**FCS15 - SOP for Quantitation of Heroin using GC-FID**

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# 1. Scope

1.1. This document establishes the procedures for quantifying heroin in samples containing heroin for use in case work and reporting out quantifiable results of heroin in test samples using Gas Chromatography Flame Ionization Detector (GC-FID) instrumentation.

# 2. Background

2.1. This method is based off the document provided by the Drug Enforcement Agency (DEA) method for *Quantitation of Heroin* (2016) using GC-FID. This document provides guidance and is in support of the *Forensic Chemistry Quality Assurance Manual (QAM)* and conforms to ISO/IEC 17025 guidelines.

# 3. Safety

3.1. Reagent Toxicity: Personnel should refer to the appropriate SDS for solvents and reagents used during analysis for any specific safety requirements.

3.1.1. For a complete review of required Health and Safety regulations of the PHL, see *DOM13 DFS Health and Safety Manual.*

3.2. Protective Equipment: Personnel should wear personal protective equipment (PPE) including: lab coat, gloves, and safety goggles when carrying out standard operating procedures.

3.2.1. Wear vinyl or nitrile gloves when handling these chemicals to prevent

absorption through the skin. If any chemicals are spilled onto gloves, discard gloves into hazardous waste.

3.3. Training: Formal training in use of instruments and software is necessary.

3.4. Personal Hygiene: Universal Precautions must be followed. Care should be taken when handling chemicals or any biological specimen. Routine use of gloves and proper hand washing should be practiced.

3.4.1. Refer to DOM13 – DFS Health and Safety Manual.

3.5. Disposal of Waste: Waste materials must be disposed of in compliance with laboratory, Federal, state, and local regulations. Solvents and reagents should always be disposed of in an appropriate container clearly marked for waste products and temporarily stored in a chemical fume hood.

3.5.1. Consult DFS Safety Officer for proper procedures.

# 4. Materials / Equipment Required

4.1. Reagent Grade Acetonitrile (ACN) and Methanol (MeOH), Chloroform, or higher purity.

4.2. Ultra-High Purity Helium tanks

4.3. Hydrogen generator or tanks for the FID

4.4. Gas Chromatography Flame Ionization Detector (GC-FID), fully assembled, including: solvent vials, injection syringe, and other consumables.

4.5. Glassware:

4.5.1. 250 mL volumetric flask (Class A)

4.5.2. 100 mL volumetric flask (Class A)

4.5.3. other volumetric glassware (Class A), as appropriate

4.6. Binder/Folder for Autotune and Standard results

4.7. Daily Maintenance Logbook

# 5. Standards and Controls

5.1. Standards may come from Cayman Chemical or the Drug Enforcement Agency (DEA), or other, approved vendor or provider.

5.2. Heroin hydrochloride (HCl) (solid powder)

5.3. Tetracosane (solid powder)

5.4. Internal Standard Solution (ISTD), 0.40 mg/mL tetracosane (exact value needed):

5.4.1. About 100mg tetracosane in a 250 mL volumetric flask, dilute to mark with chloroform/methanol (9:1). Other volumes are acceptable as long as ratio is maintained.

5.5. Heroin HCl Stock Solution, about 2mg/mL (exact value needed using purity):

5.5.1. About 200mg Heroin HCl in tared, 100mL volumetric flask

5.5.2. Dilute to mark with ISTD solution.

5.6. Heroin HCl Calibration Solutions: (dilute to mark with ISTD solution; exact value needed using purity):

| | | |
|---|---|---|
| 5.6.1. Cal 1: | 1:10 | about 0.2 mg/mL |
| 5.6.2. Cal 2: | 2:10 | about 0.4 mg/mL |
| 5.6.3. Cal 3: | 3:10 | about 0.6 mg/mL |
| 5.6.4. Cal 4: | 4:10 | about 0.8 mg/mL |
| 5.6.5. Cal 5: | 5:10 | about 1.0 mg/mL |
| 5.6.6. Cal 6: | 6:10 | about 1.2 mg/mL |
| 5.6.7. Cal 7: | 7:10 | about 1.4 mg/mL |
| 5.6.8. Cal 8: | 8:10 | about 1.6 mg/mL |
| 5.6.9. Cal 9: | Heroin HCl Stock Solution (about 2.0 mg/mL) | |

# 6. Calibration

6.1. Response to Heroin HCl Calibration Solution

6.1.1. An average of five responses are taken at each calibration level

6.1.2. The average response of each concentration level is compared to the actual concentration, with an average sensitivity calculated at each level (sensitivity is the response per concentration).

6.1.3. For the concentration curve to be acceptable, the average sensitivity of each calibration level must be within 5% of the overall average sensitivity.

6.1.4. While in use for casework, approved calibration Excel worksheets will be saved electronically.

6.1.5. Once a curve is no longer suitable for casework, the respective Excel worksheet will be archived in the appropriate electronic folder.

6.1.6. This calibration curve will be run at least once per year or as needed (based on column environment or method changes) to evaluate the suitability of the column for quantitation and a re-establishment of working range.

6.2. Monthly/Weekly Calibrant Run with Samples

6.2.1. A calibrant of heroin standard at approximately 1 mg/mL will be run for each sequence at least once each month that heroin purity analysis is performed.

6.2.2. The calibrant will be used to establish the calibration curve for that month's analysis (or until another calibrant is run).

6.2.2.1. The origin and the response from the calibrant will be the two points used to determine the new calibration curve between calibrant runs.

6.2.3. The calibrant response will be monitored in an electronic control chart.

# 7. Procedures

7.1. Method Parameters: Gas Chromatograph-Flame Ionization Detection (GC-FID)

- Split mode (60:1, split flow 60 mL/minute)
- Column: HP-5, 12mx0.20mm inner diameter (I.D.) x 0.33 µm film thickness, 5% phenyl methylpolysiloxane stationary phase, or equivalent.
- Column Flow Program: 1.0 mL/minute for 2.5 minutes, ramp 45 mL/minute to 4.5 mL/minute, hold for 1.0 minute.

- Oven Program: 270ºC for 2.5 minutes, ramp 45ºC/minute to 295ºC, hold 1.0 minute.
- Inlet (injector) Temperature: 280ºC (pressure 12.665 psi, total flow 64 mL/minute, septum purge flow 3 mL/minute)
- Detector: 280ºC at 50Hz
- Carrier Gas: Helium
- Run time: 4.05 minutes
- Injection volume: 1 µL
- Injection Solvent: Chloroform / Methanol (9:1)

7.2. Acceptable Method Parameter Variations (make mention in case notes):

7.2.1. Inlet temperature may be changed from about 270ºC to 290ºC for better improved chromatography, as per analyst's discretion.

7.2.2. Increase flow and temperature ramp at 2.5 minutes to remove late eluting compounds (*e.g.*, diltiazem, noscapine, …), as per analyst's discretion.

7.2.3. Sample preparation solvent of chloroform or chloroform/methanol (9:1), as per analyst's discretion.

7.2.4. Data sampling rate may be changed from 20 to 50Hz, as per analyst's discretion.

7.2.5. Each linearity concentration may be injected in lowest-to-highest or highest-to-lowest order, as per analyst's discretion.

7.2.6. Accuracy and recovery may be calculated from either a 3-point or 9-point curve, as per analyst's discretion.

7.3. Quality Control Standards – Two Quality Control Standards are made and evaluated over time to assess performance of the method. The bulk QC Heroin mixture is made:

7.3.1. About 50mg Heroin HCl (>99% purity) added to about 850mg sucrose.

7.3.2. Mix contents and grind in mortar

7.3.3. (Optional) Filter through approximately 20 mesh.

7.3.4. Quality Control (QC) standards (for example):

- QCLow – About 75mg bulk QC Heroin Mixture, added to 10mL volumetric flask, dilute to mark with ISTD solution
- QCHigh – About 300mg bulk QC Heroin Mixture, added to 10mL volumetric flask, dilute to mark with ISTD solution

Filter prior to injection.

7.4. Sample Preparation – Variations of this method are allowed per analyst discretion, conforming to FCU procedures on deviations, as appropriate

7.4.1. Accurately weigh the unknown sample and dissolve in the ISTD solution.

7.4.2. Add sufficient quantity to result in a concentration that is within the working range of this method.

7.4.3. Filter prior to injection.

7.5. **Acceptance Parameters**. – The following are the acceptance parameters for the GC-FID.

7.5.1. GC-FID Peak Quality

| | Acceptance Criteria | Detail |
|---|---|---|
| **GC-FID PARAMETERS** | **Retention Time** | Retention time of analyte peak must match within 2% of standard. |
| | **S/N* Cut Off** | Analyte TIC** must be more than three (3) times greater than noise. (S/N=signal-to-noise ratio) |
| | **Peak width resolution** | Analyte peak must be base peak resolved, as evaluated by the analyst. |

7.5.1. Calibration Curve Acceptability

| | Acceptance Criteria | Detail |
|---|---|---|
| **CALIBRATION CURVE ACCEPTANCE** | **Check standard falls outside of tolerance** | Tolerance set to 5% |
| | **Calibration curve is still valid** | Calibration curve (two-point recalibration) is applicable for all casework within one month of creation |

| | | |
|---|---|---|
| | **Two samples of the same item are within 20%** | Absolute difference in quant of two samples of an item must be under 20% in order to be acceptable |
| | **Range of >10% between samples requires third quantitation** | Analyst may average the sample concentrations or report the lowest value |

7.5.1. QC Check Acceptance

7.5.1.1. Positive control checks must be within 10% of each other to be acceptable.

7.5.1.2. If a QC check value does not fall within this range, a new standard solution may be prepared and injected.

7.5.1.3. If more than one QC check value does not fall within this range, a new calibration curve shall be prepared and used.

7.6. Standard Runs

7.6.1. Place a known mass of the unknown sample into a volumetric flask and dilute to mark with ISTD solution. In general, use about 0.5mg of the sample per 1mL of internal standard solution.

7.6.2. Filter and inject the solution into the GC-FID.

7.6.3. From the generated data, divide the heroin peak corrected area by the internal standard's peak area to calculate the area ration.

7.6.4. Using the two-point recalibrated calibration curve (from section 6.2), solve for the heroin concentration.

7.6.5. To calculate the percent heroin:

7.6.5.1. Slope of Calibration Curve = (Area of calibrant peak) / (Area of internal standard calibrant peak x Concentration of Calibrant (mg/mL))

7.6.5.2. Percent Purity = 100% x (Area of sample heroin peak) / (Slope of Calibration Curve x Area of sample internal standard peak x Total Concentration of Sample (mg/mL))

7.7. Control Chart Maintenance

7.7.1. As appropriate, the significant parameters appropriate for the identification of individual substances shall be recorded in the

laboratory control chart for GC-FID. Critical pieces of information include peak retention time.

# 8. Sampling

8.1. Perform sampling plan as covered under section 8 of *FCS02 – SOP for General Laboratory Procedures for FCU*.

8.2. Deviations are acceptable, but must be recorded in case notes and follow agency policy of deviations.

# 9. Calculations

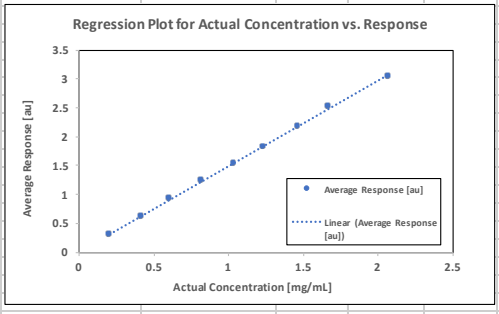
9.1. Linearity Evaluation of Calibration Standards

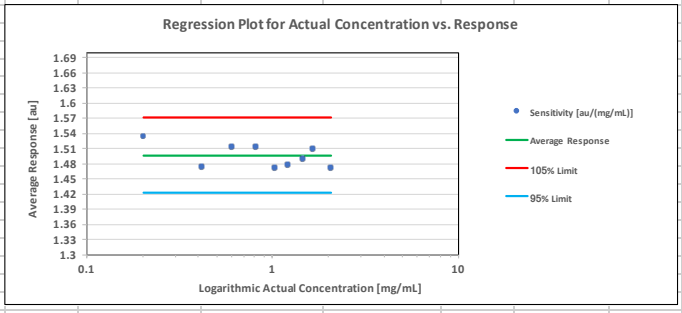
The following is an example calculation and evaluation of response values for the 9 calibrators used in this method, using Excel.

**LINEARITY EVALUATION FORM**

| | |
|---|---|
| **Method Name:** | FCSXX - SOP for the Quantitation of Heroin |
| **Target Compound:** | Heroin |
| **Location:** | Forensic Chemistry Unit, Department of Forensic Sciences |
| | 401 E.St. SW, Washington, D.C. 20024 |
| **Instrument Used:** | GC-FID XXXX |

| Approximate Concentration [mg/mL] | Actual Concentration [mg/mL] | Response 1 [au] | Response 2 [au] | Response 3 [au] | Response 4 [au] | Response 5 [au] | Average Response [au] | Sensitivity [au/(mg/mL)] | Overall Average Sensitivity [au/(mg/mL)] | Lower Acceptable Limit (95%) | Upper Acceptable Limit (105%) | Evaluation Result |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.2 | 0.2036 | 0.289 | 0.3017 | 0.3125 | 0.3259 | 0.3314 | 0.312 | 1.533 | 1.496 | 1.422 | 1.571 | PASS |
| 0.4 | 0.4181 | 0.581 | 0.5993 | 0.6047 | 0.6338 | 0.6588 | 0.616 | 1.472 | 1.496 | 1.422 | 1.571 | PASS |
| 0.6 | 0.6102 | 0.876 | 0.9010 | 0.9315 | 0.9463 | 0.9583 | 0.923 | 1.512 | 1.496 | 1.422 | 1.571 | PASS |
| 0.8 | 0.8141 | 1.17 | 1.2229 | 1.2386 | 1.2605 | 1.2647 | 1.231 | 1.513 | 1.496 | 1.422 | 1.571 | PASS |
| 1.0 | 1.0361 | 1.46 | 1.4778 | 1.5012 | 1.5635 | 1.6096 | 1.522 | 1.469 | 1.496 | 1.422 | 1.571 | PASS |
| 1.2 | 1.2295 | 1.75 | 1.7623 | 1.8226 | 1.8424 | 1.8958 | 1.815 | 1.476 | 1.496 | 1.422 | 1.571 | PASS |
| 1.4 | 1.4650 | 2.05 | 2.1254 | 2.1860 | 2.2278 | 2.3082 | 2.179 | 1.488 | 1.496 | 1.422 | 1.571 | PASS |
| 1.6 | 1.6706 | 2.35 | 2.4583 | 2.4941 | 2.6136 | 2.6838 | 2.520 | 1.508 | 1.496 | 1.422 | 1.571 | PASS |
| 2 | 2.0697 | 2.9 | 2.9605 | 3.0728 | 3.1069 | 3.1635 | 3.041 | 1.469 | 1.496 | 1.422 | 1.571 | PASS |




| | | | | |
|---|---|---|---|---|
| Correlation Coefficient: | 0.99972 | | | |
| Y-intercept: | 0.01240 | | | |
| Slope: | 1.47601 | | | |
| Linear Range: | 0.2036 | mg/mL to | 2.0697 | mg/mL |

**Figure 1.** Example calculation and evaluation of calibration standards for this method. Random data is generated from the "=RAND()" function in Excel.

# 10. Uncertainty of Measurement

10.1. Uncertainty of measurement is assessed as per the validation of this method. See corresponding validation per instrument.

# 11. Limitations

11.1. Not applicable

# 12. Documentation

12.1. Case notes

12.2. Instrument Logbooks

# 13. References

13.1. *Quantitation of Heroin*, Drug Enforcement Agency, Nov. 28th, 2016.

13.2. *Quantifying Heroin by GC-FID with Internal Standard*, Austin Police Department, Forensic Chemistry Section, Technical Manual, Jan. 1, 2014.

13.3. FCS02 – SOP for General Laboratory Procedures for FCU.

13.4. Forensic Chemistry Unit QAM (current revisions).

13.5. ISO/IEC 17025 guidelines