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. Cocaine Confirmation by GC/MS

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

1.2. Precautions/Limitations
  1.2.1. Minimum Sample Requirement
    1.2.1.1. 1 mL of blood or serum/plasma specimen for quantitative confirmation
    1.2.1.2. 250 µL of blood or serum/plasma specimen for qualitative confirmation
  1.2.2. CRMs
    1.2.2.1. CRMs used for calibrator and non-zero control stocks shall be from two different vendors, if available.
    1.2.2.2. If using CRMs from the same vendor, two different lots shall be used, if available.
    1.2.2.3. If only one lot of a CRM is available, two separate vials from the lot shall be used.
  1.2.3. BSTFA hydrolyzes easily.

1.3. Related Information
  1.3.1. Cocaine Confirmatory Analysis Method Validation (October 2015-March 2016)
  1.3.2. Stock Solution Stability (February 2020)
  1.3.3. Instrument validations
  1.3.4. Validations of calibrators, controls, and internal standards data

1.4. Instruments/Equipment
  1.4.1. Tube rack
  1.4.2. Rocker
  1.4.3. Vortex, single
  1.4.4. Sonicating water bath
  1.4.5. Centrifuge
  1.4.6. Positive pressure manifold
  1.4.7. SPE column rack
  1.4.8. SPE collection rack
  1.4.9. Waste collection rack
  1.4.10. Vial rack
  1.4.11. Dry block heater
  1.4.12. Evaporator
  1.4.13. Electronic or manual crimper
  1.4.14. Gas chromatograph
  1.4.15. Mass spectrometer, single quadrupole
  1.4.16. Pipettes

1.5. Reagents/Materials
  1.5.1. Glass tubes (e.g., 13x100 mm)
  1.5.2. Trace B SPE columns, 3 mL columns, 35 mg (Tecan #TB-335C)
  1.5.3. Tube caps (e.g., 13mm flange)

1.5.4. Pipette tips
1.5.5. Autosampler vials, inserts, and caps
1.5.6. ddH_2O
1.5.7. Negative blood (human)
1.5.8. Gas chromatograph capillary column-analytical column
    1.5.8.1. Dimensions: 15 m x 0.25 mm x 0.25 µm
    1.5.8.2. Composition: DB-5 MS UI (5%-Phenyl)-methylpolysiloxane
1.5.9. Gas chromatograph capillary column-restrictor column
    1.5.9.1. Dimensions: ~0.5 m x 150 µm
    1.5.9.2. Composition: fused silica
1.5.10. BSTFA + 1% TMCS
1.5.11. CRMs
    1.5.11.1. Benzoylecgonine
    1.5.11.2. Cocaine
    1.5.11.3. Benzoylecgonine-D3
    1.5.11.4. Cocaine-D3
1.5.12. Helium, 5.0 grade or higher
1.5.13. Nitrogen
1.5.14. Solvents shall be high quality and low residue (e.g., HPLC grade, Omnisolv, Optima, etc.) unless otherwise noted.
    1.5.14.1. Ethyl acetate
    1.5.14.2. Methylene chloride
    1.5.14.3. Isopropanol
    1.5.14.4. Glacial acetic acid, ACS grade or higher
    1.5.14.5. Ammonium hydroxide, ACS grade or higher
    1.5.14.6. Methanol, ACS grade or higher
1.5.15. Sodium phosphate monobasic
1.5.16. Sodium phosphate dibasic

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. All solutions shall conform to Solution Preparation, Validation, Verification (Doc ID: 3695).
1.7.2. COC Calibrator Stock
1.7.3. COC High Calibrator
1.7.4. COC Low Calibrator
1.7.5. COC Control Stock
1.7.6. COC High Control
1.7.7. COC Low Control
1.7.8. COC Internal Standard
1.7.9. COC Elution Solution
1.7.10. Phosphate Buffer (100 mM)
1.7.11. Acetic Acid (100 mM)

1.8. Procedures/Instructions

- 1.8.1. 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.
  - 1.8.1.1. Reinjected negative controls shall be denoted with an "RI" followed by the number of reinjections.
- 1.8.2. Mix specimens on a rocker or by inverting several times.
- 1.8.3. Add 100 µL of cocaine internal standard (resulting in a concentration of 100 ng/mL) to labeled glass tubes.
- 1.8.4. Prepare calibrator and control samples in correspondingly labeled tubes as indicated in Table 1.

Table 1: 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

- 1.8.5. Pipette 1 mL of negative blood into calibrator and control samples.
- 1.8.6. Pipette 1 mL of specimen into the correspondingly labeled tube.
- 1.8.7. Add 2 mL of 100 mM phosphate buffer to each tube. Cap and vortex each tube.
- 1.8.8. Sonicate for ~10 minutes.
- 1.8.9. Centrifuge for ~10 minutes using 3000 rpm at ~4-8 ºC.
- 1.8.10. 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:
  - 1.8.10.1. 1 mL of methanol
  - 1.8.10.2. 1 mL of ddH$_2$O
  - 1.8.10.3. 1 mL of phosphate buffer
- 1.8.11. 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.
- 1.8.12. 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:
  - 1.8.12.1. 2 mL ddH$_2$O
  - 1.8.12.2. 2 mL 100 mM acetic acid
  - 1.8.12.3. 1 mL methanol
  - 1.8.12.4. 1 mL ethyl acetate

1.8.13. Using a maximum flow of ~60 psi or greater, dry the columns for ~ 20 minutes.
1.8.14. Place empty labeled tubes into the positive pressure manifold, ensuring the placement of the tubes corresponds with the arrangement of the sample columns.
1.8.15. 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.
1.8.16. Remove tubes from the positive pressure manifold and place on the evaporator.
1.8.17. Evaporate at room temperature using nitrogen.
1.8.18. Add 50 µL ethyl acetate, then 50 µL BSTFA with 1% TMCS, to each tube and cap.
1.8.19. Vortex tubes briefly.
1.8.20. Place the tubes in a dry heat block at ~ 70 ºC for ~ 25 minutes.
1.8.21. Allow the tubes to cool. Transfer each sample to the correspondingly labeled autosampler vial and cap vial.
1.8.22. Analyze the samples by GC/MS.
    1.8.22.1. Sequence names shall be in the following format: YYYY_MM_DD_COC_Initials.
        1.8.22.1.1. The date in the sequence shall be the date of preparation of the samples.
        1.8.22.1.2. Additional information such as reinjection, validation, etc., or equivalent abbreviations should be included with the assay abbreviation.
        1.8.22.1.3. 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.
    1.8.22.2. 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 1.8.22.1. The note should list which lines are attributed to each batch.
    1.8.22.3. The extracted samples shall be stored at room temperature and analyzed for cocaine and benzoylecgonine within 4 days of completion of the extraction process.
    1.8.22.4. When a sample has a cocaine or benzoylecgonine concentration > 450 ng/mL, intelligent sequencing may be used to prevent carryover into subsequent samples.
    1.8.22.5. If the instrument sequence is paused by the acquisition software between two samples, the sequence may be restarted at the sample not yet injected.
        1.8.22.5.1. Sample stability criteria shall be met.
    1.8.22.6. 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.
        1.8.22.6.1. Sample stability criteria shall be met.
        1.8.22.6.2. Reinjection of a sample of unknown concentration may be performed once.
        1.8.22.6.3. Reinjection of a sample of known concentration may be performed multiple times.

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

1.8.22.6.3.1.1. Evidentiary samples that are skipped and do not have valid data shall be reanalyzed starting at 1.8.1.

1.8.22.6.4. 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.

1.8.22.6.5. 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.

1.8.23. GC/MS Instrument Parameters

1.8.23.1. 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

1.8.23.2. Gas chromatograph inlet

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

1.8.23.3. 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

1.8.23.4. 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

1.8.23.5. 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

1.8.23.6. Auxiliary Heaters

Thermal Aux 2 (MSD Transfer Line)
Actual: On
Set Point: 300 ºC

1.8.23.7. 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.

1.8.23.8. 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

1.9. Records

1.9.1. Pipette calibration certificate, however named

- 1.9.2. Cocaine Confirmation Calibrator and Internal Standard Solution Preparation Worksheet
- 1.9.3. Cocaine Confirmation Control Solution Preparation Worksheet
- 1.9.4. Batch Preparation Packet, however named
  - 1.9.4.1. ISDT Confirmation Worklist
  - 1.9.4.2. Retest Worksheet, as appropriate
  - 1.9.4.3. Cocaine Confirmation Preparation Worksheet
  - 1.9.4.4. Aliquot Chain of Custody
- 1.9.5. Sequence Table
- 1.9.6. Quantitative Analysis Results Summary Report
- 1.9.7. Calibrator and control chromatograms
- 1.9.8. Calibration Report
- 1.9.9. Cocaine Confirmation Ion Ratio Ranges Worksheet
- 1.9.10. Sample chromatograms
- 1.9.11. Autotune
- 1.9.12. Cocaine Confirmation Technical Review Checklist
- 1.9.13. Data comparison output, however named
- 1.9.14. Measurement Uncertainty Estimation and supporting data
- 1.9.15. 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. Chromatographic analyte and internal standard peaks shall have baseline resolution and/or shall be mass resolved in the mass spectrometer.
  - 1.10.2.1. A shoulder peak shall be < 10% of analyte peak height and area in order to report a quantitative result.
- 1.10.3. Peak filters should be set between 10% and 50% of the Cal 1 response for each analyte.
- 1.10.4. Internal standard recovery should be between 50% to 200% of the average of the calibrators in the batch.
  - 1.10.4.1. Samples with recovery less than 25% or greater than 200% shall not be accepted.
  - 1.10.4.2. Samples with recovery between 25% and 50% may be accepted at analyst discretion.
- 1.10.5. Calibration and Controls Criteria
  - 1.10.5.1. Results of samples analyzed prior to analysis of the negative control preceding the calibrators shall not be used to determine acceptability of batch data.
  - 1.10.5.2. Quantitation of calibrators and non-zero controls shall be within ± 20% of the target concentration.
  - 1.10.5.3. Generating a calibration curve
    - 1.10.5.3.1. Calibration curve shall include a minimum of five non-zero concentrations.
    - 1.10.5.3.2. Correlation coefficient (r^2) for the calibration curve shall be ≥ 0.990.
    - 1.10.5.3.3. An ion ratios shall be within ± 20% of the mean ion ratio based on all calibrators used to generate the curve.

- 1.10.5.3.4. 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.
- 1.10.5.3.5. A calibration point may be excluded if any of the following occur:
  - 1.10.5.3.5.1. An ion ratio does not meet the acceptability criteria listed in 1.10.5.3.3 or 1.10.5.3.4.
  - 1.10.5.3.5.2. The correlation coefficient (r^2) for the calibration curve is < 0.990.
  - 1.10.5.3.5.3. A quantitated value is not within ± 20% of the target concentration.
  - 1.10.5.3.5.4. A peak has poor chromatography.
- 1.10.5.3.6. 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 1.8.1.
- 1.10.5.3.7. 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 1.8.1. If unable to retest, the results for the analysis may be reported as greater than the highest calibrator used in the batch.

1.10.5.4. Each negative control shall have an analyte concentration or response < 50% of the LLOQ and/or unacceptable ion ratios as specified in 1.10.5.3.3 or 1.10.5.3.4.

- 1.10.5.4.1. 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 1.8.1. A result < 50% of the LLOQ for an evidentiary sample shall be accepted as none detected.

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

- 1.10.5.5.1. If acceptance criterion is not met, all samples in the batch shall be reanalyzed, if possible, starting at 1.8.1.

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

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

- 1.10.5.7.1. An ion ratio does not meet the acceptability criteria listed in 1.10.5.3.3 or 1.10.5.3.4.

1.10.5.7.2. A quantitated value is not within ± 20% of the target concentration.
1.10.5.7.3. A peak has poor chromatography.
1.10.5.8. Each set of one to twelve samples shall be bracketed by one low and one high non-zero control.
1.10.5.8.1. 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 1.8.1. 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 1.10.5.4 and 1.10.5.4.1.
1.10.6. If the analyte in a sample has a result > 2x ULOQ, evaluate the analyte in the subsequent sample(s) as follows:
1.10.6.1. Intelligence sequencing may be used to satisfy the following requirements.
1.10.6.2. If a > 2x ULOQ sample is immediately followed by an evidentiary sample in which the analyte is not detected, data should be accepted.
1.10.6.3. 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.
1.10.6.3.1. Additional negative controls or solvent blanks may be added to the sequence immediately following the > 2x ULOQ sample to eliminate carryover in subsequent samples.
1.10.6.4. If a > 2x ULOQ sample is immediately followed by a control and the control passes acceptance criteria, data should be accepted.
1.10.6.5. 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.
1.10.6.5.1. Additional negative controls or solvent blanks may be added to the sequence immediately following the > 2x ULOQ sample to eliminate carryover in subsequent samples.
1.10.6.6. If the MassHunter software does not provide a calculated result, the RR for the sample may be compared to the RR of Cal 8.
1.10.7. Analyte Identification (Qualitative Criteria)
1.10.7.1. Retention time shall be within ± 0.25 minutes of the mean retention time based on all calibrators used to generate the curve.
1.10.7.2. Each analyte shall have one quantitative ion and two qualitative ions monitored.
1.10.7.3. Each internal standard shall be present and have one quantitative ion and one qualitative ion monitored.
1.10.7.4. Each ion ratio shall meet the acceptability criteria listed in 1.10.5.3.3 or 1.10.5.3.4.
1.10.7.4.1. 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.

1.10.7.5. 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.

1.10.8. Analyte Stability

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

1.10.9. Retesting Samples

1.10.9.1. 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.

1.10.9.1.1. If a quantitative value cannot be reported from any analysis, the first acceptable qualitative data according to analyte identification in 1.10.6 shall be used. (ref. 1.11.4).

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

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 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”).

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”).

1.11. Report Writing

1.11.1. 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.

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

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

1.11.3. Quantitative Reporting

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

1.11.3.1.1. 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.

1.11.3.2. 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.

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

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

1.11.3.3. 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.

1.11.3.3.1. 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.

1.11.3.3.1.1. 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.

1.11.3.4. A quantitative result shall only be reported if analysis occurred within the established sample stability window (ref. 1.10.8).

1.11.3.5. 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.

1.11.4. Qualitative Reporting

1.11.4.1. A result should be reported as “Positive” when the analyte identification criteria (ref. 1.10.6) have been met, the quantitative result is > LLOQ, and the quantitative criteria have not been met.

1.11.4.1.1. 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.

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

1.11.4.2. 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).

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

1.11.4.3.1. Interference(s); or

1.11.4.3.2. 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.

1.12. References

1.12.1. 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.

1.12.2. 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.

1.12.3. United Chemical Technologies Applications Manual (2004).

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

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

2. Opioids Drug Confirmation by LC/QQQ

2.1. Scope
  2.1.1. This method shall be used for confirmation analysis of specimens requiring confirmation of opioids. Sample preparation shall be by SLE.

2.2. Precautions/Limitations
  2.2.1. Minimum Sample Requirement
    2.2.1.1. 0.200 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.
  2.2.3. Mobile phases should be kept in amber bottles to increase stability.

2.3. Related Information
  2.3.1. Opioids Confirmation Method Validation (November 2019 – May 2020)
  2.3.2. Stability and Reinjection (2022)
  2.3.3. Instrument validations
  2.3.4. 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
  2.4.4. 96-well plate positive pressure manifold
  2.4.5. 96-well plate evaporator
  2.4.6. Liquid chromatograph
  2.4.7. Mass spectrometer, triple quadrupole
  2.4.8. Pipettes

2.5. Reagents/Materials
  2.5.1. ToxBox 96-well plate (Opioids)
  2.5.2. ISOLUTE SLE 96-well plate (Biotage: SLE-B96)
  2.5.3. 2 mL 96-well collection plate and cover
  2.5.4. 96-well plate with vial inserts and cover
  2.5.5. Autosampler vials, inserts, and caps
  2.5.6. Pipette tips
  2.5.7. ddH_2O
  2.5.8. Negative blood (human)
  2.5.9. Liquid chromatograph column
    2.5.9.1. Dimensions: 2.1 mm x 100 mm
    2.5.9.2. Composition: Phenyl Hexyl, 2.7 µm particles
  2.5.10. Liquid chromatograph guard column
    2.5.10.1. Dimensions: 2.1 mm x 5 mm
    2.5.10.2. Composition: Phenyl Hexyl, 2.7 µm particles
  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. MTBE, ACS grade or higher
  2.5.12.3. Formic acid
2.5.13. Sodium carbonate
2.5.14. Sodium bicarbonate

Table 2: 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

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. Solutions

- 2.7.1. All solutions shall conform to Solution Preparation, Validation, Verification (Doc ID: 3695).
- 2.7.2. Carbonate Buffer (300 mM)
- 2.7.3. Mobile Phases
  - 2.7.3.1. Aqueous (A)
  - 2.7.3.2. Organic (B)
- 2.7.4. OPI 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. Pipette 200 µL of negative blood into each calibrator and control well on the ToxBox plate.
- 2.8.4. Pipette 200 µL of specimen into the corresponding well on the ToxBox plate.
- 2.8.5. Add 200 µL of carbonate buffer to each well of the ToxBox plate.
- 2.8.6. Vortex ToxBox plate.
- 2.8.7. Transfer 400 µL from each well of the ToxBox plate to the corresponding well of the SLE plate.
- 2.8.8. Allow samples to load onto the SLE plate for ~5 – 10 minutes. Positive pressure (<25 psi) may be applied, if necessary.
- 2.8.9. 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.
- 2.8.10. Repeat the previous elution with an additional 1 mL aliquot of MTBE.
- 2.8.11. Remove collection plate from the positive pressure manifold and place on the evaporator.
- 2.8.12. Evaporate at room temperature using nitrogen.
- 2.8.13. Add 150 µL of reconstitution solution to each well of the collection plate.
- 2.8.14. Cap collection plate and vortex.
- 2.8.15. Transfer each sample to the corresponding well within the 96-well plate with vial inserts or into labeled autosampler vials.
- 2.8.16. Cap the 96-well vial plate (or autosampler vial) and move it to the LC/QQQ for analysis.
- 2.8.17. Analyze the samples by LC/QQQ.
  - 2.8.17.1. Sequence names shall be in the following format: YYYY_MM_DD_OPI_Initials.
    - 2.8.17.1.1. The date in the sequence shall be the date of preparation of the samples.

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

2.8.17.1.3. 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.

2.8.17.2. 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 2.8.17.1. The note should list which lines are attributed to each batch.

2.8.17.3. 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.

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

2.8.17.4.1. Sample stability criteria shall be met.

2.8.17.5. 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.

2.8.17.5.1. Sample stability criteria shall be met.

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

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

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

2.8.17.5.3.1.1. Evidentiary samples that are skipped and do not have valid data shall be reanalyzed starting at 2.8.1.

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

2.8.17.5.5. 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.

2.8.18. LC/QQQ Acquisition Parameters

2.8.18.1. Liquid chromatograph sampler

Needle wash	Standard wash

Injection Volume 1 µL

2.8.18.2. 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

2.8.18.3. Liquid chromatograph column compartment
Temperature 55 ºC

2.8.18.4. Mass spectrometer
Ion Source AJS ESI
Scan Type Dynamic MRM

2.8.18.5. dMRM Parameters
MS1 Resolution Unit/Enh (6490)
MS2 Resolution Unit/Enh (6490)
Cell Acc. 5 V
Polarity Positive

Table 3: 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.

2.8.18.6. Quantitation Parameters

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

2.9. Records

2.9.1. Pipette calibration certificate, however named

2.9.2. Batch Preparation Packet

        - 2.9.2.1. ISDT Confirmation Worklist
        - 2.9.2.2. Retest Worksheet, as appropriate
        - 2.9.2.3. Opioids Drug Confirmation Preparation Worksheet
        - 2.9.2.4. Aliquot Chain of Custody
        - 2.9.2.5. Opioids Drug Confirmation Plate Layout Worksheet
    - 2.9.3. MassHunter Worklist Report
    - 2.9.4. MassHunter Ion Ratio and RRT Verification, however named
    - 2.9.5. QA/QC Packet, however named
        - 2.9.5.1. Batch summary
        - 2.9.5.2. Analyte calibration curves
        - 2.9.5.3. Calibrator and control chromatograms
    - 2.9.6. Sample chromatograms
    - 2.9.7. QQQ Check Tune Report
    - 2.9.8. Opioids Drug Confirmation Technical Review Checklist
    - 2.9.9. Data comparison output, however named.
    - 2.9.10. Measurement Uncertainty Estimation and supporting data
    - 2.9.11. 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 for quantitatively reported analytes (see Table 41) 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.
        - 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 calibrators and controls used to generate the curve.

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 analytical results for negative controls in the batch pass 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 calibrators and controls used to generate the curve.
- 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: Opioids MS Parameters 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.
- 2.10.7.5. 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 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.
- 2.10.8.2. 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.

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 opioids analysis is equal to the LLOQ for each analyte. The LLOQ and ULOQ are listed in Table 41: Opioids LLOQ and ULOQ

Table 41: 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.

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 of Group 2 and Group 3 Analytes (Except EDDP)

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 results between the target concentration of the lowest and highest calibrators that meet acceptability criteria.

2.11.3.2.1. Results 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 two decimal places for quantitative values less than 1.

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

2.11.3.2.1.3. A result shall be reported as a whole number for quantitative values 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 used to generate the curve and controls 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. 2.10.8).
- 2.11.3.5. If a specimen is analyzed more than once, the first quantitative result that meets quantitative acceptability criteria for a specific analyte shall be reported.

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

- 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 used to generate the curve and controls.

2.11.5. Qualitative Reporting of Group 1 Analytes and EDDP

- 2.11.5.1. A result shall be reported as “Positive” when the analyte identification criteria (ref. 2.10.7) have been met, the quantitative result is > LLOQ, and the calibrator and control criteria (ref. 2.10.5.) have been met.
  - 2.11.5.1.1. If quantitative criteria have not been met, an analyte may be reported “Positive” with supervisory approval.
  - 2.11.5.1.2. 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.
    - 2.11.5.1.2.1. The preparation date of last analysis shall be used as the analysis date.
- 2.11.5.2. A result may be reported as “Positive” with supervisory approval if any of the following occur:
  - 2.11.5.2.1. Interference(s); or

2.11.5.2.2. 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.

2.12. References

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

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

3. 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

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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
12/4/2025	19	Removal of BNZ, CAN, STM, and VOL testing methods and Technical and Administrative Review to stand alone test methods in QMS. Archival of Blood Drug Screening by LC/TOF and Cannabinoids on GC/MS,	Christina Beymer Kathleen Toomey