FCS16 - SOP for Quantitation of Phencyclidine using GC-FID

Table of Contents

1. Scope

1.1. This document establishes the procedures for quantifying phencyclidine in samples containing phencyclidine base or phencyclidine hydrochloride in the presence of tenocyclidine for use in case work and reporting out quantifiable results 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 Phencyclidine by Gas Chromatography* (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. LCMS Grade 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. Phencyclidine (PCP) hydrochloride (HCl) (solid powder)

5.3. Tetracosane (solid powder)

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

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. PCP HCl Stock Solution, about 1mg/mL (exact value needed using purity):

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

5.5.2. Dilute to mark with ISTD solution.

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

5.6.1.	Cal 1:	4:100	about 0.04 mg/mL
5.6.2.	Cal 2:	1:10	about 0.1 mg/mL
5.6.3.	Cal 3:	2:10	about 0.2 mg/mL
5.6.4.	Cal 4:	3:10	about 0.3 mg/mL
5.6.5.	Cal 5:	4:10	about 0.4 mg/mL
5.6.6.	Cal 6:	5:10	about 0.5 mg/mL
5.6.7.	Cal 7:	6:10	about 0.6 mg/mL
5.6.8.	Cal 8:	7:10	about 0.7 mg/mL
5.6.9.	Cal 9:	9:10	about 0.9 mg/mL
5.6.10.	Cal 10:	PCP HCl Stock Solution	about 1.0 mg/mL

6. Calibration

6.1. Response to PCP 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 average sensitivity calculated at each level (sensitivity is 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.

7. Procedures

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

7.1.1.1. Split mode (60:1)

7.1.1.2. Column: DB-1, 30mx0.250mm inner diameter (I.D.) x 0.25 µm film thickness, or equivalent.

7.1.1.3. Column Flow Program: 1.0 mL/minute

7.1.1.4. Oven Program: 125ºC starting temperature, ramp 6ºC/minute to 165ºC, ramp 4ºC/minute to 200ºC, ramp 30ºC/minute to 240ºC, hold 4.0 minute.

7.1.1.5. Inlet (injector) Temperature: 270ºC

7.1.1.6. Detector: 310ºC at 50Hz

7.1.1.7. Carrier Gas: Helium

7.1.1.8. Run time: 20.75 minutes

7.1.1.9. Injection volume: 1 µL

7.1.1.10. Injection Solvent: Chloroform

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

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

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

7.3. Quality Control Standards – Three Quality Control Standards are made and evaluated over time to assess performance of the method. Different values are acceptable, as long as recorded. The bulk QC PCP mixture is made:

7.3.1. About 25mg PCP HCl (>98% purity), with about 5mg Tenocyclidine (TCP) HCL, 5mg 1-piperidinocyclohexanecarbonitrile (PCC). About 70% pure PCP.

7.3.2. Mix contents and grind in mortar

7.3.3. Filter through approximately 20 mesh.

7.3.4. Quality Control (QC) standards (or their equivalents):

7.3.4.1. QCLow – About 0.8mg bulk QC PCP Mixture, added to 10mL volumetric flask, dilute to mark with ISTD solution. Perform 1:2 dilution. About 0.1 mg/mL PCP, or

7.3.4.2. QCMed – About 1.5mg bulk QC PCP Mixture, added to 10mL volumetric flask, dilute to mark with ISTD solution. Perform 1:5

dilution. About 0.5 mg/mL PCP.

7.3.4.3. QCHigh – About 40mg bulk QC PCP Mixture, added to 25mL volumetric flask, dilute to mark with ISTD solution. About 1 mg/mL PCP.

7.3.4.4. 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.

	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.
	Peak width resolution	Analyte peak must be base peak resolved, as evaluated by the analyst.

*S/N = Signal-to-Noise Ratio

7.6. Standard Runs

7.6.1. In order to conclusively identify a substance in a sample, a pure standard corresponding to the analyte of interest must be run within 24 hours of the sample and must be listed on the same sequence file as the sample. The substance must be run on the same method as the standard.

7.6.2. A separate standard will be run for each analyte of interest being reported, but multiple samples may be matched up with the same standard.

7.6.3. A blank run must be performed before the standard as if it were a sample.

7.6.4. The retention time of the sample must be within 2% of the retention time of the standard in order to be declared a match. Because concentrations of analytes can affect their retention times, if the retention time is outside this range, the sample or standard may be diluted with corresponding solvent and rerun.

7.6.5. Both the sample and standard must have similar mass spectra and must match a result in a Mass Spectrometry (MS) reference library.

7.6.6. A copy of the matching standard printout and the blank run immediately before it shall be included with the printout of the sample.

7.6.7. A copy of the printout for each standard used to match casework and the blank run immediately before it shall be retained in the Standards Logbook.

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

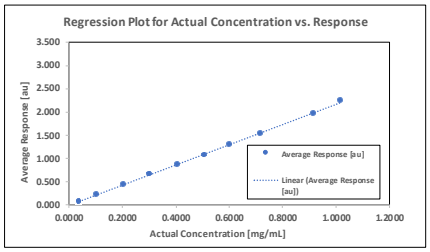
9.1. Linearity Evaluation of Calibration Standards

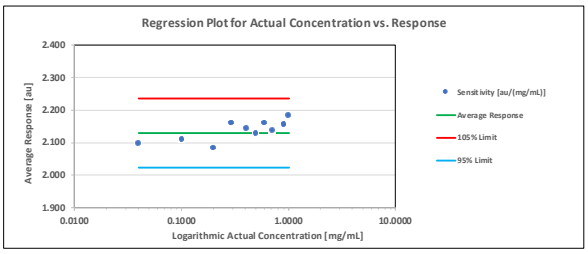
The following is an example calculation and evaluation of response values for the 9 calibrators used in this method, using Excel.

LINEARITY EVALUATION FORM

Method Name: FCS16 - SOP for the Quantitation of PCP GCFID
Target Compound: Phencyclidine (PCP)
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
0.04	0.0401	0.0819	0.0834	0.0843	0.0853	0.0854	0.084	2.098	2.129	2.023	2.236	PASS
0.1	0.1021	0.2100	0.2140	0.2144	0.2162	0.2216	0.215	2.108	2.129	2.023	2.236	PASS
0.2	0.2038	0.4200	0.4212	0.4244	0.4258	0.4299	0.424	2.082	2.129	2.023	2.236	PASS
0.3	0.3017	0.6300	0.6452	0.6573	0.6582	0.6656	0.651	2.158	2.129	2.023	2.236	PASS
0.4	0.4056	0.8400	0.8584	0.8703	0.8813	0.8958	0.869	2.143	2.129	2.023	2.236	PASS
0.5	0.5068	1.0500	1.0583	1.0787	1.0950	1.1077	1.078	2.127	2.129	2.023	2.236	PASS
0.6	0.6002	1.2700	1.2827	1.2906	1.3005	1.3325	1.295	2.158	2.129	2.023	2.236	PASS
0.7	0.7170	1.5000	1.5050	1.5338	1.5498	1.5664	1.531	2.135	2.129	2.023	2.236	PASS
0.9	0.9143	1.9300	1.9619	1.9661	1.9842	1.9999	1.968	2.153	2.129	2.023	2.236	PASS
1	1.0182	2.1400	2.1837	2.2188	2.2670	2.3074	2.223	2.184	2.129	2.023	2.236	PASS





Correlation Coefficient:	0.99988		
Y-intercept:	-0.01142		
Slope:	2.17347		
Linear Range:	0.0401 mg/mL	to	1.0182 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 Phencyclidine by Gas Chromatography (2016), Drug Enforcement Agency, Sept. 8th, 2016.

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

13.3. Forensic Chemistry Unit QAM (current revisions).

13.4. ISO/IEC 17025 guidelines