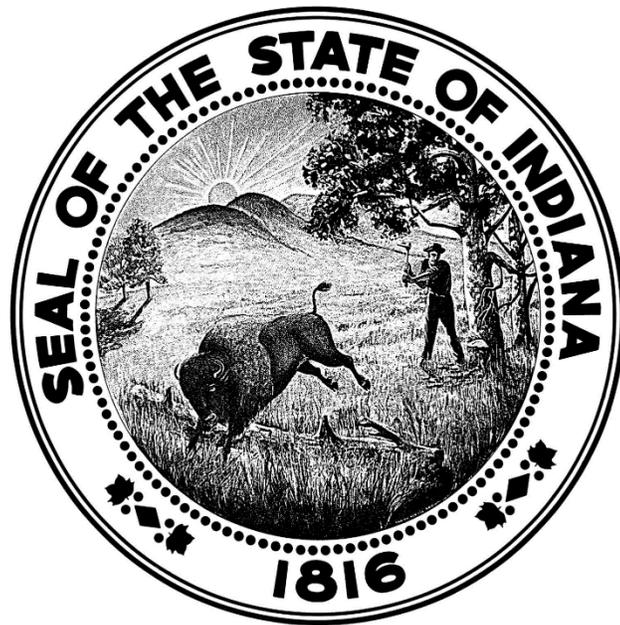


Indiana State Department of Toxicology



Laboratory Test Methods

Table of Contents

Introduction	7
Acknowledgements	7
1. Instrument and Equipment Maintenance and Operation	8
1.1. Scope	8
1.2. GC/MS.....	8
1.3. LC/QQQ	11
1.4. LC/TOF	13
1.5. HS/GC/FID.....	16
1.6. Pipettes and Auto-Diluters	18
1.7. pH Meters	19
1.8. Nitrogen Generators	19
1.9. Hydrogen Generators	20
1.10. Air Generators	20
1.11. Water Purification System (Milli-Q).....	20
1.12. Chemical Hoods	21
1.13. Biological Hoods	21
1.14. Refrigerators	21
1.15. Freezers	21
1.16. Walk-in Refrigerator	21
1.17. Heat Blocks	22
1.18. Sonicating Probe.....	22
1.19. Sonicating Bath	22
1.20. Circulating Bath	22
1.21. Centrifuges	23
1.22. Positive Pressure Manifolds	23
1.23. Evaporators.....	23
1.24. Balances.....	23
1.25. Records.....	24
2. Method Validation	25
2.1. Scope	25
2.2. Responsibilities	25
2.3. Equations	25
2.4. Validation Requirements	26
2.5. Validation Experiments (Parameters)	27
2.6. Records	45
2.7. References	45
3. Solution Verification/Validation	46
3.1. Scope	46
3.2. Precautions/Limitations.....	46

3.3.	Related Information.....	46
3.4.	Instruments/Equipment	46
3.5.	Hazards/Safety.....	46
3.6.	Reagents/Materials	46
3.7.	Reference Materials/Controls/Calibrators/Solutions.....	46
3.8.	Procedures/Instructions	46
3.9.	Records.....	51
3.10.	Interpretation of Results	52
3.11.	Report Writing.....	52
3.12.	References	52
4.	Evidence Handling.....	53
4.1.	Scope	53
4.2.	Precautions/Limitations.....	53
4.3.	Related Information.....	53
4.4.	Instruments/Equipment	53
4.5.	Reagents/Materials	53
4.6.	Hazards/Safety.....	53
4.7.	Reference Materials/Controls/Calibrators/Solutions.....	53
4.8.	Procedures/Instructions	53
4.9.	Records.....	71
4.10.	Interpretation of Results	72
4.11.	Report Writing.....	72
4.12.	References	72
5.	Specimen and Sample Preparation	73
5.1.	Scope	73
5.2.	Precautions/Limitations.....	73
5.3.	Related Information.....	73
5.4.	Instruments/Equipment	73
5.5.	Reagents/Materials	73
5.6.	Hazards/Safety.....	73
5.7.	Reference Materials/Controls/Calibrators/Solutions.....	73
5.8.	Procedure/Instructions.....	73
5.9.	Records.....	77
5.10.	Interpretation of Results	77
5.11.	Report Writing.....	77
5.12.	References	78
6.	Blood Drug Screening by LC/TOF.....	79
6.1.	Scope	79
6.2.	Precautions/Limitations.....	79
6.3.	Related Information.....	79

6.4.	Instruments/Equipment	79
6.5.	Reagents/Materials	79
6.6.	Hazards/Safety.....	80
6.7.	Reference Materials/Controls/Calibrators/Solutions.....	80
6.8.	Procedures/Instructions	80
6.9.	Records.....	83
6.10.	Interpretation of Results	83
	<i>Table 1: Blood Drug Screen by LC/TOF: Analytes, Corresponding Internal Standards, and Concentration of Non-Zero Controls and Internal Standard.....</i>	<i>85</i>
6.11.	Report Writing.....	87
6.12.	References	87
7.	Benzodiazepines and Z-Drugs Confirmation by LC/QQQ.....	88
7.1.	Scope	88
7.2.	Precautions/Limitations.....	88
7.3.	Related Information.....	88
7.4.	Instruments/Equipment	88
7.5.	Reagents/Materials	88
7.6.	Hazards/Safety.....	89
7.7.	Reference Materials/Controls/Calibrators/Solutions.....	90
7.8.	Procedures/Instructions	91
	<i>Table 2: Benzodiazepines and Z-Drugs Calibrator and Control Preparation</i>	<i>91</i>
	<i>Table 3: Benzodiazepines and Z-Drugs MS Parameters</i>	<i>93</i>
7.9.	Records.....	95
7.10.	Interpretation of Results	95
7.11.	Report Writing.....	98
	<i>Table 4: Benzodiazepines and Z-Drugs LLOQ and ULOQ</i>	<i>99</i>
7.12.	References	100
8.	Cannabinoids Confirmation by GC/MS	101
8.1.	Scope	101
8.2.	Precautions/Limitations.....	101
8.3.	Related Information.....	101
8.4.	Instruments/Equipment	101
8.5.	Reagents/Materials	101
8.6.	Hazards/Safety.....	102
8.7.	Reference Materials/Controls/Calibrators/Solutions.....	102
8.8.	Procedures/Instructions	103
	<i>Table 5: Cannabinoids Calibrator and Control Sample Preparation</i>	<i>103</i>
8.9.	Records.....	108
8.10.	Interpretation of Results	109
8.11.	Report Writing.....	112
8.12.	References	113

9.	Cocaine Confirmation by GC/MS	115
9.1.	Scope	115
9.2.	Precautions/Limitations	115
9.3.	Related Information.....	115
9.4.	Instruments/Equipment	115
9.5.	Reagents/Materials	115
9.6.	Hazards/Safety.....	116
9.7.	Reference Materials/Controls/Calibrators/Solutions.....	116
9.8.	Procedures/Instructions	117
	<i>Table 6: Cocaine Calibrator and Control Sample Preparation</i>	<i>117</i>
9.9.	Records.....	121
9.10.	Interpretation of Results	121
9.11.	Report Writing.....	125
9.12.	References	126
10.	Opioids Drug Confirmation by LC/QQQ.....	127
10.1.	Scope	127
10.2.	Precautions/Limitations.....	127
10.3.	Related Information.....	127
10.4.	Instruments/Equipment	127
10.5.	Reagents/Materials	127
10.6.	Hazards/Safety.....	128
10.7.	Solutions.....	129
10.8.	Procedures/Instructions	129
	<i>Table 7: Opioids Prepared Sample Stability and ReInjection Stability.....</i>	<i>130</i>
	<i>Table 8: Opioids MS Parameters</i>	<i>132</i>
10.9.	Records.....	133
10.10.	Interpretation of Results	134
10.11.	Report Writing.....	137
	<i>Table 9: Opioids LLOQ and ULOQ.....</i>	<i>137</i>
10.12.	References	138
11.	Stimulants Confirmation by LC/QQQ.....	140
11.1.	Scope	140
11.2.	Precautions/Limitations.....	140
11.3.	Related Information.....	140
11.4.	Instruments/Equipment	140
11.5.	Reagents/Materials	140
11.6.	Hazards/Safety.....	141
11.7.	Reference Materials/Controls/Calibrators/Solutions.....	142
11.8.	Procedures/Instructions	143
	<i>Table 10: Stimulants Calibrator and Control Sample Preparation.....</i>	<i>143</i>
	<i>Table 11: Stimulants Analyte MS Parameters</i>	<i>145</i>

11.9.	Records	146
11.10.	Interpretation of Results	147
11.11.	Report Writing.....	150
	<i>Table 12: Stimulants LLOQ and ULOQ.....</i>	<i>150</i>
11.12.	References	151
12.	Volatile Screening and Confirmation by HS/GC/FID.....	152
12.1.	Scope	152
12.2.	Precautions/Limitations.....	152
12.3.	Related Information.....	152
12.4.	Instruments/Equipment	152
12.5.	Reagents/Materials	152
12.6.	Hazards/Safety.....	153
12.7.	Reference Materials/Controls/Calibrators/Solutions.....	153
12.8.	Procedures/Instructions	153
12.9.	Records	157
12.10.	Interpretation of Results	157
12.11.	Report Writing.....	161
12.12.	References	162
13.	Technical and Administrative Review	164
13.1.	Scope	164
13.2.	Technical Review	164
13.3.	Administrative Review.....	168
13.4.	Records.....	172
14.	Appendix.....	173
14.1.	Glossary.....	173
14.2.	Abbreviations used in this document and/or associated records	174
15.	Document History	177

Introduction

The Department's mission is to provide quality forensic toxicological services and education for the state of Indiana.

The Indiana State Department of Toxicology provides science-based support to the state's criminal justice system, including impaired driving enforcement programs. We hold that quality results are obtained through professional, ethical, and unbiased analyses of evidentiary specimens entrusted to the Department for testing. The Department facilitates the interpretation and understanding of its results through open communication, technical assistance, and instruction on the science of forensic toxicology.

Deviations to the following procedures may be employed with supervisory approval.

Acknowledgements

Multiple people have contributed to the writing of these procedures. David Hall (SPEware) provided guidance on the extraction used in the stimulants test method.

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 operative 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 shall be documented in the instrument maintenance log.
 - 1.2.1.2. Autotune
 - 1.2.1.2.1. Prior to analysis of each analytical batch containing case samples, on the same day or within 24 hours of beginning analysis of an analytical batch, an autotune shall be performed.
 - 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 shall 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: $\leq 5 \times 10^{-5}$ 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: $\leq 10\%$

- 1.2.1.3. Carrier gas supply
 - 1.2.1.3.1. Carrier gas (helium) pressure shall 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 shall 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 the full 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. 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 control coordinator.
 - 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 shall be documented in the instrument maintenance log.
 - 1.3.1.2. Purge the LC 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: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 shall 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. Prior to analysis of each analytical batch containing case samples, on the same day or within 24 hours of beginning analysis of an analytical batch, a check tune using ESI-L Low Concentration Tuning Mix (Part Number G1969-85000 or equivalent) shall be performed in each ion mode (positive or negative) to be used.
 - 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 shall 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 the full 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 control coordinator.
- 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 shall be documented in the instrument maintenance log.
 - 1.4.1.2. Purge the LC 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: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 shall 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. Prior to analysis of each analytical batch containing case samples, on the same day or within 24 hours of beginning analysis of an analytical batch, a tune shall be performed in the 4 GHz high resolution mode in positive and/or negative ion mode, whichever is being used for the analysis, using positive or negative tune mix, respectively.
 - 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 μ L 0.1 mM for 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 ddH₂O, 1.0 mL 100 mM TFANH₄, 0.8 mL 2.5 mM Hexakis (1H,1H,3H-perfluoropropoxy) phosphazene (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 shall 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 control coordinator.
 - 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. An autotune 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 shall be documented in the instrument maintenance log.
 - 1.5.1.2. Supply of gases shall 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 shall 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 the full calibration curve, three replicates of a mixed volatiles control, and three replicates of either an 80 or 150 mg/dL CRM.
 - 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 control coordinator.
 - 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 that may be an extracted or a neat sample.

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 single-channel 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 diluter or pipette shall be labelled as out of service and shall not be used for casework until serviced and/or calibrated.

1.6.1.6.1. The laboratory supervisor and/or quality control coordinator should be notified of any pipette or autodiluter 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 ddH₂O).

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-dilutor 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-dilutors shall be labelled 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.

1.7.1.1.1. Buffer solutions may be used past the expiration date printed on the bottle.

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

1.7.1.3. The slope of the calibration shall be between 90 and 110.

1.7.1.4. All buffers used to calibrate the pH meter shall be within ± 0.1 of the target value.

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

1.7.1.6. Electrodes shall be filled with electrode solution (KCl).

1.7.1.7. See individual pH meter manuals 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 ddH₂O.

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 (Milli-Q)

1.11.1. Operating Maintenance

1.11.1.1. Prior to use of water, 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 A10 TOC monitor may be cleaned periodically or as necessary when the TOC values fluctuate.

1.11.2.2. Q-Gard pack, quantum cartridge, POD pak, UV lamp, A10 lamp, and inlet strainer 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. Refrigerators
 - 1.14.1. Operating Maintenance
 - 1.14.1.1. Refrigerator doors should be closed promptly after opening.
 - 1.14.2. Periodic and Preventive Maintenance
 - 1.14.2.1. Refrigerator temperatures shall be recorded ~weekly on a temperature log.
 - 1.14.2.1.1. Certified thermometers used to monitor the refrigerator shall be calibrated and certified every two years, at a minimum.
 - 1.14.2.2. Refrigerators may be cleaned periodically or as necessary.

- 1.15. Freezers
 - 1.15.1. Operating Maintenance
 - 1.15.1.1. Freezer doors should be closed promptly after opening.
 - 1.15.2. Periodic and Preventive Maintenance
 - 1.15.2.1. Freezer temperatures shall be recorded ~weekly on a temperature log.
 - 1.15.2.1.1. Certified thermometers used to monitor the freezer shall be calibrated and certified within the past two years, at a minimum.
 - 1.15.2.2. Freezers may be defrosted periodically or as necessary.

- 1.16. Walk-in Refrigerator
 - 1.16.1. Operating Maintenance
 - 1.16.1.1. The door should be closed promptly after opening.
 - 1.16.2. Periodic and Preventive Maintenance

- 1.16.2.1. Temperature and humidity shall be recorded on a log chart. The log chart shall be replaced weekly.
- 1.16.2.2. The walk-in refrigerator should be swept and mopped periodically or as necessary.
- 1.17. Heat Blocks
 - 1.17.1. Operating Maintenance
 - 1.17.1.1. Heat blocks should be used with all blocks in place.
 - 1.17.1.2. Heat blocks should be set to the appropriate temperature and allowed to reach temperature. Temperature may be verified with an external thermometer.
 - 1.17.1.3. Turn off heat block after use.
 - 1.17.1.4. See individual manuals for further information.
 - 1.17.2. Periodic and Preventive Maintenance
 - 1.17.2.1. Heat blocks should be cleaned periodically or as necessary.
- 1.18. Sonicating Probe
 - 1.18.1. Operating Maintenance
 - 1.18.1.1. 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.
 - 1.18.1.2. See individual manual for further information.
 - 1.18.2. Periodic and Preventive Maintenance
 - 1.18.2.1. The probe tip should be examined periodically and may be replaced when worn (visible corrosion) or as necessary.
 - 1.18.2.2. Threaded parts may be cleaned with alcohol.
- 1.19. Sonicating Bath
 - 1.19.1. Operating Maintenance
 - 1.19.1.1. Ensure water level is at or around the operating line.
 - 1.19.2. Periodic and Preventive Maintenance
 - 1.19.2.1. Drain water periodically and refill with fresh water.
 - 1.19.2.2. Clean water reservoir as needed.
- 1.20. Circulating Bath
 - 1.20.1. Operating Maintenance
 - 1.20.1.1. Ensure liquid level is above the highest coil.
 - 1.20.1.2. Liquid in bath may be water and ethylene glycol or propylene glycol, or a combination of these solvents. To achieve temperatures of -30 °C, a mixture of 50:50 ethylene glycol:water may be used.
 - 1.20.2. Periodic and Preventive Maintenance
 - 1.20.2.1. Periodically add liquid to bath. Typically, it is sufficient to add only water, as ethylene or propylene glycol is not likely to have evaporated.
 - 1.20.2.2. Drain liquid periodically and refill with fresh liquid.
 - 1.20.2.3. Clean bath reservoir as needed.

- 1.21. Centrifuges
 - 1.21.1. Operating Maintenance
 - 1.21.1.1. Test tubes should be capped prior to use to prevent contamination of the interior compartment.
 - 1.21.1.2. Ensure the rotors are balanced prior to use.
 - 1.21.1.3. Do not open centrifuge until the program has completed.
 - 1.21.1.4. When not in use, centrifuge lid should be closed.
 - 1.21.1.5. See individual manuals for further information.
 - 1.21.2. Periodic and Preventive Maintenance
 - 1.21.2.1. Centrifuge, rotors, rotor lids, and other parts should be cleaned periodically or as necessary.

- 1.22. Positive Pressure Manifolds
 - 1.22.1. Operating Maintenance
 - 1.22.1.1. The manifold should be wiped with water, solvent, or a cleaning agent (e.g., methanol, isopropanol) as necessary.
 - 1.22.1.2. See individual manuals for further information.
 - 1.22.2. Periodic and Preventive Maintenance
 - 1.22.2.1. The in-line air filter should be monitored periodically for condensation and wear and should be replaced as necessary.
 - 1.22.2.2. The rubber gaskets should be inspected periodically and replaced as necessary.
 - 1.22.2.3. The restrictor plate may be cleaned or replaced when the manifold becomes clogged.
 - 1.22.2.4. The gasket on the restrictor plate may be replaced as necessary.

- 1.23. Evaporators
 - 1.23.1. Operating Maintenance
 - 1.23.1.1. Evaporator tips should be cleaned prior to and promptly after each use.
 - 1.23.1.2. Open vent assembly to allow ventilation through the flexible extension into the chemical hood, if applicable.
 - 1.23.1.3. Ensure nitrogen gas flow is directed to the appropriate rows once in use.
 - 1.23.1.4. See individual manuals for further information.
 - 1.23.2. Periodic and Preventive Maintenance
 - 1.23.2.1. The manifold seal may be wiped clean with methanol or replaced periodically or as necessary.
 - 1.23.2.2. Evaporator tips shall be replaced as needed.

- 1.24. Balances
 - 1.24.1. Operating Maintenance
 - 1.24.1.1. The balance plate should be wiped clean prior to and after each use, if necessary.
 - 1.24.1.2. Ensure balance doors are closed when making measurements, if applicable.
 - 1.24.1.3. Ensure level bubble is centered in the circle prior to use, if applicable.
 - 1.24.1.3.1. Adjust using the leveling thumbwheels, as necessary.

- 1.24.1.4. After use, close balance doors and cover with plastic cover, if applicable.
- 1.24.1.5. See individual manuals for further information.
- 1.24.2. Periodic and Preventive Maintenance
 - 1.24.2.1. Analytical balances shall be calibrated annually if used for critical measurements (e.g., weighing reference materials for controls or calibrators).
 - 1.24.2.2. Analytical balances not used for critical measurements and accessioning balances may be calibrated periodically.
- 1.25. Records
 - 1.25.1. Maintenance log, if applicable
 - 1.25.2. Autotune or Tune report, if applicable
 - 1.25.3. Certificate of calibration, however named, if applicable
 - 1.25.4. Solution log, if applicable
 - 1.25.5. Buffer log, if applicable
 - 1.25.6. Temperature log, if applicable
 - 1.25.7. Temperature and humidity log chart, if applicable
 - 1.25.8. Autodilutor Maintenance Log, if applicable
 - 1.25.9. 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 supervisor and quality control coordinator 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 of methods listed in 2.4.2.

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:

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

$$\% \text{ ACC}_{\text{Sel}} = \frac{\text{Mean RR of matrix A}}{\text{Mean RR of matrix B}} \times 100$$

2.3.2. Response ratio:

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

2.3.3. Percent coefficient of variance:

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

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

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

2.3.4. Percent ion suppression/enhancement:

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

2.3.5. Relative percent difference:

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

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

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

$$\text{RPD}_{\text{Cal}} = \frac{|\text{Lot X mean concentration} - \text{Lot 1 mean concentration}|}{\text{Mean concentration}} \times 100$$

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

$$\text{RPD}_{\text{sample dilution}} = \frac{|\text{Mean undiluted} - \text{Mean diluted concentration}|}{\text{Mean of undiluted and diluted concentration}} \times 100$$

$$\text{RPD}_{\text{dil-neg blood}} = \frac{|\text{Mean ctl conc. (dil. cal.)} - \text{Mean ctl conc. (undil. cal.)}|}{\text{Mean control concentration}} \times 100$$

2.3.6. Relative Standard Deviation:

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

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

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 the laboratory supervisor and quality control coordinator.

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. Screening/qualitative identification (chromatographic)

2.4.6.1.1. Accuracy and Precision (at cutoff concentration)

2.4.6.1.2. Lower Limit of Detection (at cutoff concentration)

- 2.4.6.1.3. Carryover
- 2.4.6.1.4. Selectivity, if applicable
- 2.4.6.1.5. Matrix Interference
- 2.4.6.1.6. Non-Matrix Interference
- 2.4.6.1.7. Ion Suppression/Enhancement, if applicable (e.g., liquid chromatography/mass spectrometry)
- 2.4.6.1.8. Correlation
- 2.4.6.1.9. Stability of Extracted/Prepared Samples*
- 2.4.6.1.10. Stability of Stock Solutions*, if applicable (e.g., solutions prepared in-house from reference materials)
- 2.4.6.1.11. Reinjection Integrity*
- 2.4.6.2. Confirmation/quantitative analysis
 - 2.4.6.2.1. Accuracy and Precision
 - 2.4.6.2.2. Linearity and Lower Limit of Quantitation
 - 2.4.6.2.3. Carryover
 - 2.4.6.2.4. Selectivity, if applicable
 - 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*
 - 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. Drug analysis

- 2.5.1.2.1. Accuracy and precision shall be evaluated for whole blood in multiple batch preparations (runs). Accuracy and precision shall be evaluated for serum/plasma, in addition to whole blood, in at least one run, if applicable.
- 2.5.1.2.2. Fortified matrix samples may be evaluated using specified concentrations of non-zero controls and calibrators.
 - 2.5.1.2.2.1. A minimum of five replicates of at least two non-zero controls per run shall be evaluated. (For qualitative methods, a minimum of four replicates of the cutoff control per run, at a minimum, shall be evaluated.)
 - 2.5.1.2.2.1.1. A total of 20 whole blood replicates (12 whole blood replicates for qualitative methods), at minimum, shall be evaluated.
 - 2.5.1.2.2.1.2. Replicates may only be excluded (max. 3) for a documented reason (e.g., internal standard not detected or problems with the extraction).
 - 2.5.1.2.2.2. A minimum of four runs (three runs for qualitative methods) shall be evaluated for whole blood and one run for serum/plasma (if serum/plasma testing is applicable).
- 2.5.1.3. Volatile analysis
 - 2.5.1.3.1. Accuracy and precision shall be evaluated in multiple batch preparations (runs). Controls in addition to the aqueous CRMs may be included.
 - 2.5.1.3.2. CRMs at 80 and 150 mg/dL shall be evaluated.
 - 2.5.1.3.2.1. If a mixture of all volatiles analyzed in the method is not available at concentrations of 80 and/or 150 mg/dL, an additional non-zero control containing all mixed volatiles shall be evaluated.
 - 2.5.1.3.2.2. A minimum of five replicates of each non-zero control per run shall be evaluated.
 - 2.5.1.3.2.2.1. A total of 20 replicates, at a

- minimum, shall be evaluated for each non-zero control.
- 2.5.1.3.2.2.2. Replicates may only be excluded (max. 3) for a documented reason (e.g., internal standard not detected or problems with the extraction).
- 2.5.1.3.2.3. A minimum of four runs shall be evaluated for the method.
- 2.5.1.3.3. If accuracy and precision data from this experiment is going to be used for measurement uncertainty, volatile confirmation instruments shall be used.
- 2.5.1.4. Acceptability
- 2.5.1.4.1. 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.
- 2.5.1.4.2. % ACC and % CV shall be evaluated for quantitative analysis and may be evaluated for qualitative analysis, if applicable.
- 2.5.1.4.2.1. 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, 90 and 110% for volatile analysis, and 70 and 130% (60-140% for concentrations <10 ng/mL) for drug qualitative analysis.
- 2.5.1.4.2.1.1. For concentrations < 10 ng/ml or 10 mg/dL the acceptable range may be set as a numerical range instead of a percentage.
- 2.5.1.4.2.2. For each non-zero control, % CV (ref. 2.3.3) shall be $\leq 20\%$ (10 % for volatile analysis) for each run (within-run) and for the entire experiment (between-run).
- 2.5.1.4.2.3. If accuracy and precision are evaluated for serum/plasma, the % ACC and % CV shall be better than the % ACC and % CV for whole blood or within 2% of the % ACC and % CV for whole blood.
- 2.5.1.4.2.3.1. If the % ACC or % CV is not better than

or within the %ACC or %CV for whole blood by more than 2%, three additional runs (two additional runs for qualitative analysis) shall be performed using serum/plasma controls.

- 2.5.2. Linearity and Lower Limit of Quantitation
 - 2.5.2.1. Linearity evaluates the range of concentrations and the calibration model (e.g., linear versus quadratic calibration, equal versus 1/x weighting) used in the method.
 - 2.5.2.1.1. The LLOQ is the lowest concentration that the method can reliably and reproducibly quantitate. The LLOQ may be set administratively but shall be achievable by the method.
 - 2.5.2.2. At a minimum, fortified matrix samples shall be evaluated using the desired calibrator concentrations (ref. 2.5.2.2.1), 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.
 - 2.5.2.2.4. The first replicate of each calibrator concentration should be used to define the calibration curve.
 - 2.5.2.2.4.1. If a different replicate is used, the reason shall be stated.
 - 2.5.2.2.4.1.1. Each calibration level does not have to use the same replicate to generate the calibration curve.
 - 2.5.2.2.5. Injection volume may be evaluated to determine if the linearity of the analyte is affected by changes in the injection volume

- 2.5.2.2.6. Decreased injection volume may be evaluated to determine if the LLOQ is maintained using a lower injection volume.
- 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 curve shall have $r^2 \geq 0.99$ for each analyte.
 - 2.5.2.3.3. Negative controls should be $\leq \frac{1}{2}$ LLOQ concentration.
 - 2.5.2.3.4. % ACC and % CV shall be evaluated for quantitative analysis and may be evaluated for qualitative analysis, if applicable.
 - 2.5.2.3.4.1. % ACC (ref. 2.3.1) shall be between 80-120% (90-110% for volatile analysis) for the replicates.
 - 2.5.2.3.4.2. % CV (ref. 2.3.3) shall be $\leq 20\%$ (10% for volatile analysis) for the replicates.
 - 2.5.2.3.4.3. The LLOQ shall be determined by the lowest target calibrator concentration with acceptable % ACC and % CV.
- 2.5.3. Lower Limit of Detection
 - 2.5.3.1. The LLOD is the lowest concentration of the analyte of interest that the method can reliably and reproducibly detect.
 - 2.5.3.2. The LLOD 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.
 - 2.5.3.3.1.1. 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 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.
- 2.5.4. 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. A minimum of one matrix sample fortified at twice the desired ULOQ concentration (or physiologically relevant concentration) followed by at least one negative control 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 $\leq \frac{1}{2}$ 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. Selectivity
 - 2.5.5.1. Selectivity assesses whether the method has acceptable bias and precision for all matrices intended to be analyzed or used in calibrators and/or controls.
 - 2.5.5.2. Fortified matrices (whole blood, serum/plasma, alternative matrices, and water source, if applicable) shall be evaluated at three concentrations across the quantitative range (e.g., 3x LLOQ, 50% ULOQ, and 80% ULOQ) of the method (only LLOD for qualitative methods).
 - 2.5.5.3. A minimum of 3 sources per matrix (or replicates, if less than 3 sources are available) shall be used.
 - 2.5.5.3.1. A minimum of one sample at each concentration and one negative sample for each matrix shall be evaluated.
 - 2.5.5.3.2. The mean RR (ref. 2.3.2) shall be calculated for each matrix and used for % ACC and % CV.
 - 2.5.5.4. Acceptability
 - 2.5.5.4.1. 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.
 - 2.5.5.4.2. % ACC and % CV shall be evaluated for quantitative analysis and may be evaluated for qualitative analysis, if applicable.
 - 2.5.5.4.2.1. For each non-zero control, % ACC_{Sel} (ref. 2.3.1) shall be between 80-120% for drug quantitative analysis, 90-110% for volatile analysis, and 70-130% (60-140% for concentrations <10 ng/mL) for drug qualitative analysis for each run (within-run).
 - 2.5.5.4.2.2. Each of the following combinations should be evaluated: whole blood and serum/plasma, whole blood and water,

and serum/plasma and water, as applicable. For each matrix at each concentration, % CV (ref. 2.3.3) shall be $\leq 20\%$ for drug quantitative analysis, 10% for volatile analysis, and 30% for drug qualitative analysis.

2.5.6. Matrix Interference

2.5.6.1. 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.

2.5.6.2. Unfortified matrix samples from a minimum of 10 different sources (e.g., five serum/plasma and five whole blood) using purchased and/or closed case specimens that previously screened “negative” shall be evaluated for matrix interference.

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

2.5.6.4. Acceptability

2.5.6.4.1. 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.

2.5.6.4.2. 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.

2.5.7. Non-Matrix Interference

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

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

2.5.7.2.1. Internal standard interference

2.5.7.2.1.1. One negative control with internal standard and one negative control without internal standards shall be extracted and analyzed.

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

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

2.5.7.2.2. Drug interference

2.5.7.2.2.1. Three replicate samples containing no analyte(s) of interest or internal

standard shall be fortified with NTA(s), extracted, and analyzed.

2.5.7.2.2.2. For quantitative methods, a minimum of one non-zero control 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). These samples shall be analyzed as either extracted samples or neat solutions.

2.5.7.2.2.3. For qualitative methods, a minimum of one non-zero control 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). These samples shall be analyzed as either extracted samples or neat solutions.

2.5.7.2.2.4. 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.

2.5.7.3. Acceptability

2.5.7.3.1. Internal standard interference

2.5.7.3.1.1. The negative control 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.

2.5.7.3.1.2. The negative control 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.

2.5.7.3.1.3. 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.

- 2.5.7.3.2. Drug interference
 - 2.5.7.3.2.1. 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.
 - 2.5.7.3.2.2. $RPD_{\text{drug inter}}$ (ref. 2.3.5) of each result from the non-zero controls with and without NTA (containing internal standard) shall be $\leq 20\%$ for drug quantitative analysis, $\leq 10\%$ for volatile analysis, and $\leq 30\%$ for drug qualitative analysis.
 - 2.5.7.3.2.3. 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.
- 2.5.8. Ion Suppression/Enhancement
 - 2.5.8.1. Ion suppression/enhancement is the suppression or enhancement of analyte ionization in the presence of co-eluting compounds (LC methods only).
 - 2.5.8.2. Evaluation of a minimum of three different sources of each matrix intended to be analyzed by the method, if available, shall be fortified post-extraction with the analytes of interest and internal standards.
 - 2.5.8.2.1. 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.
 - 2.5.8.2.2. 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).
 - 2.5.8.2.3. 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.
 - 2.5.8.2.4. 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.
 - 2.5.8.3. Acceptability
 - 2.5.8.3.1. 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.
- 2.5.8.3.2. % S/E and % CV shall be evaluated for quantitative analysis and may be evaluated for qualitative analysis, if applicable.
- 2.5.8.3.2.1. % S/E (ref. 2.3.4) should be $\leq 20\%$ (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.
- 2.5.8.3.2.2. % CV (ref. 2.3.3) shall be $\leq 20\%$ (30% for qualitative analysis) for the matrix samples.
- 2.5.9. Correlation
- 2.5.9.1. Correlation is the comparison of analytical results obtained from different analyses of the same specimen.
- 2.5.9.2. 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.
- 2.5.9.3. Specimens not containing any analyte of interest above the LLOD shall be included.
- 2.5.9.4. Correlation between instruments may be performed by reinjection of previously analyzed samples if it is within the reinjection timeframe of the method.
- 2.5.9.4.1. 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).
- 2.5.9.4.1.1. For qualitative methods, there shall be at least one specimen that is presumptively positive for each analyte being validated in the method.
- 2.5.9.4.1.2. For quantitative methods, there should be at least three specimens positive for each analyte being validated in the method.
- 2.5.9.5. Acceptability
- 2.5.9.5.1. For quantitative analysis, the RPD (ref. 2.3.5) of the result from the previous analysis compared to the new result should be $\leq 30\%$ ($\leq 10\%$ 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.

- 2.5.9.5.1.1. For samples with a concentration less than 2 ng/mL, a range may be utilized instead of the RPD.
- 2.5.9.5.2. 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.
- 2.5.10. Stability of Extracted/Prepared Samples
 - 2.5.10.1. Extracted/prepared sample stability is the length of time an extracted/prepared sample can be stored prior to analysis without producing unacceptable results.
 - 2.5.10.2. Experiment should be performed with whole blood, serum/plasma, and alternative matrix samples, if applicable.
 - 2.5.10.3. 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).
 - 2.5.10.4. Drug analysis
 - 2.5.10.4.1. 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.
 - 2.5.10.4.2. All stability samples shall be aliquoted from the fortified matrix stocks and extracted on the same day.
 - 2.5.10.5. Volatile analysis
 - 2.5.10.5.1. 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.
 - 2.5.10.5.1.1. All stability samples shall be aliquoted on the same day.
 - 2.5.10.6. 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.
 - 2.5.10.6.1. One set of stability samples consists of three replicates of each non-zero control concentration.
 - 2.5.10.7. Stability samples shall be stored under conditions expected to be encountered during casework (e.g., room temperature or refrigeration).
 - 2.5.10.8. On subsequent days, calibrators (or cutoff) and controls shall be extracted/prepared and analyzed with one set of stability samples.
 - 2.5.10.9. Acceptability
 - 2.5.10.9.1. 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.

- 2.5.10.9.2. RPD_{stability} shall be evaluated for quantitative analysis and may be evaluated for qualitative analysis, if applicable.
 - 2.5.10.9.2.1. 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.
- 2.5.11. Stability of Stock Solutions
 - 2.5.11.1. Calibrator and control stock solutions stability is the length of time non-CRM stock solutions are stable without producing unacceptable results.
 - 2.5.11.2. Multiple lots of calibrator and control stock solutions shall be prepared over the course of several weeks and/or months.
 - 2.5.11.2.1. Lot 1 of calibrator and control stock solutions shall be validated according to Solution Verification/Validation (ref. 3).
 - 2.5.11.2.2. 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).
 - 2.5.11.2.2.1. 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.
 - 2.5.11.2.2.2. 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.
 - 2.5.11.3. Acceptability
 - 2.5.11.3.1. Calibrator stock solutions
 - 2.5.11.3.1.1. RPD_{Cal} (ref. 2.3.5) of lot X control concentrations processed with each lot of calibrators shall be $\leq 20\%$ (10% for volatile analysis).
 - 2.5.11.3.2. Control stock solutions
 - 2.5.11.3.2.1. 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 $\leq 20\%$ ($\leq 10\%$ for volatile analysis).

2.5.11.3.3. % ACC (ref. 2.3.1) should be between 80-120% (90-110% for volatile analysis).

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

2.5.12. Dilution Integrity of Samples

2.5.12.1. Dilution integrity of samples is the ability to produce accurate results using a smaller specimen volume than the preferred volume.

2.5.12.1.1. The effect of specimen dilution on the method's accuracy and precision shall be evaluated.

2.5.12.2. Accuracy and precision for dilutions shall be evaluated using whole blood (and serum/plasma, as applicable) as the diluent.

2.5.12.3. Samples shall be fortified with the analyte(s) of interest at concentrations that are detectable by the method when diluted (e.g., for a method with an LLOD of 20 ng/mL, a sample fortified to 50 ng/mL may be diluted 1:2 to create a diluted sample that is above the LLOD, but a 1:4 dilution would be below the LLOD). Samples shall be diluted using expected dilution ratios of 1:2 and 1:4.

2.5.12.4. For quantitative methods, a minimum of two different undiluted concentrations shall be evaluated in a minimum of two runs. For each undiluted concentration, a CRM or a single fortified matrix stock should be prepared at a volume sufficient to extract enough samples for the entire experiment.

2.5.12.4.1. For example:

2.5.12.4.1.1. Make fortified stock specimen at each control level. Add approximately 5 mL of negative blood to each 10 mL volumetric flask. Then slowly or incrementally add the appropriate amount of methanol stock for each respective control level to each volumetric flask. Last, fill to 10 mL with negative blood and mix well to make homogenous.

2.5.12.4.1.2. Prepare undiluted samples: Pipette 1 mL of the control into a test tube. Repeat for remaining 4 samples.

2.5.12.4.1.2.1. Repeat for second concentration.

2.5.12.4.1.3. Prepare 1:2 dilution samples: Aliquot 0.5 mL of the control made in 2.5.12.4.1.1 and 0.5 mL of negative blood into a test tube. Repeat for the remaining 4 test tubes.

2.5.12.4.1.3.1. Repeat for second concentration.

2.5.12.4.1.4. Prepare 1:4 dilution samples: Aliquot 0.25 mL of the control made in

- 2.5.12.4.1.1 and 0.75 mL of negative blood into a test tube. Repeat for the remaining 4 test tubes.
- 2.5.12.4.1.4.1. Repeat for second concentration.
- 2.5.12.4.2. Fortified matrix stocks may be prepared separately for each run if the analyte(s) of interest are not stable in the fortified blood matrix.
- 2.5.12.5. Diluted samples shall be prepared from the fortified matrix samples using the procedure outlined in Specimen and Sample Preparation (ref. 5).
- 2.5.12.6. Undiluted and diluted samples shall be extracted and analyzed using the method being evaluated.
- 2.5.12.7. Each replicate shall be a separate dilution except for duplicate analysis for volatile confirmation.
- 2.5.12.8. For drug confirmation, a minimum of 10 replicates with at least five replicates per analyst of each non-zero control should be evaluated at a 1:2 and 1:4 dilution, as applicable.
 - 2.5.12.8.1. Each replicate shall be a separate dilution.
 - 2.5.12.8.2. Replicates may only be excluded for a documented reason (e.g., internal standard not detected or problems with the extraction).
- 2.5.12.9. For volatile confirmation, a minimum of 20 replicates with at least six replicates per analyst of each non-zero control should be evaluated at a 1:2 and 1:4 dilution.
 - 2.5.12.9.1. For 1:2 dilutions, 25 mixed volatiles, 80 ethanol, and 150 ethanol mg/dL shall be used.
 - 2.5.12.9.2. For 1:4 dilutions, 200 mixed volatiles, 80 ethanol, and 150 ethanol mg/dL shall be used.
 - 2.5.12.9.3. A minimum of 10 dilutions analyzed in duplicate for method validation and a minimum of three dilutions analyzed in duplicate per analyst shall be performed as outlined in 12.8.4.1.3.1 for confirmation analysis.
 - 2.5.12.9.4. Replicates may only be excluded for a documented reason (e.g., internal standard not detected or problems with the extraction).
 - 2.5.12.9.5. If dilution integrity data from this experiment is going to be used for measurement uncertainty, volatile confirmation instruments shall be used.
- 2.5.12.10. Acceptability
 - 2.5.12.10.1. 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.
 - 2.5.12.10.2. $RPD_{\text{sample dilution}}$, % ACC, and % CV shall be evaluated for quantitative analysis and may be evaluated for qualitative analysis, if applicable.
 - 2.5.12.10.2.1. $RPD_{\text{sample dilution}}$ (ref. 2.3.5) of results from undiluted and diluted samples,

- using the average of the replicates, shall be $\leq 10\%$.
- 2.5.12.10.2.2. For each non-zero control, % ACC (ref. 2.3.1) shall be between 80-120% for drug quantitative analysis and 90-110% for volatile analysis.
- 2.5.12.10.2.3. For each non-zero control, % CV (ref. 2.3.3) shall be $\leq 20\%$ (10 % for volatile analysis).
- 2.5.13. Alternative Matrix Integrity (Drug Analysis Only)
- 2.5.13.1. Alternative Matrix Integrity is evaluation of a non-human matrix for use as calibrators and controls.
- 2.5.13.2. Samples shall be fortified to concentrations of each calibrator and control level (non-zero and negative) using both non-human matrix and human whole blood.
- 2.5.13.2.1. One sample of each calibrator and control level shall be prepared using a non-human matrix.
- 2.5.13.2.1.1. A minimum of eight calibrator concentrations for drug analysis or four calibrator concentrations for volatile analysis shall be evaluated.
- 2.5.13.2.2. A minimum of six replicates of each calibrator and non-zero-control level shall be prepared in human whole blood and evaluated using a calibration curve generated from the non-human matrix calibrators.
- 2.5.13.2.2.1. 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).
- 2.5.13.2.3. A minimum of nine negative controls for drug analysis or seven negative controls for volatile analysis shall be evaluated.
- 2.5.13.3. Acceptability
- 2.5.13.3.1. 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.
- 2.5.13.3.2. The calibration curve shall have $r^2 \geq 0.99$ for each analyte.
- 2.5.13.3.3. Negative controls should be $\leq \frac{1}{2}$ LLOQ concentration.
- 2.5.13.3.4. % ACC and % CV shall be evaluated for quantitative analysis and may be evaluated for qualitative analysis, if applicable.
- 2.5.13.3.4.1. % ACC (ref. 2.3.1) shall be between 90-110% (95-105% for volatile analysis) for the replicates.
- 2.5.13.3.4.2. % CV (ref. 2.3.3) shall be $\leq 20\%$ (10% for volatile analysis) for the replicates.

- 2.5.13.3.5. The non-human matrix controls shall only be used to evaluate the non-human calibration curve acceptability.
 - 2.5.13.3.5.1. % ACC (ref. 2.3.1) shall be between 80-120% (90-110% for volatile analysis) for the replicates.
- 2.5.14. Dilution Integrity of Negative Blood
 - 2.5.14.1. Dilution integrity of negative blood is the ability to produce accurate results using negative blood that has been diluted. Negative blood may be diluted with ddH₂O to increase the volume of negative blood available for use.
 - 2.5.14.1.1. The effect of diluted negative blood on the method's accuracy and precision shall be evaluated.
 - 2.5.14.2. Samples shall be fortified to concentrations of each calibrator and control level (non-zero and negative) using undiluted and 1:2 diluted negative blood. A minimum of five replicates each of non-zero and negative controls should be prepared. Samples shall be extracted and analyzed using the method being evaluated.
 - 2.5.14.3. Acceptability
 - 2.5.14.3.1. Diluted and undiluted controls shall be processed and quantitated using the diluted calibrators as well as the undiluted calibrators.
 - 2.5.14.3.2. RPD_{dil-neg blood} (ref. 2.3.5) of results from undiluted and diluted samples, using the average of the replicates for each run, shall be $\leq 10\%$.
- 2.5.15. Reinjection Integrity
 - 2.5.15.1. Reinjection integrity is the ability to produce accurate results using a sample that was previously injected.
 - 2.5.15.1.1. 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.
 - 2.5.15.2. 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.
 - 2.5.15.3. Acceptability
 - 2.5.15.3.1. 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.
 - 2.5.15.3.2. RPD shall be evaluated for quantitative analysis and may be evaluated for qualitative analysis, if applicable.
 - 2.5.15.3.2.1. For volatile methods, RPD (ref. 2.3.5) of results from originally injected samples and the reinjected samples shall be $\leq 5\%$ or 5 mg/dL, whichever is greater.

- 2.5.15.3.2.2. For qualitative drug analysis, the RPD (ref. 2.3.5) of results from originally injected samples and the reinjected samples should be $\leq 30\%$ ($\leq 40\%$ for concentrations <10 ng/mL) or be consistent with the previous result of “Positive” or “Negative.”
- 2.5.15.3.2.3. For quantitative drug methods, RPD (ref. 2.3.5) of results from the originally injected samples and the reinjected samples shall be $\leq 10\%$ ($\leq 5\%$ or 5 mg/dL, whichever is greater, for volatile analysis) for each analyte of interest.
 - 2.5.15.3.2.3.1. For concentrations < 10 ng/ml the acceptable range may be set as a numerical range instead of a percentage.
- 2.5.16. Measurement Uncertainty
 - 2.5.16.1. MU is a non-negative parameter characterizing the dispersion of the values attributed to a measured quantity.
 - 2.5.16.2. MU shall only be calculated for quantitative methods.
 - 2.5.16.3. The measureand is the concentration of the analyte measured using the method.
 - 2.5.16.4. The quantity value is the quantitative result for an analyte.
 - 2.5.16.5. Traceability for each method is established by using CRM for preparation of calibrators and controls, calibrated pipettes and autodilutors, and class A glassware for volumetric flasks, if applicable.
 - 2.5.16.6. The following contributing factors or uncertainty components shall be assessed per analyte, if applicable, when calculating the MU for each analyte in a method:
 - 2.5.16.6.1. Pipettes or autodilutors used for sample delivery and preparation of controls and calibrators;
 - 2.5.16.6.2. The sum of the CRM uncertainty for all calibrators used to calculate the calibration curve;
 - 2.5.16.6.3. The highest CRM uncertainty for controls;
 - 2.5.16.6.4. Volumetric flasks for dilutions;
 - 2.5.16.6.5. All instruments used for the specific analysis, and;
 - 2.5.16.6.6. Measurement Process Reproducibility data from all analysts trained in the method.
 - 2.5.16.6.6.1. A minimum number of 10 replicates shall be used to determine the % RSD (ref. 2.3.6) for calculations for the measurement process reproducibility.
 - 2.5.16.6.6.2. All values for the uncertainty components shall be reported in percent

- by calculating the % RSD (ref. 2.3.6) for each component.
- 2.5.16.6.3. The standard uncertainty shall be calculated for each uncertainty component by dividing the value of the uncertainty component by the divisor.
- 2.5.16.6.3.1. 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.
- 2.5.16.7. The sum of all standard uncertainty for the method shall be calculated and multiplied by the k value.
- 2.5.16.7.1. 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.
- 2.5.16.8. 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.
- 2.5.16.8.1. 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.
- 2.5.16.8.2. All measurement uncertainties shall be reported in the same unit as the quantity value and in the format of quantity value \pm the MU.
- 2.5.16.9. MU shall be evaluated and calculated after any of the following:
- 2.5.16.9.1. Validation of a method;
- 2.5.16.9.2. Modification of an existing method requiring full or partial revalidation;
- 2.5.16.9.3. New analyst being trained on the method;
- 2.5.16.9.3.1. 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.
- 2.5.16.9.4. New or modified instrument being used for the method; or,
- 2.5.16.9.5. 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 and solutions containing 7-aminoclonazepam should be sonicated prior to sampling or use if a precipitant is present.
- 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.
 - 3.8.2. 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 may be prepared and included in a casework batch as a sample. Calibrator and control solutions may be prepared and analyzed concurrently with casework but should be calibrated and processed separately from the casework batch (run).
 - 3.8.2.1. CRMs used “as is” need not be validated. The target concentration is the nominal concentration on the CoA, however named.
 - 3.8.3. Solutions other than calibrators, controls, and internal standards may be verified concurrently with casework (e.g., buffers, mobile phases, elution solutions).
 - 3.8.4. See method for specific assay for procedures for preparation of solutions.
 - 3.8.5. Validation of Negative Blood or Serum/Plasma
 - 3.8.5.1. If an expiration date is given for a negative matrix, the negative blood or serum/plasma may be used past the expiration date.
 - 3.8.5.2. Volatile analysis
 - 3.8.5.2.1. At least one negative control shall be prepared without internal standard and one negative control should be prepared with internal standard for each unvalidated negative blood lot. The analysis shall be completed on

- one screening and one confirmation instrument, at minimum. The run shall include negative blood controls prepared using a previously validated negative blood or serum/plasma lot, calibrator(s), and non-zero and negative aqueous controls.
- 3.8.5.2.2. Acceptability
- 3.8.5.2.2.1. Acceptable criteria/chromatography as stated in Volatile Screening and Confirmation by HS/GC/FID (ref. 12.10) for a negative control.
- 3.8.5.3. Drug analysis
- 3.8.5.3.1. Screening: At least one negative control shall be prepared without internal standard and one negative control should be prepared with internal standard for each unvalidated negative blood lot and 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.
- 3.8.5.3.2. Acceptability
- 3.8.5.3.2.1. Acceptable criteria/chromatography as stated in Blood Drug Screening by LC/TOF (ref. 6.10) for a negative control.
- 3.8.5.3.2.2. 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.
- 3.8.5.3.3. Confirmation: Prior to its use as a control in a drug confirmation analysis, at least one negative control shall be prepared without internal standard and one negative control should be prepared with internal standard for each unvalidated negative blood lot and 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.
- 3.8.5.3.4. Acceptability
- 3.8.5.3.4.1. Acceptable criteria/chromatography as stated in the specific drug confirmation test method under Interpretation of Results for a negative control.
- 3.8.5.3.4.2. 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.

- 3.8.6. Validation of In-House LC/TOF Drug Screening Solutions
 - 3.8.6.1. Non-CRM Calibrator and Control Solutions
 - 3.8.6.1.1. A minimum of three replicates of the calibrator/cutoff control shall be analyzed as extracted or neat samples. The run may be calibrated using one of the new calibrator replicates and/or compared to a historical calibration.
 - 3.8.6.1.2. Acceptability
 - 3.8.6.1.2.1. Acceptability criteria for the analysis as stated in Blood Drug Screening by LC/TOF (ref. 6.10).
 - 3.8.6.2. Internal standard solutions
 - 3.8.6.2.1. A minimum of two replicates of neat samples or negative controls prepared with the newly prepared internal standard shall be extracted and analyzed using any instrument validated for the method for the specific assay. The run shall include calibrator(s) and non-zero and negative controls as required by the method for the specific assay and should be prepared using a previously validated internal standard lot.
 - 3.8.6.2.2. Acceptability
 - 3.8.6.2.2.1. Acceptable criteria for the analysis as stated in Blood Drug Screening by LC/TOF (ref. 6.10).
- 3.8.7. Validation of Volatile Solutions
 - 3.8.7.1. Non-CRM calibrator solutions
 - 3.8.7.1.1. A minimum of six replicates of each newly prepared calibrator shall be analyzed on one screening and one confirmation instrument, at minimum. The run shall be calibrated using mixed volatiles CRMs as the calibrator(s) and include non-zero and negative controls as required by the method for the specific assay.
 - 3.8.7.1.2. Acceptability
 - 3.8.7.1.2.1. Acceptability criteria for the analysis as stated in Volatile Screening and Confirmation by HS/GC/FID (ref. 12.10).
 - 3.8.7.1.2.2. % ACC (ref. 2.3.1) shall be between 90-110%.
 - 3.8.7.1.2.3. % CV (ref. 2.3.3) shall be $\leq 10\%$.
 - 3.8.7.1.3. The target concentration of each calibrator shall be used for analysis.
 - 3.8.7.2. Internal standard solutions
 - 3.8.7.2.1. A minimum of two replicates of negative controls prepared with the newly prepared internal standard shall be analyzed on one screening and one confirmation instrument, at minimum. The run shall include calibrator(s) and non-zero and negative controls as required by the method for the specific assay and

should be prepared using a previously validated internal standard lot.

3.8.7.2.2. Acceptability

3.8.7.2.2.1. Acceptability criteria for the analysis as stated in Volatile Screening and Confirmation by HS/GC/FID (ref. 12.10).

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 control level 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.

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

3.8.8.1.2.1.1. If a different replicate is used, the reason shall be stated in the Analyst's Notes on the Solution Validation Technical Review Checklist.

3.8.8.1.2.1.2. Each calibration level does not have to use the same replicate for each analyte to generate the calibration curve.

3.8.8.1.2.2. If neat solutions are used, the replicate 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. 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).

- 3.8.8.1.2.3. 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.
- 3.8.8.1.3. Acceptability
 - 3.8.8.1.3.1. Acceptability criteria/chromatography for calibrators and controls as stated in Interpretation of Results for the specific test method.
 - 3.8.8.1.3.2. Between-run % ACC (ref. 2.3.1) shall be between 85-115%.
 - 3.8.8.1.3.3. Between-run % CV (ref. 2.3.3) shall be $\leq 20\%$.
- 3.8.8.1.4. The target concentration of each calibrator and control level shall be used for analysis.
 - 3.8.8.1.4.1. Actual concentrations for each calibrator and control level may only be used for analysis with supervisory approval.
- 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. Buffer Log
- 3.9.5. Validation of calibrator, control, and internal standard solutions shall be documented in a summary of data (ref. 3.9.5.2.1.5). The validation summary shall be saved electronically on a network drive, verified, and signed by the verifier.
 - 3.9.5.1. 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.
 - 3.9.5.2. 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.

- 3.9.5.2.1. The validation documentation shall include:
 - 3.9.5.2.1.1. Calibrator, control, and internal standard solution preparation worksheet(s), if applicable;
 - 3.9.5.2.1.2. Non-CRM volatile control CoA, if applicable;
 - 3.9.5.2.1.3. Analysis preparation worksheet(s);
 - 3.9.5.2.1.4. Chromatograms and/or raw data; and
 - 3.9.5.2.1.5. Tune report, if applicable;
 - 3.9.5.2.1.6. Table of responses and/or concentrations with applicable calculations for the acceptability criteria.
- 3.9.6. Material name, vendor, lot #, 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).
 - 3.9.6.1. Solutions that are made daily do not need to be documented in a log and shall be documented on the appropriate preparation worksheet.
- 3.9.7. 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
- 4.4. Instruments/Equipment
 - 4.4.1. Heat sealers
 - 4.4.2. Balance
- 4.5. Reagents/Materials
 - 4.5.1. Label
 - 4.5.2. Empty blood tube or urine bottle
 - 4.5.3. Bubble wrap
 - 4.5.4. Tape
 - 4.5.5. Marker/pen
 - 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. Select “New Case,” “01 Agency Unknown,” and leave case number blank.
 - 4.8.1.2.2. When prompted to create a new case, select “Yes.”
 - 4.8.1.2.3. Open Evidence tab, select “Add Evidence” from the Evidence menu.
 - 4.8.1.2.4. Enter evidence packaging information for Kit and/or Description.
 - 4.8.1.2.5. Document any tracking information or packaging notes in the Notes section.
 - 4.8.1.2.5.1. If there are any shipping documents containing case-specific information

that will be discarded at receiving (e.g., shipping bag/box with sticker or writing), scan information and upload the image to the electronic case file.

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

4.8.1.2.5.2. Tracking numbers shall be documented by scanning the tracking number into the Notes section or scanning the information and uploading the image to the electronic case file at the time of receiving.

4.8.1.2.6. Fill out Initial Transfer, as shown in the examples below:

Initial Transfer			
From	Courier or Drop Box		<input type="button" value="X"/>
Time	06/22/2015 11:34 AM	VIA <input type="button" value="v"/> Note Receiving	<input type="button" value="X"/>
To	ISDT staff name		<input type="button" value="X"/>
Time	06/22/2015 11:34 AM	VIA <input type="button" value="v"/> Note Storage	<input type="button" value="X"/>
Then To	Walk-In		<input checked="" type="checkbox"/> Lock <input type="button" value="X"/>

From	Courier or Drop Box		<input type="button" value="X"/>
Time	11/21/2017 01:06 PM	VIA <input type="button" value="v"/> Note Receiving/Accessioning	<input type="button" value="X"/>
To	ISDT staff name		<input type="button" value="X"/>
Time	11/21/2017 01:06 PM	VIA <input type="button" value="v"/> Note	<input type="button" value="X"/>
Then To			<input type="button" value="X"/>

4.8.1.2.7. Generate barcode.

4.8.1.2.8. Remove primary container from shipping container, if applicable, and place barcode on primary container.

4.8.1.3. Label each associated document with the case number, scan document, and upload to the electronic case file, if applicable.

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

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

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

4.8.1.4.1.1. 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, or proficiency test.

4.8.1.4.1.2. STAT status should be designated for the following: 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 for expedited analysis.

4.8.2.2. Document transfer of evidence in LIMS as shown in the example below:

From	SCAN BAR CODE for the person or place the evidence is being transferred from. (Walk-in)			×
Time	07/26/2017 11:26 AM	VIA	▼ Note	Accessioning
To	ISDT Staff Name			×
Time	07/26/2017 11:26 AM	VIA	▼ Note	
Then To				×

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. Document the ISDT case number, name of person performing accessioning, and date on the EDW.

4.8.2.6. Inspect the exterior of the primary container and complete the Primary section in the Enclosures box.

4.8.2.6.1. Describe primary container if different from ISDT kit or envelope.

4.8.2.6.2. If any case-identifying information is on the primary container, scan an image of the identifying information into the electronic case file. Verify the scanned image for completeness and legibility.

4.8.2.6.2.1. If identical case-identifying information is duplicated on multiple sides of the primary container, document the information by scanning at least one side of the primary container and document on the EDW the information that was not scanned (e.g., “The agency case number, subject’s name, and date of birth were hand written on 4 of the 6 sides of the kit.”).

4.8.2.7. Open the primary container; inspect the secondary container and complete the Secondary section in the Enclosures box.

4.8.2.7.1. If a TAR is not included with the primary container, or other documentation does not contain sufficient information (sections 1, 2, and 3 of the TAR), do not complete accessioning until the submitting agency is contacted and supplies additional information.

4.8.2.7.1.1. If there is any information on the primary container or evidence, enter applicable information into LIMS.

4.8.2.7.1.2. If additional information is not obtained during accessioning, reseal evidence

- inside the primary container. Initial and date seal on the primary container and transfer evidence to the walk-in refrigerator with the transfer note of "Storage/Not Accessioned."
- 4.8.2.7.1.3. If the requested information is not provided within a reasonable time, consult laboratory supervisor.
- 4.8.2.8. Remove evidence from the secondary container.
- 4.8.2.9. Scan an image of the TAR and any other documentation into the electronic case file, if applicable (ref. 4.8.11).
- 4.8.2.10. Ensure that any shipping documentation is scanned into the electronic case file.
 - 4.8.2.10.1. The electronic file shall be verified against the original for completeness and legibility prior to discarding the hardcopy.
- 4.8.2.11. Inspect specimens and complete Specimens box on the EDW.
 - 4.8.2.11.1. Assign each specimen a letter, starting with A.
 - 4.8.2.11.2. Letters should be assigned to specimens in the following order: whole blood, serum/plasma, urine. Multiple items of the same specimen type should be itemized in order by volume (largest volume first).
 - 4.8.2.11.2.1. If there are multiple specimen collection times, the specimen collection time should be noted in the Additional Information column of the EDW for each specimen. See also 4.8.2.17.1 and 4.8.2.18.8.1.
 - 4.8.2.11.2.2. For proficiency test specimens, itemization should be in numerical order by specimen identification number. The specimen identification number shall be documented in the Additional Information column of the EDW.
 - 4.8.2.11.2.3. If there are more than five specimens submitted, a second EDW shall be completed.
 - 4.8.2.11.2.4. If there are no whole blood or serum/plasma specimens submitted, reseal evidence inside the primary container. Initial and date seal on the primary container and transfer evidence to the walk-in refrigerator. Notify laboratory supervisor.
 - 4.8.2.11.3. Estimate the volume of each specimen by weighing the specimen container on a balance that has been tared with an equivalent empty specimen container, if

- possible. If an equivalent specimen container is not available, visually estimate the volume of the specimen.
- 4.8.2.11.3.1. If specimen volume is minimal (e.g., < 3mL per draw time) and alcohol and drug analyses are requested or only drug analysis is requested, do not relate any specimens with the request.
- 4.8.2.11.3.1.1. Notify the laboratory supervisor (or forensic toxicologist if the laboratory supervisor is unavailable).
- 4.8.2.11.4. If a container marked with case information is empty, a second person shall verify that the container is empty prior to disposal. The verifier's initials shall be recorded on the EDW.
- 4.8.2.11.5. If a specimen container has leaked, but is not broken, capture an image of the leaky container, if possible, to document the condition in which it was received.
- 4.8.2.11.5.1. Document the condition of the specimen tube at the time of accessioning on the EDW.
- 4.8.2.11.5.2. A note should be affixed across the lid of the specimen tube indicating that it should not be used for analysis without supervisory approval.
- 4.8.2.11.5.3. If all specimen tubes received for a case have leaked, notify the laboratory supervisor or quality control coordinator.
- 4.8.2.11.6. If, upon receipt, a specimen container is cracked, broken, or not securely capped, capture an image of the container, if possible, to document the condition in which it was received.
- 4.8.2.11.6.1. Transfer the contents of the container to an unused tube and document the transfer of the contents of the original container to a new tube on the EDW.
- 4.8.2.11.6.1.1. Mark the new tube with the case number, date/time of transfer, and initials of the person transferring the specimen.
- 4.8.2.11.6.2. The original specimen container shall be maintained inside a larger container (e.g., a conical tube) and itemized as the parent specimen container. Itemize the new tube in LIMS as being created

from the parent specimen container (e.g., if specimen container 1-B (parent specimen container) is a broken whole blood tube, the new tube shall be itemized by leaving the inherit field blank, the evidence number assigned as 1-B-1 (child specimen container), evidence type listed as “Blood,” and the item description listed as “Whole Blood”) ref. 4.8.2.17.

4.8.2.11.6.3. The CoC should be documented as shown below:

ITEM # / DESCRIPTION: OTHER ID #:		1-A	Whole Blood			
<u>Date/Time of Transfer</u>	<u>From</u>		<u>PIN</u>	<u>To</u>	<u>PIN</u>	<u>Purpose</u>
5/5/2020 3:24:59PM	DROP BOX,		[]	ISDT Staff Name	[X]	Receiving
5/5/2020 3:25:00PM	ISDT Staff Name		[X]	Walk-In	[]	Storage
5/6/2020 8:23:43AM	Walk-In		[]	ISDT Staff Name	[X]	Accessioning
5/6/2020 8:45:19AM	ISDT Staff Name		[X]	Sample Prep Area	[]	Decant Specimen
5/6/2020 9:01:27AM	Sample Prep Area		[]	ISDT Staff Name	[X]	Transfer
5/6/2020 9:01:29AM	ISDT Staff Name		[X]	Walk-In	[]	Storage

ITEM # / DESCRIPTION: OTHER ID #:		1-A-1	Whole Blood			
<u>Date/Time of Transfer</u>	<u>From</u>		<u>PIN</u>	<u>To</u>	<u>PIN</u>	<u>Purpose</u>
5/6/2020 8:50:43AM	Sample Prep Area		[]	ISDT Staff Name	[X]	Accessioning
5/6/2020 9:01:29AM	ISDT Staff Name		[X]	Walk-In	[]	Storage

4.8.2.11.6.4. A note should be affixed across the lid of the child specimen tube indicating that it should not be used for analysis without supervisory approval.

4.8.2.11.6.5. When the specimen containers are stored in the walk-in, the child specimen tube (e.g., 1-B-1) should be placed in the test tube rack in sequential case number order. The parent specimen container should be placed in the designated location within the walk-in for oversized tubes.

4.8.2.12. Use “Y” to document on the EDW that the subject name, date, time, collector’s initials/name, and/or officer’s initials/name are present on the specimen container(s). Verify that the information on the evidence specimen container(s) matches the information on the TAR, if applicable.

4.8.2.12.1. If there is any discrepancy, the information on the evidence shall be documented in the “Additional Information” section of the EDW. If there is a discrepancy in the subject name, contact the submitting agency or look up the case in [myCase](#) to verify the subject name.

4.8.2.13. Use “N” to document on the EDW that the subject name, date, time, collector’s initials/name, and/or officer’s initials/name are not present on the specimen container(s)

- 4.8.2.14. For proficiency specimens, indicate “N/A” on the EDW for the subject name, date, time, collector’s initials/name, and/or officer’s initials/name.
- 4.8.2.15. Upload the EDW into the electronic case file.
- 4.8.2.16. Enter information in LIMS from the TAR, including, but not limited to, the following:
 - 4.8.2.16.1. In the Agency tab, update agency to the agency listed on the TAR.
 - 4.8.2.16.1.1. If the agency is listed as Pokagon Tribal Police, documentation that officer is a deputy of the St. Joseph County Police (sheriff’s office) is required for acceptance of the evidence. If so, the agency shall be added as St. Joseph County Police Department.
 - 4.8.2.16.1.1.1. If the TAR does not list this relationship with St. Joseph County Police Department, maintain the primary container, follow the procedure for a kit without a TAR (ref. 4.8.2.7.1), and notify the laboratory supervisor.
 - 4.8.2.16.1.2. Add agency case number.
 - 4.8.2.16.1.3. Verify that the county prosecutor was added, or add county prosecutor, if applicable.
 - 4.8.2.16.1.4. If the TAR indicates that a DRE evaluation was performed, add Drug Recognition Expert as an agency.
 - 4.8.2.16.1.5. Enter the agency case number of the submitting agency as the agency case number for each agency associated with the case.
 - 4.8.2.16.2. In the Offense tab, add offense and:
 - 4.8.2.16.2.1. Select the appropriate offense type(s) from dropdown list that matches the information provided on the TAR;
 - 4.8.2.16.2.2. If an offense area is not marked on the TAR and there is no indication as to the type of case on the TAR, select “Unknown” as the offense type.
 - 4.8.2.16.2.3. Ensure that the county field is populated.
 - 4.8.2.16.3. In the Individuals tab, add an individual and:
 - 4.8.2.16.3.1. Enter the subject name;

- 4.8.2.16.3.2. Select “Subject” as the Type;
 - 4.8.2.16.3.3. Select the gender indicated on the TAR or “Unknown” if not indicated; and
 - 4.8.2.16.3.4. Enter date of birth if indicated on the TAR.
- 4.8.2.17. In the Evidence tab, select “Itemize Evidence” from the Evidence menu.
- 4.8.2.17.1. Record the specimen type in the Description field.
 - 4.8.2.17.1.1. Specimen types: Whole Blood, Serum/Plasma, Urine, Homogenized Blood, Supernatant, and Postmortem.
 - 4.8.2.17.1.2. When there are multiple draw times (>15 minutes apart), the time should be noted in 24-hour format in the item descriptions after the specimen type, e.g., “Whole Blood (15:45).”
 - 4.8.2.17.1.3. For coroner cases, if more than one specimen type is submitted, identify the sample type in the item description followed by any additional information provided, e.g., “Postmortem (Subclavian),” “Whole Blood (Admission),” “Postmortem (Femoral),” as appropriate.
 - 4.8.2.17.1.4. For each proficiency test specimen, the corresponding specimen identification shall be recorded in the Description field.
 - 4.8.2.17.2. In the Inherit dropdown list, select the primary container.
 - 4.8.2.17.3. In the Source dropdown list, select the subject name.
 - 4.8.2.17.4. Generate barcodes and affix to the specimen containers.
 - 4.8.2.17.4.1. For a proficiency test specimen, a second person shall verify that the identification on the specimen corresponds with its barcode description and the information listed on the EDW. The verification shall be documented by the verifier’s initials on the EDW.
- 4.8.2.18. In the Requests tab, add requests as indicated on the TAR.
- 4.8.2.18.1. Exception: If alcohol analysis is requested by Indianapolis Metropolitan Police Department, Beech Grove Police Department, Speedway Police Department, or Lawrence Police Department, do not enter a request for alcohol analysis unless directed by laboratory supervisor or quality control coordinator.
 - 4.8.2.18.2. Select the appropriate submitting agency and officer.

4.8.2.18.2.1. If the submitting officer is not listed, add the officer to the correct agency.
4.8.2.18.2.1.1. If an officer is listed under the wrong agency or their name is misspelled, notify a supervisor or quality control coordinator for correction. Duplicate entries should be marked as "Inactive."

- 4.8.2.18.3. For Laboratory, verify "Indiana State Department of Toxicology" is selected.
- 4.8.2.18.4. For "Section," select "Toxicology."
- 4.8.2.18.5. For an alcohol request, the unit shall be "Alcohol Analysis" and assigned to "Analyst, Volatile."
- 4.8.2.18.6. For a drug request, the unit shall be "Drug Analysis" and assigned to "Analyst, Drug."
- 4.8.2.18.7. Under Reason, select Priority or STAT, if applicable.
- 4.8.2.18.8. When prompted, relate one item of evidence and the subject name to the request. Do not relate evidence to a non-preferred analysis request if the specimen volume is minimal (ref. 4.8.2.11.3.1).
4.8.2.18.8.1. If specimens were collected at different times (>15 minutes apart), do not relate evidence to any requests. Notify the laboratory supervisor (or forensic toxicologist if the laboratory supervisor is unavailable) for follow-up on testing preferences (ref. 4.8.2.11.2.1).

4.8.2.19. Document disposal of primary container in LIMS as shown in the example below:

From	ISDT Staff Name			
Time	07/28/2017 09:00 AM	VIA		Note Disposal
To	Trash			
Time	07/28/2017 09:00 AM	VIA		Note
Then To				

4.8.2.20. Dispose of primary container, secondary container, and any other non-evidentiary contents.

4.8.2.21. Document transfer of evidence to the walk-in refrigerator in LIMS as shown in the example below:

From	ISDT Staff Name			
Time	07/26/2017 11:26 AM	VIA		Note Storage
To	Walk-In			
Time	07/26/2017 11:26 AM	VIA		Note
Then To				

- 4.8.2.22. Transfer specimens to the walk-in refrigerator.
- 4.8.2.23. File TAR and any other paperwork in the appropriate file folder numerically by case number.
- 4.8.3. Specimen Transfer Within ISDT
 - 4.8.3.1. Each specimen transfer shall be documented in LIMS contemporaneously with the specimen transfer.
 - 4.8.3.2. If specimen transfer is not documented at the correct time, submit a chain-of-custody correction to the quality control coordinator.
 - 4.8.3.3. Document transfer of evidence in LIMS as shown in the examples in 4.8.2.2 and 4.8.2.21, and enter a short explanation of the purpose of the transfer in the note section (e.g., Transfer, STM, Storage, Convert to S/P).
- 4.8.4. Containerizing Evidence
 - 4.8.4.1. Generate a list of specimens to be containerized.
 - 4.8.4.2. Retrieve specimens from the walk-in refrigerator and note time of retrieval.
 - 4.8.4.3. Create Container in LIMS.
 - 4.8.4.3.1. Select Transfer, then Containers
 - 4.8.4.3.2. Add a new container and name the container, as appropriate.
 - 4.8.4.3.3. In the Evidence to Add section, select Barcode and scan the evidence barcode on each specimen being containerized.
 - 4.8.4.3.4. Generate barcode for the container and affix to the container.
 - 4.8.4.4. Place specimens in the container
 - 4.8.4.4.1. Perform Container Verification and save verification in the designated location.
 - 4.8.4.4.2. Seal box with tape. Initial and date across the tape edge.
 - 4.8.4.5. Document evidence transfer in LIMS using the container barcode.
 - 4.8.4.5.1. 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 4.8.4.2.
 - 4.8.4.5.2. Select “No” when the Evidence Transfer box appears asking if you want to empty the container.
 - 4.8.4.6. Place container in the location documented above.
- 4.8.5. Sample Aliquot Transfer
 - 4.8.5.1. Sample aliquot transfers from sample preparation to completion of sample analysis and disposal of sample aliquots shall be documented in the chain of custody section on the Aliquot Chain of Custody.
 - 4.8.5.1.1. Document ISDT sequence name on the Aliquot Chain of Custody.
 - 4.8.5.1.2. Document the date of transfer, transfer from location, transfer to location, and purpose of the transfer for the batch.
 - 4.8.5.2. Verifications shall be performed prior to sample preparation, prior to instrument analysis, and after instrument analysis.
 - 4.8.5.2.1. Specimen sample preparation verification

- 4.8.5.2.1.1. Verify that the specimen containers being transferred match the worklist, including case number and evidence item ID.
 - 4.8.5.2.1.1.1. This verification may be completed electronically by an analyst using the excel template for electronic specimen verification, which compares the LIMS worklist to the blood tubes scanned out of the walk-in for analysis.
 - 4.8.5.2.1.2. This verification shall be properly documented on the Aliquot Chain of Custody.
- 4.8.5.2.2. Second-person pre-run verification
 - 4.8.5.2.2.1. 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.
 - 4.8.5.2.2.2. Verify that a batch preparation worksheet is completed.
 - 4.8.5.2.2.3. This verification shall be properly documented on the Aliquot Chain of Custody.
- 4.8.5.2.3. Second-person post-run verification
 - 4.8.5.2.3.1. 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.
 - 4.8.5.2.3.2. This verification shall be documented on the Aliquot Chain of Custody.
- 4.8.6. Sequestration of Evidence
 - 4.8.6.1. Save electronic copy of the sequestration request in the electronic case file and document correspondence in case synopsis.
 - 4.8.6.2. Sequestration should:
 - 4.8.6.2.1. Be by written request;
 - 4.8.6.2.2. Include documentation with case-identifying information (e.g., ISDT case number, subject name, agency case number); and
 - 4.8.6.2.3. Include a sequestration end date.
 - 4.8.6.3. File case folder in location designated for sequestered cases, if applicable.

- 4.8.7.1.3.3. Check the “2nd Transfer Receipt” box and print the Evidence Transfer Receipt or perform verification using Excel Specimen Verification template.
 - 4.8.7.1.3.3.1. 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.
- 4.8.7.1.4. Place or wrap each specimen in bubble wrap.
- 4.8.7.1.5. Place each bubble-wrapped specimen in its own plastic bag containing an absorbent pad.
 - 4.8.7.1.5.1. Multiple specimens of the same matrix from one case may be placed in the same plastic bag.
- 4.8.7.1.6. Heat-seal each plastic bag. The person who seals the bag shall initial and date across the seal.
- 4.8.7.1.7. 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.
- 4.8.7.1.8. Each inner shipping container shall be numbered sequentially (e.g., “Box 1 of 16”), sealed with tape, and initialed and dated across the tape. Package all inner shipping containers in an outer shipping container.
- 4.8.7.1.9. Document chain of custody.
 - 4.8.7.1.9.1. 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.
- 4.8.7.1.10. 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.
 - 4.8.7.1.10.1. The electronic file shall be verified against the original for completeness and legibility prior to discarding the hardcopy.

4.8.7.1.11. 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. Releasing specimens

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

4.8.8.1.1. Fill out evidence transfer as shown in the example below:

From	Walk-in (ISDT)	<input type="checkbox"/>
Time	08/18/2015 12:25 PM	VIA <input type="checkbox"/> Note Transfer
To	ISDT staff name <input type="checkbox"/>	
Time	08/18/2015 12:25 PM	VIA <input type="checkbox"/> Note Returned to (Name)
Then To	Courier (e.g., Fed Ex, Courier, USPS) <input type="checkbox"/>	

4.8.8.1.2. Check the “Returned” box.

4.8.8.1.2.1. Check the “2nd Transfer Receipt” box and print the Evidence Transfer Receipt or perform verification using Excel Specimen Verification template.

4.8.8.1.2.1.1. 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.

4.8.8.1.3. Place or wrap each specimen in bubble wrap.

4.8.8.1.4. 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.

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

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

4.8.8.1.6. Release or mail sealed container to authorized personnel.

4.8.8.1.6.1. 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.

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

- 4.8.8.1.7. Scan transfer documentation into the electronic case file, and note transfer in case synopsis notes.
 - 4.8.8.1.7.1. 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.

The screenshot displays two windows from a LIMS application. The top window is a form for specimen accessioning with fields for Agency, Badge Rep, Source, Inherit, Container, Evidence No (1-A-1), Other ID, Origin, Kit (Homogenate Container), Description, Bar Code (ISDT Evidence Submission Label), Evidence Type (NCIC Evid Lbl), Notes, and Extraction type. There are also checkboxes for 'Submit for Analysis' and 'Requested Unit', 'Reason', and 'Due Date'. The bottom window is titled 'Initial Transfer' and contains a table with columns for From, Time, To, and Note. The table entries are: From Postal Carrier Name, Time 10/16/2017 03:43 PM VIA, Note Rec'd from (Name of Lab); From ISDT Staff Name, Time 10/16/2017 03:43 PM VIA, Note Storage; and From Walk-In, Time (blank), Note (blank). There are checkboxes for 'Lock' and 'Lock Via', and buttons for 'Apply', 'Clear', and 'Close'.

- 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. File Shipping Manifest in the designated location.
 - 4.8.9.8.1. 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. Specimen Destruction

4.8.10.1. A specimen may be destroyed one year after completion of all case analysis unless it has been sequestered.

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.

From Walk-In

Time 10/12/2017 01:58 PM VIA [dropdown] Note Transfer

To ISDT Staff Name

Time 10/12/2017 01:58 PM VIA [dropdown] Note Disposal

Then To Trash

Changes made to the Transfer information above will apply only to newly selected evidence.

Lab Case No.	Container	Sub. #	Description	From	Time

1st Transfer Receipt 2nd Transfer Receipt

Returned Chain of Custody Reports

Apply Clear Close

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 4.8.10.4.2.

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.

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 Destruction
 - 4.8.11.1. An original TAR 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 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 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. Evidence Description Worksheet
 - 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, if applicable

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. Autodilutor
 - 5.4.5. Pipette
- 5.5. Reagents/Materials
 - 5.5.1. Bleach
 - 5.5.2. ddH₂O
 - 5.5.3. Pipette tips
 - 5.5.4. Microcentrifuge tubes
 - 5.5.5. 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.
 - 5.8.2.1. Serum/plasma specimens
 - 5.8.2.1.1. A serum/plasma specimen that contained a gel separator or beads and has not already been separated from the blood shall be centrifuged (other serum/plasma tubes shall be sonicated, starting at 5.8.2.2) prior to analysis.

- 5.8.2.1.2. Centrifuge the specimen tube for ~10 minutes at ~3000 rpm and ~8 °C.
 - 5.8.2.1.2.1. It may be necessary to repeat centrifugation to achieve separation.
- 5.8.2.1.3. 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.
- 5.8.2.1.4. 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., if specimen 1-A (parent specimen) is centrifuged, itemize the resulting serum/plasma specimen as item 1-A-1 with the Description “Supernatant”). Add a note to the LIMS case synopsis documenting the centrifuge speed used and the length of time the specimen was centrifuged.
- 5.8.2.1.5. The chain of custody of the parent specimen shall be updated, if necessary, to reflect the specimen separation. The chain of custody of the supernatant tube shall begin with the date and time it was created.
- 5.8.2.1.6. When the tubes are placed in the walk-in refrigerator, the supernatant 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.
- 5.8.2.2. Clotted and/or difficult-to-aliquot specimens
 - 5.8.2.2.1. A clotted and/or difficult-to-aliquot specimen shall be homogenized in the original tube using a sonicating probe.
 - 5.8.2.2.2. Clean the sonicating probe with bleach, followed by water, and dry it with a laboratory tissue or allow it to air-dry.
 - 5.8.2.2.3. Place probe into the middle of the specimen. Refrain from touching the walls of the container.
 - 5.8.2.2.4. 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.
 - 5.8.2.2.5. Clean the sonicating probe with bleach, followed by water, and dry it with a laboratory tissue or allow it to air-dry.
 - 5.8.2.2.6. Decant homogenized 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.
 - 5.8.2.2.7. 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.”

- 5.8.2.2.8. 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.
- 5.8.2.2.9. The chain of custody of the parent specimen shall be updated, if necessary, to reflect the specimen homogenization.
- 5.8.2.2.10. 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 #:						
<u>Date/Time of Transfer</u>	<u>From</u>		<u>PIN</u>	<u>To</u>	<u>PIN</u>	<u>Purpose</u>
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

- 5.8.2.2.11. 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.
- 5.8.2.3. Specimen dilutions
 - 5.8.2.3.1. Specimen dilution is only permissible in cases where the specimen volume is minimal and only if the test method is validated and approved for analysis of diluted samples.
 - 5.8.2.3.2. Specimens shall be placed on a rocker or inverted several times prior to removal of an aliquot from the specimen.
 - 5.8.2.3.3. Dilute the sample with negative matrix (matrix matched to the sample) and mix. If dilutions are needed for both screening and confirmatory analyses, separately prepared sample dilutions shall be used.
 - 5.8.2.3.4. If an autodilutor is not used for sample preparation, the sample shall be diluted to the final volume required by the test method.
 - 5.8.2.3.4.1. For example, to prepare a 1:2 sample dilution for a test method requiring a total volume of 1 mL, pipette 500 µL of specimen into a test tube and add 500

- 5.8.2.3.4.2. For example, to prepare a 1:4 sample dilution for a test method requiring a total volume of 1 mL, pipette 250 μ L of specimen into a test tube and add 750 μ L of an appropriate negative matrix, for a total volume of 1 mL.
- 5.8.2.3.5. If an autodilutor is used for sample preparation, the sample shall be diluted to a final volume larger than the volume required by the test method.
- 5.8.2.3.5.1. For example, to prepare a 1:2 sample dilution for a test method requiring a total volume of 200 μ L, pipette 125 μ L of specimen into a microcentrifuge tube (or equivalent) and add 125 μ L of an appropriate negative matrix, for a total volume of 250 μ L.
- 5.8.2.3.5.1.1. The diluted sample shall be placed on a rocker or inverted several times prior to removal of an aliquot from the diluted sample.
- 5.8.2.3.6. If the specimen volume is too low to dilute and/or conduct analysis, return it to the walk-in refrigerator and indicate under Purpose in the LIMS chain of custody that no aliquot was removed from the specimen and/or testing was not performed (e.g., Storage-No Aliquot/Test) and enter the results as “Quantity Not Sufficient” (ref. 13.3.4.1).
- 5.8.2.4. Proficiency test specimens
- 5.8.2.4.1. Each specimen in the proficiency survey shall be analyzed by approved ISDT method(s) and the analysis shall not be outsourced.
- 5.8.2.4.2. Specimens should be prepared per manufacturer’s recommendations prior to analysis, if applicable.
- 5.8.2.4.2.1. If the manufacturer’s recommendations are not consistent with analysis of specimens for the method being performed, seek direction from the laboratory supervisor or quality control coordinator.
- 5.8.2.4.3. Specimens shall be placed on a rocker or inverted several times prior to removal of an aliquot from the specimen.
- 5.8.2.4.4. 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.

- 5.8.2.5. Broken, cracked, or leaking specimen container
 - 5.8.2.5.1. If a specimen tube has a label indicating it was received broken, cracked, or leaking, seek supervisory approval prior to use of the specimen for analysis.
 - 5.8.2.5.2. If a tube is broken, cracked, or found to be leaking at any point during analysis, follow 4.8.2.11.6 through 4.8.2.11.6.5.

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. N/A

5.11. Report Writing

5.11.1. Testing Not Completed

- 5.11.1.1. The toxicology report shall state the reason the analysis was not completed (e.g., quantity (or quality) not sufficient to complete analysis) (ref. 13.3.4.1).

5.11.2. Dilutions

- 5.11.2.1. The LLOD and LLOQ shall be noted on the toxicology report.
 - 5.11.2.1.1. The LLOD and LLOQ shall be equal to the limit stated in the method multiplied by the dilution factor (e.g., for a 1:4 sample dilution tested using a method with a LLOQ of 10 ng/mL, the LLOQ for the diluted sample is 40 ng/mL) (ref. 13.3.4.2).

5.11.3. Proficiency Tests

- 5.11.3.1. A toxicology report shall be issued for each proficiency test received.
- 5.11.3.2. If analysis cannot be completed, each analysis (or drug category) not completed shall be listed on the toxicology report.
- 5.11.3.3. The administrative reviewer shall notify the quality control coordinator of completion of the administrative review of a proficiency test.
- 5.11.3.4. Results shall be reported to the proficiency test provider on the provider's form or electronically.
- 5.11.3.5. Results reported to the proficiency test provider may include more significant figures than reported on a toxicology report for an evidentiary sample.
- 5.11.3.6. 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.

5.11.4. Broken, cracked, or leaking specimen containers

5.11.4.1. 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. 13.3.4.8)

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 LLE.
- 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. Instrument validations
 - 6.3.5. ToxB_{OX}® 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. ToxB_{OX} 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. ddH₂O
 - 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. Formic acid
 - 6.5.10.3. Methyl tert-butyl ether
 - 6.5.10.4. 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 ddH₂O.
 - 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 ddH₂O. 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 μ L of formic acid and 2.52 g ammonium formate to LCMS grade H₂O to make 4 L.
 - 6.7.4.2. Organic (0.01% formic acid in acetonitrile; mobile phase B) – For example, add 400 μ L of formic acid to acetonitrile to make 4 L.
 - 6.7.5. Reconstitution Solution (95:5 water:acetonitrile)
 - 6.7.5.1. For example, add 50 mL of acetonitrile to 950 mL of ddH₂O 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). Each set of one to 21 samples shall be bracketed by calibrators or non-zero controls. The batch 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 μ L of negative blood to the calibrator and control well positions.
 - 6.8.4. Add 600 μ L of each specimen to its corresponding well position.
 - 6.8.5. Add 400 μ L 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 ddH₂O 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 °C.

- 6.8.12. Chill samples in circulating bath at -30 °C 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 °C.
- 6.8.18. Chill samples in circulating bath at -30 °C 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 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.
 - 6.8.23.3. If the instrument sequence is paused by the acquisition software between two samples, the sequence may be restarted at the sample not yet injected.
 - 6.8.23.3.1. Four-day sample stability for negative mode and five-day sample stability criteria shall be met.
 - 6.8.23.4. Reinjection of samples may be performed if initiated within four days (for negative mode) or five days (for positive mode) of the first injection of the sequence when samples are stored in the instrument autosampler or at equivalent temperature.
 - 6.8.23.4.1. A reinjection sequence shall contain, at a minimum, two calibrators and a negative control bracketing the sample(s) to be reinjected.
 - 6.8.23.4.2. A reinjection of a sample of unknown concentration may be performed once.
 - 6.8.23.4.3. Reinjection of a sample of known concentration may be performed multiple times.
 - 6.8.23.4.3.1. If a reinjection is needed more than once, the evidentiary samples that have

already been reinjected may be skipped in a bracket.

6.8.23.4.3.1.1. Evidentiary samples that are skipped shall be reanalyzed starting at 6.8.1.

6.8.24. LC/TOF Acquisition Parameters

6.8.24.1. Liquid chromatograph sampler

Injection Mode Injection with needle wash

Injection Volume 2.0 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 °C	Column Temp	55 °C
MS Parameters			
Gas Temp	300 °C	Gas Temp	350 °C
Drying Gas	10 L/min	Drying Gas	11 L/min
Nebulizer	50 psig	Nebulizer	15 psig
Sheath Gas Temp	350 °C	Sheath Gas Temp	350 °C
Sheath Gas Flow	12 L/min	Sheath Gas Flow	12 L/min
Vcap	5000 V	Vcap	4500 V
Nozzle Voltage	2000 V	Nozzle Voltage	2000 V
Time/Experiment Setup			
Time = 0	Fragmentor	Time = 0	Fragmentor
Expt (1, 2, 3)	(120, 160, 180)	Expt (1, 2, 3)	(120, 160, 180)
		Time = 1.8	Fragmentor
		Expt (1, 2)	(250, 190)
		Time = 3.1	Fragmentor
		Expt (1)	(185)

6.8.24.3. Mass spectrometer

Ion Source Dual AJS ESI

Scan Type Scan

Data Collection Centroid

Mode 4 GHz High Resolution

- 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. LC/TOF BDS Preparation Worksheet
 - 6.9.3.3. 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 except for the following:
 - 6.10.2.1. Pseudoephedrine and ephedrine do not have chromatographic or mass resolution. Detection of either analyte would result in the specimen being directed to confirmatory analysis for stimulants; consequently, it is not necessary to distinguish them from each other.
 - 6.10.2.2. Amobarbital and pentobarbital do not have chromatographic or mass resolution. Detection of either analyte would result in the specimen being directed to confirmatory analysis for barbiturates; consequently, it is not necessary to distinguish them from each other.
 - 6.10.3. Confirmation drug classes referred to in this method are listed on the ISDT website at <http://in.gov/isdt/2330.htm>.
 - 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 with no more than 21 samples between calibrators and/or controls.

- 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 negative 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: $((50 - |\text{mass accuracy}|) / 50) \times 33.3333$.
 - 6.10.6.1.1.1. Mass accuracy (expressed in ppm) will be calculated based on the monoisotopic mass of the analyte of interest plus a proton, except for codeine, for which mass accuracy may be calculated based on the monoisotopic mass of codeine plus a sodium ion (322.1419 m/z) or plus a proton (300.1599 m/z)..

- 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: (signal to noise/10) x 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: ((0.10 -|retention time difference|)/0.10) x 33.3333.
 - 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 a control being outside acceptability criteria. AB XX/XX/XX”).
 - 6.10.10.2. If data was not generated for a sample, a case synopsis note should be added to the case file explaining the lack of data obtained from the analysis.

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	4	10	Acetylfentanyl-D5	1
Alprazolam	10	40	100	Alprazolam-D5	10

Indiana State Department of Toxicology
Laboratory Test Methods

Drug	Cal (ng/mL)	HC (ng/mL)	UHC (ng/mL)	Internal Standard	ISTD (ng/mL)
7-Aminoclonazepam	10	40	100	7-Aminoclonazepam-D4	10
Amphetamine	10	40	100	(±)-Amphetamine-D11	10
Benzoylcegonine	20	80	200	Cocaine-D3	10
Buprenorphine	10	40	100	Buprenorphine-D4	10
Butabarbital*	200	800	2000	Butabarbital-D5	200
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
Desalkylflurazepam	10	40	100	Desalkylflurazepam-D4	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
Dihydrocodeine	10	40	100	Dihydrocodeine-D6	10
EDDP	10	40	100	EDDP-D3 (perchlorate)	10
Ephedrine	10	40	100	Ephedrine-D3	10
Fentanyl	1	4	10	Norfentanyl-D5	1
Flunitrazepam	10	40	100	Flunitrazepam-D7	10
Hydrocodone	10	40	100	Hydrocodone-D6	10
Hydromorphone	10	40	100	Hydromorphone-D6	10
α-Hydroxyalprazolam	10	40	100	α-Hydroxyalprazolam-D5	10
Lorazepam	10	40	100	Clonazepam-D4	10
MDEA	10	40	100	(±)-MDEA-D6	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
Midazolam	10	40	100	Oxazepam-D5	10
Morphine	10	40	100	Morphine-D3	10
Naloxone	1	4	10	Naloxone-D5	1
Naltrexone	1	4	10	Naltrexone-D3	1
Norbuprenorphine	10	40	100	Norbuprenorphine-D3	10
Nordiazepam	10	40	100	Nordiazepam-D5	10
Norfentanyl	1	4	10	Norfentanyl-D5	1
Oxazepam	10	40	100	Oxazepam-D5	10
Oxycodone	10	40	100	Oxycodone-D6	10
Oxymorphone	10	40	100	Oxymorphone-D3	10
Pentobarbital*	200	800	2000	Pentobarbital-D5	200
Phencyclidine	10	40	100	Phencyclidine-D5	10
Phenobarbital*	200	800	2000	Phenobarbital-D5	200

Drug	Cal (ng/mL)	HC (ng/mL)	UHC (ng/mL)	Internal Standard	ISTD (ng/mL)
Phentermine	10	40	100	Phentermine-D5	10
Phenylpropanolamine	10	40	100	Norephedrine-D3	10
Propoxyphene	10	40	100	(±)-Propoxyphene-D5	10
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
Zaleplon	10	40	100	Zaleplon-D4	10
Zolpidem	10	40	100	Zolpidem-D6	10
Zopiclone	10	40	100	Zopiclone-D4	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 calibrator and negatives 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. *J Anal Toxicol* 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. ddH₂O
 - 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. α-Hydroxyalprazolam (alpha-Hydroxyalprazolam)
 - 7.5.10.3. Alprazolam
 - 7.5.10.4. Clonazepam
 - 7.5.10.5. Desalkylflurazepam
 - 7.5.10.6. Diazepam
 - 7.5.10.7. Lorazepam
 - 7.5.10.8. Midazolam
 - 7.5.10.9. Nordiazepam
 - 7.5.10.10. Oxazepam
 - 7.5.10.11. Temazepam
 - 7.5.10.12. Zolpidem
 - 7.5.10.13. 7-Aminoclonazepam-D4
 - 7.5.10.14. Alprazolam-D5
 - 7.5.10.15. Diazepam-D5
 - 7.5.10.16. Lorazepam-D4
 - 7.5.10.17. Oxazepam-D5
 - 7.5.10.18. Temazepam-D5
 - 7.5.10.19. 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 shall be made by different analysts.
- 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 - α -Hydroxyalprazolam, Alprazolam, Clonazepam, Desalkylflurazepam, Lorazepam, Midazolam, 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 - α -Hydroxyalprazolam, Alprazolam, Clonazepam, Desalkylflurazepam, Lorazepam, Midazolam, 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, α -Hydroxyalprazolam, Alprazolam, Clonazepam, Desalkylflurazepam, Lorazepam, Midazolam, 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 - α -Hydroxyalprazolam, Alprazolam, Clonazepam, Desalkylflurazepam, Lorazepam, Midazolam, 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 ddH₂O.

- 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 ddH₂O.
- 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 ddH₂O.
- 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 ddH₂O.
- 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:organic mobile phase solution (or 70:30 ddH₂O:acetonitrile).
- 7.7.7.1.1. For example, add 30 mL of organic mobile phase (ref. 7.7.6.1) 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 controls. The batch shall contain a negative control at the beginning of the batch, following the highest calibrator, and at the end of the batch.
- 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 °C.
- 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 ddH₂O
 - 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 ddH₂O 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 in the case synopsis of each case in the batch and not corrected on the chromatograms.
 - 7.8.21.2. Prepared whole blood 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 and 7.11.3.4).
 - 7.8.21.3. If the instrument sequence is paused by the acquisition software between two samples, the sequence may be restarted at the sample not yet injected.
 - 7.8.21.3.1. Sample stability criteria shall be met.
 - 7.8.21.4. If the instrument sequence is interrupted during analysis of a sample or the sequence is aborted or stopped and unable to be restarted without creating a new sequence, the sequence may be restarted at the last passing control.
 - 7.8.21.4.1. Reinjection and sample stability criteria shall be met.

- 7.8.21.4.2. Reinjection of a sample of unknown concentration may be performed once.
- 7.8.21.4.3. Reinjection of a sample of known concentration may be performed multiple times.
- 7.8.21.4.3.1. If a reinjection is needed more than once, the evidentiary samples that have already been reinjected may be skipped in a bracket.
- 7.8.21.4.3.1.1. Evidentiary samples that are skipped shall be reanalyzed starting at 7.8.1.
- 7.8.21.4.4. A reinjection shall be performed by either restarting the sequence from the last passing non-zero control or reinjecting the entire sequence.
- 7.8.22. LC/QQQ Acquisition Parameters
- 7.8.22.1. Liquid chromatograph sampler
- Injection Mode Injection with needle wash
- Injection Volume 0.10 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 °C
- 7.8.22.4. Mass spectrometer
- Ion Source 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	
α-Hydroxyalprazolam	No	325	297.1	150	31	1.86

Indiana State Department of Toxicology
Laboratory Test Methods

Compound Name	Internal Standard	Precursor Ion	Product Ion	Fragmentor (V)	CE* (V)	RT** (min)
			216.1		47	
Alprazolam	No	309	281.1	145	29	4.02
			205.1		49	
Clonazepam	No	316	270.1	145	27	3.72
			214		51	
Desalkylflurazepam	No	289	226.1	145	31	4.47
			140		33	
Diazepam	No	285	222.1	155	29	5.27
			193.1		35	
Lorazepam	No	321	275	130	23	3.81
			229		33	
Midazolam	No	326	291.1	135	31	0.92
			249.1		43	
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, α -Hydroxyalprazolam, Alprazolam, Lorazepam, Oxazepam, and Temazepam

Curve fit – Quadratic

Alprazolam, Diazepam, Clonazepam, Desalkylflurazepam, Midazolam, 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. Benzodiazepines and Z-Drugs Confirmation Preparation Worksheet
 - 7.9.5.3. Aliquot Chain of Custody
 - 7.9.6. MassHunter Worklist Report
 - 7.9.7. QA/QC Packet, however named
 - 7.9.7.1. Batch summary
 - 7.9.7.2. Analyte calibration curves
 - 7.9.7.3. Calibrator and control chromatograms
 - 7.9.8. Sample chromatograms
 - 7.9.9. Reinjection Worksheet, if applicable
 - 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 $\pm 20\%$ 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 $\geq 20\%$ shall be within $\pm 20\%$ of the mean ion ratio based on all calibrators used to generate the curve.

- 7.10.3.3.4. An ion ratio with a relative abundance < 20% shall be within $\pm 30\%$ of the mean ion ratio based on all calibrators used to generate the curve.
- 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 $\pm 20\%$ 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. The negative 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 $\geq 50\%$ of the LLOQ shall not be used and shall be reanalyzed, if possible, starting at 7.8.1. Samples with a result < 50 % of the LLOQ for an evidentiary sample shall be accepted as none detected.
- 7.10.3.5. At least one negative shall have the corresponding internal standard present for the associated analyte.
 - 7.10.3.5.1.1. 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 control and one high 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 $\pm 20\%$ 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 $\pm 5\%$ of the mean relative retention time based on all calibrators used to generate the curve.
- 7.10.3.8. Each set of one to twelve samples shall be bracketed by one low control and one high 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 $\pm 5\%$ of the mean relative retention time based on all calibrators used to generate the curve.
 - 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.
 - 7.10.4.5. 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. Reinjection
 - 7.10.6.1. Results from a reinjected sample shall be acceptable if the sample results have an RPD (ref. 2.3.5) $\leq 10\%$ and the data for each bracketing control sample is within the acceptable range.
 - 7.10.6.1.1. Reinjected calibrators and controls shall meet criteria in 7.10.3.

- 7.10.6.1.2. If a calibrator result does not meet the criteria for reinjection, the calibrator may be excluded, and the data reprocessed with the remaining calibrators.
 - 7.10.6.1.2.1. Results from the reprocessed data for the reinjected cases shall be used for determining RPD.
 - 7.10.6.1.3. If a control result does not meet the criteria for reinjection, the data bracketed by the failed reinjected control shall not be used for quantitation. For samples in the bracket prior to and following the failed reinjected control, analysis shall be repeated for any analyte that was presumptive positive on the screen or has an analyte response greater than half the response of the LLOQ, if possible, starting at 7.8.1.
 - 7.10.6.1.4. Samples with an original concentration of greater than or equal to 5 ng/mL but less than 10 ng/mL shall be within ± 1 ng/mL.
 - 7.10.6.1.5. If a result for a non-control sample does not meet the criteria for reinjection or requirements in 7.8.21.2 and 7.8.21.3, the data from that sample shall not be used, and the analysis shall be repeated, if possible, starting at 7.8.1.
- 7.10.7. Retesting Samples
- 7.10.7.1. 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.
 - 7.10.7.1.1. 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).
 - 7.10.7.1.2. If data is not generated, that analysis does not count as an analysis or retest under this section.
- 7.10.8. Unacceptable Data
- 7.10.8.1. 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”).
 - 7.10.8.2. If data was not generated for a sample, a case synopsis note should be added to the case file explaining the lack of data obtained from the analysis.
- 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	250
α -Hydroxyalprazolam	10	500
Alprazolam	10	500
Clonazepam	10	500
Desalkylflurazepam	10	500
Diazepam	50	1000
Lorazepam	10	500
Midazolam	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. Results shall be reported as the quantitative value \pm the expanded measurement uncertainty.
- 7.11.3.2.1.1. Results shall be reported to one decimal place for quantitative values equal to or greater than 1 and less than 10.
- 7.11.3.2.1.2. Results shall be reported without a decimal place for quantitative values equal to or greater than 10.
- 7.11.3.3. A result that is above the ULOQ and has an ion ratio within \pm 30% of the mean ion ratio based on all calibrators 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. 7.8.21.2).

- 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) has been met, the quantitative result is > LLOQ, and the quantitative criteria has 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. 13.3.4.3).
 - 7.11.4.2.1. Interference(s) ; or
 - 7.11.4.2.2. Quantitative result > LLOQ with an ion ratio greater than $\pm 20\%$, but less than $\pm 30\%$, of the mean ion ratio based on all calibrators 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 THC and 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. Instrument validations
 - 8.3.7. 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. ddH₂O
- 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. THC
 - 8.5.12.2. THC-D9
 - 8.5.12.3. THC-COOH
 - 8.5.12.4. 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. Working stock solutions are stable for up to 5 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 shall be made by different analysts.
 - 8.7.2.1. Low Calibrator
 - 8.7.2.1.1. 100 ng/mL - THC
 - 8.7.2.1.2. 250 ng/mL - THC-COOH
 - 8.7.2.2. High Calibrator
 - 8.7.2.2.1. 500 ng/mL - THC
 - 8.7.2.2.2. 1,000 ng/mL - THC-COOH
 - 8.7.2.3. Low Control
 - 8.7.2.3.1. 200 ng/mL - THC
 - 8.7.2.3.2. 500 ng/mL - THC-COOH

- 8.7.2.4. High Control
 - 8.7.2.4.1. 500 ng/mL - THC
 - 8.7.2.4.2. 1,000 ng/mL - THC-COOH
 - 8.7.2.5. Internal Standard
 - 8.7.2.5.1. 400 ng/mL - THC-D9
 - 8.7.2.5.2. 1,000 ng/mL - THC-COOH-D9
 - 8.7.3. THC Wash Solution
 - 8.7.3.1. On the day of extraction, make an 85:15:1 ddH₂O, acetonitrile, and ammonium hydroxide solution.
 - 8.7.3.1.1. For example, 85 mL of ddH₂O, 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 controls. The batch shall contain a negative control at the beginning of the batch, following the highest calibrator, and at the end of the batch. An ethyl acetate wash may be included between the highest calibrator and negative.
 - 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 THC-D9 and 25 or 50 ng/mL 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: Cannabinoids Calibrator and Control Sample Preparation. 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

Level	Sample Identification [^]	Stock Solution	Volume of stock (µL)
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 °C.
- 8.8.10. Decant each sample's top (organic) layer into a correspondingly labeled glass tube.
- 8.8.11. Add 2 mL of ddH₂O 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 °C 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 for the batch and not corrected on the chromatograms.
 - 8.8.30.2. The extracted samples shall be stored at room temperature and analyzed for THC and THC-COOH within 7 days of completion of the extraction process.
 - 8.8.30.3. When a sample has a THC concentration > 45 ng/mL or THC-COOH concentration > 60 ng/mL, intelligent sequencing may be used to prevent carryover into subsequent samples.
 - 8.8.30.4. Samples for THC and THC-COOH analyses may have different sequence names and be analyzed on different GC/MS instruments.
 - 8.8.30.5. If the instrument sequence is paused by the acquisition software between two samples, the sequence may be restarted at the sample not yet injected.
 - 8.8.30.5.1. Sample stability criteria shall be met.
 - 8.8.30.6. If the instrument sequence is interrupted during analysis of a sample or the sequence is aborted or stopped and unable to be restarted without creating a new sequence, the sequence may be restarted at the last passing control.
 - 8.8.30.6.1. Reinjection and sample stability criteria shall be met.
 - 8.8.30.6.2. Reinjection of a sample of unknown concentration may be performed once.
 - 8.8.30.6.3. Reinjection of a sample of known concentration may be performed multiple times.
 - 8.8.30.6.3.1. If a reinjection is needed more than once, the evidentiary samples that have already been reinjected may be skipped in a bracket.
 - 8.8.30.6.3.1.1. Evidentiary samples that are skipped shall

be reanalyzed starting
at 8.8.1.

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

8.8.31. GC/MS Instrument Parameters: THC

8.8.31.1. Gas chromatograph oven

8.8.31.2. Temperature ramps:

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

Post temperature: 315 °C
Post time: 2.00 min
Run time: 9.50 min

8.8.31.3. Gas chromatograph inlet

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

8.8.31.4. Gas chromatograph capillary column 1

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

8.8.31.5. Gas chromatograph capillary column 2

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

8.8.31.6. 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
 8.8.31.7. Mass spectrometer parameters
 Thermal auxiliary: MSD transfer line heater
 Temperature: 300 °C
 Maximum solvent delay: 3.00 min
 EMV mode: Gain Factor 2
 MS source temperature: 230 °C
 MS quadrupole temperature: 150 °C
 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 (°C /min)	Final Temperature (°C)	Hold time (min)	Final Time (min)
1		180	1	1.0
2	20.0	300	3.5	10.5

- Post temperature: 315 °C
 Post time: 2.00 min
 Run time: 10.50 min
 8.8.32.2. Gas chromatograph inlet
 Mode: Splitless
 Initial temperature: 250 °C
 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 °C
 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 °C
 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. Mass spectrometer parameters
Thermal auxiliary: MSD transfer line heater
Temperature: 300 °C
Maximum solvent delay: 3.00 min
EMV mode: Gain Factor 2
MS source temperature: 230 °C
MS quadrupole temperature: 150 °C
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. Cannabinoids Confirmation Preparation Worksheet
8.9.4.3. 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. Reinjection Worksheet, if applicable
 - 8.9.12. Autotune
 - 8.9.13. Cannabinoids Confirmation Technical Review Checklist
 - 8.9.14. Data comparison output, however named
 - 8.9.15. Measurement Uncertainty Estimation and supporting data
 - 8.9.16. 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 $\pm 20\%$ of the target value ($\pm 30\%$ 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 $\geq 20\%$ shall be within $\pm 20\%$ 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 $\pm 30\%$ 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 $\pm 20\%$ of the target concentration ($\pm 30\%$ 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. All negatives 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 in the batch, samples with a concentration $< 50\%$ of the LLOQ shall be accepted as “None Detected” and samples with a concentration $\geq 50\%$ of the LLOQ shall be reanalyzed, if possible, starting at 8.8.1.
- 8.10.3.5. At least one negative 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 control and one high 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 $\pm 20\%$ 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 control and one high 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 8.10.3.5.

- 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 $\pm 30\%$ 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.
- 8.10.6. Reinjection
 - 8.10.6.1. Results from a reinjected sample shall be acceptable if the sample results have an RPD (ref. 2.3.5) $\leq 10\%$ and the data for each bracketing control sample is within the acceptable range.
 - 8.10.6.1.1. Reinjected calibrators and controls shall meet criteria in 8.10.3.
 - 8.10.6.1.2. If a calibrator result does not meet the criteria for reinjection, the calibrator may be excluded and the data reprocessed with the remaining calibrators.
 - 8.10.6.1.2.1. Results from the reprocessed data for the reinjected samples shall be used for determining RPD.
 - 8.10.6.1.3. If a control result does not meet the criteria for reinjection, the data bracketed by the failed reinjected control shall not be used for quantitation. For samples in the bracket prior to and following the failed reinjected control, analysis shall be repeated for any analyte that was presumptive positive on the screen or has an analyte response greater than half the response of the LLOQ, if possible, starting at 8.8.1.

- 8.10.6.1.4. Samples with an original concentration of greater than or equal to 5 ng/mL but less than 10 ng/mL shall be within ± 1 ng/mL.
 - 8.10.6.1.5. Samples with an original concentration of greater than or equal to 1 ng/mL but less than 5 ng/mL shall be within ± 0.5 ng/mL.
 - 8.10.6.1.6. If a result for a non-control sample does not meet the criteria for reinjection or requirements of 8.8.30.5 and 8.8.30.6, the data from analysis of that sample shall not be used, and the analysis shall be repeated, if possible, starting at 8.8.1.
- 8.10.7. Retesting Samples
- 8.10.7.1. 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.
 - 8.10.7.1.1. 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).
 - 8.10.7.1.2. If data is not generated, that analysis does not count as an analysis or retest under this section.
- 8.10.8. Unacceptable Data
- 8.10.8.1. 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”).
 - 8.10.8.2. If data was not generated for a sample, a case synopsis note should be added to the case file explaining the lack of data obtained from the analysis.
- 8.11. Report Writing
- 8.11.1. The LLOD for THC and THC-COOH analysis is equal to the LLOQ for each analyte. The LLOQ is 1 ng/mL for THC and 5 ng/mL for THC-COOH, and the ULOQ is 50 ng/mL for THC and 100 ng/mL for 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. Results shall be reported as the quantitative value \pm the expanded measurement uncertainty.
 - 8.11.3.2.1.1. Results shall be reported to one decimal place for quantitative values equal to or greater than 1 and less than 10.
 - 8.11.3.2.1.2. Results shall be reported without a decimal place for quantitative values equal to or greater than 10.
- 8.11.3.3. A result that is above the ULOQ and has an ion ratio within \pm 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 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) has been met, the quantitative result is > LLOQ, and the quantitative criteria has 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. 13.3.4.3):
 - 8.11.4.2.1. Interference(s); or
 - 8.11.4.2.2. Quantitative result > LLOQ with an ion ratio greater than \pm 20%, but less than \pm 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 Δ 9-Tetrahydrocannabinol (THC) and 11-nor-9-Carboxy- Δ 9-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. ddH₂O
 - 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. Benzoyllecgonine
 - 9.5.11.2. Cocaine
 - 9.5.11.3. Benzoyllecgonine-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 shall be made by different analysts.
 - 9.7.2.1. Calibrator Stock
 - 9.7.2.1.1. 100,000 ng/mL - Benzoyllecgonine, Cocaine
 - 9.7.2.2. High Calibrator
 - 9.7.2.2.1. 10,000 ng/mL - Benzoyllecgonine, Cocaine (1:10 dilution from 9.7.2.1)
 - 9.7.2.3. Low Calibrator
 - 9.7.2.3.1. 1,000 ng/mL - Benzoyllecgonine, 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 ddH₂O.
 - 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 ddH₂O.
- 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 controls. The batch shall contain a negative control at the beginning of the batch, following the highest calibrator, and at the end of the batch. Batches including samples from evidentiary specimens that require dilution under 9.8.6.1 shall include an additional control using the same dilution factor.
 - 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 **Error! Reference source not found.** 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
Cal 4	250 ng/mL Calibrator	High Calibrator	25
Cal 5	500 ng/mL Calibrator	High Calibrator	50

Level	Sample Identification	Stock Solution	Volume (µL)
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.
- 9.8.6.1. Dilutions may be necessary if the total specimen volume is < 1 mL (ref. 5.8.2.3).
- 9.8.6.1.1. A 1:2 or 1:4 dilution shall be prepared by diluting the evidentiary specimen with matrix-matched negative matrix. (e.g., for a 1:4 dilution, pipette 250 µL of evidentiary specimen and 750 µL of negative blood into the correspondingly labeled tube for a total volume of 1 mL.) The dilution control shall be prepared by starting with 1 ml of control (i.e., 940 µL of negative blood and 60 µL of one of the control working stocks). The dilution of the control shall be prepared subsequently to the preparation of the dilution of a sample from the evidentiary specimen using the same pipette settings for the dilution.
- 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 °C.
- 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 ddH₂O
- 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 ddH₂O
- 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 °C 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 for the batch and not corrected on the chromatograms.
 - 9.8.22.2. The extracted samples shall be stored at room temperature and analyzed for cocaine and benzoylecgonine within 4 days of completion of the extraction process.
 - 9.8.22.3. When a sample has a cocaine or benzoylecgonine concentration > 450 ng/mL, intelligent sequencing may be used to prevent carryover into subsequent samples.
 - 9.8.22.4. If the instrument sequence is paused by the acquisition software between two samples, the sequence may be restarted at the sample not yet injected.
 - 9.8.22.4.1. Sample stability criteria shall be met.
 - 9.8.22.5. If the instrument sequence is interrupted during analysis of a sample or the sequence is aborted or stopped and unable to be restarted without creating a new sequence, the sequence may be restarted at the last passing control.
 - 9.8.22.5.1. Reinjection and sample stability criteria shall be met.
 - 9.8.22.5.2. Reinjection of a sample of unknown concentration may be performed once.
 - 9.8.22.5.3. Reinjection of a sample of known concentration may be performed multiple times.
 - 9.8.22.5.3.1. If a reinjection is needed more than once, the evidentiary samples that have already been reinjected may be skipped in a bracket.
 - 9.8.22.5.3.1.1. Evidentiary samples that are skipped shall be reanalyzed starting at 9.8.1.
 - 9.8.22.5.4. A reinjection shall be performed by restarting the sequence from the last passing non-zero control or reinjecting the entire sequence within 24 hours of the original injection.

9.8.23. GC/MS Instrument Parameters

9.8.23.1. Gas chromatograph oven

Temperature ramps:

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

Post temperature: 315 °C
Post time: 2.00 min
Run time: 5.00 min

9.8.23.2. Gas chromatograph inlet

Mode: Splitless
Initial temperature: 250 °C
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 °C
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: 325 °C
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: 1
Post injection solvent A washes: 2
Post injection solvent B washes: 2
Plunger speed: Fast
Preinjection dwell: 0.00 min
Post injection dwell: 0.00 min

9.8.23.6. Mass spectrometer parameters

Thermal auxiliary: MSD transfer line heater
Temperature: 300 °C

Maximum solvent delay: 3.00 min
EMV mode: Gain Factor 2
MS source temperature: 230 °C
MS quadrupole temperature: 150 °C
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
Benzoyllecgonine	240	82, 361
Benzoyllecgonine-D3	243	364

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

9.8.23.7. 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. Cocaine Confirmation Preparation Worksheet
 - 9.9.4.3. 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. Reinjection Worksheet, if applicable
- 9.9.14. Data comparison output, however named
- 9.9.15. Measurement Uncertainty Estimation and supporting data
- 9.9.16. 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 $\pm 20\%$ 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 $\geq 20\%$ shall be within $\pm 20\%$ 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 $\pm 30\%$ 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 $\pm 20\%$ 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. All negatives 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 in the batch, samples with a concentration < 50% of the LLOQ shall be accepted as “None Detected” and samples with a concentration \geq 50% of the LLOQ shall be reanalyzed, if possible, starting at 9.8.1.
- 9.10.3.5. At least one negative 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 control and one high 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 \pm 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 control and one high 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 9.10.3.5.
- 9.10.3.9. If a diluted sample is included in the batch, an additional control with the same dilution factor as the diluted sample shall be included directly following the diluted sample (ref. 9.8.6.1.1).
- 9.10.3.9.1. A dilution control fails if the requirements of either 9.10.3.7.1 or 9.10.3.7.3 is not met.
- 9.10.3.9.1.1. The failed dilution control only affects acceptance of the analytical data for the diluted evidentiary sample and shall not be used as a control for bracketing in 9.10.3.8.
- 9.10.4. Analyte Identification (Qualitative Criteria)
- 9.10.4.1. Retention time shall be within \pm 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 $\pm 30\%$ 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. Reinjection
 - 9.10.6.1. Results from a reinjected sample shall be acceptable if the sample results have an RPD (ref. 2.3.5) $\leq 10\%$ and the data for each bracketing control sample is within the acceptable range.
 - 9.10.6.1.1. Reinjected calibrators and controls shall meet criteria in 9.10.3.
 - 9.10.6.1.2. If a calibrator result does not meet the criteria for reinjection, the calibrator may be excluded, and the data reprocessed with the remaining calibrators.
 - 9.10.6.1.2.1. Results from the reprocessed data for the reinjected cases shall be used for determining RPD.
 - 9.10.6.1.3. If a control result does not meet the criteria for reinjection, the data bracketed by the failed reinjected control shall not be used for quantitation. For samples in the bracket prior to and following the failed reinjected control, analysis shall be repeated for any analyte that was presumptive positive on the screen or has an analyte response greater than half the response of the LLOQ, if possible, starting at 9.8.1.
 - 9.10.6.1.4. If a result for a non-control sample does not meet the criteria for reinjection or requirements of 9.8.22.4 and 9.8.22.5, the data from that sample shall not be used, and the analysis shall be repeated, if possible, starting at 9.8.1.
 - 9.10.6.1.4.1. For sample results reported qualitatively due to sample dilution, the qualitative results of the analyte of interest shall be the same for the original injection and the reinjection.

- 9.10.7. Retesting Samples
 - 9.10.7.1. 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.
 - 9.10.7.1.1. 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).
 - 9.10.7.1.2. If data is not generated, that analysis does not count as an analysis or retest under this section.
 - 9.10.8. Unacceptable Data
 - 9.10.8.1. 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”).
 - 9.10.8.2. If data was not generated for a sample, a case synopsis note should be added to the case file explaining the lack of data obtained from the analysis.
- 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. Results shall be reported as the quantitative value \pm the expanded measurement uncertainty.
 - 9.11.3.2.1.1. Results shall be reported without a decimal place.
 - 9.11.3.3. A result that is above the ULOQ and has an ion ratio within \pm 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) has been met, the quantitative result is > LLOQ, and the quantitative criteria has 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 may be reported as “Positive” if a diluted sample was analyzed and the quantitative result was \geq 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. 13.3.4.3):

9.11.4.3.1. Interference(s); or

9.11.4.3.2. Quantitative result > LLOQ with an ion ratio greater than $\pm 20\%$, but less than $\pm 30\%$, 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. Instrument validations
 - 10.3.3. 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. ToxBio 96-well plate (Opioids)
 - 10.5.2. Isoelute 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 insertion and cover
 - 10.5.5. Autosampler vials, inserts, and caps
 - 10.5.6. Pipette tips
 - 10.5.7. ddH₂O
 - 10.5.8. Negative blood (human)
 - 10.5.9. Negative serum/plasma (human)
 - 10.5.10. Liquid chromatograph column
 - 10.5.10.1. Dimensions: 2.1 mm x 100 mm
 - 10.5.10.2. Composition: Phenyl Hexyl, 2.7 µm particles
 - 10.5.11. Liquid chromatograph guard column
 - 10.5.11.1. Dimensions: 2.1 mm x 5 mm
 - 10.5.11.2. Composition: Phenyl Hexyl, 2.7 µm particles

- 10.5.12. CRMs
 - 10.5.12.1. 6-Monoacetylmorphine
 - 10.5.12.2. Acetylfentanyl
 - 10.5.12.3. Codeine
 - 10.5.12.4. Dihydrocodeine
 - 10.5.12.5. Dextromethorphan
 - 10.5.12.6. EDDP
 - 10.5.12.7. Fentanyl
 - 10.5.12.8. Hydromorphone
 - 10.5.12.9. Hydrocodone
 - 10.5.12.10. Methadone
 - 10.5.12.11. Morphine
 - 10.5.12.12. Norfentanyl
 - 10.5.12.13. O-Desmethyltramadol
 - 10.5.12.14. Oxycodone
 - 10.5.12.15. Oxymorphone
 - 10.5.12.16. Propoxyphene
 - 10.5.12.17. Tramadol
 - 10.5.12.18. 6- Monoacetylmorphine -D3
 - 10.5.12.19. Acetylfentanyl-D5
 - 10.5.12.20. Codeine-D3
 - 10.5.12.21. Dextromethorphan-D3
 - 10.5.12.22. Dihydrocodeine-D6
 - 10.5.12.23. EDDP-D3
 - 10.5.12.24. Fentanyl-D5
 - 10.5.12.25. Hydrocodone-D6
 - 10.5.12.26. Hydromorphone-D6
 - 10.5.12.27. Methadone-D3
 - 10.5.12.28. Morphine-D6
 - 10.5.12.29. Norfentanyl-D5
 - 10.5.12.30. O-Desmethyltramadol-D6
 - 10.5.12.31. Oxycodone-D3
 - 10.5.12.32. Oxymorphone-D3
 - 10.5.12.33. Propoxyphene-D5
 - 10.5.12.34. Tramadol-13C-D3
- 10.5.13. Nitrogen
- 10.5.14. Solvents shall be high quality and low residue (e.g., HPLC grade, Omnisolv, Optima, etc.) unless otherwise noted.
 - 10.5.14.1. Acetonitrile, LCMS grade
 - 10.5.14.2. MTBE, ACS grade or higher
 - 10.5.14.3. Formic acid
- 10.5.15. Sodium carbonate
- 10.5.16. Sodium bicarbonate

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 40 g potassium bicarbonate and 20 g potassium carbonate into 2 L of ddH₂O.

10.7.1.2. Adjust pH to 9.0 ± 0.1.

10.7.1.3. For example, mix 50.4 g sodium bicarbonate and 63.6 g sodium carbonate into 2 L of ddH₂O.

10.7.1.4. 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 ddH₂O.

10.7.2.2. Organic (B) – Add 1 mL of formic acid per 1 L acetonitrile.

10.7.3. Reconstitution Solution (95:5 Water:Acetonitrile)

10.7.3.1. Make a 95:5 water and acetonitrile solution, respectively. For example, add 950 mL of ddH₂O 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 controls. The batch shall contain a negative control at the beginning of the batch, following the highest calibrator, and at the end of the batch.

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

10.8.3. Pipette 0.200 mL of negative blood into each calibrator and control well on the ToxBox plate.

10.8.4. Pipette 0.200 mL of specimen into the corresponding well on the ToxBox plate.

10.8.5. Add 0.200 mL of carbonate buffer to each well of the ToxBox plate.

10.8.6. Vortex ToxBox plate.

10.8.7. Transfer 0.400 mL 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 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 light positive pressure 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 or into labelled 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 in the case synopsis of each case in the batch and not corrected on the chromatograms.
- 10.8.17.2. Prepared whole blood samples may be analyzed up to the number of days and times listed in Table 7: Opioids Prepared Sample Stability and Reinjection Stability for the type of sample container when stored in the instrument autosampler or at equivalent temperature.
- 10.8.17.3. If the instrument sequence is paused by the acquisition software between two samples, the sequence may be restarted at the sample not yet injected.
 - 10.8.17.3.1. Sample stability criteria shall be met.

Table 7: Opioids Prepared Sample Stability and Reinjection Stability

Analyte	Well Plate		Autosampler Vial	
	RI Stability	# of RI	RI Stability	# of RI
6-Monoacetylmorphine	up to 24 hours w/ original Cals	1	8 days	2
	up to 9 days w/ reinjected Cals	3		
Acetylfentanyl	up to 24 hours w/ original Cals	1	8 days	2
	up to 5 days w/ reinjected Cals	2		
Codeine	9 days	3	8 days	2
Dextromethorphan	up to 24 hours w/ original Cals	1	8 days	2
	up to 5 days w/ reinjected Cals	2		
Dihydrocodeine	9 days	3	8 days	2
EDDP	5 days	2	8 days	2
Fentanyl	5 days	2	8 days	2
Hydrocodone	up to 5 days w/ original Cals	2	8 days	2
	up to 9 days w/ reinjected Cals	3		
Hydromorphone	9 days	3	8 days	2
Methadone	up to 5 days w/ original Cals	2	8 days	2
	up to 9 days w/ reinjected. Cals	3		
Morphine	9 days	3	8 days	2
Norfentanyl	9 days	3	8 days	2
O-Desmethyltramadol	9 days	3	8 days	2
Oxycodone	9 days	3	8 days	2
Oxymorphone	9 days	3	8 days	2
Propoxyphene	up to 24 hours w/ original Cals	1	8 days	2

Analyte	Well Plate		Autosampler Vial	
	RI Stability	# of RI	RI Stability	# of RI
Tramadol	up to 24 hours w/ original Cals	1	8 days	2
	up to 9 days w/ reinjected Cals	3		

10.8.17.4. If the instrument sequence is interrupted during analysis of a sample or the sequence is aborted or stopped and unable to be restarted without creating a new sequence, the sequence may be restarted at the last passing control.

10.8.17.4.1. Reinjection and sample stability criteria shall be met.

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

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

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

10.8.17.4.3.1.1. Evidentiary samples that are skipped shall be reanalyzed starting at 10.8.1.

10.8.17.4.4. A reinjection shall be performed by either restarting the sequence from the last passing non-zero control or reinjecting the entire sequence (ref. Table 7). Table 1

10.8.18. LC/QQQ Acquisition Parameters

10.8.18.1. Liquid chromatograph sampler

Injection Mode Injection with needle 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 °C

10.8.18.4. Mass spectrometer

Ion Source ESI

Scan Type Dynamic MRM

10.8.18.5. dMRM Parameters

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

Table 8: Opioids MS Parameters

Compound Name	Internal Standard	Precursor Ion	Product Ion	Fragmentor (V)	CE* (V)
6-Monoacetylmorphine	No	328	211	125	25
			165		46
Acetylfentanyl	No	323	188	125	23
			105		44
Codeine	No	300	215	115	25
			183		21
Dihydrocodeine	No	302	245	125	25
			199		36
Dextromethorphan	No	272	215	120	25
			171		46
EDDP	No	278	249	120	25
			234		34
Fentanyl	No	337	188	125	23
			105		44
Hydromorphone	No	286	185	125	36
			157		46
Hydrocodone	No	300	241	125	27
			199		34
Methadone	No	310	265	125	13
			105		29
Morphine	No	286	229	125	23
			211		27
Norfentanyl	No	233	150	85	19
			84		21
O-Desmethyltramadol	No	250	232	115	9
			58		19
Oxycodone	No	316	256	125	27
			241		34
Oxymorphone	No	302	284	120	19
			227		29
Propoxyphene	No	340	266	60	5
			58		21
Tramadol	No	264	246	105	9
			58		17

Compound Name	Internal Standard	Precursor Ion	Product Ion	Fragmentor (V)	CE* (V)
6- Monoacetylmorphine -D3	Yes	331	211	180	29
Acetylfentanyl-D5	Yes	328	105	125	46
Codeine-D3	Yes	303	215	125	29
Dextromethorphan-D3	Yes	275	147	125	36
Dihydrocodeine-D6	Yes	308	202	125	40
EDDP-D3	Yes	281	234	120	34
Fentanyl-D5	Yes	342	105	125	46
Hydrocodone-D6	Yes	306	202	125	36
Hydromorphone-D6	Yes	292	185	125	38
Methadone-D3	Yes	313	268	120	13
Morphine-D6	Yes	292	202	125	27
Norfentanyl-D5	Yes	238	84	120	19
O-Desmethyltramadol-D6	Yes	256	64	115	21
Oxycodone-D3	Yes	319	301	120	21
Oxymorphone-D3	Yes	305	287	120	21
Propoxyphene-D5	Yes	345	58	90	29
Tramadol-13C-D3	Yes	268	58	105	19

* Collision Energy

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

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. Batch Preparation Packet
 - 10.9.1.1. ISDT Confirmation Worklist
 - 10.9.1.2. Opioids Drug Confirmation Preparation Worksheet
 - 10.9.1.3. Aliquot Chain of Custody
 - 10.9.1.4. Opioids Drug Confirmation Plate Layout Worksheet
- 10.9.2. MassHunter Worklist Report
- 10.9.3. QA/QC Packet, however named
 - 10.9.3.1. Batch summary
 - 10.9.3.2. Analyte calibration curves
 - 10.9.3.3. Calibrator and control chromatograms
- 10.9.4. Sample chromatograms
- 10.9.5. Reinjection Worksheet, if applicable
- 10.9.6. QQQ Check Tune Report
- 10.9.7. Opioids Drug Confirmation Technical Review Checklist
- 10.9.8. Data comparison output, however named
- 10.9.9. Measurement Uncertainty Estimation and supporting data

- 10.9.10. 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 $\pm 20\%$ 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 $\geq 20\%$ shall be within $\pm 20\%$ of the mean ion ratio based on all calibrators used to generate the curve.
 - 10.10.3.3.4. An ion ratio with a relative abundance $< 20\%$ shall be within $\pm 30\%$ of the mean ion ratio based on all calibrators used to generate the curve.
 - 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 $\pm 20\%$ 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 (or response or RR) greater than half the LLOQ but less than the batch LLOQ shall be reanalyzed, if possible, starting at 10.8.1.
 - 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 results between the defined ULOQ and 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.

- 10.10.3.4. Each set of one to twelve samples shall be bracketed by a negative control. The negative 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 \geq 50% of the LLOQ shall not be used and shall be reanalyzed, if possible, starting at 10.8.1. Samples with a result < 50% of the LLOQ for an evidentiary sample shall be accepted as none detected.
- 10.10.3.5. At least one negative 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 control and one high 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 \pm 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 \pm 5% of the mean relative retention time based on all calibrators used to generate the curve.
- 10.10.3.8. Each set of one to twelve samples shall be bracketed by one low control and one high 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 \pm 5% of the mean relative retention time based on all calibrators 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 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 the number of days listed in Table 7 for sample container utilized when stored in the instrument auto sampler or at equivalent temperature.
- 10.10.6. Reinjection
 - 10.10.6.1. Results from a reinjected sample shall be acceptable if the sample results have an RPD (ref. 2.3.5) $\leq 10\%$ and the data for each bracketing control sample is within the acceptable range.
 - 10.10.6.1.1. Analytical results for reinjected calibrators and controls shall meet criteria in 10.10.3.
 - 10.10.6.1.2. If a calibrator result does not meet the criteria for reinjection, the calibrator may be excluded, and the data reprocessed with the remaining calibrators.
 - 10.10.6.1.2.1. Results from the reprocessed data for the reinjected cases shall be used for determining RPD.
 - 10.10.6.1.3. If a control result does not meet the criteria for reinjection, the data bracketed by the failed reinjected control shall not be used for quantitation. For samples in the bracket prior to and following the failed reinjected control, analysis shall be repeated for any analyte that was presumptive positive on the screen or has an analyte response greater than half the response of the LLOQ, if possible, starting at 10.8.1.
 - 10.10.6.1.4. Analytical results for samples with an original concentration of greater than or equal to 5 ng/mL but less than 10 ng/mL shall be within ± 1 ng/mL.
 - 10.10.6.1.5. If a result for a non-control sample does not meet the criteria for reinjection or requirements in 10.8.17.3 and 10.8.17.4, the data from that sample shall not be used, and the analysis shall be, if possible, starting at 10.8.1.
- 10.10.7. Retesting Samples
 - 10.10.7.1. 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.
 - 10.10.7.1.1. 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).
 - 10.10.7.1.2. If data is not generated, that analysis does not count as an analysis or retest under this section.

10.10.8. Unacceptable Data

10.10.8.1. 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”).

10.10.8.2. If data was not generated for a sample, a case synopsis note should be added to the case file explaining the lack of data obtained from the analysis.

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	500
Codeine	5	500
Dextromethorphan	5	500
Dihydrocodeine	5	500
Hydrocodone	5	500
Hydromorphone	5	500
Morphine	5	500
Oxycodone	5	500
Oxymorphone	5	500
Acetylfentanyl	0.50	50
Fentanyl	0.50	50
Norfentanyl	0.50	50
EDDP	10	1000
Methadone	10	1000
O-Desmethyltramadol	10	1000
Propoxyphene	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 reported as the quantitative value \pm the expanded measurement uncertainty.
 - 10.11.3.2.1.1. Results shall be reported to two decimal places for quantitative values less than 1.
 - 10.11.3.2.1.2. Results shall be reported to one decimal place for quantitative values equal to or greater than 1 and less than 10.
 - 10.11.3.2.1.3. Results shall be reported without a decimal place for quantitative values equal to or greater than 10.
- 10.11.3.3. A result that is above the ULOQ and has an ion ratio within \pm 30% of the mean ion ratio based on all calibrators used to generate the curve 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) has been met, the quantitative result is $>$ LLOQ, and the quantitative criteria has 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. 13.3.4.3).
 - 10.11.4.2.1. Interference(s); or
 - 10.11.4.2.2. Quantitative result $>$ LLOQ with an ion ratio greater than \pm 20%, but less than \pm 30%, of the mean ion ratio based on all calibrators used to generate the curve.

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.

Indiana State Department of Toxicology
Laboratory Test Methods

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. ddH₂O
 - 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. Ephedrine
 - 11.5.10.3. MDA
 - 11.5.10.4. MDEA
 - 11.5.10.5. MDMA
 - 11.5.10.6. Methamphetamine
 - 11.5.10.7. Phencyclidine
 - 11.5.10.8. Phentermine
 - 11.5.10.9. Phenylpropanolamine
 - 11.5.10.10. Pseudoephedrine
 - 11.5.10.11. Amphetamine-D11
 - 11.5.10.12. Ephedrine-D3
 - 11.5.10.13. MDA-D5
 - 11.5.10.14. MDEA-D5
 - 11.5.10.15. MDMA-D5
 - 11.5.10.16. Methamphetamine-D14
 - 11.5.10.17. Phencyclidine-D5
 - 11.5.10.18. Phentermine-D5
 - 11.5.10.19. Phenylpropanolamine-D3 or Norephedrine-D3
 - 11.5.10.20. Pseudoephedrine-D3
 - 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 shall be made by different analysts.
 - 11.7.2.1. Low Calibrator
 - 11.7.2.1.1. 500 ng/mL - Amphetamine, Ephedrine, MDA, MDMA, Phencyclidine, Phenylpropanolamine, Pseudoephedrine
 - 11.7.2.1.2. 1,000 ng/mL - MDEA, Methamphetamine
 - 11.7.2.2. High Calibrator
 - 11.7.2.2.1. 5,000 ng/mL - Amphetamine, Ephedrine, MDA, MDEA, MDMA, Methamphetamine, Phencyclidine, Phenylpropanolamine, Pseudoephedrine
 - 11.7.2.3. Phentermine Low Calibrator
 - 11.7.2.3.1. 2,000 ng/mL – Phentermine
 - 11.7.2.4. Phentermine High Calibrator
 - 11.7.2.4.1. 5,000 ng/mL - Phentermine
 - 11.7.2.5. Low Control
 - 11.7.2.5.1. 500 ng/mL - Amphetamine, Ephedrine, MDA, MDEA, MDMA, Methamphetamine, Phencyclidine, Phenylpropanolamine, Pseudoephedrine
 - 11.7.2.5.2. 600 ng/mL - Phentermine
 - 11.7.2.6. High Control
 - 11.7.2.6.1. 2,500 ng/mL - Amphetamine, Ephedrine, MDA, MDEA, MDMA, Methamphetamine, Phencyclidine, Phenylpropanolamine, Pseudoephedrine
 - 11.7.2.6.2. 3,000 ng/mL - Phentermine
 - 11.7.2.7. Internal Standard
 - 11.7.2.7.1. 2,000 ng/mL - Amphetamine-D11, Ephedrine-D3, MDA-D5, MDEA-D5, MDMA-D5, Methamphetamine-D14, Phencyclidine-D5, Phentermine-D5, Phenylpropanolamine-D3, Pseudoephedrine-D3
 - 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 ddH₂O.
 - 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 ddH₂O.

- 11.7.6. Hydrochloric Acid (25 mM)
11.7.6.1. For example, dilute 208 µL hydrochloric acid to 100 mL with ddH₂O.
- 11.7.7. Mobile Phases
11.7.7.1. Aqueous (A) – Add 1 mL of formic acid per 1 L ddH₂O.
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 controls. The batch shall contain a negative control at the beginning of the batch, following the highest calibrator, and at the end of the batch.
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)	Stock Solution	Volume (µL)
Cal 1	Low Cal	10	PHN Low Cal	10
Cal 2	Low Cal	20	PHN Low Cal	20
Cal 3	Low Cal	60	PHN Low Cal	30
Cal 4	High Cal	15	PHN High Cal	20
Cal 5	High Cal	25	PHN High Cal	40
Cal 6	High Cal	45	PHN High Cal	60
Cal 7	High Cal	70	PHN High Cal	80
Cal 8	High Cal	100	PHN 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 °C.
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 ddH₂O
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 ddH₂O
 - 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 ddH₂O 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 in the case synopsis of each case in the batch and not corrected on the chromatograms.
 - 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 and 11.11.3.4).
 - 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, the sequence may be restarted at the last passing control.
 - 11.8.22.4.1. Reinjection and 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 shall

be reanalyzed starting
at 11.8.1.

11.8.23. LC/QQQ Acquisition Parameters

11.8.23.1. Liquid chromatograph sampler

Injection Mode Injection with needle 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 °C

11.8.23.4. Mass spectrometer

Ion Source ESI

Scan Type Dynamic MRM

11.8.23.5. dMRM Parameters

MS1 Resolution Unit

MS2 Resolution Unit

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	
Ephedrine	No	166	148	75	11	2.48
			133		25	
MDA	No	180	163	80	7	4.48
			105		25	
MDEA	No	208	163	85	11	7.26
			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	

Compound Name	Internal Standard	Precursor Ion	Product Ion	Fragmentor (V)	CE* (V)	RT** (min)
Phentermine	No	150	91	70	25	5.79
			133		7	
Phenylpropanolamine	No	152	134	70	9	1.68
			117		19	
Pseudoephedrine	No	166	148	75	11	2.76
			133		25	
Amphetamine-D11	Yes	147	98	80	25	3.08
Ephedrine-D3	Yes	169	151	75	11	2.46
MDA-D5	Yes	185	110	70	27	4.39
MDEA-D5	Yes	213	105	90	31	7.23
MDMA-D5	Yes	199	107	95	29	5.99
Methamphetamine-D14	Yes	164	98	80	25	4.35
Phencyclidine-D5	Yes	249	96	75	45	10.11
Phentermine-D5	Yes	155	96	75	25	5.63
Phenylpropanolamine-D3	Yes	155	137	65	9	1.66
Pseudoephedrine-D3	Yes	169	151	75	11	2.71

* 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. Stimulants Confirmation Preparation Worksheet
 - 11.9.5.3. Aliquot Chain of Custody
- 11.9.6. MassHunter Worklist Report
- 11.9.7. QA/QC Packet, however named
 - 11.9.7.1. Batch summary
 - 11.9.7.2. Analyte calibration curves
 - 11.9.7.3. Calibrator and control chromatograms
- 11.9.8. Sample chromatograms
- 11.9.9. QQQ Check Tune Report
- 11.9.10. Reinjection Worksheet, if applicable
- 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 $\pm 20\%$ 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 $\geq 20\%$ shall be within $\pm 20\%$ of the mean ion ratio based on all calibrators used to generate the curve.
 - 11.10.3.3.4. An ion ratio with a relative abundance < 20% shall be within $\pm 30\%$ of the mean ion ratio based on all calibrators 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 $\pm 20\%$ 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 (or response or RR) greater than half the LLOQ but less than the batch LLOQ shall be reanalyzed, if possible, starting at 11.8.1.
 - 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 (or response or RR) above 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.

- 11.10.3.4. Each set of one to twelve samples shall be bracketed by a negative control. The negative 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 \geq 50% of the LLOQ shall not be used and shall be reanalyzed, if possible, starting at 11.8.1. Samples with a result < 50% of the LLOQ for an evidentiary sample shall be accepted as none detected.
- 11.10.3.5. At least one negative 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 control and one high 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 \pm 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 \pm 5% of the mean relative retention time based on all calibrators used to generate the curve.
- 11.10.3.8. Each set of one to twelve samples shall be bracketed by one low control and one high 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 \pm 5% of the mean relative retention time based on all calibrators 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 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.

- 11.10.4.5. 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. Reinjection
 - 11.10.6.1. The samples may be reinjected up to 6 times over 10 days when stored in the instrument autosampler or at equivalent temperature, if needed.
 - 11.10.6.2. Results from a reinjected sample shall be acceptable if the sample results have an RPD (ref. 2.3.5) $\leq 10\%$ and the data for each bracketing control sample is within the acceptable range.
 - 11.10.6.2.1. Reinjected calibrators and controls shall meet criteria in 11.10.3.
 - 11.10.6.2.2. If a calibrator result does not meet the criteria for reinjection, the calibrator may be excluded, and the data reprocessed with the remaining calibrators.
 - 11.10.6.2.2.1. Results from the reprocessed data for the reinjected cases shall be used for determining RPD.
 - 11.10.6.2.3. If a control result does not meet the criteria for reinjection, the data bracketed by the failed reinjected control shall not be used for quantitation. For samples in the bracket prior to and following the failed reinjected control, analysis shall be repeated for any analyte that was presumptive positive on the screen or has an analyte response greater than half the response of the LLOQ, if possible, starting at 11.8.1.
 - 11.10.6.2.4. Samples with an original concentration of greater than or equal to 5 ng/mL but less than 10 ng/mL shall be within ± 1 ng/mL.
 - 11.10.6.2.5. If a result for a non-control sample does not meet the criteria for reinjection or requirements in 11.8.22.3 and 11.8.22.4, the data from that sample shall not be used, and the analysis shall be repeated, if possible, starting at 11.8.1.
- 11.10.7. Retesting Samples
 - 11.10.7.1. 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.
 - 11.10.7.1.1. 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).

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

11.10.8. Unacceptable Data

- 11.10.8.1. 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”).
- 11.10.8.2. If data was not generated for a sample, a case synopsis note should be added to the case file explaining the lack of data obtained from the analysis.

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	500
Ephedrine	5	500
MDA	5	500
MDEA	10	500
MDMA	5	500
Methamphetamine	10	500
Phencyclidine	5	500
Phentermine	20	500
Phenylpropanolamine	5	500
Pseudoephedrine	5	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. Results shall be reported as the quantitative value ± the expanded measurement uncertainty.

11.11.3.2.1.1. Results shall be reported to one decimal place for quantitative values less than 10.

- 11.11.3.2.1.2. Results shall be reported without a decimal for quantitative values equal to or greater than 10.
- 11.11.3.3. A result that is above the ULOQ and has an ion ratio within $\pm 30\%$ of the mean ion ratio based on all calibrators 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 has 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. 13.3.4.3):
 - 11.11.4.2.1. Interference(s); or
 - 11.11.4.2.2. Quantitative result $>$ LLOQ with an ion ratio greater than $\pm 20\%$, but less than $\pm 30\%$, of the mean ion ratio based on all calibrators 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 Screening and Confirmation by HS/GC/FID

- 12.1. Scope
 - 12.1.1. This method shall be used for screening and confirmation analysis of specimens for the presence of volatiles (acetone, ethanol, isopropanol, and methanol).
- 12.2. Precautions/Limitations
 - 12.2.1. Minimum Sample Requirements – Screening
 - 12.2.1.1. Routine analysis requires 200 µL of blood or serum/plasma sample.
 - 12.2.1.2. Limited volume analysis requires no less than 125 µL of blood or serum/plasma sample.
 - 12.2.2. Minimum Sample Requirements – Confirmation
 - 12.2.2.1. Routine analysis requires 400 µL of blood or serum/plasma sample.
 - 12.2.2.2. Limited volume analysis requires no less than 225 µL of blood or serum/plasma sample.
 - 12.2.3. CRMs
 - 12.2.3.1. 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 Analysis Dilution Study-Uncertainty of Measurement (May 2016)
 - 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. Gas chromatograph capillary column-screening
 - 12.5.2.1. Dimensions: 30 m x 530 µm x 3.0 µm
 - 12.5.2.2. Composition: DB-ALC1
 - 12.5.3. Gas chromatograph capillary column-confirmation
 - 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. ddH₂O
- 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. A minimum of four calibrators shall be made or purchased. The calibrators should contain ethanol, methanol, isopropanol, and acetone in ddH₂O. Ethanol-only calibrators may be used if only screening or confirming for ethanol. The concentrations of the calibrators shall range from 10 mg/dL to 400 mg/dL.
 - 12.7.1.1. Volatiles calibrator solution made in-house is stable for 6 months when stored in a refrigerator.
 - 12.7.2. Internal standard solution shall be made to contain 12.8 mg/dL n-propanol in ddH₂O (e.g., for 4 L, dilute 640 µL of n-propanol with ddH₂O 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 screening and confirmation batch shall consist of concurrently prepared calibrators (minimum of 4), negative controls, non-zero controls, and samples. Sample preparations for confirmation analysis shall be performed after the batch screening results are obtained.
 - 12.8.1.1. Each set of one to twelve samples shall be bracketed by a pair of controls consisting of one non-zero control and one negative control.
 - 12.8.1.1.1. The same non-zero control should not be used for both sides of the bracket of one to twelve samples.
 - 12.8.1.1.2. **For screening:** One of the two non-zero controls for the bracket should be a mixed volatile.
 - 12.8.1.2. Each batch shall include at least two different concentrations of non-zero ethanol controls that are within the quantitative range.
 - 12.8.1.2.1. If methanol, acetone, or isopropanol is present in an evidentiary sample in a screening batch, at least one

- negative blood or serum/plasma, as applicable, using the same pipette setting used in 12.8.4.1.3 (e.g., for a 1:2 dilution, dilute 225 μL of CRM with 225 μL of negative blood or serum/plasma, as applicable, for a total volume of 450 μL).
- 12.8.4.1.3.3. A diluted non-zero control may be used as a dilution control for more than one evidentiary sample if the same pipette, pipette settings, and dilution were used for the diluted non-zero control and evidentiary samples.
- 12.8.4.1.3.3.1. Diluted evidentiary samples and their corresponding dilution control shall be placed in the same bracket.
- 12.8.4.1.3.4. A diluted non-zero control shall only be used as a control for the diluted sample created in 12.8.4.1.3.
- 12.8.4.1.3.5. The diluted control shall not be used as a bracketing control.
- 12.8.5. Dispense sample with 2000 μL of internal standard into a headspace vial labelled with corresponding specimen identification and cap.
- 12.8.5.1. Prior to transfer of vials to the instrument, caps shall be crimped to ensure they do not twist.
- 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.
- 12.8.6.1. Sequence names shall be in the following format:
YYYY_MM_DD_Screen_Initials or
YYYY_MM_DD_Confirm_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 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, a single 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. Reinjection of samples with an unknown concentration (evidentiary samples) may only be reinjected once.
 - 12.8.6.3.4. Reinjection of samples with a known concentration may be performed multiple times.
 - 12.8.6.3.4.1. If a reinjection is needed more than once, the evidentiary samples that have already been reinjected may be skipped in a bracket.
 - 12.8.6.3.4.1.1. Evidentiary samples that are skipped shall be reanalyzed starting at 12.8.1.
- 12.8.7. Volatile Analysis Method
- 12.8.7.1. Headspace parameters
 - Vial size: 20 mL
 - GC cycle time: 5.0 min
 - Loop fill time: 0.20 min
 - Loop temperature: 80 °C
 - Oven temperature: 70 °C
 - Shake: Low or level 1
 - Injection duration: 0.50 min
 - Fill volume: 2.2 mL
 - Loop equilibration time: 0.05 min
 - Transfer line temperature: 90 °C
 - Vial equilibration time: 6.00 min
 - Vial pressurization time: 0.2 min
 - 12.8.7.2. Gas chromatograph inlet
 - Inlet mode: Split
 - Split ratio: 10:1
 - Heater: 200 °C
 - Septum purge flow: 3 mL/min
 - Carrier gas: Helium
 - Gas saver: Off
 - 12.8.7.3. Gas chromatograph capillary column
 - Screening dimensions: 30m x 530µm x 3µm
 - Confirmation dimensions: 30m x 530µm x 2µm
 - Initial flow: 9.6 mL/min
 - Post run flow: 9.6 mL/min
 - Screening composition: DB-ALC1
 - Confirmation composition: DB-ALC2
 - 12.8.7.4. Gas chromatograph oven
 - Oven temperature: 40 °C
 - Run time: 3.5 min
 - 12.8.7.5. FID
 - Heater: 250 °C
 - H₂ flow: 40 mL/min
 - Air flow: 450 mL/min

Makeup flow: 10 mL/min
Carrier gas flow correction: Constant makeup and fuel flow
Flame: On
Electrometer: 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 12.8.8 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 (C)	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 °C
H₂ flow: Off
Air flow: Off
Makeup flow: 1 mL/min
Carrier gas flow correction: Constant makeup and fuel flow
Flame: Off
Electrometer: Off

12.9. Records

- 12.9.1. Pipette calibration certificate, however named, if applicable
- 12.9.2. Autodilutor calibration certificate, however named
- 12.9.3. Batch Preparation Packet, however named
 - 12.9.3.1. Tox Screen Worklist
 - 12.9.3.2. Sequence Tables
 - 12.9.3.3. Volatile Analysis Preparation Worksheet
 - 12.9.3.4. Aliquot Chain of Custody
- 12.9.4. Calibrator and control chromatograms
- 12.9.5. Sample chromatograms
- 12.9.6. Volatile Analysis Screen Batch Summary
- 12.9.7. Volatile Analysis Confirmation Batch Summary, if applicable
- 12.9.8. Reinjection Worksheet, if applicable
- 12.9.9. Measurement Uncertainty Estimation and supporting data
- 12.9.10. 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.

- 12.10.1.1. For dilutions, the “Amount” listed on the chromatogram shall be multiplied by the dilution factor to determine the “Final Amount.” The “Final Amount” is used for RPD calculations for acceptability, but the “Amount” is used to determine whether the two confirmation results or the screen result and lowest confirmation result are within 5 mg/dL.
- 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. Samples that are analyzed prior to a calibrator shall not be used to determine acceptability of batch data.
- 12.10.5. Non-zero controls with a target concentration below the LLOQ shall not be used to determine acceptability of batch data.
- 12.10.6. Calibration and Controls Criteria
 - 12.10.6.1. A linear curve (1/x weighting) shall be established by using a minimum of four aqueous mixed volatiles calibrators for screening analysis. A linear curve shall be established by a minimum of four aqueous calibrators containing the volatiles to be confirmed.
 - 12.10.6.1.1. The correlation coefficient (R^2) for the calibration curve shall be at least 0.99.
 - 12.10.6.2. Quantitation of calibrators shall be within $\pm 10\%$ of the nominal concentrations on the CoA or target concentration.
 - 12.10.6.3. Quantitation of non-zero controls shall be within $\pm 10\%$ or 5 mg/dL, whichever is greater, of:
 - 12.10.6.3.1. The nominal concentration on the CoA; or
 - 12.10.6.3.2. The target concentration, in the absence of a CoA.
 - 12.10.6.4. A negative control shall follow each non-zero control.
 - 12.10.6.5. Negative controls shall have an analyte response $< 50\%$ of the LLOQ.
 - 12.10.6.6. Each set of one to twelve samples shall be bracketed by a pair of controls consisting of one non-zero control and one negative control.
 - 12.10.6.7. Partial batch acceptance criteria
 - 12.10.6.7.1. Partial batch acceptance shall not occur unless the batch contains at least one passing matrix-matched negative control and one passing non-zero control containing the analyte of interest.
 - 12.10.6.7.1.1. **For screening:** If the analytical results of the negative controls in the bracketing control pairs are < 5 mg/dL, only samples with an analytical result ≥ 5 mg/dL for an analyte shall be repeated.
 - 12.10.6.7.2. If a calibrator point is excluded from the calibration curve and there are at least 4 calibrator points in the calibration curve, results to be reported as “None Detected” for samples with a quantitative value < 5 mg/dL and results for samples with a quantitative value between the lowest and highest calibrator concentrations used may be accepted.

- 12.10.6.7.3. Analytical results for each set of one to twelve samples bracketed by a pair of passing controls consisting of one non-zero control and one negative control may be accepted.
- 12.10.6.7.4. Analysis shall be repeated, if possible, for each set of one to twelve samples not bracketed by a pair of passing controls consisting of one non-zero control and one negative control starting at 12.8.1. If the analysis was a confirmation analysis, the screening analysis does not need to be repeated.
- 12.10.7. Analyte Identification
 - 12.10.7.1. Retention time shall be within $\pm 5\%$ of the retention time based on the calibrator used to generate the curve.
 - 12.10.7.2. Internal standard shall be present in each sample.
- 12.10.8. Analyte Stability
 - 12.10.8.1. Prepared samples are stable for 72 hours when refrigerated or stored on the instrument auto sampler or at equivalent temperature.
- 12.10.9. Screening Analysis
 - 12.10.9.1. A specimen is presumptively positive if the following analyte-specific criteria are met:
 - 12.10.9.1.1. Calibration, controls, and analyte identification criteria are met for the specific analyte; and
 - 12.10.9.1.2. Screening result is ≥ 10 mg/dL.
 - 12.10.9.2. Presumptive positive specimens shall be directed for confirmatory testing.
- 12.10.10. Confirmation Results Evaluation
 - 12.10.10.1. A quantitated result is reported for a specimen if the following analyte-specific criteria are met:
 - 12.10.10.1.1. Calibration, controls, and analyte identification criteria are met for the specific analyte;
 - 12.10.10.1.2. $RPD \leq 10.0\%$ or 5 mg/dL, whichever is greater, for both replicate sample confirmation results (ref. 2.3.5 and 12.10.1.1); and
 - 12.10.10.1.2.1. If the RPD is $> 10\%$ or 5 mg/dL, whichever is greater, confirmation testing shall be repeated.
 - 12.10.10.1.3. The screening result and lower result of the replicate confirmations have an $RPD \leq 10\%$ or 5 mg/dL, whichever is greater (ref. 2.3.5 and 12.10.1.1).
 - 12.10.10.1.3.1. The screening result and the lower result of the replicate confirmations may have an $RPD > 10\%$ when both results are > 400 mg/dL or when one result (screening or confirmation) is between 360 mg/dL and 400 mg/dL and the other result (screening or confirmation) is > 400 mg/dL.

- 12.10.10.1.3.2. If the above criteria are not met, both screening and confirmation testing shall be repeated for the specimen.
- 12.10.10.1.4. The screening and confirmation analysis should be performed on the same specimen but may be performed on a different tube of the same matrix type and the same draw time.
 - 12.10.10.1.4.1. If a blood tube with the same matrix type and draw time is not available, the screen shall be repeated.
- 12.10.10.2. If one or both replicate confirmation results are < 10 mg/dL, a quantitated result shall not be reported.
- 12.10.11. Diluted Samples
 - 12.10.11.1. The diluted non-zero controls shall reflect only the quality of the associated diluted evidentiary sample(s) and not any other sample(s) in the bracket.
 - 12.10.11.2. The diluted non-zero controls shall meet acceptability criteria in 12.10.6.
 - 12.10.11.3. For confirmation, the analytical results of the two diluted non-zero controls shall be within 10% of each other.
 - 12.10.11.4. If each diluted control result does not meet the above criteria, the diluted sample(s) shall be reanalyzed, if possible.
 - 12.10.11.4.1. If insufficient volume is left to repeat analysis, a result of “positive – unable to quantitate” may be reported for the specimen for the specific analyte able to be confirmed as present.
- 12.10.12. Reinjection
 - 12.10.12.1. Results from a reinjected sample shall be acceptable if the sample results have an RPD (ref. 2.3.5) $\leq 5\%$ or 5 mg/dL, whichever is greater, and the data for each bracketing control sample is within the acceptable range.
 - 12.10.12.1.1. Reinjected calibrators and controls shall meet criteria in 12.10.6.
 - 12.10.12.1.2. If a control result does not meet the criteria for reinjection, the data bracketed by the failed reinjected control shall not be used for quantitation. For samples in the bracket prior to and following the failed reinjected control, analysis shall be repeated for any analyte that was presumptive positive on the screen, if possible, starting at 12.8.1.
 - 12.10.12.1.2.1. **For screening:** if analytical results for negative controls in each control pair are < 5 mg/dL, only the analysis of samples ≥ 5 mg/dL for an analyte shall be repeated.
 - 12.10.12.1.3. If a result for a non-control sample does not meet the RPD criteria for reinjection or requirements in 12.8.6.3, the data from that sample shall not be used for

quantitative purposes, and the analysis shall be repeated, if possible, starting at 12.8.1.

12.10.13. Retesting Samples

12.10.13.1. When a sample requires retesting, the sample shall be retested at least once per screen or duplicate confirmation, if possible. A sample may be retested up to two times per screen or duplicate confirmation without supervisory approval.

12.10.13.1.1. If a quantitative value cannot be reported from any analysis, the first acceptable qualitative confirmation data according to analyte identification in 12.10.7 shall be used. (ref. 12.11.4).

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

12.10.14. Unacceptable Data

12.10.14.1. 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 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 reinjected. AB XX/XX/XX”).

12.10.14.2. If data was not generated for a sample, a case synopsis note should be added to the case file explaining the lack of data obtained from the analysis.

12.11. Report Writing

12.11.1. The LLOQ is 10 mg/dL unless sample dilution is necessary under 12.8.4.1 (e.g., LLOQ for a 1:2 dilution is 20 mg/dL and LLOQ for a 1:4 dilution is 40 mg/dL.).

12.11.1.1. If a dilution is necessary under 12.8.4.1, the report shall state that a dilution was made and note the LLOQ for the analysis (ref. 13.3.4.2.1).

12.11.2. All accepted screening data for each specimen shall be summarized in the Volatile Analysis Screen Batch Summary, technically reviewed, and entered into LIMS.

12.11.2.1. The results shall be reported as “None Detected” if all analytical results are < 10 mg/dL or 0.010 g/100 mL for a specimen.

12.11.3. Quantitative Confirmation Reporting

12.11.3.1. All accepted confirmatory data for each specimen shall be summarized in the Volatile Analysis Confirmation Batch Summary, technically reviewed, and entered into LIMS.

12.11.3.1.1. The preparation date of analysis shall be used as the analysis date.

12.11.3.2. If one of the replicate confirmation results is below the LLOQ, the result shall be reported as “None Detected.”

12.11.3.3. A quantitated value shall be reported for results between the certified concentration of the lowest and highest calibrators.

12.11.3.3.1. The lower of the two replicate confirmation results shall be reported in g/100 mL truncated to three decimal places (divide mg/dL value by 1000).

- 12.11.3.3.2. Quantitative values between 0.010 and 0.400 g/100 mL shall be reported \pm the expanded measurement uncertainty to three decimal places.
 - 12.11.3.3.2.1. If a dilution was performed, the lowest of the two confirmation results shall be multiplied by the dilution factor and reported as the result. The MU shall be calculated using the MU for dilutions and entered into LIMS (ref. 2.6.9).
 - 12.11.3.3.3. 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).
- 12.11.3.4. A quantitative result shall only be reported if analysis occurred within the established sample stability window (ref. 12.10.8).
- 12.11.3.5. If a specimen is analyzed more than once, the first set of duplicate quantitative results with data that meets acceptability criteria for quantitation of a specific analyte shall be reported (ref. 12.11.3.3.1).
- 12.11.4. Qualitative Confirmation Reporting
 - 12.11.4.1. A result should be reported as “Positive” when both replicates meet the analyte identification criteria (ref. 7.10.4), the quantitative result is > LLOQ, and the quantitative criteria has not been met.
 - 12.11.4.1.1. If a specimen is analyzed more than once, the totality of the duplicate 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. 12.3.4.3):
- 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. Rechtsmed.* 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 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 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, LIMS worklist (if applicable), an instrument sequence list, a Batch Preparation Worksheet, tune report (if applicable), Specimen Verification Worksheet (if applicable), and results of the analysis (e.g., batch summary sheets, chromatograms of calibrators and controls).
 - 13.2.4. Each note on a technical record shall be signed.
 - 13.2.4.1. 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.
 - 13.2.5. 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.
 - 13.2.6. The technical review shall follow whichever is appropriate of the following:
 - 13.2.6.1, 13.2.6.2, or 13.2.6.3.
 - 13.2.6.1. Drug Screening Analysis by LC/TOF
 - 13.2.6.1.1. Verify following information:
 - 13.2.6.1.1.1. Header information (analyst name, sequence name, instrument, analysis date, etc.) is consistent on all documentation;
 - 13.2.6.1.1.2. Each document of the QA/QC packet containing a sample result shall identify who performed the analysis and date of sample preparation;
 - 13.2.6.1.1.3. The Aliquot Chain of Custody and Batch Preparation Worksheet are accurately completed;

- 13.2.6.1.1.4. ToxBBox® Plate (or each control and internal standard solution) used is before its expiration date;
 - 13.2.6.1.1.5. Mass spectrometer tune is acceptable according to 1.4.1.5;
 - 13.2.6.1.1.6. Sample names are consistent on the LIMS Worklist, Aliquot Chain of Custody, and Instrument Worklist, as applicable;
 - 13.2.6.1.1.7. Each sample was analyzed with the appropriate method(s) (e.g., positive and negative mode);
 - 13.2.6.1.1.8. Each sample acquisition date/time is before the calibration date/time;
 - 13.2.6.1.1.9. Any chromatogram that was processed using manual integration is appropriately documented according to 6.10.7;
 - 13.2.6.1.1.10. 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
 - 13.2.6.1.1.11. Any note regarding a deviation from the method is signed by the laboratory supervisor or quality control coordinator.
- 13.2.6.2. Drug Confirmation Analysis by GC/MS or LC/QQQ
- 13.2.6.2.1. Verify following information:
 - 13.2.6.2.1.1. Header information (analyst name, sequence name, instrument, analysis date, etc.) is consistent on all documentation;
 - 13.2.6.2.1.2. Each document of the QA/QC packet containing a sample result shall identify who performed the analysis and date of sample preparation;
 - 13.2.6.2.1.3. The Batch Summary, Aliquot Chain of Custody, and Batch Preparation Worksheet are completed accurately;
 - 13.2.6.2.1.4. Each calibrator, control, and internal standard solution used is before its expiration date;
 - 13.2.6.2.1.5. Mass spectrometer tune is signed by the analyst and acceptable according to 1.2.1.2 or 1.3.1.5, as applicable;

- 13.2.6.2.1.6. Each chromatogram specifies the tune date as the same date/time as the Tune Report (GC/MS only);
 - 13.2.6.2.1.7. Sample names (including dilution ratio, if applicable) are consistent on the LIMS Worklist and Instrument Worklist/Sequence, however named.
 - 13.2.6.2.1.8. Sample names are listed in the correct order on the Instrument Worklist/Sequence and Batch Summary;
 - 13.2.6.2.1.9. If the sequence was reinjected (ref. 8.8.30.6 or 9.8.22.5), the position number and sample name match on the original sequence and reinjection sequence (GC/MS only).
 - 13.2.6.2.1.10. Each sample was analyzed with the appropriate method for the instrument used (e.g., Cocaine_MS1.M);
 - 13.2.6.2.1.11. Sample acquisition date/times of all calibrators are before the calibration date/time;
 - 13.2.6.2.1.12. 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);
 - 13.2.6.2.1.13. 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;
 - 13.2.6.2.1.14. 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; and
 - 13.2.6.2.1.15. Any note regarding a deviation from the method is signed by the laboratory supervisor or quality control coordinator.
- 13.2.6.3. Volatile Analysis
- 13.2.6.3.1. Verify the following information:
 - 13.2.6.3.1.1. Header information (analyst name, sequence name, instrument, analysis date, etc.) is consistent on all documentation;

- 13.2.6.3.1.2. Each document of the QA/QC packet containing a sample result shall identify who performed the analysis and date of sample preparation;
 - 13.2.6.3.1.3. The Batch Summary, Aliquot Chain of Custody, and Batch Preparation Worksheet are completed accurately;
 - 13.2.6.3.1.4. Each calibrator, control, and internal standard solution used is before its expiration date;
 - 13.2.6.3.1.5. Chromatogram injection date is the same as the sample prep date (or within method limitations);
 - 13.2.6.3.1.6. Sample names (including dilution ratio, if applicable) are consistent on the LIMS Worklist, Instrument Worklist, and Batch Summary, if applicable;
 - 13.2.6.3.1.7. Sample names are listed in the correct order on the Instrument Worklist and Batch Summary;
 - 13.2.6.3.1.8. Each sample was analyzed with the appropriate method for the instrument used (e.g., EtOH_HS1.M);
 - 13.2.6.3.1.9. Each sample injection date/time is before the calibration date/time;
 - 13.2.6.3.1.10. The result for each calibrator, control, and evidentiary sample meets the acceptability criteria for the method used, or the appropriate chromatogram for a failed sample is documented with the reason for the failure; and
 - 13.2.6.3.1.11. Any note regarding a deviation from the method is signed by the laboratory supervisor or quality control coordinator.
- 13.2.7. 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.
- 13.2.7.1. 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.
 - 13.2.7.1.1. Each addition or correction shall be made on the relevant page of the data and signed.
- 13.2.8. The technical reviewer shall sign the technical review worksheet to document the technical review.
- 13.2.9. If any modification to the contents of QA/QC files and/or individual chromatograms occurs after a technical review has been completed, a note shall

be added to the technical review worksheet documenting the modification and shall be technically reviewed by the original technical reviewer or another analyst trained in technical review for the assay.

- 13.2.9.1. Modifications to specimen type or replicate number that are identified after technical review has been completed do not require another technical review.
 - 13.2.10. After completion of a technical review, results shall be entered into LIMS if they meet acceptability criteria.
 - 13.2.11. Analyst verification of data entry
 - 13.2.11.1. 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.
 - 13.2.11.1.1. Correct any typographical errors in the LIMS data entry.
 - 13.2.11.1.2. Document the verification by signing the data comparison output and saving the file in the appropriate assay QA/QC folder for the batch.
 - 13.2.11.1.2.1. If the verification includes multiple batches, data comparison should be saved with data for the first batch analyzed and a shortcut to the data comparison saved with the data for the other batches.
- 13.3. Administrative Review
- 13.3.1. Verify the following information:
 - 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. EDW is complete and accurate, as appropriate (e.g., EDW description of primary container shall match the description entered into LIMS);
 - 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 EDW, if applicable.
 - 13.3.1.5.1. Agency tab shall include the submitting agency and appropriate prosecutor's office (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.
 - 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 "Other."

- 13.3.1.5.4. Evidence tab shall include the appropriate item(s) as described in the EDW.
- 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. Exception: If alcohol analysis is requested by Indianapolis Metropolitan Police Department, Beech Grove Police Department, Speedway Police Department, or Lawrence Police Department, alcohol analysis should not be completed unless directed by laboratory supervisor or quality control coordinator.
 - 13.3.1.5.5.2. 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. Officer name;
 - 13.3.1.6.3. Submitting agency name;
 - 13.3.1.6.4. Evidence received date;
 - 13.3.1.6.5. Evidence received courier;
 - 13.3.1.6.6. Evidence item(s) received;
 - 13.3.1.6.6.1. Lists the draw time (HH:MM) of the blood tubes, if there is more than one draw time submitted (> 15 minutes apart).
 - 13.3.1.6.6.2. Identifies the sample type if more than one type of sample is submitted for a coroner case (ref. 4.8.2.17.1.3).
 - 13.3.1.6.7. Subject name;
 - 13.3.1.6.8. Submitting agency case number (if applicable); and
 - 13.3.1.6.9. County of occurrence.
- 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 test report.
 - 13.3.1.8.1. The screening method and date of first sample preparation shall be correctly identified, if applicable.

- 13.3.1.8.2. If the screening was outsourced, the report should indicate “See NMS Report*” and identify the item(s) sent to NMS.
- 13.3.1.8.3. The evidence item(s) analyzed shall be correctly identified for the screening analysis, if applicable.
- 13.3.1.8.4. If there are no positive findings to report, the results section shall state “None Detected.”
- 13.3.1.8.5. If there are positive findings to report,
 - 13.3.1.8.5.1. The first acceptable data for each analyte shall be reported;
 - 13.3.1.8.5.2. Each confirmation result shall:
 - 13.3.1.8.5.2.1. Identify the analyte confirmed;
 - 13.3.1.8.5.2.2. Report the quantity found as specified in the assay test method, report the concentration as >ULOQ, or “Positive” for qualitative results;
 - 13.3.1.8.5.2.3. Report the MU for quantitative results unless the result is > ULOQ;
 - 13.3.1.8.5.2.4. Indicate the evidence item analyzed;
 - 13.3.1.8.5.2.5. Identify the instrument type used to perform the analysis;
 - 13.3.1.8.5.2.6. Indicate the date of sample preparation for the analysis; and
 - 13.3.1.8.5.2.7. Identify the analyst who performed the analysis.
- 13.3.1.8.6. 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 iResults.

- 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 the laboratory supervisor or quality control coordinator.
- 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 iResults.
- 13.3.4. Ensure the following notations are made when required:
 - 13.3.4.1. Quantity (or Quality) was not sufficient
 - 13.3.4.1.1. Specimen quantity (or quality) was not sufficient to complete testing.
 - 13.3.4.2. Sample Dilution
 - 13.3.4.2.1. Due to limited specimen volume, a dilution was used for sample analysis, resulting in a limit of quantitation of X (method LLOQ multiplied by the dilution factor). Please contact ISDT if there are any questions.
 - 13.3.4.3. Positive unable to quantitate
 - 13.3.4.3.1. This specimen was confirmed positive, but the result was not quantitated due to X (reason result could not be quantitated, e.g., interferant). Please contact ISDT if there are any questions.
 - 13.3.4.4. Request withdrawn
 - 13.3.4.4.1. Testing was not completed. Request for analysis was withdrawn by X (agency that withdrew the analysis request).
 - 13.3.4.5. Partial report
 - 13.3.4.5.1. 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.
 - 13.3.4.5.2. Partial toxicology report issued, and further testing canceled as requested by X (agency that requested partial report and withdrew request for further testing). Please contact ISDT to request completion of testing.
 - 13.3.4.6. Corrected report
 - 13.3.4.6.1. This is a corrected toxicology report for X (alcohol analysis or drug analysis). The (original, partial, corrected, or amended) report dated X (date of original report), (reason for the correction). See (original, partial, corrected, or amended) report.
 - 13.3.4.6.1.1. For example, “The original report dated January 1, 2018, incorrectly listed the subject last name as X. See original report.”
 - 13.3.4.6.1.2. If there are multiple previous reports, the language above may be modified to include references to all the previous reports.

- 13.3.4.7. Amended report
 - 13.3.4.7.1. This is an amended toxicology report for X (alcohol analysis or drug analysis). The (original, partial, corrected, or amended) report dated X (date of report), (reason for the amendment). See (original, partial, corrected, or amended) report.
 - 13.3.4.7.1.1. For example, “The original report dated January 1, 2018, did not include ethanol testing for item 2-A. See original report.”
 - 13.3.4.7.1.2. If there are multiple previous reports, the language above may be modified to include references to all the previous reports.
 - 13.3.4.8. Broken, cracked, or leaking specimen tube
 - 13.3.4.8.1. The specimen container for evidence item # (item number) was (leaking, cracked, broken, etc.) upon receipt.
- 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.”
- 13.4. Records
 - 13.4.1. Technical Review Worksheet, however named
 - 13.4.2. Case Synopsis notes
 - 13.4.3. Toxicology Report – Alcohol Analysis, if applicable
 - 13.4.4. Toxicology Report – Drug Analysis, if applicable
 - 13.4.5. Administrative Review Worksheet, if applicable

14. Appendix

14.1. Glossary

- 14.1.1. Actual concentration – Quantitative value obtained through testing.
- 14.1.2. Amended Report – A report that can be 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 – The target concentration of the lowest calibrator used to generate the calibration curve for the batch.
- 14.1.6. Batch ULOQ – 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) with an associated measurement uncertainty of a compound or compounds (CoA). CRMs may be used as calibrators or controls, or used 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 that can be issued in order 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. iResults – Online program for retrieval of toxicology reports.
- 14.1.17. 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.
- 14.1.18. Mass-to-charge ratio – The mass of an ion divided by its charge, often abbreviated as m/z.
- 14.1.19. Matrix – Biological fluid or water.
- 14.1.20. May – An option.
- 14.1.21. Neat sample – Unextracted solvent containing analyte(s) of interest.
- 14.1.22. Negative blood – Blood verified by screening/confirmation to be free of analyte(s) of interest.
- 14.1.23. Negative blood control – Negative blood containing internal standard.

- 14.1.24. Negative control – Control that is free of the analyte(s) of interest, which may be made from water, negative blood, or negative serum/plasma.
 - 14.1.25. Negative serum/plasma – Serum/plasma verified by screening/confirmation to be free of analyte(s) of interest.
 - 14.1.26. Negative serum/plasma control – Negative serum/plasma containing internal standard.
 - 14.1.27. Parameter – Setting on chromatography instrument or detector specific to the testing of the analyte in question.
 - 14.1.28. 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.
 - 14.1.29. Presumptive positive – Initial result indicating the presence of an analyte of interest obtained using a screening method.
 - 14.1.30. Retention time – The length of time required for an analyte to pass through a chromatographic column and be detected by the detector.
 - 14.1.31. 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 each sample based upon the difference between the expected and measured internal standard retention time of the sample.
 - 14.1.32. Sample – Specimen aliquot, calibrator, or control being prepared or ready for testing.
 - 14.1.33. 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.
 - 14.1.34. Serum/plasma specimen – Serum or plasma specimen.
 - 14.1.35. Shall – A requirement.
 - 14.1.36. Should – A recommendation.
 - 14.1.37. Sign or signed – Handwritten signature or initials and date (or secure electronic signature).
 - 14.1.38. 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.
 - 14.1.39. Specimen – Tube containing blood or serum/plasma collected from a subject.
 - 14.1.40. Supernatant – Liquid lying above a solid residue after centrifugation.
 - 14.1.41. Target concentration – Expected quantitated value.
 - 14.1.42. Working stock – Concentrated solution used to prepare calibrators and controls.
- 14.2. Abbreviations used in this document and/or associated records
- 14.2.1. 6-MAM – 6-Monoacetylmorphine
 - 14.2.2. % ACC – Percent Accuracy
 - 14.2.3. ACS – American Chemical Society
 - 14.2.4. AJS – Agilent Jet Stream
 - 14.2.5. AMP – Amphetamine
 - 14.2.6. BDS – Blood drug screen
 - 14.2.7. BE – Benzoylcegonine
 - 14.2.8. BNZ-Z or BNZ – Benzodiazepines, their metabolites, and zolpidem
 - 14.2.9. BSTFA – N,O-Bis(trimethylsilyl)trifluoroacetamide
 - 14.2.10. Cal – Calibrator
 - 14.2.11. CE – Collision energy
 - 14.2.12. CoA – Certificate of analysis

- 14.2.13. COC – Cocaine
- 14.2.14. CoC – Chain of custody
- 14.2.15. COM – Communication
- 14.2.16. CRM – Certified reference material
- 14.2.17. Ctrl – Control
- 14.2.18. % CV – Coefficient of variance
- 14.2.19. D – Deuterated
- 14.2.20. ddH₂O – Double distilled water
- 14.2.21. DIS – Discovery
- 14.2.22. dL – Deciliter
- 14.2.23. dMRM – Dynamic multiple reaction monitoring
- 14.2.24. EA – Ethyl Acetate
- 14.2.25. EDDP – 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
- 14.2.26. EDW – Evidence Description Worksheet
- 14.2.27. ESI – Electrospray ionization
- 14.2.28. FID – Flame ionization detector
- 14.2.29. g – Grams
- 14.2.30. GC – Gas chromatography
- 14.2.31. HPLC – High-performance liquid chromatography
- 14.2.32. HS – Headspace
- 14.2.33. HQC – High Quality Control
- 14.2.34. ISDT – Indiana State Department of Toxicology
- 14.2.35. ISTD – Internal standard
- 14.2.36. KCl – Potassium chloride
- 14.2.37. L – Liter
- 14.2.38. LC – Liquid chromatography
- 14.2.39. LIMS – Laboratory information management system (JusticeTrax)
- 14.2.40. LLE – Liquid-liquid extraction
- 14.2.41. LLOQ – Lower limit of quantitation
- 14.2.42. LLOD – Lower limit of detection
- 14.2.43. LQC – Low Quality Control
- 14.2.44. MAMP or MTH – Methamphetamine
- 14.2.45. MAN – Manifest
- 14.2.46. MDA – 3,4-Methylenedioxyamphetamine
- 14.2.47. MDEA – 3,4-Methylenedioxy-N-ethylamphetamine
- 14.2.48. MDMA – 3,4-Methylenedioxymethamphetamine
- 14.2.49. MDN or MTD – Methadone
- 14.2.50. mg – Milligrams
- 14.2.51. mL – Milliliter
- 14.2.52. MS – Mass spectrometry
- 14.2.53. MSD – Mass spectrometry detector
- 14.2.54. MTBE – Methyl tert-butyl ether (2-methoxy-2-methylpropane)
- 14.2.55. MTBSTFA – N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide
- 14.2.56. MU – Measurement uncertainty
- 14.2.57. m/z – mass to charge ratio
- 14.2.58. NaOH – Sodium hydroxide
- 14.2.59. ng – Nanogram
- 14.2.60. NTA – Non-target analyte
- 14.2.61. PCP – Phencyclidine

- 14.2.62. PFTBA – Perfluorotributylamine
- 14.2.63. PHN – Phentermine
- 14.2.64. PM – Preventive maintenance
- 14.2.65. PPA – Phenylpropanolamine
- 14.2.66. ppm – Parts per million
- 14.2.67. psi – Pounds per square inch
- 14.2.68. psig – Pounds per square inch, gauge
- 14.2.69. QA – Quality Assurance
- 14.2.70. QC – Quality control
- 14.2.71. QQQ – Triple quadrupole mass spectrometer
- 14.2.72. RI – Reinjection
- 14.2.73. RT – Retention time
- 14.2.74. RRT – Relative retention time
- 14.2.75. RPD – Relative percent difference
- 14.2.76. RR – Response ratio
- 14.2.77. RSD – Relative standard deviation
- 14.2.78. SDS – Safety data sheet
- 14.2.79. S/E – Ion suppression or enhancement
- 14.2.80. SIM – Selected ion mode
- 14.2.81. S/P – Serum/plasma
- 14.2.82. SPE – Solid phase extraction
- 14.2.83. STM – Stimulants
- 14.2.84. TAR – Toxicology Analysis Request form
- 14.2.85. TBDMCS – tert-Butyldimethylchlorosilane
- 14.2.86. THC – Delta-9-tetrahydrocannabinol
- 14.2.87. THC-COOH – Tetrahydrocannabinol-9-carboxylic acid
- 14.2.88. THC-COOH-D3 – Deuterated tetrahydrocannabinol-9-carboxylic acid
- 14.2.89. THC-D3 – Deuterated delta-9-tetrahydrocannabinol
- 14.2.90. TMCS – Trimethylsilyl chloride
- 14.2.91. TOF – Time of flight mass spectrometer
- 14.2.92. UHC – Ultra high control
- 14.2.93. ULOQ – Upper limit of quantitation

15. Document History

Effective Date	Version	Description of Activity or Revision	Approved By
02/01/18	1	<p>Initial issue: Combined laboratory methods into one document.</p> <p>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.</p> <p>New methods/sections: Method Validation, Solution Verification/Validation, Technical and Administrative Review, and Appendix</p>	<p>Ed Littlejohn Sheila A. Arnold, PhD</p>
04/16/18	2	<p>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.</p> <p>Stimulants Confirmation by LC/QQQ was updated to include prepared sample stability and reinjection stability.</p> <p>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.</p> <p>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.</p> <p>Minor edits were made throughout the document.</p>	<p>Ed Littlejohn Sheila A. Arnold, PhD</p>
01/28/19	3	<p>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)</p> <p>Incorporated the following MFRs: 2018_MFR_0525 LC3 Validation for Stimulants and Benzodiazepines</p>	<p>Ed Littlejohn Sheila A. Arnold, PhD</p>

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		<p>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</p> <p>Added additional definitions and abbreviations</p> <p>Additional minor edits were made throughout the document.</p>	
04/29/19	4	<p>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.</p> <p>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.</p> <p>Changed “value” to “concentration” when the value meant a numerical concentration.</p>	<p style="text-align: center;">Ed Littlejohn Sheila A. Arnold, PhD</p>
09/25/19	5	<p>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.</p>	<p style="text-align: center;">Ed Littlejohn Sheila A. Arnold, PhD</p>
08/4/20	6	<p>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 occurred to</p>	<p style="text-align: center;">Ed Littlejohn Sheila A. Arnold, PhD</p>

Indiana State Department of Toxicology
Laboratory Test Methods

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		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.	