Indiana State Department of Toxicology

Laboratory Test Methods

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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. Instrument and Equipment Maintenance and Operation

1.1. Scope

1.1.1. This procedure shall be used to perform maintenance at the time of operation and preventive maintenance. This procedure shall be followed to ensure proper operating conditions of equipment and instrumentation used for preparation and analysis of specimens and samples.

1.2. GC/MS

1.2.1. Operating Maintenance

1.2.1.1. All maintenance performed should be documented in the instrument maintenance log.

1.2.1.2. Autotune

1.2.1.2.1. An autotune shall be performed within 24 hours of initiation of a sequence containing one or more analytical batches.

1.2.1.2.1.1. The tune report shall be electronically signed by the analyst preparing an analytical batch for analysis.

1.2.1.2.2. Autotune parameters should be noted in the maintenance log.

1.2.1.2.3. Autotune Parameter Specifications

1.2.1.2.3.1. Tuning Compound: PFTBA

1.2.1.2.3.2. Repeller Range: 10-35 V

1.2.1.2.3.3. Electron Multiplier Voltage: max 2900V

1.2.1.2.3.4. Turbo pump: 100%

1.2.1.2.3.5. High vacuum: $\leq 5x10^{-5}$ Torr

1.2.1.2.3.6. Mass Assignments and Relative Abundances of the Tune Masses:

1.2.1.2.3.6.1. 69.0 ± 0.20 m/z ≥ 100

1.2.1.2.3.6.2. 219.0 ± 0.20 m/z ≥ 40

1.2.1.2.3.6.3. 502.0 ± 0.20 m/z ≥ 2.4

1.2.1.2.3.6.4. Base peak shall be 69.0 ± 0.20 m/z or 219.0 ± 0.20 m/z

1.2.1.2.3.7. Peak Width Range: 0.5 – 0.7 m/z

1.2.1.2.3.8. Isotope Ratios of the Tune Masses:

1.2.1.2.3.8.1. 70 m/z: 0.5-1.60%

1.2.1.2.3.8.2. 220 m/z: 3.2-5.4%

1.2.1.2.3.8.3. 503 m/z: 7.9-12.3%

1.2.1.2.3.9. Number of peaks in the spectrum scan: ≤ 400 (preferably ≤ 200)

1.2.1.2.3.10. Tune mass peaks are Gaussian in shape

1.2.1.2.3.11. Air/water abundances: ≤ 10%

1.2.1.3. Carrier gas supply
1.2.1.3.1. Carrier gas (helium) pressure should be recorded in the maintenance log and should be > 200 psi for an instrument to run an overnight batch.
1.2.1.4. Solvent wash vials
1.2.1.4.1. Solvent wash vials should be filled with ethyl acetate at or above the minimum solvent line.
1.2.1.5. Syringe
1.2.1.5.1. The autosampler syringe should be checked to ensure proper operation. Replace syringe as necessary.

1.2.2. Periodic and Preventive Maintenance
1.2.2.1. All maintenance performed shall be documented in the instrument maintenance log.
1.2.2.2. A PM should be performed on each GC/MS instrument at least annually. The PM shall be performed by the instrument manufacturer, an instrument service agency, or ISDT staff. An instrument shall only be used for casework if it has had a PM within the last 12 months.
1.2.2.2.1. Following the PM, a QC check for each assay shall be performed prior to using the instrument for analysis of evidentiary samples using that assay.
1.2.2.2.1.1. A QC check shall include all calibrators used to generate the calibration curve and two replicates of each control used by the applicable method for the assay.
1.2.2.2.1.1.1. Neat or extracted samples may be used.
1.2.2.2.1.1.2. Only extracted samples may be used for QC checks for the Cannabinoids Confirmation by GC/MS method.
1.2.2.2.1.1.3. The analytical data shall pass all applicable requirements for the method.
1.2.2.3. The frequency of periodic maintenance will depend on the frequency of instrument use and may vary.
1.2.2.3.1. A check shall be performed following maintenance other than listed in 1.2.
1.2.2.3.1.1. The check shall be appropriate for the maintenance performed and at the discretion of the analyst, supervisor, or quality assurance manager.
1.2.2.3.1.2. A check may be performed concurrently with casework, if appropriate.

1.2.2.4. If an instrument is turned off and there is a loss of vacuum for the MS, a QC check for each assay shall be performed prior to using the instrument for analysis of evidentiary samples using that assay (ref. 1.2.2.2.1.1.)

1.2.2.5. GC inlet

1.2.2.5.1. Single taper splitless inlet liners (4 mm) with glass wool should be used in the GC inlet.

1.2.2.5.2. Inlet liners, septa, and gold seals should be replaced monthly or as necessary (e.g., when poor chromatography occurs).

1.2.2.5.3. The GC inlet should be cleaned periodically and/or during a PM.

1.2.2.6. GC column

1.2.2.6.1. Instruments with backflush enabled have two columns installed: the analytical column and a fused-silica non-analytical column.

1.2.2.6.2. Columns should be replaced when chromatography is poor or as necessary.

1.2.2.6.2.1. A QC check for each assay shall be performed following replacement of the analytical column (ref. 1.2.2.2.1.1) prior to using the instrument for analysis of evidentiary samples.

1.2.2.6.3. Column ends may be trimmed periodically or as necessary.

1.2.2.6.3.1. Column lengths listed in the methods are approximate due to column trimming.

1.2.2.6.3.2. A retention time check to verify the retention time for each analyte shall be performed for each assay and prior to using the instrument for analysis of evidentiary samples.

1.2.2.6.3.2.1. A retention time check includes one calibrator or control that may be an extracted or a neat sample.

1.2.2.7. Carrier gas filter

1.2.2.7.1. Gas filter should be replaced when desiccant indicates it needs to be replaced, and/or during a PM.

1.2.2.8. Rough pump

1.2.2.8.1. The rough pump ballast valve, if so equipped, should be purged periodically (~weekly). Open valve until oil drains (~2-5 minutes) and close valve.

1.2.2.8.2. The oil in the rough pump should be checked periodically to ensure the level is acceptable and that the oil is not saturated or dirty.

1.2.2.8.3. The oil in the rough pump should be drained and replenished as needed, and/or during a PM.

1.2.2.9. Ion source

1.2.2.9.1. The ion source may be cleaned on a periodic basis, when autotune results are unacceptable, when chromatography quality is poor, and/or during a PM.

1.2.2.9.1.1. A QC check for each assay shall be performed following cleaning and/or replacement of the ion source (ref. 1.2.2.2.1.1) prior to using the instrument for analysis of evidentiary samples.

1.2.2.10. Solvent wash vials

1.2.2.10.1. Solvent wash vials should be emptied and washed monthly.

1.2.2.10.2. Vial septa should be replaced as necessary.

1.3. LC/QQQ

1.3.1. Operating Maintenance

1.3.1.1. All maintenance performed should be documented in the instrument maintenance log.

1.3.1.2. LC should be purged prior to operation.

1.3.1.2.1. Open purge valve.

1.3.1.2.2. Purge flow for ~15-20 column volumes.

1.3.1.2.3. Reduce flow to instrument method flow (if at a high flow) and close purge valve.

1.3.1.3. Spray chamber should be checked and cleaned when necessary.

1.3.1.3.1. Wipe down chamber with solvent (e.g., 50:50 isopropanol to water).

1.3.1.4. Mobile phase

1.3.1.4.1. Ensure the volume of mobile phase is sufficient for the sequence.

1.3.1.4.2. Mobile phases should be made as needed, but fresh aqueous mobile phase should be made at least monthly and the old aqueous mobile phase discarded.

1.3.1.4.2.1. Mobile phase bottles should be emptied and cleaned monthly.

1.3.1.4.3. Reset mobile phase meter in instrument software.

1.3.1.4.3.1. Acquisition → Instrument Status Panel → Binary Pump

1.3.1.5. Tune

1.3.1.5.1. A check tune shall be performed using ESI-L Low Concentration Tuning Mix (Part Number G1969-85000 or equivalent) in each applicable ion mode (positive and/or negative) within 24 hours of initiation of a sequence containing one or more analytical batches.

1.3.1.5.1.1. The tune report shall be electronically signed by the analyst preparing an analytical batch for analysis.

1.3.1.5.1.2. All deltas shall be flagged as “Pass.”

1.3.1.5.1.2.1. The m/z delta shall be within the following tolerances for each width listed below:

Unit	0.10
Wide	0.30
Widest	0.50

1.3.1.5.1.2.2. The FWHM delta shall be within the following tolerances for each width listed below:

Unit	0.14
Wide	0.60
Widest	1.25

1.3.1.6. Nitrogen collision gas

1.3.1.6.1. Nitrogen tank shall be replaced when empty.

1.3.1.7. Nitrogen nebulization gas

1.3.1.7.1. If nitrogen is supplied by a nitrogen generator, the generator should be verified to be in normal working order prior to use (ref. 1.8).

1.3.1.7.2. If nitrogen is supplied by a nitrogen tank, the pressure in the tank should be recorded in the maintenance log.

1.3.2. Periodic and Preventive Maintenance

1.3.2.1. All maintenance performed shall be documented in the instrument maintenance log.

1.3.2.2. A PM should be performed on each LC/QQQ at least annually. The PM shall be performed by the instrument manufacturer, an instrument service agency, or ISDT staff. An instrument shall only be used for casework if it has had a PM within the last 12 months.

1.3.2.2.1. Following the PM, a QC check for each assay shall be performed prior to using the instrument for analysis of evidentiary samples using that assay.

1.3.2.2.1.1. A QC check shall include all calibrators used to generate the calibration curve and two replicates of each control used by the applicable method for the assay.

1.3.2.2.1.1.1. Neat or extracted samples may be used.

1.3.2.2.1.1.2. The analytical data shall pass all applicable requirements for the method.

1.3.2.3. The frequency of periodic maintenance depends on the frequency of instrument use and may vary.

1.3.2.3.1. A check shall be performed following maintenance other than listed in 1.3.
1.3.2.3.1.1. The check shall be appropriate for the maintenance performed and at the discretion of the analyst, supervisor, or quality assurance manager.
1.3.2.3.1.2. A check may be performed concurrently with casework, if appropriate.
1.3.2.4. If an instrument is turned off and there is a loss of vacuum for the QQQ, a QC check for each assay shall be performed prior to using the instrument for analysis of evidentiary samples using that assay (ref. 1.3.2.2.1.1)
1.3.2.5. An autotune should be performed as needed and/or during a PM.
1.3.2.6. Rough pump
1.3.2.6.1. The rough pump ballast valve, if so equipped, should be purged periodically (~weekly). Open valve until oil drains (~2-5 minutes) and close valve.
1.3.2.6.2. The oil in the rough pump should be checked periodically to ensure the level is acceptable and that the oil is not saturated or dirty.
1.3.2.6.3. The oil in the rough pump should be drained and replenished as needed, and/or during a PM.
1.3.2.7. Analytical and guard columns
1.3.2.7.1. Analytical and guard columns should be replaced periodically and/or when chromatography quality is poor.
1.3.2.7.1.1. A retention time check to verify the retention time for each analyte should be performed for each assay and prior to using the instrument for analysis of evidentiary samples.
1.3.2.7.1.1.1. A retention time check includes one calibrator or control that may be an extracted or a neat sample.
1.3.2.8. Variable Wavelength Detector, if applicable
1.3.2.8.1. The deuterium lamp should be replaced as a part of a PM, when noise or drift is observed, or the lamp does not ignite.

1.4. LC/TOF
1.4.1. Operating Maintenance
1.4.1.1. All maintenance performed should be documented in the instrument maintenance log.
1.4.1.2. LC should be purged prior to operation.
1.4.1.2.1. Open purge valve.

1.4.1.2.2. Purge flow for ~15-20 column volumes.
1.4.1.2.3. Reduce flow to instrument method flow (if at a high flow) and close purge valve.
1.4.1.3. Spray chamber should be checked and cleaned when necessary.
1.4.1.3.1. Wipe down chamber with solvent (e.g., 50:50 isopropanol to water).
1.4.1.3.2. Source components should be removed, sonicated, cleaned, and polished, as necessary.
1.4.1.4. Mobile phase
1.4.1.4.1. Ensure the volume of mobile phase is sufficient for the sequence.
1.4.1.4.2. Mobile phases should be made as needed, but fresh aqueous mobile phase should be made at least monthly and the old aqueous mobile phase discarded.
1.4.1.4.2.1. Mobile phase bottles should be emptied and cleaned monthly.
1.4.1.4.3. Reset mobile phase meter in instrument software.
1.4.1.4.3.1. Acquisition → Instrument Status Panel → Binary Pump
1.4.1.5. Tune and mass calibration
1.4.1.5.1. A tune shall be performed in the 4 GHz high resolution mode in positive and/or negative ion mode using positive or negative tune mix, as appropriate, within 24 hours of initiation of a sequence containing one or more analytical batches.
1.4.1.5.1.1. The tune report shall be electronically signed by the analyst preparing an analytical batch for analysis.
1.4.1.5.1.2. The absolute value of each delta ppm or corrected residual shall be ≤ 1.0.
1.4.1.5.1.3. Mass calibration occurs as a component of a tune.
1.4.1.5.2. Prior to tune and mass calibration, verify there is sufficient volume (~5 mL) of the positive and negative tune mix to complete the tune. If not, prepare mixes and record preparation in solution log.
1.4.1.5.2.1. Positive Tune Mix preparation
1.4.1.5.2.1.1. For example, combine 85.5 mL acetonitrile, 4.5 mL water, 10 mL ESI-L Low Concentration Tuning Mix (Agilent Part Number G1969-85000 or equivalent), and 5 µL 0.1 mM Hexamethoxyphosphazine (HP-0321). Additional betaine

should be added until an abundant ion at m/z 118.08 is produced.

1.4.1.5.2.2. Negative Tune Mix preparation

1.4.1.5.2.2.1. For example, combine 95.6 mL acetonitrile, 1.9 mL water, 2.5 mL ESI-L Low Concentration Tuning Mix (Agilent Part Number G1969-85000 or equivalent).

1.4.1.5.3. Verify there is sufficient volume (~100 mL) of the reference solution mix to complete the analysis of the analytical batch. If not, prepare reference solution mix and record preparation in solution log.

1.4.1.5.3.1. For example, combine 950 mL acetonitrile, 50 mL ddH_2O, 1.0 mL 100 mM TFANH_4, 0.8 mL 2.5 mM Hexakis(1H,1H,3H-tetrafluoropropoxy) phosphazine (HP-0921), and 2.0 mL 5.0 mM purine.

1.4.1.6. Nitrogen supply

1.4.1.6.1. If nitrogen is supplied by a nitrogen generator, the generator should be verified to be in normal working order prior to use (ref. 1.8).

1.4.1.6.2. If nitrogen is supplied by a nitrogen tank, the pressure in the tank should be recorded in the maintenance log.

1.4.2. Periodic and Preventative Maintenance

1.4.2.1. All maintenance performed shall be documented in the instrument maintenance log.

1.4.2.2. A PM should be performed on each LC/TOF at least annually. The PM shall be performed by the instrument manufacturer, an instrument service agency, or ISDT staff. An instrument shall only be used for casework if it has had a PM within the last 12 months.

1.4.2.2.1. Following the PM, a QC check shall be performed prior to using the instrument for analysis for evidentiary samples using that assay.

1.4.2.2.1.1. A QC check shall include two cutoff controls from a previous screening batch.

1.4.2.2.1.1.1. If a previous screening batch is not available, the QC check may be performed concurrently with casework.

1.4.2.2.1.1.2. Neat or extracted samples may be used.

1.4.2.2.1.1.3. The analytical data shall pass all applicable requirements for the method.

1.4.2.3. The frequency of periodic maintenance will depend on the frequency of instrument use and may vary.

1.4.2.3.1. A check shall be performed following maintenance other than listed in 1.4.

1.4.2.3.1.1. The check shall be appropriate for the maintenance performed and at the discretion of the analyst, supervisor, or quality assurance manager.

1.4.2.3.1.2. A check may be performed concurrently with casework, if appropriate.

1.4.2.4. If an instrument is turned off and there is a loss of vacuum for the TOF, a QC check for each assay shall be performed prior to using the instrument for analysis of evidentiary samples using that assay (ref. 1.4.2.2.1.1).

1.4.2.5. A tune should be performed as needed and/or during a PM.

1.4.2.6. Rough pump

1.4.2.6.1. The rough pump ballast valve, if so equipped, should be purged periodically (~weekly). Open valve until oil drains (~2-5 minutes) and close valve.

1.4.2.6.2. The oil in the rough pump should be checked periodically to ensure the level is acceptable and that the oil is not saturated or dirty.

1.4.2.6.3. The oil in the rough pump should be drained and replenished as needed, and/or during a PM.

1.4.2.7. Analytical and guard columns

1.4.2.7.1. Analytical and guard columns should be replaced periodically and/or when chromatography quality is poor.

1.5. HS/GC/FID

1.5.1. Operating Maintenance

1.5.1.1. All maintenance performed should be documented in the instrument maintenance log.

1.5.1.2. Supply of gases should be checked and documented in the instrument maintenance log.

1.5.1.2.1. Carrier gas supply

1.5.1.2.1.1. Carrier gas (helium) pressure should be recorded in the maintenance log and should be > 500 psi for an instrument to run an overnight batch.

1.5.1.2.2. Air supply

1.5.1.2.2.1. Air may be supplied by either an air generator or a tank.

1.5.1.2.2.2. Prior to use, verification should be made that an air generator is in normal working order (ref. 1.10).

1.5.1.2.2.3. When air is supplied by tanks, the pressure should be recorded in the maintenance log.

1.5.1.2.3. Hydrogen supply

1.5.1.2.3.1. Hydrogen is supplied by a hydrogen generator. Prior to use, it should be verified that the generator is in normal working order (ref. 1.9).

1.5.2. Periodic and Preventive Maintenance

1.5.2.1. All maintenance performed shall be documented in the instrument maintenance log.

1.5.2.2. A PM should be performed on each HS/GC/FID instrument at least annually. The PM shall be performed by the instrument manufacturer, an instrument service agency, or ISDT staff. An instrument shall only be used for casework if it has had a PM within the last 12 months.

1.5.2.2.1. Following the PM, a QC check shall be performed prior to using the instrument for analysis of evidentiary samples using that assay.

1.5.2.2.1.1. A QC check shall include all calibrators used to generate the calibration curve, three replicates of a high concentration mixed volatiles control, and three replicates of a low concentration mixed volatiles control.

1.5.2.2.1.1.1. The analytical data shall pass all applicable requirements for the method.

1.5.2.3. The frequency of periodic maintenance depends on the frequency of instrument use and may vary between instruments.

1.5.2.3.1. A check shall be performed following maintenance other than listed in 1.5.

1.5.2.3.1.1. The check shall be appropriate for the maintenance performed and at the discretion of the analyst, supervisor, or quality assurance manager.

1.5.2.3.1.2. A check may be performed concurrently with casework, if appropriate.

1.5.2.4. GC column

1.5.2.4.1. Column should be replaced when chromatography is poor or as necessary.

1.5.2.4.1.1. A QC check for each assay shall be performed following replacement of the analytical column (ref. 1.5.2.2.1.1) prior to using the instrument for analysis of evidentiary samples.

1.5.2.4.2. Column ends may be trimmed periodically or as necessary.

1.5.2.4.2.1. Column lengths listed in the methods are approximate due to column trimming.

1.5.2.4.2.2. A retention time check to verify the retention time for each analyte shall be performed for each assay and prior to using the instrument for analysis of evidentiary samples.

1.5.2.4.2.2.1. A retention time check includes one calibrator or control.

1.6. Pipettes and Auto-Diluters

1.6.1. Operating Maintenance

1.6.1.1. Pipettes and auto-diluters should be wiped with a solvent (e.g., bleach, methanol, isopropanol) prior to and after use to prevent contamination.

1.6.1.2. A pipette should not be placed on its side with a wet tip affixed to the pipette.

1.6.1.3. Auto-diluter probes and tubing should be replaced as needed.

1.6.1.4. Prior to use of a pipette or auto-diluter, ensure that documentation of its calibration has not expired.

1.6.1.5. Serial numbers of pipettes and auto-diluters used for casework shall be documented on batch preparation worksheets.

1.6.1.6. Pipettes and auto-diluters suspected or found not to meet manufacturer specifications shall be documented on the appropriate maintenance log, and the auto-dilutor or pipette shall be labeled as out of service and shall not be used for casework until serviced and/or calibrated.

1.6.1.6.1. A laboratory supervisor and/or quality assurance manager should be notified of any pipette or auto-dilutor that is taken out of service.

1.6.1.7. Auto-diluter probe tips shall be rinsed after each aliquot (e.g., rinse tubing with 10% bleach solution followed by ddH_2O).

1.6.1.7.1. If the probe tip or tubing is changed, it should be documented on the maintenance log.

1.6.2. Periodic and Preventive Maintenance

1.6.2.1. Pipettes and auto-diluters used for critical measurements should be calibrated twice annually by a third-party vendor.

1.6.2.1.1. Pipette calibration documentation shall identify the pipette by serial number, identify the date of calibration, and report the calibration of the pipette

prior to maintenance (e.g., “as found”), if possible, and after each adjustment, if any (e.g., “as left,” “as calibrated”).

1.6.2.1.2. Auto-dilutor calibration documentation shall identify the auto-diluter by serial number, identify the date of calibration, and report the calibration of the auto-dilutor prior to maintenance (e.g., “as found”), if possible, and after maintenance, if any (e.g., “as left” or “as found”).

1.6.2.1.2.1. A calibration shall be performed if a syringe is changed.

1.6.2.2. Calibration documentation shall be maintained on a network drive.

1.6.2.3. Pipettes and auto-diluters shall be labeled with the date of the last calibration and the due date for the next calibration.

1.7. pH Meters

1.7.1. Operating Maintenance

1.7.1.1. pH meters shall be calibrated prior to each use using a minimum of two buffer solutions. The pH of buffer solutions used should encompass the desired pH to be measured.

1.7.1.2. pH meter calibrations shall be documented in the buffer log.

1.7.1.3. Electrodes shall be stored in electrode storage solution when not in use.

1.7.1.4. Electrodes shall be filled with electrode solution.

1.7.1.5. See individual manual for further information.

1.7.2. Periodic and Preventive Maintenance

1.7.2.1. Electrodes should be periodically checked for cracks and electrode solution level.

1.7.2.2. Any solids that accumulate in an electrode storage bottle or on an electrode surface should be cleaned with ddH_2O.

1.8. Nitrogen Generators

1.8.1. Operating Maintenance

1.8.1.1. Nitrogen generators are used to supply nitrogen to the LC/QQQ, the LC/TOF, and the laboratory hoods. Generators should be inspected prior to use to ensure they are in proper working order.

1.8.1.2. See individual generator manuals for further information.

1.8.2. Periodic and Preventive Maintenance

1.8.2.1. A PM should be performed annually. The PM shall be performed by the instrument manufacturer, an instrument service agency, or ISDT staff.

1.8.2.1.1. The PM consists of replacing the filter cartridges, activated carbon filter, and oxygen sensor, if applicable.

1.8.2.2. The air compressor should be replaced as necessary or during a PM, if applicable.

1.8.2.3. Any maintenance should be documented on the generator maintenance log.

1.9. Hydrogen Generators

1.9.1. Daily Maintenance and Operation

1.9.1.1. Hydrogen generators are used to supply hydrogen to the HS/GC/FID instruments. Generators should be inspected prior to use to ensure they are in proper working order (e.g., checking the water level).
1.9.1.2. See individual generator manuals for further information.
1.9.2. Periodic and Preventive Maintenance
1.9.2.1. A PM should be performed annually. The PM shall be performed by the instrument manufacturer, an instrument service agency, or ISDT staff.
1.9.2.1.1. The PM consists of replacing the filters, cartridges, electrolyte solution, desiccant, and deionizer, if applicable.
1.9.2.2. Any maintenance should be documented on the generator maintenance log.

1.10. Air Generators
1.10.1. Operating Maintenance
1.10.1.1. Air generators are used to supply air to the HS/GC/FID instruments. Generators should be inspected prior to use to ensure they are in proper working order.
1.10.1.2. See individual generator manuals for further information.
1.10.2. Periodic and Preventive Maintenance
1.10.2.1. A PM should be performed annually. The PM shall be performed by the instrument manufacturer, an instrument service agency, or ISDT staff.
1.10.2.1.1. The PM consists of replacing the filters and cartridges.
1.10.2.2. The catalyst module should be replaced when indicated by the generator.
1.10.2.3. Fuses may be replaced as necessary.
1.10.2.4. Any maintenance should be documented on the generator maintenance log.

1.11. Water Purification System
1.11.1. Operating Maintenance
1.11.1.1. Prior to first use of water purification system each day, allow water to flow and document water quality (resistivity, total organic carbon, and temperature) on the maintenance log.
1.11.1.2. See individual manual for further information.
1.11.2. Periodic and Preventive Maintenance
1.11.2.1. The system should be sanitized once per year. Refer to the manual for instructions.
1.11.2.2. UV lamp and standard filter should be replaced or cleaned when prompted by an LCD message or as necessary.
1.11.2.3. Any maintenance should be documented on the maintenance log.

1.12. Chemical Hoods
1.12.1. Operating Maintenance
1.12.1.1. Workspace in each chemical hood should be cleaned before and after each use.

1.12.1.2. The sash should be placed at or below the maximum operating height during use.

1.12.2. Periodic and Preventive Maintenance

1.12.2.1. A PM should be performed annually. The PM shall be performed by the hood manufacturer or a hood service agency. Records for PMs of hoods are retained by building maintenance.

1.13. Biological Hoods

1.13.1. Operating Maintenance

1.13.1.1. Workspace in each biological hood should be cleaned before and after each use.

1.13.1.2. The sash should be placed at or below the maximum operating height during use.

1.13.1.3. Hood blowers shall be clear of any obstruction in order to ensure proper flow.

1.13.2. Periodic and Preventive Maintenance

1.13.2.1. A PM should be performed annually. The PM shall be performed by the hood manufacturer or a hood service agency. Records for PMs of hoods are retained by building maintenance.

1.14. Ductless Hood

1.14.1. Operating Maintenance

1.14.1.1. Workspace in each hood should be cleaned before and after each use.

1.14.1.2. The sash should be placed down when in use.

1.14.1.3. Hood blowers shall be clear of any obstruction in order to ensure proper flow.

1.14.2. Periodic and Preventive Maintenance

1.14.2.1. The main filter should be changed every 2 years and the prefilter changed every year.

1.15. Refrigerators

1.15.1. Operating Maintenance

1.15.1.1. Refrigerator doors should be closed promptly after opening.

1.15.2. Periodic and Preventive Maintenance

1.15.2.1. Refrigerator temperatures should be recorded approximately weekly on a temperature log.

1.15.2.1.1. Certified thermometers used to monitor the refrigerator shall be calibrated and certified within the past two years, at a minimum.

1.15.2.2. Refrigerators may be cleaned periodically or as necessary.

1.16. Freezers

1.16.1. Operating Maintenance

1.16.1.1. Freezer doors should be closed promptly after opening.

1.16.2. Periodic and Preventive Maintenance

1.16.2.1. Freezer temperatures should be recorded approximately weekly on a temperature log.

1.16.2.1.1. Certified thermometers used to monitor the freezer shall be calibrated and certified within the past two years, at a minimum.

1.16.2.2. Freezers may be defrosted periodically or as necessary.

1.17. Walk-in Refrigerator
- 1.17.1. Operating Maintenance
  - 1.17.1.1. The door should be closed promptly after opening.
- 1.17.2. Periodic and Preventive Maintenance
  - 1.17.2.1. Temperature and humidity shall be recorded electronically and downloaded once a month.
  - 1.17.2.2. The walk-in refrigerator should be swept and mopped periodically or as necessary.

1.18. Heat Blocks
- 1.18.1. Operating Maintenance
  - 1.18.1.1. Heat blocks should be used with all blocks in place.
  - 1.18.1.2. Heat blocks should be set to the appropriate temperature and allowed to reach temperature. Temperature may be verified with an external thermometer.
  - 1.18.1.3. Turn off heat block after use.
  - 1.18.1.4. See individual manuals for further information.
- 1.18.2. Periodic and Preventive Maintenance
  - 1.18.2.1. Heat blocks should be cleaned periodically or as necessary.

1.19. Sonicating Probe
- 1.19.1. Operating Maintenance
  - 1.19.1.1. Prior to and after each use, clean the sonicating probe with bleach, followed by water, and dry with a laboratory tissue or allow to air-dry.
  - 1.19.1.2. See individual manual for further information.
- 1.19.2. Periodic and Preventive Maintenance
  - 1.19.2.1. The probe tip should be examined periodically and may be replaced when worn (visible corrosion) or as necessary.
  - 1.19.2.2. Threaded parts may be cleaned with alcohol.

1.20. Sonicating Bath
- 1.20.1. Operating Maintenance
  - 1.20.1.1. Ensure water level is at or around the operating line.
- 1.20.2. Periodic and Preventive Maintenance
  - 1.20.2.1. Drain water periodically and refill with fresh water.
  - 1.20.2.2. Clean water reservoir as needed.

1.21. Circulating Bath
- 1.21.1. Operating Maintenance
  - 1.21.1.1. Ensure liquid level is above the highest coil.
  - 1.21.1.2. Liquid in bath may be water and ethylene glycol or propylene glycol, or a combination of these solvents. To achieve temperatures of -30 ºC, a mixture of 50:50 ethylene glycol to water may be used.
- 1.21.2. Periodic and Preventive Maintenance
  - 1.21.2.1. Periodically add liquid to bath. Typically, it is sufficient to add only water, as ethylene or propylene glycol is not likely to have evaporated.

1.21.2.2. Drain liquid periodically and refill with fresh liquid.
1.21.2.3. Clean bath reservoir as needed.

1.22. Centrifuges
1.22.1. Operating Maintenance
1.22.1.1. Test tubes should be capped prior to use to prevent contamination of the interior compartment.
1.22.1.2. Ensure the rotors are balanced prior to use.
1.22.1.3. Do not open centrifuge until the program has completed.
1.22.1.4. When not in use, centrifuge lid should be closed.
1.22.1.5. See individual manuals for further information.
1.22.2. Periodic and Preventive Maintenance
1.22.2.1. Centrifuge, rotors, rotor lids, and other parts should be cleaned periodically or as necessary.

1.23. Positive Pressure Manifolds
1.23.1. Operating Maintenance
1.23.1.1. The manifold should be wiped with water, solvent, or a cleaning agent (e.g., methanol, isopropanol) as necessary.
1.23.1.2. See individual manuals for further information.
1.23.2. Periodic and Preventive Maintenance
1.23.2.1. The in-line air filter, if so equipped, should be monitored periodically for condensation and wear and should be replaced as necessary.
1.23.2.2. The rubber gaskets should be inspected periodically and replaced as necessary.
1.23.2.3. The restrictor plate may be cleaned or replaced when the manifold becomes clogged.
1.23.2.4. The gasket on the restrictor plate may be replaced as necessary.

1.24. Evaporators
1.24.1. Operating Maintenance
1.24.1.1. Evaporator tips should be cleaned prior to and promptly after each use.
1.24.1.2. Open vent assembly to allow ventilation through the flexible extension into the chemical hood, if applicable.
1.24.1.3. Ensure nitrogen gas flow is directed to the appropriate rows once in use.
1.24.1.4. See individual manuals for further information.
1.24.2. Periodic and Preventive Maintenance
1.24.2.1. The manifold seal may be wiped clean with methanol or replaced periodically or as necessary.
1.24.2.2. Evaporator tips shall be replaced as needed.

1.25. Balances
1.25.1. Operating Maintenance
1.25.1.1. The balance plate should be wiped clean prior to and after each use, if necessary.
1.25.1.2. Ensure balance doors are closed when making measurements, if applicable.
1.25.1.3. Ensure level bubble is centered in the circle prior to use, if applicable.

1.25.1.3.1. Adjust using the leveling thumbwheels, as necessary.
1.25.1.4. After use, close balance doors and cover with plastic cover, if applicable.
1.25.1.5. See individual manuals for further information.
1.25.2. Periodic and Preventive Maintenance
1.25.2.1. Analytical balances shall be calibrated annually if used for critical measurements (e.g., weighing reference materials for controls or calibrators).
1.25.2.2. Analytical balances not used for critical measurements and accessioning balances may be calibrated periodically.

1.26. Records
1.26.1. Maintenance log, if applicable
1.26.2. Autotune or Tune report, if applicable
1.26.3. Certificate of calibration, however named, if applicable
1.26.4. Solution log, if applicable
1.26.5. Buffer log, if applicable
1.26.6. Temperature log, if applicable
1.26.7. Temperature and humidity log chart, if applicable
1.26.8. Auto-dilutor Maintenance Log, if applicable
1.26.9. Generator Maintenance Log, if applicable

2. Method Validation

2.1. Scope

2.1.1. This method shall be used to validate analytical testing methods. The objective of method validation is to provide evidence that the method is suitable for its intended purpose and to demonstrate that the method is accurate, reliable, and reproducible.

2.2. Responsibilities

2.2.1. Analysts are responsible for:

2.2.1.1. Performing method development;

2.2.1.2. Performing method validation experiments;

2.2.1.3. Processing, evaluating, and summarizing the data; and

2.2.1.4. Writing a summary of the experiments performed.

2.2.2. The laboratory supervisors and quality assurance manager are responsible for:

2.2.2.1. Documenting authorization of development of new methods;

2.2.2.2. Documenting authorization of the validation plan prior to initiation of a full method validation;

2.2.2.3. Ensuring that appropriate method parameters, validation experiments, and acceptance criteria are used to evaluate method validations;

2.2.2.4. Reviewing written summaries of the data and experiments; and

2.2.2.5. Documenting approval of each validation experiment.

2.2.3. The director is responsible for reviewing the validation documentation and documenting approval for newly developed methods and new analytical technology.

2.2.4. The estimation of measurement uncertainty shall be determined for quantitative methods prior to the reporting of analytical results.

2.3. Equations

2.3.1. Percent accuracy:

$$\%ACC = \frac{\text{Mean concentration}}{\text{Target concentration}} \text{ x } 100$$

2.3.2. Response ratio:

$$RR = \frac{\text{Analyte area}}{\text{Internal standard area}}$$

2.3.3. Percent coefficient of variance:

$$\%CV = \frac{|\text{Standard deviation}|}{\text{Mean concentration}} \text{ x } 100$$

$$\%CV_{\text{within-run}} = \frac{|\text{Standard deviation of a single run for each concentration}|}{\text{Mean concentration}} \text{ x } 100$$

$$\%CV_{\text{between-run}} = \frac{|\text{Standard deviation of all samples for each concentration}|}{\text{Mean concentration}} \text{ x } 100$$

2.3.4. Percent ion suppression/enhancement:

$$\%S/E = \left(\frac{\text{Mean area of post-extraction samples}}{\text{Mean area of neat samples}} - 1\right) \text{ x } 100$$

2.3.5. Relative percent difference:

$$RPD = \frac{|\text{Previous concentration - New concentration}|}{\text{Mean concentration}} \text{ x } 100$$

$$RPD_{drug\ inter} = \frac{|\text{Non-zero control w/ NTA - Non-zero control w/o NTA}|}{\text{Mean result}} \times 100$$

$$RPD_{stability} = \frac{|\text{Day X Result - Day 0 mean result}|}{\text{Mean result}} \times 100$$

$$RPD_{Cal} = \frac{|\text{Lot X mean concentration – Lot 1 mean concentration}|}{\text{Mean concentration}} \times 100$$

$$RPD_{Control} = \frac{|\text{Lot X mean conc. - Lot 1 validation mean conc.}|}{\text{Mean concentration}} \times 100$$

2.3.6. Relative Standard Deviation:

$$\%RSD = \frac{\text{Standard Deviation}}{\text{Mean concentration}} \times 100$$

$$\%RSD = \frac{\text{Reported Uncertainty}}{\text{Concentration or Volume}} \times 100$$

2.4. Validation Requirements

2.4.1. Methods shall be validated prior to use for analysis of evidentiary samples.

2.4.2. Method validation shall be completed for:

2.4.2.1. Newly developed methods

2.4.2.2. New analytical technology

2.4.3. Validation experiments (parameters) for method revalidation should be used to confirm that method changes do not have an adverse effect on a method's performance. Method revalidation should be considered for:

2.4.3.1. Expanding the scope of an existing method beyond the original validation (e.g., addition of a new compound or matrix to a validated method)

2.4.3.2. Improving performance of an existing method (e.g., extraction procedure, changes in the dynamic range tested)

2.4.3.3. Demonstrating equivalence between established method/instrument and new method/instrument

2.4.4. A validation plan shall be established prior to starting validation experiments. The validation plan shall provide the instructions for validating the method or method change. As the validation process proceeds, the validation plan may be updated and/or modified with the approval of a laboratory supervisor and quality assurance manager.

2.4.5. The validation experiments required for method validation shall be determined based on the intended use of the method, the instrumentation used for analysis, the reason for the validation, and/or the specific parameters affected by a method modification, if applicable.

2.4.6. Validation experiments based on scope of method:

2.4.6.1. Qualitative identification (chromatographic)

2.4.6.1.1. Limit of Detection

2.4.6.1.2. Carryover

2.4.6.1.3. Matrix Interference

2.4.6.1.4. Non-Matrix Interference

2.4.6.1.5. Ion Suppression/Enhancement, if applicable (e.g., liquid chromatography/mass spectrometry)

2.4.6.1.6. Correlation

2.4.6.1.7. Stability of Extracted/Prepared Samples*

2.4.6.1.8. Stability of Stock Solutions*, if applicable (e.g., solutions prepared in-house from reference materials)
2.4.6.1.9. Reinjection Integrity*
2.4.6.2. Quantitative analysis
2.4.6.2.1. Accuracy and Precision
2.4.6.2.2. Calibration Model
2.4.6.2.3. Lower Limit of Detection/Quantitation
2.4.6.2.4. Carryover
2.4.6.2.5. Matrix Interference
2.4.6.2.6. Non-Matrix Interference
2.4.6.2.7. Ion Suppression/Enhancement (if applicable; e.g., liquid chromatography/mass spectrometry)
2.4.6.2.8. Correlation
2.4.6.2.9. Stability of Extracted/Prepared Samples*
2.4.6.2.10. Stability of Stock Solutions*, if applicable (e.g., solutions prepared in-house from reference materials)
2.4.6.2.11. Dilution Integrity (if applicable)*
2.4.6.2.12. Reinjection Integrity*
2.4.6.2.13. Measurement Uncertainty

*Not required for initial method validation for analysis of evidentiary specimens. After approval, the results of these experiments may be incorporated in the validated method (e.g., evidentiary specimens may be diluted after the appropriate Dilution Integrity experiment has been approved).

2.4.7. Validation experiments shall be performed using CRMs and/or fortified samples of the intended matrix of the method, and samples shall be extracted/prepared using the intended procedure of the method, unless otherwise noted.
2.4.8. All samples analyzed shall be fortified with internal standard, unless otherwise noted.
2.4.9. Validation experiments may be combined or performed concurrently.
2.4.10. The method shall be validated for each analyte intended to be included.

2.5. Validation Experiments (Parameters)
2.5.1. Accuracy and Precision
2.5.1.1. Accuracy (bias) evaluates the closeness of a control result to its nominal or target concentration, as applicable. Precision evaluates the agreement between a series of measurements obtained from multiple samplings of the same specimen.
2.5.1.2. Accuracy and precision shall be evaluated for each matrix (i.e. antemortem blood, postmortem blood, serum/plasma) in multiple batch preparations (runs).
2.5.1.2.1. Fortified matrix samples may be evaluated using specified concentrations of non-zero controls and calibrators.
2.5.1.2.1.1. When possible pooled fortified matrices shall be used for accuracy and precision studies.

2.5.1.2.1.1.1. A minimum of four replicates of at least three non-zero controls per run shall be evaluated per matrix. The three non-zero controls shall be low (≤3X LLOQ), mid (approximate midpoint of working range), and high (≥ 80% ULOQ)

2.5.1.2.1.2. A total of 20 whole blood replicates at minimum, shall be evaluated.

2.5.1.2.1.2.1. Replicates may only be excluded (max. 3) for a documented reason (e.g., internal standard not detected or problems with the extraction).

2.5.1.2.1.3. A minimum of five runs shall be evaluated for each matrix.

2.5.1.3. Acceptability

2.5.1.3.1. Qualitative criteria listed in the test method or method validation plan for analyte identification (e.g., retention time, peak shape, ion ratios) shall be met and reproducible.

2.5.1.3.2. %ACC and %CV shall be evaluated for quantitative analysis

2.5.1.3.2.1. For each non-zero control, %ACC (ref. 2.3.1) for each run (within-run) and for the entire experiment (between-run) shall be between 80 and 120% for drug quantitative analysis, and, 90 and 110% for volatile analysis

2.5.1.3.2.1.1. For concentrations < 10 ng/ml (drug analysis) or 10 mg/dL (volatile analysis), the acceptable range may be set as a numerical range instead of a percentage.

2.5.1.3.2.2. For each non-zero control, %CV (ref. 2.3.3) shall be ≤ 20% (10 % for volatile analysis) for each run (within-run) and for the entire experiment (between-run).

2.5.2. Calibration Model

2.5.2.1. Calibration model evaluates the range of concentrations and the calibration model (e.g., linear versus quadratic calibration, equal versus 1/x weighting) used in the method.

2.5.2.2. At a minimum, fortified matrix samples shall be evaluated using the desired calibrator concentrations, half the desired LLOQ concentration, twice the desired ULOQ concentration (or physiologically relevant concentration), and negative controls.

2.5.2.2.1. A minimum of eight calibrator concentrations for drug analysis or four calibrator concentrations for volatile analysis shall be evaluated.

2.5.2.2.2. A minimum of six replicates of each concentration shall be evaluated.

2.5.2.2.2.1. Replicates may only be excluded (max. 1 per analyte concentration) for a documented reason (e.g., internal standard not detected, problems with the extraction).

2.5.2.2.3. A minimum of nine negative controls for drug analysis or seven negative controls for volatile analysis shall be evaluated.

2.5.2.3. Acceptability

2.5.2.3.1. Qualitative criteria listed in the test method or method validation plan for analyte identification (e.g., retention time, peak shape, ion ratios) shall be met and reproducible.

2.5.2.3.2. The calibration model shall be evaluated using residual plots.

2.5.2.3.3. The calibration curve shall have $r^2 \geq 0.99$ for each analyte.

2.5.2.3.4. Negative controls should be ≤ ½ LLOQ concentration.

2.5.3. Lower Limit of Detection/Quantitation

2.5.3.1. The LLOD is the lowest concentration of the analyte of interest that the method can reliably and reproducibly detect.
The LLOQ is the lowest concentration that the method can reliably and reproducibly quantitate.

2.5.3.2. The LLOD/LLOQ may be set administratively but shall be reliably and reproducibly achievable by the method.

2.5.3.3. Fortified matrix samples shall be evaluated using the desired LLOD or cutoff concentration(s).

2.5.3.3.1. A minimum of five replicates of each concentration being evaluated shall be used.
Replicates may only be excluded (max. 1 per analyte concentration) for a documented reason (e.g., internal standard not detected or problems with the extraction).

2.5.3.3.2. Decreased injection volume may be evaluated to determine if the LLOD/LLOQ is maintained using a lower injection volume.

2.5.3.4. Acceptability

2.5.3.4.1. Qualitative criteria listed in the test method or method validation plan for analyte identification (e.g., retention time, peak shape, ion ratios) shall be met and reproducible.

2.5.3.4.2. For LLOQ %ACC shall be ≤ 20% (10% for volatile analysis) for the replicates

2.5.3.4.3. For LLOQ %CV shall be ≤ 20% (10% for volatile analysis) for the replicates

2.5.4. Carryover

2.5.4.1. Carryover occurs when residual analyte response is detected in a sample following a highly concentrated sample.

2.5.4.2. Carryover is evaluated by analyzing negative controls following highly concentrated samples.

2.5.4.3. Samples in each desired matrix shall be fortified at increasing concentrations up to five times the desired ULOQ concentration (or physiologically relevant concentration), with each concentration followed by at least one negative control, and shall be evaluated at the desired injection volume.

2.5.4.3.1. Increased injection volumes may be evaluated to determine if increasing the injection volume results in carryover or saturation of the detector.

2.5.4.4. Acceptability

2.5.4.4.1. Negative controls should be quantitated ≤ ½ LLOQ (or RR < LLOD (ref. 2.3.2), for qualitative methods, qualitative criteria listed in the test method or method validation plan for negative controls). If carryover exists, additional experiments may be performed to determine the concentration at which carryover occurs and/or additional procedures shall be implemented to prevent carryover in casework.

2.5.5. Matrix Interference

2.5.5.1. Matrix interference is a combined effect of all components in the matrix on the result obtained in the absence of the analyte(s) of interest.

2.5.5.2. Unfortified matrix samples from a minimum of 10 different sources for each matrix type when possible (e.g., serum/plasma, antemortem whole blood, postmortem whole blood) using purchased and/or closed case specimens that previously screened “negative” shall be evaluated for matrix interference. A minimum of 5 samples of each matrix type shall by used.

2.5.5.3. Unfortified samples shall not include the drugs of interest or internal standards.

2.5.5.4. Samples shall be run with at least the lowest calibrator or cutoff control to obtain LLOQ areas for acceptability criteria comparisons.

2.5.5.5. Acceptability

2.5.5.5.1. The internal standard area in each sample shall be < 10% of the lowest calibrator or cutoff control, whichever is applicable, for each analyte of interest.

2.5.5.5.2. The analyte of interest area in each unfortified sample shall be less than half the area of the lowest calibrator or cutoff control, whichever is applicable, for each analyte of interest.

2.5.6. Non-Matrix Interference

2.5.6.1. Non-matrix interference occurs when an NTA affects the ability to detect, identify, or quantitate an analyte of interest.

2.5.6.2. Potential interference (e.g., chromatographic, mass spectral) from internal standards and other analytes shall be evaluated.

2.5.6.3. A calibration curve shall be prepared in order to quantify non-zero control concentrations.

2.5.6.3.1. Internal standard interference

2.5.6.3.1.1. One negative control with internal standard and one negative control without internal standard shall be extracted and analyzed per matrix type.

2.5.6.3.1.2. For quantitative methods, a minimum of one non-zero control at ~80% of the ULOQ without internal standard shall be extracted and analyzed for each matrix the method will be used to analyze.

2.5.6.3.1.3. For qualitative methods, a minimum of one non-zero control at the highest control level without internal standard shall be extracted and analyzed for each matrix the method will be used to analyze.

2.5.6.3.2. Drug interference

2.5.6.3.2.1. Three replicate samples per matrix type containing no analyte(s) of interest or internal standard shall be fortified with NTA(s), extracted, and analyzed.

2.5.6.3.2.2. For quantitative methods, a minimum of one non-zero control per matrix at both a low and high concentration (e.g., 2x LLOQ and ~80% of the ULOQ) with internal standard shall be fortified with NTA (concentration of NTA to be determined based on the method being validated). Additionally, a minimum of one non-zero control per matrix at both a low and high concentration (e.g., 2x LLOQ and ~80% of the ULOQ) with internal standard and without NTA shall be fortified.

2.5.6.3.2.3. For qualitative methods, a minimum of one non-zero control per matrix at both a low and high concentration (e.g., at

cutoff and high control concentration) with internal standard shall be fortified with NTA (concentration of NTA to be determined based on the method being validated).

2.5.6.3.2.4. Multiple groups of NTAs may be evaluated depending on the method. At a minimum, all analytes currently included in ISDT's panel, except the analytes included in the method being validated, should be included in the NTA evaluation. Other commonly used drugs may be included in the NTAs.

2.5.6.4. Acceptability

2.5.6.4.1. Internal standard interference

2.5.6.4.1.1. The negative controls without internal standard shall have an area less than half the lowest calibrator or cutoff control for each analyte of interest and < 10% of the internal standard.

2.5.6.4.1.2. The negative controls with internal standard shall have an RR (ref. 2.3.2) less than half the lowest calibrator or cutoff control for each analyte of interest.

2.5.6.4.1.3. Each non-zero control without internal standard shall have an internal standard area < 10% of the internal standard area in the lowest calibrator or cutoff control for each analyte of interest.

2.5.6.4.2. Drug interference

2.5.6.4.2.1. Negative controls without internal standard and fortified with NTA shall have an area less than half the lowest calibrator or cutoff control for each analyte of interest and < 10% of the internal standard.

2.5.6.4.2.2. $RPD_{drug\ inter}$ (ref. 2.3.5) of each result from the non-zero controls with and without NTA (containing internal standard) shall be ≤ 20% for drug quantitative analysis, ≤ 10% for volatile analysis, and ≤ 30% for drug qualitative analysis.

2.5.6.4.2.3. Qualitative criteria listed in the test method or method validation plan for analyte identification (e.g., retention time, peak shape, ion ratios) shall be met and reproducible.

2.5.7. Ion Suppression/Enhancement

2.5.7.1. Ion suppression/enhancement is the suppression or enhancement of analyte ionization in the presence of co-eluting compounds (LC methods only).

2.5.7.2. Evaluation of ten different sources of each matrix (minimum of 5) intended to be analyzed by the method, if possible, shall be fortified post-extraction with the analytes of interest and internal standards.

2.5.7.2.1. For a quantitative method, samples shall include a negative sample and samples fortified at both a low and high concentration within the dynamic range (e.g., 2X-3X the LLOQ and 80% of the highest calibrator) for each matrix source.

2.5.7.2.2. For a qualitative method, samples shall be fortified at both a low and high concentration within the dynamic range (e.g., 2X-3X the cutoff and 80% of the highest control).

2.5.7.2.3. To prepare post-extraction fortified samples, fortify samples with each analyte of interest and its associated internal standard after the samples are extracted, but before the samples are reconstituted in the reconstitution solution.

2.5.7.2.4. At least one neat sample shall be fortified with the analyte(s) of interest and internal standard(s) per concentration and reconstituted in the reconstitution solution. Each neat sample shall be injected a minimum of six times to determine a mean analyte RR (ref. 2.3.2) for each concentration.

2.5.7.3. Acceptability

2.5.7.3.1. Qualitative criteria listed in the test method or method validation plan for analyte identification (e.g., retention time, peak shape, ion ratios) shall be met and reproducible.

2.5.7.3.2. % S/E and % CV shall be evaluated for quantitative analysis and may be evaluated for qualitative analysis, if applicable.

2.5.7.3.2.1. % S/E (ref. 2.3.4) should be ≤ 20% (30% for qualitative analysis). If analyte response is suppressed or enhanced, the internal standard response shall compensate for this and demonstrate that there is no impact on other required validation experiments.

2.5.7.3.2.2. % CV (ref. 2.3.3) shall be ≤ 20% (30% for qualitative analysis) for the matrix samples.

2.5.8. Correlation

2.5.8.1. Correlation is the comparison of analytical results obtained from different analyses of the same specimen.

2.5.8.2. Samples shall be analyzed using the method or instrument being validated, and those results shall be compared to the results from another validated method, a previously validated instrument, target concentration, certificate of analysis, or a reference laboratory, as appropriate.

2.5.8.3. Specimens not containing any analyte of interest above the LLOD shall be included.

2.5.8.4. Correlation between instruments may be performed by reinjection of previously analyzed samples if it is within the reinjection timeframe of the method.

2.5.8.4.1. The number of specimens required for the correlation experiment may be determined based on instrument capacity and type of method being validated (i.e., qualitative or quantitative).

2.5.8.4.1.1. For qualitative methods, there shall be at least one specimen that is presumptively positive for each analyte being validated in the method.

2.5.8.4.1.2. For quantitative methods, there should be at least three specimens positive for each analyte being validated in the method.

2.5.8.5. Acceptability

2.5.8.5.1. For quantitative analysis, the RPD (ref. 2.3.5) of the result from the previous analysis compared to the new result should be ≤ 30% (≤ 10% or 5 mg/dL, whichever is greater, for volatile analysis), but may be greater, depending on the stability of the analyte of interest or length of time since the previous analysis, etc.

2.5.8.5.1.1. For samples with a concentration less than 2 ng/mL, a range may be utilized instead of the RPD.

2.5.8.5.2. For qualitative analysis, the result should be consistent with the previous qualitative or quantitative result unless the analyte cutoff level changed significantly or the previous result was at or near the cutoff level.

2.5.9. Stability of Extracted/Prepared Samples

2.5.9.1. Extracted/prepared sample stability is the length of time an extracted/prepared sample can be stored prior to analysis without producing unacceptable results.

2.5.9.2. Experiment should be performed with antemortem whole blood, postmortem whole blood, serum/plasma, and alternative matrix samples, if applicable.

2.5.9.3. Experiment should evaluate samples stored in the location most likely used while waiting for analysis for the instrument type used in the method (e.g., room temperature autosampler, chilled autosampler, refrigerator, benchtop).

2.5.9.4. Drug analysis

2.5.9.4.1. For each non-zero control concentration, a single fortified matrix stock shall be prepared at a volume sufficient to extract three replicates for each day of stability being examined. For example, if three-day stability is being examined, the volume of each fortified matrix stock shall be sufficient to extract nine samples of each non-zero control concentration.

2.5.9.4.2. All stability samples shall be aliquoted from the fortified matrix stocks and extracted on the same day.

2.5.9.5. Volatile analysis

2.5.9.5.1. Two non-zero mixed volatiles matrix reference materials shall be used. Aliquots for each concentration should be taken from a single vial of each control.

2.5.9.5.1.1. All stability samples shall be aliquoted on the same day.

2.5.9.6. One set of stability samples shall be analyzed on the day of extraction/preparation with calibrators (or cutoff) and controls that are concurrently extracted/prepared.

2.5.9.6.1. One set of stability samples consists of three replicates of each non-zero control concentration.

2.5.9.7. Stability samples shall be stored under conditions expected to be encountered during casework (e.g., room temperature or refrigeration).

2.5.9.8. On subsequent days, calibrators (or cutoff) and controls shall be extracted/prepared and analyzed with one set of stability samples.

2.5.9.9. Prepared sample stability and reinjection studies may be combined. It is assumed that the reinjection window (e.g., hours, days) for punctured vials would be less stable than for unpunctured vials, so the reinjection window would apply to prepared samples.

2.5.9.10. Acceptability

2.5.9.10.1. Qualitative criteria listed in the test method or method validation plan for analyte identification (e.g., retention time, peak shape, ion ratios) shall be met and reproducible.

2.5.9.10.2. $RPD_{stability}$ shall be evaluated for quantitative analysis and may be evaluated for qualitative analysis, if applicable.

2.5.9.10.2.1. $RPD_{stability}$ (ref. 2.3.5) of results from samples analyzed on day 0 and day X shall be $\leq$ 15% for drug analysis and $\leq$ 10% for volatile analysis. When the percent difference exceeds the acceptable percentage above, the samples are no longer considered stable.

2.5.9.10.2.2. For each non-zero control, %ACC (ref. 2.3.1) shall be between 80-120% for drug quantitative analysis, 90-110% for volatile analysis, and pass all qualitative

criteria for drug qualitative analysis for each run (within-run).

2.5.9.10.2.2.1. For concentrations < 10 ng/ml (drug analysis) or 10 mg/dL (volatile analysis), the acceptable range may be set as a numerical range instead of a percentage.

2.5.10. Stability of Stock Solutions

2.5.10.1. Calibrator and control stock solutions stability is the length of time non-CRM stock solutions are stable without producing unacceptable results.

2.5.10.2. Multiple lots of calibrator and control stock solutions shall be prepared over the course of several weeks and/or months.

2.5.10.2.1. Lot 1 of calibrator and control stock solutions shall be validated according to Solution Verification/Validation (ref. 3).

2.5.10.2.2. Approximately 30, 60, and 90 days (or other time frame) after the preparation of the first stock solution lots, a new lot (lot X) shall be prepared and validated according to Solution Verification/Validation (ref. 3).

2.5.10.2.2.1. A set of calibrators and a minimum of five replicates of each non-zero control shall be prepared from each lot of stock solutions (e.g., lot 1 and lot X) being evaluated.

2.5.10.2.2.2. Calibrators and control samples from both lots shall be processed and quantitated using each lot as calibrators. This will result in two sets of concentrations for each sample: one set processed with the first lot as calibrators and one set processed with lot X as calibrators.

2.5.10.3. Acceptability

2.5.10.3.1. Calibrator stock solutions

2.5.10.3.1.1. RPD_{Cal} (ref. 2.3.5) of lot X control concentrations processed with each lot of calibrators shall be ≤ 15% (10% for volatile analysis).

2.5.10.3.2. Control stock solutions

2.5.10.3.2.1. $RPD_{Control}$ (ref. 2.3.5) of lot 1 control concentrations processed with mean lot X and the initial validation mean of lot 1 shall be ≤ 15% (≤ 10% for volatile analysis).

2.5.10.3.3. %ACC (ref. 2.3.1) shall be between 85-115% (90-110% for volatile analysis).

2.5.10.3.3.1. %ACC may be determined using the target concentration or the average from the original validation for that lot.

2.5.11. Reinjection Integrity

2.5.11.1. Reinjection integrity is the ability to produce accurate results using a sample that was previously injected.

2.5.11.1.1. Reinjections may occur during casework due to instrument issues (e.g., clogged injection needle, loss of carrier gas), network communication issues (e.g., loss of network connection, miscommunication between instruments), or other issues that cause a sequence to halt or not acquire data.

2.5.11.2. Fortified matrix samples at two different concentrations shall be prepared/extracted in triplicate, analyzed, and reinjected to determine if samples remain stable after a previous injection.

2.5.11.3. Acceptability

2.5.11.3.1. Qualitative criteria listed in the test method or method validation plan for analyte identification (e.g., retention time, peak shape, ion ratios) shall be met and reproducible.

2.5.11.3.2. RPD shall be evaluated for quantitative analysis and may be evaluated for qualitative analysis, if applicable.

2.5.11.3.2.1. For volatile methods, RPD (ref. 2.3.5) of results from originally injected samples and the reinjected samples shall be ≤ 5% or 5 mg/dL, whichever is greater.

2.5.11.3.2.2. For qualitative drug analysis, the RPD (ref. 2.3.5) of results from originally injected samples and the reinjected samples should be ≤ 30% (≤ 40% for concentrations <10 ng/mL) or be consistent with the previous result of "Positive" or "Negative."

2.5.11.3.2.3. For quantitative drug methods, RPD (ref. 2.3.5) of results from the originally injected samples and the reinjected samples shall be ≤ 10% (≤ 5% or 5 mg/dL, whichever is greater, for volatile analysis) for each analyte of interest.

2.5.11.3.2.3.1. For concentrations < 10 ng/ml the acceptable range may be set as a numerical range instead of a percentage.

2.5.12. Measurement Uncertainty

2.5.12.1. MU is a non-negative parameter characterizing the dispersion of the values attributed to a measured quantity.

2.5.12.2. MU shall only be calculated for quantitative methods.

2.5.12.3. The measureand is the concentration of the analyte measured using the method.

2.5.12.4. The quantity value is the quantitative result for an analyte.

2.5.12.5. Traceability for each method is established by using CRM for preparation of calibrators and controls, calibrated pipettes and auto-dilutors, and class A glassware for volumetric flasks, if applicable.

2.5.12.6. The following contributing factors or uncertainty components shall be assessed per analyte, if applicable, when calculating the MU for each analyte in a method:

2.5.12.6.1. Pipettes or auto-dilutors used for sample delivery and preparation of controls and calibrators;

2.5.12.6.2. The sum of the CRM uncertainty for all calibrators used to calculate the calibration curve;

2.5.12.6.3. The highest CRM uncertainty for controls;

2.5.12.6.4. Volumetric flasks for dilutions;

2.5.12.6.5. All instruments used for the specific analysis, and;

2.5.12.6.6. Measurement Process Reproducibility data from all analysts trained in the method.

2.5.12.6.6.1. A minimum number of 10 replicates shall be used to determine the % RSD (ref. 2.3.6) for calculations for the measurement process reproducibility.

2.5.12.6.6.2. All values for the uncertainty components shall be reported in percent by calculating the % RSD (ref. 2.3.6) for each component.

2.5.12.6.6.3. The standard uncertainty shall be calculated for each uncertainty component by dividing the value of the uncertainty component by the divisor.

2.5.12.6.6.3.1. The divisor is determined based on the type of distribution (normal or rectangular) and evaluation method (Type A or Type B) of the uncertainty component.

2.5.12.7. The sum of all standard uncertainty for the method shall be calculated and multiplied by the k value.

2.5.12.7.1. The k value is calculated by taking the two-tailed inverse of the Student’s t-distribution using a probability of either 95.45% (drug confirmation) or

99.73 % (volatile analysis). The degrees of freedom are equivalent to the number of replicates used to calculate the Measurement Process Reproducibility minus one.

2.5.12.8. The MU shall be calculated as a percentage that is applied to all quantitative data. The percentage shall be rounded up to the closest tenth of a percent.

2.5.12.8.1. When the MU percentage is applied to the quantitative value, the resulting MU shall be rounded using normal rounding rules (i.e., 0-4, round down and 5-9, round up) and reported to the same level of significance as the quantitative value.

2.5.12.8.2. All measurement uncertainties shall be reported in the same unit as the quantity value and in the format of quantity value ± the MU.

2.5.12.9. MU shall be evaluated and calculated after any of the following:

2.5.12.9.1. Validation of a method;

2.5.12.9.2. Modification of an existing method requiring full or partial revalidation;

2.5.12.9.3. New analyst being trained on the method;

2.5.12.9.3.1. Six months after completion of training, the analyst training data may be replaced with more recent data from the analyst and the MU reevaluated for the method.

2.5.12.9.4. New or modified instrument being used for the method; or,

2.5.12.9.5. At least once per accreditation cycle.

2.6. Records

2.6.1. Validation Plan
2.6.2. Summary spreadsheet
2.6.3. Calibrator solution preparation worksheet
2.6.4. Internal standard solution preparation worksheet
2.6.5. Control solution preparation worksheet
2.6.6. Sample chromatograms
2.6.7. Tune report
2.6.8. Assay preparation worksheet
2.6.9. Measurement Uncertainty Estimation and supporting data

2.7. References

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

3. Solution Verification/Validation

3.1. Scope
- 3.1.1. This method shall be used to validate or verify solutions prepared in the laboratory and purchased reference materials that will be used in casework.

3.2. Precautions/Limitations
- 3.2.1. See method for specific assay.
- 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. CRMs and solutions containing 7-aminoclonazepam should be sonicated prior to sampling or use if a precipitant is present.
- 3.2.4. Expiration of any solutions or material occurs at 11:59 pm on that date. Analysis must be completed before 11:59 pm on the date of expiration.

3.3. Related Information
- 3.3.1. See method for specific assay.

3.4. Instruments/Equipment
- 3.4.1. See method for specific assay.

3.5. Hazards/Safety
- 3.5.1. See Safety Manual.
- 3.5.2. See SDS for each chemical in method for specific assay.

3.6. Reagents/Materials
- 3.6.1. See method for specific assay.

3.7. Reference Materials/Controls/Calibrators/Solutions
- 3.7.1. See method for specific assay.

3.8. Procedures/Instructions
- 3.8.1. Chemicals and reagents beyond their expiration date shall not be used in laboratory casework. Expired chemicals and reagents may only be used during training or stability experiments.
  - 3.8.1.1. Exception: expired solid chemicals may be used for the creation of buffers as long as the solution is pH adjusted.
- 3.8.2. In-house calibrator solutions expire when the stability window is met or on expiration date of a component CRM, whichever occurs first. In-house control and internal standard solutions expire when stability window is met and may be used past the expiration date of the component CRMs.
- 3.8.3. Calibrator, control, and internal standard solutions shall be validated, and the validation data shall be technically reviewed prior to use of the solutions in casework. Internal standard solutions and negative blood or serum/plasma may be prepared and included in a casework batch as samples. Calibrator and control

solutions may be prepared and analyzed concurrently with casework but should be calibrated and processed separately from the casework batch (run).

3.8.3.1. CRMs used “as is” need not be validated. The target concentration is the nominal concentration on the CoA, however named.

3.8.4. Solutions other than calibrators, controls, and internal standards may be verified concurrently with casework (e.g., buffers, mobile phases, elution solutions).

3.8.5. See method for specific assay for procedures for preparation of solutions.

3.8.6. Validation of Negative Blood (Whole Blood or Serum/Plasma)

3.8.6.1. If an expiration date is given for a negative matrix, the negative blood or serum/plasma lot may be used past the expiration date.

3.8.6.2. Volatile analysis

3.8.6.2.1. At least one negative blood sample shall be prepared without internal standard, and one negative blood sample should be prepared with internal standard for each unvalidated negative blood lot. Analysis of the samples shall be completed on one ALC1 and one ALC2 instrument, at minimum. The run shall include previously validated negative blood controls of a different lot, calibrator(s), and non-zero and negative aqueous controls.

3.8.6.2.2. Acceptability

3.8.6.2.2.1. Acceptable criteria/chromatography as stated in Volatile Analysis by HS/GC/FID (ref. 12) for a negative control.

3.8.6.3. Drug analysis

3.8.6.3.1. Screening: At least one negative blood sample shall be prepared without internal standard, and one negative blood sample should be prepared with internal standard for each unvalidated negative blood lot. The samples shall be analyzed by the drug screening method(s) in use. The run shall include negative blood controls, calibrator(s), and non-zero controls prepared using a previously validated negative blood lot.

3.8.6.3.2. Acceptability

3.8.6.3.2.1. Acceptable criteria/chromatography as stated in Blood Drug Screening by LC/TOF (ref. 6.10) for a negative control.

3.8.6.3.2.2. The negative control without internal standard shall have an area less than half that of the lowest calibrator or cutoff control for each analyte of interest and < 10% that of the internal standard.

3.8.6.3.3. Confirmation: Prior to its use as a control in a drug confirmation analysis, at least one negative blood sample shall be prepared without internal standard, and one negative blood sample should be prepared with

internal standard for each unvalidated negative blood lot. The samples shall be analyzed using the confirmation method for the specific assay. The validation shall include negative blood controls, calibrator(s), and non-zero controls prepared using a previously validated negative blood lot.

3.8.6.3.4. Acceptability

3.8.6.3.4.1. Acceptable criteria/chromatography as stated in the specific drug confirmation test method under Interpretation of Results for a negative control.

3.8.6.3.4.2. The negative control without internal standard shall have an area less than half the lowest calibrator for each analyte of interest and < 10% of the internal standard.

3.8.7. Validation of Volatile Solutions

3.8.7.1. Internal standard solutions

3.8.7.1.1. A minimum of two replicates of negative controls prepared with the newly prepared internal standard shall be analyzed. One replicate each shall be run on ALC1 and ALC2, at minimum.

3.8.7.1.2. Acceptability

3.8.7.1.2.1. Acceptability criteria for the analysis as stated in Volatile Analysis by HS/GC/FID (ref. 12).

3.8.8. Validation of Drug Solutions for Confirmation Analysis

3.8.8.1. Calibrator and control solutions

3.8.8.1.1. A minimum of nine replicates of each calibrator and/or control level being validated shall be analyzed using any instrument validated for the method for the specific assay. Samples may be prepared as neat solutions or extractions from a fortified matrix as provided by the sample preparation section of the method for the specific assay. A minimum of 3 replicates shall be prepared for each run if the analysis is split into multiple runs.

3.8.8.1.1.1. Neat calibrator and control samples shall not be used for validation of solutions used in the Cannabinoids Confirmation by GC/MS method.

3.8.8.1.1.2. The run(s) should be calibrated using the first replicate of each calibrator level.

3.8.8.1.1.2.1. Each calibration level does not have to use the same replicate for each analyte to

generate the calibration curve.

3.8.8.1.1.3. If neat solutions are used, the calibrator and control samples shall be prepared by aliquoting the appropriate volumes of each solution into test tubes as outlined in the calibrator and control sample preparation table in the test method for the assay. Internal standard shall be included. If the test method uses an additive to avoid loss of analyte during sample evaporation, the appropriate volume of additive (e.g., HCl) shall be added. The samples shall then be evaporated to dryness and reconstituted into the reconstitution solution used in the test method (LC methods) or derivatized following the procedure in the test method (GC/MS methods).

3.8.8.1.1.4. Replicates shall only be excluded for documented reasons (e.g., internal standard not detected). A minimum of 8 replicates shall be used for determining acceptability. If the minimum number of replicates is not met, additional run(s) consisting of at least one of each calibrator and a minimum of 3 replicates of each level requiring additional replicates shall be included.

3.8.8.1.2. If only calibrators are being validated, a minimum of 3 replicates of each control level shall be included in the validation.

3.8.8.1.3. If only controls are being validated, a minimum of one replicate of each calibrator level shall be included in the validation for the calibration curve.

3.8.8.1.4. Acceptability

3.8.8.1.4.1. Acceptability criteria/chromatography for calibrators and controls as stated in Interpretation of Results for the specific test method.

3.8.8.1.4.2. Between-run %ACC (ref. 2.3.1) shall be between 85-115%.

3.8.8.1.4.3. Between-run %CV (ref. 2.3.3) shall be ≤ 20%.

3.8.8.1.5. The target concentration of each calibrator and control level shall be used for analysis.

3.8.8.2. Internal standard solutions

3.8.8.2.1. A minimum of two replicates of internal standard shall be prepared and analyzed using any instrument validated for the method for the specific assay. Samples may be prepared as neat solutions or extractions from a fortified matrix as provided by the sample preparation section of the method for the specific assay.

3.8.8.2.2. Acceptability

3.8.8.2.2.1. Each internal standard analyte shall be present and chromatographic peaks shall have baseline resolution and/or analytes shall be resolved in the mass spectrometer. Peaks for the target analytes shall not be present with a response > 10% of the response for the corresponding internal standard peak.

3.8.9. Validation of Other Solutions

3.8.9.1. Prepared solutions other than calibrator, control, and internal standard solutions, including, but not limited to, elution solutions, mobile phases, and reconstitution solutions, may be validated concurrently with casework.

3.8.9.1.1. Acceptability

3.8.9.1.1.1. Acceptable criteria/chromatography for the solution used as stated in Interpretation of Results for the specific test method.

3.8.10. Nomenclature for Solutions Prepared In-House

3.8.10.1. Drug confirmation, LC/TOF, and volatile calibrator, control, and internal standard solutions

3.8.10.1.1. Methanol working stocks shall be labeled with the following information: assay, two digit month, day, and year the solution was made ("lot number"), expiration date, initials of preparer, solution type (e.g., calibrator, control, or internal standard), and level, if applicable.

3.8.10.1.1.1. When identifying solutions used in an assay on a preparation worksheet, the date the solution was made is sufficient to identify the working stock used.

3.8.10.1.1.2. A letter or number may be added to the end of the lot name to distinguish between two stocks made on the same day.

3.8.10.2. Each negative blood lot shall be identified by the lot or identifying number assigned by the supplier. When blood is not uniquely identified by the supplier, it shall be labeled with the two-digit month, day, and year of the blood collection or receipt.

3.8.10.2.1. A letter may be added after the date to distinguish between two lots.

3.8.10.3. Other solutions

3.8.10.3.1. Buffers, mobile phases, and other solutions shall be labeled with the solution name or abbreviation. If a solution is stored past the date it was prepared, the bottle shall be labeled with the solution name or abbreviation, preparer's initials, and expiration date or date prepared, as applicable.

3.9. Records

3.9.1. Pipette calibration certificate, however named

3.9.2. Volumetric flask calibration certificate, however named

3.9.3. CRM certificate of analysis, however named

3.9.4. Validation of calibrator, control, and internal standard solutions shall be documented in a summary of data (ref. 3.9.4.2.1.6). The validation summary shall be saved electronically on a network drive, verified, and signed by the verifier.

3.9.4.1. Prior to verification, an analyst who is trained to perform technical review of data for the assay shall technically review the analytical data from each batch included in a validation, which shall be documented on the Solution Validation Technical Review Worksheet.

3.9.4.2. If any data from the validation is not acceptable, the verifier of the validation shall ensure a note is added documenting the reason(s) for rejecting the specific data or the validation in its entirety prior to signing the validation summary.

3.9.4.2.1. The validation documentation shall include:

3.9.4.2.1.1. Calibrator, control, and internal standard solution preparation worksheet(s), if applicable;

3.9.4.2.1.2. Non-CRM volatile control CoA, if applicable;

3.9.4.2.1.3. Analysis preparation worksheet(s);

3.9.4.2.1.4. Chromatograms and/or raw data; and

3.9.4.2.1.5. Tune report, if applicable;

3.9.4.2.1.6. Table of responses and/or concentrations with applicable calculations for the acceptability criteria.

3.9.5. Material name, vendor, lot number, name of preparer, and amounts of materials used to prepare other solutions shall be documented in the appropriate log (e.g., Solution Log, Buffers Log, or Instrument Maintenance Log).

3.9.5.1. Solutions that are made daily do not need to be documented in a log and shall be documented on the appropriate preparation worksheet.

3.9.6. Negative blood lots shall be documented in the negative blood validation log along with the associated chromatograms or reports for each assay analyzed.

3.10. Interpretation of Results

3.10.1. N/A

3.11. Report Writing

3.11.1. N/A

3.12. References

3.12.1. N/A

4. Evidence Handling

4.1. Scope
- 4.1.1. This procedure shall be used for receiving, accessioning, sequestering, transferring, and destroying evidence submitted to ISDT.

4.2. Precautions/Limitations
- 4.2.1. Proficiency test specimens shall be handled as evidence.

4.3. Related Information
- 4.3.1. Toxicology Analysis Request Instructions
- 4.3.2. Nomenclature for file storage in JusticeTrax
- 4.3.3. Evidence Submission Guidelines

4.4. Instruments/Equipment
- 4.4.1. Heat sealers
- 4.4.2. Scanner/Camera

4.5. Reagents/Materials
- 4.5.1. Label
- 4.5.2. Empty blood tube
- 4.5.3. Conical vial, with lid
- 4.5.4. Bubble wrap
- 4.5.5. Tape
- 4.5.6. Absorbent pad
- 4.5.7. Plastic bag
- 4.5.8. Box
- 4.5.9. Shipping label

4.6. Hazards/Safety
- 4.6.1. N/A

4.7. Reference Materials/Controls/Calibrators/Solutions
- 4.7.1. N/A

4.8. Procedures/Instructions
- 4.8.1. Receiving
  - 4.8.1.1. Evidence shall be received by ISDT staff from a courier or the secure ISDT drop box.
  - 4.8.1.2. Each new case shall be assigned a case number in LIMS.
    - 4.8.1.2.1. Enter primary container information.
    - 4.8.1.2.2. Remove primary container from shipping container, if applicable.
    - 4.8.1.2.3. Generate barcode and place barcode on primary container.
  - 4.8.1.3. Create a Chain of Custody for each item with transfers noted.
  - 4.8.1.4. If the TAR is on the outside of the primary container, it shall be labeled with the associated case number and uploaded to the electronic case file.
    - 4.8.1.4.1. Upload may occur during accessioning (ref. 4.8.2).

4.8.1.4.2. TAR shall be placed in the designated location to await accessioning (ref. 4.8.2).

4.8.1.4.3. Additional paperwork submitted with the evidence shall also be labeled with a case number, uploaded to the electronic case file, and stored with the TAR.

4.8.1.5. Place the primary container in the walk-in refrigerator or proceed directly to accessioning (ref. 4.8.2).

4.8.1.5.1. A case identified as a priority case should be placed in the priority location in the Walk-in refrigerator.

4.8.1.5.1.1. Priority status should be designated for the following: death investigation, fatal crash, serious bodily injury, elder/child abuse or neglect, juvenile subject, sexual assault, overdose, officer-involved incident, or proficiency test.

4.8.1.5.1.2. Stat status should be designated for a high-profile case, speedy trial, or customer request.

4.8.2. Accessioning

4.8.2.1. Evidence is typically accessioned in batches. Evidence should be accessioned in the order received unless there are cases designated as a priority.

4.8.2.1.1. If accessioning cannot be completed (e.g., no TAR is included, the TAR includes insufficient information, or no blood or serum/plasma specimen(s) are submitted), proceed as follows:

4.8.2.1.1.1. To be considered sufficient, the TAR shall contain the subject name, submitting agency, and request(s).

4.8.2.1.1.2. A case synopsis note shall be made to record any potential case identifying information (e.g., agency listed on the kit, subject name listed on the tubes) and to indicate why accessioning could not be completed.

4.8.2.1.1.2.1. Subject name, submitting agency, and agency case number should be entered into the appropriate fields.

4.8.2.1.1.2.2. If the TAR is present but contains insufficient information, upload the insufficient version.

4.8.2.1.1.3. Reseal the secondary container and specimen inside the primary container.

Initial and date the seal on the primary container and transfer the primary container back to the walk-in refrigerator with the transfer note of "Storage/Not Accessioned."

4.8.2.1.1.4. Contact the submitting agency for additional information. All communications should be added to the case file by the end of the business day in which they were received.

4.8.2.1.1.4.1. If the requested information is not provided within 3 business days, follow up on the initial request for additional information.

4.8.2.1.1.4.2. If the requested information is not provided within 7 business days, escalate to laboratory supervisor.

4.8.2.2. Document transfer of evidence in LIMS.

4.8.2.3. Retrieve any documents associated with the item(s) of evidence being accessioned that were filed at receiving, if applicable.

4.8.2.4. Open the case being accessioned in LIMS.

4.8.2.5. Open the IRIScan Desk 5 Pro camera application.

4.8.2.5.1. For each case, the below list of seven (7) images constitutes a normal representation of an evidence submission. Ensure the ruler is visible in each image.

4.8.2.5.2. Take the following images for each case:

4.8.2.5.2.1. The primary container barcode (1);

4.8.2.5.2.2. The primary container, at an angle that showcases the seal (1);

4.8.2.5.2.3. Two (2) images of the secondary enclosure (if present), both front and back of the seal with initials visible;

4.8.2.5.2.3.1. If the information on the seal is not legible in the image, record the initials in a case synopsis note.

4.8.2.5.2.3.2. If the seal is not complete, take enough images to fully capture the state of the seal.

4.8.2.5.2.3.3. A case synopsis note may be included to further describe the evidence if needed.

4.8.2.5.2.4. Three (3) images of the blood tubes containing the expiration date of the tube, if visible, and all tube label information (subject name, date, time, and collector initials and officer initials, if included).

4.8.2.5.2.4.1. Images of tubes shall have an item number associated with each tube.

4.8.2.5.3. Item numbers shall be assigned starting at A and proceeding alphabetically, by the following hierarchy:

4.8.2.5.3.1. Antemortem specimens shall be assigned before postmortem specimens;

4.8.2.5.3.2. Antemortem specimens shall be assigned with whole blood before serum/plasma unless there are draw times >15 minutes apart, then the earlier draw time shall be first;

4.8.2.5.3.3. Postmortem specimens shall be assigned with extremity blood before central or cavity blood.

4.8.2.5.3.4. Item numbers for proficiency test cases shall be assigned sequentially by test identifier, beginning with item A.

4.8.2.5.4. Make any notes about abnormal tubes in the case synopsis (i.e., broken, leaking, or cracked tubes), and in the request(s) if required (ref 13.3.4).

4.8.2.5.4.1. If no blood or serum/plasma specimen are submitted, reseal evidence and notify a supervisor.

4.8.2.5.4.2. Additional tubes shall be photographed to ensure the same information is visible.

4.8.2.5.4.3. Upload all images of evidence as one PDF file, named with the structure YY-XXXXX_EVI_V1_Initials_.

4.8.2.5.4.3.1. Automatically append the date and time to the end of the file name using the camera software.

4.8.2.5.5. Take an image of the TAR and upload, named with the structure YY-XXXXX_TAR_V1_Initials_.

4.8.2.5.5.1. Automatically append the date and time to the end of the file name using the camera software.

4.8.2.5.5.2. To be considered sufficient, the TAR shall contain the subject name, submitting agency, and request(s).

4.8.2.5.5.3. If the TAR is insufficient, stop accessioning and contact the agency for updated information. All communications should be added to the case file by the end of the business day in which they were received.

4.8.2.5.6. Additional paperwork submitted with the evidence shall also be labeled with a case number, uploaded to the electronic case file, and stored with the TAR.

4.8.2.6. Fill out the following fields in LIMS, using information from the TAR when applicable:

4.8.2.6.1. Submitting agency and (if provided) agency case number.

4.8.2.6.1.1. Ensure appropriate county prosecutor is added and (if provided) agency case number.

4.8.2.6.1.2. Add DRE as an agency if an oral fluid test and/or DRE evaluation is indicated on the TAR and (if provided) agency case number.

4.8.2.6.1.3. If submitting agency is not in LIMS, contact laboratory supervisor.

4.8.2.6.2. Offense type(s) and date, if provided.

4.8.2.6.2.1. If no offense type is indicated, mark “Unknown.”

4.8.2.6.2.2. Ensure county is populated in each offense line item.

4.8.2.6.3. Subject name and type

4.8.2.6.3.1. Enter date of birth and gender, if provided.

4.8.2.6.3.1.1. Mark gender “Unknown” if not provided.

4.8.2.6.3.2. Enter “Subject” as the subject type.

4.8.2.6.3.3. If no subject name is provided, contact the submitting agency.

4.8.2.6.4. Itemize the specimen(s) as labeled in the evidence images:

4.8.2.6.4.1. Include in the “Description” field:

4.8.2.6.4.1.1. The time on each tube, in 24-hour format, when there

are draw times >15 minutes apart.

4.8.2.6.4.1.2. The draw site for each postmortem tube (e.g., “Postmortem-Femoral”).

4.8.2.6.4.1.3. For proficiency specimens, include the specimen identifier after the specimen type (e.g., Whole Blood (FTC-01)).

4.8.2.6.4.2. Select the primary container as the “inherited from” location.

4.8.2.6.4.3. Identify the subject as the source.

4.8.2.6.5. Generate barcodes and affix to each specimen tube.

4.8.2.6.5.1. For a proficiency test specimen, a second person shall verify that the identification on the specimen corresponds with its barcode. The verification shall be documented in the case synopsis note.

4.8.2.6.6. In the Requests tab, add requests as indicated on the TAR.

4.8.2.6.6.1. Select the appropriate submitting agency and officer.

4.8.2.6.6.1.1. If no officer is listed, select “1,.”

4.8.2.6.6.2. Select “Indiana State Department of Toxicology” as the Lab, “Toxicology” as Dept, and the appropriate service.

4.8.2.6.6.3. Select “Priority” or “Stat” in the Reason field, if applicable.

4.8.2.6.6.4. Select “Oral Fluid” in the Complex field, if applicable.

4.8.2.6.6.5. Relate the 1-A item of evidence and the subject name to the request.

4.8.2.6.6.5.1. All blood or serum/plasma specimen shall be related for a proficiency test scenario.

4.8.2.6.6.6. Additional notes shall be added to the “Reviewer” section, as appropriate. Refer to 13.3.4.

4.8.2.6.6.7. If both alcohol and drug analyses are requested and case is not a priority, pend the drug request.

4.8.2.7. Document transfer of specimens to the walk-in refrigerator in LIMS and transfer specimens to the walk-in refrigerator.

4.8.2.8. Document the disposal of the primary container in LIMS and dispose of it along with the secondary container and any other non-evidentiary contents.

4.8.2.9. File TAR and any other case paperwork in the appropriate file folder numerically by case number.

4.8.3. Specimen Transfer Within ISDT

4.8.3.1. Document each specimen transfer in LIMS contemporaneously with the specimen transfer.

4.8.3.1.1. If specimen transfer is not documented contemporaneously, submit a chain-of-custody correction.

4.8.3.2. Each transfer shall contain a short explanation of the purpose of the transfer in the note section.

4.8.4. Resealing

4.8.4.1. Seal all evidence after completion of all requested testing using plastic wrap or tape/parafilm.

4.8.4.1.1. The date of resealing and the initials of the person completing the resealing shall be written on the plastic wrap.

4.8.4.1.1.1. If the resealing with plastic wrap obscures the range of case numbers listed as being contained within the evidence rack, a label with the range of case numbers shall be affixed to the plastic wrap.

4.8.4.1.2. If wrapping the evidence rack occurs within the walk-in refrigerator, a chain of custody transfer is not necessary.

4.8.4.1.3. If wrapping the evidence rack occurs outside the walk-in refrigerator, a chain of custody transfer shall be performed with the purpose of “Resealing Evidence.”

4.8.4.1.4. Evidence that is stored outside the sequentially-ordered evidence racks (e.g., broken or leaking, sequestered, or odd-sized containers) shall be sealed with either tape/parafilm on the specimen or placed inside a closed conical tube sealed with tape/parafilm. The person sealing the evidence shall initial and date the seal.

4.8.5. Containerizing Evidence

4.8.5.1. Evidence that is to be transferred from the walk-in refrigerator to a temporary storage location outside the secured laboratory shall be containerized prior to transfer.

4.8.5.1.1. Generate a list of specimens to be containerized.

4.8.5.1.2. Retrieve specimens from the walk-in refrigerator and note time of retrieval.

4.8.5.1.3. Create Container in LIMS.

4.8.5.1.3.1. Select Transfer, then Containers.
4.8.5.1.3.2. Add a new container and name the container, as appropriate.
4.8.5.1.3.3. In the Evidence to Add section, select Barcode and scan the evidence barcode on each specimen being containerized.
4.8.5.1.3.4. Generate barcode for the container and affix to the container.
4.8.5.1.4. Place specimens in the container.
4.8.5.1.4.1. Perform Container Verification and save verification in the designated location.
4.8.5.1.4.2. Seal container with tape. Initial and date across the tape edge.
4.8.5.1.5. Document evidence transfer in LIMS using the container barcode.
4.8.5.1.5.1. The purpose of the transfer from the walk-in should be “Containerization at XX:XX XM” where XX:XX XM is the time evidence was removed from the walk-in (e.g., 12:23 PM) in 4.8.5.1.2.
4.8.5.1.5.2. Select “No” when the Evidence Transfer box appears asking to empty the container.
4.8.5.1.6. Place container in the location documented above.

4.8.6. Sample Aliquot Transfer

4.8.6.1. Document all sample aliquot transfers in the chain of custody section on the Aliquot Chain of Custody.
4.8.6.1.1. Document ISDT sequence name on the Aliquot Chain of Custody.
4.8.6.1.2. Document the date of transfer, transfer from location, transfer to location, and purpose of the transfer for the batch.

4.8.6.2. Verifications shall be performed and documented prior to sample preparation, prior to instrument analysis, and after instrument analysis.
4.8.6.2.1. Specimen sample preparation verification
4.8.6.2.1.1. Verify that the specimen tube being transferred matches the worklist, including case number and evidence item ID.
4.8.6.2.1.1.1. This verification may be completed electronically by an analyst using the Excel Specimen Verification template.
4.8.6.2.2. Second-person pre-run verification

4.8.6.2.2.1. Verify that the samples are in the correct tray position according to the sequence table/worklist or verify that the plate orientation in instrument is correct.

4.8.6.2.2.2. Verify that a batch preparation worksheet is completed.

4.8.6.2.3. Second-person post-run verification

4.8.6.2.3.1. Verify that the samples are in the correct tray position according to the sequence table/worklist or verify that the plate orientation in instrument is correct.

4.8.7. Sequestration of Evidence

4.8.7.1. Save electronic copy of the sequestration request in the electronic case file and document correspondence in case synopsis.

4.8.7.2. Sequestration:

4.8.7.2.1. Shall be by written request;

4.8.7.2.2. Should include documentation with case-identifying information (e.g., ISDT case number, subject name, agency case number); and

4.8.7.2.3. Should include a sequestration end date.

4.8.7.3. In LIMS, transfer specimens to “Walk-in, Sequestered,” and place in sequestered rack in the walk-in refrigerator.

4.8.7.4. Mark an empty tube with the case number and “Sequestered,” and store the tube in the test tube rack location where the specimen was previously stored.

4.8.7.5. When sequestration ends:

4.8.7.5.1. Save documentation terminating sequestration in the electronic case file, if applicable.

4.8.7.5.2. Dispose of samples following proper disposal procedures (ref. 4.8.10).

4.8.8. Transfer of Specimens from ISDT

4.8.8.1. Transfer of evidentiary specimens shall be by hand delivery or by tracked courier shipment.

4.8.8.2. External testing

4.8.8.2.1. Generate a Shipping Manifest listing all specimens to be transferred along with their corresponding vendor test codes, if applicable.

4.8.8.2.2. Collect specimens listed on the Shipping Manifest.

4.8.8.2.2.1. If the “A” specimen has limited volume, select an alternate specimen with larger volume or select multiple specimens if applicable.

4.8.8.2.2.1.1. If the “A” specimen is not selected or multiple specimens are selected, update the Shipping Manifest

to document the specimen(s) selected (e.g., update item number to "1-B" on the manifest).

4.8.8.2.3. Remove specimens from walk-in refrigerator and scan out in LIMS.

4.8.8.2.3.1. Uncheck the "selected evidence being returned" box.

4.8.8.2.3.2. Check the "2nd Transfer Receipt" box and print the Evidence Transfer Receipt or perform verification using Excel Specimen Verification template.

4.8.8.2.3.2.1. If using the "2nd Transfer Receipt," a second person shall verify that the specimens removed from the walk-in refrigerator were scanned and shall document the verification by initialing and dating the receipt.

4.8.8.2.4. Place or wrap each specimen in bubble wrap.

4.8.8.2.5. Place each bubble-wrapped specimen in its own plastic bag containing an absorbent pad.

4.8.8.2.5.1. Multiple specimens of the same matrix from one case may be placed in the same plastic bag.

4.8.8.2.6. Heat-seal each plastic bag. The person who seals the bag shall initial and date across the seal.

4.8.8.2.7. Someone other than the person who documented the evidence transfer in LIMS shall verify that each specimen is packaged in the inner shipping container as listed on the Shipping Manifest.

4.8.8.2.8. If more than one container is shipped, each inner shipping container shall be:

4.8.8.2.8.1. numbered sequentially (e.g., "Box 1 of 16"),

4.8.8.2.8.2. sealed with tape,

4.8.8.2.8.3. initialed and dated across the tape, and

4.8.8.2.8.4. packaged in an outer shipping container.

4.8.8.2.9. Document chain of custody on Shipping Manifest.

4.8.8.2.9.1. The Relinquished By section of the chain of custody on each page of the Shipping Manifest shall be completed

by the person who transferred the evidence in LIMS.

4.8.8.2.10. Place Shipping Manifest in the outer shipping container. Seal the outer shipping container, affix shipping label on the outside, and place in dock area.

4.8.8.2.11. Scan the Shipping Manifest into the appropriate electronic case file(s) and scan the Evidence Transfer Receipt or Specimen Verification Worksheet, a copy of the Shipping Manifest, and shipping label into the appropriate electronic file.

4.8.8.2.11.1. The electronic file shall be verified against the original for completeness and legibility prior to discarding the hardcopy.

4.8.8.3. Releasing specimens

4.8.8.3.1. Remove specimen(s) to be released from walk-in refrigerator and document transfer in LIMS.

4.8.8.3.1.1. Check the "2nd Transfer Receipt" box and print the Evidence Transfer Receipt or perform verification using Excel Specimen Verification template.

4.8.8.3.1.1.1. If using the "2nd Transfer Receipt," a second person shall verify that each specimen removed from the walk-in refrigerator was scanned and shall document the verification by initialing and dating the receipt.

4.8.8.3.2. Place or wrap each specimen in bubble wrap.

4.8.8.3.3. Place each bubble-wrapped specimen in a plastic bag containing an absorbent pad, heat seal the plastic bag, and initial and date across the seal.

4.8.8.3.3.1. Multiple specimens of the same matrix from one case may be placed in the same plastic bag.

4.8.8.3.4. Place sealed plastic bag in an outer container and seal.

4.8.8.3.5. Release or mail sealed container to authorized personnel.

4.8.8.3.5.1. If evidence is to be released in person, make a photocopy of the recipient's photo identification, and have the recipient sign the Evidence Transfer Receipt.

4.8.8.3.5.2. If evidence is to be picked up by shipping courier, place shipping label on the outside, and place in dock area.

4.8.8.3.6. Scan transfer documentation into the electronic case file, and note transfer in case synopsis notes.

4.8.8.3.6.1. The electronic file shall be verified against the original for completeness and legibility prior to discarding the hardcopy.

4.8.9. Receipt and Accessioning of Transferred Specimens

4.8.9.1. Verify that each inner shipping container is sealed.

4.8.9.2. Document receipt of specimens on the chain of custody section of the Shipping Manifest.

4.8.9.3. Place corresponding Shipping Manifest with each inner shipping container.

4.8.9.3.1. If inner shipping containers will not be accessioned the same day as receipt, store the shipping container in the walk-in refrigerator, and file the Shipping Manifest in the designated location in the accessioning area.

4.8.9.3.2. Document transfer from the walk-in refrigerator for accessioning on Shipping Manifest.

4.8.9.4. Open each inner shipping container individually, verify specimens against Shipping Manifest, and document the verification with initials.

4.8.9.5. Document each evidence transfer in LIMS.

4.8.9.5.1. If the specimen returned was homogenized, itemize the homogenized specimen (do not inherit the chain of custody from the parent tube). The transfer of the specimen being itemized may be documented at the time of itemization or with other specimens received in the shipment.

4.8.9.6. Return specimens to original walk-in refrigerator storage location.

4.8.9.7. Include a copy of the applicable page of the Shipping Manifest in the electronic case file (e.g., MAN_V2).

4.8.9.8. Scan the original hardcopy and file electronically.

4.8.9.8.1. The electronic file shall be verified against the original for completeness and legibility prior to discarding the hardcopy.

4.8.10. Specimen Destruction

4.8.10.1. Unless it has been sequestered, a specimen may be destroyed one year after completion of all case analysis.

4.8.10.1.1. A sequestered specimen may be destroyed after the sequestration has been terminated or the sequestration period has elapsed.

4.8.10.2. Identify specimens to be destroyed.

4.8.10.3. If evidence has not been containerized, remove specimens from the walk-in refrigerator and document transfer in LIMS. Check the “2nd Transfer Receipt” box and print the Evidence Transfer Receipt or perform verification using Excel Specimen Verification template.

4.8.10.3.1. If using the "2nd Transfer Receipt," a second person shall verify that the specimens removed from the walk-in refrigerator were scanned and shall document the verification by initialing and dating the receipt.

4.8.10.3.2. Deposit specimens in a biohazard container.

4.8.10.3.3. Save Evidence Transfer Receipt or Specimen Verification Worksheet, as applicable, in the designated location.

4.8.10.3.3.1. If original hardcopy is scanned and filed electronically, the electronic file shall be verified against the original for completeness and legibility prior to discarding the hardcopy.

4.8.10.4. If evidence is containerized and the seal is not broken, verify that the date on the evidence container seal(s) matches the date the evidence was containerized (date of containerization may be found via chain of custody and/or date the Container Verification was electronically signed). If the seal has been broken or the dates do not match proceed to 4.8.10.5.

4.8.10.4.1. Once the evidence container is removed from the walk-in refrigerator, document the evidence transfer in LIMS by scanning the evidence container barcode.

4.8.10.4.1.1. Select "No" when asked to empty the evidence container with the transfer.

4.8.10.4.1.1.1. If "Yes" is inadvertently selected, note the time of the evidence transfer and proceed to 4.8.10.4.3.

4.8.10.4.2. Using the "Container Contents" Ad Hoc Query, generate a list of case numbers, item numbers, container name, and evidence location for the evidence container being disposed.

4.8.10.4.2.1. Document the disposal by exporting the query to a PDF file and saving it in the designated location with the nomenclature of ContainerName_Disposal_DisposalDate_Initials. Electronically sign the first page of the disposal documentation. Proceed to 4.8.10.4.4.

4.8.10.4.3. If "Yes" was inadvertently selected in 4.8.10.4.1.1, use the "Transfer Receipt" Ad Hoc Query to generate a list of evidence transferred to "Trash."

4.8.10.4.3.1. Export list as an XLS file.

4.8.10.4.3.2. Filter the list to include only the appropriate transfers for date/time of the evidence container being disposed.

4.8.10.4.3.3. Print the XLS file to PDF and save in the designated location with the nomenclature of ContainerName_Disposal_DisposalDate_Initials. Electronically sign the first page of the disposal documentation.

4.8.10.4.4. Break the seal on the evidence container, remove specimens from the evidence container, deposit specimens in a biohazard container, and dispose of the evidence container.

4.8.10.4.5. Delete the evidence container in LIMS.

4.8.10.5. If the evidence container seal has been broken or the date of the seal does not match the containerization date, remove evidence container from the walk-in refrigerator.

4.8.10.5.1. Remove specimens from the evidence container for disposal, document transfer in LIMS by scanning each specimen barcode. Check the "2nd Transfer Receipt" box and print the Evidence Transfer Receipt or perform verification using a verification template.

4.8.10.5.1.1. If using the "2nd Transfer Receipt," a second person shall verify that the specimens removed from the evidence container were scanned and shall document the verification by initialing and dating the receipt.

4.8.10.5.2. Deposit specimens in a biohazard container and dispose of the evidence container.

4.8.10.5.3. Save Evidence Transfer Receipt or verification worksheet in the designated location.

4.8.10.5.3.1. If original hardcopy is scanned and filed electronically, the electronic file shall be verified against the original for completeness and legibility prior to discarding the hardcopy.

4.8.10.5.4. Delete the evidence container in LIMS.

4.8.11. TAR and Other Document Destruction

4.8.11.1. Paper documents in a case with a 10-year record retention requirement may be destroyed after administrative review of the last toxicology report issued in the case (i.e., after completion of all requested testing).

4.8.11.1.1. For identification of 10-year case records, see the state records retention and disposition schedules at http://www.in.gov/apps/icpr/retention/icpr_retention (ref. Records Management Manual).

4.8.11.2. Paper documents for cases that must be retained for 25 years shall be maintained and filed by case number or may be transferred to the Records Center two years after the date of completion of all requested testing.

4.8.11.2.1. For identification of 25-year case records, see the state records retention and disposition schedules at http://www.in.gov/apps/icpr/retention/icpr_retention (ref. Records Management Manual).

4.8.12. Kit or Tube Distribution

4.8.12.1. Fill requests for kits/tubes in the order received.

4.8.12.2. Reply to each email request for kits/tubes to inform customer of shipping/available pickup date.

4.8.12.2.1. Maintain each email request in inbox until a reply has been sent. Once the reply has been sent and the Kit Distribution spreadsheet has been updated, move the email to the designated folder in the mailbox.

4.8.12.3. Document each request for kits/tubes in the Kit Distribution spreadsheet maintained on the lab drive.

4.8.12.3.1. Document date of request, quantity of kits/tubes requested, whether request is for shipment or pickup, requesting agency, agency contact, agency address, and distribution status.

4.8.12.3.2. Document any correspondence in the Notes column.

4.8.12.3.3. If a request can only be partially filled, notify the customer, and advise that additional kits/tubes can be requested as needed or backordered. Fill the order as appropriate and make an entry for any backordered kits/tubes on the spreadsheet.

4.8.12.4. Fill kits/tubes request

4.8.12.4.1. If kits/tubes are to be picked up, place in a container and label container with agency name. Place container in dock area.

4.8.12.4.2. If kits/tubes are to be shipped, place in a container and print a shipping label. Place container in dock area.

4.8.12.5. Document the date the request is filled, whether the kits/tubes were shipped or placed in the dock area for pickup, quantity of kits/tubes provided, lot number, and expiration date of kits/tubes.

4.8.12.6. Update the “Order Status” in the Kit Distribution spreadsheet as appropriate.

4.8.12.6.1. Document any additional information in the comments section.

4.9. Records

4.9.1. Images of evidence
4.9.2. Container or tube photocopies
4.9.3. Sequestration request document(s)
4.9.4. ISDT Case Chain of Custody Report
4.9.5. Shipping Manifest
4.9.6. Evidence Transfer Receipt
4.9.7. Photocopy of recipient identification
4.9.8. Shipping documentation
4.9.9. Aliquot Chain of Custody
4.9.10. Toxicology Analysis Request form

4.9.11. Specimen Verification Worksheet

4.10. Interpretation of Results
4.10.1. N/A

4.11. Report Writing
4.11.1. N/A

4.12. References
4.12.1. N/A

5. Specimen and Sample Preparation

5.1. Scope

5.1.1. This procedure shall be used to prepare specimens and samples for volatile and drug analysis. This procedure shall be followed to ensure specimens are homogenous, aliquots are representative of the entire specimen, and specimens from a proficiency test are treated as an evidentiary case.

5.2. Precautions/Limitations

5.2.1. Proper personal protective equipment shall be used to reduce the possibility of blood coming into contact with eyes, skin, mouth, nasal passages, and clothing.

5.2.2. Spilled or splattered blood shall be cleaned up promptly using the appropriate decontamination solution.

5.3. Related Information

5.3.1. N/A

5.4. Instruments/Equipment

5.4.1. Rocker

5.4.2. Centrifuge

5.4.3. Sonicating probe

5.4.4. Auto-dilutor

5.4.5. Pipette

5.5. Reagents/Materials

5.5.1. Bleach

5.5.2. ddH_2O

5.5.3. Pipette tips

5.5.4. Test tubes

5.6. Hazards/Safety

5.6.1. N/A

5.7. Reference Materials/Controls/Calibrators/Solutions

5.7.1. N/A

5.8. Procedure/Instructions

5.8.1. Preference for analysis should be given to whole blood specimens over serum/plasma specimens. Serum/plasma specimens shall only be tested using analytical method(s) validated and approved for a serum/plasma matrix.

5.8.2. If multiple specimens of the same matrix are available, preference should be given to the “A” tube. If the “A” tube is unavailable, the tube with the greatest volume of specimen available should be used, when practical.

5.8.3. Regardless of matrix type, the earliest draw time should be tested for antemortem blood specimens when tube draw times are >15 minutes apart. The preference in postmortem cases should be to test the extremity blood first.

5.8.4. Serum/plasma samples may need to be prepared before testing.

5.8.4.1. A serum/plasma specimen that has already been decanted shall be used as received.

5.8.4.2. A serum/plasma specimen that has already been separated should be used after decanting the supernatant.

5.8.4.2.1. Decant the serum/plasma specimen into an unused tube. Mark the tube with the case number, date/time of specimen creation, and initials of the person who created the specimen.

5.8.4.2.2. Itemize the serum/plasma specimen in LIMS (deselect the option to inherit information from the parent tube), and assign it a barcode and the Description "Supernatant" (e.g., itemize the resulting serum/plasma specimen as item 1-A-1 with the Description "Supernatant"). Set the source as the subject.

5.8.4.2.3. The chain of custody of the parent specimen shall be updated, if necessary, to reflect the specimen separation. This may be done with a chain of custody correction. The chain of custody of the supernatant tube shall begin with the date and time it was created at the Sample Prep Location.

5.8.4.2.4. When the tubes are placed in the walk-in refrigerator, the supernatant specimen should be placed in the test tube rack location. The parent specimen should be placed in the designated location within the walk-in refrigerator.

5.8.4.3. A serum/plasma specimen that contained beads shall be centrifuged prior to analysis.

5.8.4.3.1. Centrifuge the specimen tube for ~10 minutes at ~3000 rpm and ~8 ºC.

5.8.4.3.1.1. It may be necessary to repeat centrifugation to achieve separation.

5.8.4.3.2. Once separated, decant the serum/plasma specimen into an unused tube. Mark the tube with the case number, date/time of specimen creation, and initials of the person who created the specimen.

5.8.4.3.3. Itemize the serum/plasma specimen in LIMS (deselect the option to inherit information from the parent tube), and assign it a barcode and the Description "Supernatant" (e.g., itemize the resulting serum/plasma specimen as item 1-A-1 with the Description "Supernatant"). Set the source as the subject.

5.8.4.3.4. The chain of custody of the parent specimen shall be updated, if necessary, to reflect the specimen separation. This may be done with a chain of custody correction. The chain of custody of the supernatant tube shall begin with the date and time it was created at the Sample Prep Location.

5.8.4.3.5. When the tubes are placed in the walk-in refrigerator, the supernatant specimen should be placed in the test tube rack location. The parent specimen should be

placed in the designated location within the walk-in refrigerator.

5.8.4.4. A serum/plasma specimen that contained a gel separator and has not already been separated or a tube that does not contain anti-coagulants (e.g., red top tube) shall be decanted, if necessary, and sonicated starting at 5.8.5.2 prior to analysis, as applicable.

5.8.5. Homogenizing clotted and/or difficult-to-aliquot specimens

5.8.5.1. A clotted and/or difficult-to-aliquot specimen should be homogenized in the original tube using a sonicating probe.

5.8.5.1.1. If sonicating probe does not fit in the original tube decant before sonicating.

5.8.5.2. Clean the sonicating probe with bleach, followed by water, and dry it with a laboratory tissue or allow it to air-dry.

5.8.5.3. Place probe into the middle of the specimen. Refrain from touching the walls of the container.

5.8.5.4. Sonicate at ~40% amplitude for ~10 seconds while ensuring the probe does not touch the walls of the container. Increase amplitude or length of time as needed to homogenize the specimen.

5.8.5.5. Clean the sonicating probe with bleach, followed by water, and dry it with a laboratory tissue or allow it to air-dry.

5.8.5.6. Decant homogenized specimen into an unused tube. Specimen should be decanted into a tube with an opening wide enough to fit the sonicating probe. Mark the tube with the case number, date/time of specimen creation, and initials of the person who created the specimen.

5.8.5.7. Itemize the homogenate tube in LIMS (deselect the option to inherit information from the parent tube), and assign it a barcode and the Description “Homogenized Blood” (e.g., if specimen 1-A (parent specimen) is homogenized, itemize the resulting homogenized specimen as item 1-A-1 with the Description “Homogenized Blood”). Select the evidence type as “Blood.”

5.8.5.8. Document the sonicating amplitude used and the length of time the specimen was sonicated in the batch preparation packet and in the LIMS case synopsis.

5.8.5.9. The chain of custody of the parent specimen shall be updated, if necessary, to reflect the specimen homogenization.

5.8.5.10. The chain of custody of the homogenate tube should begin with the transfer from sample prep to the ISDT Staff Name and then from the ISDT Staff Name to sample prep area with the type of analysis listed in the purpose or note field as shown below:

ITEM # / DESCRIPTION: 1-A-1 Homogenized Blood
OTHER ID #:

Date/Time of Transfer	From	PIN	To	PIN	Purpose
3/5/2018 9:42:15AM	Sample Prep Area	[ ]	ISDT Staff Name	[X]	Transfer
3/5/2018 9:45:08AM	ISDT Staff Name	[X]	Sample Prep Area	[ ]	TOF BDS Prep
3/5/2018 9:57:04AM	Sample Prep Area	[ ]	ISDT Staff Name	[X]	Transfer
3/5/2018 9:57:06AM	ISDT Staff Name	[X]	Walk-In	[ ]	Storage

5.8.5.11. When the tubes are returned to the walk-in refrigerator, the homogenized specimen should be placed in the test tube rack location

where the parent specimen was previously stored. The parent specimen should be placed in the designated location within the walk-in refrigerator.

5.8.5.12. If a previously homogenized specimen requires additional homogenization, the homogenization may be done in the existing homogenate tube (e.g. the 1-A-1 tube). Further itemization of a new homogenate tube is not necessary.

5.8.5.12.1. If homogenization cannot be done in the existing homogenate tube, homogenization may occur in a 16 mm test tube and the specimen decanted back into the original homogenate tube.

5.8.6. Proficiency test specimens

5.8.6.1. Each specimen in the proficiency survey shall be analyzed by approved ISDT method(s) and the analysis shall not be outsourced.

5.8.6.2. Specimens should be prepared per manufacturer's recommendations prior to analysis, if applicable.

5.8.6.2.1. If the manufacturer's recommendations are not consistent with analysis of specimens for the method being performed, seek direction from a laboratory supervisor or quality assurance manager.

5.8.6.3. Specimens shall be placed on a rocker or inverted several times prior to removal of an aliquot from the specimen.

5.8.6.4. Specimens shall be analyzed with evidentiary samples in a batch whenever possible. Specimens may be analyzed without evidentiary samples in a batch with supervisory approval.

5.8.7. Broken, cracked, or leaking specimen tube

5.8.7.1. If a specimen tube has leaked, the cap is not secured, or the specimen tube is broken or cracked, capture an image of the tube, if possible, to document the condition.

5.8.7.2. A case synopsis note shall be made if broken, cracked, or leaking tubes are found at accessioning.

5.8.7.3. If a tube is not securely capped, secure the tube cap.

5.8.7.4. Place the tube in a larger container (e.g., conical tube) and affix a note across the lid of the specimen tube indicating that it shall not be used if the following occur:

5.8.7.4.1. Tube cap is missing;

5.8.7.4.2. Tube has leaked;

5.8.7.4.3. Tube is cracked, or;

5.8.7.4.4. Tube is broken.

5.8.7.4.5. If all tubes in a kit are broken, maintain the tubes in a secondary container, or enclose the kit and tubes in a sealable bag.

5.8.7.4.6. Notify supervisor if all evidence items are broken.

5.8.7.5. Add a note describing the condition of the tube to each request (ref. 13.3.4.6).

5.8.7.6. Leaking or cracked tubes may be used for testing if an aliquot can be taken and all other items of evidence for that case have insufficient volume for testing.

5.8.8. Contaminated specimen during analysis

5.8.8.1. Affix a note across the lid of the specimen tube indicating that it shall not be used for analysis without supervisory approval.
5.8.8.2. Add a note to the batch preparation worksheet stating the specimen was contaminated, what the specimen was contaminated with, and how this occurred.
5.8.8.3. Document on the chain of custody that the specimen was not used for analysis (e.g., Storage/Contaminated).
5.8.8.4. Add a note to the case synopsis stating the specimen was contaminated, what the specimen was contaminated with, and how this occurred.
5.8.8.5. Add a note describing the condition of the tube to each request (ref. 13.3.4.7). The note does not need to be added to requests in which the report has already been released.
5.8.8.6. Record the contamination event in the contamination log.

5.9. Records
5.9.1. Batch preparation worksheet
5.9.2. LIMS case synopsis
5.9.3. Chain of custody

5.10. Interpretation of Results
5.10.1. If the result is to be reported qualitatively only, a note shall be placed on the chromatogram stating the result is to be reported as “Positive” instead of using the quantitative result (ref. 13.3.4.1).

5.11. Report Writing
5.11.1. Testing Not Completed due to Quantity or Quality Not Sufficient
5.11.1.1. The toxicology report shall list Quantity or Quality Not Sufficient as the analyte. No date shall be reported for Quantity or Quality Not Sufficient results.
5.11.2. Proficiency Tests
5.11.2.1. A toxicology report shall be issued for each proficiency test received.
5.11.2.2. If analysis cannot be completed, each analysis (or drug category) not completed shall be listed on the toxicology report.
5.11.2.3. The administrative reviewer shall notify the quality assurance manager of completion of the administrative review of a proficiency test.
5.11.2.4. Results shall be reported to the proficiency test provider on the provider’s form or electronically.
5.11.2.5. Results reported to the proficiency test provider may include more significant figures than reported on a toxicology report for an evidentiary sample.
5.11.2.6. A proficiency test sample shall not be diluted in order to bring the sample concentration into the quantitative range of the method. If allowed by the proficiency test provider, results should be reported as greater than the highest calibrator or reported following directions of the proficiency test provider.

5.11.3. Broken, cracked, or leaking specimen tube

5.11.3.1. If a specimen tube was received or found in the walk-in to be broken, cracked, or leaking, a note shall be added to the report indicating the tube status. If supervisory approval is given to use the specimen for analysis, a note should be added to the report to indicate that a broken, cracked, or leaking tube was received and used for analysis (ref. 13.3.4.6)

5.12. References

5.12.1. N/A

6. Blood Drug Screening by LC/TOF

6.1. Scope

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

6.2. Precautions/Limitations

6.2.1. Minimum Sample Requirement

6.2.1.1. 600 µL of blood or serum/plasma specimen

6.2.2. Mobile phase solutions should be kept in amber bottles to increase stability.

6.3. Related Information

6.3.1. Blood Drug Screening by LC/TOF Validation (September 2017-April 2018)

6.3.2. 4 GHz High Resolution and Injection Volume Update (July 2019)

6.3.3. Reinjection Stability (June 2020)

6.3.4. Injection Volume Study (October-December, 2020)

6.3.5. TOF New Recon Solution Evaluation (2024)

6.3.6. Instrument validations

6.3.7. ToxBox® Plate – Indiana State Dept. of Toxicology

6.4. Instruments/Equipment

6.4.1. Tube rack

6.4.2. Rocker

6.4.3. Vortex, single and multi-tube

6.4.4. Centrifuge

6.4.5. Evaporator

6.4.6. Circulating bath

6.4.7. Liquid chromatograph

6.4.8. Mass spectrometer, time of flight

6.4.9. Vial rack

6.4.10. Pipettes

6.5. Reagents/Materials

6.5.1. ToxBox custom 96-well plate (Multidrug TOF Screen)

6.5.2. Pipette tips

6.5.3. Autosampler vials, inserts, and caps

6.5.4. 13 mm test tubes and caps

6.5.5. ddH_2O

6.5.6. Negative blood (human)

6.5.7. Liquid chromatograph column

6.5.7.1. Dimensions: 4.6 x 50 mm

6.5.7.2. Composition: Zorbax Eclipse Plus C18, Rapid Resolution HT, 1.8 µm

6.5.8. Liquid chromatograph guard column

6.5.8.1. Dimensions: 3.0 mm x 5 mm

6.5.8.2. Composition: Poroshell C18, 2.7 µm particles

6.5.9. Nitrogen

6.5.10. Solvents shall be high quality and low residue (e.g., HPLC grade, Omnisolv, Optima, etc.) unless otherwise noted.

6.5.10.1. Acetonitrile, LCMS grade or higher

6.5.10.2. Methanol, LCMS grade or higher

6.5.10.3. Formic acid
6.5.10.4. Methyl tert-butyl ether
6.5.10.5. Methylene chloride
6.5.11. Ammonium formate
6.5.12. Sodium carbonate
6.5.13. Sodium bicarbonate
6.5.14. Hydrochloric acid

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. Reference Materials/Controls/Calibrators/Solutions
6.7.1. Carbonate Buffer (300 mM), pH 9
6.7.1.1. For example, mix 50.4 g sodium bicarbonate and 63.6 g sodium carbonate into 2 L of ddH_20.
6.7.1.1.1. Adjust pH to 9.0 ± 0.1.
6.7.2. Hydrochloric Acid (0.5M)
6.7.2.1. For example, add 4.106 mL of concentrated hydrochloric acid slowly to approximately 25 mL of ddH_2O, then dilute to 100 mL.
6.7.3. Extraction Solution (60:40 methyl tert-butyl ether: methylene chloride)
6.7.3.1. For example, add 2.4 L methyl tert-butyl ether to 1.6 L methylene chloride for a total volume of 4 L.
6.7.4. Mobile Phase Solutions
6.7.4.1. Aqueous (10 mM ammonium formate and 0.01% formic acid; mobile phase A) – For example, add 400 µL of formic acid and 2.52 g ammonium formate to LCMS grade H_2O to make 4 L.
6.7.4.2. Organic (0.01% formic acid in acetonitrile; mobile phase B) – For example, add 400 µL of formic acid to acetonitrile to make 4 L.
6.7.5. Reconstitution Solution (50:50 water to methanol)
6.7.5.1. For example, add 500 mL of methanol to 500 mL of ddH_2O for a total volume of 1 L.

6.8. Procedures/Instructions
6.8.1. 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.
6.8.2. Mix specimens on a rocker or by inverting several times.
6.8.3. Add 600 µL of negative blood to the calibrator and control well positions.
6.8.4. Add 600 µL of each specimen to its corresponding well position.
6.8.5. Add 400 µL of carbonate buffer to each well position.
6.8.6. Cap and vortex plate.
6.8.7. Pipette 1 mL of sample to a correspondingly labeled test tube.
6.8.8. Add 1 mL ddH_2O to each sample.

6.8.9. Add 4 mL methyl tert-butyl ether to each sample.
6.8.10. Cap tubes and vortex on multi-tube vortexer for 15 minutes.
6.8.11. Centrifuge for 10 minutes using 3000 rpm at 4-8 ºC.
6.8.12. Chill samples in circulating bath at -30 ºC for ~2 minutes, or until the aqueous/blood layer is frozen.
6.8.13. Transfer organic layer of each sample into a correspondingly labeled test tube.
6.8.14. Add 200 µL 0.5 M hydrochloric acid to each aqueous sample and vortex briefly.
6.8.15. Add 4 mL extraction solution to each aqueous sample.
6.8.16. Cap tubes and vortex on multi-tube vortexer for 15 minutes.
6.8.17. Centrifuge for 10 minutes using 3000 rpm at 4-8 ºC.
6.8.18. Chill samples in circulating bath at -30 ºC for ~2 minutes, or until the aqueous/blood layer is frozen.
6.8.19. Transfer organic layer of each sample into the correspondingly labeled test tube from step 6.8.13 and place test tubes on the evaporator.
6.8.20. Evaporate at room temperature using nitrogen.
6.8.21. Add 150 µL of reconstitution solution to each tube and vortex.
6.8.22. Transfer each sample to a correspondingly labeled autosampler vial (with insert) and cap vial.
6.8.23. Analyze the samples by LC/TOF.
6.8.23.1. Sequence names shall be in the following format: YYYY_MM_DD_TOF BDS_Initials.
6.8.23.1.1. The date in the sequence shall be the date of preparation of the samples.
6.8.23.1.2. Additional information such as reinjection, validation, etc., or equivalent abbreviations should be included with the assay abbreviation.
6.8.23.1.3. 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.
6.8.23.2. 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 6.8.23.1. The note should list which lines are attributed to each batch.
6.8.23.3. 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.
6.8.23.4. If the instrument sequence is paused by the acquisition software between two samples, the sequence may be restarted at the sample not yet injected.
6.8.23.4.1. Seven-day sample stability for negative mode and positive mode criteria shall be met.
6.8.23.5. 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.
6.8.23.5.1. A reinjection sequence shall contain, at a minimum, two calibrators and a negative control bracketing the sample(s) to be reinjected.

6.8.23.5.2. A reinjection of a sample of unknown concentration may be performed once per mode of analysis.

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

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

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

6.8.23.6. When the entire sequence is being reinjected or is being injected on another instrument, a tune (ref. 1.4.1.5) 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.

6.8.24. LC/TOF Acquisition Parameters

6.8.24.1. Liquid chromatograph sampler

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

6.8.24.2. 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

Positive Method		Negative Method	
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)

6.8.24.3. Mass spectrometer

Ion Source	Dual AJS ESI
Scan Type	Scan
Data Collection	Centroid
Mode	4 GHz High Resolution
Reference Mass	Enabled

6.9. Records

6.9.1. Pipette calibration certificate, however named
6.9.2. ToxBox Analytical Plate Certificate of Analysis
6.9.3. Batch Preparation Packet
6.9.3.1. Tox Screen Worklist
6.9.3.2. Retest Worksheet, if applicable
6.9.3.3. LC/TOF BDS Preparation Worksheet
6.9.3.4. Aliquot Chain of Custody
6.9.4. MassHunter Worklist Report
6.9.5. TOF Tune Report for each mode analyzed
6.9.6. Calibrator and Control chromatograms
6.9.7. Sample chromatograms
6.9.8. BDS QA/QC Report for each mode analyzed
6.9.9. LC/TOF BDS Technical Review Checklist
6.9.10. 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. Each analyte shall be chromatographically resolved with baseline separation and/or mass resolved
6.10.3. Confirmation drug classes referred to in this method are listed on the ISDT website.
6.10.4. 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.
6.10.4.1. Internal standard mass accuracy shall be within 200 ppm of the target mass.
6.10.4.2. If the corresponding internal standard is not detected, samples may be reinjected (ref. 6.10.8) or, if possible, reanalyzed starting at 6.8.1 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.

6.10.5. Calibrator and Controls Criteria

6.10.5.1. Results of samples analyzed prior to analysis of the first calibrator shall not be used to determine acceptability of batch data.

6.10.5.2. Non-zero controls and/or calibrators shall be placed throughout the batch.

6.10.5.3. A linear calibration curve (no weighting) shall be generated by using at least two calibrators at the cutoff concentration and the origin.

6.10.5.3.1. If two calibrators are used, the lowest RR of the two calibrators shall be used to set the cutoff RR.

6.10.5.3.2. 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.

6.10.5.3.3. Calibrators shall only be excluded for an analyte if 6.10.2 is not met or the analyte and/or corresponding internal standard was not detected.

6.10.5.3.3.1. 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 6.8.1.

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

6.10.5.4.1. 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. 6.10.8) or, if possible, starting at 6.8.1, unless the sample is already presumptive positive for another analyte in the same confirmation drug class.

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

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

6.10.5.5.1. 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.

6.10.6. Analyte Identification

6.10.6.1. 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.

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

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

6.10.6.1.1.1. 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.

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

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

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

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

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

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

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

6.10.6.2. For each analyte with an analyte score ≥ 50, an RR is obtained (ref. 2.3.2).

6.10.6.3. 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.

6.10.6.4. If any analyte in the confirmation drug class (ref. 6.10.3) is considered presumptive positive, the evidentiary specimen shall be moved to confirmation analysis for the drug class.

6.10.7. 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.

6.10.8. Reinjection of Samples

6.10.8.1. The analytical results for a reinjected batch shall meet all acceptability requirements listed in 6.10.

6.10.9. If any criteria listed in 6.10 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. 6.10.3).

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 TOF BDS data due to missing internal standard. 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”).

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

Drug	Cal (ng/mL)	HC (ng/mL)	UHC (ng/mL)	Internal Standard	ISTD (ng/mL)
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.

6.11. Report Writing

6.11.1. 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 6.10.6.

6.11.1.1. The calibration curve and negative controls shall pass acceptability criteria in order to report findings for an evidentiary sample (ref. 6.10.5).

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

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

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

6.12. References

6.12.1. 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).

6.12.2. 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).

6.12.3. 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).

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

6.12.5. 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).

6.12.6. 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).

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

7.1. Scope

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

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.

7.2.2.4. 7-aminoclonazepam should be sonicated prior to use.

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

7.3. Related Information

7.3.1. Benzodiazepines Confirmation Method Validation (September 2015-March 2016)
7.3.2. Stability of Stock Solutions (December 2015-June 2016, January 2017)
7.3.3. Diazepam Update (September 2017)
7.3.4. Calibration Model Update-Quadratic (August 2018)
7.3.5. Stock Solution Stability (January 2020)
7.3.6. Retention Time Versus Relative Retention Time (February 2020)
7.3.7. Instrument validations
7.3.8. Validations 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 columns, 3 mL columns, 35 mg (Tecan #TB-335C)
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 column
7.5.8.1. Dimensions: 3.0 mm x 50 mm
7.5.8.2. Composition: Poroshell C18, 2.7 µm particles
7.5.9. Liquid chromatograph guard column
7.5.9.1. Dimensions: 3.0 mm x 5 mm
7.5.9.2. Composition: Poroshell C18, 2.7 µm particles
7.5.10. CRMs
7.5.10.1. 7-Aminoclonazepam
7.5.10.2. Alprazolam
7.5.10.3. Clonazepam
7.5.10.4. Diazepam
7.5.10.5. Lorazepam
7.5.10.6. Nordiazepam
7.5.10.7. Oxazepam
7.5.10.8. Temazepam
7.5.10.9. Zolpidem
7.5.10.10. 7-Aminoclonazepam-D4
7.5.10.11. Alprazolam-D5
7.5.10.12. Diazepam-D5
7.5.10.13. Lorazepam-D4
7.5.10.14. Oxazepam-D5
7.5.10.15. Temazepam-D5
7.5.10.16. Zolpidem-D7
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. Acetonitrile, LCMS grade
7.5.12.2. Ethyl acetate
7.5.12.3. Ammonium hydroxide, ACS grade or higher
7.5.12.4. Methanol, ACS grade or higher
7.5.12.5. Formic acid
7.5.12.6. Glacial acetic acid, ACS grade or higher
7.5.13. Sodium acetate
7.5.14. Potassium or sodium carbonate
7.5.15. Potassium or sodium bicarbonate

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. Working stock solutions for calibrators are stable for up to 4 months. Control and internal standard solutions are stable for up to 9 months. Calibrators, controls, and internal standard solutions should be stored in a freezer.

7.7.2. All working stock solutions shall be made by dilution of CRMs in methanol. The calibrator working stock solutions and non-zero control working stock solutions should be made by different analysts. If these solutions are made by the same analyst, calibrator and control working stock solutions shall be made in separate and distinct preparation sessions.

7.7.2.1. Low Calibrator 1

7.7.2.1.1. 500 ng/mL - 7-Aminoclonazepam

7.7.2.1.2. 1,000 ng/mL - Alprazolam, Clonazepam, Lorazepam, Zolpidem

7.7.2.2. Low Calibrator 2

7.7.2.2.1. 2,500 ng/mL - Diazepam, Nordiazepam, Oxazepam, Temazepam

7.7.2.3. High Calibrator 1

7.7.2.3.1. 2,000 ng/mL - 7-Aminoclonazepam

7.7.2.3.2. 4,000 ng/mL - Alprazolam, Clonazepam, Lorazepam, Zolpidem

7.7.2.4. High Calibrator 2

7.7.2.4.1. 10,000 ng/mL - Diazepam, Nordiazepam, Oxazepam, Temazepam

7.7.2.5. Low Control

7.7.2.5.1. 500 ng/mL - 7-Aminoclonazepam, Alprazolam, Clonazepam, Lorazepam, Zolpidem

7.7.2.5.2. 1,500 ng/mL - Diazepam, Nordiazepam, Oxazepam, Temazepam

7.7.2.6. High Control

7.7.2.6.1. 2,500 ng/mL - 7-Aminoclonazepam

7.7.2.6.2. 5,000 ng/mL - Alprazolam, Clonazepam, Lorazepam, Zolpidem

7.7.2.6.3. 10,000 ng/mL - Diazepam, Nordiazepam, Oxazepam, Temazepam

7.7.2.7. Internal Standard

7.7.2.7.1. 2,000 ng/mL-7-Aminoclonazepam-D4, Alprazolam-D5, Diazepam-D5, Lorazepam-D4, Oxazepam-D5, Temazepam-D5, Zolpidem-D7

7.7.3. Elution Solution

7.7.3.1. On the day of extraction, make a 98:2 ethyl acetate and ammonium hydroxide solution.

7.7.3.1.1. For example, 294 mL of ethyl acetate with 6 mL of ammonium hydroxide will be sufficient for a batch of 96 samples.

7.7.4. Acetate Buffer (300 mM)

7.7.4.1. For example, mix 49.2 g sodium acetate and 34.4 mL acetic acid into 2 L of ddH_2O.

7.7.4.1.1. Adjust pH to 4.6 ± 0.1.

7.7.5. Carbonate Buffer (300 mM)

7.7.5.1. For example, mix 40 g potassium bicarbonate and 20 g potassium carbonate into 2 L of ddH_2O.
7.7.5.1.1. Adjust pH to 9.0 ± 0.1.
7.7.5.2. For example, mix 50.4 g sodium bicarbonate and 63.6 g sodium carbonate into 2 L of ddH_2O.
7.7.5.2.1. Adjust pH to 9.0 ± 0.1.

7.7.6. Mobile Phases
7.7.6.1. Aqueous (A) – Add 1 mL of formic acid per 1 L ddH_2O.
7.7.6.2. Organic (B) – Add 1 mL of formic acid per 1 L acetonitrile.

7.7.7. Reconstitution Solution
7.7.7.1. Make a 70:30 aqueous mobile phase to organic mobile phase solution (or 70:30 ddH_2O to acetonitrile).
7.7.7.1.1. For example, add 30 mL of organic mobile phase (ref. 7.7.6.2) to 70 mL of aqueous mobile phase (ref. 7.7.6.1).

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 each tube.

7.8.4. Prepare calibrator and control samples in correspondingly labeled tubes as indicated in Table 2. For batch analysis, the calibrator and non-zero control working stocks used shall conform to 7.7.2.

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		

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 acetate 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 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 methanol
  7.8.10.2. 1 mL ddH_2O
  7.8.10.3. 1 mL acetate buffer
7.8.11. 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.
7.8.12. Add 3 mL of carbonate buffer to each column and allow to flow completely through at ~1 mL per minute.
7.8.13. Add 3 mL ddH_2O to each column and allow to flow completely through at ~1 mL per minute.
7.8.14. Using a maximum flow of ~60 psi or greater, dry the columns for at least 30 minutes.
7.8.15. Place empty labeled tubes into the positive pressure manifold, ensuring the placement of the tubes corresponds with the arrangement of the sample columns.
7.8.16. Add 3 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 reconstitution solution 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_BNZ-Z_Initials.
    7.8.21.1.1. The date in 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.
  7.8.21.2. 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 7.8.21.1. The note should list which lines are attributed to each batch.
  7.8.21.3. 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. 7.10.5).
  7.8.21.4. If the instrument sequence is paused by the acquisition software between two samples, the sequence may be restarted at the sample not yet injected.
    7.8.21.4.1. Sample stability criteria shall be met.
  7.8.21.5. 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.

7.8.21.5.1. Sample stability criteria shall be met.

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

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

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

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

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

7.8.21.5.5. If an entire sequence is reinjected or a reinjection includes the calibrators used to determine the calibration curve, a check tune (ref. 1.3.1.5) 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 – 5.0 µL

7.8.22.2. 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

7.8.22.3. Liquid chromatograph column compartment

Temperature	45 ºC

7.8.22.4. Mass spectrometer

Ion Source	AJS ESI
Scan Type	Dynamic MRM

7.8.22.5. 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.

7.8.22.6. 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

7.9. Records
- 7.9.1. Pipette calibration certificate, however named
- 7.9.2. Benzodiazepines and Z-Drugs Confirmation Calibrator Solution Preparation Worksheet
- 7.9.3. Benzodiazepines and Z-Drugs Confirmation Internal Standard Solution Preparation Worksheet
- 7.9.4. Benzodiazepines and Z-Drugs 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. Benzodiazepines and Z-Drugs 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. Benzodiazepines and Z-Drugs 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 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. Calibration and Controls Criteria
  - 7.10.3.1. Results of samples analyzed prior to analysis of the negative control preceding the calibrators shall not be used to determine acceptability of batch data.
  - 7.10.3.2. Quantitation of calibrators and non-zero controls shall be within ± 20% of the target concentration.
  - 7.10.3.3. Generating a calibration curve
    - 7.10.3.3.1. Calibration curve shall include a minimum of five non-zero concentrations.
    - 7.10.3.3.2. Correlation coefficient (r^2) for the calibration curve shall be ≥ 0.990.
    - 7.10.3.3.3. 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.

7.10.3.3.4. 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.

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

7.10.3.3.5.1. An ion ratio does not meet the acceptability criteria listed in 7.10.3.3.3 or 7.10.3.3.4.

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

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

7.10.3.3.5.4. A peak has poor chromatography.

7.10.3.3.6. 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.

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

7.10.3.3.7. 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.

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

7.10.3.4. 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 7.10.3.3.3 or 7.10.3.3.4.

7.10.3.4.1. 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.

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

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

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

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

7.10.3.7.1. An ion ratio does not meet the acceptability criteria listed in 7.10.3.3.3 or 7.10.3.3.4.

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

7.10.3.7.3. A peak has poor chromatography.

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

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

7.10.3.8.1. 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 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 7.10.3.4 and 7.10.3.5.

7.10.4. Analyte Identification (Qualitative Criteria)

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

7.10.4.2. 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.

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

7.10.4.4. Each ion ratio shall meet the acceptability criteria listed in 7.10.3.3.3 or 7.10.3.3.4.

7.10.4.4.1. 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.

7.10.4.5. 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.

7.10.5. Analyte Stability

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

7.10.6. Retesting Samples

7.10.6.1. 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.

7.10.6.1.1. If a quantitative value cannot be reported from any analysis, the first acceptable qualitative data according to analyte identification in 7.10.4 shall be used. (ref. 7.11.4).

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

7.10.7. Unacceptable Data

7.10.7.1. 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”).

7.10.8. No Data Generated for a Sample

7.10.8.1. 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 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

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

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 greater than or equal to 1 and less than 10.

7.11.3.2.1.2. A result shall be reported as a whole number for a quantitative value 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. A quantitative result shall only be reported if analysis occurred within the established sample stability window (ref. 7.8.21.3).

7.11.3.5. 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.

7.11.4. Qualitative Reporting

7.11.4.1. A result should be reported as “Positive” when the analyte identification criteria (ref. 7.10.4) have 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 (ref. 13.3.4.1).

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

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

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

8. Cannabinoids Confirmation by GC/MS

8.1. Scope

8.1.1. 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.

8.2. Precautions/Limitations

8.2.1. Minimum Sample Requirement

8.2.1.1. 1 mL of blood or serum/plasma specimen

8.2.2. CRMs

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

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

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

8.2.3. BSTFA and MTBSTFA hydrolyze easily.

8.3. Related Information

8.3.1. THC Confirmatory Analysis Method Validation (October 2016 - February 2017)
8.3.2. THC Stock Solution Stability Supplemental (April 2017)
8.3.3. THC Reinjection Supplemental (July 2017)
8.3.4. Stock Solution Stability (February 2020)
8.3.5. Injection Volume Supplemental for THC-COOH (March 2020)
8.3.6. THC Screw Cap Stability (2021)
8.3.7. ISTD Solution Stability (2022)
8.3.8. ISTD Solution Stability (2023)
8.3.9. Instrument validations
8.3.10. Validation of calibrators, controls, and internal standards data

8.4. Instruments/Equipment

8.4.1. Tube rack
8.4.2. Rocker
8.4.3. Vortex, single and multi-tube
8.4.4. Centrifuge
8.4.5. Positive pressure manifold
8.4.6. SPE column rack
8.4.7. SPE collection rack
8.4.8. Waste collection rack
8.4.9. Evaporator
8.4.10. Dry block heater
8.4.11. Vial rack
8.4.12. Electronic or manual crimper
8.4.13. Gas chromatograph
8.4.14. Mass spectrometer, single quadrupole
8.4.15. Pipettes

8.5. Reagents/Materials

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

8.5.2. Cerex Polychrom THC SPE columns, 6 mL columns, 65 mg (Tecan #682-0506, or equivalent)
8.5.3. Tube caps (e.g., 13 mm flange)
8.5.4. Pipette tips
8.5.5. Autosampler vials, inserts, and caps
8.5.6. ddH_2O
8.5.7. Negative blood (human)
8.5.8. Gas chromatograph capillary column-analytical column
8.5.8.1. Dimensions: 15 m x 0.25 mm x 0.25 µm
8.5.8.2. Composition: DB-5 MS UI (5%-Phenyl)-methylpolysiloxane
8.5.9. Gas chromatograph capillary column-restrictor column
8.5.9.1. Dimensions: ~0.5 m x 150 µm
8.5.9.2. Composition: Fused silica
8.5.10. BSTFA + 1% TMCS
8.5.11. MTBSTFA + 1% TBDMCS
8.5.12. CRMs
8.5.12.1. Delta-9 THC
8.5.12.2. Delta-9 THC-D9
8.5.12.3. Delta-9 THC-COOH
8.5.12.4. Delta-9 THC-COOH-D9
8.5.13. Helium, 5.0 grade or higher
8.5.14. Nitrogen
8.5.15. Solvents shall be high quality and low residue (e.g., HPLC grade, Omnisolv, Optima, etc.) unless otherwise noted.
8.5.15.1. Acetonitrile
8.5.15.2. Ethyl acetate
8.5.15.3. Hexane(s)
8.5.15.4. Glacial acetic acid, ACS grade or higher
8.5.15.5. Ammonium hydroxide, ACS grade or higher
8.5.15.6. Methanol, ACS grade or higher

8.6. Hazards/Safety
8.6.1. See Safety Manual.
8.6.2. See SDS for each chemical in this method.
8.6.3. Add acids to approximately half the volume of the less acidic liquid, then dilute to final volume.

8.7. Reference Materials/Controls/Calibrators/Solutions
8.7.1. Calibrator and control working stock solutions are stable for up to 5 months and should be stored in a freezer. Internal standard working stock solutions are stable for up to 25 months and should be stored in a freezer.
8.7.2. All working stock solutions shall be made by dilution of CRMs in methanol. The calibrator working stock solutions and non-zero control working stock solutions should be made by different analysts. If these solutions are made by the same analyst, calibrator and control working stock solutions shall be made in separate and distinct preparation sessions.
8.7.2.1. Low Calibrator
8.7.2.1.1. 100 ng/mL - Delta-9 THC
8.7.2.1.2. 250 ng/mL - Delta-9 THC-COOH

8.7.2.2. High Calibrator
8.7.2.2.1. 500 ng/mL - Delta-9 THC
8.7.2.2.2. 1,000 ng/mL - Delta-9 THC-COOH
8.7.2.3. Low Control
8.7.2.3.1. 200 ng/mL - Delta-9 THC
8.7.2.3.2. 500 ng/mL - Delta-9 THC-COOH
8.7.2.4. High Control
8.7.2.4.1. 500 ng/mL - Delta-9 THC
8.7.2.4.2. 1,000 ng/mL - Delta-9 THC-COOH
8.7.2.5. Internal Standard
8.7.2.5.1. 400 ng/mL - Delta-9 THC-D9
8.7.2.5.2. 1,000 ng/mL - Delta-9 THC-COOH-D9

8.7.3. THC Wash Solution
8.7.3.1. On the day of extraction, make an 85:15:1 ddH_2O, acetonitrile, and ammonium hydroxide solution.
8.7.3.1.1. For example, 85 mL of ddH_2O, 15 mL of acetonitrile, and 1 mL of ammonium hydroxide will be sufficient for a batch of 48 samples.

8.7.4. THC Elution Solution
8.7.4.1. On the day of extraction, make a 50:50 solution of ethyl acetate and hexane.
8.7.4.1.1. For example, 75 mL of ethyl acetate and 75 mL hexane will be sufficient for a batch of 48 samples.

8.7.5. THC-COOH Elution Solution
8.7.5.1. On the day of extraction, make a 90:10:3 solution of hexane, ethyl acetate, and glacial acetic acid.
8.7.5.1.1. For example, 135 mL hexane, 15 mL ethyl acetate, and 4.5 mL glacial acetic acid will be sufficient for a batch of 48 samples.

8.8. Procedures/Instructions

8.8.1. 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.
8.8.1.1. Reinjected negative controls shall be denoted with an “RI” followed by the number of reinjections.

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

8.8.3. 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.

8.8.4. Prepare calibrator and control samples in correspondingly labeled tubes as indicated in Table 5. For batch analysis, the calibrator and non-zero control working stocks used shall conform to 8.7.2.

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 8.8.19)

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

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

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

8.8.7. 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.

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

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

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

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

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

8.8.13. 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.

8.8.14. Add 2 mL of THC Wash Solution to each column and allow to flow completely through each column at ~2 mL per minute.

8.8.15. Using a maximum flow of ~30 psi on the positive pressure manifold, dry the columns for ~10 minutes.

8.8.16. Add 2 mL of hexane to each column and allow to flow completely through each column at ~2 mL per minute.

8.8.17. Using a maximum flow of ~10 psi on the positive pressure manifold, dry the columns for ~5 minutes.

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

8.8.19. Add 3 mL of THC elution solution to each column and allow to flow completely through each column at ~2 mL per minute.

8.8.20. Remove tubes with THC eluent from the positive pressure manifold and set aside until step 8.8.25, or place on evaporator and evaporate at room temperature using nitrogen.

8.8.21. Add 2 mL of ethyl acetate to each column and allow to flow completely through each column at ~2 mL per minute.
8.8.22. Using a maximum flow of ~30 psi on the positive pressure manifold, dry the columns for ~5 minutes.
8.8.23. Place empty labeled tubes into the positive pressure manifold, ensuring the placement of the tubes corresponds with the arrangement of the sample columns.
8.8.24. Add 3 mL of THC-COOH elution solution to each column and allow to flow completely through each column at ~2 mL per minute.
8.8.25. Remove THC-COOH tubes from the positive pressure manifold and place on the evaporator. Evaporate at room temperature using nitrogen.
8.8.26. Add 50 µL BSTFA with 1% TMCS to each THC tube, cap, and vortex.
8.8.27. Add 50 µL MTBSTFA with 1% TBDMCS to each THC-COOH tube, cap, and vortex.
8.8.28. Place the tubes in a dry heat block at ~70 ºC for ~25 minutes.
8.8.29. Allow the tubes to cool. Transfer each sample to a correspondingly labeled autosampler vial and cap vial.
8.8.30. Analyze the samples by GC/MS.
8.8.30.1. Sequence names shall be in the following format: YYYY_MM_DD_THC_Initials and/or YYYY_MM_DD_COOH_Initials.
8.8.30.1.1. The date in the sequence shall be the date of preparation of the samples.
8.8.30.1.2. Additional information such as reinjection, validation, etc., or equivalent abbreviations should be included with the assay abbreviation.
8.8.30.1.3. 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.
8.8.30.2. 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 8.8.30.1. The note should list which lines are attributed to each batch.
8.8.30.3. 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.
8.8.30.4. 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.
8.8.30.5. Samples for Delta-9 THC and Delta-9 THC-COOH analyses may have different sequence names and be analyzed on different GC/MS instruments.
8.8.30.6. If the instrument sequence is paused by the acquisition software between two samples, the sequence may be restarted at the sample not yet injected.
8.8.30.6.1. Sample stability criteria shall be met.
8.8.30.7. 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.

8.8.30.7.1. Sample stability criteria shall be met.

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

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

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

8.8.30.7.3.1.1. Evidentiary samples that are skipped and do not have valid data shall be reanalyzed starting at 8.8.1.

8.8.30.7.4. 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.

8.8.30.7.5. If an entire sequence is reinjected or a reinjection includes the calibrators used to generate the calibration curve, an autotune (ref. 1.2.1.2) 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.

8.8.31. GC/MS Instrument Parameters: THC

8.8.31.1. 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

8.8.31.2. Gas chromatograph inlet

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

8.8.31.3. 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

8.8.31.4. 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

8.8.31.5. 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

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

8.8.31.7. 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.

8.8.32. GC/MS Instrument Parameters: THC-COOH

8.8.32.1. 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

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

8.8.32.3. 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

8.8.32.4. 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

8.8.32.5. 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

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

8.8.32.7. 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.

8.8.33. 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

8.9. Records

8.9.1. Pipette calibration certificate, however named
8.9.2. Cannabinoids Confirmation Calibrator and Internal Standard Solution Preparation
8.9.3. Cannabinoids Confirmation Control Solution Preparation
8.9.4. Batch Preparation Packet, however named
8.9.4.1. ISDT Confirmation Worklist
8.9.4.2. Retest Worksheet, as appropriate
8.9.4.3. Cannabinoids Confirmation Preparation Worksheet
8.9.4.4. Aliquot Chain of Custody
8.9.5. Sequence Table
8.9.6. Quantitative Analysis Results Summary Report
8.9.7. Calibration Report
8.9.8. Calibrator and control chromatograms
8.9.9. Cannabinoids Confirmation Ion Ratio Ranges Worksheet
8.9.10. Sample chromatograms
8.9.11. Autotune
8.9.12. Cannabinoids Confirmation Technical Review Checklist
8.9.13. Data comparison output, however named
8.9.14. Measurement Uncertainty Estimation and supporting data
8.9.15. Specimen Verification Worksheet, if applicable

8.10. Interpretation of Results

8.10.1. Interpretation of results for each analyte shall occur independent of the other analytes in the method.
8.10.2. Chromatographic analyte and internal standard peaks shall have baseline resolution and/or analytes shall be mass resolved in the mass spectrometer.
8.10.2.1. A shoulder peak shall be < 10% of analyte peak height and area in order to report a quantitative result.
8.10.3. Calibration and Controls Criteria
8.10.3.1. Results of samples analyzed prior to analysis of the negative control preceding the calibrators shall not be used to determine acceptability of batch data.

8.10.3.2. 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).

8.10.3.3. Generating a calibration curve

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

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

8.10.3.3.3. 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.

8.10.3.3.4. 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.

8.10.3.3.5. 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.

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

8.10.3.3.6.1. An ion ratio does not meet the acceptability criteria listed in 8.10.3.3.3 or 8.10.3.3.4;

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

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

8.10.3.3.6.4. A peak has poor chromatography.

8.10.3.3.7. 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 8.8.1.

8.10.3.3.8. 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 8.8.1. If unable to retest, the results for the analysis may be reported as greater than the highest calibrator used in the batch.

8.10.3.4. Each negative control shall have an analyte concentration or response < 50% of the LLOQ and/or unacceptable ion ratios as specified in 8.10.3.3.3 or 8.10.3.3.4.

8.10.3.4.1. 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 8.8.1. A result < 50% of the LLOQ for an evidentiary sample shall be accepted as none detected.

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

8.10.3.5.1. If acceptance criterion is not met, all samples in the batch shall be reanalyzed, if possible, starting at 8.8.1.

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

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

8.10.3.7.1. An ion ratio does not meet the acceptability criteria listed in 8.10.3.3.3 or 8.10.3.3.4;

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

8.10.3.7.3. A peak has poor chromatography.

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

8.10.3.8.1. 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 8.8.1. 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 8.10.3.4 and 8.10.3.4.1.

8.10.3.9. If the acceptability criteria are not met for one analyte, analysis may be repeated for only the failed analyte, starting at 8.8.1. The eluent for the analyte that passed may be discarded prior to reanalysis for the failed analyte.

8.10.4. Analyte Identification (Qualitative Criteria)

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

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

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

8.10.4.4. Each ion ratio shall meet the acceptability criteria listed in 8.10.3.3.3 or 8.10.3.3.4.

8.10.4.4.1. 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.

8.10.4.5. 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.

8.10.5. Analyte Stability

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

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

8.10.6. Retesting Samples

8.10.6.1. 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.

8.10.6.1.1. If a quantitative value cannot be reported from any analysis, the first acceptable qualitative data according to analyte identification in 8.10.4 shall be used. (ref. 8.11.4).

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

8.10.7. Unacceptable Data

8.10.7.1. 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”).

8.10.8. No Data Generated for a Sample

8.10.8.1. 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 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.

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

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

8.11.3. Quantitative Reporting

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

8.11.3.1.1. 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.

8.11.3.2. 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.

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

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

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

8.11.3.3. 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.

8.11.3.3.1. 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.

8.11.3.3.1.1. 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.

8.11.3.4. A quantitative result shall only be reported if analysis occurred within the established sample stability window (ref. 8.10.5).

8.11.3.5. 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.

8.11.4. Qualitative Reporting

8.11.4.1. A result should be reported as “Positive” when the analyte identification criteria (ref. 8.10.4) have been met, the quantitative result is > 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 any of the following occur (ref. 13.3.4.1):

8.11.4.2.1. Interference(s); or

8.11.4.2.2. 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.

8.12. References

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

8.12.2. 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).

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

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

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

8.12.6. United Chemical Technologies Applications Manual (2004).

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

9. Cocaine Confirmation by GC/MS

9.1. Scope

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

9.2. Precautions/Limitations

9.2.1. Minimum Sample Requirement

9.2.1.1. 1 mL of blood or serum/plasma specimen for quantitative confirmation

9.2.1.2. 250 µL of blood or serum/plasma specimen for qualitative confirmation

9.2.2. CRMs

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

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

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

9.2.3. BSTFA hydrolyzes easily.

9.3. Related Information

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

9.3.2. Stock Solution Stability (February 2020)

9.3.3. Instrument validations

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

9.4. Instruments/Equipment

9.4.1. Tube rack

9.4.2. Rocker

9.4.3. Vortex, single

9.4.4. Sonicating water bath

9.4.5. Centrifuge

9.4.6. Positive pressure manifold

9.4.7. SPE column rack

9.4.8. SPE collection rack

9.4.9. Waste collection rack

9.4.10. Vial rack

9.4.11. Dry block heater

9.4.12. Evaporator

9.4.13. Electronic or manual crimper

9.4.14. Gas chromatograph

9.4.15. Mass spectrometer, single quadrupole

9.4.16. Pipettes

9.5. Reagents/Materials

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

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

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

9.5.4. Pipette tips
9.5.5. Autosampler vials, inserts, and caps
9.5.6. ddH_2O
9.5.7. Negative blood (human)
9.5.8. Gas chromatograph capillary column-analytical column
9.5.8.1. Dimensions: 15 m x 0.25 mm x 0.25 µm
9.5.8.2. Composition: DB-5 MS UI (5%-Phenyl)-methylpolysiloxane
9.5.9. Gas chromatograph capillary column-restrictor column
9.5.9.1. Dimensions: ~0.5 m x 150 µm
9.5.9.2. Composition: fused silica
9.5.10. BSTFA + 1% TMCS
9.5.11. CRMs
9.5.11.1. Benzoylecgonine
9.5.11.2. Cocaine
9.5.11.3. Benzoylecgonine-D3
9.5.11.4. Cocaine-D3
9.5.12. Helium, 5.0 grade or higher
9.5.13. Nitrogen
9.5.14. Solvents shall be high quality and low residue (e.g., HPLC grade, Omnisolv, Optima, etc.) unless otherwise noted.
9.5.14.1. Ethyl acetate
9.5.14.2. Methylene chloride
9.5.14.3. Isopropanol
9.5.14.4. Glacial acetic acid, ACS grade or higher
9.5.14.5. Ammonium hydroxide, ACS grade or higher
9.5.14.6. Methanol, ACS grade or higher
9.5.15. Sodium phosphate monobasic
9.5.16. Sodium phosphate dibasic

9.6. Hazards/Safety
9.6.1. See Safety Manual.
9.6.2. See SDS for each chemical in this method.
9.6.3. Add acids to approximately half the volume of the less acidic liquid, then dilute to final volume.

9.7. Reference Materials/Controls/Calibrators/Solutions
9.7.1. Working stock solutions are stable for up to 6 months and should be stored in the freezer.
9.7.2. All working stock solutions shall be made by dilution of CRMs in methanol. The calibrator working stock solutions and non-zero control working stock solutions should be made by different analysts. If these solutions are made by the same analyst, calibrator and control working stock solution shall be made in separate and distinct preparation sessions.
9.7.2.1. Calibrator Stock
9.7.2.1.1. 100,000 ng/mL - Benzoylecgonine, Cocaine
9.7.2.2. High Calibrator
9.7.2.2.1. 10,000 ng/mL - Benzoylecgonine, Cocaine (1:10 dilution from 9.7.2.1)
9.7.2.3. Low Calibrator

9.7.2.3.1. 1,000 ng/mL - Benzoylecgonine, Cocaine (1:10 dilution from 9.7.2.2)

9.7.2.4. Control Stock

100,000 ng/mL - Benzoylecgonine, Cocaine

9.7.2.5. High Control

9.7.2.5.1. 10,000 ng/mL - Benzoylecgonine, Cocaine (1:10 dilution from 9.7.2.4)

9.7.2.6. Low Control

9.7.2.6.1. 1,000 ng/mL - Benzoylecgonine, Cocaine (1:10 dilution from 9.7.2.5)

9.7.2.7. Internal Standard

9.7.2.7.1. 1,000 ng/mL – Benzoylecgonine-D3, Cocaine-D3

9.7.3. Cocaine Elution Solution

9.7.3.1. On the day of extraction, make a 20:2:78 isopropanol, ammonium hydroxide, and methylene chloride solution.

9.7.3.1.1. For example, 20 mL isopropanol and 2 mL ammonium hydroxide, diluted to 100 mL with methylene chloride, will be sufficient for a batch of 48 samples.

9.7.4. Phosphate Buffer (100 mM)

9.7.4.1. For example, dissolve 12.14 g sodium phosphate monobasic and 1.70 g sodium phosphate dibasic to 1 L with ddH_2O.

9.7.4.1.1. Adjust pH to 6.0 ± 0.1.

9.7.5. Acetic Acid (100 mM)

9.7.5.1. For example, dilute 2.86 mL glacial acetic acid to 500 mL with ddH_2O.

9.8. Procedures/Instructions

9.8.1. 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.

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

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

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

9.8.4. Prepare calibrator and control samples in correspondingly labeled tubes as indicated in Table 6. For batch analysis, the calibrator and non-zero control working stocks used shall conform to 9.7.2.

Table 6: 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

Level	Sample Identification	Stock Solution	Volume (µL)
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

9.8.5. Pipette 1 mL of negative blood into calibrator and control samples.
9.8.6. Pipette 1 mL of specimen into the correspondingly labeled tube.
9.8.7. Add 2 mL of 100 mM phosphate buffer to each tube. Cap and vortex each tube.
9.8.8. Sonicate for ~10 minutes.
9.8.9. Centrifuge for ~10 minutes using 3000 rpm at ~4-8 ºC.
9.8.10. 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:
  9.8.10.1. 1 mL of methanol
  9.8.10.2. 1 mL of ddH_2O
  9.8.10.3. 1 mL of phosphate buffer
9.8.11. 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.
9.8.12. 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:
  9.8.12.1. 2 mL ddH_2O
  9.8.12.2. 2 mL 100 mM acetic acid
  9.8.12.3. 1 mL methanol
  9.8.12.4. 1 mL ethyl acetate
9.8.13. Using a maximum flow of ~60 psi or greater, dry the columns for ~ 20 minutes.
9.8.14. Place empty labeled tubes into the positive pressure manifold, ensuring the placement of the tubes corresponds with the arrangement of the sample columns.
9.8.15. 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.
9.8.16. Remove tubes from the positive pressure manifold and place on the evaporator.
9.8.17. Evaporate at room temperature using nitrogen.
9.8.18. Add 50 µL ethyl acetate, then 50 µL BSTFA with 1% TMCS, to each tube and cap.
9.8.19. Vortex tubes briefly.
9.8.20. Place the tubes in a dry heat block at ~ 70 ºC for ~ 25 minutes.
9.8.21. Allow the tubes to cool. Transfer each sample to the correspondingly labeled autosampler vial and cap vial.
9.8.22. Analyze the samples by GC/MS.
  9.8.22.1. Sequence names shall be in the following format: YYYY_MM_DD_COC_Initials.
    9.8.22.1.1. The date in the sequence shall be the date of preparation of the samples.

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

9.8.22.1.3. 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.

9.8.22.2. 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 9.8.22.1. The note should list which lines are attributed to each batch.

9.8.22.3. The extracted samples shall be stored at room temperature and analyzed for cocaine and benzoylecgonine within 4 days of completion of the extraction process.

9.8.22.4. When a sample has a cocaine or benzoylecgonine concentration > 450 ng/mL, intelligent sequencing may be used to prevent carryover into subsequent samples.

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

9.8.22.5.1. Sample stability criteria shall be met.

9.8.22.6. 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.

9.8.22.6.1. Sample stability criteria shall be met.

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

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

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

9.8.22.6.3.1.1. Evidentiary samples that are skipped and do not have valid data shall be reanalyzed starting at 9.8.1.

9.8.22.6.4. 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.

9.8.22.6.5. If an entire sequence is reinjected or a reinjection includes the calibrators used to generate the calibration curve, an autotune (ref. 1.2.1.2) 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.

9.8.23. GC/MS Instrument Parameters

9.8.23.1. 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

9.8.23.2. Gas chromatograph inlet

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

9.8.23.3. 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

9.8.23.4. 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

9.8.23.5. 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

9.8.23.6. Auxiliary Heaters

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

9.8.23.7. 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.

9.8.23.8. 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

9.9. Records
- 9.9.1. Pipette calibration certificate, however named
- 9.9.2. Cocaine Confirmation Calibrator and Internal Standard Solution Preparation Worksheet
- 9.9.3. Cocaine Confirmation Control Solution Preparation Worksheet
- 9.9.4. Batch Preparation Packet, however named
  - 9.9.4.1. ISDT Confirmation Worklist
  - 9.9.4.2. Retest Worksheet, as appropriate
  - 9.9.4.3. Cocaine Confirmation Preparation Worksheet
  - 9.9.4.4. Aliquot Chain of Custody
- 9.9.5. Sequence Table
- 9.9.6. Quantitative Analysis Results Summary Report
- 9.9.7. Calibrator and control chromatograms
- 9.9.8. Calibration Report
- 9.9.9. Cocaine Confirmation Ion Ratio Ranges Worksheet
- 9.9.10. Sample chromatograms
- 9.9.11. Autotune
- 9.9.12. Cocaine Confirmation Technical Review Checklist
- 9.9.13. Data comparison output, however named
- 9.9.14. Measurement Uncertainty Estimation and supporting data
- 9.9.15. Specimen Verification Worksheet, if applicable

9.10. Interpretation of Results

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

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

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

9.10.3. Calibration and Controls Criteria

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

9.10.3.2. Quantitation of calibrators and non-zero controls shall be within ± 20% of the target concentration.

9.10.3.3. Generating a calibration curve

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

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

9.10.3.3.3. 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.

9.10.3.3.4. 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.

9.10.3.3.5. 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 Cocaine Confirmation Ion Ratio Ranges Worksheet may differ in the tenths decimal place from the chromatogram.

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

9.10.3.3.6.1. An ion ratio does not meet the acceptability criteria listed in 9.10.3.3.3 or 9.10.3.3.4.

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

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

9.10.3.3.6.4. A peak has poor chromatography.

9.10.3.3.7. 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 9.8.1.

9.10.3.3.8. 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 9.8.1. If unable to retest, the results for the analysis may be reported as greater than the highest calibrator used in the batch.

9.10.3.4. Each negative control shall have an analyte concentration or response < 50% of the LLOQ and/or unacceptable ion ratios as specified in 9.10.3.3.3 or 9.10.3.3.4.

9.10.3.4.1. 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 9.8.1. A result < 50% of the LLOQ for an evidentiary sample shall be accepted as none detected.

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

9.10.3.5.1. If acceptance criterion is not met, all samples in the batch shall be reanalyzed, if possible, starting at 9.8.1.

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

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

9.10.3.7.1. An ion ratio does not meet the acceptability criteria listed in 9.10.3.3.3 or 9.10.3.3.4.

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

9.10.3.7.3. A peak has poor chromatography.

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

9.10.3.8.1. 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 9.8.1. 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 9.10.3.4 and 9.10.3.4.1.

9.10.4. Analyte Identification (Qualitative Criteria)

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

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

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

9.10.4.4. Each ion ratio shall meet the acceptability criteria listed in 9.10.3.3.3 or 9.10.3.3.4.

9.10.4.4.1. 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.

9.10.4.5. 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.

9.10.5. Analyte Stability

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

9.10.6. Retesting Samples

9.10.6.1. 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.

9.10.6.1.1. If a quantitative value cannot be reported from any analysis, the first acceptable qualitative data according to analyte identification in 9.10.4 shall be used. (ref. 9.11.4).

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

9.10.7. Unacceptable Data

9.10.7.1. 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”).

9.10.8. No Data Generated for a Sample

9.10.8.1. 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”).

9.11. Report Writing

9.11.1. 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.

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

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

9.11.3. Quantitative Reporting

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

9.11.3.1.1. 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.

9.11.3.2. 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.

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

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

9.11.3.3. 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.

9.11.3.3.1. 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.

9.11.3.3.1.1. 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.

9.11.3.4. A quantitative result shall only be reported if analysis occurred within the established sample stability window (ref. 9.10.5).

9.11.3.5. 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.

9.11.4. Qualitative Reporting

9.11.4.1. A result should be reported as “Positive” when the analyte identification criteria (ref. 9.10.4) have been met, the quantitative result is > LLOQ, and the quantitative criteria have not been met.

9.11.4.1.1. 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.

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

9.11.4.2. 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).

9.11.4.3. A result may be reported as “Positive” with supervisory approval if any of the following occur (ref. 13.3.4.1):

9.11.4.3.1. Interference(s); or

9.11.4.3.2. 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.

9.12. References

9.12.1. 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.

9.12.2. 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.

9.12.3. United Chemical Technologies Applications Manual (2004).

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

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

10. Opioids Drug Confirmation by LC/QQQ

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

10.2. Precautions/Limitations
- 10.2.1. Minimum Sample Requirement
  - 10.2.1.1. 0.200 mL of blood or serum/plasma specimen.
- 10.2.2. CRMs
  - 10.2.2.1. CRMs used for calibrator and non-zero control stocks shall be from two different vendors, if available.
  - 10.2.2.2. If using CRMs from the same vendor, two different lots shall be used, if available.
  - 10.2.2.3. If only one lot of a CRM is available, two separate vials from the lot shall be used.
- 10.2.3. Mobile phases should be kept in amber bottles to increase stability.

10.3. Related Information
- 10.3.1. Opioids Confirmation Method Validation (November 2019 – May 2020)
- 10.3.2. Stability and Reinjection (2022)
- 10.3.3. Instrument validations
- 10.3.4. Validations of calibrators, controls, and internal standards data

10.4. Instruments/Equipment
- 10.4.1. Tube rack
- 10.4.2. Rocker
- 10.4.3. Vortex
- 10.4.4. 96-well plate positive pressure manifold
- 10.4.5. 96-well plate evaporator
- 10.4.6. Liquid chromatograph
- 10.4.7. Mass spectrometer, triple quadrupole
- 10.4.8. Pipettes

10.5. Reagents/Materials
- 10.5.1. ToxBox 96-well plate (Opioids)
- 10.5.2. ISOLUTE SLE 96-well plate (Biotage: SLE-B96)
- 10.5.3. 2 mL 96-well collection plate and cover
- 10.5.4. 96-well plate with vial inserts and cover
- 10.5.5. Autosampler vials, inserts, and caps
- 10.5.6. Pipette tips
- 10.5.7. ddH_2O
- 10.5.8. Negative blood (human)
- 10.5.9. Liquid chromatograph column
  - 10.5.9.1. Dimensions: 2.1 mm x 100 mm
  - 10.5.9.2. Composition: Phenyl Hexyl, 2.7 µm particles
- 10.5.10. Liquid chromatograph guard column
  - 10.5.10.1. Dimensions: 2.1 mm x 5 mm
  - 10.5.10.2. Composition: Phenyl Hexyl, 2.7 µm particles
- 10.5.11. Nitrogen

10.5.12. Solvents shall be high quality and low residue (e.g., HPLC grade, Omnisolv, Optima, etc.) unless otherwise noted.
- 10.5.12.1. Acetonitrile, LCMS grade
- 10.5.12.2. MTBE, ACS grade or higher
- 10.5.12.3. Formic acid

10.5.13. Sodium carbonate

10.5.14. Sodium bicarbonate

Table 7: 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

10.6. Hazards/Safety
- 10.6.1. See Safety Manual.
- 10.6.2. See SDS for each chemical in this method.

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

10.7. Solutions

10.7.1. Carbonate Buffer (300 mM)

10.7.1.1. For example, mix 50.4 g sodium bicarbonate and 63.6 g sodium carbonate into 2 L of ddH_2O.

10.7.1.2. Adjust pH to 9.0 ± 0.1.

10.7.2. Mobile Phases

10.7.2.1. Aqueous (A) – Add 1 mL of formic acid per 1 L ddH_2O.

10.7.2.2. Organic (B) – Add 1 mL of formic acid per 1 L acetonitrile.

10.7.3. Reconstitution Solution (95:5 Water to Acetonitrile)

10.7.3.1. Make a 95:5 water to acetonitrile solution. For example, add 950 mL of ddH_2O to 50 mL of acetonitrile.

10.8. Procedures/Instructions

10.8.1. 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.

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

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

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

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

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

10.8.6. Vortex ToxBox plate.

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

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

10.8.9. 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.

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

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

10.8.12. Evaporate at room temperature using nitrogen.

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

10.8.14. Cap collection plate and vortex.

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

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

10.8.17. Analyze the samples by LC/QQQ.

10.8.17.1. Sequence names shall be in the following format: YYYY_MM_DD_OPI_Initials.
- 10.8.17.1.1. The date in the sequence shall be the date of preparation of the samples.
- 10.8.17.1.2. Additional information such as reinjection, validation, etc., or equivalent abbreviations should be included with the assay abbreviation.
- 10.8.17.1.3. 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.

10.8.17.2. 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 10.8.17.1. The note should list which lines are attributed to each batch.

10.8.17.3. Prepared samples may be analyzed up to 15 days after date of preparation when stored in the instrument autosampler or at equivalent temperature.

10.8.17.4. If the instrument sequence is paused by the acquisition software between two samples, the sequence may be restarted at the sample not yet injected.
- 10.8.17.4.1. Sample stability criteria shall be met.

10.8.17.5. 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.
- 10.8.17.5.1. Sample stability criteria shall be met.
- 10.8.17.5.2. Reinjection of a sample of unknown concentration may be performed up to two times.
- 10.8.17.5.3. Reinjection of a sample of known concentration may be performed multiple times.
  - 10.8.17.5.3.1. If a reinjection is needed more than twice, the evidentiary samples that have already been reinjected may be skipped in a bracket.
    - 10.8.17.5.3.1.1. Evidentiary samples that are skipped and do not have valid data shall be reanalyzed starting at 10.8.1.
- 10.8.17.5.4. A reinjection shall be performed by restarting the sequence from the last bracketing non-zero control or reinjecting the entire sequence.
- 10.8.17.5.5. If an entire sequence is reinjected or a reinjection includes the calibrators used to generate the calibration curve, a check tune (ref. 1.3.1.5) 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.

10.8.18. LC/QQQ Acquisition Parameters

10.8.18.1. Liquid chromatograph sampler

Needle wash	Standard wash
Injection Volume	1 µL

10.8.18.2. 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

10.8.18.3. Liquid chromatograph column compartment

Temperature	55 ºC

10.8.18.4. Mass spectrometer

Ion Source	AJS ESI
Scan Type	Dynamic MRM

10.8.18.5. dMRM Parameters

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

Table 8: 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

Compound Name	Internal Standard	Precursor Ion	Product Ion	Fragmentor (V)	CE* (V)	RT** (min)
			199		34	
Hydromorphone	No	286	**185**	125	36	2.39
			157		46	
Methadone	No	310	**265**	125	13	7.64
			105		29	
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.

10.8.18.6. Quantitation Parameters

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

Internal standard concentration 100

10.9. Records
- 10.9.1. Pipette calibration certificate, however named
- 10.9.2. Batch Preparation Packet
  - 10.9.2.1. ISDT Confirmation Worklist
  - 10.9.2.2. Retest Worksheet, as appropriate
  - 10.9.2.3. Opioids Drug Confirmation Preparation Worksheet
  - 10.9.2.4. Aliquot Chain of Custody
  - 10.9.2.5. Opioids Drug Confirmation Plate Layout Worksheet
- 10.9.3. MassHunter Worklist Report
- 10.9.4. MassHunter Ion Ratio and RRT Verification, however named
- 10.9.5. QA/QC Packet, however named
  - 10.9.5.1. Batch summary
  - 10.9.5.2. Analyte calibration curves
  - 10.9.5.3. Calibrator and control chromatograms
- 10.9.6. Sample chromatograms
- 10.9.7. QQQ Check Tune Report
- 10.9.8. Opioids Drug Confirmation Technical Review Checklist
- 10.9.9. Data comparison output, however named.
- 10.9.10. Measurement Uncertainty Estimation and supporting data
- 10.9.11. Specimen Verification Worksheet, if applicable

10.10. Interpretation of Results
- 10.10.1. Interpretation of results for each analyte shall occur independent of the other analytes in the method.
- 10.10.2. Chromatographic analyte and internal standard peaks shall have baseline resolution and/or shall be mass resolved in the mass spectrometer.
  - 10.10.2.1. A shoulder peak shall be < 10% of analyte peak height and area in order to report a quantitative result.
- 10.10.3. Calibration and Controls Criteria
  - 10.10.3.1. Results of samples analyzed prior to analysis of the negative control preceding the calibrators shall not be used to determine acceptability of batch data.
  - 10.10.3.2. Quantitation of calibrators and non-zero controls shall be within ± 20% of the target concentration.
  - 10.10.3.3. Generating a calibration curve
    - 10.10.3.3.1. Calibration curve shall include a minimum of five non-zero concentrations.
    - 10.10.3.3.2. Correlation coefficient (r^2) for the calibration curve shall be ≥ 0.990.
    - 10.10.3.3.3. 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.
    - 10.10.3.3.4. 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.
    - 10.10.3.3.5. A calibration point may be excluded if any of the following occur:

10.10.3.3.5.1. An ion ratio does not meet the acceptability criteria listed in 10.10.3.3.3 or 10.10.3.3.4.
10.10.3.3.5.2. The correlation coefficient (r^2) for the calibration curve is < 0.990.
10.10.3.3.5.3. A quantitated value is not within ± 20% of the target concentration.
10.10.3.3.5.4. A peak has poor chromatography.
10.10.3.3.6. 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 10.8.1.
10.10.3.3.6.1. RR or response may be used to determine which specimens require reanalysis, if any.
10.10.3.3.7. 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 10.8.1. If unable to retest, the results for the analysis may be reported as greater than the highest calibrator used in the batch.
10.10.3.3.7.1. RR or response may be used to determine which specimens require reanalysis, if any.
10.10.3.4. 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 10.10.3.3.3 or 10.10.3.3.4.
10.10.3.4.1. 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 10.8.1. A result < 50% of the LLOQ for an evidentiary sample shall be accepted as none detected.
10.10.3.5. At least one negative control shall have the corresponding internal standard present for the associated analyte.
10.10.3.5.1. If acceptance criterion is not met, all samples in the batch shall be reanalyzed, if possible, starting at 10.8.1.
10.10.3.6. At least one low and one high non-zero control shall be included in each batch.
10.10.3.7. A non-zero control for an analyte fails if any of the following occur:
10.10.3.7.1. An ion ratio does not meet the acceptability criteria listed in 10.10.3.3.3 or 10.10.3.3.4.

10.10.3.7.2. The quantitated value is not within ± 20% of the target concentration.
10.10.3.7.3. A peak has poor chromatography.
10.10.3.7.4. 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.
10.10.3.8. Each set of one to twelve samples shall be bracketed by one low and one high non-zero control.
10.10.3.8.1. 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 10.8.1. 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 10.10.3.4 and 10.10.3.5.

10.10.4. Analyte Identification (Qualitative Criteria)
10.10.4.1. Relative retention time shall be within ± 5% of the mean relative retention time based on all calibrators and controls used to generate the curve.
10.10.4.2. Each analyte shall have two ion transitions monitored. The ion transition from the precursor to the product ion listed in **bold** type in Table 8: Opioids MS Parameters is used for quantitation.
10.10.4.3. Each internal standard shall be present and have one ion transition monitored.
10.10.4.4. Each ion ratio shall meet the acceptability criteria listed in 10.10.3.3.3 or 10.10.3.3.4.
10.10.4.5. 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.

10.10.5. Analyte Stability
10.10.5.1. Prepared samples are stable for 15 day when stored in the instrument auto sampler (or at equivalent temperature) or at room temperature.

10.10.6. Retesting Samples
10.10.6.1. 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.
10.10.6.1.1. If a quantitative value cannot be reported from any analysis, the first acceptable qualitative data according to analyte identification in 10.10.4 shall be used. (ref. 10.11.4).
10.10.6.1.2. If data is not generated, that analysis does not count as an analysis or retest under this section.

10.10.7. Unacceptable Data

10.10.7.1. 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”).

10.10.8. No Data Generated for a Sample

10.10.8.1. 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”).

10.11. Report Writing

10.11.1. The LLOD for opioids analysis is equal to the LLOQ for each analyte. The LLOQ and ULOQ are listed in Table 9:

Table 9: Opioids LLOQ and ULOQ

Analyte	LLOQ (ng/mL)	ULOQ (ng/mL)
6-Monoacetylmorphine	5.0	500
Codeine	5.0	500
Dextromethorphan	5.0	500
Hydrocodone	5.0	500
Hydromorphone	5.0	500
Morphine	5.0	500
Oxycodone	5.0	500
Oxymorphone	5.0	500
Acetylfentanyl	0.50	50
Fentanyl	0.50	50
Norfentanyl	0.50	50
EDDP	10	1000
Methadone	10	1000
O-Desmethyltramadol	10	1000
Tramadol	10	1000

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

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

10.11.3. Quantitative Reporting

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

10.11.3.1.1. 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.

10.11.3.2. 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.

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

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

10.11.3.2.1.2. A result shall be reported to one decimal place for quantitative values ≥ to 1 and < 10.

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

10.11.3.3. 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.

10.11.3.3.1. 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.

10.11.3.3.1.1. 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.

10.11.3.4. A quantitative result shall only be reported if analysis occurred within the established sample stability window (ref. 10.10.5).

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

10.11.4. Qualitative Reporting

10.11.4.1. A result should be reported as "Positive" when the analyte identification criteria (ref. 10.10.4) have been met, the quantitative result is > LLOQ, and the quantitative criteria have not been met.

10.11.4.1.1. 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.

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

10.11.4.2. A result may be reported as "Positive" with supervisory approval if any of the following occur (ref. 13.3.4.1):

10.11.4.2.1. Interference(s); or

10.11.4.2.2. 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.

10.12. References

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

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

11. Stimulants Confirmation by LC/QQQ

11.1. Scope
- 11.1.1. This method shall be used for confirmation analysis of specimens requiring confirmation of stimulant drugs. Sample preparation shall be by SPE.

11.2. Precautions/Limitations
- 11.2.1. Minimum Sample Requirement
  - 11.2.1.1. 1 mL of blood or serum/plasma specimen
- 11.2.2. CRMs
  - 11.2.2.1. CRMs used for calibrator and non-zero control stocks shall be from two different vendors, if available.
  - 11.2.2.2. If using CRMs from the same vendor, two different lots shall be used, if available.
  - 11.2.2.3. If only one lot of a CRM is available, two separate vials from the lot shall be used.
- 11.2.3. Mobile phases should be kept in amber bottles to increase stability.

11.3. Related Information
- 11.3.1. Stimulant Confirmatory Analysis Method Validation (September 2016-January 2017)
- 11.3.2. Stimulants Linearity Supplemental (October –December 2017)
- 11.3.3. Stimulants Stock and Prepared Sample Stability (April 2017)
- 11.3.4. Stimulants Reinjection Stability (April 2017, April 2018, January 2019)
- 11.3.5. Calibration Model Update-Quadratic (January-February 2019)
- 11.3.6. Reinjection Stability Supplemental (January-February 2019)
- 11.3.7. Injection Volume Supplemental (August-September 2019)
- 11.3.8. Stock Solution Stability (January 2020)
- 11.3.9. Retention time versus relative retention time Evaluation (January 2020)
- 11.3.10. Instrument validations
- 11.3.11. Validation of calibrators, controls, and internal standards data

11.4. Instruments/Equipment
- 11.4.1. Tube rack
- 11.4.2. Rocker
- 11.4.3. Vortex, single
- 11.4.4. Sonicating water bath
- 11.4.5. Centrifuge
- 11.4.6. Positive pressure manifold
- 11.4.7. SPE column rack
- 11.4.8. SPE collection rack
- 11.4.9. Waste collection rack
- 11.4.10. Evaporator
- 11.4.11. Vial rack
- 11.4.12. Liquid chromatograph
- 11.4.13. Mass spectrometer, triple quadrupole
- 11.4.14. Pipettes

11.5. Reagents/Materials
- 11.5.1. Glass tubes (e.g., 13x100 mm)

11.5.2. Trace B SPE columns, 3 mL columns, 35 mg (Tecan #TB-335C, or equivalent)
11.5.3. Tube caps (e.g., 13mm flange)
11.5.4. Pipette tips
11.5.5. Autosampler vials, inserts, and caps
11.5.6. ddH_2O
11.5.7. Negative blood (human)
11.5.8. Liquid chromatograph analytical column
11.5.8.1. Dimensions: 2.1 mm x 100 mm
11.5.8.2. Composition: Phenyl Hexyl, 2.7 µm particles
11.5.9. Liquid chromatograph guard column
11.5.9.1. Dimensions: 2.1 mm x 5 mm
11.5.9.2. Composition: Phenyl Hexyl, 2.7 µm particles
11.5.10. CRMs
11.5.10.1. Amphetamine
11.5.10.2. MDA
11.5.10.3. MDMA
11.5.10.4. Methamphetamine
11.5.10.5. Phencyclidine
11.5.10.6. Amphetamine-D11
11.5.10.7. MDA-D5
11.5.10.8. MDMA-D5
11.5.10.9. Methamphetamine-D14
11.5.10.10. Phencyclidine-D5
11.5.11. Nitrogen
11.5.12. Solvents shall be high quality and low residue (e.g., HPLC grade, Omnisolv, Optima, etc.) unless otherwise noted.
11.5.12.1. Ethyl acetate
11.5.12.2. Isopropanol
11.5.12.3. Glacial acetic acid, ACS grade or higher
11.5.12.4. Ammonium hydroxide, ACS grade or higher
11.5.12.5. Methanol, ACS grade or higher
11.5.12.6. Hydrochloric acid, ACS grade or higher
11.5.12.7. Formic acid
11.5.12.8. Acetonitrile
11.5.13. Sodium Phosphate Monobasic
11.5.14. Sodium Phosphate Dibasic

11.6. Hazards/Safety
11.6.1. See Safety Manual.
11.6.2. See SDS for each chemical in this method.
11.6.3. Add acids to approximately half the volume of the less acidic liquid, then dilute to final volume.

11.7. Reference Materials/Controls/Calibrators/Solutions
11.7.1. Working stock solutions are stable for up to 9 months and should be stored in a freezer.
11.7.2. All working stock solutions shall be made by dilution of CRMs in methanol. The calibrator working stock solutions and non-zero control working stock solutions should be made by different analysts. If these solutions are made by the same

analyst, calibrator and control working stock solution shall be made in separate and distinct preparation sessions.

11.7.2.1. Low Calibrator
- 11.7.2.1.1. 500 ng/mL - Amphetamine, MDA, MDMA, Phencyclidine
- 11.7.2.1.2. 1,000 ng/mL - Methamphetamine

11.7.2.2. High Calibrator
- 11.7.2.2.1. 5,000 ng/mL - Amphetamine, MDA, MDMA, Methamphetamine, Phencyclidine,

11.7.2.3. Low Control
- 11.7.2.3.1. 500 ng/mL - Amphetamine, MDA, MDMA, Methamphetamine, Phencyclidine,

11.7.2.4. High Control
- 11.7.2.4.1. 2,500 ng/mL - Amphetamine, MDA, MDMA, Methamphetamine, Phencyclidine,

11.7.2.5. Internal Standard
- 11.7.2.5.1. 2,000 ng/mL - Amphetamine-D11, MDA-D5, MDMA-D5, Methamphetamine-D14, Phencyclidine-D5

11.7.3. Elution Solution
- 11.7.3.1. On the day of extraction, make a 90:6:4 ethyl acetate, isopropanol, and ammonium hydroxide solution.
  - 11.7.3.1.1. For example, 180 mL of ethyl acetate, 12 mL of isopropanol and 8 mL of ammonium hydroxide will be sufficient for a batch of 96 samples.

11.7.4. Phosphate Buffer (100 mM)
- 11.7.4.1. For example, mix 12.14 g sodium phosphate monobasic and 1.70 g sodium phosphate dibasic into 1 L of ddH_2O.
  - 11.7.4.1.1. Adjust pH to 6.0 ± 0.1

11.7.5. Acetic Acid (100 mM)
- 11.7.5.1. For example, dilute 5.72 mL glacial acetic acid to 1L with ddH_2O.

11.7.6. Hydrochloric Acid (25 mM)
- 11.7.6.1. For example, dilute 208 µL hydrochloric acid to 100 mL with ddH_2O.

11.7.7. Mobile Phases
- 11.7.7.1. Aqueous (A) – Add 1 mL of formic acid per 1 L ddH_2O.
- 11.7.7.2. Organic (B) – Add 1 mL of formic acid per 1 L acetonitrile.

11.8. Procedures/Instructions

11.8.1. 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.
- 11.8.1.1. Reinjected negative controls shall be denoted with an “RI” followed by the number of reinjections.

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

11.8.3. Add 50 µL of internal standard (resulting in a concentration of 100 ng/mL) to labeled glass tubes.

11.8.4. Prepare calibrator and control samples in correspondingly labeled tubes as indicated in Table 10.
11.8.5. For batch analysis, the calibrator and non-zero control working stocks used shall conform to 11.7.2.

Table 10: 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

11.8.6. Pipette 1 mL of negative blood into each calibrator and control tube.
11.8.7. Pipette 1 mL of specimen into the correspondingly labeled tube.
11.8.8. Add 2 mL of phosphate buffer to each tube. Cap and vortex each tube.
11.8.9. Sonicate for ~10 minutes.
11.8.10. Centrifuge for ~10 minutes using 3000 rpm at 4-8 ºC.
11.8.11. 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:
  11.8.11.1. 1 mL of methanol
  11.8.11.2. 1 mL of ddH_2O
  11.8.11.3. 1 mL of phosphate buffer
11.8.12. 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.
11.8.13. 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:
  11.8.13.1. 2 mL ddH_2O
  11.8.13.2. 2 mL acetic acid
  11.8.13.3. 1 mL methanol
  11.8.13.4. 1 mL ethyl acetate
11.8.14. Using a maximum flow of ~60 psi, dry the columns for ~20 minutes.
11.8.15. Place empty labeled tubes into the positive pressure manifold, ensuring the placement of the tubes corresponds with the arrangement of the sample columns.
11.8.16. Add 100 µL of hydrochloric acid to each tube prior to 11.8.19.
11.8.17. Add 2 mL of elution solution to each column and allow to flow completely through into tube at ~1 mL per minute.
11.8.18. Remove tubes from the positive pressure manifold and place on the evaporator.
11.8.19. Evaporate at room temperature using nitrogen.
11.8.20. Add 100 µL of ddH_2O to each tube and vortex.
11.8.21. Transfer each sample to a correspondingly labeled autosampler vial and cap vial.

11.8.22. Analyze the samples by LC/QQQ.
- 11.8.22.1. Sequence names shall be in the following format: YYYY_MM_DD_STM_Initials.
  - 11.8.22.1.1. The date of the sequence shall be the date of preparation of the samples.
  - 11.8.22.1.2. Additional information such as reinjection, validation, etc., or equivalent abbreviations should be included with the assay abbreviation.
  - 11.8.22.1.3. 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.
  - 11.8.22.1.4. 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 11.8.22.1. The note should list which lines are attributed to each batch.
- 11.8.22.2. 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. 11.10.5).
- 11.8.22.3. If the instrument sequence is paused by the acquisition software between two samples, the sequence may be restarted at the sample not yet injected.
  - 11.8.22.3.1. Sample stability criteria shall be met.
- 11.8.22.4. 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.
  - 11.8.22.4.1. Sample stability criteria shall be met.
  - 11.8.22.4.2. Reinjection of a sample of unknown concentration may be performed six times.
  - 11.8.22.4.3. Reinjection of a sample of known concentration may be performed multiple times.
    - 11.8.22.4.3.1. If a reinjection is needed more than six times, the evidentiary samples that have already been reinjected may be skipped in a bracket.
      - 11.8.22.4.3.1.1. Evidentiary samples that are skipped and do not have valid data shall be reanalyzed starting at 11.8.1.
  - 11.8.22.4.4. A reinjection shall be performed by restarting the sequence from the last bracketing non-zero control or reinjecting the entire sequence.
  - 11.8.22.4.5. If an entire sequence is reinjected or a reinjection includes the calibrators used to generate the calibration curve, a check tune (ref. 1.3.1.5) 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.

11.8.23. LC/QQQ Acquisition Parameters

11.8.23.1. Liquid chromatograph sampler

Needle Wash	Standard wash
Injection Volume	0.10 to 1 µL

11.8.23.2. 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

11.8.23.3. Liquid chromatograph column compartment

Temperature	55 ºC

11.8.23.4. Mass spectrometer

Ion Source	AJS ESI
Scan Type	Dynamic MRM

11.8.23.5. dMRM Parameters

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

Table 11: 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

Compound Name	Internal Standard	Precursor Ion	Product Ion	Fragmentor (V)	CE* (V)	RT** (min)
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.

11.8.23.6. Quantitation Parameters

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

11.9. Records
- 11.9.1. Pipette calibration certificate, however named
- 11.9.2. Stimulants Confirmation Calibrator Solution Preparation Worksheet
- 11.9.3. Stimulants Confirmation Internal Standard Solution Preparation Worksheet
- 11.9.4. Stimulants Confirmation Control Solution Preparation Worksheet
- 11.9.5. Batch Preparation Packet, however named
  - 11.9.5.1. ISDT Confirmation Worklist
  - 11.9.5.2. Retest Worksheet, as appropriate
  - 11.9.5.3. Stimulants Confirmation Preparation Worksheet
  - 11.9.5.4. Aliquot Chain of Custody
- 11.9.6. MassHunter Worklist Report
- 11.9.7. MassHunter Ion Ratio and RRT Verification, however named
- 11.9.8. QA/QC Packet, however named
  - 11.9.8.1. Batch summary
  - 11.9.8.2. Analyte calibration curves
  - 11.9.8.3. Calibrator and control chromatograms
- 11.9.9. Sample chromatograms
- 11.9.10. QQQ Check Tune Report
- 11.9.11. Stimulants Confirmation Technical Review Checklist
- 11.9.12. Data comparison output, however named
- 11.9.13. Measurement Uncertainty Estimation and supporting data
- 11.9.14. Specimen Verification Worksheet, if applicable

11.10. Interpretation of Results
- 11.10.1. Interpretation of results for each analyte shall occur independent of the other analytes in the method.
- 11.10.2. Chromatographic analyte and internal standard peaks shall have baseline resolution and/or analytes shall be mass resolved in the mass spectrometer.
  - 11.10.2.1. A shoulder peak shall be < 10% of analyte peak height and area in order to report a quantitative result.
- 11.10.3. Calibration and Controls Criteria
  - 11.10.3.1. Results of samples analyzed prior to analysis of the negative control preceding the calibrators shall not be used to determine acceptability of batch data.
  - 11.10.3.2. Quantitation of calibrators and non-zero controls shall be within ± 20% of the target concentration.
  - 11.10.3.3. Generating a calibration curve

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

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

11.10.3.3.3. 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.

11.10.3.3.4. 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.

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

11.10.3.3.5.1. An ion ratio does not meet the acceptability criteria listed in 11.10.3.3.3 or 11.10.3.3.4.

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

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

11.10.3.3.5.4. A peak has poor chromatography.

11.10.3.3.6. 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 11.8.1.

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

11.10.3.3.7. 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 11.8.1. If unable to retest, the results for the analysis may be reported as greater than the highest calibrator used in the batch.

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

11.10.3.4. 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 11.10.3.3.3 or 11.10.3.3.4.

11.10.3.4.1. 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 11.8.1. A result < 50% of the

LLOQ for an evidentiary sample shall be accepted as none detected.

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

11.10.3.5.1. If acceptance criterion is not met, all samples in the batch shall be reanalyzed, if possible, starting at 11.8.1.

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

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

11.10.3.7.1. An ion ratio does not meet the acceptability criteria listed in 11.10.3.3.3 or 11.10.3.3.4.

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

11.10.3.7.3. A peak has poor chromatography.

11.10.3.7.4. 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.

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

11.10.3.8.1. 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 11.8.1. 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 11.10.3.4 and 11.10.3.5.

11.10.4. Analyte Identification (Qualitative Criteria)

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

11.10.4.2. 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: Stimulants Analyte MS Parameters is used for quantitation.

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

11.10.4.4. Each ion ratio shall meet the acceptability criteria listed in 11.10.3.3.3 or 11.10.3.3.4.

11.10.4.4.1. 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.

11.10.4.5. 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.

11.10.5. Analyte Stability

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

11.10.6. Retesting Samples

11.10.6.1. 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.

11.10.6.1.1. If a quantitative value cannot be reported from any analysis, the first acceptable qualitative data according to analyte identification in 11.10.4 shall be used. (ref. 11.11.4).

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

11.10.7. Unacceptable Data

11.10.7.1. 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”).

11.10.8. No Data Generated for a Sample

11.10.8.1. 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”).

11.11. Report Writing

11.11.1. The LLOD for stimulant analysis is equal to the LLOQ for each analyte. The LLOQ and ULOQ are listed in Table 12.

Table 12: 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

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

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

11.11.3. Quantitative Reporting

11.11.3.1. A result less than the LLOQ shall not be reported.
11.11.3.1.1. 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.
11.11.3.2. A quantitated result that meets acceptability criteria shall be reported for results between the target concentration of the lowest and highest calibrators.
11.11.3.2.1. A result shall be truncated to the appropriate level of significance and reported as the quantitative value ± the expanded measurement uncertainty.
11.11.3.2.1.1. A result shall be reported to one decimal place for quantitative values less than 10.
11.11.3.2.1.2. A result shall be reported as a whole number for quantitative values greater than or equal to 10.
11.11.3.3. 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.
11.11.3.3.1. 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.
11.11.3.3.1.1. 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.
11.11.3.4. Quantitative results shall only be reported if analysis occurred within the established sample stability window (ref. 11.10.5).
11.11.3.5. If a specimen is analyzed more than once, the first quantitative result that meets quantitative acceptability criteria for a specific analyte shall be reported.
11.11.4. Qualitative Reporting
11.11.4.1. A result should be reported as “Positive” when the analyte identification criteria (ref. 11.10.4) has been met, the quantitative result is > LLOQ, and the quantitative criteria have not been met.
11.11.4.1.1. 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.
11.11.4.1.1.1. The preparation date of last analysis shall be used as the analysis date.
11.11.4.2. A result may be reported as “Positive” with supervisory approval if any of the following occur (ref. 13.3.4.1):
11.11.4.2.1. Interference(s); or
11.11.4.2.2. 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.

11.12. References

11.12.1. ISDT Cocaine and Metabolite GC/MS Confirmation Method

11.12.2. ISDT Benzodiazepines and Z-Drugs LC/QQQ Confirmation Method

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

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

12. Volatile Analysis by HS/GC/FID

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

12.2. Precautions/Limitations
- 12.2.1. Minimum Sample Requirements
  - 12.2.1.1. Routine analysis requires 400 µL of blood or serum/plasma sample.
- 12.2.2. CRMs
  - 12.2.2.1. A CRM from the same lot number shall not be used for a calibrator and control in the same batch.

12.3. Related Information
- 12.3.1. Volatile Multi-point Calibration Validation (December 2015-February 2016)
- 12.3.2. Volatile Analysis Sample Reinjection Validation (June 2015)
- 12.3.3. Volatile Analysis Sample Stability Validation (July 2015)
- 12.3.4. Volatile Workflow Change Evaluation (October 2023)
- 12.3.5. Instrument validations
- 12.3.6. Validation of internal standard lot data

12.4. Instruments/Equipment
- 12.4.1. Rocker
- 12.4.2. Headspace sampler
- 12.4.3. Gas chromatograph with flame ionization detector
- 12.4.4. Auto diluter
- 12.4.5. Crimper
- 12.4.6. Tube rack
- 12.4.7. Vortex, single
- 12.4.8. Vial rack
- 12.4.9. Volumetric flasks
- 12.4.10. Pipettes

12.5. Reagents/Materials
- 12.5.1. Pipette tips
- 12.5.2. ALC1 Gas chromatograph capillary column
  - 12.5.2.1. Dimensions: 30 m x 530 µm x 3.0 µm
  - 12.5.2.2. Composition: DB-ALC1
- 12.5.3. ALC2 Gas chromatograph capillary column
  - 12.5.3.1. Dimensions: 30 m x 530 µm x 2.0 µm
  - 12.5.3.2. Composition: DB-ALC2
- 12.5.4. 20 mL headspace crimp top vials
- 12.5.5. Headspace crimp caps
- 12.5.6. Compressed air
- 12.5.7. Helium, 5.0 grade or higher
- 12.5.8. Hydrogen
- 12.5.9. ddH_2O
- 12.5.10. Negative blood (human)
- 12.5.11. Negative serum/plasma (human)
- 12.5.12. Aqueous CRMs

12.5.12.1. Ethanol
12.5.12.2. Mixed volatiles (containing ethanol, methanol, isopropanol, and acetone)
12.5.13. Solvents shall be high quality and low residue (e.g., HPLC grade, Omnisolv, Optima, etc.).
12.5.13.1. Ethanol
12.5.13.2. Methanol
12.5.13.3. Acetone
12.5.13.4. Isopropanol
12.5.13.5. n-propanol

12.6. Hazards/Safety
12.6.1. See Safety Manual.
12.6.2. See SDS for each chemical in this method.

12.7. Reference Materials/Controls/Calibrators/Solutions
12.7.1. 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.
12.7.2. Internal standard solution shall be made to contain 12.8 mg/dL n-propanol in ddH_2O (e.g., for 4 L, dilute 640 µL of n-propanol with ddH_2O in a 4 L volumetric flask).
12.7.2.1. Internal standard solution is stable for 6 months when stored at room temperature.

12.8. Procedures/Instructions
12.8.1. An evidentiary batch shall consist of two preparations. Each preparation shall consist of concurrently prepared calibrators, negative controls, non-zero controls, and specimen aliquots.
12.8.1.1. Each preparation shall be a unique event between which the samples are sealed.
12.8.1.1.1. ALC1 samples shall be verified onto the instrument prior to the start of ALC2 preparation.
12.8.1.2. 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.
12.8.1.2.1. The same non-zero control concentration shall not be used for both sides of the bracket of one to twelve specimen aliquots.
12.8.1.3. Each preparation shall include at least two different concentrations of non-zero mixed controls that are within the quantitative range.
12.8.1.3.1. Negative aqueous controls shall be included in each batch.
12.8.1.3.2. At least one matrix matched negative shall be included for each specimen matrix represented in each preparation.
12.8.2. Mix calibrators, controls, and specimens, i.e., on a rocker or by vortexing.
12.8.3. When using an auto-diluter, tubing shall be primed with internal standard.
12.8.4. Aspirate 200 µL of each calibrator, control, or specimen and 2000 µL of ISTD.

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

Note: 12.8.4 and 12.8.5 may be completed by manual pipetting.

12.8.6. 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.

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

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

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

12.8.6.1.3. 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.

12.8.6.2. 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.

12.8.6.3. 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:

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

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

12.8.6.3.3. Specimen aliquots may only be reinjected once.

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

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

12.8.6.3.4.1.1. The specimen aliquots that are skipped and do not have valid data shall be reanalyzed starting at 12.8.1.

12.8.7. Volatile Analysis Method

12.8.7.1. 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
12.8.7.2.	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
12.8.7.3.	Gas chromatograph capillary column	
	ALC1 dimensions:	30m x 530µm x 3µm
	ACL2 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
12.8.7.4.	Gas chromatograph oven	
	Oven temperature:	40 ºC
	Run time:	3.5 min
12.8.7.5.	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

12.8.8. 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 12.8.7 as follows:

12.8.8.1.	Gas chromatograph inlet	
	Split ratio:	50:1
	Gas saver:	20 mL after 16 min
12.8.8.2.	Gas chromatograph capillary column	
	Initial flow:	9.6 mL/min
	Post run flow:	1.0 mL/min

12.8.8.3. 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

12.8.8.4.	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

12.9. Records
- 12.9.1. Pipette calibration certificate, however named, if applicable
- 12.9.2. Auto-dilutor calibration certificate, however named
- 12.9.3. Batch Preparation Packet, named as: YYYY_MM_DD_VOL_Initials
  - 12.9.3.1. Tox Screen Worklist and/or Retest Worksheet, as appropriate
  - 12.9.3.2. Sequence Tables
  - 12.9.3.3. Volatile Analysis Preparation Worksheet
  - 12.9.3.4. Aliquot Chain of Custodies
- 12.9.4. Calibrator and control chromatograms
- 12.9.5. Sample chromatograms
- 12.9.6. Volatile Analysis Batch Summary
- 12.9.7. Measurement Uncertainty Estimation and supporting data
- 12.9.8. Specimen Verification Worksheet, if applicable

12.10. Interpretation of Results
- 12.10.1. 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.
- 12.10.2. Interpretation of results for each analyte shall occur independent of the other analytes in the method.
- 12.10.3. Chromatographic analyte and internal standard peaks shall have baseline resolution between adjacent peaks.
- 12.10.4. Results from samples that are analyzed prior to a calibrator shall not be used to determine acceptability of batch data.
- 12.10.5. Calibration and Controls Criteria
  - 12.10.5.1. Generating a calibration curve:
    - 12.10.5.1.1. A linear curve (1/x weighting) shall be established by using four aqueous mixed volatiles calibrators.
    - 12.10.5.1.2. The correlation coefficient (R^2) for the calibration curve shall be at least 0.99.
    - 12.10.5.1.3. Quantitation of calibrators shall be within ± 10% of the nominal concentrations on the CoA or target concentration.
    - 12.10.5.1.4. If the above criteria are not met in either preparation, the entire batch shall be reprepared and reanalyzed starting at 12.8.1.
  - 12.10.5.2. Evaluating non-zero controls
    - 12.10.5.2.1. Quantitation of non-zero controls shall be within ± 10% or 5 mg/dL, whichever is greater, of the target concentration.
    - 12.10.5.2.2. 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 12.8.1, if possible.
  - 12.10.5.3. Evaluating negative controls
    - 12.10.5.3.1. A negative control shall follow each non-zero control.

12.10.5.3.2. Negative controls shall have an analyte response < 50% of the LLOQ.

12.10.5.3.3. 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 12.8.1, if possible.

12.10.5.4. 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.

12.10.5.4.1. If specimen aliquots are not bracketed in both preparations, the bracket shall be reprepared and reanalyzed starting at 12.8.1, if possible.

12.10.5.5. 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 12.8.1, if possible.

12.10.6. Analyte Identification

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

12.10.6.2. Internal standard shall be present in each sample.

12.10.7. Analyte Stability

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

12.10.8. Results Evaluation

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

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

12.10.8.1.2. The truncated ALC1 result and truncated ALC2 result have an RPD ≤ 10% or 5 mg/dL, whichever is greater (ref. 2.3.5).

12.10.8.1.2.1. 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.

12.10.8.1.2.2. If the above criteria are not met, testing shall be repeated for the specimen unless either results is less than 10 mg/dL (ref. 12.10.8.2).

12.10.8.1.3. The ALC1 and ACL2 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.

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

12.10.9. Retesting Specimens

12.10.9.1. When a specimen requires retesting, the specimen shall be retested starting at 12.8.1, if possible. A specimen may be retested up to two times without supervisory approval.
12.10.9.1.1. If a quantitative value cannot be reported from any analysis, the first acceptable qualitative data according to analyte identification in 12.10.6 shall be used. (ref. 12.11.4148).
12.10.9.1.2. If data is not generated, that analysis does not count as an analysis or retest under this section.
12.10.10. Unacceptable Data
12.10.10.1. 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 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”).
12.10.11. No Data Generated for a Sample
12.10.11.1. 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”).

12.11. Report Writing
12.11.1. 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.
12.11.2. Data for each specimen shall be technically reviewed prior to entering the result into LIMS.
12.11.2.1. The preparation date of analysis shall be used as the analysis date.
12.11.3. Quantitative Reporting
12.11.3.1. If one of the replicate results is below the LLOQ, the result shall be reported as “None Detected.”
12.11.3.2. A quantitated value shall be reported for results between the target concentration of the lowest and highest calibrators.
12.11.3.2.1. The lower of the two results shall be reported in g/100 mL truncated to three decimal places (divide mg/dL value by 1000).
12.11.3.2.2. Quantitative values between 0.010 and 0.400 g/100 mL shall be reported ± the expanded measurement uncertainty to three decimal places.
12.11.3.2.3. 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).
12.11.3.3. A quantitative result shall only be reported if analysis occurred within the established sample stability window (ref. 12.10.7).
12.11.3.4. 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. 12.11.3).

12.11.4. Qualitative Reporting

12.11.4.1. A result should be reported as “Positive” when both replicates meet the analyte identification criteria (ref. 13.3.4.1), the quantitative results are > LLOQ, and the quantitative criteria have not been met.

12.11.4.1.1. 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.

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

12.11.4.2. A result may be reported as “Positive” with supervisory approval if an interference occurs (ref. 13.3.4.1).

12.12. References

12.12.1. 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.

12.12.2. Anthony, R. M.; Sutheimer, C. A.; Sunshine, I. Acetaldehyde, Methanol, and Ethanol by Headspace Gas Chromatography. *J. Anal. Toxicol.* 1980, *4*, 43-45.

12.12.3. 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.

12.12.4. Firor, R. L., Meng, C. Static Headspace Blood Alcohol Analysis with the G1888 Network Headspace Sampler. Application Document, Agilent Technologies, Inc. 2004.

12.12.5. Machata, G. Determination of alcohol in blood by gas chromatography headspace analysis. *Perkin Elmer Clin. Chem. Newsl*. 1972, *4*, 29-32.

12.12.6. Machata, G. The advantages of automated blood alcohol determination by headspace analysis. *Z. Rechtstned*. 1975, *75*, 229-234.

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

13. Technical and Administrative Review

13.1. Scope

13.1.1. This method shall be used for technical review of analytical batch analysis and for administrative review of toxicology reports.

13.2. Technical Review

13.2.1. 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.

13.2.1.1. Failed data may be reviewed concurrently with data from the acceptable batch.

13.2.1.2. 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”).

13.2.1.3. 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”).

13.2.2. Analytical data obtained for screening results should be technically reviewed and approved prior to beginning confirmatory analysis.

13.2.3. 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).

13.2.3.1. 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.

13.2.4. 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).

13.2.5. Each note on a technical record shall be signed.

13.2.5.1. 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.

13.2.6. 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.

13.2.7. The technical review shall follow whichever is appropriate of the following:

13.2.7.1. Drug Screening Analysis by LC/TOF

13.2.7.1.1. Verify the following:

13.2.7.1.1.1. Header information (analyst name, sequence name, instrument, analysis date, etc.) is consistent on all documentation;

13.2.7.1.1.2. Each document of the QA/QC packet containing a sample result includes the analyst name and date of sample preparation;

13.2.7.1.1.3. The Aliquot Chain of Custody and Batch Preparation Worksheet are accurately completed;

13.2.7.1.1.4. ToxBox® Plate (or each control and internal standard solution) used is before its expiration date;

13.2.7.1.1.5. Mass spectrometer tune is acceptable according to 1.4.1.5;

13.2.7.1.1.6. Sample names are consistent on the LIMS Worklist, Aliquot Chain of Custody or Specimen Verification, Instrument Worklist, and Retest Worksheet, as applicable;

13.2.7.1.1.7. Each sample was analyzed with the appropriate method(s) (e.g., positive and negative mode);

13.2.7.1.1.8. Each sample acquisition date/time is before the calibration date/time;

13.2.7.1.1.9. Any chromatogram that was processed using manual integration is appropriately documented according to 6.10.7;

13.2.7.1.1.10. 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

13.2.7.1.1.11. Any note regarding a deviation from the method is signed by a laboratory supervisor or quality assurance manager.

13.2.7.2. Drug Confirmation Analysis by GC/MS or LC/QQQ

13.2.7.2.1. Verify the following:

13.2.7.2.1.1. Header information (analyst name, sequence name, instrument, analysis date, etc.) is consistent on all documentation;

13.2.7.2.1.2. Each document of the QA/QC packet containing a sample result includes the analyst name and date of sample preparation;

13.2.7.2.1.3. The Batch Summary, Aliquot Chain of Custody, and Batch Preparation Worksheet are completed accurately;

13.2.7.2.1.4. Each calibrator, control, and internal standard solution used is before its expiration date;

13.2.7.2.1.5. Mass spectrometer tune is signed by the analyst and acceptable according to 1.2.1.2 or 1.3.1.5, as applicable;

13.2.7.2.1.6. Each chromatogram specifies the tune date as the same date/time as the Tune Report (GC/MS only);

13.2.7.2.1.7. 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.

13.2.7.2.1.8. Sample names are listed in the correct order on the Instrument Worklist/Sequence and Batch Summary;

13.2.7.2.1.9. Each sample was analyzed with the appropriate method for the instrument used (e.g., Cocaine_MS1.M);

13.2.7.2.1.10. Sample acquisition date/times of all calibrators are before the calibration date/time;

13.2.7.2.1.11. 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);

13.2.7.2.1.12. The MassHunter Ion Ratio and RRT Verification sheets are saved and signed (LC/QQQ only);

13.2.7.2.1.13. 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

13.2.7.2.1.14. 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.

13.2.7.2.1.15. Any note regarding a deviation from the method is signed by a laboratory

supervisor or quality assurance manager.

13.2.7.3. Outsourced Testing, within ISDT's Scope

13.2.7.3.1. Technical review of outsourced cases should be done by a laboratory supervisor.

13.2.7.3.2. Verify the following:

13.2.7.3.2.1. Final documentation is saved in outsource folder by date

13.2.7.3.2.1.1. LIMS Worklist;

13.2.7.3.2.1.2. Signed Evidence Transfer Receipt or signed Specimen Verification, as appropriate;

13.2.7.3.2.1.3. Shipping Manifest;

13.2.7.3.2.1.4. Shipping label;

13.2.7.3.2.1.5. Signed LIMS Data Verification

13.2.7.3.2.2. Manifest document(s) are uploaded to case file(s);

13.2.7.3.2.3. Testing codes are accurate;

13.2.7.3.2.4. "Findings Entered" fields in LIMS are appropriate;

13.2.7.3.2.5. Cost tracking spreadsheet is updated.

13.2.7.3.2.6. Any note regarding a deviation from the method is signed by the quality assurance manager.

13.2.7.4. Volatile Analysis

13.2.7.4.1. Verify the following:

13.2.7.4.1.1. Header information (analyst name, batch/sequence names, instrument, analysis date, etc.) is consistent on all documentation;

13.2.7.4.1.2. Each document of the QA/QC packet containing a sample includes the analyst name and date of sample preparation;

13.2.7.4.1.3. The Batch Summary, Aliquot Chain of Custody, and Batch Preparation Worksheet are completed accurately;

13.2.7.4.1.4. Each calibrator, control, and internal standard solution used is before its expiration date;

13.2.7.4.1.5. Chromatogram injection date is the same as the sample prep date (or within method limitations);

13.2.7.4.1.6. Sample names are consistent on the LIMS Worklist, Retest Worksheet,

Instrument Worklist, and Batch Summary, if applicable;

13.2.7.4.1.7. Sample names are listed in the correct order on the Instrument Worklist and Batch Summary;

13.2.7.4.1.8. Each sample was analyzed with the appropriate method for the instrument used (e.g., EtOH_HS1.M);

13.2.7.4.1.9. Each sample injection date/time is before the calibration date/time;

13.2.7.4.1.10. 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

13.2.7.4.1.11. Any note regarding a deviation from the method is signed by a laboratory supervisor or quality assurance manager.

13.2.8. 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.

13.2.8.1. 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.

13.2.8.1.1. Each addition or correction shall be made on the relevant page of the data and signed.

13.2.9. The technical reviewer shall sign the technical review worksheet to document the technical review.

13.2.10. 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.

13.2.11. 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.

13.2.12. Analyst verification of data entry

13.2.12.1. 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.

13.2.12.1.1. Correct any typographical errors in the LIMS data entry.

13.2.12.1.2. Document the verification by saving the LIMS Data Verification file in the appropriate batch QA/QC folder.
13.2.12.1.2.1. 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.
13.2.12.1.3. 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.

13.3. Administrative Review
13.3.1. Verify the following:
13.3.1.1. Case number is documented on each electronically saved document in the electronic case file and each document has the appropriate file name.
13.3.1.2. 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).
13.3.1.3. The appropriate images of evidence are saved.
13.3.1.4. TAR is legibly scanned.
13.3.1.5. 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.
13.3.1.5.1. Agency tab shall include the submitting agency, appropriate prosecutor's office, and DRE (if applicable).
13.3.1.5.2. 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."
13.3.1.5.3. 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."
13.3.1.5.4. Evidence tab shall include the appropriate item(s) as present in the evidence images.
13.3.1.5.4.1. 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.
13.3.1.5.5. 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.

13.3.1.5.5.1. Compare screening and confirmation results and ensure the appropriate screening and confirmation tests were completed, if applicable.

13.3.1.6. Verify the following information on the Draft Report header:
- 13.3.1.6.1. Case number;
- 13.3.1.6.2. Submitting agency name;
- 13.3.1.6.3. Evidence received date;
- 13.3.1.6.4. Evidence received courier;
- 13.3.1.6.5. Evidence item(s) received;
  - 13.3.1.6.5.1. Lists the draw time(s) (HH:MM) of the blood tubes, if there is more than one draw time submitted (> 15 minutes apart).
  - 13.3.1.6.5.2. Lists the draw site(s) (Postmortem – [draw site]) of the blood tubes, if there is more than one draw site submitted.
  - 13.3.1.6.5.3. Identifies the sample type if more than one type of sample is submitted for a coroner case (ref. 4.8.2.6.4.1.2).
- 13.3.1.6.6. Subject name; and
- 13.3.1.6.7. Submitting agency case number (if applicable).

13.3.1.7. If any of the information in 13.3.1.6 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.

13.3.1.8. Perform technical review of the toxicology report.
- 13.3.1.8.1. If the screening was outsourced, the report should indicate "See NMS Report*" and identify the item(s) sent to NMS.
- 13.3.1.8.2. Ensure all presumptive positive screening results have a paired confirmation analysis or are appropriately noted.
- 13.3.1.8.3. If there are no positive findings to report, the results section shall state "None Detected."
- 13.3.1.8.4. If there are positive findings to report,
  - 13.3.1.8.4.1. The first acceptable data for each analyte shall be reported;
  - 13.3.1.8.4.2. Each confirmation result shall:
    - 13.3.1.8.4.2.1. Identify the analyte confirmed;
    - 13.3.1.8.4.2.2. Report the quantity found as specified in the assay test method, report the concentration as

>ULOQ, or "Positive" for qualitative results;

13.3.1.8.4.2.3. Report the MU for quantitative results unless the result is > ULOQ;

13.3.1.8.4.2.4. Indicate the evidence item analyzed;

13.3.1.8.4.2.5. Identify the instrument type used to perform the analysis;

13.3.1.8.4.2.6. Indicate the date of sample preparation for the analysis; and

13.3.1.8.4.2.7. Identify the analyst who performed the analysis.

13.3.1.8.5. 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.

13.3.1.9. If any of the testing was outsourced,

13.3.1.9.1. Upload the appropriate report from the outsourced testing into the electronic case file;

13.3.1.9.2. Review the report for accuracy (e.g., appropriate test(s), associated with correct evidence item); and

13.3.1.9.3. Send the report to ToxResults.

13.3.2. If any information in 13.3.1.6 was updated during the administrative review and another report for the same case has already been released, check the released report for accuracy.

13.3.2.1. If a corrected report needs to be issued, notify a laboratory supervisor or quality assurance manager.

13.3.3. 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.

13.3.4. Ensure notes are added in the following scenarios (examples provided):

13.3.4.1. Positive unable to quantitate

13.3.4.1.1. This specimen was confirmed positive, but the result was not quantitated.

13.3.4.2. Request withdrawn

13.3.4.2.1. Testing was not completed. Request for analysis was withdrawn by X (agency that withdrew the analysis request).

13.3.4.3. Partial report

13.3.4.3.1. 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.

13.3.4.3.2. Partial toxicology report issued, and further testing canceled as requested by X (agency that requested partial report and withdrew request for further testing).

13.3.4.4. Corrected report

13.3.4.4.1. 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.

13.3.4.4.1.1. For example, “The original report dated January 1, 2018, incorrectly listed the subject last name as X. See original report.”

13.3.4.4.1.2. If there are multiple previous reports, the language above may be modified to include references to all the previous reports.

13.3.4.5. Amended report

13.3.4.5.1. 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.

13.3.4.5.1.1. For example, “The original report dated January 1, 2018, did not include ethanol testing for item 2-A. See original report.”

13.3.4.5.1.2. If there are multiple previous reports, the language above may be modified to include references to all the previous reports.

13.3.4.6. Broken, cracked, or leaking specimen tube

13.3.4.6.1. 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.

13.3.4.7. Contaminated Sample

13.3.4.7.1. 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.

13.3.5. 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.

13.3.6. Update analysis request status to “Admin. Reviewed.”

13.3.6.1. 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:

13.3.6.1.1. If the ethanol concentration is ≥ 0.10 g/100 mL blood, verify that the request for drug analysis has been canceled, or,

13.3.6.1.2. If the ethanol concentration is < 0.10 g/100 mL blood, verify that the request for drug analysis is in progress.

13.4. Records

13.4.1. Technical Review Worksheet, however named

13.4.2. LIMS Data Verification

13.4.3. Case Synopsis notes

13.4.4. Toxicology Report – Alcohol Analysis, if applicable

13.4.5. Toxicology Report – Drug Analysis, if applicable

13.4.6. Administrative Review Checklist, if applicable

14. Appendix

14.1. Glossary

14.1.1. Actual concentration – Quantitative value obtained through testing.
14.1.2. Amended Report – A report issued to add testing results or other information to the original report.
14.1.3. Annually – Within the last 12 months (This definition applies to this document only.)
14.1.4. 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.
14.1.5. Batch LLOQ – A modified LLOQ equal to the target concentration of the lowest calibrator used to generate the calibration curve for the batch.
14.1.6. Batch ULOQ – A modified ULOQ equal to the target concentration of the highest calibrator used to generate the calibration curve for the batch.
14.1.7. Blood specimen – Whole blood, homogenate, or supernatant.
14.1.8. 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.
14.1.9. Certified value – Quantitative value listed on a CoA.
14.1.10. Clot – A gelatinous mass formed by a complex mechanism involving red blood cells, fibrinogen, platelets, and other clotting factors.
14.1.11. Confirmation – Testing done to verify a screening result.
14.1.12. Corrected Report – A report issued to correct an error on the original report.
14.1.13. Fortified matrix sample – A blank matrix sample spiked with target analyte and/or internal standard using reference materials.
14.1.14. 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.
14.1.15. Instrument – an implement used to analyze samples (e.g., GC/MS, HS/GC/FID, LC/QQQ, or LC/TOF).
14.1.16. 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.
14.1.17. Mass-to-charge ratio – The mass of an ion divided by its charge, often abbreviated as m/z.
14.1.18. Matrix – Biological fluid or water.
14.1.19. May – An option.
14.1.20. Neat sample – Unextracted solvent containing analyte(s) of interest.
14.1.21. Negative blood – Blood verified by screening/confirmation to be free of analyte(s) of interest.
14.1.22. Negative blood control – Negative blood containing internal standard.
14.1.23. Negative control – Control that is free of the analyte(s) of interest, which may be made from water, negative blood, or negative serum/plasma.

14.1.24. Negative serum/plasma – Serum/plasma verified by screening/confirmation to be free of analyte(s) of interest.
14.1.25. Negative serum/plasma control – Negative serum/plasma containing internal standard.
14.1.26. Parameter – Setting on chromatography instrument or detector specific to the testing of the analyte in question.
14.1.27. 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.
14.1.28. Presumptive positive – Initial result indicating the presence of an analyte of interest obtained using a screening method.
14.1.29. Retention time – The length of time required for an analyte to pass through a chromatographic column and be detected by the detector.
14.1.30. 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.
14.1.31. Sample – Specimen aliquot, calibrator, or control being prepared or ready for testing.
14.1.32. 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.
14.1.33. Serum/plasma specimen – Serum or plasma specimen.
14.1.34. Shall – A requirement.
14.1.35. Should – A recommendation.
14.1.36. Sign or signed – Handwritten signature or initials and date (or secure electronic signature).
14.1.37. 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.
14.1.38. Specimen – Tube containing blood or serum/plasma collected from a subject.
14.1.39. Supernatant – Liquid lying above a solid residue after centrifugation.
14.1.40. Target concentration – Expected quantitated value.
14.1.41. ToxResults – Online program for retrieval of toxicology reports.
14.1.42. Working stock – Concentrated solution used to prepare calibrators and controls.

15. 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