SFL5

Method: CEPHENISOMER1

Separation of Controlled and Non-controlled Substances by Capillary Electrophoresis

Scope: Limited purpose - separation of select controlled and non-controlled substances as well as optical isomer determination of select substances (see individual instrument validation reports)

Sample Preparation:

Samples in aqueous solution. Insoluble material filtered or removed from sample. Nicotinamide and/or noscapine may be used as optional internal standards. Nicotinamide was used as the fixed compound for validation purposes.

Method Parameters:

Instrument: Capillary Electrophoresis Agilent 7100 or equivalent
Capillary Type and Dimensions: 48.5 cm × 50 mm ID (40 cm effective length)
Capillary Temperature: 15ºC
Injection Parameters: 150 mbar·s (50 mbar for 3 seconds or equivalent). Optional buffer, water, or IS injections permissible
Buffer: Microsolv custom buffer (PN 05375-M2-X) or equivalent (2-Hydroxypropyl-β-cyclodextrin in Microsolv Celixer pH Accelerator B (PN 06125-CE))
Voltage: 30 kV after 0.2 minutes
Detection Wavelength: 195 nm (DAD1 method)
Minimum Run Time: 6 minutes

Limitations: See individual instrument validation reports.

Additional reproducibility testing conducted using differing batches of run buffer resulted in migration times outside of the acceptance range. As such, all samples and positive controls must be analyzed using the same batch of run buffer.

Acceptance Criteria (unless otherwise noted in individual validation report):

Selectivity: Target compounds are visually separated and observed as a single peak with a clear, non-splitting apex. Peak fronting/tailing, if observed, does not preclude the detection of closely eluting peaks. A minimum S/N =3 is observed.

Repeatability: Individual migration times measured are within 0.3 minutes of the average of all injections, and the individual relative retention times are with 1% of the average of all injections.

Reproducibility: Individual absolute and migration times measured during 6 weeks. Absolute migration times are within 0.3 minutes of the values measured on week 1, and the individual relative retention times are with 1% of the values measured on week 1.

SFL5

Method: GCGEN1H2

Separation of Controlled and Non-controlled Substances by Gas Chromatography

Scope: General purpose

Sample Preparation:

Samples dissolved or extracted into a volatile organic solvent. Insoluble material filtered or removed from sample. Injection of aqueous solutions is not permitted. Tetracosane internal standard used as fixed compound for validation purposes.

Method Parameters:

Instrument: Gas Chromatograph Agilent 7890 or equivalent equipped with an LTM module
Column Type and Dimensions: 15 m DB-CSI (or DB-5MS) × 0.25 mm × 0.25 μm film thickness LTM column
Inlet Temperature: 280 ºC
Minimum Injection Volume: 1.0 μL
Injection Mode: Split
Maximum Split Ratio: 50:1
Carrier Gas and Flow: Hydrogen at 3.7 mL/min
Make-up Gas: Nitrogen (with FID detector)
Control Mode: Constant flow
Oven Program Set Points: 280 ºC
LTM Program Set Points: 60°C hold for 0.5 min, ramp 120°C/min to 230°C, hold for 0.3 min, ramp 120°C/min to 320°C, hold for 1 min.
Minimum Run Time: 3.9667 minutes
Detector Temperature: 300ºC (FID1 method); 280ºC (FID2 method)

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Selectivity: Target compounds are visually separated and observed as a single peak with a clear, non-splitting apex. Peak fronting/tailing, if observed, does not preclude the detection of closely eluting peaks. A minimum S/N =3 is observed, including a low level (0.5%) marker compound. Earliest eluting compound met minimum retention factor criteria and/or fulfilled the repeatability and reproducibility acceptance criteria.

Repeatability: Individual retention times measured for representative compounds are within 0.1 minutes of the average of 30 injections, and/or the individual relative retention times are within 1% of the average of 30 injections.

Reproducibility: Individual retention times measured for representative compounds during 6 weeks are within 0.1 minutes of the values measured on week 1, and/or the individual relative retention times during 6 weeks are within 1% of the values measured on week 1.

SFL5

Method: GCGEN5H2

Separation of Controlled and Non-controlled Substances by Gas Chromatography

Scope: General purpose

Sample Preparation:

Samples dissolved or extracted into a volatile organic solvent. Insoluble material filtered or removed from sample. Injection of aqueous solutions is not permitted. Tetracosane internal standard used as fixed compound for validation purposes.

Method Parameters:

Instrument: Gas Chromatograph Agilent 7890 or equivalent equipped with an LTM module
Column Type and Dimensions: 10 m DB-CSI × 0.15 mm × 0.3 μm film thickness LTM column
Inlet Temperature: 280 ºC
Minimum Injection Volume: 0.5 μL
Injection Mode: Split
Maximum Split Ratio: 50:1
Carrier Gas and Flow: Hydrogen at 1.5 mL/min
Make-up Gas: Nitrogen (with FID detector)
Control Mode: Constant flow
Oven Program Set Points: 280°C
LTM Program Set Points: 100°C hold for 0.5 min, ramp 38°C/min to 320°C, hold for 4 min.
Minimum Run Time: 10.289 minutes
Detector Temperature: 300ºC (FID1 method); 280ºC (FID2 method).

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Selectivity: Target compounds are visually separated and observed as a single peak with a clear, non-splitting apex. Peak fronting/tailing, if observed, does not preclude the detection of closely eluting peaks. A minimum S/N =3 is observed, including a low level (0.5%) marker compound. Earliest eluting compound met minimum retention factor criteria and/or fulfilled the repeatability and reproducibility acceptance criteria.

Repeatability: Individual retention times measured for representative compounds are within 0.1 minutes of the average of 30 injections, and/or the individual relative retention times are within 1% of the average of 30 injections.

Reproducibility: Individual retention times measured for representative compounds during 6 weeks are within 0.1 minutes of the values measured on week 1, and/or the individual relative retention times during 6 weeks are within 1% of the values measured on week 1.

SFL5

Method: GCGEN6

Separation of Controlled and Non-controlled Substances by Gas Chromatography

Scope: General purpose

Sample Preparation:

Samples dissolved or extracted into a volatile organic solvent. Insoluble material filtered or removed from sample. Injection of aqueous solutions is not permitted. Tetracosane internal standard used as fixed compound for validation purposes.

Method Parameters:

Instrument: Gas Chromatograph Agilent 7890 or equivalent
Column Type and Dimensions: 15 m DB-1 × 0.32 mm × 0.25 μm film thickness
Inlet Temperature: 280 ºC
Minimum Injection Volume: 1.0 μL
Injection Mode: Split
Maximum Split Ratio: 50:1
Carrier Gas and Flow: Helium at 1.8 mL/min
Make-up Gas: Nitrogen (with FID detector)
Control Mode: Constant flow
Oven Program Set Points: 80°C hold for 1 min, ramp 30°C/min to 320°C, hold for 2 min.
Minimum Run Time: 11 minutes
Detector Temperature: 300ºC (FID1 method); 280ºC (FID2 method)

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Selectivity: Target compounds are visually separated and observed as a single peak with a clear, non-splitting apex. Peak fronting/tailing, if observed, does not preclude the detection of closely eluting peaks. A minimum S/N =3 is observed, including a low level (0.5%) marker compound. Earliest eluting compound met minimum retention factor criteria and/or fulfilled the repeatability and reproducibility acceptance criteria.

Repeatability: Individual retention times measured for representative compounds are within 0.1 minutes of the average of 30 injections, and/or the individual relative retention times are within 1% of the average of 30 injections.

Reproducibility: Individual retention times measured for representative compounds during 6 weeks are within 0.1 minutes of the values measured on week 1, and/or the individual relative retention times during 6 weeks are within 1% of the values measured on week 1.

SFL5

Method: GCGEN7

Separation of Controlled and Non-controlled Substances by Gas Chromatography

Scope: General purpose

Sample Preparation:

Samples dissolved or extracted into a volatile organic solvent. Insoluble material filtered or removed from sample. Injection of aqueous solutions is not permitted. Tetracosane internal standard used as fixed compound for validation purposes.

Method Parameters:

Instrument: Gas Chromatograph Agilent 7890 or equivalent
Column Type and Dimensions: 15 m DB-1 × 0.32 mm × 0.25 μm film thickness
Inlet Temperature: 280 ºC
Minimum Injection Volume: 1.0 μL
Injection Mode: Split
Maximum Split Ratio: 50:1
Carrier Gas and Flow: Helium at 1.8 mL/min
Make-up Gas: Nitrogen (with FID detector)
Control Mode: Constant flow
Oven Program Set Points: 80°C hold for 1 min, ramp 15°C/min to 320°C, hold for 2 min.
Minimum Run Time: 19 minutes
Detector Temperature: 300ºC (FID1 method); 280ºC (FID2 method)

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Selectivity: Target compounds are visually separated and observed as a single peak with a clear, non-splitting apex. Peak fronting/tailing, if observed, does not preclude the detection of closely eluting peaks. A minimum S/N =3 is observed, including a low level (0.5%) marker compound. Earliest eluting compound met minimum retention factor criteria and/or fulfilled the repeatability and reproducibility acceptance criteria.

Repeatability: Individual retention times measured for representative compounds are within 0.1 minutes of the average of 30 injections, and/or the individual relative retention times are within 1% of the average of 30 injections.

Reproducibility: Individual retention times measured for representative compounds during 6 weeks are within 0.1 minutes of the values measured on week 1, and/or the individual relative retention times during 6 weeks are within 1% of the values measured on week 1.

SFL5

Method: GCGEN8

Separation of Controlled and Non-controlled Substances by Gas Chromatography

Scope: General purpose

Sample Preparation:

Samples dissolved or extracted into a volatile organic solvent. Insoluble material filtered or removed from sample. Injection of aqueous solutions is not permitted. Tetracosane internal standard used as fixed compound for validation purposes.

Method Parameters:

Instrument: Gas Chromatograph Agilent 7890 or equivalent
Column Type and Dimensions: 12 m HP-5 × 0.2 mm × 0.33 μm film thickness
Inlet Temperature: 280 ºC
Minimum Injection Volume: 1.0 μL
Injection Mode: Split
Maximum Split Ratio: 50:1
Carrier Gas and Flow: Hydrogen at 1.0 mL/min
Make-up Gas: Nitrogen (with FID detector)
Control Mode: Constant flow
Oven Program Set Points: 80°C hold for 1 min, ramp 30°C/min to 320°C, hold for 2 min.
Minimum Run Time: 11 minutes
Detector Temperature: 300ºC (FID1 method); 280ºC (FID2 method)

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Selectivity: Target compounds are visually separated and observed as a single peak with a clear, non-splitting apex. Peak fronting/tailing, if observed, does not preclude the detection of closely eluting peaks. A minimum S/N =3 is observed, including a low level (0.5%) marker compound. Earliest eluting compound met minimum retention factor criteria and/or fulfilled the repeatability and reproducibility acceptance criteria.

Repeatability: Individual retention times measured for representative compounds are within 0.1 minutes of the average of 30 injections, and/or the individual relative retention times are within 1% of the average of 30 injections.

Reproducibility: Individual retention times measured for representative compounds during 6 weeks are within 0.1 minutes of the values measured on week 1, and/or the individual relative retention times during 6 weeks are within 1% of the values measured on week 1.

SFL5

Method: GCGEN9

Separation of Controlled and Non-controlled Substances by Gas Chromatography

Scope: General purpose

Sample Preparation:

Samples dissolved or extracted into a volatile organic solvent. Insoluble material filtered or removed from sample. Injection of aqueous solutions is not permitted. Tetracosane internal standard used as fixed compound for validation purposes.

Method Parameters:

Instrument: Gas Chromatograph Agilent 7890 or equivalent
Column Type and Dimensions: 30 m DB-1 × 0.25 mm × 0.25 μm film thickness
Inlet Temperature: 280 ºC
Minimum Injection Volume: 1.0 μL
Injection Mode: Split
Maximum Split Ratio: 50:1
Carrier Gas and Flow: Helium at 1.5 mL/min
Make-up Gas: Nitrogen (with FID detector)
Control Mode: Constant flow
Oven Program Set Points: 60°C hold for 2 min, ramp 20°C/min to 320°C, hold for 5 min.
Minimum Run Time: 20 minutes
Detector Temperature: 300ºC (FID1 method); 280ºC (FID2 method)

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Selectivity: Target compounds are visually separated and observed as a single peak with a clear, non-splitting apex. Peak fronting/tailing, if observed, does not preclude the detection of closely eluting peaks. A minimum S/N =3 is observed, including a low level (0.5%) marker compound. Earliest eluting compound met minimum retention factor criteria and/or fulfilled the repeatability and reproducibility acceptance criteria.

Repeatability: Individual retention times measured for representative compounds are within 0.1 minutes of the average of 30 injections, and/or the individual relative retention times are within 1% of the average of 30 injections.

Reproducibility: Individual retention times measured for representative compounds during 6 weeks are within 0.1 minutes of the values measured on week 1, and/or the individual relative retention times during 6 weeks are within 1% of the values measured on week 1.

SFL5

Method: GCGEN10

Separation of Controlled and Non-controlled Substances by Gas Chromatography

Scope: General purpose

Sample Preparation:

Samples dissolved or extracted into a volatile organic solvent. Insoluble material filtered or removed from sample. Injection of aqueous solutions is not permitted. Tetracosane internal standard used as fixed compound for validation purposes.

Method Parameters:

Instrument: Gas Chromatograph Agilent 7890 or equivalent
Column Type and Dimensions: 30 m DB-1 × 0.25 mm × 0.5 μm film thickness
Inlet Temperature: 280 ºC
Minimum Injection Volume: 1.0 μL
Injection Mode: Split
Maximum Split Ratio: 50:1
Carrier Gas and Flow: Hydrogen at 1.0 mL/min
Make-up Gas: Nitrogen (with FID detector)
Control Mode: Constant flow
Oven Program Set Points: 80°C hold for 2 min, ramp 20°C/min to 320°C, hold for 6 min.
Minimum Run Time: 20 minutes
Detector Temperature: 300ºC (FID1 method); 280ºC (FID2 method)

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Selectivity: Target compounds are visually separated and observed as a single peak with a clear, non-splitting apex. Peak fronting/tailing, if observed, does not preclude the detection of closely eluting peaks. A minimum S/N =3 is observed, including a low level (0.5%) marker compound. Earliest eluting compound met minimum retention factor criteria and/or fulfilled the repeatability and reproducibility acceptance criteria.

Repeatability: Individual retention times measured for representative compounds are within 0.1 minutes of the average of 30 injections, and/or the individual relative retention times are within 1% of the average of 30 injections.

Reproducibility: Individual retention times measured for representative compounds during 6 weeks are within 0.1 minutes of the values measured on week 1, and/or the individual relative retention times during 6 weeks are within 1% of the values measured on week 1.

SFL5

Method: GCGEN12

Separation of Controlled and Non-controlled Substances by Gas Chromatography

Scope: General purpose

Sample Preparation:

Samples dissolved or extracted into a volatile organic solvent. Insoluble material filtered or removed from sample. Injection of aqueous solutions is not permitted. Tetracosane internal standard used as fixed compound for validation purposes.

Method Parameters:

Instrument: Gas Chromatograph Agilent 7890 or equivalent
Column Type and Dimensions: 20 m DB-17 × 0.18 mm × 0.18 μm film thickness
Inlet Temperature: 280 ºC
Minimum Injection Volume: 1.0 μL
Injection Mode: Split
Maximum Split Ratio: 50:1
Carrier Gas and Flow: Helium at 2.5 mL/min
Make-up Gas: Nitrogen (with FID detector)
Control Mode: Constant flow
Oven Program Set Points: 100°C hold for 1.0 min, ramp 15°C/min to 320°C, hold for 5 min.
Minimum Run Time: 21 minutes
Detector Temperature: 300ºC (FID1 method); 280ºC (FID2 method)

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Selectivity: Target compounds are visually separated and observed as a single peak with a clear, non-splitting apex. Peak fronting/tailing, if observed, does not preclude the detection of closely eluting peaks. A minimum S/N =3 is observed, including a low level (0.5%) marker compound. Earliest eluting compound met minimum retention factor criteria and/or fulfilled the repeatability and reproducibility acceptance criteria.

Repeatability: Individual retention times measured for representative compounds are within 0.1 minutes of the average of 30 injections, and/or the individual relative retention times are within 1% of the average of 30 injections.

Reproducibility: Individual retention times measured for representative compounds during 6 weeks are within 0.1 minutes of the values measured on week 1, and/or the individual relative retention times during 6 weeks are within 1% of the values measured on week 1.

SFL5

Method: GCMTPA1

Separation of Controlled and Non-controlled Substances by Gas Chromatography

Scope: Limited purpose (d- and l-methamphetamine derivatized with MTPA)

Sample Preparation:

Samples extracted with 1 – 5 N NaOH into chloroform. Chloroform layer placed into an autosampler vial and MTPA reagent solution added; allow to stand for approximately 5 minutes. Injection of aqueous solutions is not permitted.

Method Parameters:

Instrument: Gas Chromatograph Agilent 7890 or equivalent
Column Type and Dimensions: 15 m DB-1 × 0.32 mm × 0.25 μm film thickness
Inlet Temperature: 280ºC
Minimum Injection Volume: 1.0 μL
Injection Mode: Split
Maximum Split Ratio: 20:1 (Variable split ratios for sample and/or positive control(s) are acceptable in order to obtain comparable peak areas for comparison.)
Carrier Gas and Flow: Helium at 1.0 mL/min
Make-up Gas: Nitrogen (with FID detector)
Control Mode: Constant flow
Oven Program Set Points: 200°C hold for 8 min, ramp 40°C/min to 280°C
Minimum Run Time: 10 minutes
Detector Temperature: 300ºC (FID1 method); 280ºC (FID2 method)

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Selectivity: Target compounds are visually separated and observed as a single peak with a clear, non-splitting apex. Peak fronting/tailing, if observed, does not preclude the detection of closely eluting peaks. A minimum S/N =3 is observed. Earliest eluting compound met minimum retention factor criteria.

Repeatability: Individual retention times measured for representative compounds are within 0.1 minutes of the average of 30 injections, and/or the individual relative retention times are within 1% of the average of 30 injections.

Reproducibility: Individual retention times measured for representative compounds during 6 weeks are within 0.1 minutes of the values measured on week 1, and/or the individual relative retention times during 6 weeks are within 1% of the values measured on week 1.

SFL5

Method: GCMTPA2

Separation of Controlled and Non-controlled Substances by Gas Chromatography

Scope: Limited purpose (d- and l-methamphetamine derivatized with MTPA)

Sample Preparation:

Samples extracted with 1 – 5 N NaOH into chloroform. Chloroform layer placed into an autosampler vial and MTPA reagent solution added; allow to stand for approximately 5 minutes. Injection of aqueous solutions is not permitted.

Method Parameters:

Instrument: Gas Chromatograph Agilent 7890 or equivalent
Column Type and Dimensions: 30 m DB-1 × 0.25 mm × 0.25 μm film thickness
Inlet Temperature: 280ºC
Minimum Injection Volume: 1.0 μL
Injection Mode: Split
Maximum Split Ratio: 20:1 (Variable split ratios for sample and/or positive control(s) are acceptable in order to obtain comparable peak areas for comparison.)
Carrier Gas and Flow: Helium at 1.5 mL/min
Make-up Gas: Nitrogen (with FID detector)
Control Mode: Constant flow
Oven Program Set Points: 200°C hold for 8 min, ramp 30°C/min to 320°C, hold for 5 min.
Minimum Run Time: 17 minutes
Detector Temperature: 300ºC (FID1 method); 280ºC (FID2 method)

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Selectivity: Target compounds are visually separated and observed as a single peak with a clear, non-splitting apex. Peak fronting/tailing, if observed, does not preclude the detection of closely eluting peaks. A minimum S/N =3 is observed. Earliest eluting compound met minimum retention factor criteria.

Repeatability: Individual retention times measured for representative compounds are within 0.1 minutes of the average of 30 injections, and/or the individual relative retention times are within 1% of the average of 30 injections.

Reproducibility: Individual retention times measured for representative compounds during 6 weeks are within 0.1 minutes of the values measured on week 1, and/or the individual relative retention times during 6 weeks are within 1% of the values measured on week 1.

SFL5

Method: GCMTPA3

Separation of Controlled and Non-controlled Substances by Gas Chromatography

Scope: Limited purpose (d- and l-methamphetamine derivatized with MTPA)

Sample Preparation:

Samples extracted with 1 – 5 N NaOH into chloroform. Chloroform layer placed into an autosampler vial and MTPA reagent solution added; allow to stand for approximately 5 minutes. Injection of aqueous solutions is not permitted.

Method Parameters:

Instrument: Gas Chromatograph Agilent 7890 or equivalent
Column Type and Dimensions: 30 m DB-1 × 0.25 mm × 0.5 μm film thickness
Inlet Temperature: 280ºC
Minimum Injection Volume: 1.0 μL
Injection Mode: Split
Maximum Split Ratio: 20:1 (Variable split ratios for sample and/or positive control(s) are acceptable in order to obtain comparable peak areas for comparison.)
Carrier Gas and Flow: Hydrogen at 1.0 mL/min
Make-up Gas: Nitrogen (with FID detector)
Control Mode: Constant flow
Oven Program Set Points: 230°C hold for 1 min, ramp 3°C/min to 250°C, ramp 30°C/min to 290°C.
Minimum Run Time: 9 minutes
Detector Temperature: 300ºC (FID1 method); 280ºC (FID2 method)

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Selectivity: Target compounds are visually separated and observed as a single peak with a clear, non-splitting apex. Peak fronting/tailing, if observed, does not preclude the detection of closely eluting peaks. A minimum S/N =3 is observed. Earliest eluting compound met minimum retention factor criteria.

Repeatability: Individual retention times measured for representative compounds are within 0.1 minutes of the average of 30 injections, and/or the individual relative retention times are within 1% of the average of 30 injections.

Reproducibility: Individual retention times measured for representative compounds during 6 weeks are within 0.1 minutes of the values measured on week 1, and/or the individual relative retention times during 6 weeks are within 1% of the values measured on week 1.

SFL5

Method: GCMTPA4

Separation of Controlled and Non-controlled Substances by Gas Chromatography

Scope: Limited purpose (d- and l-methamphetamine derivatized with MTPA)

Sample Preparation:

Samples extracted with 1 – 5 N NaOH into chloroform. Chloroform layer placed into an autosampler vial and MTPA reagent solution added; allow to stand for approximately 5 minutes. Injection of aqueous solutions is not permitted.

Method Parameters:

Instrument: Gas Chromatograph Agilent 7890 or equivalent equipped with an LTM module
Column Type and Dimensions: 15 m DB-CSI (or DB-5MS) × 0.25 mm × 0.25 μm film thickness LTM column
Inlet Temperature: 280ºC
Minimum Injection Volume: 1.0 μL
Injection Mode: Split
Maximum Split Ratio: 20:1 (Variable split ratios for sample and/or positive control(s) are acceptable in order to obtain comparable peak areas for comparison.)
Carrier Gas and Flow: Hydrogen at 1.0 mL/min
Make-up Gas: Nitrogen (with FID detector)
Control Mode: Constant flow
Oven Program Set Points: 280°C
LTM Program Set Points: 200°C hold for 8 min, ramp 40°C/min to 280°C
Minimum Run Time: 10 minutes
Detector Temperature: 300ºC (FID1 method); 280ºC (FID2 method)

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Selectivity: Target compounds are visually separated and observed as a single peak with a clear, non-splitting apex. Peak fronting/tailing, if observed, does not preclude the detection of closely eluting peaks. A minimum S/N =3 is observed. Earliest eluting compound met minimum retention factor criteria.

Repeatability: Individual retention times measured for representative compounds are within 0.1 minutes of the average of 30 injections, and/or the individual relative retention times are within 1% of the average of 30 injections.

Reproducibility: Individual retention times measured for representative compounds during 6 weeks are within 0.1 minutes of the values measured on week 1, and/or the individual relative retention times during 6 weeks are within 1% of the values measured on week 1.

SFL5

Method: GCMTPA4He

Separation of Controlled and Non-controlled Substances by Gas Chromatography

Scope: Limited purpose (d- and l-methamphetamine derivatized with MTPA)

Sample Preparation:

Samples extracted with 1 – 5 N NaOH into chloroform. Chloroform layer placed into an autosampler vial and MTPA reagent solution added; allow to stand for approximately 5 minutes. Injection of aqueous solutions is not permitted.

Method Parameters:

Instrument: Gas Chromatograph Agilent 7890 or equivalent equipped with an LTM module
Column Type and Dimensions: 15 m DB-CSI (or DB-5MS) × 0.25 mm × 0.25 μm film thickness LTM column
Inlet Temperature: 280ºC
Minimum Injection Volume: 1.0 μL
Injection Mode: Split
Maximum Split Ratio: 20:1 (Variable split ratios for sample and/or positive control(s) are acceptable in order to obtain comparable peak areas for comparison.)
Carrier Gas and Flow: Helium at 1.0 mL/min
Make-up Gas: Nitrogen (with FID detector)
Control Mode: Constant flow
Oven Program Set Points: 280°C
LTM Program Set Points: 200°C hold for 8 min, ramp 40°C/min to 280°C
Minimum Run Time: 10 minutes
Detector Temperature: 300ºC (FID1 method); 280ºC (FID2 method)

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Selectivity: Target compounds are visually separated and observed as a single peak with a clear, non-splitting apex. Peak fronting/tailing, if observed, does not preclude the detection of closely eluting peaks. A minimum S/N =3 is observed. Earliest eluting compound met minimum retention factor criteria.

Repeatability: Individual retention times measured for representative compounds are within 0.1 minutes of the average of 30 injections, and/or the individual relative retention times are within 1% of the average of 30 injections.

Reproducibility: Individual retention times measured for representative compounds during 6 weeks are within 0.1 minutes of the values measured on week 1, and/or the individual relative retention times during 6 weeks are within 1% of the values measured on week 1.

SFL5

Method: GCGEN15

Separation of Controlled and Non-controlled Substances by Gas Chromatography

Scope: General purpose – detection of low-level (0.5%) secondary substances not included in scope.

Sample Preparation:

Samples dissolved or extracted into a volatile organic solvent. Insoluble material filtered or removed from sample. Injection of aqueous solutions is not permitted. Tetracosane internal standard used as fixed compound for validation purposes.

Method Parameters:

Instrument: Gas Chromatograph Agilent 7890 or equivalent with ASAP IRDIII vapor phase infrared detector
Column Type and Dimensions: 30 m HP-5 × 0.32 mm × 0.25 μm film thickness
Inlet Temperature: 275 ºC
Minimum Injection Volume: 1.0 μL
Injection Mode: Split or Splitless
Maximum Split Ratio: 10:1
Carrier Gas and Flow: Helium at 2.0 mL/min
Control Mode: Constant flow
Oven Program Set Points: 60°C hold for 2.0 min, ramp 20°C/min to 320°C, hold for 7 min.
Minimum Run Time: 22 minutes
Detector: See IRD Vapor Phase Infrared Spectroscopy Method Parameters

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Selectivity: Target compounds are visually separated and observed as a single peak with a clear, non-splitting apex. Peak fronting/tailing, if observed, does not preclude the detection of closely eluting peaks. A minimum S/N =3 is observed. Earliest eluting compound met minimum retention factor criteria and/or fulfilled the repeatability and reproducibility acceptance criteria.

Repeatability: Individual retention times measured for representative compounds are within 0.1 minutes of the average of 30 injections, and/or the individual relative retention times are within 1% of the average of 30 injections.

Reproducibility: Individual retention times measured for representative compounds during 6 weeks are within 0.1 minutes of the values measured on week 1, and/or the individual relative retention times during 6 weeks are within 1% of the values measured on week 1.

SFL5

Method: IRD1

Identification of Controlled and Non-controlled Substances by Vapor Phase Infrared Spectroscopy

Scope: General purpose

Sample Preparation:

GC effluent

Method Parameters:

Instrument: ASAP IRDIII vapor phase infrared detector
Scan Range: 550 – 4000 cm^{-1}
Transfer Line Temperature: 280ºC
Resolution: 16 cm^{-1}
Light Pipe Temperature: 280ºC

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Accuracy: Analyte spectra are compared to a verified reference database, commercial library, published spectra or spectra from another ISO/IEC 17025 accredited laboratory. The overall sample spectral pattern corresponds to that of the reference spectrum and the prominent, well-defined, measured signals in the sample spectra between 650 – 2000 cm^{-1} were all within 4 cm^{-1} of those in the reference spectrum. No prominent extraneous signals are observed in the sample spectrum.

Repeatability and Reproducibility: The repeatability and reproducibility of confirmatory methods is established via evaluation of system-wide historical spectral data.

SFL5

Method: GCGEN1He

Separation of Controlled and Non-controlled Substances by Gas Chromatography

Scope: General purpose

Sample Preparation:

Samples dissolved or extracted into a volatile organic solvent. Insoluble material filtered or removed from sample. Injection of aqueous solutions is not permitted. Tetracosane internal standard used as fixed compound for validation purposes.

Method Parameters:

Instrument: Gas Chromatograph Agilent 7890 or equivalent equipped with an LTM module and Agilent 5977 Mass Spectrometer or equivalent
Column Type and Dimensions: 15 m DB-CSI (or DB-5MS) × 0.25 mm × 0.25 μm film thickness LTM column
Inlet Temperature: 280 ºC
Minimum Injection Volume: 1.0 μL
Injection Mode: Split
Maximum Split Ratio: 50:1
Carrier Gas and Flow: Helium at 3.7 mL/min
Control Mode: Constant flow
Oven Program Set Points: 280 ºC
LTM Program Set Points: 60°C hold for 0.5 min, ramp 120°C/min to 230°C, hold for 0.3 min, ramp 120°C/min to 320°C, hold for 1 min.
Minimum Run Time: 3.9667 minutes
Detector: See MSD2 Mass Spectrometry Method Parameters

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Selectivity: Target compounds are visually separated and observed as a single peak with a clear, non-splitting apex. Peak fronting/tailing, if observed, does not preclude the detection of closely eluting peaks. A minimum S/N =3 is observed, including a low level (0.5%) marker compound. Earliest eluting compound met minimum retention factor criteria and/or fulfilled the repeatability and reproducibility acceptance criteria.

Repeatability: Individual retention times measured for representative compounds are within 0.1 minutes of the average of 30 injections, and/or the individual relative retention times are within 1% of the average of 30 injections.

Reproducibility: Individual retention times measured for representative compounds during 6 weeks are within 0.1 minutes of the values measured on week 1, and/or the individual relative retention times during 6 weeks are within 1% of the values measured on week 1.

SFL5

Method: GCGEN2

Separation of Controlled and Non-controlled Substances by Gas Chromatography

Scope: General purpose

Sample Preparation:

Samples dissolved or extracted into a volatile organic solvent. Insoluble material filtered or removed from sample. Injection of aqueous solutions is not permitted. Tetracosane internal standard used as fixed compound for validation purposes.

Method Parameters:

Instrument: Gas Chromatograph Agilent 7890 or equivalent equipped with an LTM module and Agilent 5977 Mass Spectrometer or equivalent
Column Type and Dimensions: 15 m DB-CSI (or DB-5MS) × 0.25 mm × 0.25 μm film thickness LTM column
Inlet Temperature: 280 ºC
Minimum Injection Volume: 1.0 μL
Injection Mode: Split
Maximum Split Ratio: 50:1
Carrier Gas and Flow: Helium at 2.0 mL/min
Control Mode: Constant flow
Oven Program Set Points: 280 ºC
LTM Program Set Points: 60°C hold for 2.0 min, ramp 40°C/min to 320°C, hold for 2.5 min.
Minimum Run Time: 11 minutes
Detector: See MSD2 Mass Spectrometry Method Parameters

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Selectivity: Target compounds are visually separated and observed as a single peak with a clear, non-splitting apex. Peak fronting/tailing, if observed, does not preclude the detection of closely eluting peaks. A minimum S/N =3 is observed, including a low level (0.5%) marker compound. Earliest eluting compound met minimum retention factor criteria and/or fulfilled the repeatability and reproducibility acceptance criteria.

Repeatability: Individual retention times measured for representative compounds are within 0.1 minutes of the average of 30 injections, and/or the individual relative retention times are within 1% of the average of 30 injections.

Reproducibility: Individual retention times measured for representative compounds during 6 weeks are within 0.1 minutes of the values measured on week 1, and/or the individual relative retention times during 6 weeks are within 1% of the values measured on week 1.

SFL5

Method: GCGEN3

Separation of Controlled and Non-controlled Substances by Gas Chromatography

Scope: General purpose

Sample Preparation:

Samples dissolved or extracted into a volatile organic solvent. Insoluble material filtered or removed from sample. Injection of aqueous solutions is not permitted. Tetracosane internal standard used as fixed compound for validation purposes.

Method Parameters:

Instrument: Gas Chromatograph Agilent 7890 or equivalent with Agilent 5975 or 5977 Mass Spectrometer or equivalent
Column Type and Dimensions: 20 m HP-5MS × 0.18 mm × 0.18 μm film thickness
Inlet Temperature: 275 ºC
Minimum Injection Volume: 1.0 μL
Injection Mode: Split
Maximum Split Ratio: 50:1
Carrier Gas and Flow: Helium at 1.5 mL/min
Control Mode: Constant flow
Oven Program Set Points: 80°C hold for 1 min, ramp 30°C/min to 320°C, hold for 2 min.
Minimum Run Time: 11 minutes
Detector: See MSD1 or MSD2 Mass Spectrometry Method Parameters

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Selectivity: Target compounds are visually separated and observed as a single peak with a clear, non-splitting apex. Peak fronting/tailing, if observed, does not preclude the detection of closely eluting peaks. A minimum S/N =3 is observed, including a low level (0.5%) marker compound. Earliest eluting compound met minimum retention factor criteria and/or fulfilled the repeatability and reproducibility acceptance criteria.

Repeatability: Individual retention times measured for representative compounds are within 0.1 minutes of the average of 30 injections, and/or the individual relative retention times are within 1% of the average of 30 injections.

Reproducibility: Individual retention times measured for representative compounds during 6 weeks are within 0.1 minutes of the values measured on week 1, and/or the individual relative retention times during 6 weeks are within 1% of the values measured on week 1.

SFL5

Method: GCGEN4

Separation of Controlled and Non-controlled Substances by Gas Chromatography

Scope: General purpose

Sample Preparation:

Samples dissolved or extracted into a volatile organic solvent. Insoluble material filtered or removed from sample. Injection of aqueous solutions is not permitted. Tetracosane internal standard used as fixed compound for validation purposes.

Method Parameters:

Instrument: Gas Chromatograph Agilent 7890 or equivalent with Agilent 5975 Mass Spectrometer or equivalent
Column Type and Dimensions: 20 m DB-1MS × 0.18 mm × 0.18 μm film thickness
Inlet Temperature: 275 ºC
Minimum Injection Volume: 0.5 μL
Injection Mode: Split
Maximum Split Ratio: 50:1
Carrier Gas and Flow: Helium at 1.5 mL/min
Control Mode: Constant flow
Oven Program Set Points: 80°C hold for 1 min, ramp 30°C/min to 320°C, hold for 2 min.
Minimum Run Time: 11 minutes
Detector: See MSD1 Mass Spectrometry Method Parameters

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Selectivity: Target compounds are visually separated and observed as a single peak with a clear, non-splitting apex. Peak fronting/tailing, if observed, does not preclude the detection of closely eluting peaks. A minimum S/N =3 is observed, including a low level (0.5%) marker compound. Earliest eluting compound met minimum retention factor criteria and/or fulfilled the repeatability and reproducibility acceptance criteria.

Repeatability: Individual retention times measured for representative compounds are within 0.1 minutes of the average of 30 injections, and/or the individual relative retention times are within 1% of the average of 30 injections.

Reproducibility: Individual retention times measured for representative compounds during 6 weeks are within 0.1 minutes of the values measured on week 1, and/or the individual relative retention times during 6 weeks are within 1% of the values measured on week 1.

SFL5

Method: GCGEN16

Separation of Controlled and Non-controlled Substances by Gas Chromatography

Scope: General purpose

Sample Preparation:

Samples dissolved or extracted into a volatile organic solvent. Insoluble material filtered or removed from sample. Injection of aqueous solutions is not permitted. Tetracosane internal standard used as fixed compound for validation purposes.

Method Parameters:

Instrument: Gas Chromatograph Agilent 7890 or equivalent equipped with an LTM module and Agilent 5977 Mass Spectrometer or equivalent
Column Type and Dimensions: 10 m DB-CSI × 0.15 mm × 0.3 μm film thickness LTM column
Inlet Temperature: 280ºC
Minimum Injection Volume: 0.5 μL
Injection Mode: Split
Maximum Split Ratio: 50:1
Carrier Gas and Flow: Helium at 1.5 mL/min
Control Mode: Constant flow
Oven Program Set Points: 280°C
LTM Program Set Points: 80°C hold for 0.5 min, ramp 40°C/min to 320°C, hold for 4.0 min.
Minimum Run Time: 10.5 minutes
Detector: See MSD2 Mass Spectrometry Method Parameters

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Selectivity: Target compounds are visually separated and observed as a single peak with a clear, non-splitting apex. Peak fronting/tailing, if observed, does not preclude the detection of closely eluting peaks. A minimum S/N =3 is observed, including a low level (0.5%) marker compound. Earliest eluting compound met minimum retention factor criteria and/or fulfilled the repeatability and reproducibility acceptance criteria.

Repeatability: Individual retention times measured for representative compounds are within 0.1 minutes of the average of 30 injections, and/or the individual relative retention times are within 1% of the average of 30 injections.

Reproducibility: Individual retention times measured for representative compounds during 6 weeks are within 0.1 minutes of the values measured on week 1, and/or the individual relative retention times during 6 weeks are within 1% of the values measured on week 1.

SFL5

Method: MSD1

Identification of Controlled and Non-controlled Substances by Mass Spectrometry (EI)

Scope: General purpose

Sample Preparation:

GC effluent

Method Parameters:

Instrument: Agilent 5975 mass spectrometer or equivalent
Mass Analyzer: Single Quadrupole
Ionization Mode: Positive EI
Scan Range: 35 – 550 m/z
Scan Rate: Normal; Sampling Rate = 2
Source Temperature: 230ºC
MS Quad Temperature: 150ºC
Transfer Line Temperature: 280ºC
Tune Type: Standard tune (S tune)

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Accuracy: Analyte spectra are compared to a verified reference database, commercial library, published literature spectra or spectra from another ISO/IEC 17025 accredited laboratory. The overall sample spectral pattern corresponds to that of the reference spectrum. The measured m/z values for prominent ions in the sample spectrum are of the same nominal mass as those in the reference spectrum. The molecular ion is present in the sample spectrum if it is present in the reference spectrum. No prominent unexplainable extraneous ions are observed in the sample spectrum.

Repeatability and Reproducibility: The repeatability and reproducibility of confirmatory methods is established via evaluation of system-wide historical spectral data.

SFL5

Method: MSD2

Identification of Controlled and Non-controlled Substances by Mass Spectrometry (EI)

Scope: General purpose

Sample Preparation:

GC effluent

Method Parameters:

Instrument: Agilent 5977 mass spectrometer or equivalent
Mass Analyzer: Single Quadrupole
Ionization Mode: Positive EI
Scan Range: 35 – 550 m/z
Scan Rate: N = 1
Source Temperature: 230ºC
MS Quad Temperature: 150ºC
Transfer Line Temperature: 280ºC
Tune Type: Standard tune (S tune)

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Accuracy: Analyte spectra are compared to a verified reference database, commercial library, published literature spectra or spectra from another ISO/IEC 17025 accredited laboratory. The overall sample spectral pattern corresponds to that of the reference spectrum. The measured m/z values for prominent ions in the sample spectrum are of the same nominal mass as those in the reference spectrum. The molecular ion is present in the sample spectrum if it is present in the reference spectrum. No prominent unexplainable extraneous ions are observed in the sample spectrum.

Repeatability and Reproducibility: The repeatability and reproducibility of confirmatory methods is established via evaluation of system-wide historical spectral data.

SFL5

Methods: ATR1 and ATR1b

Identification of Controlled and Non-controlled Substances by Solid-Phase Infrared Spectroscopy

Scope: General purpose

Sample Preparation:

Sample will be analyzed either by direct analysis, sublimation, or extraction with any appropriate solvent, including but not limited to methanol, chloroform, acetone, petroleum ether, ethyl ether, methylene chloride, water, ethyl acetate, xylenes and hexane, or any combination of these.

Method Parameters:

Instrument: Thermo Scientific, Nicolet iS10 FT-IR
Number of Background Scans: 16 Scans
Minimum Number of Sample Scans: 16 Scans
Scan Range:

- *ATR1*: 400-4000 cm^{-1}
- *ATR1b*: 600 – 4000 cm^{-1}

Sample Gain: Autogain
Resolution: 4 cm^{-1}
Optical Velocity: 0.4747 cm/s
Aperture: Open
Accessory: Smart iTX or Smart Atr

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Accuracy: Analyte spectra are compared to a verified reference database, commercial library, published spectra or spectra from another ISO/IEC 17025 accredited laboratory. The overall sample spectral pattern corresponds to that of the reference spectrum and the prominent, well-defined, measured signals in the sample spectrum between 2000 - 650 cm^{-1} were all within 4 cm^{-1} of those in the reference spectrum. No prominent extraneous signals are observed in the sample spectrum.

Repeatability and Reproducibility: The repeatability and reproducibility of confirmatory methods is established via evaluation of system-wide historical spectral data.

SFL5

Method: ATR2

Identification of Controlled and Non-controlled Substances by Solid-Phase Infrared Spectroscopy

Scope: General purpose

Sample Preparation:

Sample will be analyzed either by direct analysis, sublimation, or extraction with any appropriate solvent, including but not limited to methanol, chloroform, acetone, petroleum ether, ethyl ether, methylene chloride, water, ethyl acetate, xylenes and hexane, or any combination of these.

Method Parameters:

Instrument: Thermo Scientific, Nicolet 6700 Infrared Spectrophotometer
Number of Background Scans: 16 Scans
Minimum Number of Sample Scans: 16 Scans
Scan Range: 400 – 4000 cm^{-1}
Sample Gain: Autogain
Resolution: 4 cm^{-1}
Optical Velocity: 0.4747 cm/s
Aperture: 100
Accessory: Smart Atr

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Accuracy: Analyte spectra are compared to a verified reference database, commercial library, published spectra or spectra from another ISO/IEC 17025 accredited laboratory. The overall sample spectral pattern corresponds to that of the reference spectrum and the prominent, well-defined, measured signals in the sample spectrum between 2000 - 650 cm^{-1} were all within 4 cm^{-1} of those in the reference spectrum. No prominent extraneous signals are observed in the sample spectrum.

Repeatability and Reproducibility: The repeatability and reproducibility of confirmatory methods is established via evaluation of system-wide historical spectral data.

SFL5

Methods: IRMicroARO1 and IRMicroATR1

Identification of Controlled and Non-controlled Substances by Solid-Phase Infrared Spectroscopy

Scope: General purpose

Sample Preparation:

Sample will be analyzed either by direct analysis, sublimation, or extraction with any appropriate solvent, including but not limited to methanol, chloroform, acetone, petroleum ether, ethyl ether, methylene chloride, water, ethyl acetate, xylenes and hexane, or any combination of these.

Method Parameters:

Instrument: Smiths Detection IlluminatIR FTIR with Olympus microscope attachment
Number of Background Scans:

- *IRMicroARO1*: 8 Scans
- *IRMicroATR1*: 16 Scans

Minimum Number of Sample Scans:

- *IRMicroARO1*: 8 Scans
- *IRMicroATR1*:16 Scans

Scan Range: 650 – 4000 cm^{-1}
Resolution: 4 cm^{-1}
Detector: MCT
Accessory:

- *IRMicroARO1*: ARO IR Microscope 15x objective
- *IRMicroATR1*: ATR IR Microscope 36x objective

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Accuracy: Analyte spectra are compared to a verified reference database, commercial library, published spectra or spectra from another ISO/IEC 17025 accredited laboratory. The overall sample spectral pattern corresponds to that of the reference spectrum and the prominent, well-defined, measured signals in the sample spectrum between 2000 – 650 cm^{-1} were all within 8 cm^{-1} of those in the reference spectrum. No prominent extraneous signals are observed in the sample spectrum.

Repeatability and Reproducibility: The repeatability and reproducibility of confirmatory methods is established via evaluation of system-wide historical spectral data.

SFL5

Method: LCMURSHROOM1

Separation of Controlled and Non-controlled Substances by Liquid Chromatography

Scope: Limited purpose (analysis of psilocybe mushrooms)

Sample Preparation:

Samples dissolved or extracted into solvents miscible with water. Insoluble material removed from sample prior to injection; 0.45 μm filter recommended. Psilocybin was used as the fixed compound for validation purposes.

Method Parameters:

Instrument: Liquid Chromatograph Agilent 1260, 1290, or equivalent
Column: 4.6 × 150 mm 4 μm Synergi Polar-RP
Column Temperature: 25ºC
Buffer/Mobile Phase: (A) 0.1% TFA with hexylamine in water; (B) Acetonitrile
Minimum Injection Volume: 2.5 μL
Gradient Set Points: Isocratic (85% A: 15% B)
Flow Rate: 1.0 mL/min
Detection Wavelength: 254 nm (DAD2 method)
Minimum Run Time: 7 minutes

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Selectivity: Target compounds are visually separated and observed as a single peak with a clear, non-splitting apex. Peak fronting/tailing, if observed, does not preclude the detection of closely eluting peaks. A minimum S/N = 3 is observed. Earliest eluting compound fulfilled the repeatability and reproducibility acceptance criteria.

Repeatability: Individual absolute retention times measured are within 0.1 minutes of the average of 30 injections, and the individual relative retention times are with 1% of the average of 30 injections.

Reproducibility: Individual retention times measured during 3 weeks were within 0.1 minutes of the values measured on week 1, and the individual relative retention times during 4 weeks are with 1% of the values measured on week 1.

SFL5

Method: LCOXY1

Separation of Controlled and Non-controlled Substances by Liquid Chromatography

Scope: Limited purpose (analysis of hydrocodone and oxycodone)

Sample Preparation:

Samples dissolved or extracted into solvents miscible with water. Insoluble material removed from sample prior to injection; 0.45 μm filter recommended. Oxycodone was used as the fixed compound for validation purposes.

Method Parameters:

Instrument: Liquid Chromatograph Waters UPLC Acquity I-class or equivalent
Column: 2.1 × 100 mm 1.7 μm BEH C18
Column Temperature: 25ºC ± 5ºC
Buffer/Mobile Phase: (A) 10 mM Ammonium Formate with TFA in water, pH 3.6; (B) Acetonitrile
Minimum Injection Volume: 1.0 μL
Gradient Set Points: 0 minute: 90% A: 10% B
2.0 minute: 50% A: 50% B
2.6 minute: 90% A: 10% B
3.0 minute: 90% A: 10% B
Flow Rate: 0.35 mL/min
Detection Wavelength: 235 nm (DAD5 method)
Minimum Run Time: 3 minutes

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Selectivity: Target compounds are visually separated and observed as a single peak with a clear, non-splitting apex. Peak fronting/tailing, if observed, does not preclude the detection of closely eluting peaks. A minimum S/N =3 is observed. Earliest eluting compound fulfilled the repeatability and reproducibility acceptance criteria.

Repeatability: Individual absolute retention times measured are within 0.1 minutes of the average of 30 injections, and the individual relative retention times are with 1% of the average of 30 injections.

Reproducibility: Individual retention times measured during 6 weeks were within 0.1 minutes of the values measured on week 1, and the individual relative retention times during 6 weeks are with 1% of the values measured on week 1.

SFL5 LCPHEN1

Method: LCPHEN1

Separation of Controlled and Non-controlled Substances by Liquid Chromatography

Scope: Limited purpose (analysis of phenethylamines)

Sample Preparation:

Samples dissolved or extracted into solvents miscible with water. Insoluble material removed from sample prior to injection; 0.45 μm filter recommended. Methamphetamine was used as the fixed compound for validation purposes.

Method Parameters:

Instrument: Liquid Chromatograph Waters UPLC Acquity I-class or equivalent
Column: 2.1 × 100 mm 1.7 μm BEH C18
Column Temperature: 30ºC ± 2ºC
Buffer/Mobile Phase: (A) 85 mM sodium phosphate in water, pH 1.8; (B) Acetonitrile
Minimum Injection Volume: 1.0 μL
Gradient Set Points: Isocratic (90% A: 10% B)
Flow Rate: 0.45 mL/min
Detection Wavelength: 210 nm (DAD6 method)
Minimum Run Time: 5 minutes

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Selectivity: Target compounds are visually separated and observed as a single peak with a clear, non-splitting apex. Peak fronting/tailing, if observed, does not preclude the detection of closely eluting peaks. A minimum S/N =3 is observed. Earliest eluting compound met minimum retention factor criteria.

Repeatability: Individual absolute retention times measured are within 0.1 minutes of the average of 30 injections, and the individual relative retention times are with 1% of the average of 30 injections.

Reproducibility: Individual retention times measured during 6 weeks were within 0.1 minutes of the values measured on week 1, and the individual relative retention times during 6 weeks are with 1% of the values measured on week 1.

SFL5

Method: LCPSEUDO1

Separation of Controlled and Non-controlled Substances by Liquid Chromatography

Scope: Limited purpose (analysis of pseudoephedrine and ephedrine)

Sample Preparation:

Samples dissolved or extracted into solvents miscible with water. Insoluble material removed from sample prior to injection; 0.45 μm filter recommended. Pseudoephedrine was used as the fixed compound for validation purposes.

Method Parameters:

Instrument: Liquid Chromatograph Agilent 1260, 1290, or equivalent
Column: 4.6 × 150 mm 4 μm Synergi Polar-RP
Column Temperature: Not controlled
Buffer/Mobile Phase: 0.1% TFA with hexylamine in water
Minimum Injection Volume: 2.5 μL
Gradient Set Points: Isocratic (100% Buffer)
Flow Rate: 1.5 mL/min
Detection Wavelength: 210 nm (DAD4 method)
Minimum Run Time: 10 minutes

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Selectivity: Target compounds are visually separated and observed as a single peak with a clear, non-splitting apex. Peak fronting/tailing, if observed, does not preclude the detection of closely eluting peaks. A minimum S/N = 3 is observed. Earliest eluting compound met minimum retention factor criteria.

Repeatability: Individual absolute retention times measured are within 0.1 minutes of the average of 30 injections, and the individual relative retention times are with 1% of the average of 30 injections.

Reproducibility: Individual retention times measured during 3 weeks were within 0.1 minutes of the values measured on week 1, and the individual relative retention times during 4 weeks are with 1% of the values measured on week 1.

SFL5

Method: LCFENT1

Separation of Controlled and Non-controlled Substances by Liquid Chromatography

Scope: Limited purpose (analysis of heroin and fentanyl related compounds)

Sample Preparation:

Samples dissolved or extracted into solvents miscible with water. Insoluble material removed from sample prior to injection; centrifugation recommended. Heroin was used as the fixed compound for validation purposes.

Method Parameters:

Instrument: Liquid Chromatograph Agilent 1260, 1290, or equivalent with Agilent 6545 Q-TOF Mass Spectrometer or equivalent
Column: 3.0 × 150 mm 4 μm Synergi Hydro-RP 80Å
Column Temperature: Not controlled
Buffer/Mobile Phase: (A) 0.1% Formic acid in water; (B) Acetronitrile
Minimum Injection Volume: 1 μL
Gradient Set Points: Isocratic (70% A: 30% B)
Flow Rate: 0.4 mL/min
Minimum Run Time: 13 minutes
Detector: See MSMS1 Mass Spectrometry Method Parameters

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Selectivity: Target compounds are visually separated and observed as a single peak with a clear, non-splitting apex. Peak fronting/tailing, if observed, does not preclude the detection of closely eluting peaks. A minimum S/N = 3 is observed. Earliest eluting compound met minimum retention factor criteria.

Repeatability: Individual retention times measured are within 0.3 minutes of the average of 30 injections.

Reproducibility: Individual retention times measured during 2 weeks are within 0.3 minutes of the values measured on week 1.

SFL5

Method: LCLSD2

Separation of Controlled and Non-controlled Substances by Liquid Chromatography

Scope: Limited purpose (analysis of LSD)

Sample Preparation:

Samples dissolved or extracted into solvents miscible with water. Insoluble material removed from sample prior to injection; centrifugation recommended. LSD was used as the fixed compound for validation purposes.

Method Parameters:

Instrument: Liquid Chromatograph Agilent 1260, 1290, or equivalent with Agilent 6545 Q-TOF Mass Spectrometer or equivalent
Column: 3.0 × 150 mm 4 μm Synergi Hydro-RP 80Å
Column Temperature: Not controlled
Buffer/Mobile Phase: (A) 0.1% Formic acid in water; (B) Acetonitrile
Minimum Injection Volume: 1 μL
Gradient Set Points: Isocratic (80% A: 20% B)
Flow Rate: 0.4 mL/min
Minimum Run Time: 18 minutes
Detector: See MSMS1 Mass Spectrometry Method Parameters

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Selectivity: Target compounds are visually separated and observed as a single peak with a clear, non-splitting apex. Peak fronting/tailing, if observed, does not preclude the detection of closely eluting peaks. A minimum S/N = 3 is observed. Earliest eluting compound met minimum retention factor criteria.

Repeatability: Individual retention times measured are within 0.3 minutes of the average of 30 injections and the individual relative retention times are with 1% of the average of 30 injections. However, multiple repeatability studies revealed that a positive control should be run within 15 sequential injections of the unknown sample.

Reproducibility: Individual retention times measured during 2 weeks were not within 0.3 minutes of the values measured on week 1. Based on the repeatability testing, positive controls shall be analyzed within a maximum of 15 sequential injections of the unknown sample, within the same sequence on the same day.

SFL5

Method: LCMUSHROOM2

Separation of Controlled and Non-controlled Substances by Liquid Chromatography

Scope: Limited purpose (analysis of psilocybe mushrooms)

Sample Preparation:

Samples dissolved or extracted into solvents miscible with water. Insoluble material removed from sample prior to injection; centrifugation recommended. Psilocybin was used as the fixed compound for validation purposes.

Method Parameters:

Instrument: Liquid Chromatograph Agilent 1260, 1290, or equivalent with Agilent 6545 Q-TOF Mass Spectrometer or equivalent
Column: 3.0 × 150 mm 4 μm Synergi Hydro-RP 80Å
Column Temperature: Not controlled
Buffer/Mobile Phase: (A) 0.1% Formic acid in water; (B) Acetonitrile
Minimum Injection Volume: 1 μL
Gradient Set Points: Isocratic (85% A: 15% B)
Flow Rate: 0.4 mL/min
Minimum Run Time: 5 minutes
Detector: See MSMS1 Mass Spectrometry Method Parameters

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Selectivity: Target compounds are visually separated and observed as a single peak with a clear, non-splitting apex. Peak fronting/tailing, if observed, does not preclude the detection of closely eluting peaks. A minimum S/N = 3 is observed. Earliest eluting compound fulfilled the repeatability and reproducibility acceptance criteria.

Repeatability: Individual absolute retention times measured are within 0.3 minutes of the average of 30 injections.

Reproducibility: Individual retention times measured during 4 weeks were within 0.3 minutes of the values measured on week 1. Based on this testing, positive controls shall be analyzed within 21 days of the unknown sample.

SFL5

Method: MSMS1

Identification of Controlled and Non-controlled Substances by Electrospray Ionization-Mass Spectrometry (ESI-MS)

Scope: General purpose (detection of low-level (0.5%) secondary substances not included in scope)

Sample Preparation:

LC effluent

Method Parameters:

Instrument: Agilent 6545 Q-TOF Mass Spectrometer with dual AJS ESI source
Mass Analyzer: Q-TOF
Ionization Mode: Positive ESI
Drying Gas: Nitrogen
MS Scan Range: 50 – 1000 m/z
MSMS Range: 50 – 500 m/z
MS Scan Rate: 1.5 spectra/s
MSMS Scan Rate: 1 spectra/s
Collision Gas: Nitrogen
Collision Energy: 10 – 50 eV
Tune File: TOFMassCalibration-1700mzRange
Reference Masses: 121.050873, 922.009798
Activation Type: CID

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Accuracy: Analyte spectra are compared to a verified reference database, commercial library, published literature spectra or spectra from another ISO/IEC 17025 accredited laboratory. The overall MS and MSMS (if used) fragmentation spectra corresponds to those of the reference spectrum. The measured m/z values for prominent ions in the sample MS spectrum were all within 5 ppm of those in the reference spectrum. The measured m/z values for prominent ions in the sample MSMS spectrum were all of the same nominal mass as those in the reference spectrum. No prominent unexplainable extraneous ions are observed in the sample spectrum.

Repeatability and Reproducibility: The repeatability and reproducibility of confirmatory methods is established via evaluation of system-wide historical spectral data.

SFL5

Method: PROTON

Identification of Controlled and Non-controlled Substances by Nuclear Resonance Spectroscopy

Scope: Proton

Procedure:

Samples dissolved or extracted into deuterated solvent. Insoluble material filtered or removed from sample.

Method Parameters:

Instrument: Varian MR-400 Nuclear Magnetic Resonance Spectrometer
MHz: 400
Minimum Spectral Range (ppm): -2 – 14 ppm (6410.3 Hz)
Minimum Delay between Pulses (seconds): 1 s
Minimum Pulse Angle (degrees): 1 - 90º
Minimum Acquisition Time: ≥ 2 s
Minimum Number of Scans: 8

Limitations: See individual instrument validation reports.

Acceptance Criteria (unless otherwise noted in individual validation report):

Accuracy: Analyte spectra are compared to a verified reference database, commercial library, published literature spectra, or spectra from another ISO/IEC 17025 accredited laboratory. Overall sample spectral pattern corresponds to that of the reference spectrum acquired using the same solvent. All signals in the sample spectra were within 0.2 ppm of those in the reference spectrum. No unexplainable extraneous signals are observed in the sample spectrum.

Repeatability and Reproducibility: The repeatability and reproducibility of confirmatory methods is established via evaluation of system-wide historical spectral data.

NCL-EPH-GCQ1–

Quantitation of Ephedrine by Gas Chromatography

Scope

Samples containing ephedrine.

Procedure:

Accurately weigh the sample and dissolve in 1 part methanol, 1 part internal standard solution, and 3 parts chloroform so that the concentration of the target analyte is bracketed between the high- and low-concentration QC solutions and the concentration of internal standard is 0.8 mg/mL. For samples containing a large amount of insoluble material, it may be necessary to deliver the required amount of methanol, internal standard solution, and chloroform via volumetric pipet instead of diluting to volume using the indicator line on a volumetric flask.

Base extraction: Base extract the sample with 5 N NaOH solution, vortex/shake vigorously and centrifuge or allow layers to separate. Remove the bottom layer (chloroform) for analysis. Filter the solution if necessary. (See limitations 1 and 2).

Centrifuge or filter sample solutions containing appreciable amounts of insoluble material prior to removing aliquot portions for serial dilutions or transferring solutions to injection vials.

Internal Standard Solution:

4.0 mg/mL n-tridecane in chloroform.

Standard Solution:

Accurately weigh the ephedrine hydrochloride reference material and dissolve in 1 part methanol, 1 part internal standard solution, and 3 parts chloroform so that the concentration of the ephedrine is within the working range of the method and the concentration of internal standard is 0.8 mg/mL. Perform base extraction. Target concentration: 1.6 mg/mL.

Quality Control Solutions:

Prepare two QC solutions for use as positive controls during quantitative analysis. These two solutions are prepared such that their target analyte concentrations represent the low and high ends of the method's working range and the concentration of internal standard is 0.8 mg/mL. Perform base extraction.

Chromatographic System:

Instrument: Gas Chromatograph HP/Agilent 7890 equipped with an FID detector (or equivalent)
Column: DB-1 (J&W Part No. 123-1012); 15 m × 0.32 mm I.D. × 0.25 µm film thickness, 100% methylpolysiloxane stationary phase
Inlet (Injector) Temperature: 275 °C
Mode: Split
Split Ratio: 35:1
Carrier Gas: Helium
Carrier Gas Flow Rate: 2.2 mL/min
Oven Program: Isothermal at 130 °C
Total Run Time: 7.0 min (See limitation 3)
Detector Temperature: 280 °C
Injection Volume: 1 µL
Injection Solvent: 4:1 Chloroform:Methanol base extracted with 1 – 5 N NaOH

Limitations:

1. Although ephedrine hydrochloride readily dissolves in methanol, its solubility in chloroform is limited; therefore the entire sample solution must be base extracted with 5 N NaOH. In addition, sugars and inorganic salts are not soluble. These insoluble materials may be filtered out for further analysis if necessary.
2. The pKa of ephedrine hydrochloride is 9.22. Use of a concentrated sodium hydroxide solution (pH~14) is necessary to ensure complete conversion of the ephedrine hydrochloride into the base, with subsequent extraction into the organic chloroform layer.
3. Triprolidine and chlorpheniramine are common adulterants in tablets that do not elute under the 7 minute isothermal run conditions. If these compounds are present in a sample, the temperature should be ramped after the initial 7 minute run and held until these compounds elute.
4. Ephedrine coelutes with p-methoxyamphetamine, pseudoephedrine, and isosafrole. This method is not suitable for the quantitation of ephedrine in samples containing these components.

5. Tridecane coelutes with phenylpropanolamine and piperonal. This method is not suitable for the quantitation of ephedrine in samples containing these components.

Acceptance Criteria:

Selectivity: Ephedrine and n-tridecane resolved (R ≥1.5) from each compound tested except as described in the limitations section.
Linearity: At least seven concentrations were within 95-105% overall average sensitivity (response/concentration) limits.
Repeatability: Relative Standard Deviation (RSD) for each concentration tested was less than 2%.
Accuracy: Experimentally measured purity (expressed in % w/w) within ± 5% relative to the known prepared purity.

Working Range:

0.4 – 4.0 mg/mL Ephedrine HCl

NCL-MDA-GCQ1 – Quantitation of 3,4-Methylenedioxyamphetamine (MDA) by Gas Chromatography

Scope

Samples containing 3,4-methylenedioxyamphetamine.

Procedure:

Accurately weigh the sample and dissolve in 1 part methanol, 1 part internal standard solution, and 3 parts chloroform so that the concentration of the target analyte is bracketed between the high- and low-concentration QC solutions and the concentration of internal standard is 0.8 mg/mL. For samples containing a large amount of insoluble material, it may be necessary to deliver the required amount of methanol, internal standard solution, and chloroform via volumetric pipet instead of diluting to volume using the indicator line on a volumetric flask.

Base extraction: Remove a 1.0 – 2.0 mL aliquot to a test tube and add an equal volume of 1 – 5 N NaOH solution. Vortex/shake vigorously and centrifuge or allow layers to separate. Remove the bottom layer (chloroform) for analysis. Filter the solution if necessary.

Centrifuge or filter sample solutions containing appreciable amounts of insoluble material prior to removing aliquot portions for serial dilutions or transferring solutions to injection vials.

Internal Standard Solution:

4.0 mg/mL n-tridecane in chloroform.

Standard Solution:

Accurately weigh the MDA hydrochloride reference material and dissolve in 1 part methanol, 1 part internal standard solution, and 3 parts chloroform so that the concentration of the MDA is within the working range of the method and the concentration of internal standard is 0.8 mg/mL. Perform base extraction. Target concentration: 1.0 mg/mL.

Quality Control Solutions:

Prepare two QC solutions for use as positive controls during quantitative analysis. These two solutions are prepared such that their target analyte concentrations represent the low and high ends of the method's working range and the concentration of internal standard is 0.8 mg/mL. Perform base extraction.

Chromatographic System:

Instrument: Gas Chromatograph HP/Agilent 7890 equipped with an FID detector (or equivalent)
Column: DB-1 (J&W Part No. 123-1012); 15 m × 0.32 mm I.D. × 0.25 µm film thickness, 100% methylpolysiloxane stationary phase
Inlet (Injector) Temperature: 275 °C
Mode: Split
Split Ratio: 35:1
Carrier Gas: Helium
Carrier Gas Flow Rate: 1.6 mL/min
Oven Program: Isothermal at 130 °C
Total Run Time: 7.0 min
Detector Temperature: 280 °C
Injection Volume: 1 µL
Injection Solvent: 4:1 Chloroform:Methanol base extracted with 1 – 5 N NaOH

Limitations:

1. MDA coelutes with MD-P2P. This method is not suitable for the quantitation of MDA in samples containing MD-P2P.
2. Tridecane coelutes with phenylpropanolamine and piperonal. This method is not suitable for the quantitation of MDA in samples containing these components.

Acceptance Criteria:

Selectivity: MDA and n-tridecane resolved (R ≥1.5) from each compound tested except as described in the limitations section.
Linearity: At least seven concentrations were within 95-105% overall average sensitivity (response/concentration) limits.
Repeatability: Relative Standard Deviation (RSD) for each concentration tested was less than 2%.
Accuracy: Experimentally measured purity (expressed in % w/w) within ± 5% relative to the known prepared purity.

Working Range:

0.5 – 4.0 mg/mL MDA HCl

NCL-MDM-GCQ1 – Quantitation of 3,4-Methylenedioxymethamphetamine (MDMA) by Gas Chromatography

Scope

Samples containing 3,4-MDMA

Procedure:

Accurately weigh the sample and dissolve in 1 part methanol, 1 part internal standard solution, and 3 parts chloroform so that the concentration of the target analyte is bracketed between the high- and low-concentration QC solutions and the concentration of internal standard is 0.8 mg/mL. For samples containing a large amount of insoluble material, it may be necessary to deliver the required amount of methanol, internal standard solution, and chloroform via volumetric pipet instead of diluting to volume using the indicator line on a volumetric flask.

Base extraction: Remove a 1.0 – 2.0 mL aliquot to a test tube and add an equal volume of 1 – 5 N NaOH solution. Vortex/shake vigorously and centrifuge or allow layers to separate. Remove the bottom layer (chloroform) for analysis. Filter the solution if necessary.

Centrifuge or filter sample solutions containing appreciable amounts of insoluble material prior to removing aliquot portions for serial dilutions or transferring solutions to injection vials.

Internal Standard Solution:

4.0 mg/mL n-tridecane in chloroform.

Standard Solution:

Accurately weigh the MDMA hydrochloride reference material and dissolve in 1 part methanol, 1 part internal standard solution, and 3 parts chloroform so that the concentration of the MDMA is within the working range of the method and the concentration of internal standard is 0.8 mg/mL. Perform base extraction. Target concentration: 1.0 mg/mL.

Quality Control Solutions:

Prepare two QC solutions for use as positive controls during quantitative analysis. These two solutions are prepared such that their target analyte concentrations represent the low and high ends of the method's working range and the concentration of internal standard is 0.8 mg/mL. Perform base extraction.

Chromatographic System:

Instrument: Gas Chromatograph HP/Agilent 7890 equipped with an FID detector (or equivalent)
Column: DB-1 (J&W Part No. 123-1012); 15 m × 0.32 mm I.D. × 0.25 µm film thickness, 100% methylpolysiloxane stationary phase
Inlet (Injector) Temperature: 275 °C
Mode: Split
Split Ratio: 35:1
Carrier Gas: Helium
Carrier Gas Flow Rate: 1.6 mL/min
Oven Program: Isothermal at 130 °C
Total Run Time: 7.0 min
Detector Temperature: 280 °C
Injection Volume: 1 µL
Injection Solvent: 4:1 Chloroform:Methanol base extracted with 1 – 5 N NaOH

Limitations:

1. MDMA coelutes with pentadecane. This method is not suitable for the quantitation of MDMA in samples containing pentadecane.
2. Tridecane coelutes with phenylpropanolamine and piperonal. This method is not suitable for the quantitation of MDMA in samples containing these components.

Acceptance Criteria:

Selectivity: MDMA and n-tridecane resolved (R ≥1.5) from each compound tested except as described in the limitations section.
Linearity: At least seven concentrations were within 95-105% overall average sensitivity (response/concentration) limits.
Repeatability: Relative Standard Deviation (RSD) for each concentration tested was less than 2%.
Accuracy: Experimentally measured purity (expressed in % w/w) within ± 5% relative to the known prepared purity.

Working Range: **0.2 – 4.0 mg/mL MDMA HCl**

NCL-MEM-GCQ1 – Quantitation of Methamphetamine by Gas Chromatography

Scope

Samples containing methamphetamine.

Procedure:

Prepare the sample solution so that the concentration of the target analyte is bracketed between the high- and low-concentration QC solutions and the concentration of internal standard is 0.8 mg/mL.

(A) Accurately weigh the sample and dissolve in 1 part methanol, 1 part internal standard solution (A), and 3 parts chloroform

OR

(B) Accurately weigh the sample and dissolved in internal standard solution (B).

For samples containing a large amount of insoluble material, it may be necessary to deliver the required amount of methanol, internal standard solution, and chloroform via volumetric pipet instead of diluting to volume using the indicator line on a volumetric flask.

Base extraction: Remove a 1.0 – 2.0 mL aliquot to a test tube and add an equal volume of 1 – 5 N NaOH solution. Vortex/shake vigorously and centrifuge or allow layers to separate. Remove the bottom layer (chloroform) for analysis. Filter the solution if necessary.

Centrifuge or filter sample solutions containing appreciable amounts of insoluble material prior to removing aliquot portions for serial dilutions or transferring solutions to injection vials.

Internal Standard Solution:

(A) 4.0 mg/mL n-tridecane in chloroform

OR

(B) 0.8 mg/mL n-tridecane in 4:1 chloroform:methanol.

Standard Solution:

Accurately weigh the methamphetamine hydrochloride reference material and dissolve as described in (A) or (B) in the sample procedure so that the concentration of the target analyte is within the working range of the method and the concentration of internal standard is 0.8 mg/mL. Perform base extraction. Target concentration: 1.0 mg/mL.

Quality Control Solutions:

Prepare two QC solutions for use as positive controls during quantitative analysis. These two solutions are prepared such that their target analyte concentrations represent the low and high ends of the method's working range and the concentration of internal standard is 0.8 mg/mL. Perform base extraction.

Chromatographic System:

Instrument: Gas Chromatograph HP/Agilent 7890 equipped with an FID detector (or equivalent)
Column: DB-1 (J&W Part No. 123-1012); 15 m × 0.32 mm I.D. × 0.25 µm film thickness, 100% methylpolysiloxane stationary phase
Inlet (Injector) Temperature: 275 °C
Mode: Split
Split Ratio: 35:1
Carrier Gas: Helium
Carrier Gas Flow Rate: 1.6 mL/min
Oven Program: Isothermal at 120 °C
Total Run Time: 5.0 min
Detector Temperature: 280 °C
Injection Volume: 1 µL
Injection Solvent: 4:1 Chloroform:Methanol base extracted with 1 – 5 N NaOH

Limitations:

Methamphetamine hydrochloride elutes closely with one of the birch reaction byproducts and phenylpropanolamine elutes closely with the tridecane internal standard. This method is not suitable for quantitation of samples containing birch reaction byproducts and the resolution of samples containing phenylpropanolamine must be checked.

Acceptance Criteria:

Selectivity: Methamphetamine and n-tridecane resolved (R ≥1.5) from each compound tested except as described in the limitations section.
Linearity: At least seven concentrations were within 95-105% overall average sensitivity (response/concentration) limits.
Repeatability: Relative Standard Deviation (RSD) for each concentration tested was less than 2%.
Accuracy: Experimentally measured purity (expressed in % w/w) within ± 5% relative to the known prepared purity.

Working Range:

0.1 – 4.0 mg/mL Methamphetamine HCl

NCL-MEM-GCQ6 – Quantitation of Methamphetamine by Gas Chromatography

Scope

Samples containing methamphetamine.

Procedure:

Prepare the sample solution so that the concentration of the target analyte is bracketed between the high- and low-concentration QC solutions and the concentration of internal standard is 0.8 mg/mL.

(A) Accurately weigh the sample and dissolve in 1 part methanol, 1 part internal standard solution (A), and 3 parts chloroform

OR

(B) Accurately weigh the sample and dissolved in internal standard solution (B).

For samples containing a large amount of insoluble material, it may be necessary to deliver the required amount of methanol, internal standard solution, and chloroform via volumetric pipet instead of diluting to volume using the indicator line on a volumetric flask.

Base extraction: Remove a 1.0 – 2.0 mL aliquot to a test tube and add an equal volume of 1 – 5 N NaOH solution. Vortex/shake vigorously and centrifuge or allow layers to separate. Remove the bottom layer (chloroform) for analysis. Filter the solution if necessary.

Centrifuge or filter sample solutions containing appreciable amounts of insoluble material prior to removing aliquot portions for serial dilutions or transferring solutions to injection vials.

Internal Standard Solution:

(A) 4.0 mg/mL n-tridecane in chloroform

OR

(B) 0.8 mg/mL n-tridecane in 4:1 chloroform:methanol.

Standard Solution:

Accurately weigh the methamphetamine hydrochloride reference material and dissolve as described in (A) or (B) in the sample procedure so that the concentration of the target analyte is within the working range of the method and the concentration of internal standard is 0.8 mg/mL. Perform base extraction. Target concentration: 1.0 mg/mL.

Quality Control Solutions:

Prepare two QC solutions for use as positive controls during quantitative analysis. These two solutions are prepared such that their target analyte concentrations represent the low and high ends of the method's working range and the concentration of internal standard is 0.8 mg/mL. Perform base extraction.

Chromatographic System:

Instrument: Gas Chromatograph HP/Agilent 7890 equipped with an FID detector (or equivalent)
Column: DB-1 (J&W Part No. 122-1012); 30 m × 0.25 mm I.D. × 0.25 µm film thickness, 100% methylpolysiloxane stationary phase
Inlet (Injector) Temperature: 275 °C
Mode: Split
Split Ratio: 50:1
Carrier Gas: Helium
Carrier Gas Flow Rate: 1.5 mL/min
Oven Program: Isothermal at 120 °C
Total Run Time: 10.0 min
Detector Temperature: 280 °C
Injection Volume: 1 µL
Injection Solvent: 4:1 Chloroform:Methanol base extracted with 1 – 5 N NaOH

Limitations:

None.

Acceptance Criteria:

Selectivity: Methamphetamine and n-tridecane resolved (R ≥1.5) from each compound tested.
Linearity: At least seven concentrations were within 95-105% overall average sensitivity (response/concentration) limits.
Repeatability: Relative Standard Deviation (RSD) for each concentration tested was less than 2%.

Accuracy: Experimentally measured purity (expressed in % w/w) within ± 5% relative to the known prepared purity.

Working Range:

0.2 – 4.0 mg/mL Methamphetamine HCl

NCL-PSE-GCQ1 – Quantitation of Pseudoephedrine by Gas Chromatography

Scope

Samples containing pseudoephedrine.

Procedure:

Accurately weigh the sample and dissolve in 1 part methanol, 1 part internal standard solution, and 3 parts chloroform so that the concentration of the target analyte is bracketed between the high- and low-concentration QC solutions and the concentration of internal standard is 0.8 mg/mL. For samples containing a large amount of insoluble material, it may be necessary to deliver the required amount of methanol, internal standard solution, and chloroform via volumetric pipet instead of diluting to volume using the indicator line on a volumetric flask.

Base extraction: Base extract the sample with 5 N NaOH solution, vortex/shake vigorously and centrifuge or allow layers to separate. Remove the bottom layer (chloroform) for analysis. Filter the solution if necessary. (See limitations 1 and 2).

Centrifuge or filter sample solutions containing appreciable amounts of insoluble material prior to removing aliquot portions for serial dilutions or transferring solutions to injection vials.

Internal Standard Solution:

4.0 mg/mL n-tridecane in chloroform.

Standard Solution:

Accurately weigh the pseudoephedrine hydrochloride reference material and dissolve in 1 part methanol, 1 part internal standard solution, and 3 parts chloroform so that the concentration of the pseudoephedrine is within the working range and the concentration of internal standard is 0.8 mg/mL. Perform base extraction. Target concentration: 1.6 mg/mL.

Quality Control Solutions:

Prepare two QC solutions for use as positive controls during quantitative analysis. These two solutions are prepared such that their target analyte concentrations represent the low and high ends of the method's working range and the concentration of internal standard is 0.8 mg/mL. Perform base extraction.

Chromatographic System:

Instrument: Gas Chromatograph HP/Agilent 7890 equipped with an FID detector (or equivalent)
Column: DB-1 (J&W Part No. 123-1012); 15 m × 0.32 mm I.D. × 0.25 µm film thickness, 100% methylpolysiloxane stationary phase
Inlet (Injector) Temperature: 275 °C
Mode: Split
Split Ratio: 35:1
Carrier Gas: Helium
Carrier Gas Flow Rate: 2.2 mL/min
Oven Program: Isothermal at 130 °C
Total Run Time: 7.0 min (See limitation 3)
Detector Temperature: 280 °C
Injection Volume: 1 µL
Injection Solvent: 4:1 Chloroform:Methanol base extracted with 1 – 5 N NaOH

Limitations:

1. Although pseudoephedrine hydrochloride readily dissolves in methanol, its solubility in chloroform is limited; therefore the entire sample solution must be base extracted with 5 N NaOH. In addition, sugars and inorganic salts are not soluble. These insoluble materials may be filtered out for further analysis if necessary.
2. The pKa of pseudoephedrine hydrochloride is 9.22. Use of a concentrated sodium hydroxide solution (pH~14) is necessary to ensure complete conversion of the pseudoephedrine hydrochloride into the base, with subsequent extraction into the organic chloroform layer.
3. Triprolidine and chlorpheniramine are common adulterants in tablets that do not elute under the 7 minute isothermal run conditions. If these compounds are present in a sample, the temperature should be ramped after the initial 7 minute run and held until these compounds elute.
4. Pseudoephedrine coelutes with p-methoxyamphetamine, ephedrine, and isosafrole. This method is not suitable for the quantitation

of pseudoephedrine in samples containing these components.

5. Tridecane coelutes with phenylpropanolamine and piperonal. This method is not suitable for the quantitation of pseudoephedrine in samples containing these components.

Acceptance Criteria:

Selectivity: Pseudoephedrine and n-tridecane resolved (R ≥1.5) from each compound tested except as described in the limitations section.
Linearity: At least seven concentrations were within 95-105% overall average sensitivity (response/concentration) limits.
Repeatability: Relative Standard Deviation (RSD) for each concentration tested was less than 2%.
Accuracy: Experimentally measured purity (expressed in % w/w) within ± 5% relative to the known prepared purity.

Working Range:

0.4 – 4.0 mg/mL Pseudoephedrine HCl

NCL-PSE-LCQ2 – Quantitation of Pseudoephedrine by High Performance Liquid Chromatography

Scope

Samples containing pseudoephedrine.

Procedure:

Accurately weigh the sample and dissolve in H_2O so that the concentration of the target analyte is bracketed between the high- and low-concentration QC solutions. For samples containing a large amount of insoluble material, it may be necessary to deliver the required amount of water via volumetric pipet instead of diluting to volume using the indicator line on a volumetric flask.

Centrifuge or filter sample solutions containing appreciable amounts of insoluble material prior to removing aliquot portions for serial dilutions or transferring solutions to injection vials.

Injection Solvent:

H_2O (HPLC grade or 18 MΩ deionized).

Buffer Preparation:

4000 mL HPLC grade or 18 MΩ water, 4.0 mL trifluoroacetic acid, 4.0 mL hexylamine, 4.0 mg sodium azide filtered through a Nylon 66 0.45 µm membrane. (pH ≈ 2-2.5)

Standard Solution:

Accurately weigh the pseudoephedrine hydrochloride reference material and dissolve in water so that the concentration of the pseudoephedrine is within the working range. Target concentration: 0.3 mg/mL.

Quality Control Solutions:

Prepare two QC solutions for use as positive controls during quantitative analysis. These two solutions are prepared such that their target analyte concentrations represent the low and high ends of the method's working range.

Chromatographic System:

Instrument: High Performance Liquid Chromatograph HP/Agilent 1200 Series equipped with a UV-DAD detector (or equivalent)
Column: Phenomenex Synergi 4µ Polar-RP 80Å, 150 × 4.60 mm
Mobile Phase: 100% Buffer (See limitation 3)
Flow: 1.5 mL/min
Total Run Time: 10.0 min
Detection: 210 nm
Injection Volume: 2.5 µL
Injection Solvent: Water

Limitations:

1. Although pseudoephedrine readily dissolves in H_2O, inorganic salts and some common tablet excipients are not soluble. This may require sonication in order to release all of the pseudoephedrine from the excipient matrix. These insoluble materials may be filtered out for further analysis if necessary.
2. Use extreme caution and wear the necessary personal protective equipment when working with concentrated trifluoroacetic acid (TFA) to prepare the buffer. It can cause severe burns if it comes in direct contact with the skin.
3. Triprolidine, guaifenesin, and caffeine do not elute under these conditions. A gradient ramp to 70:30 buffer:ACN must be added to the end of the method in order to elute these components. The large peak resulting from column bleed at the end of the ramp is not visible at the 254 nm, 280 nm, and 290 nm wavelengths. Caffeine is best seen with a 280 nm wavelength as it elutes closely to the column bleed peak. Always ensure to re-equilibrate the column at 100% buffer after a gradient ramp before injecting the next sample. The following gradient conditions table is designed to elute these compounds and may be modified as necessary:

Time (min)	Buffer (%)	ACN (%)
0.0	100	0
10.0	100	0
12.0	70	30
14.0	70	30
15.0	100	0
17.0	100	0

4. Loratadine requires a drop of HCl for dissolution in H_2O.

5. Pseudoephedrine coelutes with amphetamine. This method is not suitable for the quantitation of psuedoephedrine in samples containing amphetamine.
6. Methamphetamine elutes after the 10 minute isocratic run. If methamphetamine is present in the sample, use a 15 minute isocratic run.
7. Diphenhydramine is not detectable with this method.

Acceptance Criteria:

Selectivity: Pseudoephedrine resolved (R ≥1.5) from each compound tested including ephedrine, except as described in the limitations section.
Linearity: At least seven concentrations were within 95-105% overall average sensitivity (response/concentration) limits.
Repeatability: Relative Standard Deviation (RSD) for each concentration tested was less than 2%.
Accuracy: Experimentally measured purity (expressed in % w/w) within ± 5% relative to the known prepared purity.

Working Range:

0.04 – 1.0 mg/mL Pseudoephedrine HCl