DEA 101 – Quantitation of Cocaine by Gas Chromatography

Scope:

Samples containing cocaine hydrochloride and/or cocaine base

Sample Preparation:

Accurately weigh the sample and dissolve in Internal Standard Solution 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 internal standard solution 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.

Internal Standard Solution:

0.4 mg/mL n-tetracosane in chloroform/methanol (9:1).

Standard Solution:

Accurately weigh the cocaine hydrochloride reference material in Internal Standard Solution so that the concentration of the cocaine is within the working range.

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: Agilent 7890 Gas Chromatograph with Flame Ionization Detector, or equivalent
Column: HP-5 12 m × 0.20 mm I.D. × 0.33 µm, or equivalent
Injector Temperature: 270°C
Mode: Split
Split Ratio: 60:1
Carrier Gas and Flow Rate: Hydrogen, 1.0 mL/min
Oven Program: Isothermal at 230°C for 4 min, Ramp temperature 30 °C/min to 320 °C, hold for 3 min.
Total Run Time: 10 min
Detector Temperature: 280 °C
Signal Data (Sampling) Rate: 50 Hz
Injection Volume: 1 µL
Injection Solvent: chloroform/methanol (9:1)

Limitations:

Cocaine and tetracaine (critical resolution pair) do not resolve at oven temperatures greater than 240 °C. The highest oven temperature at which cocaine and tetracaine was experimentally determined to resolve at R > 1.5 was 235 °C.

Acceptance Criteria:

Selectivity: Cocaine and n-tetracosane 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:

0.195 – 2.002 mg/mL Cocaine

DEA 101S – Quantitation of Cocaine by Gas Chromatography

Scope:

Samples containing cocaine hydrochloride and/or cocaine base (DEA 101S is an approved modification of DEA 101)

Sample Preparation:

Accurately weigh the sample and dissolve in Internal Standard Solution 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 internal standard solution 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.

Internal Standard Solution:

0.4 mg/mL n-tetracosane in chloroform/methanol (9:1).

Standard Solution:

Accurately weigh the cocaine hydrochloride reference material in Internal Standard Solution so that the concentration of the cocaine is within the working range.

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: Agilent 7890 Gas Chromatograph with Flame Ionization Detector, or equivalent
Column: HP-5 12 m × 0.20 mm I.D. × 0.33 µm, or equivalent
Injector Temperature: 270 °C
Mode: Split
Split Ratio: 60:1
Carrier Gas and Flow Rate: Hydrogen, 1.0 mL/min
Oven Program: Isothermal at 230 °C for 4 min, Ramp temperature 30 °C/min to 290 °C.
Total Run Time: 6 min
Detector Temperature: 280 °C
Signal Data (Sampling) Rate: 50 Hz
Injection Volume: 1 µL
Injection Solvent: chloroform/methanol (9:1)

Limitations:

Not suitable for use with samples containing analytes eluting after 6 minutes such as hydroxyzine, diltiazem, or trazodone. Additionally, 3,4,5-trimethoxycocaine may not be observed as it elutes very close to the end of the run and may elute during the duty cycle. Cocaine and tetracaine (critical resolution pair) do not resolve at oven temperatures greater than 240 ° C. The highest oven temperature at which cocaine and tetracaine was experimentally determined to resolve at R > 1.5 was 235 °C.

Acceptance Criteria:

Selectivity: Cocaine and n-tetracosane 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:

0.195 – 2.002 mg/mL Cocaine

DEA 101L – Quantitation of Cocaine by Gas Chromatography-Low Thermal Mass Column Module

Scope:
Samples containing cocaine hydrochloride and/or cocaine base

Sample Preparation:
Accurately weigh the sample and dissolve in Internal Standard Solution 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 internal standard solution 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.

Internal Standard Solution:
0.4 mg/mL n-tetracosane in chloroform/methanol (9:1).

Standard Solution:
Accurately weigh the cocaine hydrochloride reference material in Internal Standard Solution so that the concentration of the cocaine is within the working range.

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: Agilent 7890 Gas Chromatograph equipped with Low Thermal Mass (LTM) Series II Column module and Flame Ionization Detector, or equivalent
In-segment (Pre-column): DB-5 1.0 m × 0.18 mm I.D. × 0.18 µm, or equivalent
LTM Column (Column 2): DB-5 long legs (Agilent special order) 15 m × 0.25 mm I.D. × 0.25 µm
Inlet (Injector) Temperature: 270°C
Mode: Split
Split Ratio: 50:1
Carrier Gas and Flow Rate: Hydrogen, 2.5 mL/min for 1.4 min, ramp 10 mL/min per min to 5 mL/min
Oven Program: 280°C ramp temperature 35 °C/min to 315 °C, hold for 1.5 min
LTM Temperature Program: 230°C for 1.3 min, ramp 150°C/min to 315°C
Total Run Time: 2.5 min
Detector Temperature: 300°C
Signal Data (Sampling) Rate: 50 Hz
Injection Volume: 1 µL
Injection Solvent: chloroform/methanol (9:1)

Limitations:
QC solutions prepared in internal standard solution expire 45 days after the date of preparation.

Acceptance Criteria:
Selectivity: Cocaine and n-tetracosane 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:
0.29810 – 3.0168 mg/mL Cocaine

DEA 102 – Quantitation of Heroin by Gas Chromatography

Scope:
Samples containing heroin hydrochloride

Sample Preparation:
Accurately weigh the sample and dissolve in Internal Standard Solution 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 internal standard solution 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.

Internal Standard Solution:
0.4 mg/mL n-tetracosane in chloroform/methanol (9:1).

Standard Solution:
Accurately weigh the heroin hydrochloride reference material in Internal Standard Solution so that the concentration of the heroin is within the working range.

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: Agilent 7890 Gas Chromatograph with Flame Ionization Detector, or equivalent
Column: HP-5 12 m × 0.20 mm I.D. × 0.33 µm, or equivalent
Inlet Temperature: 280 °C
Mode: Split
Split Ratio: 60:1
Carrier Gas and Flow Rate: Hydrogen, 1.0 mL/min for 2.5 min, ramp 45 mL/min to 4.5 mL/min, hold for 1.0 min
Oven Program: 270 °C for 2.5 min, ramp temperature 45 °C/min to 295 °C, hold for 1.0 min.
Total Run Time: 4.05 min
Detector Temperature: 280°C
Signal Data (Sampling) Rate: 50 Hz
Injection Volume: 1 µL
Injection Solvent: chloroform/methanol (9:1)

Limitations:
Dipyrone chromatographs as a large, broad hump at approximately 0.6 minutes. Chromatograms from samples containing dipyrone must be visually examined to ensure that there is no interference with n-tetracosane.

Acceptance Criteria:
Selectivity: Heroin and n-tetracosane 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:
0.200 – 1.996 mg/mL Heroin

DEA 102L – Quantitation of Heroin by Gas Chromatography-Low Thermal Mass Column Module

Scope:
Samples containing heroin hydrochloride

Sample Preparation:
Accurately weigh the sample and dissolve in Internal Standard Solution 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 internal standard solution 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.

Internal Standard Solution:
0.4 mg/mL n-tetracosane in chloroform/methanol (9:1).

Standard Solution:
Accurately weigh the heroin hydrochloride reference material in Internal Standard Solution so that the concentration of the heroin is within the working range.

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: Agilent 7890 Gas Chromatograph equipped with Low Thermal Mass (LTM) Series II Column Module and Flame Ionization Detector (or equivalent)
In-segment (Pre-column): DB-5 1.0 m × 0.18 mm I.D. × 0.18 µm, or equivalent
LTM Column (Column 2): DB-5 long legs (Agilent special order) 15 m × 0.25 mm I.D. × 0.25 µm
Inlet Temperature: 280°C
Mode: Split
Split Ratio: 50:1
Carrier Gas and Flow Rate: Hydrogen, 2.5 mL/min
Oven Program: 280 °C ramp temperature 35 °C/min to 315 °C, hold for 1 min
LTM Temperature Program: 225°C for 0.2 min, ramp 75°C/min to 315 °C
Total Run Time: 2.0 min
Detector Temperature: 300°C
Signal Data (Sampling) Rate: 50 Hz
Injection Volume: 1 µL
Injection Solvent: chloroform/methanol (9:1)

Limitations:
1. Samples with chloroquine must be promptly filtered after preparation. If not filtered, these samples must be run using a different instrumental technique.
2. Chromatograms from samples that contain dipyrone must be visually examined to ensure that the dipyrone is not interfering with n-tetracosane. If interference is observed, the sample must be diluted to decrease the dipyrone concentration or a different instrumental technique must be used.

Acceptance Criteria:
Selectivity: Heroin and n-tetracosane 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:
0.06020 – 2.98536 mg/mL Heroin

DEA 103 – Quantitation of Methamphetamine, 3,4-Methylenedioxymethamphetamine (MDMA), Ephedrine, and Pseudoephedrine by Gas Chromatography

Scope:

Samples containing methamphetamine hydrochloride, methamphetamine base, MDMA hydrochloride, ephedrine hydrochloride, and pseudoephedrine hydrochloride.

Sample Preparation:

Accurately weigh the sample and dissolve in Internal Standard Solution 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 internal standard solution via volumetric pipet instead of diluting to volume using the indicator line on a volumetric flask.

Basic extract the sample by removing a 1-2 mL aliquot of the solution to a test tube, then add an equal volume of a 2 N sodium hydroxide solution, vortex/shake and centrifuge or allow layers to separate. Remove the bottom layer (chloroform) for analysis. Filter the solution if necessary.

Do not perform the basic extraction procedure for methamphetamine base samples.

Internal Standard Solution:

0.4 mg/mL dimethyl phthalate in chloroform/methanol (4:1)

Standard Solution:

Accurately weigh the methamphetamine hydrochloride reference material in Internal Standard Solution so that the concentration of the methamphetamine is within the working range. Basic extract by removing a 1-2 mL aliquot of the standard solution to a test tube; add an equal volume of a 2 N sodium hydroxide solution; vortex/shake then centrifuge or allow layers to separate. Remove the bottom layer (chloroform) for analysis.

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. Perform basic extraction of QC solutions in same manner as sample or standard solutions.

Chromatographic System:

Instrument: Agilent 7890 Gas Chromatograph with Flame Ionization Detector, or equivalent
Column: HP-5 12 m × 0.20 mm I.D. × 0.33 µm, or equivalent
Inlet Temperature: 250°C
Mode: Split
Split Ratio: 60:1
Carrier Gas and Flow Rate: Hydrogen, 2.0 mL/min
Oven Program: 130 °C for 1.0 min, ramp 30 °C/min to 300°C; ramp 20 °C/min to 310°C; hold for 0.3333 min
Total Run Time: 7.5 min
Detector Temperature: 300°C
Signal Data (Sampling) Rate: 50 Hz
Injection Volume: 1 µL
Injection Solvent: chloroform/methanol (4:1)

Limitations:

1. Method does not resolve 1-(2,5-cyclohexadienyl)-2-methylaminopropane (Birch product #1) from methamphetamine and is not validated for the quantitation of methamphetamine in samples containing this compound. 1-(1,4-cyclohexadienyl)-2-methylaminopropane (CMP; Birch product #2) is readily resolved from methamphetamine with a resolution > 1.5; this method is suitable for quantitation of methamphetamine containing Birch product #2.
2. Samples of methamphetamine that also contain phentermine need to be evaluated to ensure the two peaks are resolved.
3. Method must not be used for MDMA samples that also contain 1-(3-trifluoromethylphenyl)piperazine (TFMPP).
4. Method must not be used for ephedrine and pseudoephedrine when both compounds are present in the sample.

5. Method must not be used for pseudoephedrine when nicotinamide is also present.
6. Samples of ephedrine that also contain nicotinamide need to be evaluated to ensure the two peaks are resolved.

Acceptance Criteria:

Selectivity: Target analytes and dimethyl phthalate resolved (R ≥1.5) from each compound tested, except as noted in Limitations.
Linearity: At least nine 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:

0.2448 – 2.9357 mg/mL Methamphetamine Hydrochloride
0.1967 – 2.4216 mg/mL Methamphetamine Base
0.1483 – 2.8560 mg/mL MDMA Hydrochloride
0.2972 – 2.9345 mg/mL Ephedrine Hydrochloride
0.3020 – 2.9096 mg/mL Pseudoephedrine Hydrochloride

DEA 103L – Quantitation of Methamphetamine by Gas Chromatography-Low Thermal Mass Column Module

Scope:
Samples containing methamphetamine hydrochloride and methamphetamine base

Sample Preparation:
Accurately weigh the sample and dissolve in Internal Standard Solution 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 internal standard solution via volumetric pipet instead of diluting to volume using the indicator line on a volumetric flask.

Basic extract the sample by removing a 1-2 mL aliquot of the solution to a test tube, then add an equal volume of a 2 N sodium hydroxide solution, vortex/shake and centrifuge or allow layers to separate. Remove the bottom layer (chloroform) for analysis. Filter the solution if necessary.

Do not perform the basic extraction procedure for methamphetamine base samples.

Internal Standard Solution:
0.4 mg/mL dimethyl phthalate in chloroform/methanol (4:1)

Standard Solution:
Accurately weigh the methamphetamine hydrochloride reference material in Internal Standard Solution so that the concentration of the methamphetamine is within the working range. Basic extract by removing a 1-2 mL aliquot of the standard solution to a test tube; add an equal volume of a 2 N sodium hydroxide solution; vortex/shake then centrifuge or allow layers to separate. Remove the bottom layer (chloroform) for analysis.

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. Perform basic extraction of QC solutions in same manner as sample or standard solutions.

Chromatographic System:
Instrument: Agilent 7890 Gas Chromatograph equipped with Low Thermal Mass (LTM) Series II Column Module and Flame Ionization Detector, or equivalent
In-segment (Pre-column): DB-5 1.0 m × 0.18 mm I.D. × 0.18 µm, or equivalent
LTM Column (Column 2): DB-5 MS UI long legs (Agilent special order) 15 m × 0.25 mm I.D. × 0.25 µm
Inlet Temperature: 250°C
Mode: Split
Split Ratio: 60:1
Carrier Gas and Flow Rate: Hydrogen, 2.0 mL/min for 1.5 min, ramp 10 mL/min per min to 5 mL/min
Oven Program: 230 °C for 1.0 min, ramp temperature 30 °C/min to 275 °C
LTM Temperature Program: 130°C for 1.0 min, ramp 250°C/min to 310°C
Total Run Time: 2.5 min
Detector Temperature: 300°C
Signal Data (Sampling) Rate: 50 Hz
Injection Volume: 1 µL
Injection Solvent: chloroform/methanol (4:1)

Limitations:
1. The data for samples with CMP/Birch reduction by-products must be carefully inspected to ensure sufficient resolution between methamphetamine and other by-products.
2. The methamphetamine peak shape may limit the upper concentration limit of the method, particularly when new unions (part #G3182-20580 from ultimate union kit #G3182-61581) are installed. Conditioning of the new union may improve the methamphetamine peak shape resulting in a larger working concentration range.

Acceptance Criteria:
Selectivity: Methamphetamine and dimethyl phthalate 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:
0.06068 – 3.03415 mg/mL Methamphetamine

DEA 105 – Quantitation of Oxycodone Hydrochloride by Gas Chromatography

Scope:
Samples containing oxycodone hydrochloride

Sample Preparation:
Accurately weigh the sample into a volumetric flask and dilute to approximately ½ volume using Internal Standard Solution. Sonicate the solution, then complete the dilution by adding internal standard solution. If necessary, perform a serial dilution using internal standard solution so that the concentration of the target analyte is within the acceptable working range. Filter the solution if necessary. Sonication should take place for all solutions prior to completing the dilution of the sample.

Internal Standard Solution:
0.4 mg/mL n-tetracosane in chloroform/methanol (9:1)

Standard Solution:
Accurately weigh the oxycodone hydrochloride reference material in Internal Standard Solution so that the concentration of the oxycodone is within the working range.

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: Agilent 7890 Gas Chromatograph with Flame Ionization Detector, or equivalent
Column: HP-5 12 m × 0.20 mm I.D. × 0.33 µm, or equivalent
Inlet Temperature: 280°C
Mode: Split
Split Ratio: 60:1
Carrier Gas and Flow Rate: Hydrogen, 1.0 mL/min for 2.5 min, ramp 45 mL/min to 4.5 mL/min, hold for 1.0 min
Oven Program: 270°C for 2.5 min, ramp temperature 45°C/min to 295°C, hold for 1.0 min.
Total Run Time: 4.05 min
Detector Temperature: 280°C
Signal Data (Sampling) Rate: 50 Hz
Injection Volume: 1 µL
Injection Solvent: chloroform/methanol (9:1)

Limitations:
1. This method is not appropriate for oxycodone tablets containing gelling agents (i.e., OxyContin with "OP" logo). A different instrumental technique (LC, CE, NMR) should be used for these formulations.
2. Pharmaceutical preparations of low-purity oxycodone may cause some solubility issues with oxycodone hydrochloride. These solutions should use volumetric pipettes for solution delivery, as the use of volumetric flasks will result in an incorrect volume due to the displacement caused by the insoluble material (i.e., 10 mL, 25 mL). Additionally, to effectively insure the complete solubility of oxycodone, the sample preparation of the solutions includes sonication.
3. Palmitic acid co-elutes with caffeine.
4. All samples containing O6-MAM and oxycodone must be visually examined to make sure the peaks are baseline resolved. The samples may calculate as R>1.5 but may not be visually resolved.

Acceptance Criteria:
Selectivity: Oxycodone and n-tetracosane 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:
0.05969 – 1.9127 mg/mL Oxycodone

DEA 107L – Quantitation of Fentanyl by Gas Chromatography-Low Thermal Mass Column Module

Scope:

Samples containing fentanyl hydrochloride or fentanyl citrate (with option for base extraction)

Sample Preparation:

Accurately weigh the sample and dissolve in the Internal Standard Solution. If necessary, perform a serial dilution using internal standard solution so that the concentration of the target analyte is within the low and high concentration of QC solutions. Filter the solution if an appreciable amount of insoluble material is present.

Base extraction: Pipet a 3 mL aliquot of the sample solution into a test tube. Add a 1 mL aliquot of 10% sodium hydroxide or 2 N sodium hydroxide solution to the test tube. Thoroughly mix the solution and allow the two layers to separate. Remove the chloroform layer (bottom) for analysis. The chloroform layer may be passed through sodium sulfate or filtered through a 0.2 µm filter for analysis.

Internal Standard Solution:

0.4 mg/mL n-tetracosane in chloroform/methanol (9:1)

Standard Solution:

Accurately weigh the fentanyl hydrochloride reference material in Internal Standard Solution so that the concentration of the fentanyl is within the working range. Perform base extraction if necessary.

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. Perform base extraction if necessary.

Chromatographic System:

Instrument: Agilent 7890 Gas Chromatograph equipped with Low Thermal Mass (LTM) Series II Column Module and Flame Ionization Detector, or equivalent
In-segment (Pre-column): DB-5 1.0 m × 0.18 mm I.D. × 0.18 µm, or equivalent
LTM Column (Column 2): DB-5 long legs (Agilent special order) 15 m × 0.25 mm I.D. × 0.25 µm
Inlet Temperature: 280°C
Mode: Split
Split Ratio: 50:1
Carrier Gas and Flow Rate: Hydrogen, 2.5 mL/min
Oven Program: 280°C ramp temperature 35°C/min to 315°C, hold for 1 min
LTM Temperature Program: 225°C for 0.2 min, ramp 75°C/min to 315°C
Total Run Time: 2.0 min
Detector Temperature: 300°C
Signal Data (Sampling) Rate: 50 Hz
Injection Volume: 1 µL
Injection Solvent: chloroform/methanol (9:1)

Limitations:

1. Samples with a significant amount of acetaminophen must incorporate a base extraction procedure prior to injection.
2. This method did not evaluate pharmaceutical preparations; therefore, this method is not to be used to quantitate fentanyl pharmaceutical preparations.
3. This method is not suitable for the quantitation of aqueous preparations of fentanyl citrate.
4. Chromatograms from samples that contain dipyrone must be visually examined to ensure that the dipyrone is not interfering with the n-tetracosane (ISTD) peak. If interference is observed, the sample must be diluted to decrease the dipyrone concentration, or a different instrumental technique must be used.

Acceptance Criteria:

Selectivity: Fentanyl and n-tetracosane 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:
0.04592 – 1.452546 mg/mL Fentanyl

DEA 108 – Quantitation of Hydrocodone Bitartrate by Gas Chromatography

Scope:
Samples containing hydrocodone bitartrate

Sample Preparation:
Accurately weigh the sample and dissolve in Internal Standard Solution 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 internal standard solution via volumetric pipet instead of diluting to volume using the indicator line on a volumetric flask. Centrifuge or filter sample solutions containing appreciable amount of insoluble material prior to removing aliquot portions for serial dilutions or transferring solutions to injection vials.

Internal Standard Solution:
0.4 mg/mL n-tetracosane in chloroform/methanol (1:1)

Standard Solution:
Accurately weigh the hydrocodone bitartrate reference material in Internal Standard Solution so that the concentration of the hydrocodone is within the working range.

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: Agilent 7890 Gas Chromatograph with Flame Ionization Detector, or equivalent
Column: DB-5 12 m × 0.20 mm I.D. × 0.33 µm, or equivalent
Inlet Temperature: 280°C
Mode: Split
Split Ratio: 60:1
Carrier Gas and Flow Rate: Hydrogen, 1.0 mL/min
Oven Program: 250°C for 2.0 min, ramp 30°C/min to 300°C, hold for 1.0 min
Total Run Time: 4.6 min
Detector Temperature: 280°C
Signal Data (Sampling) Rate: 50 Hz
Injection Volume: 1 µL
Injection Solvent: chloroform/methanol (1:1)

Limitations:
1. All samples containing mixtures of hydrocodone with diazepam and/or morphine must be visually examined to ensure the peaks are baseline resolved.
2. This method was not evaluated for liquid pharmaceutical preparations.
3. This method was not evaluated for extended-release hydrocodone tablets or capsules.

Acceptance Criteria:
Selectivity: Hydrocodone and n-tetracosane 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:
0.11 – 1.99 mg/mL Hydrocodone

DEA 127 – Quantitation of Heroin and Fentanyl by Gas Chromatography

Scope:

Samples containing heroin hydrochloride, fentanyl hydrochloride, and/or fentanyl citrate.

Sample Preparation:

Accurately weigh the sample and dissolve in Internal Standard Solution 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 internal standard solution via volumetric pipet instead of diluting to volume using the indicator line on a volumetric flask. Centrifuge or filter sample solutions containing appreciable amount of insoluble material prior to removing aliquot portions for serial dilutions or transferring solutions to injection vials.

Internal Standard Solution:

0.4 mg/mL n-tetracosane in chloroform/methanol (9:1)

Standard Solution:

Accurately weigh the heroin hydrochloride, fentanyl hydrochloride and/or fentanyl citrate reference material in Internal Standard Solution so that the concentration of the heroin and/or fentanyl is within the working range.

Quality Control Solutions:

Prepare two QC solutions for heroin and and/or fentanyl 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: Agilent 7890 Gas Chromatograph with Flame Ionization Detector, or equivalent
Column: HP-5 12 m × 0.20 mm I.D. × 0.33 µm, or equivalent
Inlet Temperature: 270°C
Mode: Split
Split Ratio: 50:1
Carrier Gas and Flow Rate: Hydrogen, 1.5 mL/min
Oven Program: 230°C for 1 min, ramp temperature 20°C/min to 300°C, hold for 0.5 min
Total Run Time: 5 min
Detector Temperature: 300°C
Signal Data (Sampling) Rate: 50 Hz
Injection Volume: 1 µL
Injection Solvent: chloroform/methanol (9:1)

Limitations:

1. Fentanyl and 3-Methylfentanyl (trans-isomer) co-elute on this method.
2. Dipyrone chromatographs as a large, broad hump. Chromatograms from samples containing dipyrone must be visually examined to ensure that there is no interference with n-tetracosane.
3. This method did not evaluate pharmaceutical preparations; therefore, this method is not to be used to quantitate fentanyl pharmaceutical preparations such as transdermal patches or lollipops.
4. This method did not evaluate aqueous preparations of fentanyl and/or heroin; therefore, this method is not to be used to quantitate aqueous samples.

Acceptance Criteria:

Selectivity: Heroin and n-tetracosane resolved (R ≥1.5) from each compound tested. Fentanyl resolved (R ≥1.5) from each compound tested, except 3-methylfentanyl (trans-isomer) as noted in Limitations.
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:

0.060324 – 3.00601 mg/mL Heroin
0.25 – 1.393 mg/mL Fentanyl

DEA 201 – Quantitation of Cocaine by High Performance Liquid Chromatography

Scope:
Samples containing cocaine hydrochloride and/or cocaine base

Sample Preparation:
Accurately weigh the sample into a volumetric flask and dilute to volume using Injection Solvent 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 internal standard solution via volumetric pipet instead of diluting to volume using the indicator line on a volumetric flask. If necessary, perform a serial dilution using Injection Solvent so that the concentration of the target analyte is within the acceptable working range. Filter the solution through a 0.45 µm filter.

Injection Solvent:
0.01 N hydrochloric acid (10% methanol for cocaine base)

Buffer Preparation:
To 4 L of water, add 30 mL phosphoric acid, 10.0 g sodium hydroxide, 8 mL hexylamine, and 100 mg sodium azide (pH 2.5). No pH adjustment is necessary.

Standard Solution:
Accurately weigh the cocaine hydrochloride reference material in Injection Solvent so that the concentration of the cocaine is within the working range.

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: Agilent 1200 Series High Performance Liquid Chromatograph, or equivalent
Column: Phenomenex Luna C18 Column: 50 mm x 3 mm x 3 µm, 100 Å porous size, or equivalent
Column Temperature: 50°C
Buffer Solution Shelf-life: 60 days
Gradient Program:

- 0.0 – 3.5 min: 82:18 buffer/acetonitrile (0.50 mL/min)
- 3.5 – 4.5 min: 82:18 to 75:25 buffer/acetonitrile (1.0 mL/min)
- 4.5 – 8.0 min: 75:25 buffer/acetonitrile (1.0 mL/min)
- 8.0 – 9.0 min: 75:25 to 82:18 buffer/acetonitrile (1.0 mL/min)
- 9.0 – 10.0 min: 82:18 buffer/acetonitrile (1.0 mL/min)

Total Run Time: 10.0 min
Detection: 233 nm (10 nm bandwidth); reference: 550 nm (100 nm bandwidth); peak width > 0.05 min
Signal Data (Sampling) Rate: 5.0 Hz
Injection Volume: 1 µL
Injection Solvent: 0.01 N hydrochloric acid (10% methanol for cocaine base).

Limitations:
1. Cocaine does not resolve from heroin (R < 1.5) at cocaine concentrations greater than 1.55 mg/mL.
2. Antipyrine coelutes with cocaine.
3. Cocaine base must be dissolved in methanol (10% total volume) prior to dilution in 0.01 N HCl.

Acceptance Criteria:
Selectivity: Cocaine resolved (R ≥1.5) from each compound tested except for the compounds noted in Limitations.
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:
0.222 – 2.022 mg/mL Cocaine

DEA 202 – Quantitation of Heroin by High Performance Liquid Chromatography

Scope:
Samples containing heroin hydrochloride

Sample Preparation:
Accurately weigh the sample into a volumetric flask and dilute to volume using Injection Solvent 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 internal standard solution via volumetric pipet instead of diluting to volume using the indicator line on a volumetric flask. If necessary, perform a serial dilution using Injection Solvent so that the concentration of the target analyte is within the acceptable working range. Filter the solution through a 0.45 µm filter.

Injection Solvent:
Methanol

Buffer Preparation:
To 4 L of water, add 30 mL phosphoric acid, 10.0 g sodium hydroxide, 8 mL hexylamine, and 100 mg sodium azide (pH 2.5). No pH adjustment is necessary.

Standard Solution:
Accurately weigh the heroin hydrochloride reference material in Injection Solvent so that the concentration of the heroin is within the working range.

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: Agilent 1200 Series High Performance Liquid Chromatograph, or equivalent
Column: Phenomenex Luna C18 Column: 50 mm x 3 mm x 3 µm, 100 Å porous size, or equivalent
Column Temperature: 35°C
Buffer Solution Shelf-life: 60 days
Gradient Program:

- 0.0 – 3.5 min: 89:11 buffer/acetonitrile (1.0 mL/min)
- 3.5 – 4.0 min: 89:11 to 75:25 buffer/acetonitrile (1.0 mL/min)
- 4.0 – 7.5 min: 75:25 buffer/acetonitrile (1.0 mL/min)
- 7.5 – 8.0 min: 75:25 to 89:11 buffer/acetonitrile (1.0 mL/min)
- 8.0 – 10.0 min: 89:11 buffer/acetonitrile (1.0 mL/min)

Total Run Time: 10.0 min
Detection: 210 nm (5 nm bandwidth); reference: 360 nm (100 nm bandwidth); peak width > 0.05 min
Signal Data (Sampling) Rate: 2.5 Hz
Injection Volume: 1 µL
Injection Solvent: Methanol

Limitations:
1. Heroin does not resolve from guaifenesin (R < 1.5).
2. Alprazolam and tetracaine do not elute within method run time; subsequent wash is required to prevent carryover between analyses.

Acceptance Criteria:
Selectivity: Heroin resolved (R ≥1.5) from each compound tested except for guaifenesin as noted in Limitations.
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:
0.046 – 1.077 mg/mL Heroin

DEA 250 – Quantitation of Δ^9-Tetrahydrocannabinol (THC) and Δ^9-Tetrahydrocannabinolic Acid (THCA) by Liquid Chromatography

Scope:

Samples containing Δ^9-Tetrahydrocannabinol (THC) and Δ^9-Tetrahydrocannabinolic Acid (THCA)

Sample Preparation:

1. Grind at least 200 mg of dry plant material and then sieve the material through a 40-mesh screen (425 µm particle size)
2. Weigh two separate portions of 100 mg of the material from Step 1 into two separate centrifuge tubes
3. Add 5 mL of Injection Solvent into each centrifuge tube and vortex for 2-3 seconds
4. Sonicate for 15 minutes
5. Centrifuge at 1000 rpm for 2 minutes
6. Transfer each supernatant into a 10 mL volumetric flask and dilute to mark using Injection Solvent
7. If necessary, perform a second dilution using Injection Solvent to attain target concentration
8. Pass the final solutions through a 0.45 µm filter into an injection vial.

Injection Solvent:

80% Acetonitrile / 20% Methanol

Standard Solution:

Cayman Phytocannabinoid Mixture 5 (CRM)

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: Shimadzu LC-2030C Plus Cannabis Analyzer
Column: Shimadzu Nexleaf CBX for Potency: 150 mm x 4.6 mm x 2.7 µm
Column Temperature: 35°C
Injection Volume: 5 µL
Mobile Phase: A: 0.085% H_3PO_4 in water; B: 0.085% H_3PO_4 in Acetonitrile
Gradient Program:

- 0.00 – 3.00 min: 30:70 A/B
- 3.00 – 7.00 min: 30:70 A/B to 15:85 A/B
- 7.00 – 7.01 min: 15:85 A/B to 5:95 A/B
- 7.01 – 8.00 min: 5:95 A/B
- 8.00 – 8.01 min: 5:95 A/B to 30:70 A/B
- 8.01 – 10.0 min: 30:70 A/B

Autosampler Temperature: 4 °C
Flow: 1.6 ml/min
Detection: 220 nm
Sampling Period: 200 msec
Peak Width: > 5 s

Limitations:

Δ^8-THC and Δ^9-THC are not baseline resolved when both compounds are present in similar concentration. If Δ^8-THC is present in the sample at high concentration, resolution between Δ^8-THC and Δ^9-THC should be greater than 1.3.

Acceptance Criteria:

Selectivity: Δ^9-THC and Δ^9-THCA are 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%.
Recovery: Experimentally measured within 92-102%.

Working Range:

29.2 – 125 µg/mL Δ^9-THC
13.1 – 250 µg/mL Δ^9-THCA

DEA 273 – Quantitation of Methamphetamine by Ultra-High Performance Liquid Chromatography

Scope:
Samples containing only methamphetamine hydrochloride or mixtures containing only methamphetamine hydrochloride and dimethyl sulfone

Sample Preparation:
Accurately weigh the sample into a volumetric flask and dilute to volume using Injection Solvent 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 internal standard solution via volumetric pipet instead of diluting to volume using the indicator line on a volumetric flask. If necessary, perform a serial dilution using Injection Solvent so that the concentration of the target analyte is within the acceptable working range. Filter the solution through a 0.2-0.45 µm filter.

Injection Solvent:
85 mM sodium phosphate buffer (pH ~1.8)

Buffer Preparation:
Stock: Add 44 mL H_3PO_4 (85%) and 5.4 grams sodium hydroxide (pellets) to 4 L deionized water.
Working: Dilute 530 mL of stock to 1 L deionized water. Filter working buffer through a 0.2 µm filter.

Standard Solution:
Accurately weigh the methamphetamine hydrochloride reference material in Injection Solvent so that the concentration of the methamphetamine is within the working range.

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: Waters Acquity Ultra Performance Liquid Chromatograph equipped with photo diode array detector, or equivalent
Column: Waters BEH C18, 10 cm, 2.1 mm id, 1.7 µm, or equivalent
Column Temperature: 30°C
Injection Parameters: 5.0 µL, partial loop injection with needle overfill
Injection Solvent: Buffer
Sample Loop Volume: 10 µL
Strong Needle Wash: Option 1) 200 µL acetonitrile; Option 2) 2000 µL methanol
Weak Needle Wash: Option 1) 600 µL 90% water:10% acetonitrile; Option 2) 6000 µL 90% water:10% acetonitrile
Seal Washes: 5.0 min
Autosampler Temperature: Not regulated
Mobile Phase: 80% buffer: 20% acetonitrile. Buffer prepared as listed above. Mobile phase constituents combined by instrument.
Flow: 0.45 mL/min
Gradient Program: Isocratic
Detection Wavelength: 210 nm with 4.8 nm resolution
Sampling Rate: 20 points/s
Total Run Time: 1.2 min

Limitations:
This method is not suitable for samples containing substances other than methamphetamine hydrochloride and dimethyl sulfone.

Acceptance Criteria:
Selectivity: Methamphetamine 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:
0.01642 – 0.14078 mg/mL Methamphetamine

DEA 275 – Quantitation of Oxycodone by Ultra-High Performance Liquid Chromatography

Scope:

Samples containing oxycodone hydrochloride

Sample Preparation:

Accurately weigh the sample into a volumetric flask and dilute to volume using Injection Solvent 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 internal standard solution via volumetric pipet instead of diluting to volume using the indicator line on a volumetric flask. If necessary, perform gravimetric dilutions using Injection Solvent so that the concentration of the target analyte is within the acceptable working range. Filter the solution through a 0.2-0.45 µm filter.

Injection Solvent:

10 mM ammonium formate buffer with TFA, pH 3.7

Buffer Preparation:

To prepare 1 L of buffer:
Add 0.6306 g of ammonium formate, ≥99.995% trace metals basis, to 1 L deionized water. Add 2.3 mL (3.4 g) of TFA, LC-MS Ultra, eluent additive for UHPLC-MS, to the liter of buffer. Adjust the pH to approximately 3.7 using approximately 3.7 mL of 28-30% ammonium hydroxide. Filter buffer through a 0.2µm filter.

To prepare 4 L of buffer:
Add 2.5224 g of ammonium formate to 4 L deionized water. Add 9.2 mL (13.6 g) of TFA to the liter of buffer. Adjust the pH to approximately 3.7 using approximately 8.7 mL of 28-30% ammonium hydroxide. Filter buffer through a 0.2 µm filter.

Standard Solution:

Accurately weigh the oxycodone hydrochloride reference material in Injection Solvent so that the concentration of the oxycodone is within the working range.

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: Waters Acquity I-Class Ultra Performance Liquid Chromatograph, equipped with photo diode array detector, or equivalent
Column: Waters BEH C18, 10 cm, 2.1 mm id, 1.7 µm, or equivalent
Column Temperature: 25°C
Injection Parameters: 1.0 µL (using a Flow-through needle)
Injection Solvent: Buffer
Strong Needle Wash: Methanol
Weak Needle Wash: 90% water/10% acetonitrile
Pre-Inject Wash Time: 0.0 (sec)
Post-Inject Wash Time: 6.0 (sec)
Purge Solvent Name: Acetonitrile
Seal Washes: 5.0 min
Autosampler Temperature: Not regulated
Mobile Phase: 90% buffer: 10% acetonitrile. Buffer prepared as listed above. Mobile phase constituents combined by instrument. Gradient program listed below.
Flow: 0.35 mL/min
Gradient Program:

	Time (min)	Flow Rate	%A	%B	Curve
1	Initial	0.350	90.0	10.0	Initial
2	2.00	0.350	50.0	50.0	6
3	2.60	0.350	90.0	10.0	6
4	3.00	0.350	90.0	10.0	11

Detection Wavelength: Channel 1: 235 nm with 4.8 nm resolution, Absorbance - Compensated, Compensation Reference Range 300-400 nm
Sampling Rate: 20 points/s
Total Run Time: 3.0 min

Limitations:

1. Acetaminophen tailing will interfere with oxycodone at high concentrations. Samples containing pharmaceutical preparations of oxycodone and acetaminophen should be evaluated to ensure peak tailing from acetaminophen does not interfere with the

integration of the oxycodone peak. Final concentrations of oxycodone samples containing acetaminophen are suggested to not exceed 0.18 mg/mL; this applies to the QC samples and calibrant used.

2. Oxycodone, O^3-MAM, and O^6-MAM co-elute; therefore, this method is not suitable for oxycodone samples containing heroin or heroin alkaloids.
3. This method is not suitable for oxycodone samples containing naproxen.

Acceptance Criteria:

Selectivity: Oxycodone resolved (R ≥1.5) from each compound tested except those listed in Limitations.
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:

0.01414 – 0.60085 mg/mL Oxycodone

DEA 440H/450H/460H – Quantitation by Proton Nuclear Magnetic Resonance Spectroscopy (q-HNMR)

Scope:

Validated proton NMR quantitations with the following Internal Standard/Solvent combinations:

- Maleic Acid/D2O
- Maleic Acid/CD3OD

Sample Preparation:

Internal Standard Solution:

Accurately weight the sample and add an accurate volume of internal standard solution containing a known concentration of calibrant. The internal standard solution is normally approximately 5 mg/mL maleic acid in D2O with trimethylsilylpropanoic acid (TSP) as the zero ppm reference. Thoroughly mix the solution. If no insoluble material is observable, then transfer at least 0.7 mL of solution to an NMR tube and acquire a qHNMR spectrum.

If insoluble material is present, then either prepare a second solution at a difference concentration, or do the following. Transfer at least 0.7 mL of solution is free of insoluble material, add solvent that does not contain the calibrant or zero ppm reference substances. Thoroughly mix the solution, transfer at least 0.7 mL of filtered solution to an NMR tube, and acquire a qHNMR spectrum.

OR

Calibrant Weighed:

Accurately weigh two separate portions of the sample. Add an accurately weighed amount of calibrant and appropriate amount of solvent to each to ensure the sample concentration is significantly different between the two solutions. Mix each of the solutions thoroughly. Transfer at least 0.7 mL of filtered (if insoluble material is present) solution to an NMR tube and acquire qHNMR spectra of each solution.

Quality Control Solutions:

Prepare one QC solution containing a compound with known purity that is validated for the same calibrant and solvent as the analyte.

Experimental Settings:

Instrument: Agilent NMR, or equivalent
MHz: 400, 500, 600
Experiment: Proton
Pulse Angle: ≤ 90 degrees
Pulse Width: ≤ 10 µs
Spectral Width Encompasses: -1 to 11 ppm
Acquisition Time: ≥ 5 seconds
Delay Between Pulses: ≥ 5xT1 (default is 45 seconds)

Limitations:

The analyte is not physically separate from other compounds in the solution, so signal overlap is possible. The internal calibrant signal and at least one integral region of the analyte must be free of overlapping signals.

Acceptance Criteria:

90° Pulse Width and Spectral Width: The 90° pulse width is less than 10 µs.
Quantitative Spectral Region Uniformity: Relative Standard Deviation (RSD) of peak heights between zero and 10 ppm was less than 3%.
Linearity: The correlation coefficient of the instrument method was greater than 0.998.
Repeatability: RSD for the method repeatability test was less than 2%.
Accuracy: Experimentally measured purity was within ± 5% relative to the known prepared purity.
Analyte Stability: Analyte purity results had less than 1% deviation per hour.
Selectivity: Validation is performed on each sample spectrum. The lowest integral result is believed to be the most free from possible interfering signals. This result is verified by agreement to either at least two additional integral regions, or the peak height result.
Analyte Solubility: If insoluble material is present in the sample solution, then the purity results of the two spectra must be within the uncertainty of measurement estimate for quantitative values.
Calibrant Concentration: If an internal standard solution is utilized, then the ratio of internal calibrant to the zero point compound between the sample and negative control spectra must agree to within 5%. If an internal standard solution is not utilized, then the purity result of the two sample spectra must agree to within the uncertainty of measurement estimate for quantitative values.

Working Range:

Signal to noise greater than 50:1 and sample concentration less than 100 mg/mL (preferably less than 50 mg/mL).

DEA 503 – Quantitation of Methamphetamine Hydrochloride by UV/Vis Spectroscopy

Scope:

Samples containing only methamphetamine hydrochloride or mixtures containing methamphetamine hydrochloride and the following substances: dimethyl sulfone, boric acid, and/or sodium chloride

Sample Preparation:

Dissolve samples in deionized water using class-A glassware or calibrated micro pipettes. Solutions should be prepared so that the final target analyte concentrations are bracketed by simultaneously analyzed QC solutions.

Standard Solution:

Accurately weigh the methamphetamine hydrochloride reference material in deionized water so that the concentration of the methamphetamine is within the working range. Two separate stock solutions are prepared for 18-Cell Cuvette Changer and the Fiber Optic Dip Probe configurations, respectively.

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.

Method Parameters:

Instrument: Agilent Cary 60 UV/Vis Spectrometer
Configurations: 18-Cell Cuvette Chamber or Fiber Optic Dip Probe
Detection wavelength: 267 nm
Collection time: 0.5 s
Replicates: 5
Total Run Time: 2.5 s

Limitations:

1. This method is only suitable for quantitation of methamphetamine hydrochloride in deionized water using 267 nm as the detection wavelength, when there are no other compounds with significant absorption at that wavelength, such as caffeine, cocaine hydrochloride, creatine, α-Benzyl-N-methylphenethylamine (B-compound), or phenethylamine.
2. Solutions should be analyzed within 24 hours of preparation. If solutions cannot be analyzed within such time frame, refrigeration is recommended. The absorbance of the solutions should be evaluated upon preparation and during refrigerated storage in order to monitor any response changes.

Acceptance Criteria:

Selectivity: The absorption of each tested solution (negative controls, component solutions, and mixtures of methamphetamine hydrochloride with selected compounds) at 267 nm was compared to the absorption measured for methamphetamine hydrochloride at similar concentration. Compounds with interferences measured above 2% relative to the absorption of methamphetamine are listed as a limitation.
Linearity: At least seven concentrations were within 95-105% overall average sensitivity (response/concentration) limits for each instrument configuration.
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:

18-Cell Cuvette Chamber: 1.3707 – 4.9790 mg/mL Methamphetamine

Fiber Optic Dip Probe: 0.5401 – 3.9872 mg/mL Methamphetamine

4MDMA3 – Quantitation of 3,4-MDMA by Gas Chromatography

Scope:
Samples containing 3,4-Methylenedioxymethamphetamine (3,4-MDMA)

Sample Preparation:
Accurately weigh the sample into a volumetric flask and dilute to volume with deionized water. The sample solution should be prepared such that the 3,4-MDMA concentration is within the established working range and between the high- and low-concentration QC solutions. A 2 mL aliquot of the sample solution is then extracted with 2 mL of 1 N sodium hydroxide into 2 mL of the Internal Standard Solution.

Internal Standard Solution:
0.7 mg/mL tetradecane in methylene chloride

Standard Solution:
Accurately weigh the 3,4-MDMA reference material in deionized water so that the concentration of the 3,4-MDMA is within the working range. A 2 mL aliquot of the standard solution is then extracted with 2 mL of 1 N sodium hydroxide into 2 mL of the Internal Standard Solution.

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: Agilent 6890 Gas Chromatograph with an Flame Ionization Detector, or equivalent
Column: 100% Dimethylpolysiloxane, 30m x 0.25 mm i.d. x 0.25 µm
Inlet Temperature: 270°C
Mode: Split
Split Ratio: 100:1
Carrier Gas and Flow Rate: Hydrogen, 59 cm/s
Oven Program: Isothermal at 160°C
Total Run Time: 4 min
Detector Temperature: 250°C
Signal Data (Sampling) Rate: 50 Hz
Injection Volume: 1 µL
Injection Solvent: methylene chloride

Limitations:
N/A

Acceptance Criteria:
Selectivity: 3,4-MDMA and n-tetradecane 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:
0.2075 – 2.3733 mg/mL 3,4-MDMA

6OXY-LCM – Quantitation of Oxycodone by High Performance Liquid Chromatography

Scope:

Samples containing oxycodone hydrochloride

Sample Preparation:

Accurately weigh the sample into a volumetric flask and dilute to volume using Injection Solvent 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 internal standard solution via volumetric pipet instead of diluting to volume using the indicator line on a volumetric flask. If necessary, perform a serial dilution using Injection Solvent so that the concentration of the target analyte is within the acceptable working range. Filter the solution through a 0.45 µm syringe filter.

Injection Solvent:

0.1N hydrochloric acid

Buffer Preparation:

Accurately measure out 4000mL of Millipore water then add 10.00g of sodium hydroxide pellets. Measure 30mL of phosphoric acid. Add the phosphoric acid to the solution and do not rinse out the graduated cylinder. Using a pipette measure 8mL of hexylamine and add to the solution. Using a calibrated pH meter, measure the pH of the solution and adjust the pH to 2.5 with a sodium hydroxide solution or phosphoric acid.

Standard Solution:

Accurately weigh the oxycodone hydrochloride reference material in Injection Solvent so that the concentration of the oxycodone is within the working range.

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: Liquid Chromatograph with photo diode array detector
Column: C18 150 mm x 4.60 mm x 5 µm
Column Temperature: 50°C
Injection Parameters: 10 µL
Injection Solvent: Buffer
Mobile Phase: 85% buffer/15% acetonitrile. Buffer prepared as listed above. Mobile phase constituents combined by instrument.
Flow: 1.0 mL/min
Detection Wavelength: Diode array@ 280nm, 4nm BW; 550nm Ref, 80nm BW
Total Run Time: 5.0 min

Limitations:

N/A

Acceptance Criteria:

Selectivity: Oxycodone resolved (R ≥1.5) from each compound.
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 3%.
Accuracy: Experimentally measured purity (expressed in % w/w) within ± 5% relative to the known prepared purity.

Working Range:

0.10 – 2.00 mg/mL Oxycodone

7-EPHLC2 – Quantitation of Ephedrine by High performance Liquid Chromatography

Scope:
Samples containing ephedrine hydrochloride

Sample Preparation:
Accurately weigh the sample and dilute to volume with water so that the final concentration is approximately 0.2 mg/mL ephedrine hydrochloride. Filter through a 0.45 µm filter.

Buffer Preparation:
To 4000 mL HPLC grade water, add 30 mL conc. H_3PO_4, 10g sodium hydroxide, 8 mL hexylamine, and 100 mg sodium azide.

Standard Stock Solution:
Accurately weigh ephedrine hydrochloride and dilute to volume with water to achieve a target concentration of approximately 0.2 mg/mL ephedrine hydrochloride. Filter through a 0.45 µm filter.

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. Filter through 0.45 µm filter.

Chromatographic System:
Instrument: Agilent 1200 series Liquid Chromatograph with a diode array detector, or equivalent
Column: Phenomenex Aqua C18 150 mm x 4.6 mm x 5 µm, or equivalent
Column Temperature: 35°C
Mobile Phase: NaHAP: acetonitrile (95.5:4.5)
Flow Rate: 1.5 mL/minute
Minimum Run Time: 7.0 min
Injection Volume: 2.0 – 7.0 µL injection
Detector: UV, diode array or 210 nm

Limitations:
N/A

Acceptance Criteria:
Selectivity: Ephedrine resolved (R ≥1.5) from each compound tested.
Linearity: The calculated correlation coefficient for at least seven compounds tested is 0.9996.
Repeatability: Relative Standard Deviation (RSD) for each concentration tested was less than 2.5%.
Accuracy: Experimentally measured purity (expressed in % w/w) within ± 5% relative to the known prepared purity.

Working Range:
0.10 – 0.40 mg/mL Ephedrine

7-MDALC2 – Quantitation of 3,4-Methylenedioxyamphetamine (MDA) by High Performance Liquid Chromatography

Scope:
Samples containing MDA hydrochloride

Sample Preparation:
Accurately weigh the sample and dilute to volume with water so that the final concentration is approximately 0.2 mg/mL MDA hydrochloride. Filter through a 0.45 µm filter.

Buffer Preparation:
To 4000 mL HPLC grade water, add 30 mL conc. H_3PO_4, 10g sodium hydroxide, 8 mL hexylamine, and 100 mg sodium azide.

Standard Stock Solution:
Accurately weigh MDA hydrochloride and dilute to volume with water to achieve a target concentration of approximately 0.2 mg/mL MDA hydrochloride. Filter through a 0.45 µm filter.

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. Filter through 0.45 µm filter.

Chromatographic System:
Instrument: Agilent 1200 series Liquid Chromatograph, with a diode array detector, or equivalent
Column: Phenomenex Aqua C18 150 mm x 4.6 mm x 5 µm, or equivalent
Column Temperature: 35°C
Mobile Phase: NaHAP: acetonitrile (95.5:4.5)
Flow Rate: 1.5 mL/min
Minimum Run Time: 4.0 min
Injection Volume: 2.0 – 7.0 µL injection
Detector: UV, diode array or 210 nm

Limitations:
N/A

Acceptance Criteria:
Selectivity: MDA resolved (R ≥1.5) from each compound tested.
Linearity: The calculated correlation coefficient for at least seven compounds tested is 0.9997.
Repeatability: Relative Standard Deviation (RSD) for each concentration tested was less than 2.5%.
Accuracy: Experimentally measured purity (expressed in % w/w) within ± 5% relative to the known prepared purity.

Working Range:
0.10 – 0.4 mg/mL MDA

7-MDMALC2 – Quantitation of 3,4-Methylenedioxymethamphetamine (MDMA) by High Performance Liquid Chromatography

Scope:
Samples containing MDMA hydrochloride

Sample Preparation:
Accurately weigh the sample and dilute to volume with water so that the final concentration is approximately 0.2 mg/mL MDMA hydrochloride. Filter through a 0.45 µm filter.

Buffer Preparation:
To 4000 mL HPLC grade water, add 30 mL conc. H_3PO_4, 10g sodium hydroxide, 8 mL hexylamine, and 100 mg sodium azide.

Standard Stock Solution:
Accurately weigh MDMA hydrochloride and dilute to volume with water to achieve a target concentration of approximately 0.2 mg/mL MDMA hydrochloride. Filter through a 0.45 µm filter.

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. Filter through 0.45 µm filter.

Chromatographic System:
Instrument: Agilent 1200 series Liquid Chromatograph with a diode array detector, or equivalent
Column: Phenomenex Aqua C18 150 mm x 4.6 mm x 5 µm, or equivalent
Column Temperature: 35°C
Mobile Phase: NaHAP: acetonitrile (95.5:4.5)
Flow Rate: 1.5 mL/min
Minimum Run Time: 8.0 min
Injection Volume: 2.0 – 7.0 µL injection
Detector: UV, diode array or 210 nm

Limitations:
N/A

Acceptance Criteria:
Selectivity: MDMA resolved (R ≥1.5) from each compound tested.
Linearity: The calculated correlation coefficient for at least seven compounds tested is 0.9999.
Repeatability: Relative Standard Deviation (RSD) for each concentration tested was less than 2.0%.
Accuracy: Experimentally measured purity (expressed in % w/w) within ± 5% relative to the known prepared purity.

Working Range:
0.10 – 0.4 mg/mL MDMA

7-METHLC2 – Quantitation of Methamphetamine by High Performance Liquid Chromatography

Scope:
Samples containing methamphetamine hydrochloride

Sample Preparation:
Accurately weigh the sample and dilute to volume with water so that the final concentration is approximately 0.2 mg/mL methamphetamine hydrochloride. Filter through a 0.45 µm filter.

Buffer Preparation:
To 4000 mL HPLC grade water, add 30 mL conc. H_3PO_4, 10g sodium hydroxide, 8 mL hexylamine, and 100 mg sodium azide.

Standard Stock Solution:
Accurately weigh methamphetamine hydrochloride and dilute to volume with water to achieve a target concentration of approximately 0.2 mg/mL methamphetamine hydrochloride. Filter through a 0.45 µm filter.

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. Filter through 0.45 µm filter.

Chromatographic System:
Instrument: Agilent 1200 series Liquid Chromatograph with a diode array detector, or equivalent
Column: Phenomenex Aqua C18 150 mm x 4.6 mm x 5 µm, or equivalent
Column Temperature: 35°C
Mobile Phase: NaHAP: acetonitrile (95.5:4.5)
Flow Rate: 1.5 mL/min
Minimum Run Time: 6.0 min
Injection Volume: 2.0 – 7.0 µL injection
Detector: UV, diode array or 210 nm

Limitations:
N/A

Acceptance Criteria:
Selectivity: Methamphetamine resolved (R ≥1.5) from each compound tested.
Linearity: The calculated correlation coefficient for at least seven compounds tested is 0.9999.
Repeatability: Relative Standard Deviation (RSD) for each concentration tested was less than 2.0%.
Accuracy: Experimentally measured purity (expressed in % w/w) within ± 5% relative to the known prepared purity.

Working Range:
0.10 – 0.4 mg/mL Methamphetamine

7-PSEULC2 – Quantitation of Pseudoephedrine by High Performance Liquid Chromatography

Scope:
Samples containing pseudoephedrine hydrochloride

Sample Preparation:
Accurately weigh the sample and dilute to volume with water so that the final concentration is approximately 0.2 mg/mL pseudoephedrine hydrochloride. Filter through a 0.45 µm filter.

Buffer Preparation:
To 4000 mL HPLC grade water, add 30 mL conc. H_3PO_4, 10g sodium hydroxide, 8 mL hexylamine, and 100 mg sodium azide.

Standard Stock Solution:
Accurately weigh pseudoephedrine hydrochloride and dilute to volume with water to achieve a target concentration of approximately 0.2 mg/mL pseudoephedrine hydrochloride. Filter through a 0.45 µm filter.

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. Filter through 0.45 µm filter.

Chromatographic System:
Instrument: Agilent 1200 series Liquid Chromatograph with a diode array detector, or equivalent
Column: Phenomenex Aqua C18 150mm x 4.6 mm x 5 µm, or equivalent
Column Temperature: 35°C
Mobile Phase: NaHAP: acetonitrile (95.5:4.5)
Flow Rate: 1.5 mL/min
Minimum Run Time: 4.0 min
Injection Volume: 2.0 – 7.0 µL injection
Detector: UV, diode array or 210 nm

Limitations:
N/A

Acceptance Criteria:
Selectivity: Pseudoephedrine resolved (R ≥1.5) from each compound tested.
Linearity: The calculated correlation coefficient for at least seven compounds tested is 0.9999.
Repeatability: Relative Standard Deviation (RSD) for each concentration tested was less than 2.0%.
Accuracy: Experimentally measured purity (expressed in % w/w) within ± 5% relative to the known prepared purity.

Working Range:
0.10 – 0.40 mg/mL Pseudoephedrine

G-PCPH-01 – Quantitation of Phencyclidine (PCP) by Gas Chromatography

Scope:
Samples containing PCP hydrochloride or PCP base

Sample Preparation:
Accurately weigh the sample and dissolve in Internal Standard Solution 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 internal standard solution 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.

Internal Standard Solution:
0.4 mg/mL docosane in chloroform

Standard Solution:
Accurately weigh the PCP hydrochloride reference material in internal standard solution so that the concentration of the phencyclidine is within the working range.

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: Agilent 7890 Gas Chromatograph with Flame Ionization Detector, or equivalent
Column: HP-5 12 m × 0.20 mm I.D. × 0.33 µm, or equivalent
Inlet Temperature: 230 °C
Injection Volume: 1 µL
Mode: Split
Split Ratio: 50:1
Carrier Gas and Flow Rate: Hydrogen, 1.0 mL/min
Oven Program: 200 °C for 1.2 min., ramp temperature 30 °C/min to 270 °C, hold for 2 min
Total Run Time: 5.5333 min
Detector Temperature: 280°C
Signal Data (Sampling) Rate: 50 Hz

Limitations:
N/A

Acceptance Criteria:
Selectivity: Phencyclidine and docosane 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:
0.1168 mg/mL – 2.9359 mg/mL PCP

METH-LC – Quantitation of Methamphetamine by High Performance Liquid Chromatography

Scope:
Samples containing methamphetamine hydrochloride and/or methamphetamine base

Sample Preparation:
Accurately weigh the sample into a volumetric flask and dilute to volume using Injection Solvent 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 internal standard solution via volumetric pipet instead of diluting to volume using the indicator line on a volumetric flask. If necessary, perform a serial dilution using Injection Solvent so that the concentration of the target analyte is within the acceptable working range. Filter the solution through a 0.2-0.45 µm filter.

Injection Solvent:
0.1 N hydrochloric acid

Buffer Preparation:
To 4 L of water, add 30 mL phosphoric acid, 10.0 g sodium hydroxide, 8 mL hexylamine, and 100 mg sodium azide (pH 2.5).

Standard Solution:
Accurately weigh the methamphetamine hydrochloride reference material in Injection Solvent so that the concentration of the methamphetamine is approximately 0.6 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.

Chromatographic System:
Instrument: Agilent 1200 Series High Performance Liquid Chromatograph, or equivalent
Column: Phenomenex Luna C18 75 mm x 4.6 mm x 3 µm, or 3.5 µm, or equivalent
Column Temperature: 50°C
Injection Volume: 3 µL
Injection Solvent: 0.1 N hydrochloric acid
Mobile Phase: 95% buffer/5% acetonitrile. Buffer prepared as listed above. Mobile phase constituents combined by instrument.
Flow: 1.5 mL/min
Gradient Program: Isocratic
Total Run Time: 3 min
Detection: 210 nm (10 nm bandwidth); reference: 360 nm (100 nm bandwidth); peak width > 0.05 min

Limitations:
This method is not suitable for samples containing acetaminophen.

Acceptance Criteria:
Selectivity: Methamphetamine resolved (R ≥1.5) from each compound tested except for the compounds noted in Limitations.
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:
0.11672 mg/mL - 1.32736 mg/mL Methamphetamine

METH-LC-1-6 – Quantitation of Methamphetamine by High Performance Liquid Chromatography

Scope:
Samples containing methamphetamine hydrochloride

Sample Preparation:
Accurately weigh the sample into a volumetric flask and dilute to volume using Injection Solvent 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 internal standard solution via volumetric pipet instead of diluting to volume using the indicator line on a volumetric flask. If necessary, perform a serial dilution using Injection Solvent so that the concentration of the target analyte is within the acceptable working range. Filter the solution through a 0.45 µm syringe filter.

Injection Solvent:
0.1N hydrochloric acid or other appropriate solvent

Buffer Preparation:
4000mL distilled water, 10g sodium hydroxide, 30mL phosphoric acid, 8mL hexylamine, and 0.1g sodium azide

Standard Solution:
Accurately weigh the methamphetamine hydrochloride reference material in Injection Solvent so that the concentration of the methamphetamine is within the working range.

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: Agilent 1200 Series High Performance Liquid Chromatograph, or equivalent
Column: C18 150 mm x 4.60 mm x 5 µm
Column Temperature: 50°C
Injection Volume: 3.0 µL
Injection Solvent: 0.1 N hydrochloric acid or other appropriate solvent
Mobile Phase: 90% buffer/10% acetonitrile. Buffer prepared as listed above. Mobile phase constituents combined by instrument.
Flow: 1.0 mL/min
Detection: UV diode array @ 210nm, 10nm BW; 550nm Ref, 100nm BW
Total Run Time: 4.0 min

Limitations:
Methamphetamine and 3,4-MDMA co-elute on this method.

Acceptance Criteria:
Selectivity: Methamphetamine resolved (R ≥1.5) from each compound tested, except as noted in Limitations.
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:
0.08088 – 1.6176 mg/mL Methamphetamine

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

Scope:
Samples containing methamphetamine.

Sample Preparation:
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 sodium hydroxide 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 preparation 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: Agilent 7890 Gas Chromatograph with Flame Ionization Detector, or equivalent
Column: DB-1 15 m × 0.32 mm I.D. × 0.25 µm, or equivalent
Inlet Temperature: 275°C
Mode: Split
Split Ratio: 35:1
Carrier Gas and Flow Rate: Helium, 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 sodium hydroxide

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 with birch reaction byproducts.

Sample Preparation:
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.

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

(D) 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 sodium hydroxide 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:
(C) 4.0 mg/mL n-tridecane in chloroform OR

(D) 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 preparation 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: Agilent 7890 Gas Chromatograph H with Flame Ionization Detector, or equivalent
Column: DB-1 30 m × 0.25 mm I.D. × 0.25 µm, or equivalent
Inlet Temperature: 275°C
Mode: Split
Split Ratio: 50:1
Carrier Gas and Flow Rate: Helium, 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 sodium hydroxide

Limitations:
N/A

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:
0.1 – 4.0 mg/mL Methamphetamine HCl

PCP-GCQ-SWL1 – Quantitation of Phencyclidine (PCP) by Gas Chromatography

Scope:
Samples containing PCP hydrochloride

Sample Preparation:
Accurately weigh the sample and dissolve in internal standard solution 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 internal standard solution 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.

Internal Standard Solution:
0.6 mg/mL docosane in chloroform.

Standard Solution:
Accurately weigh the PCP hydrochloride reference material in internal standard solution so that the concentration of the PCP is within the working range.

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: Agilent 6890 Gas Chromatograph with Flame Ionization Detector, or equivalent
Column: HP-5 10 m × 0.32 mm I.D. × 0.25 µm, or equivalent
Inlet Temperature: 240°C
Mode: Split
Split Ratio: 50:1
Carrier Gas and Flow Rate: Hydrogen, 1.7 mL/min
Oven Program: Isothermal at 200°C
Total Run Time: 5 min
Detector Temperature: 280°C
Signal Data (Sampling) Rate: 50 Hz
Injection Volume: 1 µL
Injection Solvent: chloroform

Limitations:
N/A

Acceptance Criteria:
Selectivity: PCP and docosane 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 3%.
Accuracy: Experimentally measured purity (expressed in % w/w) within ± 5% relative to the known prepared purity.

Working Range:
0.16 -1.59 mg/mL PCP

PCPQ1 – Quantitation of Phencyclidine (PCP) by Gas Chromatography

Scope:
Samples containing PCP

Sample Preparation:
Accurately weigh the sample and dissolve in internal standard solution. If necessary, perform a serial dilution using internal standard solution so that the concentration of the target analyte is within the low and high concentration QC solutions. Filter the solution if an appreciable amount of insoluble material is present.

Internal Standard Solution:
0.9507 mg/mL Eicosane in chloroform/methanol (80:20)

Standard Solution:
Accurately weigh the PCP hydrochloride reference material in internal standard solution so that the concentration of the PCP is within the working range.

Quality Control Solutions:
Prepare two QC solutions for PCP 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: Agilent 7890 Gas Chromatography with a Flame Ionization Detector, or equivalent
Column: HP-5 30 m × 0.320 mm I.D. × 0.25 µm film thickness,
Inlet Temperature: 220°C
Injection Volume: 1 µL
Mode: Split
Split Ratio: 100:1
Carrier Gas and Flow Rate: Hydrogen, 2ml/min (1.65min); ramp @1.5ml/min/min to 3ml/min (4min)
Oven Program: 165°C for 1.5min then 40°C/min to 250°C for 1.5 min
Total Run Time: 5.125 min
Detector Temperature: 275°C
Signal Data (Sampling) Rate: 20 Hz

Limitations:
There are no known limitations associated with this method.

Acceptance Criteria:
Selectivity: Selectivity was determined for the critical pair of PCP and lidocaine. Resolution between the PCP/Lidocaine critical pair is > 1.5.
Linearity: Eight 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:
0.16 mg/mL – 3.01 mg/mL PCP

PHEN-UPLCQ-SWL1 – Quantitation of Methamphetamine and Amphetamine by Ultra-High Performance Liquid Chromatography

Scope:

Samples containing methamphetamine hydrochloride and/or amphetamine sulfate

Sample Preparation:

Accurately weigh the sample into a volumetric flask and dilute to volume using Injection Solvent. Perform a gravimetric dilution using Injection Solvent. The final concentration of the substance must be within the working range. Filter to 0.2 μm.

Injection Solvent:

85 mM sodium phosphate buffer (pH ~1.8)

Buffer Preparation:

To prepare 2 L of buffer:
Add 22 mL H_3PO_4 (85%) to 2 L deionized water.
Add 2.7 g sodium hydroxide pellets to this solution.
Filter buffer through a 0.22 μm filter.

To prepare 4 L of buffer:
Add 44 mL H_3PO_4 (85%) to 4 L deionized water.
Add 5.4 g sodium hydroxide pellets to this solution.
Filter buffer through a 0.22 μm filter.

Standard Solution:

Accurately weigh an amount of the appropriate standard(s) into a volumetric flask and dilute to final volume with 85 mM sodium phosphate buffer. Perform a gravimetric dilution in 85 mM sodium phosphate buffer. The final concentration of each standard in solution must be within the working range. Filter to 0.2 μm.

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: Waters Acquity Ultra Performance Liquid Chromatograph with photo diode array detector, or equivalent
Column: Waters BEH C18 100 mm x 2.1 mm x 1.7 μm, or equivalent
Column Temperature: 30°C
Injection Parameters: 5.0 μL (partial loop injection with needle overfill; if available)
Injection Solvent: Buffer
Strong Needle Wash: Methanol
Weak Needle Wash: 90% water/10% acetonitrile
Pre-Inject Wash Time: 3.0 s
Post-Inject Wash Time: 6.0 s
Purge Solvent Name: 10:90 acetonitrile/water
Seal Washes: 5.0 minutes
Autosampler Temperature: Not controlled
Mobile Phase: 90% buffer: 10% acetonitrile. Buffer prepared as listed above. Mobile phase constituents combined by instrument. Gradient program listed below.
Flow: 0.45 mL/min
Gradient Program: Isocratic
Detection Wavelength: Channel 1: 210 nm with 4.8 nm resolution, Absorbance
Sampling Rate: 20 points/s
Total Run Time: 4.0 min

Limitations:

See individual instrument validation reports.

Acceptance Criteria:

Selectivity: Methamphetamine and amphetamine 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:

0.01398-0.1256 mg/mL Amphetamine
0.01008 – 0.10006 mg/mL Methamphetamine

TCPPCP – Quantitation of Phencyclidine (PCP) by Gas Chromatography

Scope:
Samples containing PCP base and/or PCP hydrochloride in the presence of tenocyclidine.

Sample Preparation:
Accurately weigh the sample and dissolve in Internal Standard Solution 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 internal standard solution 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.

Internal Standard Solution:
0.4 mg/mL docosane in chloroform

Standard Solution:
Accurately weigh the PCP hydrochloride reference material in Internal Standard Solution so that the concentration of the PCP is within the working range.

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: Agilent 7890 Gas Chromatograph with Flame Ionization Detector, or equivalent
Column: DB-1 30 m × 0.25 mm I.D. × 0.25 µm, or equivalent
Inlet Temperature: 270°C
Mode: Split
Split Ratio: 60:1
Carrier Gas and Flow Rate: Hydrogen, 1.0 mL/min
Oven Program: 125°C initial, ramp 6°C/min to 165 °C, 4°C/min to 200°C, 30°C/min to 240°C, hold for 4 min.
Total Run Time: 20.75 min
Detector Temperature: 310°C
Signal Data (Sampling) Rate: 50 Hz
Injection Volume: 1 µL
Injection Solvent: chloroform

Limitations:
N/A

Acceptance Criteria:
Selectivity: Phencyclidine and docosane 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:
0.1141 – 0.9512 mg/mL PCP