL methanol. A minimum of four compounds must be used.

Stock Solution	Volume (µL)
Acetaminophen	20
Butalbital	20
Carbamazepine	20
Carisoprodol	20
Meprobamate	20
Phenobarbital	20
Secobarbital	20

3.3.1.3 Solution is stable for 6-months when stored at room temperature. Remake solution if deterioration is noted.

3.3.2 Internal Standard Mix

3.3.2.1 Stock Solution

3.3.2.1.1 1 mg/mL Aprobarbital

3.3.2.2 Working Internal Standard Solution [50 ng/µL]

3.3.2.2.1 Add 500 µL Aprobarbital stock solution to 10 mL volumetric ball flask. QS with methanol.

3.3.2.2.2 Solution is stable for 3 months when stored at room temperature.

3.3.3 Negative Control

3.3.3.1 Negative Whole Blood

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4.0 Procedure

4.1 Extraction Procedure

4.1.1 Initial set-up

4.1.1.1 Label ALS vials, with micro-inserts, and two extraction tubes for each control and case sample.

4.1.2 Positive Control

Note: The same lot of negative blood must be used for the preparation of both negative and positive spiked controls.

4.1.2.1 Prepare or use commercially obtained positive control. To prepare: add 100 µL mixed working control solution to 1 mL negative whole blood.

4.1.2.2 Positive control may be run in duplicate.

4.1.3 Negative Control

4.1.3.1 Transfer 1 mL negative whole blood to screw top extraction tube.

4.1.4 Casework Samples

4.1.4.1 Transfer 1 mL casework samples to screw top extraction tube.

4.1.4.2 To all samples, including controls, add 20 µL of internal standard working solution.

4.1.4.3 Vortex.

4.1.4.4 Add 1 mL saturated ammonium chloride and vortex.

4.1.5 Extraction

4.1.5.1 Pipet 4 mL ethyl acetate into each tube, cap.

4.1.5.2 Place tube on rocker for 10 minutes.

4.1.5.3 Centrifuge for 10 minutes at 3400 rpm.

4.1.5.4 Transfer the ethyl acetate (top) layer to second tube.

4.1.5.5 *If necessary, this is potential overnight stopping point. Tubes must be capped and refrigerated.*

4.1.6 Evaporation

4.1.6.1 Evaporate to dryness under a gentle stream of nitrogen at approximately 37°C.

4.1.7 Hexane Wash

4.1.7.1 Pipet 500 µL hexane into each tube and vortex.

4.1.7.2 Place tube on rocker for 5 minutes.

4.1.7.3 Pipet 50 µL Acetonitrile. Vortex briefly.

4.1.7.4 Centrifuge for 5 minutes at 3400 rpm

4.1.7.5 Discard the hexane (top) layer.

4.1.7.6 Transfer acetonitrile extract to labeled ALS vial with micro-insert.

4.1.8 Preparation for Analysis Run

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

4.1.8.2 Load samples, standards, blank and controls into the quadrant rack as noted in the sequence table.

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4.1.9 Acquisition Parameters

4.1.9.1 Refer to instrument method printouts for acquisition parameters.

4.1.9.1.1 Instrument Run Parameters

- Inlet at 250 degrees Celsius, splitless injection, injection volume: 1µL
- Oven at 120 degrees Celsius, hold 2 minutes
- Ramp 35 degrees Celsius, hold 3 minutes
- Final temperature: 290 degrees Celsius, hold at least 9.143 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.

4.1.9.2 Current acquisition method must be stored centrally as a hard or electronic copy.

4.1.10 GC-MSD Qualitative Detection and Identification Criteria

4.1.10.1 For the identification of compounds not included in positive control, analyze appropriate non-extracted reference materials.

4.1.10.2 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.

4.2 Quality Assurance Requirements

4.2.1 General

4.2.1.1 Blood samples are to be stored under refrigeration after aliquots are removed for analysis.

4.2.1.2 Refer to Toxicology AM #17, AM #19and AM #21 for quality assurance and reference material authentication requirements.

4.3 Analysis Documentation

4.3.1 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 LIMS system.

4.3.2 A packet containing 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 destruction.

4.3.3 A copy of controls need not be included in individual case files. When necessary, a copy of control printouts can be prepared from the centrally stored document.

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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

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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.

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.

Table 1B

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

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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.

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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 with weighing paper or weigh boat.

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 for compliance with ISO/IEC 17025, and initialed before the balance is placed back in use.

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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 with 0.005 g of their expected weight. Calibration certificates will be checked for compliance with ISO/IEC 17025 and 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.

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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 When reported, quantitative values must be within the estimated uncertainty of measurement.

4.1.3 If the analyst does not correctly identify all target analytes and/or quantitative values do not fall within 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.

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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., and 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).*

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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 controls and analytical plates may be utilized for qualitative and/or quantitative analysis after the manufacturer's expiration date *provided* all method control requirements are met AND an additional in-house control that is not expired be run. This limited permission is not applicable to expired controls of a unique nature (e.g. EMIT level 0 pre-run control).

4.3.1.1.5 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.

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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 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.

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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.
- LCMSMS targeted screen (analytical methods 6, 25 and 26).
- LC-QTOF targeted screen (analytical methods 29 and 30).
- LCMSMS 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)
- LCMSMS targeted confirmation methods (analytical methods 27, and 28)

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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

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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. 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 charges pending

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 LCMSMS, 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 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 not necessary to pursue confirmatory testing for metabolites or to report the metabolite was indicated.

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Example 1. A targeted LCMSMS screen is run and is positive for zolpidem and zopiclone. Zolpidem is confirmed by LCMSMS. 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 LCMSMS screen is run and is positive for hydrocodone, norhydrocodone, and dihydrocodeine. An LCMSMS confirmation is run and is positive for hydrocodone but the norhydrocodone is too weak and dihydrocodeine could not be evaluated. The analyst would report Drug(s) confirmed: Hydrocodone.

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 LCMSMS 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 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.

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4.6.10 All examples of reporting criteria are guidelines and can be altered to fit the circumstance. The exact wording specified does not need to be followed but is merely a guideline.

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.

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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 GC/MS and liquid/liquid extraction methods
- Methods that utilize the LC/MS/MS and SLE or liquid/liquid extraction

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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.

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.

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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.

4.2 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.

4.2.1.1.1 Reference material used for LCMS analytical methods may be authenticated using the appropriate acquisition method. Documentation of the authentication will be the calibration curve print out for the compound of interest. The calibration curve and COA should be filed.

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.

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4.2.2 Reference materials used for qualitative purposes do not have expiration dates; if the compound breaks down and is no longer performing as intended, the reference material will be discarded (or clearly marked invalid for casework since it may be used for training purposes).

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 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.

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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.

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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)

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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

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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. Store in a brown glass bottle.
- *Solution is stable for six-months. A positive and negative control will be run with each use. Remake as indicated by control data, or every 6 months.*

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)**

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- 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.*

4.1.8 Sodium Acetate Buffers

4.1.8.1 **2.0M Acetate Buffer, pH 4.8 (1 L)**

- Dissolve 141.4g sodium acetate trihydrate in approximately 800 mL DI water. Add 55.2 mL glacial acetic acid. Adjust to pH 4.8 and QS to 1 L.
- *A positive and negative control will be run with each use. Remake as indicated by control data.*

4.1.9 Sodium Bicarbonate

4.1.9.1 **50mM Sodium Bicarbonate, pH 11 (500 mL)**

- Dissolve 2.1g sodium bicarbonate in 500 mL DI water. Adjust to pH 11 as needed.
- *A positive and negative control will be run with each use. Remake as indicated by control data.*

4.1.10 Sodium Hydroxide (NaOH)

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

4.1.10.1 **2N Sodium Hydroxide (2N NaOH) (1 L)**

- Place approximately 250 mL DI water into a 1 L beaker. Gradually add 80g NaOH**.** Transfer to 500 mL volumetric flask and QS to 500 mL. (Caution: Exothermic)
- 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.

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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 Quadruple 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 Quadruple 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.

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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 LC/MS/MS or LC-QTOF on, using the prepare pump function, purge the LC pump and set flow to 1 mL/min. Let run for approximately 10-15 minutes, this will remove any bubbles that may have accumulated since last use. Monitor the pressure curves. If it appears there may be any solvent bubbles, the analyst may use the prime or condition function.

4.2.1.4 Verify the Column to be used for the desired method.

4.2.1.4.1 If a column change is needed, remove the column that is installed on the instrument, and replace with the dead volume connector.

4.2.1.4.2 Run correct mobile phases at a 50/50 ratio with 1 mL/min flow for approximately 5 minutes to flush any previous mobile phase from the system.

4.2.1.4.3 Install the desired column, if necessary, set solvent flow to appropriate method flow rate and solvent ratio, then let the system run for approximately 5-10 minutes.

4.2.1.5 Select Tune in MassHunter Acquisition and run a checktune (LC/MS/MS) or mass calibration (LC-QTOF), to check the calibration of the instrument. 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 (LC/MS/MS) or system tune and/or transmission tune (LC-QTOF).

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4.2.1.5.1 NOTE: A successful checktune must have been run within one week prior to running case samples for LC/MS/MS. A successful transmission tune must have been run within one week prior to running cases and a successful mass calibration must be run prior to the start of the run. A system tune should be performed when the transmission and/or calibration check dictates the need.

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.

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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%. For values below 10 ng/mL the results should be within ± 30% of the target value.

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.

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

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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 5 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

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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.

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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

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

3.1.11 Appropriate sized pipette tips

3.1.12 Heat sealing foil covers for 96 well plate

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

3.1.14 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)

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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)

3.2.9.10 20% Methanol in water (Reconstitution Solvent) (LCMS, Optima LCMS grade, or equivalent)

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

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

3.2.11 Instant Buffer I (provided with BGTurbo)

3.2.12 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 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.

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. For urine samples/controls only: Add 250 µl of blank urine to a clean 48 or 96 well plate. Add 40 µl of BGTurbo and 100 µl of Instant Buffer I to the wells containing urine. Place a cover on the plate and shake for a minimum of 5 minutes at ambient temperature.

4.1.3 Using a **calibrated** single channel pipette, add 250 µL of blank blood into the appropriate wells of the analytical plate to wells containing internal positive and negative controls. Pipette the appropriate sample/case blood into the appropriate wells containing only internal standards. For urine samples/controls: after shaking, transfer 250 µl of the urine mix to the appropriate wells containing only internal standard on the analytical plate.

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

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

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

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

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

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

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

4.1.13 Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approximately 35 degrees Celsius. Optional step: if run contains urine samples, add 50 µl of 1% HCl in MeOH to the wells 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 degrees Celsius). Temperatures for this step are not critical and it is appropriate to use the equipment temperature readings. The temperatures 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, 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.

4.1.14 Reconstitute in 100 µL 20% methanol in water (LCMS, Optima LCMS grade, or equivalent) and heat seal plate with foil.

4.1.15 Analyze samples or freeze for future analysis.

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

4.2.2.1.3: 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

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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.

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 analytes 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.

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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, methamphetamine, and pseudoephedrine of less than 32 (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. 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 print out (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.

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

1.0 Background/References

1.1 Background

Δ9-THC (Figure 1) is the chief psychoactive cannabinoid resulting from exposure to marijuana. Δ9-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 Δ9-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. Δ9-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 Δ9-THC inactive metabolite, carboxy-THC, gradually increases and may plateau for several hours.[5] There is poor correlation between blood Δ9-THC and psychoactive effects since the Δ9-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 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 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.

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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

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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

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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)

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)

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)

3.2.9 Saturated Phosphate Buffer (if run includes urine samples)

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 positive urine control must also be included.

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. For urine samples/controls only: 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 degrees Celsius.

4.1.3 Using a **calibrated** single channel pipette, add 1.0 mL of blank blood to the appropriate sample wells containing internal positive and negative controls. Pipette the appropriate sample/case blood into the appropriate wells containing only internal standards. For urine samples/controls: after shaking, transfer 1.0 mL of the urine/buffer to the appropriate wells containing internal standard on the analytical plate.

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

4.1.5 Add 0.5 mL 0.1% formic acid in LCMS water to all of the wells containing blood. Add 0.5 mL of saturated phosphate buffer to all of the wells containing urine.

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

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

4.1.8 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.

4.1.10 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*)*

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

4.1.12 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*)*

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

4.1.14 Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approximately 35 degrees Celsius. If run contains urine samples, place ACT cover on top of plate prior to drying. Temperatures for this step are not critical and it is appropriate to use the equipment temperature readings. The temperatures 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 degrees Celsius). 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.

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

4.1.16 Analyze samples or freeze for future analysis.

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

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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.

4.3.1.3 Retention time 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 sample may be evaluated as positive if the other criteria are met.

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.

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 re-inject or re-extract that 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.

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4.5.2 The print out (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 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.

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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] 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 quantitative blood levels.

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.

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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)

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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 Acetonitrile (LCMS, Optima LCMS grade, or equivalent)

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)

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 external urine control will also be included in the run.

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.

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. For urine samples/controls only: 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 degrees Celsius.

4.1.3 Using a **calibrated** single channel pipette, add 1.0 mL of blank blood to the appropriate sample wells containing internal positive and negative controls. Pipette the appropriate sample/case blood into the appropriate wells containing only internal standards. For urine samples/controls: after shaking, transfer 1.0 mL of the urine/buffer to the appropriate wells containing internal standard on the analytical plate.

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

4.1.5 Add 0.5 mL 0.1% formic acid in LCMS water to all of the wells containing blood. Add 0.5 mL of saturated phosphate buffer to all of the wells containing urine.

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

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

4.1.8 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.

4.1.10 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)

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

4.1.12 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)

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

4.1.14 Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approximately 35 degrees Celsius. If run contains urine samples, 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 degrees Celsius). Temperatures for this step are not critical and it is appropriate to use the equipment temperature readings. The temperatures 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.

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

4.1.16 Analyze samples or freeze for future analysis.

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.

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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 Non-extracted solvent blank will be run directly before a case sample.

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 - 50^0C

4.2.5.1.2 Injection volume –0.5-10 µL

4.2.5.1.3 Mobile Phase Flow rate - 0.6 mL/min

4.2.5.1.3: 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	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 limit of detection (LOD) is the same as the limit of quantitation (LOQ) for blood quantitative analysis results. The uncertainty of measurement budgets for each compound have been established and will be updated when new instrumentation is approved for use and will be evaluated annually by the discipline lead. The LODs are as follows:

Analyte	LOD
THC	3 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 sample must have a concentration no less than the established LOD for that analyte.

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4.3.2.2 Samples with a higher response than the 50 ng/mL will be reported out as greater than 50 ng/mL. The uncertainty of measurement has currently only been evaluated between 3 and 50 ng/mL for each compound. 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.

4.3.2.3 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 sample may be evaluated as positive if the other criteria are met.

4.3.2.4 For calibrators and controls 10 ng and below, the accuracy must be within 30%, for calibrators and controls greater than 10 ng/mL the accuracy must be within 20%. If a control falls outside the accuracy range, at the analyst's discretion, the compound may be reported qualitatively.

4.3.2.5 If any points are dropped from the approved quantitative range of the curve, the compound will be reported qualitatively. For calibrators and controls 10 ng and below, the accuracy must be within 30%, for calibrators and controls greater than 10 ng/mL the accuracy must be within 20%. If a control falls outside the accuracy range, at the analyst's discretion, the compound may be reported qualitatively.

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

4.3.2.7 S/N of detected analyte must be greater than 10.

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 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.

4.3.2.10 No quantitative values will be reported for urine cases.

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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.

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

3.1.11 Appropriate sized pipette tips

3.1.12 Heat sealing foil covers

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

3.1.14 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)

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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)

3.2.9.10 20% Methanol in Water (LCMS, Optima LCMS grade, or equivalent) (Reconstitution Solvent)

3.2.9.11 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)

3.2.11 Instant Buffer I (provided with BGTurbo)

3.2.12 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.

3.3.4 Blanks

3.3.4.1 A non-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. 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.

3.3.4.2 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.

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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. For urine samples/controls only: Add 250 µl of blank urine to a clean 48 or 96-well plate. Add 40 µl of BGTurbo and 100 µl of Instant Buffer I to the wells containing urine. Place a cover on the plate and shake for a minimum of 5 minutes.

4.1.3 Using a **calibrated** single channel pipette, add 250 µL of blank blood into the appropriate wells of the analytical (standards) plate (wells containing internal positive and negative controls). Pipette the appropriate sample/case blood into the appropriate wells containing only internal standards. For urine samples/controls: after shaking, transfer 250 µl of the urine mix to the appropriate wells containing internal standard on the analytical plate.

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

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

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

4.1.7 Transfer 300 µL of blood+base or urine+base mixture (if applicable) to corresponding wells of SLE+ plate.

4.1.8 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)

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

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

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4.1.13 Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approximately 35 degrees Celsius. If run contains urine samples, at analysts' discretion, add 50 µl of 1% HCl in MeOH to the wells. Place ACT cover on top of plate prior to drying if run contains urine samples. The samples may also be dried in another type of heated nitrogen dryer (the temperature should be set at approximately 30-40 degrees Celsius). Temperatures for this step are not critical and it is appropriate to use the equipment temperature readings. The temperatures 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.

4.1.13 Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approximately 35 degrees Celsius. If run contains urine samples, at analysts' discretion, add 50 µl of 1% HCl in MeOH to the wells. Place ACT cover on top of plate prior to drying if run contains urine samples. The samples may also be dried in another type of heated nitrogen dryer (the temperature should be set at approximately 30-40 degrees Celsius). Temperatures for this step are not critical and it is appropriate to use the equipment temperature readings. The temperatures 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 in 100 µL 20% LCMS grade methanol in LCMS Water and heat seal plate with foil.

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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^{0}C

4.2.4.1.2 Injection volume – 0.5-10 µL

4.2.4.1.3 Mobile Phase Flow rate - 0.5 mL/min

4.2.4.1.3: 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

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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 limit of detection (LOD) is defined in the scope of this method. Compounds listed as "Qualitative only" will only be reported out qualitatively, no reference to a quantitative value will be given until further review of data to establish Uncertainty of Measurement. Urine samples will be reported qualitatively only.

4.3.1.2.1 Since urine samples are diluted during the hydrolysis process, samples that are over 32 ng/mL for amphetamine and methamphetamine and may be reported as positive.

4.3.1.2.2 (Blood only) Compounds that have been evaluated and are approved for quantitative reporting will have a quantitative range value in the chart 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.

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

4.3.1.2.2.2 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.

4.3.2 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. Found in this AM section 2.2.

4.3.2.1.1 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.

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4.3.2.1.2 For quantitative reporting: All points in the approved range defined in section 2.2 of this method must be used. In addition, the accuracy for all controls (QC's) for that compound must be within the appropriate tolerances (+/- 30% for 10 ng/ml and lower and +/- 20% for above 10 ng/ml). If a QC fails to meet the requirements, the compound will be reported qualitatively (see exceptions below).

4.3.2.1.2.1 If any points are dropped from the approved quantitative range of the curve, the compound will be reported qualitatively. The exception is if the highest point is dropped. In addition, if the highest control (QC) fails. The quantitative range for that compound in that range will be adjusted to the next highest calibrator level. Any samples exceeding that level will be reported as greater than (upper limit) ng/ml.

4.3.2.1.2.2 The quantitative range for fentanyl is 5-100. Samples with concentrations between 0.5 and 5 will be reported qualitatively.

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 sample may be evaluated as positive if the other criteria are met.

4.3.2.3 For calibrators 10 ng and below the accuracy must be within 30%, for calibrators greater than 10 ng/mL the accuracy must be within 20%.

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

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

4.3.2.6 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.

4.3.2.7 Controls must give a positive response for each compound if the concentration of the control is greater than the LOD for any given compound. It is not necessary for the control to give a positive response if a particular compound is not being evaluated in a run. In those instances, a notation will be made on the control indicating that compound is not being evaluated. For compounds that are approved for quantitative reporting controls 10 ng and below the accuracy must be within 30%, for controls greater than 10 ng/mL the accuracy must be within 20%. If any control within the evaluated quantitative range falls outside the accuracy range, at the analyst's discretion, the compound may be reported qualitatively.

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4.3.2.8 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.

4.3.2.9 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.

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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

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

3.1.11 Appropriate sized pipette tips

3.1.12 Heat sealing foil covers for 96 well plate

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

3.1.14 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)

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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)

3.2.9.10 20% Methanol in water (Reconstitution Solvent) (LCMS, Optima LCMS grade, or equivalent)

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

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

3.2.12 Instant Buffer I (provided with BGTurbo)

3.2.13 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 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.

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. For urine samples/controls only: Add 250 µl of blank urine to a clean 48 or 96 well plate. Add 40 µl of BGTurbo and 100 µl of 500mM sodium phosphate (pH 6.8) to the wells containing urine. Place a cover on the plate and shake for a minimum of 5 minutes at ambient temperature.

4.1.3 Using a **calibrated** single channel pipette, add 250 µL of blank blood into the appropriate wells of the analytical plate to wells containing internal positive and negative controls. Pipette the appropriate sample/case blood into the appropriate wells containing only internal standards. For urine samples/controls: after shaking, transfer 250 µl of the urine mix to the appropriate wells containing only internal standard on the analytical plate.

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

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

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

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

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

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

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

4.1.13 Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approximately 35 degrees Celsius. Optional step: if run contains urine samples, add 50 µl of 1% HCl in MeOH to the wells 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 degrees Celsius). Temperatures for this step are not critical and it is appropriate to use the equipment temperature readings. The temperatures 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, 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.

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

4.1.15 Analyze samples or freeze for future analysis.

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

4.2.2.1.3: 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

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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 analytes 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. If the calculated concentration for amphetamine, methamphetamine, and/or pseudoephedrine is less than 32 (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.

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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 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 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 print out (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.

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

1.0 Background/References

1.1 Background

Δ9-THC (Figure 1) is the chief psychoactive cannabinoid resulting from exposure to marijuana. Δ9-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 Δ9-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. Δ9-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 Δ9-THC inactive metabolite, carboxy-THC, gradually increases and may plateau for several hours.[5] There is poor correlation between blood Δ9-THC and psychoactive effects since the Δ9-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 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.

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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

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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

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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)

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)

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

3.2.8 1N KOH (if run includes urine samples)

3.2.9 Saturated Phosphate Buffer (if run includes urine samples)

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 positive urine control must also be included.

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. For urine samples/controls only: 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 degrees Celsius.

4.1.3 Using a **calibrated** single channel pipette, add 1.0 mL of blank blood to the appropriate sample wells containing internal positive and negative controls. Pipette the appropriate sample/case blood into the appropriate wells containing only internal standards. For urine samples/controls: after shaking, transfer 1.0 mL of the urine/buffer to the appropriate wells containing internal standard on the analytical plate.

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

4.1.5 Add 0.5 mL 0.1% formic acid in LCMS water to all of the wells containing blood. To all wells containing urine: add 0.5 mL of saturated phosphate buffer.

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

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

4.1.8 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.

4.1.10 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*)*

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

4.1.12 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*)*

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

4.1.14 Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approximately 35 degrees Celsius. If run contains urine samples, place ACT cover on top of plate prior to drying. Temperatures for this step are not critical and it is appropriate to use the equipment temperature readings. The temperatures 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 degrees Celsius). 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.

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

4.1.16 Analyze samples or freeze for future analysis.

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

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

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.

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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 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.

4.4 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 print out (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.

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