Validated Quantitative Methods

DEA 101 – Quantitation of Cocaine by Gas Chromatography

Scope

Samples containing cocaine hydrochloride and/or cocaine base

Procedure:

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: Gas Chromatograph HP/Agilent 7890 equipped with an FID detector (or equivalent)
Column: HP-5 (Agilent Part No. 19091J-101 SN USB409713B); 12 m × 0.20 mm I.D. × 0.33 µm film thickness, 5% phenylmethylpolysiloxane stationary phase
Inlet (Injector) Temperature: 270 °C
Mode: Split
Split Ratio: 60:1
Carrier Gas: Hydrogen
Carrier Gas Flow Rate: 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 101L – Quantitation of Cocaine by Gas Chromatography-Low Thermal Mass Column Module

Scope

Samples containing cocaine hydrochloride and/or cocaine base

Procedure:

Accurately weigh the sample into a volumetric flask and dilute to volume using 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 an appreciable amount of insoluble material is present.

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: Gas Chromatograph HP/Agilent 7890 equipped with Low Thermal Mass Series II Column Module and an FID detector (or equivalent)
In-segment (Pre-column): DB-5 (cut from SN US9146037H) 1.0 m × 0.18 mm I.D. × 0.18 µm film thickness; 5% phenylmethylpolysiloxane stationary phase
LTM Column (Column 2): DB-5 long legs (Agilent special order; SN US15260934) 15 m × 0.25 mm I.D. × 0.25 µm film thickness; 5% phenylmethylpolysiloxane stationary phase
Inlet (Injector) Temperature: 270 °C
Mode: Split
Split Ratio: 50:1
Carrier Gas: Hydrogen
Carrier Gas Flow Rate: 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 or 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

Procedure:

Accurately weigh the sample into a volumetric flask and dilute to volume using 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 an appreciable amount of insoluble material is present.

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: Gas Chromatograph HP/Agilent 7890 equipped with an FID detector (or equivalent)
Column: HP-5 (Agilent Part No. 19091J-101 SN USB409713B); 12 m × 0.20 mm I.D. × 0.33 µm film thickness, 5% phenylmethylpolysiloxane stationary phase
Inlet (Injector) Temperature: 280 °C
Mode: Split
Split Ratio: 60:1
Carrier Gas: Hydrogen
Column Flow Program: 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

Procedure:

Accurately weigh the sample into a volumetric flask and dilute to volume using 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 an appreciable amount of insoluble material is present.

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: Gas Chromatograph HP/Agilent 7890 equipped with Low Thermal Mass Series II Column Module and an FID detector (or equivalent)
In-segment (Pre-column): DB-5 (cut from SN US9146037H) 1.0 m × 0.18 mm I.D. × 0.18 µm film thickness; 5% phenylmethylpolysiloxane stationary phase
LTM Column (Column 2): DB-5 long legs (Agilent special order; SN US15260934) 15 m × 0.25 mm I.D. × 0.25 µm film thickness; 5% phenylmethylpolysiloxane stationary phase
Inlet (Injector) Temperature: 280 °C
Mode: Split
Split Ratio: 50:1
Carrier Gas: Hydrogen
Carrier Gas Flow Rate: 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 103L – Quantitation of Methamphetamine by Gas Chromatography-Low Thermal Mass Column Module

Scope

Samples containing methamphetamine hydrochloride and methamphetamine base

Procedure:

Accurately weigh the sample into a volumetric flask and dilute to volume using internal standard (ISTD) solution. If necessary, perform a serial dilution using ISTD solution so that the concentration of the target analyte is within the acceptable working range. 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 NaOH 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 NaOH 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: Gas Chromatograph HP/Agilent 7890 equipped with Low Thermal Mass Series II Column Module and an FID detector (or equivalent)
In-segment (Pre-column): DB-5 (cut from SN US9146037H) 1.0 m × 0.18 mm I.D. × 0.18 µm film thickness; 5% phenylmethylpolysiloxane stationary phase
LTM Column (Column 2): DB-5 MS UI long legs (Agilent special order; SN US15260934) 15 m × 0.25 mm I.D. × 0.25 µm film thickness; 5% phenylmethylpolysiloxane stationary phase
Inlet (Injector) Temperature: 250 °C
Mode: Split
Split Ratio: 60:1
Carrier Gas: Hydrogen
Carrier Gas Flow Rate: 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

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

Procedure:

Accurately weigh the sample into a volumetric flask and dilute to approximately ½ volume using ISTD 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: Gas Chromatograph HP/Agilent 7890 equipped with an FID detector (or equivalent)
Column: HP-5 (Agilent Part No. 19091J-101); 12 m × 0.20 mm I.D. × 0.33 µm film thickness, 5% phenylmethylpolysiloxane stationary phase
Inlet (Injector) Temperature: 280 °C
Mode: Split
Split Ratio: 60:1
Carrier Gas: Hydrogen
Column Flow Program: 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 – 1.91276 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)

Procedure:

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% NaOH or 2 N NaOH 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 heroin 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: Gas Chromatograph HP/Agilent 7890 equipped with Low Thermal Mass Series II Column Module and an FID detector (or equivalent)
In-segment (Pre-column): DB-5 (cut from longer column SN USF395211B) 1.0 m × 0.18 mm I.D. × 0.18 µm film thickness; 5% phenylmethylpolysiloxane stationary phase
LTM Column (Column 2): DB-5 long legs (Agilent special order; SN US16120404) 15 m × 0.25 mm I.D. × 0.25 µm film thickness; 5% phenylmethylpolysiloxane stationary phase
Inlet (Injector) Temperature: 280 °C
Mode: Split
Split Ratio: 50:1
Carrier Gas: Hydrogen
Carrier Gas Flow Rate: 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 127 – Quantitation of Heroin and Fentanyl by Gas Chromatography

Scope

Samples containing heroin hydrochloride and/or fentanyl hydrochloride

Procedure:

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:

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

Standard Solution:

Accurately weigh the Heroin Hydrochloride and/or Fentanyl Hydrochloride 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: Gas Chromatograph HP/Agilent 7890 equipped with an FID detector (or equivalent)
Column: HP-5 (Agilent Part No. 19091J-101); 12 m × 0.20 mm I.D. × 0.33 µm film thickness, 5% phenylmethylpolysiloxane stationary phase
Inlet (Injector) Temperature: 270 °C
Mode: Split
Split Ratio: 50:1
Carrier Gas: Hydrogen
Carrier Gas Flow Rate: 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. This method did not evaluate fentanyl citrate samples and/or pharmaceutical preparations; therefore, this method is not to be used to quantitate fentanyl citrate.
3. 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

0.03082 – 1.48168 mg/mL Fentanyl

DEA 201 – Quantitation of Cocaine by High Performance Liquid Chromatography

Scope

Samples containing cocaine hydrochloride and/or cocaine base

Procedure:

Accurately weigh the sample into a volumetric flask and dilute to volume using Injection Solvent. 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:

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

Buffer Preparation:

To 4 L of water, add 30 mL phosphoric acid, 10.0 g NaOH, 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: High Performance Liquid Chromatograph HP/Agilent 1200 Series (or equivalent)
Column: Phenomenex Luna C18 Column: 50 mm x 3 mm x 3 µm, 100 Å porous size
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

Procedure:

Accurately weigh the sample into a volumetric flask and dilute to volume using *Injection Solvent*. 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:

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: High Performance Liquid Chromatograph HP/Agilent 1200 Series (or equivalent)
Column: Phenomenex Luna C18 Column: 50 mm x 3 mm x 3 μm, 100 Å porous size
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 guafenesin (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 guafenesin 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 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

Procedure:

Accurately weigh the sample into a volumetric flask and dilute to volume using Injection Solvent. 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 NaOH (pellets) to 4 L de-ionized H_2O.
Working: Dilute 530 mL of stock to 1 L de-ionized H_2O. 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 UPLC equipped with photo diode array detector (or equivalent)
Column: Waters BEH C18, 10 cm, 2.1 mm id, 1.7 µm particle size
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% H_2O:10% acetonitrile; Option 2) 6000 µL 90% H_2O:10% acetonitrile
Seal Washes: 5.0 minutes
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/second
Total Run Time: 1.2 minutes

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

Procedure:

Accurately weigh the sample into a volumetric flask and dilute to volume using Injection Solvent. 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 (pH ~1.8)

Buffer Preparation:

To prepare 1 L of buffer:
Add 0.6306 g of ammonium formate, ≥99.995% trace metals basis, to 1 L de-ionized H_2O. 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, NH_4OH. Filter buffer through a 0.2µm filter.

To prepare 4 L of buffer:
Add 2.5224 g of ammonium formate to 4 L de-ionized H_2O. 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% NH_4OH. 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 UPLC equipped with photo diode array detector (or equivalent)
Column: Waters BEH C18, 10 cm, 2.1 mm id, 1.7 µm particle size
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% H_2O:10% acetonitrile
Pre-Inject Wash Time: 0.0 (sec)
Post-Inject Wash Time: 6.0 (sec)
Purge Solvent Name: Acetonitrile
Seal Washes: 5.0 minutes
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/second
Total Run Time: 3.0 minutes

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 ($R \geq 1.5$) 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 - Quantitation by Proton Nuclear Magnetic Resonance Spectroscopy (qHNMR)

Scope

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

- Maleic Acid/D2O
- Maleic Acid/CD3OD
- Maleic Acid/DMSO-d6
- Dimethylfumarate/CDCl3
- Dimethylfumarate/CD3OD
- Dimethylfumarate/DMSO-d6
- 1,4-BTMSB-d4/CDCl3
- 1,4-BTMSB-d4/CD3OD
- 1,4-Dioxane/D2O
- DSS-d6/D2O
- Dimethylsulfone/CDCl3
- Dimethylsulfone/CD3OD
- Methenamine/CDCl3

Procedure:

Internal Standard Solution:
Accurately weigh the sample and add an accurate volume of internal standard solution containing a known concentration of calibrant. The internal standard solution is normally approximately 5mg/mL maleic acid in D_2O with trimethylsilylpropanoic acid (TSP) as the zero ppm reference. Thoroughly mix the solution. If no insoluble material is observable, then transfer at least 0.7mL of solution to an NMR tube and acquire a qHNMR spectrum.
If insoluble material is present, then either prepare a second solution at a different concentration, or do the following. Transfer at least 0.7mL of solution that is free of insoluble material to an NMR tube and acquire a qHNMR spectrum. To the remaining solution containing the 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 0.7mL of filtered (if insoluble material is present) solution to an NMR tube and acquire qHNMR spectra of each solution.

Quality Control Solution:

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

Experimental Settings:

Instrument: Varian/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 separated 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: The relative standard deviation of peak heights between zero and ten ppm was less than 3%.
Linearity: The correlation coefficient of the instrument method was greater than 0.998.
Repeatability: The relative standard deviation for the method repeatability test was less than 2%.
Accuracy: The experimentally measured purity was within 5% relative to the known analyte's purity.

Analyte Stability: The 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 blank 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 50mg/mL).