Indiana State Department of Toxicology

Laboratory Test Methods

Table of Contents

Introduction

The methods in this document outline the procedures to be followed in the forensic toxicology testing laboratory.

Deviations to the following procedures may be employed with supervisory or quality assurance manager approval.

1. Blood Drug Screening by LC/TOF

1.1. Scope
- 1.1.1. This method shall be used for screening specimens for the presence of drugs and/or metabolites. Sample preparation shall be by liquid-liquid extraction.

1.2. Precautions/Limitations
- 1.2.1. Minimum Sample Requirement
  - 1.2.1.1. 600 µL of blood or serum/plasma specimen
- 1.2.2. Mobile phase solutions should be kept in amber bottles to increase stability.

1.3. Related Information
- 1.3.1. Blood Drug Screening by LC/TOF Validation (September 2017-April 2018)
- 1.3.2. 4 GHz High Resolution and Injection Volume Update (July 2019)
- 1.3.3. Reinjection Stability (June 2020)
- 1.3.4. Injection Volume Study (October-December 2020)
- 1.3.5. TOF New Recon Solution Evaluation (2024)
- 1.3.6. TOF Centrifuging Samples Validation (2024)
- 1.3.7. Instrument validations
- 1.3.8. ToxBox® Plate – Indiana State Dept. of Toxicology

1.4. Instruments/Equipment
- 1.4.1. Tube rack
- 1.4.2. Rocker
- 1.4.3. Vortex, single and multi-tube
- 1.4.4. Centrifuge
- 1.4.5. Evaporator
- 1.4.6. Circulating bath
- 1.4.7. Liquid chromatograph
- 1.4.8. Mass spectrometer, time of flight
- 1.4.9. Vial rack
- 1.4.10. Pipettes

1.5. Reagents/Materials
- 1.5.1. ToxBox custom 96-well plate (Multidrug TOF Screen)
- 1.5.2. Pipette tips
- 1.5.3. Autosampler vials, inserts, and caps
- 1.5.4. 13 mm test tubes and caps
- 1.5.5. ddH_2O
- 1.5.6. Negative blood (human)
- 1.5.7. Liquid chromatograph column
  - 1.5.7.1. Dimensions: 4.6 x 50 mm
  - 1.5.7.2. Composition: Zorbax Eclipse Plus C18, Rapid Resolution HT, 1.8 µm
- 1.5.8. Liquid chromatograph guard column
  - 1.5.8.1. Dimensions: 3.0 mm x 5 mm
  - 1.5.8.2. Composition: Poroshell C18, 2.7 µm particles
- 1.5.9. Nitrogen
- 1.5.10. Solvents shall be high quality and low residue (e.g., HPLC grade, Omnisolv, Optima, etc.) unless otherwise noted.
  - 1.5.10.1. Acetonitrile, LCMS grade or higher

1.5.10.2. Methanol, LCMS grade or higher
1.5.10.3. Formic acid
1.5.10.4. Methyl tert-butyl ether
1.5.10.5. Methylene chloride
1.5.11. Ammonium formate
1.5.12. Sodium carbonate
1.5.13. Sodium bicarbonate
1.5.14. Hydrochloric acid

1.6. Hazards/Safety
1.6.1. See Safety Manual.
1.6.2. See SDS for each chemical in this method.
1.6.3. Add acids to approximately half the volume of the less acidic liquid, then dilute to final volume.

1.7. Reference Materials/Controls/Calibrators/Solutions
1.7.1. Carbonate Buffer (300 mM), pH 9
1.7.2. Hydrochloric Acid (0.5M)
1.7.3. Extraction Solution (60:40 methyl tert-butyl ether: methylene chloride)
1.7.4. Mobile Phase Solutions
1.7.4.1. Aqueous (A)
1.7.4.2. Organic (B)
1.7.5. Reconstitution Solution

1.8. Procedures/Instructions
1.8.1. An evidentiary batch shall consist of concurrently prepared negative blood controls, calibrators, HC, UHC, and samples (ref. Table 1). The sequence shall be set up so that calibrators and/or non-zero controls are evenly spread through the batch (e.g., each set of one to 21 samples is bracketed by calibrators or non-zero controls). The sequence shall begin with a negative control, two calibrators at the cutoff concentrations, and a negative control.
1.8.2. Mix specimens on a rocker or by inverting several times.
1.8.3. Add 600 µL of negative blood to the calibrator and control well positions.
1.8.4. Add 600 µL of each specimen to its corresponding well position.
1.8.5. Add 400 µL of carbonate buffer to each well position.
1.8.6. Cap and vortex plate.
1.8.7. Pipette 1 mL of sample to a correspondingly labeled test tube.
1.8.8. Add 1 mL ddH_2O to each sample.
1.8.9. Add 4 mL methyl tert-butyl ether to each sample.
1.8.10. Cap tubes and vortex on multi-tube vortexer for 15 minutes.
1.8.11. Centrifuge for 10 minutes using 3000 rpm at 4-8 ºC.
1.8.12. Chill samples in circulating bath at -30 ºC for ~2 minutes, or until the aqueous/blood layer is frozen.
1.8.13. Transfer organic layer of each sample into a correspondingly labeled test tube.
1.8.14. Add 200 µL 0.5 M hydrochloric acid to each aqueous sample and vortex briefly.
1.8.15. Add 4 mL extraction solution to each aqueous sample.
1.8.16. Cap tubes and vortex on multi-tube vortexer for 15 minutes.
1.8.17. Centrifuge for 10 minutes using 3000 rpm at 4-8 ºC.

1.8.18. Chill samples in circulating bath at -30 ºC for ~2 minutes, or until the aqueous/blood layer is frozen.
1.8.19. Transfer organic layer of each sample into the correspondingly labeled test tube from step 1.8.13 and place test tubes on the evaporator.
1.8.20. Evaporate at room temperature using nitrogen.
1.8.21. Add 150 µL of reconstitution solution to each tube and vortex.
1.8.22. Centrifuge for 10 minutes using 3000 rpm 4-8 ºC.
1.8.23. Transfer 100 µL of each sample to a correspondingly labeled autosampler vial (with insert) and cap vial.
1.8.24. Analyze the samples by LC/TOF.
1.8.24.1. Sequence names shall be in the following format: YYYY_MM_DD_TOF BDS_Initials.
1.8.24.1.1. The date in the sequence shall be the date of preparation of the samples.
1.8.24.1.2. Additional information such as reinjection, validation, etc., or equivalent abbreviations should be included with the assay abbreviation.
1.8.24.1.3. If the sequence is run with the wrong sequence name, it shall be noted in the case synopsis of each case in the batch and not corrected on the chromatograms.
1.8.24.2. If multiple batches are included in one sequence, add a note on the MassHunter Worklist report that includes the batch names of each batch combined in the sequence in the format listed in 1.8.24.1. The note should list which lines are attributed to each batch.
1.8.24.3. If both modes need to be analyzed, negative mode should be analyzed first, followed by positive mode. Analytes marked with an asterisk in Table 1 are analyzed in negative mode. All other analytes are analyzed in positive mode.
1.8.24.4. If the instrument sequence is paused by the acquisition software between two samples, the sequence may be restarted at the sample not yet injected.
1.8.24.4.1. Seven-day sample stability for negative mode and positive mode criteria shall be met.
1.8.24.5. Reinjection of samples may be performed if initiated within seven days of the first injection of the sequence when samples are stored in the instrument autosampler or at equivalent temperature.
1.8.24.5.1. A reinjection sequence shall contain, at a minimum, two calibrators and a negative control bracketing the sample(s) to be reinjected.
1.8.24.5.2. A reinjection of a sample of unknown concentration may be performed once per mode of analysis.
1.8.24.5.3. Reinjection of a sample of known concentration may be performed multiple times.
1.8.24.5.3.1. If a reinjection is needed more than once, the evidentiary samples that have already been reinjected may be skipped in a bracket.
1.8.24.5.3.1.1. Evidentiary samples that are skipped and

do not have valid data shall be reanalyzed starting at 1.8.1.

1.8.24.6. When the entire sequence is being reinjected or is being injected on another instrument, a tune (Doc ID: 2843) shall be performed within 24 hours of initiation of the reinjection sequence. Resuming a sequence or reinjecting a partial sequence does not require a tune.

1.8.25. LC/TOF Acquisition Parameters

1.8.25.1. Liquid chromatograph sampler

Injection Mode	Standard wash, or equivalent
Injection Volume	2.0-10 µL for positive mode 5.0 µL for negative mode

1.8.25.2. Instrument Parameters

Positive Method		**Negative Method**	
LC Gradient			
Time (minutes)	**%B**	**Time (minutes)**	**%B**
0	5	0	55
6.5	60	3.1	67
7.5	95	3.2	95
8.0	95	3.7	95
LC Parameters			
Stop Time	8 min	Stop Time	3.7 min
Post Time	0.5 min (LC1) 1.0 min (LC4)	Post Time	0.5 min (LC1) 1.0 min (LC4)
Flow Rate	1.5 mL/min	Flow Rate	1.5 mL/min
Polarity	Positive	Polarity	Negative
Column Temp	55 ºC	Column Temp	55 ºC
MS Parameters			
Gas Temp	300 ºC	Gas Temp	350 ºC
Drying Gas	10 L/min	Drying Gas	11 L/min
Nebulizer	50 psi	Nebulizer	15 psi
Sheath Gas Temp	350 ºC	Sheath Gas Temp	350 ºC
Sheath Gas Flow	12 L/min	Sheath Gas Flow	12 L/min
VCap	5000 V	VCap	4500 V
Nozzle Voltage	2000 V	Nozzle Voltage	2000 V
Time/Experiment Setup			
Time = 0	Fragmentor	Time = 0	Fragmentor
Expt (1, 2, 3)	(120, 160, 180)	Expt (1, 2, 3)	(120, 160, 180)
		Time = 1.8	Fragmentor
		Expt (1, 2)	(250, 190)
		Time = 3.1	Fragmentor
		Expt (1)	(185)

1.8.25.3. Mass spectrometer

Ion Source	Dual AJS ESI
Scan Type	Scan
Data Collection	Centroid

Mode	4 GHz High Resolution
Reference Mass	Enabled

1.9. Records
- 1.9.1. Pipette calibration certificate, however named
- 1.9.2. ToxBox Analytical Plate Certificate of Analysis
- 1.9.3. Batch Preparation Packet
  - 1.9.3.1. Tox Screen Worklist
  - 1.9.3.2. Retest Worksheet, if applicable
  - 1.9.3.3. LC/TOF BDS Preparation Worksheet
  - 1.9.3.4. Aliquot Chain of Custody
- 1.9.4. MassHunter Worklist Report
- 1.9.5. TOF Tune Report for each mode analyzed
- 1.9.6. Calibrator and Control chromatograms
- 1.9.7. Sample chromatograms
- 1.9.8. BDS QA/QC Report for each mode analyzed
- 1.9.9. LC/TOF BDS Technical Review Checklist
- 1.9.10. Specimen Verification Worksheet, if applicable

1.10. Interpretation of Results
- 1.10.1. Interpretation of results for each analyte shall occur independent of the other analytes in the method.
- 1.10.2. Each analyte shall be chromatographically resolved with baseline separation and/or mass resolved
- 1.10.3. Confirmation drug classes referred to in this method are listed on the ISDT website.
- 1.10.4. The corresponding internal standard (Table 1) for each analyte shall be detected in each evidentiary sample and calibrator and should be detected in negative controls and non-zero controls.
  - 1.10.4.1. Internal standard mass accuracy shall be within 200 ppm of the target mass.
  - 1.10.4.2. If the corresponding internal standard is not detected, samples may be reinjected (ref. 1.10.8) or, if possible, reanalyzed starting at 1.8.1 for the analyte(s) with the internal standard that was not detected, unless the sample is already presumptive positive for another analyte in the same confirmation drug class.
- 1.10.5. Calibrator and Controls Criteria
  - 1.10.5.1. Results of samples analyzed prior to analysis of the first calibrator shall not be used to determine acceptability of batch data.
  - 1.10.5.2. Non-zero controls and/or calibrators shall be placed throughout the batch.
  - 1.10.5.3. A linear calibration curve (no weighting) shall be generated by using at least two calibrators at the cutoff concentration and the origin.
    - 1.10.5.3.1. If two calibrators are used, the lowest RR of the two calibrators shall be used to set the cutoff RR.
    - 1.10.5.3.2. If more than two calibrators are used in the calibration curve, the average RR of the calibrators shall be used to set the cutoff RR.

1.10.5.3.3. Calibrators shall only be excluded for an analyte if 1.10.2 is not met or the analyte and/or corresponding internal standard was not detected.

1.10.5.3.3.1. If fewer than two calibrators have acceptable analytical results, the samples shall be re-analyzed if the sample was not positive for another analyte in that confirmation drug class for the failed analyte, if possible, starting at 1.8.1.

1.10.5.4. Each negative control shall be negative for each analyte.

1.10.5.4.1. If any negative control result is positive, each sample with an RR less than half the RR cutoff for the analyte shall be considered "none detected" for the analyte, and each sample between half the RR cutoff and RR cutoff shall be reanalyzed for the failed analyte either by reinjection (ref. 1.10.8) or, if possible, starting at 1.8.1, unless the sample is already presumptive positive for another analyte in the same confirmation drug class.

1.10.5.4.2. The corresponding internal standard should be present for the associated analyte.

1.10.5.5. Each non-zero control should be positive for each analyte.

1.10.5.5.1. Non-zero controls shall be used to assess saturation of the detector, drift in retention times, and peak accuracy within the batch and shall not be used for batch acceptability.

1.10.6. Analyte Identification

1.10.6.1. An analyte score is obtained for each drug in each sample analyzed. This score is composed of three individual scores: a mass accuracy score, a signal to noise score, and a retention time score. These three scores are summed to obtain an analyte score of up to 99.9999.

1.10.6.1.1. The mass accuracy score is obtained by the following formula:

$$Score = \frac{50 - |\text{mass accuracy}|}{50} * 33.3333$$

1.10.6.1.1.1. Mass accuracy (expressed in ppm) is calculated based on the monoisotopic mass of the analyte of interest plus a proton (positive mode) or minus a proton (negative mode), except for codeine and THC-COOH. For codeine, the mass accuracy may be calculated based on the monoisotopic mass of codeine plus a sodium cation (322.1419 m/z) or plus a proton (300.1599 m/z). For THC-COOH, the mass accuracy is calculated based on the monoisotopic

mass of THC-COOH minus a proton and a water molecule.

1.10.6.1.1.2. If the mass accuracy is greater than 50 ppm, the mass accuracy score shall be zero.

1.10.6.1.2. The signal to noise score is obtained by the following formula:

$$Score = \frac{\text{signal to noise}}{10} * 33.3333$$

1.10.6.1.2.1. If the signal to noise is greater than 10, the signal to noise score shall be 33.3333.

1.10.6.1.3. The retention time score is obtained by the following formula:

$$Score = \frac{0.10 - |\text{retention time difference}|}{0.10} * 33.333$$

1.10.6.1.3.1. If the retention time difference is greater than 0.10 minutes, the retention time score shall be 0.

1.10.6.2. For each analyte with an analyte score ≥ 50, an RR is obtained.

1.10.6.3. Any analyte with an analyte score ≥ 50 and an RR greater than or equal to the RR cutoff is considered presumptive positive for the analyte.

1.10.6.4. If any analyte in the confirmation drug class (ref. 1.10.3) is considered presumptive positive, the evidentiary specimen shall be moved to confirmation analysis for the drug class.

1.10.7. Data analysis software manual integration tools (Snap Baseline and Drop Baseline) may be utilized to adjust the integration algorithm after manual selection of the peak. Use of software manual integration shall be documented on the chromatogram.

1.10.8. Reinjection of Samples

1.10.8.1. The analytical results for a reinjected batch shall meet all acceptability requirements listed in 1.10.

1.10.9. If any criteria listed in 1.10 are not met for an analyte, the sample does not require reanalysis if the sample is already presumptive positive for the same drug confirmation class based on the results for another analyte in the drug confirmation class (ref. 1.10.3).

1.10.10. Unacceptable Data

1.10.10.1. Data found to be unacceptable shall be marked with a signed note identifying the specific analytical data that should not be used and the reason for not using the data (e.g., “Do not use this TOF BDS data due to missing internal standard. AB XX/XX/XX”).

1.10.11. No Data Generated for a Sample

1.10.11.1. Cases with no generated data should have a case synopsis note to explain the lack of data associated with the chain of custody preparation date (e.g., “ XX/XX/XX No data was collected from [batch name] due to the instrument stopping. AB”).

Table 1: Blood Drug Screen by LC/TOF: Analytes, Corresponding Internal Standards, and Concentration of Non-Zero Controls and Internal Standard.

Drug	Cal (ng/mL)	HC (ng/mL)	UHC (ng/mL)	Internal Standard	ISTD (ng/mL)
Acetylfentanyl	1.0	4.0	10	Acetylfentanyl-D5	1.0
Alprazolam	10	40	100	Alprazolam-D5	10
7-Aminoclonazepam	10	40	100	7-Aminoclonazepam-D4	10
Amphetamine	10	40	100	(±)-Amphetamine-D11	10
Benzoylecgonine	20	80	200	Cocaine-D3	10
Buprenorphine	10	40	100	Buprenorphine-D4	10
Butalbital*	200	800	2000	Butalbital-D5	200
Carisoprodol	500	2000	5000	Carisoprodol-D7	500
Clonazepam	10	40	100	Clonazepam-D4	10
Cocaine	10	40	100	Cocaine-D3	10
Codeine	10	40	100	Dihydrocodeine-D6	10
Cyclobenzaprine	10	40	100	Cyclobenzaprine-D3	10
O-Desmethyltramadol	10	40	100	O-desmethyl-cis-tramadol-D6	10
Dextromethorphan	10	40	100	Dextromethorphan-D3	10
Diazepam	10	40	100	Diazepam-D5	10
EDDP	10	40	100	EDDP-D3 (perchlorate)	10
Fentanyl	1.0	4.0	10	Norfentanyl-D5	1.0
Hydrocodone	10	40	100	Hydrocodone-D6	10
Hydromorphone	10	40	100	Hydromorphone-D6	10
Lorazepam	10	40	100	Clonazepam-D4	10
MDMA	10	40	100	(±)-MDMA-D5	10
Meprobamate	500	2000	5000	Meprobamate-D7	500
Methadone	10	40	100	(±)-Methadone-D3	10
Methamphetamine	10	40	100	(±)-Methamphetamine-D11	10
Morphine	10	40	100	Morphine-D3	10
Norbuprenorphine	10	40	100	Norbuprenorphine-D3	10
Nordiazepam	10	40	100	Nordiazepam-D5	10
Norfentanyl	1.0	4.0	10	Norfentanyl-D5	1.0
Oxazepam	10	40	100	Oxazepam-D5	10
Oxycodone	10	40	100	Oxycodone-D6	10
Oxymorphone	10	40	100	Oxymorphone-D3	10
Pentobarbital*	200	800	2000	Pentobarbital-D5	200
Phencyclidine	10	40	100	Phencyclidine-D5	10
Phenobarbital*	200	800	2000	Phenobarbital-D5	200
Secobarbital*	200	800	2000	Secobarbital-D5	200
Temazepam	10	40	100	Temazepam-D5	10
THC-COOH*	10	40	100	THC-COOH-D3	10
Tramadol	10	40	100	Tramadol-13C, D3	10
Zolpidem	10	40	100	Zolpidem-D6	10

Analytes that are analyzed in negative mode are denoted by an asterisk.

1.11. Report Writing

1.11.1. A sample is presumptive positive for a confirmation drug class if one or more analytes in the confirmation drug class are identified per criteria outlined in 1.10.6.

1.11.1.1. The calibration curve and negative controls shall pass acceptability criteria in order to report findings for an evidentiary sample (ref. 1.10.5).

1.11.2. All accepted screening data for each specimen shall be technically reviewed prior to being entered into LIMS.

1.11.2.1. A presumptive positive for any analyte within a confirmation drug class will direct the specimen for confirmatory testing of the confirmation drug class.

1.11.2.2. If all confirmation drug classes screen negative, the result shall be reported as “None Detected.”

1.12. References

1.12.1. Marin, S. J., Hughes, J. M., Lawlor, B. G., Clark, C. J. & McMillin, G. A. Rapid Screening for 67 Drugs and Metabolites in Serum or Plasma by Accurate-Mass LC–TOF-MS. *Journal of Analytical Toxicology* bks061 (2012).

1.12.2. Logan, B. K. *et al.* Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities. *Journal of Analytical Toxicology* 37, 552–558 (2013).

1.12.3. Winek, C. L., Wahba, W. W., Winek Jr., C. L. & Balzer, T. W. Drug and chemical blood-level data 2001. *Forensic Science International* 122, 107–123 (2001).

1.12.4. Standard Practices for Method Validation in Forensic Toxicology. ANSI/ASB Standard 036, 1st edition, 2019, 1-46.

1.12.5. Vincenti, M. *et al.* Fast screening of 88 pharmaceutical drugs and metabolites in whole blood by ultrahigh-performance liquid chromatography–tandem mass spectrometry. *Anal Bioanal Chem* 405, 863–879 (2012).

1.12.6. Roman, M., Ström, L., Tell, H. & Josefsson, M. Liquid chromatography/time-of-flight mass spectrometry analysis of postmortem blood samples for targeted toxicological screening. *Anal Bioanal Chem* 405, 4107–4125 (2013).

2. Benzodiazepines and Z-Drugs Confirmation by LC/QQQ

2.1. Scope

2.1.1. This method shall be used for confirmation analysis of specimens requiring confirmation of benzodiazepines, their metabolites, and zolpidem. Sample preparation shall be by SPE.

2.2. Precautions/Limitations

2.2.1. Minimum Sample Requirement

2.2.1.1. 1 mL of blood or serum/plasma specimen.

2.2.2. CRMs

2.2.2.1. CRMs used for calibrator and non-zero control stocks shall be from two different vendors, if available.

2.2.2.2. If using CRMs from the same vendor, two different lots shall be used, if available.

2.2.2.3. If only one lot of a CRM is available, two separate vials from the lot shall be used.

2.2.2.4. 7-aminoclonazepam should be sonicated prior to use.

2.2.3. Mobile phases should be kept in amber bottles to increase stability.

2.3. Related Information

2.3.1. Benzodiazepines Confirmation Method Validation (September 2015-March 2016)
2.3.2. Stability of Stock Solutions (December 2015-June 2016, January 2017)
2.3.3. Diazepam Update (September 2017)
2.3.4. Calibration Model Update-Quadratic (August 2018)
2.3.5. Stock Solution Stability (January 2020)
2.3.6. Retention Time Versus Relative Retention Time (February 2020)
2.3.7. Instrument validations
2.3.8. Validations of calibrators, controls, and internal standards data

2.4. Instruments/Equipment

2.4.1. Tube rack
2.4.2. Rocker
2.4.3. Vortex, single
2.4.4. Sonicating water bath
2.4.5. Centrifuge
2.4.6. Positive pressure manifold
2.4.7. SPE column rack
2.4.8. SPE collection rack
2.4.9. Waste collection rack
2.4.10. Evaporator
2.4.11. Vial rack
2.4.12. Liquid chromatograph
2.4.13. Mass spectrometer, triple quadrupole
2.4.14. Pipettes

2.5. Reagents/Materials

2.5.1. Glass tubes (e.g., 13x100 mm)
2.5.2. Trace-B columns, 3 mL columns, 35 mg (Tecan #TB-335C)
2.5.3. Tube caps (e.g., 13mm flange)

2.5.4. Pipette tips
2.5.5. Autosampler vials, inserts, and caps
2.5.6. ddH_2O
2.5.7. Negative blood (human)
2.5.8. Liquid chromatograph column
2.5.8.1. Dimensions: 3.0 mm x 50 mm
2.5.8.2. Composition: Poroshell C18, 2.7 µm particles
2.5.9. Liquid chromatograph guard column
2.5.9.1. Dimensions: 3.0 mm x 5 mm
2.5.9.2. Composition: Poroshell C18, 2.7 µm particles
2.5.10. CRMs
2.5.10.1. 7-Aminoclonazepam
2.5.10.2. Alprazolam
2.5.10.3. Clonazepam
2.5.10.4. Diazepam
2.5.10.5. Lorazepam
2.5.10.6. Nordiazepam
2.5.10.7. Oxazepam
2.5.10.8. Temazepam
2.5.10.9. Zolpidem
2.5.10.10. 7-Aminoclonazepam-D4
2.5.10.11. Alprazolam-D5
2.5.10.12. Diazepam-D5
2.5.10.13. Lorazepam-D4
2.5.10.14. Oxazepam-D5
2.5.10.15. Temazepam-D5
2.5.10.16. Zolpidem-D7
2.5.11. Nitrogen
2.5.12. Solvents shall be high quality and low residue (e.g., HPLC grade, Omnisolv, Optima, etc.) unless otherwise noted.
2.5.12.1. Acetonitrile, LCMS grade
2.5.12.2. Ethyl acetate
2.5.12.3. Ammonium hydroxide, ACS grade or higher
2.5.12.4. Methanol, ACS grade or higher
2.5.12.5. Formic acid
2.5.12.6. Glacial acetic acid, ACS grade or higher
2.5.13. Sodium acetate
2.5.14. Potassium or sodium carbonate
2.5.15. Potassium or sodium bicarbonate

2.6. Hazards/Safety
2.6.1. See Safety Manual.
2.6.2. See SDS for each chemical in this method.
2.6.3. Add acids to approximately half the volume of the less acidic liquid, then dilute to final volume.

2.7. Reference Materials/Controls/Calibrators/Solutions
2.7.1. All solutions shall conform to Solution Preparation, Validation, Verification (Doc ID: 3695).

2.7.2. BNZ Low Calibrator 1
2.7.3. BNZ Low Calibrator 2
2.7.4. BNZ High Calibrator 1
2.7.5. BNZ High Calibrator 2
2.7.6. BNZ Low Control
2.7.7. BNZ High Control
2.7.8. BNZ Internal Standard
2.7.9. BNZ Elution Solution
2.7.10. Acetate Buffer (300 mM)
2.7.11. Carbonate Buffer (300 mM)
2.7.12. Mobile Phases
2.7.12.1. Aqueous (A)
2.7.12.2. Organic (B)
2.7.13. BNZ Reconstitution Solution

2.8. Procedures/Instructions

2.8.1. An evidentiary confirmation batch shall consist of concurrently prepared calibrators, negative blood controls, non-zero controls, and samples. Each set of one to twelve samples shall be bracketed by non-zero controls. The batch shall contain alternating low and high non-zero controls. The batch shall contain at least three prepared negative controls. Negative controls may be reinjected multiple times throughout the batch.
2.8.1.1. Reinjected negative controls shall be denoted with an "RI" followed by the number of reinjections.
2.8.2. Mix specimens on a rocker or by inverting several times.
2.8.3. Add 50 µL of internal standard (resulting in a concentration of 100 ng/mL) to each tube.
2.8.4. Prepare calibrator and control samples in correspondingly labeled tubes as indicated in Table 2.

Table 2: Benzodiazepines and Z-Drugs Calibrator and Control Preparation

Level	Stock Solution	Volume (µL)	Stock Solution	Volume (µL)
Cal 1	Low Cal 1	10	Low Cal 2	20
Cal 2	Low Cal 1	20	Low Cal 2	30
Cal 3	Low Cal 1	50	Low Cal 2	40
Cal 4	Low Cal 1	100	Low Cal 2	60
Cal 5	High Cal 1	50	High Cal 2	25
Cal 6	High Cal 1	75	High Cal 2	50
Cal 7	High Cal 1	100	High Cal 2	75
Cal 8	High Cal 1	125	High Cal 2	100
Low Control	Low Ctrl	60		
High Control	High Ctrl	70		

2.8.5. Pipette 1 mL of negative blood into each calibrator and control tube.
2.8.6. Pipette 1 mL of specimen into the correspondingly labeled tube.
2.8.7. Add 2 mL of acetate buffer to each tube. Cap and vortex each tube.
2.8.8. Sonicate for ~10 minutes.
2.8.9. Centrifuge for ~10 minutes using 3000 rpm at ~4-8 ºC.

2.8.10. In the order listed, condition columns with each of the following solutions, allowing each solution to flow completely through each column before proceeding to the next solution:
  2.8.10.1. 1 mL methanol
  2.8.10.2. 1 mL ddH_2O
  2.8.10.3. 1 mL acetate buffer
2.8.11. While the sorbent bed is still wet, decant each sample into the SPE column and allow to flow completely through at ~1 mL per minute.
2.8.12. Add 3 mL of carbonate buffer to each column and allow to flow completely through at ~1 mL per minute.
2.8.13. Add 3 mL ddH_2O to each column and allow to flow completely through at ~1 mL per minute.
2.8.14. Using a maximum flow of ~60 psi or greater, dry the columns for at least 30 minutes.
2.8.15. Place empty labeled tubes into the positive pressure manifold, ensuring the placement of the tubes corresponds with the arrangement of the sample columns.
2.8.16. Add 3 mL of elution solution to each column and allow to flow completely through into tube at ~1 mL per minute.
2.8.17. Remove tubes from the positive pressure manifold and place on the evaporator.
2.8.18. Evaporate at room temperature using nitrogen.
2.8.19. Add 100 µL of reconstitution solution to each tube and vortex.
2.8.20. Transfer each sample to a correspondingly labeled autosampler vial and cap vial.
2.8.21. Analyze the samples by LC/QQQ.
  2.8.21.1. Sequence names shall be in the following format: YYYY_MM_DD_BNZ-Z_Initials.
    2.8.21.1.1. The date in the sequence shall be the date of preparation of the samples.
    2.8.21.1.2. Additional information such as reinjection, validation, etc., or equivalent abbreviations should be included with the assay abbreviation.
    2.8.21.1.3. If the sequence is run with the wrong sequence name, it shall be noted on the Technical Review Worksheet and in the case synopsis of each case in the batch and not corrected on the chromatograms.
  2.8.21.2. If multiple batches are included in one sequence, add a note on the MassHunter Worklist report that includes the batch name of each batch included in the sequence in the format listed in 2.8.21.1. The note should list which lines are attributed to each batch.
  2.8.21.3. The extracted samples may be analyzed up to 6 days after the date of preparation when stored at room temperature or in the instrument autosampler or at equivalent temperature (ref. 2.10.8).
  2.8.21.4. If the instrument sequence is paused by the acquisition software between two samples, the sequence may be restarted at the sample not yet injected.
    2.8.21.4.1. Sample stability criteria shall be met.
  2.8.21.5. If the instrument sequence is interrupted during analysis of a sample or the sequence is aborted or stopped, the sequence should be restarted at the last bracketing non-zero control or may be resumed by beginning at the next sample not yet injected.

2.8.21.5.1. Sample stability criteria shall be met.
2.8.21.5.2. Reinjection of a sample of unknown concentration may be performed once.
2.8.21.5.3. Reinjection of a sample of known concentration may be performed multiple times.
2.8.21.5.3.1. If a reinjection is needed more than once, the evidentiary samples that have already been reinjected may be skipped in a bracket.
2.8.21.5.3.1.1. Evidentiary samples that are skipped and do not have valid data shall be reanalyzed starting at 2.8.1.
2.8.21.5.4. A reinjection shall be performed by restarting the sequence from the last bracketing non-zero control or reinjecting the entire sequence.
2.8.21.5.5. If an entire sequence is reinjected or a reinjection includes the calibrators used to determine the calibration curve, a check tune (Doc ID: 2842) shall be performed within 24 hours of initiation of the reinjection sequence. Resuming a sequence or reinjecting a sequence starting with the last bracketing control does not require a check tune.

2.8.22. LC/QQQ Acquisition Parameters

2.8.22.1. Liquid chromatograph sampler

Needle Wash	Standard Wash
Injection Volume	0.10 – 5.0 µL

2.8.22.2. Liquid chromatograph binary pump

	Time	Gradient A %	Gradient B %
1	0.0	70	30
2	1.5	90	10
3	1.75	70	30
4	3.0	70	30
5	5.0	25	75

Flow	0.8 mL/min
Stoptime	6.00 min
Posttime	2.00 min

2.8.22.3. Liquid chromatograph column compartment

Temperature	45 ºC

2.8.22.4. Mass spectrometer

Ion Source	AJS ESI
Scan Type	Dynamic MRM

2.8.22.5. dMRM Parameters

MS1 Resolution	Wide/Unit
MS2 Resolution	Wide/Unit

Cell Acc.	4 V
Polarity	Positive

Table 3: Benzodiazepines and Z-Drugs MS Parameters

Compound Name	Internal Standard	Precursor Ion	Product Ion	Fragmentor (V)	CE* (V)	RT** (min)
7-Aminoclonazepam	No	286	222.1	130	27	0.49
			121		35	
Alprazolam	No	309	281.1	145	29	4.02
			205.1		49	
Clonazepam	No	316	**270.1**	145	27	3.72
			214		51	
Diazepam	No	285	222.1	155	29	5.27
			193.1		35	
Lorazepam	No	321	**275**	130	23	3.81
			229		33	
Nordiazepam	No	271	208.1	150	33	3.71
			140		31	
Oxazepam	No	287	**241**	130	23	2.53
			103.9		41	
Temazepam	No	301	**255.1**	115	25	4.84
			193		39	
Zolpidem	No	308	263.1	135	29	0.56
			235.1		39	
7-Aminoclonazepam-D4	Yes	290	226	140	27	0.49
Alprazolam-D5	Yes	314	210.2	135	49	3.94
Diazepam-D5	Yes	290	198.1	140	35	5.22
Lorazepam-D4	Yes	325	233	130	37	3.76
Oxazepam-D5	Yes	292	246.1	135	25	2.35
Temazepam-D5	Yes	306	260.1	125	25	4.79
Zolpidem-D7	Yes	315	242.1	160	41	0.55

* Collision Energy Ions in **bold** are used to quantitate.
**RTs are based on the average analyte retention times of calibrators and may be updated in the acquisition method and/or quantitation method, as necessary.

2.8.22.6. Quantitation Parameters

RRT Max % Deviation	5 percent
Curve fit – Linear	7-Aminoclonazepam, Lorazepam, Oxazepam, and Temazepam
Curve fit – Quadratic	Alprazolam, Clonazepam, Diazepam, Nordiazepam, and Zolpidem
Data point weight	1/x
Units of concentration	ng/mL
Internal standard concentration	100

2.9. Records
- 2.9.1. Pipette calibration certificate, however named
- 2.9.2. Benzodiazepines and Z-Drugs Confirmation Calibrator Solution Preparation Worksheet
- 2.9.3. Benzodiazepines and Z-Drugs Confirmation Internal Standard Solution Preparation Worksheet
- 2.9.4. Benzodiazepines and Z-Drugs Confirmation Control Solution Preparation Worksheet
- 2.9.5. Batch Preparation Packet, however named
  - 2.9.5.1. ISDT Confirmation Worklist
  - 2.9.5.2. Retest Worksheet, as appropriate
  - 2.9.5.3. Benzodiazepines and Z-Drugs Confirmation Preparation Worksheet
  - 2.9.5.4. Aliquot Chain of Custody
- 2.9.6. MassHunter Worklist Report
- 2.9.7. MassHunter Ion Ratio and RRT Verification, however named
- 2.9.8. QA/QC Packet, however named
  - 2.9.8.1. Batch summary
  - 2.9.8.2. Analyte calibration curves
  - 2.9.8.3. Calibrator and control chromatograms
- 2.9.9. Sample chromatograms
- 2.9.10. QQQ Check Tune Report
- 2.9.11. Benzodiazepines and Z-Drugs Confirmation Technical Review Checklist
- 2.9.12. Data comparison output, however named
- 2.9.13. Measurement Uncertainty Estimation and supporting data
- 2.9.14. Specimen Verification Worksheet, if applicable

2.10. Interpretation of Results
- 2.10.1. Interpretation of results for each analyte shall occur independent of the other analytes in the method.
- 2.10.2. Chromatographic analyte and internal standard peaks shall have baseline resolution and/or shall be mass resolved in the mass spectrometer.
  - 2.10.2.1. A shoulder peak shall be < 10% of analyte peak height and area in order to report a quantitative result.
- 2.10.3. Peak filters should be set between 10% and 50% of the Cal 1 response for each analyte.
- 2.10.4. Internal standard recovery should be between 50% to 200% of the average of the calibrators in the batch.
  - 2.10.4.1. Samples with recovery less than 25% or greater than 200% shall not be accepted.
  - 2.10.4.2. Samples with recovery between 25% and 50% may be accepted at analyst discretion.
- 2.10.5. Calibration and Controls Criteria
  - 2.10.5.1. Results of samples analyzed prior to analysis of the negative control preceding the calibrators shall not be used to determine acceptability of batch data.
  - 2.10.5.2. Quantitation of calibrators and non-zero controls shall be within ± 20% of the target concentration.
  - 2.10.5.3. Generating a calibration curve

2.10.5.3.1. Calibration curve shall include a minimum of five non-zero concentrations.

2.10.5.3.2. Correlation coefficient (r^2) for the calibration curve shall be ≥ 0.990.

2.10.5.3.3. An ion ratio with a relative abundance ≥ 20% shall be within ± 20% of the mean ion ratio based on all calibrators used to generate the curve and controls.

2.10.5.3.4. An ion ratio with a relative abundance < 20% shall be within ± 30% of the mean ion ratio based on all calibrators used to generate the curve and controls.

2.10.5.3.5. A calibration point may be excluded if any of the following occur:

2.10.5.3.5.1. An ion ratio does not meet the acceptability criteria listed in 2.10.5.3.3 or 2.10.5.3.4.

2.10.5.3.5.2. The correlation coefficient (r^2) for the calibration curve is < 0.990.

2.10.5.3.5.3. A quantitated value is not within ± 20% of the target concentration.

2.10.5.3.5.4. A peak has poor chromatography.

2.10.5.3.6. If the lowest calibrator used to generate the calibration curve is not equal to the defined LLOQ, all samples with an analyte concentration greater than half the LLOQ but less than the target concentration of the lowest calibrator used to generate the calibration curve shall be reanalyzed, if possible, starting at 2.8.1.

2.10.5.3.6.1. RR or response may be used to determine which specimens require reanalysis, if any.

2.10.5.3.7. If the highest calibrator used to generate the calibration curve is not equal to the defined ULOQ, all samples with an analyte concentration above the target concentration of the highest calibrator used to generate the calibration curve shall be reanalyzed, if possible, starting at 2.8.1. If unable to retest, the results for the analysis may be reported as greater than the highest calibrator used in the batch.

2.10.5.3.7.1. RR or response may be used to determine which specimens require reanalysis, if any.

2.10.5.4. Each set of one to twelve samples shall be bracketed by a negative control for partial batch acceptance. The negative control shall have an analyte concentration or response < 50% of the LLOQ and/or unacceptable ion ratios as specified in 2.10.5.3.3 or 2.10.5.3.4.

2.10.5.4.1. If the above acceptance criterion is not met, the analytical data for the samples bracketed by the failed negative control with a concentration ≥ 50% of the LLOQ shall not be used and shall be reanalyzed, if possible, starting at 2.8.1. A result < 50% of the LLOQ

for an evidentiary sample shall be accepted as none detected.

2.10.5.5. At least one negative control shall have the corresponding internal standard present for the associated analyte.

2.10.5.5.1. If acceptance criterion is not met, all samples in the batch shall be reanalyzed, if possible, starting at 2.8.1.

2.10.5.6. At least one low and one high non-zero control shall be included in each batch.

2.10.5.7. A non-zero control for an analyte fails if any of the following occur:

2.10.5.7.1. An ion ratio does not meet the acceptability criteria listed in 2.10.5.3.3 or 2.10.5.3.4.

2.10.5.7.2. The quantitated value is not within ± 20% of the target concentration.

2.10.5.7.3. A peak has poor chromatography.

2.10.5.7.4. The relative retention time is greater than ± 5% of the mean relative retention time based on all calibrator and control retention times.

2.10.5.8. Each set of one to twelve samples shall be bracketed by one low and one high non-zero control.

2.10.5.8.1. If a control result does not meet the above criteria, the analytical data for the samples bracketed by the failed control shall not be used, and samples in the bracket prior to and following the failed control that are positive for the analyte that failed shall be reanalyzed, if possible, starting at 2.8.1. A result below the LLOQ for an evidentiary sample shall be accepted as none detected if the negative controls for the batch pass the acceptability criteria in 2.10.5.4 and 2.10.5.5.

2.10.6. If the analyte in a sample has a result > 2x ULOQ, evaluate the analyte in the subsequent sample(s) as follows:

2.10.6.1. If a > 2x ULOQ sample is immediately followed by an evidentiary sample in which the analyte is not detected, data should be accepted.

2.10.6.2. If a > 2x ULOQ sample is immediately followed by an evidentiary sample in which the analyte is detected, the sample following the > 2x ULOQ sample shall be retested.

2.10.6.2.1. Additional negative controls or solvent blanks may be added to the sequence immediately following the > 2x ULOQ sample to eliminate carryover in subsequent samples.

2.10.6.3. If a > 2x ULOQ sample is immediately followed by a control and the control passes acceptance criteria, data should be accepted.

2.10.6.4. If a > 2x ULOQ sample is immediately followed by a control and it does not pass control acceptance criteria, all samples in the preceding and following brackets shall be reinjected or retested.

2.10.6.4.1. Additional negative controls or solvent blanks may be added to the sequence immediately following the > 2x ULOQ sample to eliminate carryover in subsequent samples.

2.10.6.5. If the MassHunter software does not provide a calculated result, the RR for the sample may be compared to the RR of Cal 8.

2.10.7. Analyte Identification (Qualitative Criteria)

2.10.7.1. Relative retention time shall be within ± 5% of the mean relative retention time based on all calibrator and control retention times.

2.10.7.2. Each analyte shall have two ion transitions monitored. The ion transition from the precursor to the product ion listed in **bold** type in Table 3 is used for quantitation.

2.10.7.3. Each internal standard shall be present and have one ion transition monitored.

2.10.7.4. Each ion ratio shall meet the acceptability criteria listed in 2.10.5.3.3 or 2.10.5.3.4.

2.10.7.4.1. If the ion ratio is greater than 30% due to detector saturation for an analyte in one or more case samples, the calibrators, three negative controls, and at least one low and high control bracketing the case sample(s) may be reinjected at a lower injection volume.

2.10.7.5. Data analysis software manual integration tools (Merge Right Peak, Merge Left Peak, Split Peak and Pick Left, Split Peak and Pick Right, Snap Baseline, Drop Baseline, Apply ISTD RTs to Target, Apply Target RTs to Qualifier) may be used to adjust the integration algorithm to select the correct peak or adjust the baseline. Use of software manual integration tools shall be documented on the chromatogram.

2.10.8. Analyte Stability

2.10.8.1. Prepared samples are stable for 6 days when stored at room temperature, in the auto sampler, or at equivalent temperature.

2.10.9. Retesting Samples

2.10.9.1. When a sample requires retesting, the sample shall be retested at least once, if possible. A sample may be retested up to two times without supervisory approval.

2.10.9.1.1. If a quantitative value cannot be reported from any analysis, the first acceptable qualitative data according to analyte identification in 2.10.7 shall be used. (ref. 2.11.4).

2.10.9.1.2. If data is not generated, that analysis does not count as an analysis or retest under this section.

2.10.10. Unacceptable Data

2.10.10.1. Data found to be unacceptable shall be marked with a signed note identifying the specific analytical data that should not be used and the reason for not using the data (e.g., “Do not use this quantitative alprazolam data due to a bracketing control being outside acceptability criteria. AB XX/XX/XX” or “Do not use any data from this batch due to sequence interruption. Samples will be retested. AB XX/XX/XX”).

2.10.11. No Data Generated for a Sample

2.10.11.1. Cases with no generated data should have a case synopsis note to explain the lack of data associated with the chain of custody

preparation date (e.g., “XX/XX/XX No data was collected from [batch name] due to the instrument stopping. AB”).

2.11. Report Writing

2.11.1. The LLOD for benzodiazepine analysis is equal to the LLOQ for each analyte. The LLOQ and ULOQ are listed in Table 4:

Table 4: Benzodiazepines and Z-Drugs LLOQ and ULOQ

Analyte	LLOQ (ng/mL)	ULOQ (ng/mL)
7-Aminoclonazepam	5.0	250
Alprazolam	10	500
Clonazepam	10	500
Diazepam	50	1000
Lorazepam	10	500
Nordiazepam	50	1000
Oxazepam	50	1000
Temazepam	50	1000
Zolpidem	10	500

2.11.2. Confirmatory data for each specimen shall be technically reviewed prior to entering the result into LIMS.

2.11.2.1. The preparation date of the analysis being reported shall be entered as the analysis date.

2.11.3. Quantitative Reporting

2.11.3.1. A result less than the LLOQ shall not be reported.

2.11.3.1.1. If a batch LLOQ is used, a quantitative result less than the target concentration for the lowest calibrator used in the calibration curve shall not be reported.

2.11.3.2. A quantitated result that meets acceptability criteria shall be reported for a result between the target concentration of the lowest and highest calibrators that meet acceptability criteria.

2.11.3.2.1. A result shall be truncated to the appropriate level of significance and reported as the quantitative value ± the expanded measurement uncertainty.

2.11.3.2.1.1. A result shall be reported to one decimal place for quantitative values greater than or equal to 1 and less than 10.

2.11.3.2.1.2. A result shall be reported as a whole number for a quantitative value greater than or equal to 10.

2.11.3.3. A result that is above the ULOQ and has an ion ratio within ± 30% of the mean ion ratio based on all calibrators and controls used to generate the curve shall be reported as > the ULOQ in ng/mL.

2.11.3.3.1. If a batch ULOQ is used, a quantitative result greater than the target concentration for the highest calibrator used in the calibration curve shall not be reported.

2.11.3.3.1.1. A result greater than the target concentration of the highest calibrator used in the calibration curve may be reported if retesting of a specimen is not feasible.

2.11.3.4. A quantitative result shall only be reported if analysis occurred within the established sample stability window (ref. 2.8.21.3).

2.11.3.5. If a specimen is analyzed more than once, the first quantitative result that meets acceptability criteria for quantitation of a specific analyte shall be reported.

2.11.4. Qualitative Reporting

2.11.4.1. A result should be reported as "Positive" when the analyte identification criteria (ref. 2.10.7) have been met, the quantitative result is > LLOQ, and the quantitative criteria have not been met.

2.11.4.1.1. If a specimen is analyzed more than once, the totality of the qualitative data shall be evaluated by the analyst for acceptability criteria for analyte identification of a specific analyte.

2.11.4.1.1.1. The preparation date of last analysis shall be used as the analysis date.

2.11.4.2. A result may be reported as "Positive" with supervisory approval if any of the following occur:

2.11.4.2.1. Interference(s); or

2.11.4.2.2. Quantitative result > LLOQ with an ion ratio greater than ± 20%, but less than ± 30%, of the mean ion ratio based on all calibrators and controls used to generate the curve.

2.12. References

2.12.1. SPEware Application Note: Benzodiazepines From Whole Blood For GC/MS or LC/MS Confirmations Using: Extraction Column: TRACE-B 35mg, TB-335.

2.12.2. Standard Practices for Method Validation in Forensic Toxicology. ANSI/ASB Standard 036, 1st edition, 2019, 1-46.

2.12.3. Standard for Mass Spectral Data Acceptance for Definitive Identification. Scientific Working Group for Forensic Toxicology (SWGTOX). 2014, 1-11.

3. Cannabinoids Confirmation by GC/MS

3.1. Scope
- 3.1.1. This method shall be used for confirmation analysis of specimens requiring confirmation of Delta-9 THC and Delta-9 THC-COOH. Sample preparation shall be by SPE and derivatization.

3.2. Precautions/Limitations
- 3.2.1. Minimum Sample Requirement
  - 3.2.1.1. 1 mL of blood or serum/plasma specimen
- 3.2.2. CRMs
  - 3.2.2.1. CRMs used for calibrator and non-zero control stocks shall be from two different vendors, if available.
  - 3.2.2.2. If using CRMs from the same vendor, two different lots shall be used, if available.
  - 3.2.2.3. If only one lot of a CRM is available, two separate vials from the lot shall be used.
- 3.2.3. BSTFA and MTBSTFA hydrolyze easily.

3.3. Related Information
- 3.3.1. THC Confirmatory Analysis Method Validation (October 2016 - February 2017)
- 3.3.2. THC Stock Solution Stability Supplemental (April 2017)
- 3.3.3. THC Reinjection Supplemental (July 2017)
- 3.3.4. Stock Solution Stability (February 2020)
- 3.3.5. Injection Volume Supplemental for THC-COOH (March 2020)
- 3.3.6. THC Screw Cap Stability (2021)
- 3.3.7. ISTD Solution Stability (2022)
- 3.3.8. ISTD Solution Stability (2023)
- 3.3.9. Instrument validations
- 3.3.10. Validation of calibrators, controls, and internal standards data

3.4. Instruments/Equipment
- 3.4.1. Tube rack
- 3.4.2. Rocker
- 3.4.3. Vortex, single and multi-tube
- 3.4.4. Centrifuge
- 3.4.5. Positive pressure manifold
- 3.4.6. SPE column rack
- 3.4.7. SPE collection rack
- 3.4.8. Waste collection rack
- 3.4.9. Evaporator
- 3.4.10. Dry block heater
- 3.4.11. Vial rack
- 3.4.12. Electronic or manual crimper
- 3.4.13. Gas chromatograph
- 3.4.14. Mass spectrometer, single quadrupole
- 3.4.15. Pipettes

3.5. Reagents/Materials
- 3.5.1. Glass tubes (e.g., 13x100 mm and 16x100 mm)

3.5.2. Cerex Polychrom THC SPE columns, 6 mL columns, 65 mg (Tecan #682-0506, or equivalent)
3.5.3. Tube caps (e.g., 13 mm flange)
3.5.4. Pipette tips
3.5.5. Autosampler vials, inserts, and caps
3.5.6. ddH_2O
3.5.7. Negative blood (human)
3.5.8. Gas chromatograph capillary column-analytical column
3.5.8.1. Dimensions: 15 m x 0.25 mm x 0.25 µm
3.5.8.2. Composition: DB-5 MS UI (5%-Phenyl)-methylpolysiloxane
3.5.9. Gas chromatograph capillary column-restrictor column
3.5.9.1. Dimensions: ~0.5 m x 150 µm
3.5.9.2. Composition: Fused silica
3.5.10. BSTFA + 1% TMCS
3.5.11. MTBSTFA + 1% TBDMCS
3.5.12. CRMs
3.5.12.1. Delta-9 THC
3.5.12.2. Delta-9 THC-D9
3.5.12.3. Delta-9 THC-COOH
3.5.12.4. Delta-9 THC-COOH-D9
3.5.13. Helium, 5.0 grade or higher
3.5.14. Nitrogen
3.5.15. Solvents shall be high quality and low residue (e.g., HPLC grade, Omnisolv, Optima, etc.) unless otherwise noted.
3.5.15.1. Acetonitrile
3.5.15.2. Ethyl acetate
3.5.15.3. Hexane(s)
3.5.15.4. Glacial acetic acid, ACS grade or higher
3.5.15.5. Ammonium hydroxide, ACS grade or higher
3.5.15.6. Methanol, ACS grade or higher

3.6. Hazards/Safety
3.6.1. See Safety Manual.
3.6.2. See SDS for each chemical in this method.
3.6.3. Add acids to approximately half the volume of the less acidic liquid, then dilute to final volume.

3.7. Reference Materials/Controls/Calibrators/Solutions
3.7.1. All solutions shall conform to Solution Preparation, Validation, Verification (Doc ID: 3695).
3.7.2. THC Low Calibrator
3.7.3. THC High Calibrator
3.7.4. THC Low Control
3.7.5. THC High Control
3.7.6. THC Internal Standard
3.7.7. THC Wash Solution
3.7.8. THC Elution Solution
3.7.9. THC-COOH Elution Solution

3.8. Procedures/Instructions

3.8.1. An evidentiary confirmation batch shall consist of concurrently prepared calibrators, negative blood controls, non-zero controls, and samples. Each set of one to twelve samples shall be bracketed by non-zero controls. The batch shall contain alternating low and high non-zero controls. The batch shall contain at least three prepared negative controls. Negative controls may be reinjected multiple times throughout the batch. An ethyl acetate wash may be included between the highest calibrator and negative control.

3.8.1.1. Reinjected negative controls shall be denoted with an "RI" followed by the number of reinjections.

3.8.2. Mix specimens on a rocker or by inverting several times.

3.8.3. Add 25 µL or 50 µL of internal standard (resulting in a concentration of 10 or 20 ng/mL Delta-9 THC-D9 and 25 or 50 ng/mL Delta-9 THC-COOH-D9, respectively) to labeled glass tubes. The volume of internal standard that is chosen shall be used for the entire batch.

3.8.4. Prepare calibrator and control samples in correspondingly labeled tubes as indicated in Table 5.

Table 5: Cannabinoids Calibrator and Control Sample Preparation

Level	Sample Identification^	Stock Solution	Volume of stock (µL)
Cal 1	**1** / 2.5* ng/mL Calibrator	Low Calibrator	10
Cal 2	**2** / 5 ng/mL Calibrator	Low Calibrator	20
Cal 3	**4** / 10 ng/mL Calibrator	Low Calibrator	40
Cal 4	**6** / 15 ng/mL Calibrator	Low Calibrator	60
Cal 5	**10** / 25 ng/mL Calibrator	Low Calibrator	100
Cal 6	**20** / 40 ng/mL Calibrator	High Calibrator	40
Cal 7	**30** / 60 ng/mL Calibrator	High Calibrator	60
Cal 8	**40** / 80 ng/mL Calibrator	High Calibrator	80
Cal 9	**50** / 100 ng/mL Calibrator	High Calibrator	100
	8 / 20 ng/mL Control	Low Control	40
	35 / 70 ng/mL Control	High Control	70

* 2.5 ng/mL calibrator for THC-COOH not used (discarded after step 3.8.19)

^ Concentrations referenced are written with the THC concentration listed first in bold and the THC-COOH concentration listed second (**THC** / THC-COOH)

3.8.5. Pipette 1 mL of negative blood into each calibrator and control tube.

3.8.6. Pipette 1 mL of specimen into the correspondingly labeled tube.

3.8.7. Place tubes on a multi-tube vortex. While the tubes are vortexing at low speed, add 2 mL of cold acetonitrile (stored in refrigerator) slowly to each sample.

3.8.8. Cap each sample, then vortex for approximately one minute at high speed. The sample should not reach the cap.

3.8.9. Centrifuge for ~10 minutes using 3000 rpm at 4-8 ºC.

3.8.10. Decant each sample's top (organic) layer into a correspondingly labeled glass tube.

3.8.11. Add 2 mL of ddH_2O to the tube and vortex.

3.8.12. Condition the SPE columns with 3 mL of methanol and allow to flow completely through each column.

3.8.13. While the sorbent bed is still wet, decant each sample into the SPE column and allow the sample to flow completely through each column at ~2 mL per minute.
3.8.14. Add 2 mL of THC Wash Solution to each column and allow to flow completely through each column at ~2 mL per minute.
3.8.15. Using a maximum flow of ~30 psi on the positive pressure manifold, dry the columns for ~10 minutes.
3.8.16. Add 2 mL of hexane to each column and allow to flow completely through each column at ~2 mL per minute.
3.8.17. Using a maximum flow of ~10 psi on the positive pressure manifold, dry the columns for ~5 minutes.
3.8.18. Place empty labeled tubes into the positive pressure manifold, ensuring the placement of the tubes corresponds with the arrangement of the sample columns.
3.8.19. Add 3 mL of THC elution solution to each column and allow to flow completely through each column at ~2 mL per minute.
3.8.20. Remove tubes with THC eluent from the positive pressure manifold and set aside until step 3.8.25, or place on evaporator and evaporate at room temperature using nitrogen.
3.8.21. Add 2 mL of ethyl acetate to each column and allow to flow completely through each column at ~2 mL per minute.
3.8.22. Using a maximum flow of ~30 psi on the positive pressure manifold, dry the columns for ~5 minutes.
3.8.23. Place empty labeled tubes into the positive pressure manifold, ensuring the placement of the tubes corresponds with the arrangement of the sample columns.
3.8.24. Add 3 mL of THC-COOH elution solution to each column and allow to flow completely through each column at ~2 mL per minute.
3.8.25. Remove THC-COOH tubes from the positive pressure manifold and place on the evaporator. Evaporate at room temperature using nitrogen.
3.8.26. Add 50 µL BSTFA with 1% TMCS to each THC tube, cap, and vortex.
3.8.27. Add 50 µL MTBSTFA with 1% TBDMCS to each THC-COOH tube, cap, and vortex.
3.8.28. Place the tubes in a dry heat block at ~70 ºC for ~25 minutes.
3.8.29. Allow the tubes to cool. Transfer each sample to a correspondingly labeled autosampler vial and cap vial.
3.8.30. Analyze the samples by GC/MS.
3.8.30.1. Sequence names shall be in the following format:
YYYY_MM_DD_THC_Initials and/or
YYYY_MM_DD_COOH_Initials.
3.8.30.1.1. The date in the sequence shall be the date of preparation of the samples.
3.8.30.1.2. Additional information such as reinjection, validation, etc., or equivalent abbreviations should be included with the assay abbreviation.
3.8.30.1.3. If the sequence is run with the wrong sequence name, it shall be noted on the Technical Review Worksheet and in the case synopsis of each case in the batch and not corrected on the chromatograms.
3.8.30.2. If multiple batches are included in one sequence, add a note on the sequence table that includes the batch name of each batch included in

the sequence in the format listed in 3.8.30.1. The note should list which lines are attributed to each batch.

3.8.30.3. The extracted samples shall be stored at room temperature and analyzed for Delta-9 THC and Delta-9 THC-COOH within 7 days of completion of the extraction process.

3.8.30.4. When a sample has a Delta-9 THC concentration > 45 ng/mL or Delta-9 THC-COOH concentration > 60 ng/mL, intelligent sequencing may be used to prevent carryover into subsequent samples.

3.8.30.5. Samples for Delta-9 THC and Delta-9 THC-COOH analyses may have different sequence names and be analyzed on different GC/MS instruments.

3.8.30.6. If the instrument sequence is paused by the acquisition software between two samples, the sequence may be restarted at the sample not yet injected.

3.8.30.6.1. Sample stability criteria shall be met.

3.8.30.7. If the instrument sequence is interrupted during analysis of a sample or the sequence is aborted or stopped, the sequence should be restarted at the last bracketing non-zero control or may be resumed by beginning at the next sample not yet injected.

3.8.30.7.1. Sample stability criteria shall be met.

3.8.30.7.2. Reinjection of a sample of unknown concentration may be performed once.

3.8.30.7.3. Reinjection of a sample of known concentration may be performed multiple times.

3.8.30.7.3.1. If a reinjection is needed more than once, the evidentiary samples that have already been reinjected may be skipped in a bracket.

3.8.30.7.3.1.1. Evidentiary samples that are skipped and do not have valid data shall be reanalyzed starting at 3.8.1.

3.8.30.7.4. A reinjection shall be performed by restarting the sequence from the last bracketing non-zero control or reinjecting the entire sequence within 5 days of the original injection.

3.8.30.7.5. If an entire sequence is reinjected or a reinjection includes the calibrators used to generate the calibration curve, an autotune (Doc ID: 2841) shall be performed within 24 hours of initiation of the reinjection sequence. When reinjecting a sequence starting with the last bracketing control, an autotune is not required to be performed.

3.8.31. GC/MS Instrument Parameters: THC

3.8.31.1. Gas chromatograph oven Temperature ramps:

	Rate (ºC /min)	Final Temperature (ºC)	Hold time (min)	Final Time (min)
1		150	1	1.0
2	20.0	240	4	9.5

Post temperature: 315 ºC
Post time: 2.00 min
Run time: 9.50 min

3.8.31.2. Gas chromatograph inlet
Mode: Splitless
Initial temperature: 250 ºC
Purge flow: 15 mL/min
Purge time: 0.75 min
Gas type: Helium

3.8.31.3. Gas chromatograph capillary column 1
Dimensions: 15 m x 0.25 mm x 0.25 µm
Composition: (5%-Phenyl) methylpolysiloxane
Max temperature: 325 ºC
Mode: Constant flow
Flow: 0.8 mL/min
Post run flow: -4.2765 mL/min
Outlet: AUX EPC 1

3.8.31.4. Gas chromatograph capillary column 2
Dimensions: ~0.5 m x 150 µm x 0 µm
Composition: Fused silica
Max temperature: 350 ºC
Mode: Constant flow
Flow: 2.5 mL/min
Post run flow: 30 mL/min
Outlet: MSD

3.8.31.5. Gas chromatograph injector
Sample washes: 0
Sample pumps: 3
Injection volume: 2.0
Syringe: 10 µL with beveled needle
Solvent A and B: Ethyl acetate
Preinjection solvent A washes: 2
Preinjection solvent B washes: 2
Post injection solvent A washes: 8
Post injection solvent B washes: 8
Plunger speed: Fast
Preinjection dwell: 0.00 min
Post injection dwell: 0.00 min

3.8.31.6. Auxiliary Heater
Thermal Aux 2 (MSD Transfer Line)
Actual: On
Set Point: 300 ºC

3.8.31.7. Mass spectrometer parameters
Maximum solvent delay: 3.00 min
EM setting: Gain Factor

Gain factor: 2.000
MS source temperature: 230 ºC
MS quadrupole temperature: 150 ºC
Acquisition mode: SIM
SIM resolution: High
SIM dwell time: 40 ms
Ions monitored:

Analyte	Quantitative Ions (m/z)	Qualitative Ions (m/z)
THC	386	303, 371
THC-D9	380	352

Note: Exact ion masses may vary from instrument to instrument within +/- 0.5 m/z.

3.8.32. GC/MS Instrument Parameters: THC-COOH

3.8.32.1. Gas chromatograph oven

Temperature ramps:

	Rate (ºC /min)	Final Temperature (ºC)	Hold time (min)	Final Time (min)
1		180	1	1.0
2	20.0	300	3.5	10.5

Post temperature: 315 ºC
Post time: 2.00 min
Run time: 10.50 min

3.8.32.2. Gas chromatograph inlet

Mode: Splitless
Initial temperature: 250 ºC
Purge flow: 15 mL/min
Purge time: 0.75 min
Gas type: Helium

3.8.32.3. Gas chromatograph capillary column 1

Dimensions: 15 m x 0.25 mm x 0.25 µm
Composition: (5%-Phenyl)-methylpolysiloxane
Max temperature: 325 ºC
Mode: Constant flow
Flow: 0.8 mL/min
Post run flow: -4.2765 mL/min
Outlet: AUX EPC 1

3.8.32.4. Gas chromatograph capillary column 2

Dimensions: ~0.5 m x 150 µm x 0 µm
Composition: Fused silica
Max temperature: 350 ºC
Mode: Constant flow
Flow: 2.5 mL/min
Post run flow: 30 mL/min
Outlet: MSD

3.8.32.5. Gas chromatograph injector

Sample washes: 0
Sample pumps: 3
Injection volume: 2.00 µL to 4.0 µL

Syringe:	10 µL with beveled needle
Solvent A and B:	Ethyl acetate
Preinjection solvent A washes:	2
Preinjection solvent B washes:	2
Post injection solvent A washes:	8
Post injection solvent B washes:	8
Plunger speed:	Fast
Preinjection dwell:	0.00 min
Post injection dwell:	0.00 min

3.8.32.6. Auxiliary Heater

Thermal Aux 2 (MSD Transfer Line)

Actual:	On
Set Point:	300 ºC

3.8.32.7. Mass spectrometer parameters

Maximum solvent delay:	3.00 min
EM setting:	Gain Factor
Gain Factor	2.000
MS source temperature:	230 ºC
MS quadrupole temperature:	150 ºC
Acquisition mode:	SIM
SIM resolution:	High
SIM dwell time:	40 ms

Ions monitored:

Analyte	Quantitative Ions (m/z)	Qualitative Ions (m/z)
THC-COOH	515	557, 572
THC-COOH-D9	524	422

Note: Exact ion masses may vary from instrument to instrument within +/- 0.5 m/z.

3.8.33. Quantitation Parameters

RT reference window	1 min
RT non-reference window	0.5 min
Curve fit	Linear
Data point weight	1/x
Units of concentration	ng/mL

3.9. Records

- 3.9.1. Pipette calibration certificate, however named
- 3.9.2. Cannabinoids Confirmation Calibrator and Internal Standard Solution Preparation
- 3.9.3. Cannabinoids Confirmation Control Solution Preparation
- 3.9.4. Batch Preparation Packet, however named
  - 3.9.4.1. ISDT Confirmation Worklist
  - 3.9.4.2. Retest Worksheet, as appropriate
  - 3.9.4.3. Cannabinoids Confirmation Preparation Worksheet
  - 3.9.4.4. Aliquot Chain of Custody
- 3.9.5. Sequence Table
- 3.9.6. Quantitative Analysis Results Summary Report
- 3.9.7. Calibration Report
- 3.9.8. Calibrator and control chromatograms

3.9.9. Cannabinoids Confirmation Ion Ratio Ranges Worksheet
3.9.10. Sample chromatograms
3.9.11. Autotune
3.9.12. Cannabinoids Confirmation Technical Review Checklist
3.9.13. Data comparison output, however named
3.9.14. Measurement Uncertainty Estimation and supporting data
3.9.15. Specimen Verification Worksheet, if applicable

3.10. Interpretation of Results
3.10.1. Interpretation of results for each analyte shall occur independent of the other analytes in the method.
3.10.2. Chromatographic analyte and internal standard peaks shall have baseline resolution and/or analytes shall be mass resolved in the mass spectrometer.
3.10.2.1. A shoulder peak shall be < 10% of analyte peak height and area in order to report a quantitative result.
3.10.3. Peak filters should be set between 10% and 50% of the Cal 1 response for each analyte.
3.10.4. Internal standard recovery should be between 50% to 200% of the average of the calibrators in the batch.
3.10.4.1. Samples with recovery less than 25% or greater than 200% shall not be accepted.
3.10.4.2. Samples with recovery between 25% and 50% may be accepted at analyst discretion.
3.10.5. Calibration and Controls Criteria
3.10.5.1. Results of samples analyzed prior to analysis of the negative control preceding the calibrators shall not be used to determine acceptability of batch data.
3.10.5.2. Quantitation of calibrators and non-zero controls shall be within ± 20% of the target value (± 30% of the target value for concentrations < 2 ng/mL).
3.10.5.3. Generating a calibration curve
3.10.5.3.1. Calibration curve shall include a minimum of five non-zero concentrations.
3.10.5.3.2. Correlation coefficient (r^2) for the calibration curve shall be ≥ 0.990.
3.10.5.3.3. An ion ratio with a relative abundance ≥ 20% shall be within ± 20% of the mean ion ratio based on all calibrators used to generate the curve.
3.10.5.3.4. An ion ratio with a relative abundance < 20% shall be within ± 30% of the mean ion ratio based on all calibrators used to generate the curve.
3.10.5.3.5. The ion ratio range listed on the chromatogram as calculated by the software shall be used to determine ion ratio acceptability. The mean ion ratio calculated on the Cannabinoids Confirmation Ion Ratio Ranges Worksheet may differ in the tenths decimal place from the chromatogram.
3.10.5.3.6. A calibration point may be excluded if any of the following occur:

3.10.5.3.6.1. An ion ratio does not meet the acceptability criteria listed in 3.10.5.3.3 or 3.10.5.3.4;

3.10.5.3.6.2. The correlation coefficient (r^2) for the calibration curve is < 0.990;

3.10.5.3.6.3. A quantitated value is not within ± 20% of the target concentration (± 30% for concentrations < 2 ng/mL); or

3.10.5.3.6.4. A peak has poor chromatography.

3.10.5.3.7. If the lowest calibrator used to generate the calibration curve is not equal to the defined LLOQ, all samples with an analyte concentration (or response) greater than half the LLOQ but less than the batch LLOQ shall be reanalyzed, if possible, starting at 3.8.1.

3.10.5.3.8. If the highest calibrator used to generate the calibration curve is not equal to the defined ULOQ, all samples with an analyte concentration (or response) above the highest calibrator used to generate the calibration curve shall be reanalyzed, if possible, starting at 3.8.1. If unable to retest, the results for the analysis may be reported as greater than the highest calibrator used in the batch.

3.10.5.4. Each negative control shall have an analyte concentration or response < 50% of the LLOQ and/or unacceptable ion ratios as specified in 3.10.5.3.3 or 3.10.5.3.4.

3.10.5.4.1. If the above acceptance criterion is not met, the analytical data for the samples bracketed by the failed negative control with a concentration ≥ 50% of the LLOQ shall not be used and shall be reanalyzed, if possible, starting at 3.8.1. A result < 50% of the LLOQ for an evidentiary sample shall be accepted as none detected.

3.10.5.5. At least one negative control shall have the corresponding internal standard present for the associated analyte.

3.10.5.5.1. If acceptance criterion is not met, all samples in the batch shall be reanalyzed, if possible, starting at 3.8.1.

3.10.5.6. At least one low and one high non-zero control shall be included in each batch.

3.10.5.7. A non-zero control for an analyte fails if any of the following occur:

3.10.5.7.1. An ion ratio does not meet the acceptability criteria listed in 3.10.5.3.3 or 3.10.5.3.4;

3.10.5.7.2. A quantitated value is not within ± 20% of the target concentration; or

3.10.5.7.3. A peak has poor chromatography.

3.10.5.8. Each set of one to twelve samples shall be bracketed by one low and one high non-zero control.

3.10.5.8.1. If a control result does not meet the above criteria, the analytical data for the samples bracketed by the failed control shall not be used, and analysis of the samples in

the bracket prior to and following the failed control shall be repeated for samples positive for the analyte that failed, if possible, starting at 3.8.1. A result below the LLOQ for an evidentiary sample shall be accepted as none detected if the negative controls for the batch pass the acceptability criteria in 3.10.5.4 and 3.10.5.4.1.

3.10.5.9. If the acceptability criteria are not met for one analyte, analysis may be repeated for only the failed analyte, starting at 3.8.1. The eluent for the analyte that passed may be discarded prior to reanalysis for the failed analyte.

3.10.6. Analyte Identification (Qualitative Criteria)

3.10.6.1. Retention time shall be within ± 0.25 minutes of the mean retention time based on all calibrators used to generate the curve.

3.10.6.2. Each analyte shall have one quantitative ion and two qualitative ions monitored.

3.10.6.3. Each internal standard shall be present and have one quantitative ion and one qualitative ion monitored.

3.10.6.4. Each ion ratio shall meet the acceptability criteria listed in 3.10.5.3.3 or 3.10.5.3.4.

3.10.6.4.1. If the concentration of the analyte is > the ULOQ, the ion ratio shall be less than or equal to ± 30% of the mean ion ratio based on all calibrators used to generate the curve.

3.10.6.5. Data analysis software manual integration tools (Zero Peak, Merge Right Peak, Merge Left Peak, Split Peak and Pick Left, Split Peak and Pick Right, Snap Baseline, Drop Baseline, Apply ISTD RTs to Target, Apply Target RTs to Qualifier) may be used to adjust the integration algorithm to select the correct peak or adjust the baseline. Use of software manual integration tools shall be documented on the chromatogram.

3.10.7. Analyte Stability

3.10.7.1. Prepared samples are stable for 7 days when stored on the instrument auto sampler or at equivalent temperature with crimp caps.

3.10.7.2. Prepared samples are stable for 4 days when stored on the instrument auto sampler or at equivalent temperature with screw caps.

3.10.8. Retesting Samples

3.10.8.1. When a sample requires retesting, the sample shall be retested at least once, if possible. A sample may be retested up to two times without supervisory approval.

3.10.8.1.1. If a quantitative value cannot be reported from any analysis, the first acceptable qualitative data according to analyte identification in 3.10.6 shall be used. (ref. 3.11.4).

3.10.8.1.2. If data is not generated, that analysis does not count as an analysis or retest under this section.

3.10.9. Unacceptable Data

3.10.9.1. Data found to be unacceptable shall be marked with a signed note identifying the specific analytical data that should not be used and the reason for not using the data (e.g., “Do not use this quantitative THC

data due to a bracketing control being outside acceptability criteria. AB XX/XX/XX" or "Do not use any data from this batch due to sequence interruption. Samples will be retested. AB XX/XX/XX").

3.10.10. No Data Generated for a Sample

3.10.10.1. Cases with no generated data should have a case synopsis note to explain the lack of data associated with the chain of custody preparation date (e.g., "XX/XX/XX No data was collected from [batch name] due to the instrument stopping. AB").

3.11. Report Writing

3.11.1. The LLOD for Delta-9 THC and Delta-9 THC-COOH analysis is equal to the LLOQ for each analyte. The LLOQ is 1.0 ng/mL for Delta-9 THC and 5.0 ng/mL for Delta-9 THC-COOH, and the ULOQ is 50 ng/mL for Delta-9 THC and 100 ng/mL for Delta-9 THC-COOH.

3.11.2. Confirmatory data for each specimen shall be technically reviewed prior to entering the result into LIMS.

3.11.2.1. The preparation date of the analysis being reported shall be entered as the analysis date.

3.11.3. Quantitative Reporting

3.11.3.1. A result less than the LLOQ shall not be reported.

3.11.3.1.1. If a batch LLOQ is used, a quantitative result less than the target concentration for the lowest calibrator used in the calibration curve shall not be reported.

3.11.3.2. A quantitated result that meets acceptability criteria shall be reported for results between the target concentration of the lowest and highest calibrators that meet acceptability criteria.

3.11.3.2.1. A result shall be truncated to the appropriate level of significance and reported as the quantitative value ± the expanded measurement uncertainty.

3.11.3.2.1.1. A result shall be reported to one decimal place for quantitative values greater than or equal to 1 and less than 10.

3.11.3.2.1.2. A result shall be reported as a whole number for a quantitative value greater than or equal to 10.

3.11.3.3. A result that is above the ULOQ and has an ion ratio within ± 30% of the mean ion ratio based on all calibrators used to generate the curve shall be reported as greater (>) than the ULOQ in ng/mL.

3.11.3.3.1. If a batch ULOQ is used, a quantitative result greater than the target concentration for the highest calibrator used in the calibration curve shall not be reported.

3.11.3.3.1.1. A result greater than the target concentration of the highest calibrator used in the calibration curve may be reported as greater (>) than the ULOQ in ng/mL if retesting of a specimen is not feasible.

3.11.3.4. A quantitative result shall only be reported if analysis occurred within the established sample stability window (ref. 3.10.7).

3.11.3.5. If a specimen is analyzed more than once, the first quantitative result that meets acceptability criteria for quantitation of a specific analyte shall be reported.

3.11.4. Qualitative Reporting

3.11.4.1. A result should be reported as "Positive" when the analyte identification criteria (ref. 3.10.6) have been met, the quantitative result is > LLOQ, and the quantitative criteria have not been met.

3.11.4.1.1. If a specimen is analyzed more than once, the totality of the qualitative data shall be evaluated by the analyst for acceptability criteria for analyte identification of a specific analyte.

3.11.4.1.1.1. The preparation date of last analysis shall be used as the analysis date.

3.11.4.2. A result may be reported as "Positive" with supervisory approval if any of the following occur:

3.11.4.2.1. Interference(s); or

3.11.4.2.2. Quantitative result > LLOQ with an ion ratio greater than ± 20%, but less than ± 30%, of the mean ion ratio based on all calibrators used to generate the curve.

3.12. References

3.12.1. Standard for Mass Spectral Data Acceptance for Definitive Identification. Scientific Working Group for Forensic Toxicology (SWGTOX). 2014, 1-11.

3.12.2. RD Scurlock, GB Ohlson, DA Worthen. The Detection of Δ9-Tetrahydrocannabinol (THC) and 11-nor-9-Carboxy-Δ9-Tetrahydrocannabinol (THCA) in Whole Blood Using Two-Dimensional Gas Chromatography and EI-Mass Spectrometry. J. Anal. Toxicol. 30:262-266 (2006).

3.12.3. SPEware Corporation Application Method for the Extraction of THC and Metabolite from Blood, 2004.

3.12.4. Clarke's Isolation and Identification of Drugs, The Pharmaceutical Press, London, 1986.

3.12.5. Principles of Forensic Toxicology, American Association for Clinical Chemistry, 1999.

3.12.6. United Chemical Technologies Applications Manual (2004).

3.12.7. Standard Practices for Method Validation in Forensic Toxicology. ANSI/ASB Standard 036, 1st edition, 2019, 1-46.

4. Cannabinoids Confirmation by LC/QQQ

4.1. Scope

4.1.1. This method shall be used for confirmation analysis of specimens requiring confirmation of Delta-9 THC, Delta-9 THC-COOH, CBD and Delta-8 THC. Sample preparation shall be by SPE.

4.2. Precautions/Limitations

4.2.1. Minimum Sample Requirement

4.2.1.1. 0.5 mL of blood or serum/plasma specimen

4.2.2. CRMs

4.2.2.1. CRMs used for calibrator and non-zero control stocks shall be from two different vendors, if available.

4.2.2.2. If using CRMs from the same vendor, two different lots shall be used, if available.

4.2.2.3. If only one lot of a CRM is available, two separate vials from the lot shall be used.

4.2.3. Mobile phases should be kept in amber bottles to increase stability.

4.2.4. Serum/plasma and postmortem whole blood samples shall only be reported qualitatively.

4.2.5. CBD and Delta-8-THC shall only be reported qualitatively.

4.3. Related Information

4.3.1. CAN Method Development Notes (December 2023-August 2024)

4.3.2. CAN Confirmatory Analysis Method Validation (August 2024-February 2025)

4.3.3. Instrument validations

4.3.4. Validation of calibrators, controls, and internal standards data

4.4. Instruments/Equipment

4.4.1. Tube rack

4.4.2. Rocker

4.4.3. Vortex, single and multi-tube

4.4.4. Centrifuge

4.4.5. Positive pressure manifold

4.4.6. SPE column rack

4.4.7. SPE collection rack

4.4.8. Waste collection rack

4.4.9. Evaporator

4.4.10. Vial rack

4.4.11. Liquid chromatograph

4.4.12. Mass spectrometer, triple quadrupole

4.4.13. Pipettes

4.5. Reagents/Materials

4.5.1. Glass tubes (e.g., 13x100 mm and 16x125 mm)

4.5.2. UCT Clean Screen® THC Extraction Columns, 6 mL columns, 200 mg sorbent (part# CSTHC206 or equivalent)

4.5.3. Tube caps (e.g., 13 mm flange)

4.5.4. Pipette tips

4.5.5. Autosampler vials, inserts, and caps

4.5.6. ddH_2O
4.5.7. Negative blood (human)
4.5.8. Liquid chromatograph guard column
4.5.8.1. Dimensions: 2.1 x 5 mm
4.5.8.2. Composition: SelectraCore C18, 2.7 µm
4.5.9. Liquid chromatograph analytical column
4.5.9.1. Dimensions: 2.1 x 100 mm
4.5.9.2. Composition: SelectraCore C18, 2.7 µm
4.5.10. CRMs
4.5.10.1. Delta-8 THC
4.5.10.2. Delta-9 THC
4.5.10.3. Delta-9 THC-COOH
4.5.10.4. CBD
4.5.10.5. Delta-8 THC-D9
4.5.10.6. Delta-9 THC-D3
4.5.10.7. Delta-9 THC-COOH-D3
4.5.10.8. CBD-D3
4.5.11. Nitrogen
4.5.12. Solvents shall be high quality and low residue (e.g., HPLC grade, Omnisolv, Optima, etc.) unless otherwise noted.
4.5.12.1. Acetonitrile
4.5.12.2. Ethyl acetate
4.5.12.3. Glacial acetic acid, ACS grade or higher
4.5.12.4. Ammonium hydroxide, ACS grade or higher
4.5.12.5. Methanol, LCMS grade
4.5.12.6. Formic Acid

4.6. Hazards/Safety
4.6.1. See Safety Manual.
4.6.2. See SDS for each chemical in this method.
4.6.3. Add acids to approximately half the volume of the less acidic liquid, then dilute to final volume.

4.7. Reference Materials/Controls/Calibrators/Solutions
4.7.1. All solutions shall conform to Solution Preparation, Validation, Verification (Doc ID: 3695).
4.7.2. CAN Low Calibrator
4.7.3. CAN High Calibrator 1
4.7.4. CAN High Calibrator 2
4.7.5. CAN Low Control
4.7.6. CAN High Control
4.7.7. CAN Internal Standard
4.7.8. CAN Wash Solution
4.7.9. CAN Elution Solution
4.7.10. Mobile Phases
4.7.10.1. Aqueous (A)
4.7.10.2. Organic (B)

4.8. Procedures/Instructions

4.8.1. An evidentiary confirmation batch shall consist of concurrently prepared calibrators, negative blood controls, non-zero controls, and samples. Each set of one to twelve samples shall be bracketed by non-zero controls. The batch shall contain alternating low and high non-zero controls. The batch shall contain at least three prepared negative controls. Negative controls may be reinjected multiple times throughout the batch.

4.8.1.1. Reinjected negative controls shall be denoted with an "RI" followed by the number of reinjections.

4.8.2. Mix specimens on a rocker or by inverting several times.

4.8.3. Add 50 µL of internal standard (resulting in a concentration of 25 ng/mL) to each tube.

4.8.4. Prepare calibrator and control samples in correspondingly labeled tubes as indicated in Table 6.

Table 6: Cannabinoids Calibrator and Control Sample Preparation

Level	Stock Soln.	CAN Conc. (ng/mL)	Spike Vol. (µL)	Delta-9 THC COOH Stock Soln.	Delta-9 THC COOH Conc. (ng/mL)	Spike Vol. (µL)
Cal 1	Low Cal	1	10	-	**5**	-
Cal 2	Low Cal	2	20	-	**10**	-
Cal 3	Low Cal	4	40	-	**20**	-
Cal 4	Low Cal	6	60	-	**30**	-
Cal 5	High Cal 1	10	10	**High Cal 2**	**40**	**20**
Cal 6	High Cal 1	20	20	**High Cal 2**	**60**	**30**
Cal 7	High Cal 1	30	30	**High Cal 2**	**80**	**40**
Cal 8	High Cal 1	50	50	**High Cal 2**	**100**	**50**
LQC	Low Ctrl	3	50	-	**15**	-
HQC	High Ctrl	25	50	-	**70**	-

4.8.5. Pipette 0.5 mL of negative blood into each calibrator and control tube.

4.8.6. Pipette 0.5 mL of specimen into the correspondingly labeled tube.

4.8.7. Add 2 mL of cold acetonitrile (stored in refrigerator) to each sample.

4.8.8. Cap each sample, then vortex for approximately one minute at high speed on a multi tube vortex. The sample should not reach the cap.

4.8.9. Centrifuge for ~10 minutes using 3000 rpm at 4-8 ºC.

4.8.10. Add 3 mL of pH 7 phosphate buffer to a clean, correspondingly labeled test tube.

4.8.11. Decant each sample's top (organic) layer into the correspondingly labeled glass tube and vortex.

4.8.12. In the order listed, condition columns with each of the following solutions, allowing each solution to flow completely through each column before proceeding to the next solution:

4.8.12.1. 2 mL methanol

4.8.12.2. 2 mL pH 7 phosphate buffer

4.8.13. While the sorbent bed is still wet, decant each sample into an SPE column and allow the sample to filter by gravity completely through each column.

4.8.14. In the order listed, wash the columns with each of the following solutions, allowing each solution to flow completely through each column at ~1-2 mL per minute before proceeding to the next solution:

4.8.14.1. 3 mL ddH_2O
4.8.14.2. 3 mL ddH_2O
4.8.14.3. 3 mL Wash Solution
4.8.14.4. 3 mL Wash Solution

4.8.15. Using a maximum flow of ~60 psi or greater, dry the columns for at least 15 minutes.
4.8.16. Place empty labeled tubes into the positive pressure manifold, ensuring the placement of the tubes corresponds with the arrangement of the sample columns.
4.8.17. Add 3 mL of elution solution to each column and allow to filter by gravity completely through each column. Apply positive pressure to elute the last few drops of solution, if needed.
4.8.18. Remove the tubes from the positive pressure manifold and place on the evaporator. Evaporate at room temperature using nitrogen.
4.8.19. Add 150 µL MeOH to each tube and vortex.
4.8.20. Transfer each sample to a correspondingly labeled autosampler vial and cap vial.
4.8.21. Analyze the samples by LC/QQQ.
4.8.21.1. Sequence names shall be in the following format: YYYY_MM_DD_CAN_Initials.
4.8.21.1.1. The date in the sequence shall be the date of preparation of the samples.
4.8.21.1.2. Additional information such as reinjection, validation, etc., or equivalent abbreviations should be included with the assay abbreviation.
4.8.21.1.3. If the sequence is run with the wrong sequence name, it shall be noted on the Technical Review Worksheet and in the case synopsis of each case in the batch and not corrected on the chromatograms.
4.8.21.2. If multiple batches are included in one sequence, add a note on the MassHunter Worklist report that includes the batch name of each batch included in the sequence in the format listed in 4.8.21. The note should list which lines are attributed to each batch.
4.8.21.3. The extracted samples shall be stored in the instrument autosampler or at equivalent temperature and analyzed within 72 hours of completion of the extraction process.
4.8.21.4. If the instrument sequence is paused by the acquisition software between two samples, the sequence may be restarted at the sample not yet injected.
4.8.21.4.1. Sample stability criteria shall be met.
4.8.21.5. If the instrument sequence is interrupted during analysis of a sample or the sequence is aborted or stopped, the sequence should be restarted at the last bracketing non-zero control or may be resumed by beginning at the next sample not yet injected.
4.8.21.5.1. Sample stability criteria shall be met.
4.8.21.5.2. Reinjection of a sample of unknown concentration may be performed once.
4.8.21.5.3. Reinjection of a sample of known concentration may be performed multiple times.
4.8.21.5.3.1. If a reinjection is needed more than once, the evidentiary samples that have

already been reinjected may be skipped in a bracket.

4.8.21.5.3.1.1. Evidentiary samples that are skipped and do not have valid data shall be reanalyzed starting at 4.8.1

4.8.21.5.4. A reinjection shall be performed by restarting the sequence from the last bracketing non-zero control or reinjecting the entire sequence within 72 hours of the original injection.

4.8.21.5.5. If an entire sequence is reinjected or a reinjection includes the calibrators used to generate the calibration curve, an autotune (Doc ID: 2842) shall be performed within 24 hours of initiation of the reinjection sequence. When reinjecting a sequence starting with the last bracketing control, an autotune is not required to be performed.

4.8.22. LC/QQQ Acquisition Parameters

4.8.22.1. Liquid chromatograph sampler

Needle Wash	Standard Wash
Injection Volume	10 µL

4.8.22.2. Liquid chromatograph binary pump

	Time	Gradient A %	Gradient B %
1	0	86	14
2	1.5	86	14
3	4.5	45	55
4	4.6	35	65
5	10.6	15	85
6	14.0	15	85

Flow	0.6 mL/min
Stoptime	14.00 min
Posttime	2.50 min

4.8.22.3. Liquid chromatograph column compartment

Temperature	55 ºC

4.8.22.4. Mass spectrometer

Ion Source	AJS ESI
Scan Type	Dynamic MRM

4.8.22.5. dMRM Parameters

MS1 Resolution	Unit/Unit
MS2 Resolution	Unit/Unit
Cell Acc.	4 V
Polarity	Positive

Table 7: Cannabinoid MS Parameters

Compound Name	Internal Standard	Precursor Ion	Product Ion	Fragmentor (V)	CE* (V)	RT** (min)
Δ9-THC	No	315	**123.3**	125	39	11.780
			135.3	155	21	
Δ9-THC-COOH	No	345	**327**	140	15	9.942
			299	145	21	
Δ8-THC	No	315	**123.3**	140	37	11.990
			193.4	135	25	
CBD	No	315	**193**	145	23	10.223
			123	100	40	
Δ9-THC-D3	Yes	318	196	140	25	11.761
Δ9-THC-COOH-D3	Yes	348	330	130	15	9.921
Δ8-THC-D9	Yes	324	202	130	25	11.945
CBD-D3	Yes	318	196	115	23	10.211

* Collision Energy Ions in **bold** are used to quantitate.

**RTs are based on the average analyte retention times of calibrators and may be updated in the acquisition method and/or quantitation method, as necessary.

4.8.22.6. Quantitation Parameters

RRT Max % Deviation	5 percent
Curve fit – Quadratic	Δ9-THC, Δ9-THC-COOH, Δ8-THC, CBD
Data point weight	1/x
Units of concentration	ng/mL
Internal standard concentration	25

4.9. Records

4.9.1. Pipette calibration certificate, however named
4.9.2. Cannabinoids Confirmation Calibrator Preparation Worksheet
4.9.3. Cannabinoids Confirmation Control Solution Preparation Worksheet
4.9.4. Cannabinoids Confirmation Internal Standard Solution Preparation Worksheet
4.9.5. Batch Preparation Packet, however named
4.9.5.1. ISDT Confirmation Worklist
4.9.5.2. Retest Worksheet, as appropriate
4.9.5.3. Cannabinoids Confirmation Preparation Worksheet
4.9.5.4. Aliquot Chain of Custody
4.9.6. MassHunter Worklist Report
4.9.7. MassHunter Ion Ratio and RRT Verification, however named
4.9.8. QA/QC Packet, however named
4.9.8.1. Batch summary
4.9.8.2. Analyte calibration curves
4.9.8.3. Calibrator and control chromatograms
4.9.9. Sample chromatograms
4.9.10. QQQ Check Tune Report
4.9.11. Cannabinoids Confirmation Technical Review Checklist
4.9.12. Data comparison output, however named
4.9.13. Measurement Uncertainty Estimation and supporting data

4.9.14. Specimen Verification Worksheet, if applicable

4.10. Interpretation of Results

4.10.1. Interpretation of results for each analyte shall occur independent of the other analytes in the method.

4.10.2. Chromatographic analyte and internal standard peaks shall have baseline resolution and/or analytes shall be mass resolved in the mass spectrometer.

4.10.2.1. A shoulder peak shall be < 10% of analyte peak height and area in order to report a quantitative result.

4.10.3. Peak filters should be set between 10% and 50% of the Cal 1 response for each analyte.

4.10.4. Internal standard recovery should be between 50% to 200% of the average of the calibrators in the batch.

4.10.4.1. Samples with recovery less than 25% or greater than 200% shall not be accepted.

4.10.4.2. Samples with recovery between 25% and 50% may be accepted at analyst discretion.

4.10.5. Calibration and Controls Criteria

4.10.5.1. Results of samples analyzed prior to analysis of the negative control preceding the calibrators shall not be used to determine acceptability of batch data.

4.10.5.2. Quantitation of calibrators and non-zero controls for quantitatively reported analytes (see Table 8: Cannabinoids LLOQ and ULOQ) shall be within ± 20% of the target value (± 30% of the target value for concentrations < 2 ng/mL). Quantitation of calibrators and non-zero controls for qualitatively reported analytes shall be within ± 30% of the target concentration (± 40% of the target value for concentrations < 2 ng/mL).

4.10.5.3. Generating a calibration curve

4.10.5.3.1. Calibration curve shall include a minimum of five non-zero concentrations.

4.10.5.3.2. Correlation coefficient (r^2) for the calibration curve shall be ≥ 0.990.

4.10.5.3.3. An ion ratio with a relative abundance ≥ 20% shall be within ± 20% of the mean ion ratio based on all calibrators used to generate the curve.

4.10.5.3.4. An ion ratio with a relative abundance < 20% shall be within ± 30% of the mean ion ratio based on all calibrators used to generate the curve.

4.10.5.3.5. A calibration point may be excluded if any of the following occur:

4.10.5.3.5.1. An ion ratio does not meet the acceptability criteria listed in 4.10.5.3.3 or 4.10.5.3.4;

4.10.5.3.5.2. The correlation coefficient (r^2) for the calibration curve is < 0.990;

4.10.5.3.5.3. A quantitated value is not within ± 20% of the target concentration (± 30% for concentrations < 2 ng/mL); or

4.10.5.3.5.4. A peak has poor chromatography.

4.10.5.3.6. If the lowest calibrator used to generate the calibration curve is not equal to the defined LLOQ, all samples with an analyte concentration (or response) greater than half the LLOQ but less than the batch LLOQ shall be reanalyzed, if possible, starting at 4.8.1.

4.10.5.3.6.1. RR or response may be used to determine which specimens require reanalysis, if any.

4.10.5.3.7. If the highest calibrator used to generate the calibration curve is not equal to the defined ULOQ, all samples with an analyte concentration (or response) above the highest calibrator used to generate the calibration curve shall be reanalyzed, if possible, starting at 4.8.1 If unable to retest, the results for the analysis may be reported as greater than the highest calibrator used in the batch.

4.10.5.3.7.1. RR or response may be used to determine which specimens require reanalysis, if any.

4.10.5.4. Each set of one to twelve samples shall be bracketed by a negative control for partial batch acceptance. The negative control shall have an analyte concentration or response < 50% of the LLOQ and/or unacceptable ion ratios as specified in 4.10.5.3.3 or 4.10.5.3.4.

4.10.5.4.1. If the above acceptance criterion is not met, the analytical data for the samples bracketed by the failed negative control with a concentration ≥ 50% of the LLOQ shall not be used and shall be reanalyzed, if possible, starting at4.8.1. A result < 50% of the LLOQ for an evidentiary sample shall be accepted as none detected.

4.10.5.5. At least one negative control shall have the corresponding internal standard present for the associated analyte.

4.10.5.5.1. If acceptance criterion is not met, all samples in the batch shall be reanalyzed, if possible, starting at 4.8.1.

4.10.5.6. At least one low and one high non-zero control shall be included in each batch.

4.10.5.7. A non-zero control for an analyte fails if any of the following occur:

4.10.5.7.1. An ion ratio does not meet the acceptability criteria listed in 4.10.5.3.3 or 4.10.5.3.4;

4.10.5.7.2. A quantitated value is not within ± 20% of the target concentration; or

4.10.5.7.3. A peak has poor chromatography; or

4.10.5.7.4. The relative retention time is greater than ± 5% of the mean relative retention time based on all calibrator and control retention times.

4.10.5.8. Each set of one to twelve samples shall be bracketed by one low and one high non-zero control.

4.10.5.8.1. If a control result does not meet the above criteria, the analytical data for the samples bracketed by the failed control shall not be used, and samples in the bracket prior to and following the failed control that are positive for the analyte that failed shall be reanalyzed, if possible, starting at 4.8.1. A result below the LLOQ for an evidentiary sample shall be accepted as none detected if the negative controls for the batch pass the acceptability criteria in 4.10.5.4 and 4.10.5.5.

4.10.6. If the analyte in a sample has a result > 5x ULOQ, evaluate the analyte in the subsequent sample(s) as follows:

4.10.6.1. If a > 5x ULOQ sample is immediately followed by an evidentiary sample in which the analyte is not detected, data should be accepted.

4.10.6.2. If a > 5x ULOQ sample is immediately followed by an evidentiary sample in which the analyte is detected, the sample following the > 5x ULOQ sample shall be retested.

4.10.6.2.1. Additional negative controls or solvent blanks may be added to the sequence immediately following the > 5x ULOQ sample to eliminate carryover in subsequent samples.

4.10.6.3. If a > 5x ULOQ sample is immediately followed by a control and the control passes acceptance criteria, data should be accepted.

4.10.6.4. If a > 5x ULOQ sample is immediately followed by a control and it does not pass control acceptance criteria, all samples in the preceding and following brackets shall be retested.

4.10.6.4.1. Additional negative controls or solvent blanks may be added to the sequence immediately following the > 5x ULOQ sample to eliminate carryover in subsequent samples.

4.10.6.5. If the MassHunter software does not provide a calculated result, the RR for the sample may be compared to the RR of Cal 8.

4.10.7. Analyte Identification (Qualitative Criteria)

4.10.7.1. Relative retention time shall be within ± 5% of the mean relative retention time based on all calibrators used to generate the curve.

4.10.7.2. Each analyte shall have two ion transitions monitored. The ion transition listed in **bold** type in Table 7 is used for quantitation

4.10.7.3. Each internal standard shall be present and have one ion transition monitored.

4.10.7.4. Each ion ratio shall meet the acceptability criteria listed in 4.10.5.3.3 or 4.10.5.3.4.

4.10.7.4.1. If the concentration of the analyte is > the ULOQ, the ion ratio shall be less than or equal to ± 30% of the mean ion ratio based on all calibrators used to generate the curve.

4.10.7.5. Data analysis software manual integration tools (Zero Peak, Merge Right Peak, Merge Left Peak, Split Peak and Pick Left, Split Peak and Pick Right, Snap Baseline, Drop Baseline, Apply ISTD RTs to

Target, Apply Target RTs to Qualifier) may be used to adjust the integration algorithm to select the correct peak or adjust the baseline. Use of software manual integration tools shall be documented on the chromatogram.

4.10.8. Analyte Stability

4.10.8.1. Prepared samples are 72 hours when stored on the instrument autosampler or at equivalent temperature with screw caps.

4.10.9. Retesting Samples

4.10.9.1. When a sample requires retesting, the sample shall be retested at least once, if possible. A sample may be retested up to two times without supervisory approval.

4.10.9.1.1. If a quantitative value cannot be reported from any analysis, the first acceptable qualitative data according to analyte identification in 4.10.7 shall be used. (ref. 4.11.4).

4.10.9.1.2. If data is not generated, that analysis does not count as an analysis or retest under this section.

4.10.10. Unacceptable Data

4.10.10.1. Data found to be unacceptable shall be marked with a signed note identifying the specific analytical data that should not be used and the reason for not using the data (e.g., “Do not use this quantitative THC data due to a bracketing control being outside acceptability criteria. AB XX/XX/XX” or “Do not use any data from this batch due to sequence interruption. Samples will be retested. AB XX/XX/XX”).

4.10.11. No Data Generated for a Sample

4.10.11.1. Cases with no generated data should have a case synopsis note to explain the lack of data associated with the chain of custody preparation date (e.g., “XX/XX/XX No data was collected from [batch name] due to the instrument stopping. AB”).

4.11. Report Writing

4.11.1. Serum/plasma and postmortem whole blood samples shall be reported qualitatively only.

4.11.1.1. The reporting cutoff is equal the LLOQ listed in Table 8: Cannabinoids LLOQ and ULOQ.

4.11.2. The LLOD for cannabinoid analysis is equal to the LLOQ for each analyte. The LLOQ and ULOQ are listed in Table 8:

Table 8: Cannabinoids LLOQ and ULOQ

Analyte	LLOQ (ng/mL)	ULOQ (ng/mL)
Δ9-THC	1	50
Δ9-THC-COOH	5	100
Δ8-THC*	1	-
CBD*	1	-

*Analytes shall only be reported qualitatively with the LLOQ as the cutoff.

4.11.3. Confirmatory data for each specimen shall be technically reviewed prior to entering the result into LIMS.

4.11.3.1. The preparation date of the analysis being reported shall be entered as the analysis date.

4.11.4. Quantitative Reporting of Delta-9 THC and Delta-9 THC-COOH

4.11.4.1. A result less than the LLOQ shall not be reported.

4.11.4.1.1. If a batch LLOQ is used, a quantitative result less than the target concentration for the lowest calibrator used in the calibration curve shall not be reported.

4.11.4.2. A quantitated result that meets acceptability criteria shall be reported for results between the target concentration of the lowest and highest calibrators that meet acceptability criteria.

4.11.4.2.1. A result shall be truncated to the appropriate level of significance and reported as the quantitative value ± the expanded measurement uncertainty.

4.11.4.2.1.1. A result shall be reported to one decimal place for quantitative values greater than or equal to 1 and less than 10.

4.11.4.2.1.2. A result shall be reported as a whole number for a quantitative value greater than or equal to 10.

4.11.4.3. A result that is above the ULOQ and has an ion ratio within ± 30% of the mean ion ratio based on all calibrators used to generate the curve shall be reported as greater (>) than the ULOQ in ng/mL.

4.11.4.3.1. If a batch ULOQ is used, a quantitative result greater than the target concentration for the highest calibrator used in the calibration curve shall not be reported.

4.11.4.3.1.1. A result greater than the target concentration of the highest calibrator used in the calibration curve may be reported as greater (>) than the ULOQ in ng/mL if retesting of a specimen is not feasible.

4.11.4.4. A quantitative result shall only be reported if analysis occurred within the established sample stability window (ref. 4.10.8).

4.11.4.5. If a specimen is analyzed more than once, the first quantitative result that meets acceptability criteria for quantitation of a specific analyte shall be reported.

4.11.5. Qualitative Reporting of Delta-9 THC and Delta-9 THC-COOH

4.11.5.1. A result should be reported as “Positive” when the analyte identification criteria (ref. 4.10.7) have been met, the quantitative result is > LLOQ, and the quantitative criteria have not been met.

4.11.5.1.1. If a specimen is analyzed more than once, the totality of the qualitative data shall be evaluated by the analyst for acceptability criteria for analyte identification of a specific analyte.

4.11.5.1.1.1. The preparation date of last analysis shall be used as the analysis date.

4.11.5.2. A result may be reported as “Positive” with supervisory approval if any of the following occur:

4.11.5.2.1. Interference(s); or
4.11.5.2.2. Quantitative result > LLOQ with an ion ratio greater than ± 20%, but less than ± 30%, of the mean ion ratio based on all calibrators used to generate the curve.

4.11.6. Qualitative Reporting of Delta-8 THC and CBD
4.11.6.1. A result shall be reported as "Positive" when the analyte identification criteria (ref. 4.10.7) have been met, the quantitative result is > LLOQ, and the calibrator and control criteria (ref. 4.10.5) have been met.
4.11.6.1.1. If quantitative criteria have not been met, an analyte may be reported "Positive" with supervisory approval.
4.11.6.1.2. If a specimen is analyzed more than once, the totality of the qualitative data shall be evaluated by the analyst for acceptability criteria for analyte identification of a specific analyte. The preparation date of last analysis shall be used as the analysis date.
4.11.6.2. A result may be reported as "Positive" with supervisory approval if any of the following occur:
4.11.6.2.1. Interference(s); or
4.11.6.2.2. Quantitative result > LLOQ with an ion ratio greater than ± 20%, but less than ± 30%, of the mean ion ratio based on all calibrators used to generate the curve and controls.

4.12. References
4.12.1. United Chemical Technologies. (2022, May 4). *Analysis of Natural Cannabinoids and Metabolites from Blood Using Clean Screen® THC and SelectraCore® C18 Column on LC-MSMS*. https://www.unitedchem.com/wp-content/uploads/2022/05/Natural_Cannabinoids_Metabolites_From_Blood_Application_Note_2023-2.pdf
4.12.2. American National Standards Institute-Academy Standards Board. *Standard for Mass Spectral Analysis in Forensic, First Edition.* (2023). https://www.aafs.org/asb-standard/standard-mass-spectral-analysis-forensic-toxicology.

5. Cocaine Confirmation by GC/MS

5.1. Scope

5.1.1. This method shall be used for confirmation analysis of specimens requiring confirmation of cocaine and benzoylecgonine. Sample preparation shall be by SPE and derivatization.

5.2. Precautions/Limitations

5.2.1. Minimum Sample Requirement

5.2.1.1. 1 mL of blood or serum/plasma specimen for quantitative confirmation

5.2.1.2. 250 µL of blood or serum/plasma specimen for qualitative confirmation

5.2.2. CRMs

5.2.2.1. CRMs used for calibrator and non-zero control stocks shall be from two different vendors, if available.

5.2.2.2. If using CRMs from the same vendor, two different lots shall be used, if available.

5.2.2.3. If only one lot of a CRM is available, two separate vials from the lot shall be used.

5.2.3. BSTFA hydrolyzes easily.

5.3. Related Information

5.3.1. Cocaine Confirmatory Analysis Method Validation (October 2015-March 2016)

5.3.2. Stock Solution Stability (February 2020)

5.3.3. Instrument validations

5.3.4. Validations of calibrators, controls, and internal standards data

5.4. Instruments/Equipment

5.4.1. Tube rack

5.4.2. Rocker

5.4.3. Vortex, single

5.4.4. Sonicating water bath

5.4.5. Centrifuge

5.4.6. Positive pressure manifold

5.4.7. SPE column rack

5.4.8. SPE collection rack

5.4.9. Waste collection rack

5.4.10. Vial rack

5.4.11. Dry block heater

5.4.12. Evaporator

5.4.13. Electronic or manual crimper

5.4.14. Gas chromatograph

5.4.15. Mass spectrometer, single quadrupole

5.4.16. Pipettes

5.5. Reagents/Materials

5.5.1. Glass tubes (e.g., 13x100 mm)

5.5.2. Trace B SPE columns, 3 mL columns, 35 mg (Tecan #TB-335C)

5.5.3. Tube caps (e.g., 13mm flange)

5.5.4. Pipette tips
5.5.5. Autosampler vials, inserts, and caps
5.5.6. ddH_2O
5.5.7. Negative blood (human)
5.5.8. Gas chromatograph capillary column-analytical column
5.5.8.1. Dimensions: 15 m x 0.25 mm x 0.25 µm
5.5.8.2. Composition: DB-5 MS UI (5%-Phenyl)-methylpolysiloxane
5.5.9. Gas chromatograph capillary column-restrictor column
5.5.9.1. Dimensions: ~0.5 m x 150 µm
5.5.9.2. Composition: fused silica
5.5.10. BSTFA + 1% TMCS
5.5.11. CRMs
5.5.11.1. Benzoylecgonine
5.5.11.2. Cocaine
5.5.11.3. Benzoylecgonine-D3
5.5.11.4. Cocaine-D3
5.5.12. Helium, 5.0 grade or higher
5.5.13. Nitrogen
5.5.14. Solvents shall be high quality and low residue (e.g., HPLC grade, Omnisolv, Optima, etc.) unless otherwise noted.
5.5.14.1. Ethyl acetate
5.5.14.2. Methylene chloride
5.5.14.3. Isopropanol
5.5.14.4. Glacial acetic acid, ACS grade or higher
5.5.14.5. Ammonium hydroxide, ACS grade or higher
5.5.14.6. Methanol, ACS grade or higher
5.5.15. Sodium phosphate monobasic
5.5.16. Sodium phosphate dibasic

5.6. Hazards/Safety
5.6.1. See Safety Manual.
5.6.2. See SDS for each chemical in this method.
5.6.3. Add acids to approximately half the volume of the less acidic liquid, then dilute to final volume.

5.7. Reference Materials/Controls/Calibrators/Solutions
5.7.1. All solutions shall conform to Solution Preparation, Validation, Verification (Doc ID: 3695).
5.7.2. COC Calibrator Stock
5.7.3. COC High Calibrator
5.7.4. COC Low Calibrator
5.7.5. COC Control Stock
5.7.6. COC High Control
5.7.7. COC Low Control
5.7.8. COC Internal Standard
5.7.9. COC Elution Solution
5.7.10. Phosphate Buffer (100 mM)
5.7.11. Acetic Acid (100 mM)

5.8. Procedures/Instructions

5.8.1. An evidentiary confirmation batch shall consist of concurrently prepared calibrators, negative blood controls, non-zero controls, and samples. Each set of one to twelve samples shall be bracketed by non-zero controls. The batch shall contain alternating low and high non-zero controls. The batch shall contain at least three prepared negative controls. Negative controls may be reinjected multiple times throughout the batch.

5.8.1.1. Reinjected negative controls shall be denoted with an "RI" followed by the number of reinjections.

5.8.2. Mix specimens on a rocker or by inverting several times.

5.8.3. Add 100 µL of cocaine internal standard (resulting in a concentration of 100 ng/mL) to labeled glass tubes.

5.8.4. Prepare calibrator and control samples in correspondingly labeled tubes as indicated in Table 9.

Table 9: Cocaine Calibrator and Control Sample Preparation

Level	Sample Identification	Stock Solution	Volume (µL)
Cal 1	20 ng/mL Calibrator	Low Calibrator	20
Cal 2	50 ng/mL Calibrator	Low Calibrator	50
Cal 3	100 ng/mL Calibrator	Low Calibrator	100
Cal 4	250 ng/mL Calibrator	High Calibrator	25
Cal 5	500 ng/mL Calibrator	High Calibrator	50
Cal 6	750 ng/mL Calibrator	High Calibrator	75
Cal 7	1000 ng/mL Calibrator	High Calibrator	100
Low Control	60 ng/mL Control	Low Control	60
High Control	600 ng/mL Control	High Control	60

5.8.5. Pipette 1 mL of negative blood into calibrator and control samples.

5.8.6. Pipette 1 mL of specimen into the correspondingly labeled tube.

5.8.7. Add 2 mL of 100 mM phosphate buffer to each tube. Cap and vortex each tube.

5.8.8. Sonicate for ~10 minutes.

5.8.9. Centrifuge for ~10 minutes using 3000 rpm at ~4-8 ºC.

5.8.10. In the order listed, condition the SPE columns with each of the following solutions, allowing each solution to flow completely through each column before proceeding to the next solution:

5.8.10.1. 1 mL of methanol

5.8.10.2. 1 mL of ddH_2O

5.8.10.3. 1 mL of phosphate buffer

5.8.11. While the sorbent bed is still wet, decant each sample into the SPE column. Allow the sample to flow completely through each column at ~1 mL per minute.

5.8.12. In the order listed, wash columns with each of the following solutions, allowing each wash solution to flow completely through each column before proceeding to the next solution:

5.8.12.1. 2 mL ddH_2O

5.8.12.2. 2 mL 100 mM acetic acid

5.8.12.3. 1 mL methanol

5.8.12.4. 1 mL ethyl acetate

5.8.13. Using a maximum flow of ~60 psi or greater, dry the columns for ~ 20 minutes.
5.8.14. Place empty labeled tubes into the positive pressure manifold, ensuring the placement of the tubes corresponds with the arrangement of the sample columns.
5.8.15. Add 2 mL of cocaine elution solution to each column. Allow the cocaine elution solution to flow completely through each column into each correspondingly labeled tube at ~1 mL per minute.
5.8.16. Remove tubes from the positive pressure manifold and place on the evaporator.
5.8.17. Evaporate at room temperature using nitrogen.
5.8.18. Add 50 µL ethyl acetate, then 50 µL BSTFA with 1% TMCS, to each tube and cap.
5.8.19. Vortex tubes briefly.
5.8.20. Place the tubes in a dry heat block at ~ 70 ºC for ~ 25 minutes.
5.8.21. Allow the tubes to cool. Transfer each sample to the correspondingly labeled autosampler vial and cap vial.
5.8.22. Analyze the samples by GC/MS.
5.8.22.1. Sequence names shall be in the following format: YYYY_MM_DD_COC_Initials.
5.8.22.1.1. The date in the sequence shall be the date of preparation of the samples.
5.8.22.1.2. Additional information such as reinjection, validation, etc., or equivalent abbreviations should be included with the assay abbreviation.
5.8.22.1.3. If the sequence is run with the wrong sequence name, it shall be noted on the Technical Review Worksheet and in the case synopsis of each case in the batch and not corrected on the chromatograms.
5.8.22.2. If multiple batches are included in one sequence, add a note on the sequence table that includes the batch name of each batch included in the sequence in the format listed in 5.8.22.1. The note should list which lines are attributed to each batch.
5.8.22.3. The extracted samples shall be stored at room temperature and analyzed for cocaine and benzoylecgonine within 4 days of completion of the extraction process.
5.8.22.4. When a sample has a cocaine or benzoylecgonine concentration > 450 ng/mL, intelligent sequencing may be used to prevent carryover into subsequent samples.
5.8.22.5. If the instrument sequence is paused by the acquisition software between two samples, the sequence may be restarted at the sample not yet injected.
5.8.22.5.1. Sample stability criteria shall be met.
5.8.22.6. If the instrument sequence is interrupted during analysis of a sample or the sequence is aborted or stopped, the sequence should be restarted at the last bracketing non-zero control or may be resumed by beginning at the next sample not yet injected.
5.8.22.6.1. Sample stability criteria shall be met.
5.8.22.6.2. Reinjection of a sample of unknown concentration may be performed once.
5.8.22.6.3. Reinjection of a sample of known concentration may be performed multiple times.

5.8.22.6.3.1. If a reinjection is needed more than once, the evidentiary samples that have already been reinjected may be skipped in a bracket.

5.8.22.6.3.1.1. Evidentiary samples that are skipped and do not have valid data shall be reanalyzed starting at 5.8.1.

5.8.22.6.4. A reinjection shall be performed by restarting the sequence from the last bracketing non-zero control or reinjecting the entire sequence within 24 hours of the original injection.

5.8.22.6.5. If an entire sequence is reinjected or a reinjection includes the calibrators used to generate the calibration curve, an autotune (Doc ID: 2841) shall be performed within 24 hours of initiation of the reinjection sequence. When reinjecting a sequence starting with the last bracketing control, an autotune is not required to be performed.

5.8.23. GC/MS Instrument Parameters

5.8.23.1. Gas chromatograph oven

Temperature ramps:

	Rate (ºC /min)	Final Temperature (ºC)	Hold Time (min)	Final Time (min)
1		150	0	0
2	30.0	300	0	5.0

Post temperature: 315 ºC
Post time: 2.00 min
Run time: 5.00 min

5.8.23.2. Gas chromatograph inlet

Mode: Splitless
Initial temperature: 250 ºC
Purge flow: 50 mL/min
Purge time: 0.75 min
Gas type: Helium

5.8.23.3. Gas chromatograph capillary column 1

Dimensions: 15 m x 0.25 mm x 0.25 µm
Composition: (5%-Phenyl)-methylpolysiloxane
Max temperature: 325 ºC
Mode: Constant flow
Flow: 1.0 mL/min
Post run flow: -4.2765 mL/min
Outlet: AUX EPC 1

5.8.23.4. Gas chromatograph capillary column 2

Dimensions: ~0.5 m x 150 µm
Composition: Fused silica
Max temperature: 350 ºC

Mode:	Constant flow
Flow:	2.5 mL/min
Post run flow:	30 mL/min
Outlet:	MSD

5.8.23.5. Gas chromatograph injector

Sample washes:	0
Sample pumps:	3
Injection volume:	2.00 µL
Syringe:	10 µL with beveled needle
Solvent A and B:	Ethyl acetate
Preinjection solvent A washes:	1
Preinjection solvent B washes:	2
Post injection solvent A washes:	1
Post injection solvent B washes:	2
Plunger speed:	Fast
Preinjection dwell:	0.00 min
Post injection dwell:	0.00 min

5.8.23.6. Auxiliary Heaters

Thermal Aux 2 (MSD Transfer Line)

Actual:	On
Set Point:	300 ºC

5.8.23.7. Mass spectrometer parameters

Maximum solvent delay:	3.00 min
EM setting:	Gain Factor
Gain factor:	2.000
MS source temperature:	230 ºC
MS quadrupole temperature:	150 ºC
Acquisition mode:	SIM
SIM resolution:	High
SIM dwell time:	30 ms

Ions monitored:

Analyte	Quantitative Ions (m/z)	Qualitative Ions (m/z)
Cocaine	182	82, 303
Cocaine-D3	185	306
Benzoylecgonine	240	82, 361
Benzoylecgonine-D3	243	364

Note: Exact ion masses may vary from instrument to instrument within +/- 0.5 m/z.

5.8.23.8. Quantitation Parameters

RT reference window	1 min
RT non-reference window	0.5 min
Curve fit	Linear
Data point weight	1/x
Units of concentration	ng/mL

5.9. Records

5.9.1. Pipette calibration certificate, however named

5.9.2. Cocaine Confirmation Calibrator and Internal Standard Solution Preparation Worksheet
5.9.3. Cocaine Confirmation Control Solution Preparation Worksheet
5.9.4. Batch Preparation Packet, however named
5.9.4.1. ISDT Confirmation Worklist
5.9.4.2. Retest Worksheet, as appropriate
5.9.4.3. Cocaine Confirmation Preparation Worksheet
5.9.4.4. Aliquot Chain of Custody
5.9.5. Sequence Table
5.9.6. Quantitative Analysis Results Summary Report
5.9.7. Calibrator and control chromatograms
5.9.8. Calibration Report
5.9.9. Cocaine Confirmation Ion Ratio Ranges Worksheet
5.9.10. Sample chromatograms
5.9.11. Autotune
5.9.12. Cocaine Confirmation Technical Review Checklist
5.9.13. Data comparison output, however named
5.9.14. Measurement Uncertainty Estimation and supporting data
5.9.15. Specimen Verification Worksheet, if applicable

5.10. Interpretation of Results
5.10.1. Interpretation of results for each analyte shall occur independent of the other analytes in the method.
5.10.2. Chromatographic analyte and internal standard peaks shall have baseline resolution and/or shall be mass resolved in the mass spectrometer.
5.10.2.1. A shoulder peak shall be < 10% of analyte peak height and area in order to report a quantitative result.
5.10.3. Peak filters should be set between 10% and 50% of the Cal 1 response for each analyte.
5.10.4. Internal standard recovery should be between 50% to 200% of the average of the calibrators in the batch.
5.10.4.1. Samples with recovery less than 25% or greater than 200% shall not be accepted.
5.10.4.2. Samples with recovery between 25% and 50% may be accepted at analyst discretion.
5.10.5. Calibration and Controls Criteria
5.10.5.1. Results of samples analyzed prior to analysis of the negative control preceding the calibrators shall not be used to determine acceptability of batch data.
5.10.5.2. Quantitation of calibrators and non-zero controls shall be within ± 20% of the target concentration.
5.10.5.3. Generating a calibration curve
5.10.5.3.1. Calibration curve shall include a minimum of five non-zero concentrations.
5.10.5.3.2. Correlation coefficient (r^2) for the calibration curve shall be ≥ 0.990.
5.10.5.3.3. An ion ratios shall be within ± 20% of the mean ion ratio based on all calibrators used to generate the curve.

5.10.5.3.4. The ion ratio range listed on the chromatogram as calculated by the software shall be used to determine ion ratio acceptability. The mean ion ratio calculated on the Cocaine Confirmation Ion Ratio Ranges Worksheet may differ in the tenths decimal place from the chromatogram.

5.10.5.3.5. A calibration point may be excluded if any of the following occur:

5.10.5.3.5.1. An ion ratio does not meet the acceptability criteria listed in 5.10.5.3.3 or 5.10.5.3.4.

5.10.5.3.5.2. The correlation coefficient (r^2) for the calibration curve is < 0.990.

5.10.5.3.5.3. A quantitated value is not within ± 20% of the target concentration.

5.10.5.3.5.4. A peak has poor chromatography.

5.10.5.3.6. If the lowest calibrator used to generate the calibration curve is not equal to the defined LLOQ, all samples with an analyte concentration (or response) greater than half the LLOQ but less than the batch LLOQ shall be reanalyzed, if possible, starting at 5.8.1.

5.10.5.3.7. If the highest calibrator used to generate the calibration curve is not equal to the defined ULOQ, all samples with an analyte concentration (or response) above the highest calibrator used to generate the calibration curve shall be reanalyzed, if possible, starting at 5.8.1. If unable to retest, the results for the analysis may be reported as greater than the highest calibrator used in the batch.

5.10.5.4. Each negative control shall have an analyte concentration or response < 50% of the LLOQ and/or unacceptable ion ratios as specified in 5.10.5.3.3 or 5.10.5.3.4.

5.10.5.4.1. If the above acceptance criterion is not met, the analytical data for the samples bracketed by the failed negative control with a concentration ≥ 50% of the LLOQ shall not be used and shall be reanalyzed, if possible, starting at 5.8.1. A result < 50% of the LLOQ for an evidentiary sample shall be accepted as none detected.

5.10.5.5. At least one negative control shall have the corresponding internal standard present for the associated analyte.

5.10.5.5.1. If acceptance criterion is not met, all samples in the batch shall be reanalyzed, if possible, starting at 5.8.1.

5.10.5.6. At least one low and one high non-zero control shall be included in each batch.

5.10.5.7. A non-zero control for an analyte fails if any of the following occur:

5.10.5.7.1. An ion ratio does not meet the acceptability criteria listed in 5.10.5.3.3 or 5.10.5.3.4.

5.10.5.7.2. A quantitated value is not within ± 20% of the target concentration.

5.10.5.7.3. A peak has poor chromatography.

5.10.5.8. Each set of one to twelve samples shall be bracketed by one low and one high non-zero control.

5.10.5.8.1. If a control result does not meet the above criteria, the analytical data from the samples bracketed by the failed control shall not be used, and analysis of the samples in the bracket prior to and following the failed control shall be repeated for samples positive for the analyte that failed, if possible, starting at 5.8.1. A result below the LLOQ for an evidentiary sample shall be accepted as none detected if the negative controls for the batch pass the acceptability criteria in 5.10.5.4 and 5.10.5.4.1.

5.10.6. If the analyte in a sample has a result > 2x ULOQ, evaluate the analyte in the subsequent sample(s) as follows:

5.10.6.1. Intelligence sequencing may be used to satisfy the following requirements.

5.10.6.2. If a > 2x ULOQ sample is immediately followed by an evidentiary sample in which the analyte is not detected, data should be accepted.

5.10.6.3. If a > 2x ULOQ sample is immediately followed by an evidentiary sample in which the analyte is detected, the sample following the > 2x ULOQ sample shall be retested.

5.10.6.3.1. Additional negative controls or solvent blanks may be added to the sequence immediately following the > 2x ULOQ sample to eliminate carryover in subsequent samples.

5.10.6.4. If a > 2x ULOQ sample is immediately followed by a control and the control passes acceptance criteria, data should be accepted.

5.10.6.5. If a > 2x ULOQ sample is immediately followed by a control and it does not pass control acceptance criteria, all samples in the preceding and following brackets shall be reinjected or retested.

5.10.6.5.1. Additional negative controls or solvent blanks may be added to the sequence immediately following the > 2x ULOQ sample to eliminate carryover in subsequent samples.

5.10.6.6. If the MassHunter software does not provide a calculated result, the RR for the sample may be compared to the RR of Cal 8.

5.10.7. Analyte Identification (Qualitative Criteria)

5.10.7.1. Retention time shall be within ± 0.25 minutes of the mean retention time based on all calibrators used to generate the curve.

5.10.7.2. Each analyte shall have one quantitative ion and two qualitative ions monitored.

5.10.7.3. Each internal standard shall be present and have one quantitative ion and one qualitative ion monitored.

5.10.7.4. Each ion ratio shall meet the acceptability criteria listed in 5.10.5.3.3 or 5.10.5.3.4.

5.10.7.4.1. If the concentration of the analyte is > the ULOQ, the ion ratio shall be less than or equal to ± 30% of the

mean ion ratio based on all calibrators used to generate the curve.

5.10.7.5. Data analysis software manual integration tools (Zero Peak, Merge Right Peak, Merge Left Peak, Split Peak and Pick Left, Split Peak and Pick Right, Snap Baseline, Drop Baseline, Apply ISTD RTs to Target, Apply Target RTs to Qualifier) may be used to adjust the integration algorithm to select the correct peak or adjust the baseline. Use of software manual integration tools shall be documented on the chromatogram.

5.10.8. Analyte Stability

5.10.8.1. Prepared samples are stable for 4 days when stored on the instrument auto sampler or at equivalent temperature.

5.10.9. Retesting Samples

5.10.9.1. When a sample requires retesting, the sample shall be retested at least once, if possible. A sample may be retested up to two times without supervisory approval.

5.10.9.1.1. If a quantitative value cannot be reported from any analysis, the first acceptable qualitative data according to analyte identification in 5.10.6 shall be used. (ref. 5.11.4).

5.10.9.1.2. If data is not generated, that analysis does not count as an analysis or retest under this section.

5.10.10. Unacceptable Data

5.10.10.1. Data found to be unacceptable shall be marked with a signed note identifying the specific analytical data that should not be used and the reason for not using the data (e.g., “Do not use this quantitative cocaine data due to a bracketing control being outside acceptability criteria. AB XX/XX/XX” or “Do not use any data from this batch due to sequence interruption. Samples will be retested. AB XX/XX/XX”).

5.10.11. No Data Generated for a Sample

5.10.11.1. Cases with no generated data should have a case synopsis note to explain the lack of data associated with the chain of custody preparation date (e.g., “XX/XX/XX No data was collected from [batch name] due to the instrument stopping. AB”).

5.11. Report Writing

5.11.1. The LLOD for cocaine and benzoylecgonine analysis is equal to the LLOQ for each analyte. The LLOQ is 20 ng/mL and the ULOQ is 1000 ng/mL.

5.11.2. Confirmatory data for each specimen shall be technically reviewed prior to entering the result into LIMS.

5.11.2.1. The preparation date of the analysis being reported shall be entered as the analysis date.

5.11.3. Quantitative Reporting

5.11.3.1. A result less than the LLOQ shall not be reported.

5.11.3.1.1. If a batch LLOQ is used, a quantitative result less than the target concentration for the lowest calibrator used in the calibration curve shall not be reported.

5.11.3.2. A quantitated result that meets acceptability criteria shall be reported for results between the target concentration of the lowest and highest calibrators that meet acceptability criteria.

5.11.3.2.1. A result shall be truncated to the appropriate level of significance and reported as the quantitative value ± the expanded measurement uncertainty.

5.11.3.2.1.1. A result shall be reported as a whole number.

5.11.3.3. A result that is above the ULOQ and has an ion ratio within ± 30% of the mean ion ratio based on all calibrators used to generate the curve shall be reported as greater (>) than the ULOQ in ng/mL.

5.11.3.3.1. If a batch ULOQ is used, a quantitative result greater than the target concentration for the highest calibrator used in the calibration curve shall not be reported.

5.11.3.3.1.1. A result greater than the target concentration of the highest calibrator used in the calibration curve may be reported if retesting of a specimen is not feasible.

5.11.3.4. A quantitative result shall only be reported if analysis occurred within the established sample stability window (ref. 5.10.8).

5.11.3.5. If a specimen is analyzed more than once, the first quantitative result that meets acceptability criteria for quantitation of a specific analyte shall be reported.

5.11.4. Qualitative Reporting

5.11.4.1. A result should be reported as “Positive” when the analyte identification criteria (ref. 5.10.6) have been met, the quantitative result is > LLOQ, and the quantitative criteria have not been met.

5.11.4.1.1. If a specimen is analyzed more than once, the totality of the qualitative data shall be evaluated by the analyst for acceptability criteria for analyte identification of a specific analyte.

5.11.4.1.1.1. The preparation date of last analysis shall be used as the analysis date.

5.11.4.2. A result should be reported as “Positive” if a diluted sample was analyzed and the quantitative result was ≥ LLOQ (e.g., 20 ng/mL multiplied by the dilution factor).

5.11.4.3. A result may be reported as “Positive” with supervisory approval if any of the following occur:

5.11.4.3.1. Interference(s); or

5.11.4.3.2. Quantitative result > LLOQ with an ion ratio greater than ± 20%, but less than ± 30%, of the mean ion ratio based on all calibrators used to generate the curve.

5.12. References

5.12.1. Abusada, G.M., Abukhalaf, I.K., Alford, D.D., Vinzon-Bautista, I., Pramanik, A.K., Manno, J.E., & Manno, B.R. (1993). Solid-phase extraction and GC/MS quantitation of cocaine, ecgonine methyl ester, benzoylecgonine, and

cocaethylene from meconium, whole blood, and plasma. Journal of Analytical Toxicology 17(6):353-8.

5.12.2. Fleming, S.W., Dasgupta, A., & Garg, U. (2010). Quantitation of cocaine, benzoylecgonine, ecgoninemethyl ester, and cocaethylene in urine and blood using gas chromatography-mass spectrometry (GC-MS). Methods in Molecular Biology Clifton, NJ, 603, 145-156.

5.12.3. United Chemical Technologies Applications Manual (2004).

5.12.4. Standard Practices for Method Validation in Forensic Toxicology. ANSI/ASB Standard 036, 1st edition, 2019, 1-46.

5.12.5. Standard for Mass Spectral Data Acceptance for Definitive Identification. Scientific Working Group for Forensic Toxicology (SWGTOX). 2014, 1-11.

6. Opioids Drug Confirmation by LC/QQQ

6.1. Scope
- 6.1.1. This method shall be used for confirmation analysis of specimens requiring confirmation of opioids. Sample preparation shall be by SLE.

6.2. Precautions/Limitations
- 6.2.1. Minimum Sample Requirement
  - 6.2.1.1. 0.200 mL of blood or serum/plasma specimen.
- 6.2.2. CRMs
  - 6.2.2.1. CRMs used for calibrator and non-zero control stocks shall be from two different vendors, if available.
  - 6.2.2.2. If using CRMs from the same vendor, two different lots shall be used, if available.
  - 6.2.2.3. If only one lot of a CRM is available, two separate vials from the lot shall be used.
- 6.2.3. Mobile phases should be kept in amber bottles to increase stability.

6.3. Related Information
- 6.3.1. Opioids Confirmation Method Validation (November 2019 – May 2020)
- 6.3.2. Stability and Reinjection (2022)
- 6.3.3. Instrument validations
- 6.3.4. Validations of calibrators, controls, and internal standards data

6.4. Instruments/Equipment
- 6.4.1. Tube rack
- 6.4.2. Rocker
- 6.4.3. Vortex
- 6.4.4. 96-well plate positive pressure manifold
- 6.4.5. 96-well plate evaporator
- 6.4.6. Liquid chromatograph
- 6.4.7. Mass spectrometer, triple quadrupole
- 6.4.8. Pipettes

6.5. Reagents/Materials
- 6.5.1. ToxBox 96-well plate (Opioids)
- 6.5.2. ISOLUTE SLE 96-well plate (Biotage: SLE-B96)
- 6.5.3. 2 mL 96-well collection plate and cover
- 6.5.4. 96-well plate with vial inserts and cover
- 6.5.5. Autosampler vials, inserts, and caps
- 6.5.6. Pipette tips
- 6.5.7. ddH_2O
- 6.5.8. Negative blood (human)
- 6.5.9. Liquid chromatograph column
  - 6.5.9.1. Dimensions: 2.1 mm x 100 mm
  - 6.5.9.2. Composition: Phenyl Hexyl, 2.7 µm particles
- 6.5.10. Liquid chromatograph guard column
  - 6.5.10.1. Dimensions: 2.1 mm x 5 mm
  - 6.5.10.2. Composition: Phenyl Hexyl, 2.7 µm particles
- 6.5.11. Nitrogen

6.5.12. Solvents shall be high quality and low residue (e.g., HPLC grade, Omnisolv, Optima, etc.) unless otherwise noted.
- 6.5.12.1. Acetonitrile, LCMS grade
- 6.5.12.2. MTBE, ACS grade or higher
- 6.5.12.3. Formic acid

6.5.13. Sodium carbonate

6.5.14. Sodium bicarbonate

Table 10: Opioids ToxBox CRM Concentrations (all concentrations in ng/mL)

Drug	Cal 1	Cal 2	Cal 3	Cal 4	Cal 5	Cal 6	Cal 7	Cal 8	LQC	HQC
Acetyl Fentanyl	0.5	1	5	10	20	30	40	50	3	35
Fentanyl	0.5	1	5	10	20	30	40	50	3	35
Norfentanyl	0.5	1	5	10	20	30	40	50	3	35
6-MAM	5	25	50	100	200	300	400	500	40	350
Codeine	5	25	50	100	200	300	400	500	40	350
Dextromethorphan	5	25	50	100	200	300	400	500	40	350
Hydrocodone	5	25	50	100	200	300	400	500	40	350
Hydromorphone	5	25	50	100	200	300	400	500	40	350
Morphine	5	25	50	100	200	300	400	500	40	350
Oxycodone	5	25	50	100	200	300	400	500	40	350
Oxymorphone	5	25	50	100	200	300	400	500	40	350
EDDP	10	50	100	200	400	600	800	1000	80	700
Methadone	10	50	100	200	400	600	800	1000	80	700
O-Desmethyltramadol	10	50	100	200	400	600	800	1000	80	700
Tramadol	10	50	100	200	400	600	800	1000	80	700
Acetyl Fentanyl-D5	100	100	100	100	100	100	100	100	100	100
Fentanyl-D5	100	100	100	100	100	100	100	100	100	100
Norfentanyl-D5	100	100	100	100	100	100	100	100	100	100
6-MAM-D3	100	100	100	100	100	100	100	100	100	100
Codeine-D3	100	100	100	100	100	100	100	100	100	100
Dextromethorphan-D3	100	100	100	100	100	100	100	100	100	100
Hydrocodone-D6	100	100	100	100	100	100	100	100	100	100
Hydromorphone-D6	100	100	100	100	100	100	100	100	100	100
Morphine-D6	100	100	100	100	100	100	100	100	100	100
Oxycodone-D3	100	100	100	100	100	100	100	100	100	100
Oxymorphone-D3	100	100	100	100	100	100	100	100	100	100
EDDP-D3	100	100	100	100	100	100	100	100	100	100
Methadone-D3	100	100	100	100	100	100	100	100	100	100
O-Desmethyltramadol-D6	100	100	100	100	100	100	100	100	100	100
Tramadol-13C-D3	100	100	100	100	100	100	100	100	100	100

6.6. Hazards/Safety
- 6.6.1. See Safety Manual.
- 6.6.2. See SDS for each chemical in this method.

6.6.3. Add acids to approximately half the volume of the less acidic liquid, then dilute to final volume.

6.7. Solutions

6.7.1. All solutions shall conform to Solution Preparation, Validation, Verification (Doc ID: 3695).

6.7.2. Carbonate Buffer (300 mM)

6.7.3. Mobile Phases

6.7.3.1. Aqueous (A)

6.7.3.2. Organic (B)

6.7.4. OPI Reconstitution Solution

6.8. Procedures/Instructions

6.8.1. An evidentiary confirmation batch shall consist of concurrently prepared calibrators, negative blood controls, non-zero controls, and samples. Each set of one to twelve samples shall be bracketed by non-zero controls. The batch shall contain alternating low and high non-zero controls. The batch shall contain at least three prepared negative controls. Negative controls may be reinjected multiple times throughout the batch.

6.8.1.1. Reinjected negative controls shall be denoted with an “RI” followed by the number of reinjections.

6.8.2. Mix specimens on a rocker or by inverting several times.

6.8.3. Pipette 200 µL of negative blood into each calibrator and control well on the ToxBox plate.

6.8.4. Pipette 200 µL of specimen into the corresponding well on the ToxBox plate.

6.8.5. Add 200 µL of carbonate buffer to each well of the ToxBox plate.

6.8.6. Vortex ToxBox plate.

6.8.7. Transfer 400 µL from each well of the ToxBox plate to the corresponding well of the SLE plate.

6.8.8. Allow samples to load onto the SLE plate for ~5 – 10 minutes. Positive pressure (<25 psi) may be applied, if necessary.

6.8.9. Add 1 mL of MTBE to each SLE plate well and allow to flow through to a collection plate; apply positive pressure (<25 psi) as necessary and allow to elute for ~ 5 – 10 minutes.

6.8.10. Repeat the previous elution with an additional 1 mL aliquot of MTBE.

6.8.11. Remove collection plate from the positive pressure manifold and place on the evaporator.

6.8.12. Evaporate at room temperature using nitrogen.

6.8.13. Add 150 µL of reconstitution solution to each well of the collection plate.

6.8.14. Cap collection plate and vortex.

6.8.15. Transfer each sample to the corresponding well within the 96-well plate with vial inserts or into labeled autosampler vials.

6.8.16. Cap the 96-well vial plate (or autosampler vial) and move it to the LC/QQQ for analysis.

6.8.17. Analyze the samples by LC/QQQ.

6.8.17.1. Sequence names shall be in the following format: YYYY_MM_DD_OPI_Initials.

6.8.17.1.1. The date in the sequence shall be the date of preparation of the samples.

6.8.17.1.2. Additional information such as reinjection, validation, etc., or equivalent abbreviations should be included with the assay abbreviation.

6.8.17.1.3. If the sequence is run with the wrong sequence name, it shall be noted on the Technical Review Worksheet and in the case synopsis of each case in the batch and not corrected on the chromatograms.

6.8.17.2. If multiple batches are included in one sequence, add a note on the MassHunter Worklist report that includes the batch name of each batch included in the sequence in the format listed in 6.8.17.1. The note should list which lines are attributed to each batch.

6.8.17.3. Prepared samples may be analyzed up to 15 days after date of preparation when stored in a well plate with sealing cap in the instrument autosampler or at equivalent temperature.

6.8.17.4. If the instrument sequence is paused by the acquisition software between two samples, the sequence may be restarted at the sample not yet injected.

6.8.17.4.1. Sample stability criteria shall be met.

6.8.17.5. If the instrument sequence is interrupted during analysis of a sample or the sequence is aborted or stopped, the sequence should be restarted at the last bracketing non-zero control or may be resumed by beginning at the next sample not yet injected.

6.8.17.5.1. Sample stability criteria shall be met.

6.8.17.5.2. Reinjection of a sample of unknown concentration may be performed up to two times.

6.8.17.5.3. Reinjection of a sample of known concentration may be performed multiple times.

6.8.17.5.3.1. If a reinjection is needed more than twice, the evidentiary samples that have already been reinjected may be skipped in a bracket.

6.8.17.5.3.1.1. Evidentiary samples that are skipped and do not have valid data shall be reanalyzed starting at 6.8.1.

6.8.17.5.4. A reinjection shall be performed by restarting the sequence from the last bracketing non-zero control or reinjecting the entire sequence.

6.8.17.5.5. If an entire sequence is reinjected or a reinjection includes the calibrators used to generate the calibration curve, a check tune (Doc ID: 2842) shall be performed within 24 hours of initiation of the reinjection sequence. Resuming a sequence or reinjecting a sequence starting with the last bracketing control does not require a check tune.

6.8.18. LC/QQQ Acquisition Parameters

6.8.18.1. Liquid chromatograph sampler

Needle wash	Standard wash

Injection Volume 1 µL

6.8.18.2. Liquid chromatograph binary pump

	Time	Gradient A %	Gradient B %
1	0.0	98	2
2	8.0	50	50
3	8.5	5	95
4	12.0	5	95

Flow 0.6 mL/min

Stoptime 12.00 min

Posttime 3.00 min

6.8.18.3. Liquid chromatograph column compartment

Temperature 55 ºC

6.8.18.4. Mass spectrometer

Ion Source AJS ESI

Scan Type Dynamic MRM

6.8.18.5. dMRM Parameters

MS1 Resolution Unit/Enh (6490)

MS2 Resolution Unit/Enh (6490)

Cell Acc. 5 V

Polarity Positive

Table 11: Opioids MS Parameters

Compound Name	Internal Standard	Precursor Ion	Product Ion	Fragmentor (V)	CE* (V)	RT** (min)
6-Monoacetylmorphine	No	328	**211**	125	25	3.85
			165		46	
Acetylfentanyl	No	323	**188**	125	23	5.94
			105		44	
Codeine	No	300	**215**	115	25	3.49
			183		21	
Dextromethorphan	No	272	215	120	25	6.36
			171		46	
EDDP	No	278	249	120	25	7.1
			234		34	
Fentanyl	No	337	**188**	125	23	6.56
			105		44	
Hydrocodone	No	300	241	120	27	4.02
			199		34	
Hydromorphone	No	286	**185**	125	36	2.39
			157		46	
Methadone	No	310	**265**	125	13	7.64
			105		29	

Compound Name	Internal Standard	Precursor Ion	Product Ion	Fragmentor (V)	CE* (V)	RT** (min)
Morphine	No	286	229	125	23	1.63
			211		27	
Norfentanyl	No	233	150	85	19	4.41
			84		21	
O-Desmethyltramadol	No	250	232	115	9	3.71
			58		19	
Oxycodone	No	316	256	125	27	3.84
			241		34	
Oxymorphone	No	302	284	120	19	1.91
			227		29	
Tramadol	No	264	246	105	9	4.89
			58		17	
6-Monoacetylmorphine-D3	Yes	331	**211**	180	29	3.84
Acetylfentanyl-D5	Yes	328	**105**	125	46	5.92
Codeine-D3	Yes	303	**215**	125	29	3.48
Dextromethorphan-D3	Yes	275	**147**	125	36	6.35
EDDP-D3	Yes	281	**234**	120	34	7.09
Fentanyl-D5	Yes	342	**105**	125	46	6.53
Hydrocodone-D6	Yes	306	**202**	125	36	3.99
Hydromorphone-D6	Yes	292	**185**	125	38	2.36
Methadone-D3	Yes	313	**268**	120	13	7.63
Morphine-D6	Yes	292	**202**	125	27	1.6
Norfentanyl-D5	Yes	238	**84**	120	19	4.38
O-Desmethyltramadol-D6	Yes	256	**64**	115	21	3.7
Oxycodone-D3	Yes	319	**301**	120	21	3.82
Oxymorphone-D3	Yes	305	**287**	120	21	1.9
Tramadol-13C-D3	Yes	268	**58**	105	19	4.86

* Collision Energy Ions in **bold** are used to quantitate.

**RTs are based on the average analyte retention times of calibrators and can be updated in the acquisition method and/or quantitation method, as necessary.

6.8.18.6. Quantitation Parameters

RRT Max % Deviation	5 percent
Curve fit	Quadratic
Data point weight	1/x
Units of concentration	ng/mL
Internal standard concentration	100

6.9. Records

6.9.1. Pipette calibration certificate, however named

6.9.2. Batch Preparation Packet

6.9.2.1. ISDT Confirmation Worklist
6.9.2.2. Retest Worksheet, as appropriate
6.9.2.3. Opioids Drug Confirmation Preparation Worksheet
6.9.2.4. Aliquot Chain of Custody
6.9.2.5. Opioids Drug Confirmation Plate Layout Worksheet
6.9.3. MassHunter Worklist Report
6.9.4. MassHunter Ion Ratio and RRT Verification, however named
6.9.5. QA/QC Packet, however named
6.9.5.1. Batch summary
6.9.5.2. Analyte calibration curves
6.9.5.3. Calibrator and control chromatograms
6.9.6. Sample chromatograms
6.9.7. QQQ Check Tune Report
6.9.8. Opioids Drug Confirmation Technical Review Checklist
6.9.9. Data comparison output, however named.
6.9.10. Measurement Uncertainty Estimation and supporting data
6.9.11. Specimen Verification Worksheet, if applicable

6.10. Interpretation of Results
6.10.1. Interpretation of results for each analyte shall occur independent of the other analytes in the method.
6.10.2. Chromatographic analyte and internal standard peaks shall have baseline resolution and/or shall be mass resolved in the mass spectrometer.
6.10.2.1. A shoulder peak shall be < 10% of analyte peak height and area in order to report a quantitative result.
6.10.3. Peak filters should be set between 10% and 50% of the Cal 1 response for each analyte.
6.10.4. Internal standard recovery should be between 50% to 200% of the average of the calibrators in the batch.
6.10.4.1. Samples with recovery less than 25% or greater than 200% shall not be accepted.
6.10.4.2. Samples with recovery between 25% and 50% may be accepted at analyst discretion.
6.10.5. Calibration and Controls Criteria
6.10.5.1. Results of samples analyzed prior to analysis of the negative control preceding the calibrators shall not be used to determine acceptability of batch data.
6.10.5.2. Quantitation of calibrators and non-zero controls for quantitatively reported analytes (see Table 12) shall be within ± 20% of the target concentration. Quantitation of calibrators and non-zero controls for qualitatively reported analytes shall be within ± 30% of the target concentration.
6.10.5.3. Generating a calibration curve
6.10.5.3.1. Calibration curve shall include a minimum of five non-zero concentrations.
6.10.5.3.2. Correlation coefficient (r^2) for the calibration curve shall be ≥ 0.990.

6.10.5.3.3. An ion ratio with a relative abundance ≥ 20% shall be within ± 20% of the mean ion ratio based on all calibrators used to generate the curve and controls.

6.10.5.3.4. An ion ratio with a relative abundance < 20% shall be within ± 30% of the mean ion ratio based on all calibrators used to generate the curve and controls.

6.10.5.3.5. A calibration point may be excluded if any of the following occur:

6.10.5.3.5.1. An ion ratio does not meet the acceptability criteria listed in 6.10.5.3.3 or 6.10.5.3.4.

6.10.5.3.5.2. The correlation coefficient (r^2) for the calibration curve is < 0.990.

6.10.5.3.5.3. A quantitated value is not within ± 20% of the target concentration.

6.10.5.3.5.4. A peak has poor chromatography.

6.10.5.3.6. If the lowest calibrator used to generate the calibration curve is not equal to the defined LLOQ, all samples with an analyte concentration greater than half the LLOQ but less than the target concentration of the lowest calibrator used to generate the calibration curve shall be reanalyzed, if possible, starting at 6.8.1.

6.10.5.3.6.1. RR or response may be used to determine which specimens require reanalysis, if any.

6.10.5.3.7. If the highest calibrator used to generate the calibration curve is not equal to the defined ULOQ, all samples with an analyte concentration above the target concentration of the highest calibrator used to generate the calibration curve shall be reanalyzed, if possible, starting at 6.8.1. If unable to retest, the results for the analysis may be reported as greater than the highest calibrator used in the batch.

6.10.5.3.7.1. RR or response may be used to determine which specimens require reanalysis, if any.

6.10.5.4. Each set of one to twelve samples shall be bracketed by a negative control for partial batch acceptance. The negative control shall have an analyte concentration or response < 50% of the LLOQ and/or unacceptable ion ratios as specified in 6.10.5.3.3 or 6.10.5.3.4.

6.10.5.4.1. If the above acceptance criterion is not met, the analytical data for the samples bracketed by the failed negative control with a concentration ≥ 50% of the LLOQ shall not be used and shall be reanalyzed, if possible, starting at 6.8.1. A result < 50% of the LLOQ for an evidentiary sample shall be accepted as none detected.

6.10.5.5. At least one negative control shall have the corresponding internal standard present for the associated analyte.

6.10.5.5.1. If acceptance criterion is not met, all samples in the batch shall be reanalyzed, if possible, starting at 6.8.1.

6.10.5.6. At least one low and one high non-zero control shall be included in each batch.

6.10.5.7. A non-zero control for an analyte fails if any of the following occur:

6.10.5.7.1. An ion ratio does not meet the acceptability criteria listed in 6.10.5.3.3 or 6.10.5.3.4.

6.10.5.7.2. The quantitated value is not within ± 20% of the target concentration.

6.10.5.7.3. A peak has poor chromatography.

6.10.5.7.4. The relative retention time is greater than ± 5% of the mean relative retention time based on all calibrators and controls used to generate the curve.

6.10.5.8. Each set of one to twelve samples shall be bracketed by one low and one high non-zero control.

6.10.5.8.1. If a control result does not meet the above criteria, the analytical data for the samples bracketed by the failed control shall not be used, and samples in the bracket prior to and following the failed control that are positive for the analyte that failed shall be reanalyzed, if possible, starting at 6.8.1. A result below the LLOQ for an evidentiary sample shall be accepted as none detected if the analytical results for negative controls in the batch pass acceptability criteria in 6.10.5.4 and 6.10.5.5.

6.10.6. If the analyte in a sample has a result > 2x ULOQ, evaluate the analyte in the subsequent sample(s) as follows:

6.10.6.1. If a > 2x ULOQ sample is immediately followed by an evidentiary sample in which the analyte is not detected, data should be accepted.

6.10.6.2. If a > 2x ULOQ sample is immediately followed by an evidentiary sample in which the analyte is detected, the sample following the > 2x ULOQ sample shall be retested.

6.10.6.2.1. Additional negative controls or solvent blanks may be added to the sequence immediately following the > 2x ULOQ sample to eliminate carryover in subsequent samples.

6.10.6.3. If a > 2x ULOQ sample is immediately followed by a control and the control passes acceptance criteria, data should be accepted.

6.10.6.4. If a > 2x ULOQ sample is immediately followed by a control and it does not pass control acceptance criteria, all samples in the preceding and following brackets shall be reinjected or retested.

6.10.6.4.1. Additional negative controls or solvent blanks may be added to the sequence immediately following the > 2x ULOQ sample to eliminate carryover in subsequent samples.

6.10.6.5. If the MassHunter software does not provide a calculated result, the RR for the sample may be compared to the RR of Cal 8.

6.10.7. Analyte Identification (Qualitative Criteria)

6.10.7.1. Relative retention time shall be within ± 5% of the mean relative retention time based on all calibrators and controls used to generate the curve.

6.10.7.2. Each analyte shall have two ion transitions monitored. The ion transition from the precursor to the product ion listed in **bold** type in Table 11: Opioids MS Parameters is used for quantitation.

6.10.7.3. Each internal standard shall be present and have one ion transition monitored.

6.10.7.4. Each ion ratio shall meet the acceptability criteria listed in 6.10.5.3.3 or 6.10.5.3.4.

6.10.7.5. Data analysis software manual integration tools (Merge Right Peak, Merge Left Peak, Split Peak and Pick Left, Split Peak and Pick Right, Snap Baseline, Drop Baseline, Apply ISTD RTs to Target, Apply Target RTs to Qualifier) may be used to adjust the integration algorithm to select the correct peak or adjust the baseline. Use of software manual integration tools shall be documented on the chromatogram.

6.10.8. Analyte Stability

6.10.8.1. Prepared samples in a capped well plate are stable for 15 days when stored in the instrument auto sampler (or at equivalent temperature) or at room temperature.

6.10.8.2. Prepared samples in autosampler vials are stable for 8 days when stored in the instrument auto sampler (or at equivalent temperature) or at room temperature.

6.10.9. Retesting Samples

6.10.9.1. When a sample requires retesting, the sample shall be retested at least once, if possible. A sample may be retested up to two times without supervisory approval.

6.10.9.1.1. If a quantitative value cannot be reported from any analysis, the first acceptable qualitative data according to analyte identification in 6.10.7 shall be used. (ref. 6.11.4).

6.10.9.1.2. If data is not generated, that analysis does not count as an analysis or retest under this section.

6.10.10. Unacceptable Data

6.10.10.1. Data found to be unacceptable shall be marked with a signed note identifying the specific analytical data that should not be used and the reason for not using the data (e.g., “Do not use this quantitative alprazolam data due to a bracketing control being outside acceptability criteria. AB XX/XX/XX” or “Do not use any data from this batch due to sequence interruption. Samples will be retested. AB XX/XX/XX”).

6.10.11. No Data Generated for a Sample

6.10.11.1. Cases with no generated data should have a case synopsis note to explain the lack of data associated with the chain of custody preparation date (e.g., “XX/XX/XX No data was collected from [batch name] due to the instrument stopping. AB”).

6.11. Report Writing

6.11.1. The LLOD for opioids analysis is equal to the LLOQ for each analyte. The LLOQ and ULOQ are listed in Table 12: Opioids LLOQ and ULOQ

Table 12: Opioids LLOQ and ULOQ

Group	Analyte	LLOQ (ng/mL)	ULOQ (ng/mL)
1	6-Monoacetylmorphine	5.0*	-
1	Codeine	5.0*	-
1	Dextromethorphan	5.0*	-
1	Hydrocodone	5.0*	-
1	Hydromorphone	5.0*	-
1	Morphine	5.0*	-
1	Oxycodone	5.0*	-
1	Oxymorphone	5.0*	-
2	Acetylfentanyl	0.50	50
2	Fentanyl	0.50	50
2	Norfentanyl	0.50	50
3	Methadone	10	1000
3	O-Desmethyltramadol	10	1000
3	Tramadol	10	1000
3	EDDP	10*	-

*Analytes shall only be reported qualitatively with the LLOQ as the cutoff.

6.11.2. Confirmatory data for each specimen shall be technically reviewed prior to entering the result into LIMS.

6.11.2.1. The preparation date of the analysis being reported shall be entered as the analysis date.

6.11.3. Quantitative Reporting of Group 2 and Group 3 Analytes (Except EDDP)

6.11.3.1. A result less than the LLOQ shall not be reported.

6.11.3.1.1. If a batch LLOQ is used, a quantitative result less than the target concentration for the lowest calibrator used in the calibration curve shall not be reported.

6.11.3.2. A quantitated result that meets acceptability criteria shall be reported for results between the target concentration of the lowest and highest calibrators that meet acceptability criteria.

6.11.3.2.1. Results shall be truncated to the appropriate level of significance and reported as the quantitative value ± the expanded measurement uncertainty.

6.11.3.2.1.1. A result shall be reported to two decimal places for quantitative values less than 1.

6.11.3.2.1.2. A result shall be reported to one decimal place for quantitative values greater than or equal to 1 and less than 10.

6.11.3.2.1.3. A result shall be reported as a whole number for quantitative values greater than or equal to 10.

6.11.3.3. A result that is above the ULOQ and has an ion ratio within ± 30% of the mean ion ratio based on all calibrators used to generate the curve and controls shall be reported as > the ULOQ in ng/mL.

6.11.3.3.1. If a batch ULOQ is used, a quantitative result greater than the target concentration for the highest calibrator used in the calibration curve shall not be reported.

6.11.3.3.1.1. A result greater than the target concentration of the highest calibrator used in the calibration curve may be reported if retesting of a specimen is not feasible.

6.11.3.4. A quantitative result shall only be reported if analysis occurred within the established sample stability window (ref. 6.10.8).

6.11.3.5. If a specimen is analyzed more than once, the first quantitative result that meets quantitative acceptability criteria for a specific analyte shall be reported.

6.11.4. Qualitative Reporting of Group 2 and Group 3 Analytes (Except EDDP)

6.11.4.1. A result should be reported as “Positive” when the analyte identification criteria (ref. 6.10.7) have been met, the quantitative result is > LLOQ, and the quantitative criteria have not been met.

6.11.4.1.1. If a specimen is analyzed more than once, the totality of the qualitative data shall be evaluated by the analyst for acceptability criteria for analyte identification of a specific analyte.

6.11.4.1.1.1. The preparation date of last analysis shall be used as the analysis date.

6.11.4.2. A result may be reported as “Positive” with supervisory approval if any of the following occur:

6.11.4.2.1. Interference(s); or

6.11.4.2.2. Quantitative result > LLOQ with an ion ratio greater than ± 20%, but less than ± 30%, of the mean ion ratio based on all calibrators used to generate the curve and controls.

6.11.5. Qualitative Reporting of Group 1 Analytes and EDDP

6.11.5.1. A result shall be reported as “Positive” when the analyte identification criteria (ref. 6.10.7) have been met, the quantitative result is > LLOQ, and the calibrator and control criteria (ref. 6.10.5.) have been met.

6.11.5.1.1. If quantitative criteria have not been met, an analyte may be reported “Positive” with supervisory approval.

6.11.5.1.2. If a specimen is analyzed more than once, the totality of the qualitative data shall be evaluated by the analyst for acceptability criteria for analyte identification of a specific analyte.

6.11.5.1.2.1. The preparation date of last analysis shall be used as the analysis date.

6.11.5.2. A result may be reported as “Positive” with supervisory approval if any of the following occur:

6.11.5.2.1. Interference(s); or

6.11.5.2.2. Quantitative result > LLOQ with an ion ratio greater than ± 20%, but less than ± 30%, of the mean ion ratio based on all calibrators used to generate the curve and controls.

6.12. References

6.12.1. Standard Practices for Method Validation in Forensic Toxicology. ANSI/ASB Standard 036, 1st edition, 2019, 1-46.

6.12.2. Standard for Mass Spectral Data Acceptance for Definitive Identification. Scientific Working Group for Forensic Toxicology (SWGTOX). 2014, 1-11.

7. Stimulants Confirmation by LC/QQQ

7.1. Scope

7.1.1. This method shall be used for confirmation analysis of specimens requiring confirmation of stimulant drugs. Sample preparation shall be by SPE.

7.2. Precautions/Limitations

7.2.1. Minimum Sample Requirement

7.2.1.1. 1 mL of blood or serum/plasma specimen

7.2.2. CRMs

7.2.2.1. CRMs used for calibrator and non-zero control stocks shall be from two different vendors, if available.

7.2.2.2. If using CRMs from the same vendor, two different lots shall be used, if available.

7.2.2.3. If only one lot of a CRM is available, two separate vials from the lot shall be used.

7.2.3. Mobile phases should be kept in amber bottles to increase stability.

7.3. Related Information

7.3.1. Stimulant Confirmatory Analysis Method Validation (September 2016-January 2017)

7.3.2. Stimulants Linearity Supplemental (October-December 2017)

7.3.3. Stimulants Stock and Prepared Sample Stability (April 2017)

7.3.4. Stimulants Reinjection Stability (April 2017, April 2018, January 2019)

7.3.5. Calibration Model Update-Quadratic (January-February 2019)

7.3.6. Reinjection Stability Supplemental (January-February 2019)

7.3.7. Injection Volume Supplemental (August-September 2019)

7.3.8. Stock Solution Stability (January 2020)

7.3.9. Retention time versus relative retention time Evaluation (January 2020)

7.3.10. Instrument validations

7.3.11. Validation of calibrators, controls, and internal standards data

7.4. Instruments/Equipment

7.4.1. Tube rack

7.4.2. Rocker

7.4.3. Vortex, single

7.4.4. Sonicating water bath

7.4.5. Centrifuge

7.4.6. Positive pressure manifold

7.4.7. SPE column rack

7.4.8. SPE collection rack

7.4.9. Waste collection rack

7.4.10. Evaporator

7.4.11. Vial rack

7.4.12. Liquid chromatograph

7.4.13. Mass spectrometer, triple quadrupole

7.4.14. Pipettes

7.5. Reagents/Materials

7.5.1. Glass tubes (e.g., 13x100 mm)

7.5.2. Trace B SPE columns, 3 mL columns, 35 mg (Tecan #TB-335C, or equivalent)
7.5.3. Tube caps (e.g., 13mm flange)
7.5.4. Pipette tips
7.5.5. Autosampler vials, inserts, and caps
7.5.6. ddH_2O
7.5.7. Negative blood (human)
7.5.8. Liquid chromatograph analytical column
7.5.8.1. Dimensions: 2.1 mm x 100 mm
7.5.8.2. Composition: Phenyl Hexyl, 2.7 µm particles
7.5.9. Liquid chromatograph guard column
7.5.9.1. Dimensions: 2.1 mm x 5 mm
7.5.9.2. Composition: Phenyl Hexyl, 2.7 µm particles
7.5.10. CRMs
7.5.10.1. Amphetamine
7.5.10.2. MDA
7.5.10.3. MDMA
7.5.10.4. Methamphetamine
7.5.10.5. Phencyclidine
7.5.10.6. Amphetamine-D11
7.5.10.7. MDA-D5
7.5.10.8. MDMA-D5
7.5.10.9. Methamphetamine-D14
7.5.10.10. Phencyclidine-D5
7.5.11. Nitrogen
7.5.12. Solvents shall be high quality and low residue (e.g., HPLC grade, Omnisolv, Optima, etc.) unless otherwise noted.
7.5.12.1. Ethyl acetate
7.5.12.2. Isopropanol
7.5.12.3. Glacial acetic acid, ACS grade or higher
7.5.12.4. Ammonium hydroxide, ACS grade or higher
7.5.12.5. Methanol, ACS grade or higher
7.5.12.6. Hydrochloric acid, ACS grade or higher
7.5.12.7. Formic acid
7.5.12.8. Acetonitrile
7.5.13. Sodium Phosphate Monobasic
7.5.14. Sodium Phosphate Dibasic

7.6. Hazards/Safety
7.6.1. See Safety Manual.
7.6.2. See SDS for each chemical in this method.
7.6.3. Add acids to approximately half the volume of the less acidic liquid, then dilute to final volume.

7.7. Reference Materials/Controls/Calibrators/Solutions
7.7.1. All solutions shall conform to Solution Preparation, Validation, Verification (Doc ID: 3695).
7.7.2. STM Low Calibrator
7.7.3. STM High Calibrator
7.7.4. STM Low Control

7.7.5. STM High Control
7.7.6. STM Internal Standard
7.7.7. STM Elution Solution
7.7.8. Phosphate Buffer (100 mM)
7.7.9. Acetic Acid (100 mM)
7.7.10. Hydrochloric Acid (25 mM)
7.7.11. Mobile Phases
7.7.11.1. Aqueous (A)
7.7.11.2. Organic (B)

7.8. Procedures/Instructions
7.8.1. An evidentiary confirmation batch shall consist of concurrently prepared calibrators, negative blood controls, non-zero controls, and samples. Each set of one to twelve samples shall be bracketed by non-zero controls. The batch shall contain alternating low and high non-zero controls. The batch shall contain at least three prepared negative controls. Negative controls may be reinjected multiple times throughout the batch.
7.8.1.1. Reinjected negative controls shall be denoted with an "RI" followed by the number of reinjections.
7.8.2. Mix specimens on a rocker or by inverting several times.
7.8.3. Add 50 µL of internal standard (resulting in a concentration of 100 ng/mL) to labeled glass tubes.
7.8.4. Prepare calibrator and control samples in correspondingly labeled tubes as indicated in Table 13.

Table 13: Stimulants Calibrator and Control Sample Preparation

Level	Stock Solution	Volume (µL)
Cal 1	Low Cal	10
Cal 2	Low Cal	20
Cal 3	Low Cal	60
Cal 4	High Cal	15
Cal 5	High Cal	25
Cal 6	High Cal	45
Cal 7	High Cal	70
Cal 8	High Cal	100
Low Control	Low Ctrl	80
High Control	High Ctrl	80

7.8.5. Pipette 1 mL of negative blood into each calibrator and control tube.
7.8.6. Pipette 1 mL of specimen into the correspondingly labeled tube.
7.8.7. Add 2 mL of phosphate buffer to each tube. Cap and vortex each tube.
7.8.8. Sonicate for ~10 minutes.
7.8.9. Centrifuge for ~10 minutes using 3000 rpm at 4-8 ºC.
7.8.10. In the order listed, condition the SPE columns with each of the following solutions, allowing each solution to flow completely through each column before proceeding to the next solution:
7.8.10.1. 1 mL of methanol
7.8.10.2. 1 mL of ddH_2O

7.8.10.3. 1 mL of phosphate buffer

7.8.11. While the sorbent bed is still wet, decant each sample into the SPE column and allow the sample to flow completely through each column at ~1 mL per minute.

7.8.12. In the order listed, wash columns with each of the following solutions, allowing each wash solution to flow completely through each column at ~1 mL per minute before proceeding to the next solution:

7.8.12.1. 2 mL ddH_2O

7.8.12.2. 2 mL acetic acid

7.8.12.3. 1 mL methanol

7.8.12.4. 1 mL ethyl acetate

7.8.13. Using a maximum flow of ~60 psi, dry the columns for ~20 minutes.

7.8.14. Place empty labeled tubes into the positive pressure manifold, ensuring the placement of the tubes corresponds with the arrangement of the sample columns.

7.8.15. Add 100 µL of hydrochloric acid to each tube prior to 7.8.18.

7.8.16. Add 2 mL of elution solution to each column and allow to flow completely through into tube at ~1 mL per minute.

7.8.17. Remove tubes from the positive pressure manifold and place on the evaporator.

7.8.18. Evaporate at room temperature using nitrogen.

7.8.19. Add 100 µL of ddH_2O to each tube and vortex.

7.8.20. Transfer each sample to a correspondingly labeled autosampler vial and cap vial.

7.8.21. Analyze the samples by LC/QQQ.

7.8.21.1. Sequence names shall be in the following format: YYYY_MM_DD_STM_Initials.

7.8.21.1.1. The date of the sequence shall be the date of preparation of the samples.

7.8.21.1.2. Additional information such as reinjection, validation, etc., or equivalent abbreviations should be included with the assay abbreviation.

7.8.21.1.3. If the sequence is run with the wrong sequence name, it shall be noted on the Technical Review Worksheet and in the case synopsis of each case in the batch and not corrected on the chromatograms.

7.8.21.1.4. If multiple batches are included in one sequence, add a note on the MassHunter Worklist report that includes the batch name of each batch combined in the sequence in the format listed in 7.8.21.1. The note should list which lines are attributed to each batch.

7.8.21.2. Prepared samples may be analyzed up to 6 days after the date of preparation when stored at room temperature or up to 10 days in the instrument autosampler or at equivalent temperature (ref. 7.10.8).

7.8.21.3. If the instrument sequence is paused by the acquisition software between two samples, the sequence may be restarted at the sample not yet injected.

7.8.21.3.1. Sample stability criteria shall be met.

7.8.21.4. If the instrument sequence is interrupted during analysis of a sample, or the sequence is aborted or stopped, the sequence should be restarted at the last bracketing non-zero control or may be resumed by beginning at the next sample not yet injected.

7.8.21.4.1. Sample stability criteria shall be met.

7.8.21.4.2. Reinjection of a sample of unknown concentration may be performed six times.

7.8.21.4.3. Reinjection of a sample of known concentration may be performed multiple times.

7.8.21.4.3.1. If a reinjection is needed more than six times, the evidentiary samples that have already been reinjected may be skipped in a bracket.

7.8.21.4.3.1.1. Evidentiary samples that are skipped and do not have valid data shall be reanalyzed starting at 7.8.1.

7.8.21.4.4. A reinjection shall be performed by restarting the sequence from the last bracketing non-zero control or reinjecting the entire sequence.

7.8.21.4.5. If an entire sequence is reinjected or a reinjection includes the calibrators used to generate the calibration curve, a check tune (Doc ID: 2842) shall be performed within 24 hours of initiation of the reinjection sequence. Resuming a sequence or reinjecting a sequence starting with the last bracketing control does not require a check tune.

7.8.22. LC/QQQ Acquisition Parameters

7.8.22.1. Liquid chromatograph sampler

Needle Wash	Standard wash
Injection Volume	0.10 to 1 µL

7.8.22.2. Liquid chromatograph binary pump

	Time	**Gradient A %**	**Gradient B %**
1	0.0	98	2
2	3.0	98	2
3	8.0	80	20
4	9.0	0	100
5	9.5	0	100
6	9.6	95	5

Flow	0.600 mL/min
Stoptime	10.75 min
Posttime	2.5 min

7.8.22.3. Liquid chromatograph column compartment

Temperature	55 ºC

7.8.22.4. Mass spectrometer

Ion Source	AJS ESI
Scan Type	Dynamic MRM

7.8.22.5. dMRM Parameters

MS1 Resolution	Unit/Enh (6490)
MS2 Resolution	Unit/Enh (6490)
Cell Acc.	4 V
Polarity	Positive

Table 14: Stimulants Analyte MS Parameters

Compound Name	Internal Standard	Precursor Ion	Product Ion	Fragmentor (V)	CE* (V)	RT** (min)
Amphetamine	No	136	**91**	75	17	3.26
			119		5	
MDA	No	180	**163**	80	7	4.48
			105		25	
MDMA	No	194	**163**	80	11	6.05
			105		25	
Methamphetamine	No	150	**119**	80	9	4.67
			65		35	
Phencyclidine	No	244	**91**	70	51	10.12
			159		13	
Amphetamine-D11	Yes	147	98	80	25	3.08
MDA-D5	Yes	185	110	70	27	4.39
MDMA-D5	Yes	199	107	95	29	5.99
Methamphetamine-D14	Yes	164	98	80	25	4.35
Phencyclidine-D5	Yes	249	96	75	45	10.11

* Collision Energy Ions in **bold** are used to quantitate.
**RTs are based on the average analyte retention times of calibrators and can be updated in the acquisition method and/or quantitation method, as necessary.

7.8.22.6. Quantitation Parameters

RRT Max % Deviation	5 percent
Curve fit	Quadratic
Data point weight	1/x
Units of concentration	ng/mL
Internal standard concentration	100

7.9. Records

- 7.9.1. Pipette calibration certificate, however named
- 7.9.2. Stimulants Confirmation Calibrator Solution Preparation Worksheet
- 7.9.3. Stimulants Confirmation Internal Standard Solution Preparation Worksheet
- 7.9.4. Stimulants Confirmation Control Solution Preparation Worksheet
- 7.9.5. Batch Preparation Packet, however named
  - 7.9.5.1. ISDT Confirmation Worklist
  - 7.9.5.2. Retest Worksheet, as appropriate
  - 7.9.5.3. Stimulants Confirmation Preparation Worksheet
  - 7.9.5.4. Aliquot Chain of Custody
- 7.9.6. MassHunter Worklist Report
- 7.9.7. MassHunter Ion Ratio and RRT Verification, however named
- 7.9.8. QA/QC Packet, however named
  - 7.9.8.1. Batch summary
  - 7.9.8.2. Analyte calibration curves
  - 7.9.8.3. Calibrator and control chromatograms
- 7.9.9. Sample chromatograms
- 7.9.10. QQQ Check Tune Report
- 7.9.11. Stimulants Confirmation Technical Review Checklist
- 7.9.12. Data comparison output, however named

7.9.13. Measurement Uncertainty Estimation and supporting data
7.9.14. Specimen Verification Worksheet, if applicable

7.10. Interpretation of Results
7.10.1. Interpretation of results for each analyte shall occur independent of the other analytes in the method.
7.10.2. Chromatographic analyte and internal standard peaks shall have baseline resolution and/or analytes shall be mass resolved in the mass spectrometer.
7.10.2.1. A shoulder peak shall be < 10% of analyte peak height and area in order to report a quantitative result.
7.10.3. Peak filters should be set between 10% and 50% of the Cal 1 response for each analyte.
7.10.4. Internal standard recovery should be between 50% to 200% of the average of the calibrators in the batch.
7.10.4.1. Samples with recovery less than 25% or greater than 200% shall not be accepted.
7.10.4.2. Samples with recovery between 25% and 50% may be accepted at analyst discretion.
7.10.5. Calibration and Controls Criteria
7.10.5.1. Results of samples analyzed prior to analysis of the negative control preceding the calibrators shall not be used to determine acceptability of batch data.
7.10.5.2. Quantitation of calibrators and non-zero controls shall be within ± 20% of the target concentration.
7.10.5.3. Generating a calibration curve
7.10.5.3.1. Calibration curve shall include a minimum of five non-zero concentrations.
7.10.5.3.2. Correlation coefficient (r^2) for the calibration curve shall be ≥ 0.990.
7.10.5.3.3. An ion ratio with a relative abundance ≥ 20% shall be within ± 20% of the mean ion ratio based on all calibrators and controls used to generate the curve.
7.10.5.3.4. An ion ratio with a relative abundance < 20% shall be within ± 30% of the mean ion ratio based on all calibrators and controls used to generate the curve.
7.10.5.3.5. A calibration point may be excluded if any of the following occur:
7.10.5.3.5.1. An ion ratio does not meet the acceptability criteria listed in 7.10.5.3.3 or 7.10.5.3.4.
7.10.5.3.5.2. The correlation coefficient (r^2) for the calibration curve is < 0.990.
7.10.5.3.5.3. A quantitated value is not within ± 20% of the target concentration.
7.10.5.3.5.4. A peak has poor chromatography.
7.10.5.3.6. If the lowest calibrator used to generate the calibration curve is not equal to the defined LLOQ, all samples with an analyte concentration greater than half the LLOQ but less than the target concentration of the

lowest calibrator used to generate the calibration curve shall be reanalyzed, if possible, starting at 7.8.1.

7.10.5.3.6.1. RR or response may be used to determine which specimens require reanalysis, if any.

7.10.5.3.7. If the highest calibrator used to generate the calibration curve is not equal to the defined ULOQ, all samples with an analyte concentration above the target concentration of the highest calibrator used to generate the calibration curve shall be reanalyzed, if possible, starting at 7.8.1. If unable to retest, the results for the analysis may be reported as greater than the highest calibrator used in the batch.

7.10.5.3.7.1. RR or response may be used to determine which specimens require reanalysis, if any.

7.10.5.4. Each set of one to twelve samples shall be bracketed by a negative control for partial batch acceptance. The negative control shall have an analyte concentration or response < 50% of the LLOQ and/or unacceptable ion ratios as specified in 7.10.5.3.3 or 7.10.5.3.4.

7.10.5.4.1. If the above acceptance criterion is not met, the analytical data for the samples bracketed by the failed negative control with a concentration ≥ 50% of the LLOQ shall not be used and shall be reanalyzed, if possible, starting at 7.8.1. A result < 50% of the LLOQ for an evidentiary sample shall be accepted as none detected.

7.10.5.5. At least one negative control shall have the corresponding internal standard present for the associated analyte.

7.10.5.5.1. If acceptance criterion is not met, all samples in the batch shall be reanalyzed, if possible, starting at 7.8.1.

7.10.5.6. At least one low and one high non-zero control shall be included in each batch.

7.10.5.7. A non-zero control for an analyte fails if any of the following occur:

7.10.5.7.1. An ion ratio does not meet the acceptability criteria listed in 7.10.5.3.3 or 7.10.5.3.4.

7.10.5.7.2. A quantitated value is not within ± 20% of the target concentration.

7.10.5.7.3. A peak has poor chromatography.

7.10.5.7.4. The relative retention time is greater than ± 5% of the mean relative retention time based on all calibrators and controls used to generate the curve.

7.10.5.8. Each set of one to twelve samples shall be bracketed by one low and one high non-zero control.

7.10.5.8.1. If a control result does not meet the above criteria, the analytical data for the samples bracketed by the failed control shall not be used, and analysis of the samples in the bracket prior to and following the failed control shall be repeated for samples positive for the analyte

that failed, if possible, starting at 7.8.1. A result below the LLOQ for an evidentiary sample shall be accepted as none detected, if the negative controls for the batch pass the acceptability criteria in 7.10.5.4 and 7.10.5.5.

7.10.6. If the analyte in a sample has a result > 2x ULOQ, evaluate the analyte in the subsequent sample(s) as follows:

7.10.6.1. If a > 2x ULOQ sample is immediately followed by an evidentiary sample in which the analyte is not detected, data should be accepted.

7.10.6.2. If a > 2x ULOQ sample is immediately followed by an evidentiary sample in which the analyte is detected, the sample following the > 2x ULOQ sample shall be retested.

7.10.6.2.1. Additional negative controls or solvent blanks may be added to the sequence immediately following the > 2x ULOQ sample to eliminate carryover in subsequent samples.

7.10.6.3. If a > 2x ULOQ sample is immediately followed by a control and the control passes acceptance criteria, data should be accepted.

7.10.6.4. If a > 2x ULOQ sample is immediately followed by a control and it does not pass control acceptance criteria, all samples in the preceding and following brackets shall be reinjected or retested.

7.10.6.4.1. Additional negative controls or solvent blanks may be added to the sequence immediately following the > 2x ULOQ sample to eliminate carryover in subsequent samples.

7.10.6.5. If the MassHunter software does not provide a calculated result, the RR for the sample may be compared to the RR of Cal 8.

7.10.7. Analyte Identification (Qualitative Criteria)

7.10.7.1. Relative retention time shall be within ± 5% of the mean relative retention time based on all calibrators and controls used to generate the curve.

7.10.7.2. Each analyte shall have two ion transitions monitored. The ion transition from the precursor to the product ion listed in **bold** type in Table 14: Stimulants Analyte MS Parameters is used for quantitation.

7.10.7.3. Each internal standard shall be present and have one ion transition monitored.

7.10.7.4. Each ion ratio shall meet the acceptability criteria listed in 7.10.5.3.3 or 7.10.5.3.4.

7.10.7.4.1. If the ion ratio is greater than 30% due to detector saturation for an analyte in one or more case samples, the calibrators used to generate the calibration curve, three negative controls, and at least one low and high control bracketing the case sample(s) may be reinjected at a lower injection volume.

7.10.7.5. Data analysis software manual integration tools (Merge Right Peak, Merge Left Peak, Split Peak and Pick Left, Split Peak and Pick Right, Snap Baseline, Drop Baseline, Apply ISTD RTs to Target, Apply Target RTs to Qualifier) may be used to adjust the integration algorithm to select the correct peak or adjust the baseline. Use of

software manual integration tools shall be documented on the chromatogram.

7.10.8. Analyte Stability

7.10.8.1. Prepared samples are stable for 6 days at room temperature or 10 days when stored in the auto sampler.

7.10.9. Retesting Samples

7.10.9.1. When a sample requires retesting, the sample shall be retested at least once, if possible. A sample may be retested up to two times without supervisory approval.

7.10.9.1.1. If a quantitative value cannot be reported from any analysis, the first acceptable qualitative data according to analyte identification in 7.10.7 shall be used. (ref. 7.11.4).

7.10.9.1.2. If data is not generated, that analysis does not count as an analysis or retest under this section.

7.10.10. Unacceptable Data

7.10.10.1. Data found to be unacceptable shall be marked with a signed note identifying the specific analytical data that should not be used and the reason for not using the data (e.g., “Do not use this quantitative amphetamine data due to a bracketing control being outside acceptability criteria. AB XX/XX/XX” or “Do not use any data from this batch due to sequence interruption. Samples will be retested. AB XX/XX/XX”).

7.10.11. No Data Generated for a Sample

7.10.11.1. Cases with no generated data should have a case synopsis note to explain the lack of data associated with the chain of custody preparation date (e.g., “XX/XX/XX No data was collected from [batch name] due to the instrument stopping. AB”).

7.11. Report Writing

7.11.1. The LLOD for stimulant analysis is equal to the LLOQ for each analyte. The LLOQ and ULOQ are listed in Table 15.

Table 15: Stimulants LLOQ and ULOQ

Analyte	LLOQ (ng/mL)	ULOQ (ng/mL)
Amphetamine	5.0	500
MDA	5.0	500
MDMA	5.0	500
Methamphetamine	10	500
Phencyclidine	5.0	500

7.11.2. Confirmatory data for each specimen shall be technically reviewed prior to entering the result into LIMS.

7.11.2.1. The preparation date of the analysis being reported shall be entered as the analysis date.

7.11.3. Quantitative Reporting

7.11.3.1. A result less than the LLOQ shall not be reported.

7.11.3.1.1. If a batch LLOQ is used, a quantitative result less than the target concentration for the lowest calibrator used in the calibration curve shall not be reported.

7.11.3.2. A quantitated result that meets acceptability criteria shall be reported for results between the target concentration of the lowest and highest calibrators.

7.11.3.2.1. A result shall be truncated to the appropriate level of significance and reported as the quantitative value ± the expanded measurement uncertainty.

7.11.3.2.1.1. A result shall be reported to one decimal place for quantitative values less than 10.

7.11.3.2.1.2. A result shall be reported as a whole number for quantitative values greater than or equal to 10.

7.11.3.3. A result that is above the ULOQ and has an ion ratio within ± 30% of the mean ion ratio based on all calibrators and controls used to generate the curve shall be reported as > the ULOQ in ng/mL.

7.11.3.3.1. If a batch ULOQ is used, a quantitative result greater than the target concentration for the highest calibrator used in the calibration curve shall not be reported.

7.11.3.3.1.1. A result greater than the target concentration of the highest calibrator used in the calibration curve may be reported if retesting of a specimen is not feasible.

7.11.3.4. Quantitative results shall only be reported if analysis occurred within the established sample stability window (ref. 7.10.8).

7.11.3.5. If a specimen is analyzed more than once, the first quantitative result that meets quantitative acceptability criteria for a specific analyte shall be reported.

7.11.4. Qualitative Reporting

7.11.4.1. A result should be reported as “Positive” when the analyte identification criteria (ref. 7.10.7) has been met, the quantitative result is > LLOQ, and the quantitative criteria have not been met.

7.11.4.1.1. If a specimen is analyzed more than once, the totality of the qualitative data shall be evaluated by the analyst for acceptability criteria for analyte identification of a specific analyte.

7.11.4.1.1.1. The preparation date of last analysis shall be used as the analysis date.

7.11.4.2. A result may be reported as “Positive” with supervisory approval if any of the following occur:

7.11.4.2.1. Interference(s); or

7.11.4.2.2. Quantitative result > LLOQ with an ion ratio greater than ± 20%, but less than ± 30%, of the mean ion ratio based on all calibrators and controls used to generate the curve.

7.12. References

7.12.1. ISDT Cocaine and Metabolite GC/MS Confirmation Method
7.12.2. ISDT Benzodiazepines and Z-Drugs LC/QQQ Confirmation Method
7.12.3. Standard Practices for Method Validation in Forensic Toxicology. ANSI/ASB Standard 036, 1st edition, 2019, 1-46.
7.12.4. Standard for Mass Spectral Data Acceptance for Definitive Identification. Scientific Working Group for Forensic Toxicology (SWGTOX). 2014, 1-11.

8. Volatile Analysis by HS/GC/FID

8.1. Scope
- 8.1.1. This method shall be used for analysis of specimens for the presence of volatiles (acetone, ethanol, isopropanol, and methanol).

8.2. Precautions/Limitations
- 8.2.1. Minimum Sample Requirements
  - 8.2.1.1. Routine analysis requires 400 µL of blood or serum/plasma sample.
- 8.2.2. CRMs
  - 8.2.2.1. A CRM from the same lot number shall not be used for a calibrator and control in the same batch.

8.3. Related Information
- 8.3.1. Volatile Multi-point Calibration Validation (December 2015-February 2016)
- 8.3.2. Volatile Analysis Sample Reinjection Validation (June 2015)
- 8.3.3. Volatile Analysis Sample Stability Validation (July 2015)
- 8.3.4. Volatile Workflow Change Evaluation (October 2023)
- 8.3.5. Instrument validations
- 8.3.6. Validation of internal standard lot data

8.4. Instruments/Equipment
- 8.4.1. Rocker
- 8.4.2. Headspace sampler
- 8.4.3. Gas chromatograph with flame ionization detector
- 8.4.4. Auto diluter
- 8.4.5. Crimper
- 8.4.6. Tube rack
- 8.4.7. Vortex, single
- 8.4.8. Vial rack
- 8.4.9. Volumetric flasks
- 8.4.10. Pipettes

8.5. Reagents/Materials
- 8.5.1. Pipette tips
- 8.5.2. ALC1 Gas chromatograph capillary column
  - 8.5.2.1. Dimensions: 30 m x 530 µm x 3.0 µm
  - 8.5.2.2. Composition: DB-ALC1
- 8.5.3. ALC2 Gas chromatograph capillary column
  - 8.5.3.1. Dimensions: 30 m x 530 µm x 2.0 µm
  - 8.5.3.2. Composition: DB-ALC2
- 8.5.4. 20 mL headspace crimp top vials
- 8.5.5. Headspace crimp caps
- 8.5.6. Compressed air
- 8.5.7. Helium, 5.0 grade or higher
- 8.5.8. Hydrogen
- 8.5.9. ddH_2O
- 8.5.10. Negative blood (human)
- 8.5.11. Negative serum/plasma (human)
- 8.5.12. Aqueous CRMs

8.5.12.1. Mixed volatiles (containing ethanol, methanol, isopropanol, and acetone)

8.5.13. Solvents shall be high quality and low residue (e.g., HPLC grade, Omnisolv, Optima, etc.).

8.5.13.1. Ethanol

8.5.13.2. Methanol

8.5.13.3. Acetone

8.5.13.4. Isopropanol

8.5.13.5. n-propanol

8.6. Hazards/Safety

8.6.1. See Safety Manual.

8.6.2. See SDS for each chemical in this method.

8.7. Reference Materials/Controls/Calibrators/Solutions

8.7.1. All solutions shall conform to Solution Preparation, Validation, Verification (Doc ID: 3695).

8.7.2. Four calibrators shall be used. The calibrators shall contain ethanol, methanol, isopropanol, and acetone in ddH_2O. The concentrations of the calibrators shall range from 10 mg/dL to 400 mg/dL.

8.7.3. VOL Internal standard

8.8. Procedures/Instructions

8.8.1. An evidentiary batch shall consist of two preparations. Each preparation shall consist of concurrently prepared calibrators, negative controls, non-zero controls, and specimen aliquots.

8.8.1.1. Each preparation shall be a unique event between which the samples are sealed.

8.8.1.1.1. ALC1 samples shall be verified onto the instrument prior to the start of ALC2 preparation.

8.8.1.2. Each set of one to twelve specimen aliquots shall be bracketed by a pair of controls consisting of one non-zero control and one negative control.

8.8.1.2.1. The same non-zero control concentration shall not be used for both sides of the bracket of one to twelve specimen aliquots.

8.8.1.3. Each preparation shall include at least two different concentrations of non-zero mixed controls that are within the quantitative range.

8.8.1.3.1. Negative aqueous controls shall be included in each batch.

8.8.1.3.2. At least one matrix matched negative shall be included for each specimen matrix represented in each preparation.

8.8.2. Mix calibrators, controls, and specimens, i.e., on a rocker or by vortexing.

8.8.3. When using an auto-diluter, tubing shall be primed with internal standard.

8.8.4. Aspirate 200 µL of each calibrator, control, or specimen and 2000 µL of ISTD.

8.8.5. Dispense 200 µL sample with 2000 µL of internal standard into a headspace vial labeled with corresponding specimen identification and cap.

8.8.6. Analyze prepared samples using headspace-gas chromatograph/FID. One of the duplicate preparations shall be run on an instrument using the ALC1 column and one preparation shall be run on an instrument using the ALC2 column.

8.8.6.1. Sequence names shall be in the following format:
YYYY_MM_DD_ALC1_Initials or
YYYY_MM_DD_ALC2_Initials.

8.8.6.1.1. The date in the sequence shall be the date of preparation of the samples.

8.8.6.1.2. Additional information such as reinjection, validation, etc., or equivalent abbreviations should be included with the assay abbreviation.

8.8.6.1.3. If the sequence is run with the wrong sequence name, it shall be noted on the Technical Review Worksheet and in the case synopsis of each case in the batch and not corrected on the chromatograms.

8.8.6.2. If samples are not to be analyzed on the day of preparation, they may be stored at room temperature or refrigerated for up to 72 hours.

8.8.6.3. If a mechanical or network interruption results in incomplete analysis of the batch, reinjection may be performed. A reinjection shall be performed by restarting the sequence from the last passing control pair or reinjecting the entire sequence:

8.8.6.3.1. Within 24 hours of the original injection for samples containing acetone or isopropanol.

8.8.6.3.2. Within 48 hours of the original injection for samples containing ethanol or methanol.

8.8.6.3.3. Specimen aliquots may only be reinjected once.

8.8.6.3.4. Calibrators and/or controls may be reinjected multiple times.

8.8.6.3.4.1. If a reinjection is needed more than once, the specimen aliquots that have already been reinjected may be skipped in a bracket.

8.8.6.3.4.1.1. The specimen aliquots that are skipped and do not have valid data shall be reanalyzed starting at 8.8.1.

8.8.7. Volatile Analysis Method

8.8.7.1. Headspace parameters

Oven temperature:	70 ºC
Loop temperature:	80 ºC
Transfer line temperature:	90 ºC
Vial equilibration time:	6.00 min
Injection duration:	0.50 min
GC cycle time:	5.0 min
Vial size:	20 mL
Shake:	Less or 18 shakes/min
Fill volume:	2.2 mL
Vial pressurization time:	0.2 min

8.8.7.2. Gas chromatograph inlet

Heater:	200 ºC
Total Flow:	108.6 mL/min
Septum purge flow:	3 mL/min
Inlet mode:	Split
Split ratio:	10:1
Carrier gas:	Helium
Gas saver:	Off

8.8.7.3. Gas chromatograph capillary column

ALC1 dimensions:	30m x 530µm x 3µm
ALC2 dimensions:	30m x 530µm x 2µm
Initial flow:	9.6 mL/min
Post run flow:	9.6 mL/min
ALC1 composition:	DB-ALC1
ALC2 composition:	DB-ALC2

8.8.7.4. Gas chromatograph oven

Oven temperature:	40 ºC
Run time:	3.5 min

8.8.7.5. FID

Heater:	250 ºC
Air flow:	450 mL/min
H_2 flow:	40 mL/min
Makeup flow:	10 mL/min
Carrier gas flow correction:	Constant makeup and fuel flow
Flame:	On

8.8.8. Following completion of analysis of a batch using the volatile analysis method, the volatile analysis post batch method may be initiated to put the instrument in idle mode. If used, the volatile analysis post batch method parameters differ from 8.8.7 as follows:

8.8.8.1. Gas chromatograph inlet

Split ratio:	50:1
Gas saver:	20 mL after 16 min

8.8.8.2. Gas chromatograph capillary column

Initial flow:	9.6 mL/min
Post run flow:	1.0 mL/min

8.8.8.3. Gas chromatograph oven

Rate (C/min)	**Value I**	**Hold Time (min)**	**Run Time (min)**
	40	1	1
25	150	10	15.4
25	40	0	19.8

8.8.8.4. FID

Heater:	175 ºC
H_2 flow:	Off
Air flow:	Off
Makeup flow:	1 mL/min
Carrier gas flow correction:	Constant makeup and fuel flow
Flame:	Off

8.9. Records
- 8.9.1. Pipette calibration certificate, however named, if applicable
- 8.9.2. Auto-dilutor calibration certificate, however named
- 8.9.3. Batch Preparation Packet, named as: YYYY_MM_DD_VOL_Initials
  - 8.9.3.1. Tox Screen Worklist and/or Retest Worksheet, as appropriate
  - 8.9.3.2. Sequence Tables
  - 8.9.3.3. Volatile Analysis Preparation Worksheet
  - 8.9.3.4. Aliquot Chain of Custodies
- 8.9.4. Calibrator and control chromatograms
- 8.9.5. Sample chromatograms
- 8.9.6. Volatile Analysis Batch Summary
- 8.9.7. Measurement Uncertainty Estimation and supporting data
- 8.9.8. Specimen Verification Worksheet, if applicable

8.10. Interpretation of Results
- 8.10.1. For determination of acceptability of the data in accordance with the acceptance criteria for this method, the "Final Amount" listed on the chromatogram truncated to one decimal place in units of mg/dL shall be used.
- 8.10.2. Interpretation of results for each analyte shall occur independent of the other analytes in the method.
- 8.10.3. Chromatographic analyte and internal standard peaks shall have baseline resolution between adjacent peaks.
- 8.10.4. Results from samples that are analyzed prior to a calibrator shall not be used to determine acceptability of batch data.
- 8.10.5. Calibration and Controls Criteria
  - 8.10.5.1. Generating a calibration curve:
    - 8.10.5.1.1. A linear curve (1/x weighting) shall be established by using four aqueous mixed volatiles calibrators.
    - 8.10.5.1.2. The correlation coefficient (r^2) for the calibration curve shall be at least 0.990.
    - 8.10.5.1.3. Quantitation of calibrators shall be within ± 10% of the nominal concentrations on the CoA or target concentration.
    - 8.10.5.1.4. If the above criteria are not met in either preparation, the entire batch shall be reprepared and reanalyzed starting at 8.8.1.
  - 8.10.5.2. Evaluating non-zero controls
    - 8.10.5.2.1. Quantitation of non-zero controls shall be within ± 10% or 5 mg/dL, whichever is greater, of the target concentration.
    - 8.10.5.2.2. If the above criterion is not met for any analyte, all bracketed specimen aliquots with results above the LLOQ for that analyte shall be reprepared and reanalyzed starting at 8.8.1, if possible.
  - 8.10.5.3. Evaluating negative controls
    - 8.10.5.3.1. A negative control shall follow each non-zero control.
    - 8.10.5.3.2. Negative controls shall have an analyte response < 50% of the LLOQ.

8.10.5.3.3. If the above criteria are not met for any analyte, all bracketed specimen aliquots with results ≥ 50% of the LLOQ for that analyte shall be reprepared and reanalyzed starting at 8.8.1, if possible.

8.10.5.4. Each set of one to twelve specimen aliquots shall be bracketed by a pair of controls consisting of one non-zero control and one negative control.

8.10.5.4.1. If specimen aliquots are not bracketed in both preparations, the bracket shall be reprepared and reanalyzed starting at 8.8.1, if possible.

8.10.5.5. Both preparations shall meet all acceptability criteria for a specimen to be reported. If either ALC1 or ALC2 fails to meet acceptability criteria, each specimen result affected by that failure shall be reprepared and reanalyzed starting at 8.8.1, if possible.

8.10.6. If the analyte in a sample has a result > 2x ULOQ, evaluate the analyte in the subsequent sample(s) as follows:

8.10.6.1. If a > 2x ULOQ sample is immediately followed by an evidentiary sample in which the analyte is not detected, data should be accepted.

8.10.6.2. If a > 2x ULOQ sample is immediately followed by an evidentiary sample in which the analyte is detected, the sample following the > 2x ULOQ sample shall be retested.

8.10.6.2.1. Additional negative controls or solvent blanks may be added to the sequence immediately following the > 2x ULOQ sample to eliminate carryover in subsequent samples.

8.10.6.3. If a > 2x ULOQ sample is immediately followed by a control and the control passes acceptance criteria, data should be accepted.

8.10.6.4. If a > 2x ULOQ sample is immediately followed by a control and it does not pass control acceptance criteria, all samples in the preceding and following brackets shall be reinjected or retested.

8.10.6.4.1. Additional negative controls or solvent blanks may be added to the sequence immediately following the > 2x ULOQ sample to eliminate carryover in subsequent samples.

8.10.7. Analyte Identification

8.10.7.1. Retention time shall be within ± 5% of the retention time based on the calibrators used to generate the curve.

8.10.7.2. Internal standard shall be present in each sample.

8.10.8. Analyte Stability

8.10.8.1. Prepared samples are stable for 72 hours when refrigerated or stored on the instrument auto sampler or at equivalent temperature.

8.10.9. Results Evaluation

8.10.9.1. A quantitated result shall be reported for a specimen if the following analyte-specific criteria are met:

8.10.9.1.1. Calibration, controls, and analyte identification criteria are met for the specific analyte in both preparations.

8.10.9.1.2. The truncated ALC1 result and truncated ALC2 result have an RPD ≤ 10% or 5 mg/dL, whichever is greater.

8.10.9.1.2.1. The results may have an RPD > 10% when both results are > 400 mg/dL or when one result (ALC1 or ALC2) is between 360 mg/dL and 400 mg/dL and the other result (ALC1 or ALC2) is > 400 mg/dL.

8.10.9.1.2.2. If the above criteria are not met, testing shall be repeated for the specimen unless either results is less than 10 mg/dL (ref. 8.10.9.2).

8.10.9.1.3. The ALC1 and ALC2 analysis should be performed on the same specimen but may be performed on a different tube of the same matrix type with the same draw time.

8.10.9.2. If either result is < 10 mg/dL, a quantitated result shall not be reported.

8.10.10. Retesting Specimens

8.10.10.1. When a specimen requires retesting, the specimen shall be retested starting at 8.8.1, if possible. A specimen may be retested up to two times without supervisory approval.

8.10.10.1.1. If a quantitative value cannot be reported from any analysis, the first acceptable qualitative data according to analyte identification in 8.10.7 shall be used. (ref. 8.11.4).

8.10.10.1.2. If data is not generated, that analysis does not count as an analysis or retest under this section.

8.10.11. Unacceptable Data

8.10.11.1. Data found to be unacceptable shall be marked with a signed note identifying the specific analytical data that should not be used and the reason for not using the data (e.g., “Do not use the quantitative data for acetone due to a bracketing control being outside acceptability criteria. AB XX/XX/XX” or “Do not use any data from this batch due to sequence interruption. Samples will be reinjected. AB XX/XX/XX”).

8.10.12. No Data Generated for a Sample

8.10.12.1. Cases with no generated data should have a case synopsis note to explain the lack of data associated with the chain of custody preparation date (e.g., “XX/XX/XX No data was collected from [batch name] due to the instrument stopping. AB”).

8.11. Report Writing

8.11.1. The LOD is equal to the LLOQ for each analyte. The LLOQ is 0.010 g/100 mL and the ULOQ is 0.400 g/100 mL for each analyte.

8.11.2. Data for each specimen shall be technically reviewed prior to entering the result into LIMS.

8.11.2.1. The preparation date of analysis shall be used as the analysis date.

8.11.3. Quantitative Reporting

8.11.3.1. If one of the replicate results is below the LLOQ, the result shall be reported as “None Detected.”

8.11.3.2. A quantitated value shall be reported for results between the target concentration of the lowest and highest calibrators.

8.11.3.2.1. The lower of the two results shall be reported in g/100 mL truncated to three decimal places (divide mg/dL value by 1000).

8.11.3.2.2. Quantitative values between 0.010 and 0.400 g/100 mL shall be reported ± the expanded measurement uncertainty to three decimal places.

8.11.3.2.3. Results with a quantitated value greater than the highest calibrator shall be reported as greater than the highest calibrator in g/100 mL truncated to three decimal places (e.g., > 0.400 g/100 mL).

8.11.3.3. A quantitative result shall only be reported if analysis occurred within the established sample stability window (ref. 8.10.8).

8.11.3.4. If a specimen is analyzed more than once, the first set of quantitative results with data that meets acceptability criteria for quantitation of a specific analyte shall be reported (ref. 8.11.3).

8.11.4. Qualitative Reporting

8.11.4.1. A result should be reported as "Positive" when both replicates meet the analyte identification criteria, the quantitative results are > LLOQ, and the quantitative criteria have not been met.

8.11.4.1.1. If a specimen is analyzed more than once, the totality of the qualitative data shall be evaluated by the analyst for acceptability criteria for analyte identification of a specific analyte.

8.11.4.1.1.1. The preparation date of last analysis shall be used as the analysis date.

8.11.4.2. A result may be reported as "Positive" with supervisory approval if an interference occurs.

8.12. References

8.12.1. Kristoffersen, L.; Stormyhr, L.; Smith-Kielland, A. Headspace gas chromatographic determination of ethanol: The use of factorial design to study effects of blood storage and headspace conditions on ethanol stability and acetaldehyde formation in whole blood and plasma. *Forensic Science International*, 2006, *161*, 151–157.

8.12.2. Anthony, R. M.; Sutheimer, C. A.; Sunshine, I. Acetaldehyde, Methanol, and Ethanol by Headspace Gas Chromatography. *J. Anal. Toxicol.* 1980, *4*, 43-45.

8.12.3. Glendening, B.L.; Harvey, R.A. A simple method using headspace gas for determination of blood alcohol by gas chromatography. *J. Forensic Sci.* 1969, *14*, 136-145.

8.12.4. Firor, R. L., Meng, C. Static Headspace Blood Alcohol Analysis with the G1888 Network Headspace Sampler. Application Document, Agilent Technologies, Inc. 2004.

8.12.5. Machata, G. Determination of alcohol in blood by gas chromatography headspace analysis. *Perkin Elmer Clin. Chem. Newsl*. 1972, *4*, 29-32.

8.12.6. Machata, G. The advantages of automated blood alcohol determination by headspace analysis. *Z. Rechtstned*. 1975, *75*, 229-234.

8.12.7. Standard Practices for Method Validation in Forensic Toxicology. ANSI/ASB Standard 036, 1st edition, 2019, 1-46.

9. Technical and Administrative Review

9.1. Scope

9.1.1. This method shall be used for technical review of analytical batch analysis and for administrative review of toxicology reports.

9.2. Technical Review

9.2.1. Each analytical result obtained for evidentiary samples, including failed data, shall be technically reviewed by a forensic scientist other than the scientist who performed the analysis.

9.2.1.1. Failed data may be reviewed concurrently with data from the acceptable batch.

9.2.1.2. If the entire batch fails and no data was collected for any evidentiary sample, the batch analytical data does not need to be reviewed, but the reason for the absence of analytical data should be documented in the case synopsis notes (e.g., “communication error caused the sequence to stop before the acquisition of data for any case samples”).

9.2.1.3. If data was not collected for an evidentiary sample, the reason for the absence of analytical data should be documented in the case synopsis notes (e.g., “communication error caused the sequence to stop before the acquisition of data for any case samples”).

9.2.2. Analytical data obtained for screening results should be technically reviewed and approved prior to beginning confirmatory analysis.

9.2.3. For each batch containing evidentiary samples, a QA/QC file shall be compiled including an Aliquot Chain of Custody, an instrument sequence list, a Batch Preparation Worksheet, a Technical Review Worksheet, a LIMS data verification, and the results of the analysis (e.g., batch summary sheets, chromatograms of calibrators and controls).

9.2.3.1. When applicable, the following shall also be included: a LIMS worklist, a Retest Worksheet, a tune report, an Ion Ratio Worksheet, and/or a Specimen Verification Worksheet.

9.2.4. For each batch of outsourced evidentiary specimens being sent for testing within ISDT’s scope of testing, a QA/QC file shall be compiled including a LIMS Worklist (if applicable), Evidence Transfer Receipt or Specimen Verification, Shipping Manifest, Shipping Label, Technical Review Worksheet (if applicable) and LIMS Data Verification (if applicable).

9.2.5. Each note on a technical record shall be signed.

9.2.5.1. A worksheet filled out concurrently with sample preparation does not need to be signed unless a note is made by someone other than the analyst.

9.2.6. After all the data has been reviewed by the analyst, the analyst shall submit the batch for technical review by an analyst trained in technical review for the assay.

9.2.7. The technical review shall follow whichever is appropriate of the following:

9.2.7.1. Drug Screening Analysis by LC/TOF

9.2.7.1.1. Verify the following:

9.2.7.1.1.1. Header information (analyst name, sequence name, instrument, analysis date, etc.) is consistent on all documentation;

9.2.7.1.1.2. Each document of the QA/QC packet containing a sample result includes the analyst name and date of sample preparation;

9.2.7.1.1.3. The Aliquot Chain of Custody and Batch Preparation Worksheet are accurately completed;

9.2.7.1.1.4. ToxBox® Plate (or each control and internal standard solution) used is before its expiration date;

9.2.7.1.1.5. Mass spectrometer tune is acceptable according to the LC/TOF Instrument Operation and Maintenance Manual (Doc ID: 2843);

9.2.7.1.1.6. Sample names are consistent on the LIMS Worklist, Aliquot Chain of Custody or Specimen Verification, Instrument Worklist, and Retest Worksheet, as applicable;

9.2.7.1.1.7. Each sample was analyzed with the appropriate method(s) (e.g., positive and negative mode);

9.2.7.1.1.8. Each sample acquisition date/time is before the calibration date/time;

9.2.7.1.1.9. Any chromatogram that was processed using manual integration is appropriately documented according to 1.10.7;

9.2.7.1.1.10. The result for each control and evidentiary sample meets the acceptability criteria for the method used, or the appropriate chromatogram for a failed sample is documented with the reason for the failure; and

9.2.7.1.1.11. Any note regarding a deviation from the method is signed by a laboratory supervisor or quality assurance manager.

9.2.7.2. Drug Confirmation Analysis by GC/MS or LC/QQQ

9.2.7.2.1. Verify the following:

9.2.7.2.1.1. Header information (analyst name, sequence name, instrument, analysis date, etc.) is consistent on all documentation;

9.2.7.2.1.2. Each document of the QA/QC packet containing a sample result includes the analyst name and date of sample preparation;

9.2.7.2.1.3. The Batch Summary, Aliquot Chain of Custody, and Batch Preparation Worksheet are completed accurately;

9.2.7.2.1.4. Each calibrator, control, and internal standard solution used is before its expiration date;

9.2.7.2.1.5. Mass spectrometer tune is signed by the analyst and acceptable according to LC/QQQ Instrument Operation and Maintenance Manual (Doc ID: 2842) or GC/MS Instrument Operation and Maintenance Manual (Doc ID: 2841), as applicable;

9.2.7.2.1.6. Each chromatogram specifies the tune date as the same date/time as the Tune Report (GC/MS only);

9.2.7.2.1.7. Sample names are consistent on the LIMS Worklist, Aliquot Chain of Custody or Specimen Verification, Instrument Worklist/Sequence, QA Summary, however named, plate layout, and Retest Worksheet, as applicable.

9.2.7.2.1.8. Sample names are listed in the correct order on the Instrument Worklist/Sequence and Batch Summary;

9.2.7.2.1.9. Each sample was analyzed with the appropriate method for the instrument used (e.g., Cocaine_MS1.M);

9.2.7.2.1.10. Sample acquisition date/times of all calibrators are before the calibration date/time;

9.2.7.2.1.11. The Ion Ratio Worksheet accurately documents each ion ratio for each calibrator used in the calibration curve, and the average ion ratio is accurately applied to each sample in the batch (GC/MS only);

9.2.7.2.1.12. The MassHunter Ion Ratio and RRT Verification sheets are saved and signed (LC/QQQ only);

9.2.7.2.1.13. Any chromatogram that was processed using the manual integration tool(s) permitted by the method is appropriately documented according to the test method for the analysis; and

9.2.7.2.1.14. The result for each calibrator, control, and evidentiary sample meets the

acceptability criteria for the method used, or the appropriate chromatogram for a failed sample is documented with the reason for the failure.

9.2.7.2.1.15. Any note regarding a deviation from the method is signed by a laboratory supervisor or quality assurance manager.

9.2.7.3. Volatile Analysis

9.2.7.3.1. Verify the following:

9.2.7.3.1.1. Header information (analyst name, batch/sequence names, instrument, analysis date, etc.) is consistent on all documentation;

9.2.7.3.1.2. Each document of the QA/QC packet containing a sample includes the analyst name and date of sample preparation;

9.2.7.3.1.3. The Batch Summary, Aliquot Chain of Custody, and Batch Preparation Worksheet are completed accurately;

9.2.7.3.1.4. Each calibrator, control, and internal standard solution used is before its expiration date;

9.2.7.3.1.5. Chromatogram injection date is the same as the sample prep date (or within method limitations);

9.2.7.3.1.6. Sample names are consistent on the LIMS Worklist, Retest Worksheet, Instrument Worklist, and Batch Summary, if applicable;

9.2.7.3.1.7. Sample names are listed in the correct order on the Instrument Worklist and Batch Summary;

9.2.7.3.1.8. Each sample was analyzed with the appropriate method for the instrument used (e.g., EtOH_HS1.M);

9.2.7.3.1.9. Each sample injection date/time is before the calibration date/time;

9.2.7.3.1.10. The result for each calibrator, control, and evidentiary sample meets the acceptability criteria for the method used, or the appropriate chromatogram for a failed sample is documented with the reason for the failure; and

9.2.7.3.1.11. Any note regarding a deviation from the method is signed by a laboratory supervisor or quality assurance manager.

9.2.8. The technical reviewer shall notify the analyst of a discrepancy between the data and any method, policy, or manual found during technical review. The analyst shall correct the record(s) and notify the technical reviewer of the action taken. The technical reviewer shall resume the technical review.

9.2.8.1. The technical reviewer shall document on the technical review worksheet the following: description of discrepancy found, date of notification of discrepancy, identity of person notified, and action taken.

9.2.8.1.1. Each addition or correction shall be made on the relevant page of the data and signed.

9.2.9. The technical reviewer shall sign the technical review worksheet to document the technical review.

9.2.10. If any amendment to the technical records of the batch (e.g., amending notes, re-processing of data) occurs after a technical review has been completed, the amendment shall be documented on the technical review worksheet and shall be technically reviewed by the original technical reviewer or another analyst trained in technical review for the assay.

9.2.11. Amendments to chromatogram notes that do not affect the acceptability of the results (e.g., specimen type or item number) that are identified after technical review has been completed do not require documentation on the technical review worksheet or additional-technical review. After completion of a technical review, results shall be entered into LIMS if they meet acceptability criteria.

9.2.12. Analyst verification of data entry

9.2.12.1. Use data comparison output to compare the results entered into LIMS with the results from the analysis and to verify the correct date of preparation was entered into LIMS.

9.2.12.1.1. Correct any typographical errors in the LIMS data entry.

9.2.12.1.2. Document the verification by saving the LIMS Data Verification file in the appropriate batch QA/QC folder.

9.2.12.1.2.1. If the verification includes multiple batches, data comparison should be saved with data for the first batch analyzed and an additional data comparison saved with each individual batch.

9.2.12.1.3. Another ISDT employee shall document the presence of the sequence LIMS Data Verification list by signing the designated area on the sequence Technical Review Worksheet.

9.3. Administrative Review

9.3.1. Verify the following:

9.3.1.1. Case number is documented on each electronically saved document in the electronic case file and each document has the appropriate file name.

9.3.1.2. CoC is accurately completed and dates/times are consistent with other documentation for the case (e.g., each CoC transfer for each item of

evidence has analytical data or other documentation with a consistent date/time).

9.3.1.3. The appropriate images of evidence are saved.

9.3.1.4. TAR is legibly scanned.

9.3.1.5. Information entered into LIMS in the Agency, Individuals, Offense, Evidence, and Requests tabs is correct and corresponds to the TAR and evidence images, if applicable.

9.3.1.5.1. Agency tab shall include the submitting agency, appropriate prosecutor's office, and DRE (if applicable).

9.3.1.5.2. Individuals tab shall include the first and last name of the subject and the type shall be selected as "Subject." The date of birth and gender of the subject should be included if provided, or gender shall be marked "Unknown."

9.3.1.5.3. Offense tab shall have at least one offense included with the correct county. If no offense is listed on the TAR, the offense shall be entered as "Unknown."

9.3.1.5.4. Evidence tab shall include the appropriate item(s) as present in the evidence images.

9.3.1.5.4.1. If specimens with different draw times or different postmortem draw sites are present, each specimen description shall include the draw time or draw site as appropriate.

9.3.1.5.5. Request tab shall include the test(s) requested on the TAR and/or as specified in communication(s) documented in the electronic case file, if applicable, and the correct officer name.

9.3.1.5.5.1. Compare screening and confirmation results and ensure the appropriate screening and confirmation tests were completed, if applicable.

9.3.1.6. Verify the following information on the Draft Report header:

9.3.1.6.1. Case number;

9.3.1.6.2. Submitting agency name;

9.3.1.6.3. Evidence received date;

9.3.1.6.4. Evidence received courier;

9.3.1.6.5. Evidence item(s) received;

9.3.1.6.5.1. Lists the draw time(s) (HH:MM) of the blood tubes, if there is more than one draw time submitted (> 15 minutes apart).

9.3.1.6.5.2. Lists the draw site(s) (Postmortem – [draw site]) of the blood tubes, if there is more than one draw site submitted.

9.3.1.6.5.3. Identifies the sample type if more than one type of sample is submitted for a coroner case (Doc ID: 3589).

9.3.1.6.6. Subject name; and
9.3.1.6.7. Submitting agency case number (if applicable).

9.3.1.7. If any of the information in 9.3.1.6 is unclear (e.g., illegible information on TAR), check the electronic case file for correspondence about the issue or verification with court records associated with the case. If the issue has not previously been addressed, verify with court records associated with the case or contact submitting agency or prosecutor's office to verify the information, and document the verification or communication in the case synopsis, including attaching a copy of any correspondence.

9.3.1.8. Perform technical review of the toxicology report.

9.3.1.8.1. If the screening was outsourced, the report should indicate "See NMS Report*" and identify the item(s) sent to NMS.

9.3.1.8.2. Ensure all presumptive positive screening results have a paired confirmation analysis or are appropriately noted.

9.3.1.8.3. If there are no positive findings to report, the results section shall state "None Detected."

9.3.1.8.4. If there are positive findings to report,

9.3.1.8.4.1. The first acceptable data for each analyte shall be reported;

9.3.1.8.4.2. Each confirmation result shall:

9.3.1.8.4.2.1. Identify the analyte confirmed;

9.3.1.8.4.2.2. Report the quantity found as specified in the assay test method, report the concentration as >ULOQ, or "Positive" for qualitative results;

9.3.1.8.4.2.3. Report the MU for quantitative results unless the result is > ULOQ;

9.3.1.8.4.2.4. Indicate the evidence item analyzed;

9.3.1.8.4.2.5. Identify the instrument type used to perform the analysis;

9.3.1.8.4.2.6. Indicate the date of sample preparation for the analysis; and

9.3.1.8.4.2.7. Identify the analyst who performed the analysis.

9.3.1.8.5. The confirmation results obtained from outsourced testing shall be entered as “Other” for analyte, indicate the item(s) sent to NMS for analysis, and “See NMS Report*” for analyst. The date of analysis shall be left blank.

9.3.1.9. If any of the testing was outsourced,

9.3.1.9.1. Upload the appropriate report from the outsourced testing into the electronic case file;

9.3.1.9.2. Review the report for accuracy (e.g., appropriate test(s), associated with correct evidence item); and

9.3.1.9.3. Send the report to ToxResults.

9.3.2. If any information in 9.3.1.6 was updated during the administrative review and another report for the same case has already been released, check the released report for accuracy.

9.3.2.1. If a corrected report needs to be issued, notify a laboratory supervisor or quality assurance manager.

9.3.3. If the report being reviewed is an amended or corrected report, verify that the original report is in the electronic case file and sent to ToxResults.

9.3.4. Ensure notes are added in the following scenarios (examples provided):

9.3.4.1. Request withdrawn

9.3.4.1.1. Testing was not completed. Request for analysis was withdrawn by X (agency that withdrew the analysis request).

9.3.4.2. Partial report

9.3.4.2.1. Partial toxicology report issued as requested by X (agency that requested partial report). An amended report will be issued upon completion of testing in this case.

9.3.4.2.2. Partial toxicology report issued, and further testing canceled as requested by X (agency that requested partial report and withdrew request for further testing).

9.3.4.3. Corrected report

9.3.4.3.1. This is a corrected toxicology report for X (alcohol analysis or drug analysis). The X (original, partial, corrected, or amended) report dated X (date of original report), incorrectly listed "X" for the Z instead of "Y." See (original, partial, corrected, or amended) report.

9.3.4.3.1.1. For example, “The original report dated January 1, 2018, incorrectly listed the subject last name as X. See original report.”

9.3.4.3.1.2. If there are multiple previous reports, the language above may be modified to include references to all the previous reports.

9.3.4.4. Amended report

9.3.4.4.1. This is an amended toxicology report for X (alcohol analysis or drug analysis). The X (original, partial, or corrected) report dated X (date of report), (reason for

the amendment). See (original, partial, corrected, or amended) report.

9.3.4.4.1.1. For example, “The original report dated January 1, 2018, did not include ethanol testing for item 2-A. See original report.”

9.3.4.4.1.2. If there are multiple previous reports, the language above may be modified to include references to all the previous reports.

9.3.4.5. Broken, cracked, or leaking specimen tube

9.3.4.5.1. The specimen tube for evidence item (item number) was (leaking, cracked, broken, etc.) (upon receipt, in the laboratory, etc.). If this item was used for testing, the results may be impacted.

9.3.4.6. Contaminated Sample

9.3.4.6.1. Item(s) “Evidence Number(s)” was contaminated during preparation of sample for testing. No further analysis will be performed on this sample. Please contact ISDT if there are any questions.

9.3.5. A discrepancy between the data or case documentation and any method, policy, or manual found during administrative review shall be corrected prior to releasing the final report. The administrative reviewer shall document the following information in the case synopsis: description of discrepancy, action taken to correct the discrepancy, date of the action, and identity of person performing the action.

9.3.6. Update analysis request status to “Admin. Reviewed.”

9.3.6.1. Upon completion of administrative review of an alcohol analysis report, if both alcohol and drug analyses were requested and the case is not a priority or stat, proceed as follows:

9.3.6.1.1. If the ethanol concentration is ≥ 0.10 g/100 mL blood, verify that the request for drug analysis has been canceled, or,

9.3.6.1.2. If the ethanol concentration is < 0.10 g/100 mL blood, verify that the request for drug analysis is in progress.

9.4. Records

9.4.1. Technical Review Worksheet, however named

9.4.2. LIMS Data Verification

9.4.3. Case Synopsis notes

9.4.4. Toxicology Report – Alcohol Analysis, if applicable

9.4.5. Toxicology Report – Drug Analysis, if applicable

9.4.6. Administrative Review Checklist, if applicable

10. Appendix

10.1. Glossary

10.1.1. Actual concentration – Quantitative value obtained through testing.

10.1.2. Amended Report – A report issued to add testing results or other information to the original report.

10.1.3. Annually – Within the last 12 months (This definition applies to this document only.)

10.1.4. Analyte score – A score used in the drug screen to determine presumptive positive for an analyte. It consists of a mass accuracy score, a signal to noise score, and a retention time score. These three scores are summed to obtain an analyte score of up to 99.9999.

10.1.5. Batch LLOQ – A modified LLOQ equal to the target concentration of the lowest calibrator used to generate the calibration curve for the batch.

10.1.6. Batch ULOQ – A modified ULOQ equal to the target concentration of the highest calibrator used to generate the calibration curve for the batch.

10.1.7. Blood specimen – Whole blood, homogenate, or supernatant.

10.1.8. Certified reference material – A purchased reference material that is certified to contain specific concentration(s) of a compound or compounds and is accompanied by a Certificate of Analysis (CoA) that contains a measurement uncertainty. A CRM may be used as a calibrator or control or to prepare calibrators and controls.

10.1.9. Certified value – Quantitative value listed on a CoA.

10.1.10. Clot – A gelatinous mass formed by a complex mechanism involving red blood cells, fibrinogen, platelets, and other clotting factors.

10.1.11. Confirmation – Testing done to verify a screening result.

10.1.12. Corrected Report – A report issued to correct an error on the original report.

10.1.13. Fortified matrix sample – A blank matrix sample spiked with target analyte and/or internal standard using reference materials.

10.1.14. Intelligent sequencing – Feature of GC/MS acquisition software that automatically adjusts the sequence running to add blank samples after a sample when its quantitative result is over a predetermined threshold.

10.1.15. Instrument – an implement used to analyze samples (e.g., GC/MS, HS/GC/FID, LC/QQQ, or LC/TOF).

10.1.16. Manual integration tools – MassHunter data analysis software features that may be used for analyte identification and/or quantification, i.e., Zero Peak, Merge Right Peak, Merge Left Peak, Split Peak and Pick Left, Split Peak and Pick Right, Snap Baseline, Drop Baseline, Apply ISTD RTs to Target, and Apply Target RTs to Qualifier.

10.1.17. Mass-to-charge ratio – The mass of an ion divided by its charge, often abbreviated as m/z.

10.1.18. Matrix – Biological fluid or water.

10.1.19. May – An option.

10.1.20. Neat sample – Unextracted solvent containing analyte(s) of interest.

10.1.21. Negative blood – Blood verified by screening/confirmation to be free of analyte(s) of interest.

10.1.22. Negative blood control – Negative blood containing internal standard.

10.1.23. Negative control – Control that is free of the analyte(s) of interest, which may be made from water, negative blood, or negative serum/plasma.

10.1.24. Negative serum/plasma – Serum/plasma verified by screening/confirmation to be free of analyte(s) of interest.
10.1.25. Negative serum/plasma control – Negative serum/plasma containing internal standard.
10.1.26. Parameter – Setting on chromatography instrument or detector specific to the testing of the analyte in question.
10.1.27. Preparation Packet or Prep Packet – LIMS worklist, preparation worksheet, and Aliquot Chain of Custody. It may also include the instrument worklist or sequence table, or any other documents generated during analysis.
10.1.28. Presumptive positive – Initial result indicating the presence of an analyte of interest obtained using a screening method.
10.1.29. Retention time – The length of time required for an analyte to pass through a chromatographic column and be detected by the detector.
10.1.30. Retention time difference – The difference between the expected retention time of the analyte and the measured retention time of the analyte. The expected retention time of the analyte is corrected for each sample based upon the difference between the expected and measured internal standard retention time of the sample.
10.1.31. Sample – Specimen aliquot, calibrator, or control being prepared or ready for testing.
10.1.32. Secure electronic signature – A picture of the signature or initials and date in electronic format generated through a secure login, or name or initials added electronically as a result of a secure login.
10.1.33. Serum/plasma specimen – Serum or plasma specimen.
10.1.34. Shall – A requirement.
10.1.35. Should – A recommendation.
10.1.36. Sign or signed – Handwritten signature or initials and date (or secure electronic signature).
10.1.37. Signal to noise – Signal of the ion of interest compared to the proximal (in time) noise at that m/z using the ASTM Noise algorithm.
10.1.38. Specimen – Tube containing blood or serum/plasma collected from a subject.
10.1.39. Supernatant – Liquid lying above a solid residue after centrifugation.
10.1.40. Target concentration – Expected quantitated value.
10.1.41. ToxResults – Online program for retrieval of toxicology reports.
10.1.42. Working stock – Concentrated solution used to prepare calibrators and controls.

11. Document History

Effective Date	Version	Description of Activity or Revision	Approved By
02/01/18	1	Initial issue: Combined laboratory methods into one document. **Replaces Existing methods:** Volatiles-Headspace GC/FID Screen and Confirmation V4, Blood Drug Screening by LC/TOF V2, THC and Metabolite GC/MS Confirmation V3, Stimulant LC/QQQ Confirmation V1, Specimen and Sample Preparation V1, Instrument and Equipment Maintenance and Operation V1, Evidence V2, Drug Screen Method Enzyme-Linked Immunosorbent Assay V2, Cocaine and Metabolite GC/MS Confirmation V2, Benzodiazepines and Z-Drugs LC/QQQ Confirmation V3, and ISDT Quality Manual V1. **New methods/sections:** Method Validation, Solution Verification/Validation, Technical and Administrative Review, and Appendix	Ed Littlejohn Sheila A. Arnold, PhD
04/16/18	2	Blood Drug Screen by LC/TOF was revised significantly to reflect a new extraction, acquisition, and data processing method, which allows for inclusion of THC-COOH in the analysis. Stimulants Confirmation by LC/QQQ was updated to include prepared sample stability and reinjection stability. Instrument and Equipment Maintenance and Operation was updated to include variable wavelength detector, QC checks after PM, and more specific information for solutions used in LC/TOF tunes. Language was added to Evidence Handling, Specimen and Sample Preparation, Technical and Administrative Review to address containers received or found to be broken or leaking. Minor edits were made throughout the document.	Ed Littlejohn Sheila A. Arnold, PhD
01/28/19	3	Removed all references to immunoassays or ELISA (Deleted: 1.6, 2.4.6.1, 3.8.5, 3.8.10.1, 6, 13.2.7, 14.2.16, 14.2.63, 14.2.64, and Tables 1-8, Modified: 2.5.1.2.1, 3.8.4.2.2.1, and 14.1.12) Incorporated the following MFRs: 2018_MFR_0525 LC3 Validation for Stimulants and Benzodiazepines	Ed Littlejohn Sheila A. Arnold, PhD

Effective Date	Version	Description of Activity or Revision	Approved By
		and Z-drugs, 2018_MFR_0615 TOF Test Method Updates, 2018_MFR_0821 TOF Test Method Updates 2, 2018_MFR_0906 BNZ-Z Confirmation Calibration Model Update, 2018_MFR_1206 Test Method Solution Validation Updates, and 2018_MFR_1214 LC4 Validation and TOF Test Method Updates 3 Added additional definitions and abbreviations. Additional minor edits were made throughout the document.	
04/29/19	4	Modified: 2.5.1.1, 7.10.2.3.6, 8.10.2.3.7, 9.10.2.3.6, 10.10.2.3.7, 11.8.6.1.2.1, 11.8.6.1.2.2, 11.10.5.3, 11.10.5.3.1, 11.10.9.1, and 11.10.9.2. Added: 11.8.6.1.2.3, 11.8.6.1.2.3.1, 11.10.5.2, 11.10.9.3, 12.3.1.8, 12.3.1.8.1, and 12.3.1.8.2. Changed “value” to “concentration” when the value meant a numerical concentration.	Ed Littlejohn Sheila A. Arnold, PhD
09/25/19	5	Major changes throughout the document, including but not limited to, adding clarity in accessioning, electronic verification between LIMS worklist and specimens scanned, Non-matrix interferences (2.5.7), Ion Suppression (2.5.8), Dilution Integrity (2.5.9), Blood Drug Screen by LC/TOF (6), reinjection procedure and acceptance criteria (7, 8, 9, 10, 11), and adding the sample preparation date on the toxicology report.	Ed Littlejohn Sheila A. Arnold, PhD
08/4/20	6	MFRs that modified the Laboratory Test Methods were incorporated in this draft (2019_MFR_1115_Laboratory Test Method Updates, 2020_MFR_0409 LC QQQ Retention Time Update, 2020_MFR_0518 Drug Confirmation Method Updates, and 2020_MFR_0615 Evidence Container Disposal). The Evidence Handling section was updated for containerization of evidence, destruction of specimens and TARs, and to clarify accessioning of specimens of different draw times. The tests methods were rearranged in order to add a new test method for Opioids Drug Confirmation by LC/QQQ and to reorder the test methods into alphabetical order. Major changes to test method	Ed Littlejohn Sheila A. Arnold, PhD

Effective Date	Version	Description of Activity or Revision	Approved By
		occurred to include addition of qualitative reporting for confirmations, retesting section, and sequence nomenclature. All drug confirmation methods had negative controls acceptance criteria clarified. Additional minor edits were made throughout the document.	
5/24/22	7	MFRs that modified the Laboratory Test Methods were incorporated (2020_MFR_0925 Clonazepam alternative ISTD, 2020_MFR_1208 TOF BDS Injection Volume, 2021_MFR_0127 BNZ Injection Volume, 2021_MFR_0202 Instrument Parameter Audit, 2021_MFR_0330 Missing Evidence, and 2021_MFR_0420 Sealing Evidence) Updated mass spectrometer tune criteria for GC/MS, LC/QQQ, and LC/TOF Updated water purification system maintenance Updates added for CRMs and use in calibrator and control stock solutions Updated Evidence chapter to align with new LIMS version and include procedure for resealing evidence Clarified procedures for specimen handling of serum/plasma Added procedures for reinjection of samples in analytical methods Additional minor edits were made throughout the document	Christina Beymer
2/15/2023	8	Incorporated MFRs 2022_MFR_0615 Evidence Receiving, 2022_MFR_102822 Test Method Updates, 2022_MFR_093022 Mobile Phase, and 2022_MFR_082622 Chemical Expiration Major changes to Chapter 4, Evidence handling, to clarify and streamline receiving and accessioning. Updates throughout to align “should” with items that are not audited or verified as completed regularly. Removal of special instructions for Reinjection in all assays Updated QQQ assays to average calibrators and controls during data processing	Christina Beymer Kathleen Toomey

Effective Date	Version	Description of Activity or Revision	Approved By
11/1/2023	9	Major revisions to Section 12 – Volatile Analysis to reflect new workflow and to Section 2 – Method Validation to reflect recommendations from ASB 036 (2021). General clarification and grammatical updates throughout. Incorporated MFRs: 2023_MFR_0630 Lab Test Method Update, QA Manual Update, Testing Policy Update, 2023_MFR_0802 Laboratory Test Method Update, 2023_MFR_0831 New Customer Agreement, 2023_MFR_0414 TOF Injection Volume, 2023_MFR_0505 Lab Test Method Updates, 2023_MFR_0627 THC Test Method Updates	Christina Beymer Kathleen Toomey
1/19/24	10	Incorporating Out of Scope Testing. Minor edits, grammar, and formatting changes throughout	Christina Beymer Kathleen Toomey
4/29/24	11	Update to remove EDW and allow photos to record evidence; clarification on when a case synopsis note is required; removed VOL replicate numbers; change to TOF reconstitution solution; and minor edits for grammar and clarification throughout.	Christina Beymer Kathleen Toomey
8/26/24	12	Edits to the procedure for and documentation of contaminated samples to the note for Broken, Cracked, and Leaking Samples, and inclusion of validation studies in Reference sections of assay procedures.	Christina Beymer Kathleen Toomey
1/18/25	13	Included TOF Centrifuging Validation information, removed ruler requirement from evidence pictures, included domestic violence as a priority case type, and included opioids autosampler vial stability	Christina Beymer Kathleen Toomey
1/22/25	14	Change EDDP to qualitative reporting only.	Christina Beymer Kathleen Toomey
1/31/25	15	Change 6-Monoacetylmorphine, Codeine, Dextromethorphan, Hydrocodone, Hydromorphone, Morphine, Oxycodone, and Oxymorphone to qualitative reporting only	Christina Beymer Kathleen Toomey
3/4/25	16	Added Cannabinoids Confirmation by LC/QQQ Removed Method Validation information to stand alone documents	Christina Beymer Kathleen Toomey

Effective Date	Version	Description of Activity or Revision	Approved By
		Updated references throughout	
3/31/25	17	Removed Instrumentation and Equipment Maintenance section to standalone documents. Updated references as needed.	Christina Beymer Kathleen Toomey
7/10/25	18	Removal of Solution Verification/Validation, Specimen and Sample Preparation, and Evidence Handling Inclusion of peak filter and internal standard recovery criteria per 2025_CAR_0331 Inclusion of suggestion log items and carryover evaluation criteria	Christina Beymer Kathleen Toomey