STANDARD OPERATING PROCEDURE

FOR

ANALYSIS OF OPIOIDS BY GC-MS TOF

PHILIS SOP L-A-505 Rev. 1

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PREPARED BY

PHILIS

PREPARED FOR

U.S. Environmental Protection Agency
Office of Emergency Management
Washington, DC 20460

Approvals:

PHILIS, Castle Rock Lead Chemist	**November 28, 2023** Date
PHILIS, Edison Lead Chemist	**November 28, 2023** Date
PHILIS, Quality Assurance Manager	**November 28, 2023** Date
PHILIS, Program Manager	**November 28, 2023** Date

Revision History

Revision	Name	Date	Description of Change
0	Tom Fowler James Travis	06/21/2021	SOP Developed
1	Tom Fowler James Travis	11/29/2022	Revision

SOP REVISION FORM

SOP Name	**Analysis of Opioids by GC-MS TOF**		
Purpose: *(Review or Revise)*	*SOP #:*	*Rev. #:* *(Being Reviewed or Revised)*	*Origination / Release Date:*
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For *Revision*: Summary of Revisions (*specify sections*)

Document	Revised from NELAP format to EPA format.
Signature Page	Changed Project Manager to Program Manager
Document	Updated reference numbers listed in document
1.1	Expanded the analyte list in this procedure

For *Review*: Comments

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1.0 Scope and Application

This standard operating procedure (SOP) is a gas chromatography/mass spectrometry Time of Flight (GC/MS TOF) technique for analysis of fentanyl at the low part per billion (ug/L or ug/Kg) level in waters and soils and nanogram levels (ng/wipe) in environmental samples. This procedure follows the general guidelines of EPA method 8270E for full scan GC/MS analysis.

1.1 This protocol is for the determination and measurement of the opioids listed below. It is based on USEPA (i.e., SW-846) Methods 8270E, 3510C, 3511, 3545A, and 3570 for the analysis and preparation of solid, wipe, and water samples.

Contaminant	CAS Number
Fentanyl	437-38-7
Acetylfentanil	3258-84-2
Alfentanil	69049-06-5
Carfentanil	61086-44-0
Heroin	561-27-3
Remifentanil	132539-07-2
Sulfentanil	69049-06-5

1.2 Procedures in this protocol have been tested for the target analytes listed in Section 1.2 in reference matrices (i.e., reagent water, Ottawa sand, dried soils, and wipes) and have not all been evaluated in field samples.

2.0 Summary of Method

2.1 This procedure involves solvent extraction of the sample followed by gas chromatography/ mass spectrometry (GC/MS TOF) analysis to determine drug concentration in environmental samples.

2.2 Prior to analysis, samples must be prepared using sample preparation techniques appropriate for each analyte and matrix type. Aqueous, solid, and wipe samples may be extracted by microscale extraction based on the required detection limits. Extracts may require a concentration step using nitrogen evaporation to achieve appropriate levels of quantitation.

2.3 Development of the method follows the same protocol as is used in the SW846 Method 8270E.

3.0 Definitions

3.1 Batch‡: Environmental samples that are prepared and/or analyzed together with the same process and personnel, using the same lot(s) of reagents. A Preparation Batch is composed of between 1 and 20 environmental samples of the same matrix, meeting the above mentioned criteria and with a maximum time between the start of processing of the first and the last sample in the batch to be 24hours. Each Preparation Batch requires; one MB, LCS, and MS/MSD pair. An Analytical Batch is composed of prepared environmental samples (extracts, digestates, or concentrates) which are analyzed together as a group. An analytical batch can include prepared samples originating from various quality system matrices and can exceed twenty (20) samples.

3.2 Holding Time: The maximum amount of time permitted between sampling and sample preparation and/or sample preparation and sample analysis. Also the period of time a sample may be stored prior to analysis when there is no preparation step. See the specific method or SW846 8000D Table 4.1.

3.3 Internal Standards (IS)‡: A known amount of standard added to a test portion of a sample as a reference for evaluating and controlling the precision and bias of the applied analytical method.

3.4 Laboratory Control Sample (LCS)‡: (however named, such as laboratory fortified blank, blank spike (BS), or QC check sample). A sample matrix, free from the analytes of interest, spiked with verified known amounts of analytes or a material containing known amounts of analytes and taken through all sample preparation and analytical steps of the procedure unless otherwise noted in a reference method. It is generally used to establish intra-laboratory or analyst specific precision and bias or to assess the performance of all or a portion of the measurement system.

The standard source can be the same as the calibration or a second source. The LCS is analyzed exactly like a sample to determine whether the method is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.5 Matrix Spike (spiked sample of fortified sample)‡: A sample prepared, taken through all sample preparation and analytical steps of the procedure unless otherwise noted in a referenced method, by adding a known amount of target analyte to a specified amount of the sample for which an independent test results of target analyte concentration is available. Matrix spikes are used, for example, to determine the effect of the matrix on a method's recovery efficiency.

3.6 Matrix Spike Duplicate (spiked sample or fortified sample duplicate)‡: A replicate matrix spike prepared in the laboratory and analyzed to obtain a measure of the precision of the recovery for each analyte.

3.7 Method Blank (MB): An aliquot of reagent water or other blank matrix that is treated exactly as a sample, including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. Method Blank analytical results are evaluated to determine the presence of contamination in the analytical method process.

3.8 Reporting Limit (RL): The reporting limit, also known as the LOQ is the minimum concentration that can be reported as a quantitated value for a target analyte in a sample. This value can be no lower than the concentration of the lowest calibration standard.

3.9 Surrogate Standard (SS): Organic compounds which are similar to the target analytes in chemical composition and mimic the behavior of the target analytes throughout the analytical process. Surrogate compounds are not normally found in environmental samples. Each calibration standard, sample, MB, LCS, MS, and MSD is spiked with surrogate standards. Surrogates are used to evaluate analytical efficiency by measuring recovery. See analytical method SOP for a list of specific surrogate compounds that are appropriate for sample-specific analysis.

ǂ EL-V1M2-ISO-2016, 2016 NELAP Standard definition.

4.0 Interferences

4.1 Matrix interferences can be caused by contaminants that are extracted from the sample during the extraction process. The amount of matrix interference varies from sample to sample. Cleanup procedures may help eliminate some of the interferences.

4.2 Contaminants in the solvents, reagents, glassware, and other extraction components may lead to matrix interferences. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running MBs with every batch. The data for all MB, LCS, MS, MSD, and samples must be evaluated for interferences. If interferences are detected, it is necessary to determine if the source of interference is in the preparation and then take corrective action to eliminate the interference. Using high purity reagents, solvents, and gases helps minimize interference problems.

4.3 Carryover contamination may occur when a sample containing low levels of analytes is injected immediately following a sample containing high levels of analytes or background. If this situation occurs during analysis, the sample containing the low concentration SVOCs may require reanalysis. A solvent blank should be run after the high level sample to ensure that the system is free of contamination. To reduce carryover, the injection syringe must be rinsed with solvent between samples.

4.4 Phthalate contamination is commonly observed, and its occurrence should be carefully evaluated as an indicator of the contamination problem in the sample preparation step of the analysis.

5.0 Safety

WARNING: The toxicity of the opioids analyzed by this method presents hazards unfamiliar to most experienced laboratory personnel. Special techniques and precautions must be used even for the simplest procedures involving these agents. If opioids are suspected target analytes, laboratory personnel must be thoroughly trained in appropriate safety procedures prior to using this method.

5.1 There are specific requirements for operations with opioids. Analysts must have read the PHILIS Chemical Hygiene Plan relating to opioids and receive all required training prior to conducting the analytical procedures described in this protocol.

5.2 At a minimum, personal protective equipment (PPE) requirements include safety glasses, lab coats, and protective gloves. All work with samples and standards shall be conducted in a fume hood. The availability of emergency response equipment and support personnel should be as indicated in a laboratory Chemical Hygiene Plan.

5.3 Exposure to drug material is possible from contact, and risk is primarily associated with compromise of protective clothing. Respiratory exposure can result from spills or improper use of ventilation controls and PPE.

5.4 Employees must abide by the policies and procedures in the Chemical Hygiene Plan and this document. This procedure involves hazardous material, operations, and equipment. This SOP does not purport to address all of the safety problems associated with its use. It is the responsibility of the user of the method to follow the appropriate safety, waste disposal, and health practices under the assumption that all samples and reagents are hazardous. Standard laboratory safety procedures should be followed when working with unknown samples.

5.5 Eye protection that satisfies ANSI Z87.1, laboratory coat, and nitrile gloves must be worn while handling samples, standards, solvents, and reagents. Disposable gloves that have been contaminated must be removed and discarded. Non-disposable gloves must be cleaned immediately. Latex and vinyl gloves provide no protection against the organic solvents used in this method, so nitrile or similar must be used.

5.6 The toxicity and/or carcinogenicity of the reagents and analytes used in this method have not been precisely defined; therefore, each chemical and sample should be treated as a potential health hazard. Exposure should be reduced to the lowest possible level. This entire extraction procedure must be performed in a fume hood.

6.0 Equipment and Supplies

6.1 Glassware

6.1.1 Autosampler vials with Teflon lined crimp tops used for analysis and storage of sample extracts. The vials may be clear or amber.

6.1.2 Mini inserts with plastic springs may be used with autosampler vials to allow for smaller extract aliquots.

6.1.3 10-mL/40-mL/60-mL vials used for storage of standards and spiking solutions

6.2 Syringes

Gas-tight micro syringes- various sizes for transferring the concentrated extracts, adding internal standards to extracts, and aliquoting the calibration standards Instrumentation.

6.3 Equipment and Supplies

6.3.1 Gas chromatograph/Mass spectrometer – Time of Flight system: an analytical system complete with a temperature-programmable gas chromatograph suitable for split/splitless injection and all required accessories, including syringes, analytical columns, and gases. The capillary column should be directly coupled to the source, and the instrument must be operated in splitless injection mode.

6.3.2 Gas Chromatography Column – Recommended length 30 m x 0.25 mm ID (or 0.32 mm) bonded phase silicon coated fused silica capillary column DB-5 (J&W Scientific); RTX-5, RTX-5Sil MS (Restek); Zebron ZB-5(Phenomenex); SPB-5 (Supelco); AT-5 (Alltech); HP-5 (Agilent); CP-Sil 8 CB (Chrompack); 007-2 (Quadrex); BP-5 (SGE); or equivalent. Although a film thickness of 1.0 micron is recommended because of its larger capacity, a film thickness of 0.25 micron may be used. A capillary column is considered equivalent if:

6.3.2.1 The column does not introduce contaminants that interfere with the identification and quantification of the compounds listed in Table 3.

6.3.2.2 The analytical results generated using the column meet the initial and continuing calibration verification technical acceptance criteria listed in the protocol and the quantitation levels determined as described in Section 12.8.

6.3.2.3 The column provides equal or better resolution of the compounds listed in Table 3, when compared to columns listed in Section 6.3.2.

6.3.3 Mass Spectrometer – Must be capable of detecting masses from 35 – 500 atomic mass unit (amu) such that 20 spectra a collected across a chromatographic peak, using 70 volts (nominal) electron energy in the electron impact ionization mode, and producing a mass spectrum that meets the tuning acceptance criteria when 50 ng or less of decafluorotriphenylphosphine (DFTPP) is injected through the GC inlet. The instrument must be vented to the outside of the facility or to a trapping system that prevents the release of contaminants into the instrument room.

6.3.4 Autosampler: Gerstel MPS-2 or equivalent.

6.3.5 GC/MS interface: Any GC/MS interface that gives acceptable calibration points and achieves acceptable tuning performance criteria may be used.

6.3.6 Data System The data system is equipped with the Leco Chromatof software for data acquisition, data processing and Gerstel's Maestro for the autosampler. Any equivalent system would work.

6.3.7 Syringe: 10-µL Gerstel syringe, or equivalent.

6.3.8 Carrier gas: ultra-high purity, equivalent or better helium.

6.3.9 Time of Flight (TOF) mass spectrometer

7.0 Reagents and Standards

7.1 Reagents

Original containers of reagents shall be labeled with expiration date when applicable. All containers of prepared reagents must bear a name, preparation date, and must be linked to the preparation records.

7.1.1 Organic-free Reagent Water – Defined as water in which an interferant is not observed at or above the RL for each analyte of interest. Reagent water may be generated by passing tap water through a filter bed containing activated carbon or may be purchased.

7.1.2 Reagent soil – TCL-free sand is used for QC samples.

7.1.3 Helium carrier gas- 99.999% (UHP) or better such as Research Grade, 99.9999%.

7.1.4 Nitrogen- purge gas, 99.999% (UHP) grade.

7.2 Solvents

7.2.1 Methylene Chloride–nano grade or equivalent.

7.2.2 Methanol—HPLC grade or equivalent.

7.3 Standards

The laboratory must be able to verify that the standards are certified. Manufacturer's certificates of analysis must be retained by the laboratory and presented upon request. Standard solutions purchased from a chemical supply house as extracts in sealed, glass ampules may be retained and used until the expiration date provided by the manufacturer. If no manufacturer's expiration date is provided, the ampouled standard solutions, if unopened, may be retained and used for two years from the preparation date. The expiration date of the ampouled standards, upon the breaking of the glass seal, is six months (or sooner, if the standard has degraded or evaporated). Note: For many of the target compounds, neat standards may be unavailable; therefore, the laboratory may need to purchase diluted standards.

7.3.1 Prepare standards for a calibration curve with a minimum of five points. Five points are valid for average response factor or linear regression curve fitting. Six calibration points are required for quadratic (second order) curve fits. The low point of the calibration curve must be at or below the reporting limit. The high standard defines the range of the calibration.

7.3.2 An internal standard (IS) solution is prepared by dissolving the compounds in MeOH or by purchasing a mixture from a commercial source. See Table 2 for a list of the internal standards used. The final concentration in the extract should be 500pg/µL.

7.3.3 Surrogate Standard Spiking Solution: Prepare as instructed in the extraction SOP (PHILIS SOP L-P-114) will result in a concentration of 500ng/mL. Other concentrations may be used provided the resulting amount in the samples and standards doesn't change. Surrogate compounds are listed in Table 2.

7.3.4 DFTPP GC/MS Tuning Standard: A solution in methylene chloride containing 50 µg/mL of decafluorotriphenylphosphine (DFTPP) is prepared.

7.3.5 Laboratory Control Spiking Solution: Prepared as instructed in the extraction SOPs. This solution must contain all target analytes.

7.3.6 Matrix Spike Solution: This is the same as the Laboratory Control Spiking Solution.

7.3.7 The standards must be stored away from any light source at 0 - 6 °C in Teflon lined screw cap amber bottles. The standard solutions expire six months after preparation date or at the earliest expiration date assigned by the vendor to any parent standard, whichever is earlier. Continuing calibration standards and other dilute standards should be checked weekly for degradation or when the standards fail to meet criteria, whichever is first.

7.3.8 Protect all standards from light. Samples, sample extracts, and standards must be stored separately.

7.3.9 The laboratory is responsible for maintaining the integrity of standard solutions and verifying the solution prior to use. The standards must be brought to room temperature prior to use, checked for losses, and checked to ensure that all components have remained in solution. Guidance on standard verification procedures can be found in EPA's Superfund Analytical Services / Contract Laboratory Program, Multi-Media, Multi-Concentration Organics Analysis, SOM01.2, Exhibit E, Section 7, May 2005. (http://www.epa.gov/superfund/programs/clp/download/som/som11e-h.pdf)

8.0 Sample Collection, Preservation, and Storage

8.1 Sample Preservation

Samples must be iced or refrigerated at 0 - 6°C from the time of collection until extraction.

8.2 Sample Storage

8.2.1 Samples must be protected from light and refrigerated at 0 - 6°C from the time of receipt until 60 days after delivery of results to the reference agency. After 60 days, the samples may be disposed of in a manner that complies with all applicable regulations.

8.2.2 Samples must be stored in an atmosphere demonstrated to be free of all potential contaminants.

8.3 Sample Extract Storage

8.3.1 Sample extracts must be protected from light and stored at 0 - 6°C until one year after delivery of results to the reference agency.

8.3.2 Samples, sample extracts, and standards must be stored separately.

8.4 Technical Holding Times

8.4.1 Certain analytes will start to degrade immediately after sample collection; therefore, it is recommended that samples be extracted immediately upon receipt in the laboratory.

8.4.2 Extracts must be analyzed within 14 days following extraction.

9.0 Quality Control

9.1 Initial Demonstration of Capability (IDC)

An initial demonstration of capability (IDC) shall be performed prior to the analysis of any samples and with each significant change in instrument type (e.g., different detection technique), personnel or method. An IDC consists of the following:

9.1.1 An initial demonstration of precision and recovery (IPR) determination (Section 9.2).

9.1.2 A method detection limit (MDL) study (Section 9.7).

9.1.3 A quantitation limit (QL) determination (Section 12.8) on a clean matrix (reagent water, Ottawa sand, pre-cleaned wipes).

The IDC consists of four replicate samples of a clean matrix spiked with opioids around the midpoint of the calibration curve and carried through the entire analytical process. Prior to performing the IDC it is required that, a valid initial calibration (Section 10.3) be established.

9.2 Initial Precision and Recovery (IPR)

9.2.1 For preparation of IDC samples, see PHILIS SOP L-P-114.

9.2.2 Calculations for IDC.

Calculate the percent recovery of each compound in the IDC sample using Equations 5-8 (Section 12.2.6). Calculate an average percent recovery for each compound.

Calculate a percent relative standard deviation (%RSD) for each compound in the IPR samples.

9.2.3 Technical Acceptance Criteria for IDC

The average percent recovery of each compound in the IDC should be within the analyte acceptance limits in Table 4 and the surrogate acceptance limits in Table 5.

9.2.4 Corrective Action for IDC

If the technical acceptance criteria in Table 4 and Table 5 are not met, inspect the system for problems and take corrective actions to achieve the acceptance criteria.

9.3 Method Blanks

A method blank is a volume of a clean reference matrix (e.g., reagent water for water samples, clean inert sand along with purified sodium sulfate or Hydromatrix drying agent for solid samples, or clean wipes for wipe samples) spiked with a sufficient amount of surrogate standard spiking solution such that the same amount of surrogate is added as for the associated samples and carried through the entire analytical procedure. Internal standard solution is added just prior to analysis by GC/MS to give a concentration of 200pg/µL for each internal standard. The volume or weight of the reference matrix must be approximately equal to the volume or weight of the samples associated with the blank.

9.3.1 Frequency of Method Blanks

A method blank must be extracted each time samples are extracted. The number of samples extracted with each method blank shall not exceed 20 field samples [excluding MS/MSDs and Performance Evaluation (PE) samples]. In addition, a method blank shall:

9.3.1.1 Be extracted by the same procedure used to extract samples.

9.3.1.2 Be analyzed on each GC/MS system used to analyze associated samples and conditions (i.e., GC/MS settings).

9.3.1.3 Under no circumstances should method blanks be analyzed at a dilution.

9.3.2 Technical Acceptance Criteria for Method Blank Analysis

9.3.2.1 All blanks must be analyzed at the frequency described in Section 10.3.1 on a GC/MS system meeting the DFTPP tuning criteria in Section 13.2.4 and Table 1, initial calibration in Section 10.3, and continuing calibration verification (CCV) technical acceptance criteria in Section 10.4.5.

9.3.2.2 The Percent Recovery (%Recovery) of each of the surrogates in the blank must be within the acceptance limits listed in Table 5.

9.3.2.3 The blank must meet the sample acceptance criteria listed in Section 9.3.

9.3.2.4 A method blank for solid, water, and wipe samples must contain less than the RL of target compounds. Note: In cases where a blank has detects above the RL, but associated samples have detects greater than 10 times the blank, consult the agency to determine if re-extraction is required.

9.3.3 Corrective Action for Method Blanks

9.3.3.1 If a method blank does not meet the technical acceptance criteria for method blank analysis, the laboratory shall consider the analytical system to be out of control.

9.3.3.2 If contamination is the problem, then the source of the contamination must be investigated and appropriate corrective measures must be taken and documented before further sample analysis proceeds. It is the laboratory's responsibility to ensure that interferences caused by contaminants in solvents, reagents, glassware, and sample storage and processing hardware that lead to discrete artifacts and/or elevated baselines in the GC/MS be eliminated. If possible, an aliquot of any sample associated with the contaminated blank must be re-extracted and reanalyzed or the data flagged.

9.3.3.3 If surrogate recoveries in the method blank do not meet the acceptance criteria listed in Table 5, first reanalyze the method blank. If the surrogate recoveries do not meet the acceptance criteria after reanalysis, the method blank and an aliquot of any sample associated with that method blank must be re-extracted, if possible, and reanalyzed or documented in the case narrative.

9.3.3.4 If the method blank does not meet internal standard response requirements listed in Section 9.3, follow the corrective action procedure outlined in Section 9.4. The laboratory shall resolve and document the resolution of the problem before proceeding with sample analysis.

9.3.3.5 If the method blank does not meet the retention time (RT) requirements for internal standards, check the instrument for malfunction and recalibrate. Reanalyze the method blank. Sample analyses cannot proceed until the method blank meets these requirements.

9.4 Matrix Spike and Matrix Spike Duplicate (MS/MSD)

To evaluate the effects of the sample matrix on the methods used for analyses, a mixture of target compounds must be spiked into two aliquots of a water or solid sample and analyzed in accordance with the appropriate method. Mixtures should be spiked at levels at a concentration near the midpoint of the calibration range.

An MS/MSD pair shall be analyzed with each sample batch of each matrix type where possible.

As part of EPA's quality assurance/quality control (QA/QC) program, water rinseate samples and/or field blanks (field QC) or PE samples may accompany solid, water, air, and/or wipe samples that are delivered to the laboratory for analysis. The laboratory must not perform MS/MSD analysis on any of the field QC or PE samples.

If the reference agency designates a sample to be used as an MS/MSD, then that sample must be used. If there is insufficient sample remaining to perform an MS/MSD, then the laboratory shall choose another sample on which to perform an MS/MSD analysis. At the time the selection is made, the laboratory shall notify the reference agency that insufficient sample was received and identify the reference agency sample selected for the MS/MSD analysis.

If there is insufficient sample remaining in any of the samples in a batch to perform the required MS/MSD, the laboratory will report this in the data narrative.

9.4.1 Dilution of MS/MSD

Before any MS/MSD analysis, analyze the original sample, then analyze the MS/MSD at the same concentration as the most concentrated extract for which the original sample results will be reported.

9.4.2 Calculations for MS/MSD

Calculate the percent recovery of each matrix spike compound in the MS/MSD sample (see EQ. 11 in Section 12.2.9).

Calculate the Relative Percent Difference (RPD) of the concentrations of each compound in the MS/MSD using EQ. 1.

Concentrations of the matrix spike compounds are calculated using the same equations as used for target compounds (Equation 5 for water samples and Equation 6 for solid samples in Section 12.2.6).

EQ. 1 Relative Percent Difference Calculation

$$RPD = \frac{|C_1 - C_2|}{\left(\frac{C_1 + C_2}{2}\right)} \times 100$$

Where:
C_1 = Measured concentration of the first sample aliquot
C_2 = Measured concentration of the second sample aliquot

9.4.3 Technical Acceptance Criteria for MS/MSD

All MS/MSDs must be analyzed on a GC/MS system meeting DFTPP, initial and continuing calibration verification technical acceptance criteria, and the method blank technical acceptance criteria.

The MS/MSD must be extracted and analyzed within the technical holding time (Section 8.4).

The retention time (RT) shift for each of the internal standards must be within ± 0.50 minutes (30 seconds) between the MS/MSD sample and the most recent CCV standard analysis.

The limits for matrix spike compound recovery and RPD are given in Tables 5 & 6.

9.4.4 Corrective Action for MS/MSD

If recovery or RPD limits are not met and the LCS, CCV and method blank are within acceptable limits, this may be an indication of matrix interferences.

If MS/MSD recovery limits cannot be met, flag the results of the associated sample.

9.5 Laboratory Control Sample (LCS)

An LCS consists of an aliquot of clean reference matrix, of the same weight or volume as the corresponding field samples, and spiked with the same compounds at the same concentrations used to spike the MS/MSD. When the results of the MS/MSD analysis indicate matrix interference may be present, the LCS results are used to verify that the interferences are due to the sample matrix and not from artifacts introduced in the laboratory.

9.5.1 Frequency of LCS Analyses

One LCS must be prepared, extracted, analyzed, and reported for every 20 field samples or fewer extracted in a batch of a similar matrix. The LCS must be extracted and analyzed concurrently with the samples, using the same extraction procedure, cleanup procedure (if required), and instrumentation.

9.5.2 Calculations for LCS

Calculate the recovery of each compound in the LCS using Equation 11 (Section 12.2.9).

9.5.3 Technical Acceptance Criteria for LCS Analysis

All laboratory control samples must be extracted and analyzed at the frequency described in Section 9.5.1 on a GC/MS system meeting the tuning, initial and continuing calibration verification, and the method blank technical acceptance criteria.

The limits for LCS compound recovery can be found in Table 4 and Table 5.

9.5.4 Corrective Action for LCS

If LCS recovery limits are not met, inspect the system for problems and take corrective actions to achieve the acceptance criteria including reanalysis.

If LCS recovery limits cannot be met, flag all associated sample and blank data accordingly.

9.6 Instrument Detection Limit (IDL) Determination

Before any field samples are analyzed, laboratories may determine an IDL for each target compound on each instrument used for analysis. While determining IDLs are not required, IDL results can be helpful in determining an appropriate spike level for use in determining the MDL (Section 9.7). It is recommended that IDLs be verified annually thereafter, or after major instrument maintenance. Major instrument maintenance includes, but is not limited to: cleaning or replacement of the mass spectrometer source, mass filters, electron multiplier, and installing a different GC column type. An IDL is instrument-specific and independent of sample matrices.

9.6.1 An IDL is determined for each compound as the concentration that produces an average signal-to-noise ratio of between 3:1 and 5:1 for at least three replicate injections.

9.6.2 All documentation for the IDL determination shall be maintained at the laboratory and provided to the reference agency or the data user upon request.

9.7 Method Detection Limit (MDL) Determination

Before any field samples are analyzed, laboratory MDLs must be determined for each target analyte in appropriate reference matrices (i.e., reagent water, Ottawa sand, or clean wipes), using the sample preparation and analytical procedures described in this protocol for each specific matrix, and following the instructions and requirements described at 40 CFR Part 136, Appendix B.

9.7.1 The laboratory must use full method procedures to prepare and analyze at least seven replicates.

9.7.2 Spike each replicate sample at concentrations of 1 – 5 times the IDL concentration for each analyte and analyze the samples following protocol procedures.

9.7.3 To determine analyte MDLs, the following equation is applied to the analytical results (Student's t-factor is dependent on the number of replicates used; 3.14 assumes seven replicates):

MDL = 3.14 x sd

Where:
sd = the standard deviation for the analytical results, and
3.14 = the Student's t-value for seven replicate samples

9.7.4 The MDL results calculated using the equation in Section 9.7.3 must meet the following requirements as well as all other requirements specified in 40 CFR Part 136, Appendix B:

9.7.5 MDL result must not be greater than the spiking level used for the MDL determination.

9.7.6 MDL results must not be less than one tenth the spiking level used for the MDL determination.

9.7.7 If either requirement is not met, the laboratory must adjust their spiking level appropriately and repeat the MDL determination.

9.7.8 See Table 7 for MDLs.

9.8 Reporting Limit (RL) Determination

Laboratory RLs can be determined by multiplying the standard deviation of the results used to determine the MDL by 10. This approach uses the variability of the results used to determine the MDL, to estimate the concentration that would yield a 10% relative standard deviation (RSD) under ideal conditions. The resulting RL should be evaluated against the criteria listed below. These criteria are provided as guidance. If any of the criteria are not met, the laboratory should consult project managers to determine if the RL is sufficient to address project needs:

9.8.1 Results from spikes at the RL should be above the MDL.

9.8.2 The RL should be at or above the lowest calibration level.

9.8.3 The RL should be at least two times the MDL.

9.8.4 The relative standard deviation of results from spikes at the RL should be less than 20%.

9.8.5 The mean recovery of spikes at the RL must be within 50 – 150%.

9.8.6 See Table 7 for RLs.

10.0 Calibration and Standardization

10.1 Instrument Operating Conditions

10.1.1 Gas Chromatograph (GC)

10.1.1.1 The following GC analytical conditions are provided for guidance (pulsed splitless injection) and may be modified if needed to optimize analytical results. Other conditions may be used, provided that all technical acceptance criteria in Sections 10.2.4, 10.3.4, 10.4.5, and 12.3 are met. Initial column temperature: 110°C for 1.5 minutes

Column temperature program: 40°C/minute to 250 °C; 30°C/minute to 300 °C; 25°C/minute to 330 °C and hold for 4.20 min.

Injector temperature: 280°C

Injector liner: 4mm Single Gooseneck

Injection mode: Pulsed splitless at 80 psi for 1.5 minutes and purge flow of 20mL/min. at 1 minute.

10.1.1.2 Sample injection volume: 1.0 µL

10.1.1.3 GC column: Agilent HP-5MS, (5%-phenyl)- methylpolysiloxane (see Section 6.4.2 for equivalent columns)

10.1.1.4 Column dimensions: 30 m x 0.25 mm x 0.25 µm

10.1.1.5 Carrier gas: Helium at 32 cm/second

Optimize GC conditions for analyte separation and sensitivity. Once optimized, the same GC conditions must be used for the analysis of all standards, samples, blanks, and MS/MSDs.

10.1.2 Mass Spectrometer (MS)

The following GCMS-TOF analytical conditions are provided in this section to optimize analytical results. Other settings may be used provided they are equivalent or better. Examples of alternate conditions using Time of Flight (TOF) MS are provided in Appendix A.

10.1.2.1 MS transfer line temperature: 280°C

10.1.2.2 Source temperature: 250°C or according to manufacturer's specifications

10.1.2.3 Electron energy: 70 eV (nominal)

10.1.2.4 Mass range: 35 to 500 m/z

10.1.2.5 Ionization mode: Electron Ionization (EI), positive

10.1.2.6 Spectra per second: -~12 at peak width half height

10.2 Library searching: NIST 05 Mass Spectral Database MS Mass Calibration (Tuning) and Ion Abundance

10.2.1 Summary of MS Instrument Performance Check

The MS system must be tuned to meet the manufacturer's specifications, using a suitable calibration compound such as perfluoro-tri-n-butylamine (FC-43) or perfluorokerosene (PFK). The mass calibration and resolution of the MS system are verified by the analysis of the instrument performance check solution (Section 10.3.4). Prior to the analysis of any samples, including MS/MSDs, blanks, or calibration standards, the laboratory must establish that the MS system meets the mass spectral ion abundance criteria for the instrument performance check solution (Table 1) containing DFTPP.

10.2.2 Frequency of GC/MS Instrument Performance Check – The instrument performance check solution must be analyzed prior to each initial calibration.

10.2.3 GC/MS Instrument Performance Check

The analysis of the instrument performance check solution may be performed as an injection of 50 ng or less of DFTPP into the MS or by adding a sufficient amount of DFTPP to the calibration standards to result in an on-column amount of 50 ng or less of DFTPP (Section 10.3.4) and analyzing the calibration standard.

10.2.4 Technical Acceptance Criteria for GC/MS Instrument Performance Check

The instrument performance check solution must be analyzed at the frequency described in Section 10.2.2.

Abundance criteria are listed in Table 1 for guidance. The mass spectrum of DFTPP must be acquired in the following manner: three scans (the peak apex scan and the scans immediately preceding and following the apex) are acquired and averaged. Background subtraction is required, and must be accomplished using a single scan acquired no more than 20 scans prior to the elution of DFTPP. The background subtraction should be designed only to eliminate column bleed or instrument background ions. Do not subtract part of the DFTPP peak.

Note: All subsequent standards, samples, MS/MSDs, and blanks associated with a DFTPP analysis must use the identical GC/MS instrument run conditions.

10.2.5 Corrective Action for GC/MS Instrument Performance Check

If the GC/MS instrument performance check technical acceptance criteria are not met, re-tune the GC/MS system. It may be necessary to perform maintenance to achieve the technical acceptance criteria.

The instrument performance check technical acceptance criteria in Section 10.2.4 must be met before any standards, samples, including MS/MSDs, or required blanks are analyzed.

10.3 Initial Calibration

Prior to sample analysis, and after instrument performance check technical acceptance criteria have been met, each GC/MS system must be calibrated at a minimum of five concentrations (Section 10.2.2 and Table 6) to determine instrument sensitivity and the linearity of GC/MS response for the target and surrogate compounds. If the RSD criteria cannot be met, a linear or quadratic curve may be used. Each initial calibration standard contains all the target compounds, surrogates, and internal standards.

10.3.1 Frequency of Initial Calibration

Each GC/MS must be calibrated whenever the laboratory takes corrective action that may change or affect the initial calibration criteria, or if the CCV technical acceptance criteria are not met.

If time remains in the 12-hour period after meeting initial calibration acceptance criteria, samples may be analyzed. It is not necessary to analyze a continuing calibration standard within this period.

10.3.2 Procedure for Initial Calibration

Prepare at least five calibration standards containing all the detected target compounds and associated surrogates at the concentrations described in Table 6.

Add a sufficient amount of internal standard solution (Section 7.3.2) to aliquots of calibration standards to result in 500pg/µL of each internal standard. Standards specified in Section 7.3.1 should permit most of the target compounds to have relative retention times (RRTs) of approximately 0.60 to 1.70, using the assignments of internal standards to target compounds given in Table 2.

Analyze each calibration standard by injecting 1.0 µL of standard.

10.3.3 Calculations for Initial Calibration

Calculate the relative response factor (RRF) for each analyte and surrogate using Equation 2 and the primary characteristic ions found in Table 3. Assign target compounds and surrogates to internal standards according to Table 2. For internal standards, use the primary ion listed in Table 3 unless interferences are present. Note: Unless otherwise stated, the area response of the primary characteristic ion is the quantitation ion.

EQ. 2 Relative Response Factor Calculation

$$RRF = \frac{A_x}{A_{is}} \times \frac{C_{is}}{C_x}$$

Where:
A_x = Area of the characteristic ion for the compound to be measured (Table 3)
A_{is} = Area of the characteristic ion for specific internal standard (Table 2)
C_{is} = Amount of the internal standard injected (ng)
C_x = Amount of the target compound or surrogate injected (ng)

The Mean Relative Response Factor ($\overline{RRF}$) for the Initial Calibration RRFs and mean RRFs must be calculated for all compounds. Calculate the percent relative standard deviation (%RSD) of the RRF values for the initial calibration. If linear regression or quadratic curve fitting is needed, consult SW-846 Method 8000D for guidance on the appropriate calculations.

10.3.4 Technical Acceptance Criteria for Initial Calibration

An initial calibration should be performed at the frequency described in Section 10.3.1 on a GC/MS system meeting the instrument performance check technical acceptance criteria (Section 10.2.4).

The RRF for each target compound and surrogate should be greater than or equal to 0.01.

10.3.5 The %RSD of the RRFs over the initial calibration range for each target compound and surrogate should be less than or equal to 20.00. If %RSD for a target analyte or surrogate cannot meet the acceptance criteria, curve fitting by linear or quadratic regression may be used provided the R^2 value is greater than or equal to 0.99.

10.3.6 Corrective Action for Initial Calibration

If technical acceptance criteria using at least one of the three optional approaches to initial calibration (%RSD of the RRFs, linear regression, or quadratic regression) are not met, inspect the system for problems, take corrective actions, and re-calibrate the system. If criteria are not met with re-calibration, the laboratory will flag all data associated with the calibration.

Initial calibration technical acceptance criteria must be met before any samples, including MS/MSDs or required blanks are analyzed and reported without data qualification.

10.4 Continuing Calibration Verification

10.4.1 Summary of Continuing Calibration Verification

Prior to the analysis of samples, each GC/MS system must be routinely checked by analyzing a CCV standard or calibration with tune to ensure that the instrument continues to meet the instrument sensitivity and linearity requirements. The CCV standard contains all the target compounds, surrogates, and internal standards. The same injection volume must be used for all standards, samples, and blanks.

10.4.2 Frequency of Continuing Calibration Verification – Each GC/MS used for analysis must be checked once every 12-hour time period of operation. The 12-hour time period begins with the injection of DFTPP prior to calibration analysis or the injection of the opening CCV on other sequences without a calibration.

10.4.3 Procedure for Continuing Calibration Verification

Add a sufficient amount of internal standard solution (Section 7.3.4.) to an aliquot of CCV standard to result in a concentration of 500pg/µL

Analyze the CCV standard by injecting 1.0 µL of standard.

10.4.4 Calculations for CCV

Calculate an RRF for each target compound and surrogate using Equation 2 and the primary characteristic ions found in Table 3.

Calculate the Percent Difference (%Difference) between the $\overline{RRF}$ from the most recent initial calibration and the continuing calibration verification RRF for each target compound and surrogate using Equation 3.

EQ. 3 Relative Response Factor Percent Difference Calculation

$$\%\text{Difference}_{RRF} = \frac{RRF_c - \overline{RRF_i}}{\overline{RRF_i}} \times 100$$

Where:
RRF_i = Mean Relative Response Factor from the most recent initial calibration meeting technical acceptance criteria.
RRF_c = Relative Response Factor from CCV standard.

10.4.5 Technical Acceptance Criteria for CCV

The CCV standard must be analyzed at or near the mid-point concentration level, at the frequency described in Section 13.4.2, on a GC/MS TOF system meeting the instrument performance check and the initial calibration technical acceptance criteria.

The RRF for each target compound and surrogate should be ≥ 0.01.

The RRF percent difference for each target compound in all CCVs should be within the range of ±50 %. (Note: This range may be updated following additional laboratory testing of the method.) If regression techniques are used for the initial calibration, the CCV must be evaluated in terms of percent drift using concentrations (See Equation 3a).

EQ. 3a Percent Drift (PD) Calculation for CCV

$$PD = \frac{\text{CalculatedConcentration} - \text{Theoretical Concentration}}{\text{Theoretical Concentration}} \text{x} 100\%$$

The percent drift (PD) for each target compound should be within the range of ±50.

Excluding those ions in the solvent front, no quantitation ion may saturate the detector.

10.4.6 Corrective Action for CCV

If the CCV technical acceptance criteria in Section 10.4.5 are not met, recalibrate the GC/MS instrument according to Section 10.3.

CCV technical acceptance criteria should be met before any samples MS/MSDs, or required blanks, are analyzed. If CCV criteria are not met, flag associated samples and blanks accordingly.

10.5 Instrument Blank

10.5.1 Summary of Instrument Blank

An instrument blank is comprised of DCM spiked with internal standards at the same concentration used for associated samples. The purpose of the instrument blank is to minimize the impact of carryover.

10.5.2 Frequency of Instrument Blank

An instrument blank is recommended for analysis following suspected carry-over or during analysis of samples containing suspected high concentrations.

10.5.3 Procedure for Instrument Blank Analysis

Add sufficient amount of internal standard solution (Section 7.3.4) to an aliquot of clean solvent to result in a concentration of 500pg/µL. Analyze each instrument blank by injecting 1.0 µL of standard.

10.5.4 Calculations for Instrument Blank

Calculate the concentrations of any observed target analyte using Equation 5, setting V_t, V_o, and DF all equal to 1.

10.5.5 Technical Acceptance Criteria for Instrument Blank

If an instrument blank is analyzed, the concentration of all target analytes in the instrument blank should be less than the concentration of the target analytes in the low calibration standard. The area response of the internal standards should be within 50 – 150% of the associated CCV or mid-level concentration of the initial calibration.

10.5.6 Corrective Action for Instrument Blank

If an instrument blank is analyzed and the instrument blank technical acceptance criteria are not met, analyze an additional instrument blank. If the problem persists, inspect the system for problems and take corrective actions to achieve the acceptance criteria. Instrument blank technical acceptance criteria should be met before samples are analyzed. Samples that are analyzed with corresponding instrument blanks that do not meet the instrument blank criteria should be reanalyzed, or the corresponding data should be flagged.

11.0 Procedure

11.1 Sample Analysis

11.1.1 Analysis is performed using an automated injection GC/MS TOF instrument.

11.1.2 In Chromatof or equivalent instrument software, load the sequence from the previous run and enter in the sequence information for the day. A typical sequence will have one or two rinses, the CCV, an instrument blank, the QC from the batch, then extracts of the samples.

11.1.3 Calibrate the instrument as described in Section 10.3.2. All instrument tuning and calibration criteria must be met prior to the analysis of samples.

11.1.4 All samples must be analyzed using the same instrument conditions as the preceding ICAL, and CCV standards.

11.1.5 Add internal standard to the sample extract to result in a 500pg/µL concentration of internal standard. Mix thoroughly before injection into the instrument.

11.1.6 If samples are to be diluted, add the internal standard after the dilution is made.

11.1.7 Inject the sample aliquot into the GC/MS TOF using the sample injection technique as used for the standards. Injection amount is 1 µL.

11.1.8 The data system will determine the concentration of each analyte in the extract using calculations based on the initial calibration, not the continuing calibration verification.

11.1.9 Identified compounds are reviewed for proper integration. Manual integrations are performed if necessary and are documented by the analyst. The minimum documentation required is a hard copy of the original data peak integration and a copy showing the manual integration with the analyst initials and date and explanation of why the manual integration was performed.

11.1.10 The internal standard response in the sample must be within 50- 200% of the response in the CCV or midpoint of the calibration curve.

11.2 Dilutions

11.2.1 If the response for any compound exceeds the working range of the GC/MS TOF system, a dilution of the extract is prepared and analyzed. An appropriate dilution should be in the upper half of the calibration range. Samples may be screened to determine the appropriate dilution for the initial run. If the initial diluted run has no hits or hits below 20% of the calibration range and the matrix allows for an analysis at a lesser dilution, the sample must be re-analyzed at a dilution targeted to bring the largest hit above 50% of the calibration range.

11.2.2 Add the internal standard to the diluted extract for a resulting concentration of 500pg/µL of each internal standard, and analyze the diluted extract.

11.2.3 Reporting Dilutions

The most concentrated dilution with no target analytes above the calibration range will be reported. Other dilutions will be reported only at the client's request.

12.0 Data Analysis and Calculations

12.1 Qualitative Identification of Target Compounds

12.1.1 The compounds listed in Table 3 must be identified by an analyst competent in the interpretation of mass spectra by comparison of the sample mass spectrum to the mass spectrum of the standard of the suspected compound. Two criteria must be satisfied to verify the identifications:

12.1.1.1 Elution of the sample analyte within the GC RRT unit window established from the 12-hour calibration standard.

12.1.1.2 Correspondence of the sample analyte and calibration standard component mass spectra.

12.1.2 For establishing correspondence of the GC RRT, the sample component must compare within ±0.06 RRT units of the standard component. For samples analyzed during the same 12 hour time period as the initial calibration standards, compare the analyte RTs to those from the midpoint initial calibration standard. Otherwise, use the corresponding CCV standard. If coelution of interfering components prohibits accurate assignment of the sample component RRT from the total ion chromatogram, the RRT should be assigned by using EICPs for ions unique to the component of interest.

12.1.3 For comparison of standard and sample component mass spectra, mass spectra obtained from a calibration standard on a GC/MS meeting the daily instrument performance requirements for DFTPP are required. Once obtained, these standard spectra may be used for identification purposes only if the GC/MS meets the DFTPP instrument performance requirements.

12.1.4 All ions present in the standard mass spectrum at a relative intensity greater than 10% (the most abundant ion in the spectrum equaling 100%) must be present in the sample spectrum. The relative intensities of ions specified in Table 3 must agree within ± 20% between the standard and sample spectra (e.g., for an ion with an abundance of 50% in the standard spectra, the corresponding sample ion abundance must be between 30 – 70%). Ions greater than 10% in the sample spectrum, but not present in the standard spectrum, must be considered and accounted for by the analyst making the comparison. The verification process should favor false positives. All compounds meeting the identification criteria must be reported with their spectra. When target compounds are below LOQs, but the spectrum meets the identification criteria, report the concentration with a "J". For example, if the LOQ is 5.0 g/L and concentration of 3.0 g/L is calculated, report as "3.0 J". Reporting below the LOQ is performed at client request.

12.1.5 If a compound cannot be verified by all of the spectral identification criteria in Sections 12.1.1 – 12.1.4, but in the technical judgment of the mass spectral interpretation specialist the identification is correct, then the laboratory must report the identification and proceed with quantitation.

12.2 Data Analysis and Calculations of Target Compounds

12.2.1 Target compounds identified shall be quantitated by the internal standard method. The internal standard used shall be the one assigned to that analyte for quantitation (Table 2). The EICP area of primary characteristic ions of analytes listed in Table 3 are used for quantitation.

12.2.2 It is expected that situations will arise when the automated quantitation procedures in the GC/MS software provide an inappropriate result. This normally occurs when there is compound coelution, baseline noise, or matrix interferences. In these circumstances, the laboratory must perform a manual quantitation. Manual integrations are performed by integrating the area of the quantitation ion of the compound. This integration shall only include the area attributable to the specific target compound. The area integrated must not include baseline background noise. The area integrated must not extend past the point where the sides of the peak intersect with the baseline noise. Manual integration is not to be used solely to meet QC criteria, nor is it to be used as a substitute for corrective action on the chromatographic system.

12.2.3 In all instances where the data system report has been edited or where manual integration or quantitation has been performed, the GC/MS operator must identify such edits or manual procedures by initialing and dating the changes made to the report and shall include the integration scan range. The GC/MS operator must also mark each integrated area on the quantitation report. In addition, a hardcopy printout of the EICP of the quantitation ion displaying the manual integration shall be included in the raw data.

12.2.4 The requirements listed in Sections 12.2.1 – 12.2.3 apply to all standards, samples, and blanks.

12.2.5 The $\overline{RRF}$ from the initial calibration is used to calculate the concentration in the sample. Secondary ion quantitation is allowed only when there are sample interferences with the primary ion. If linear regression is used, a regression curve must be used to calculate the concentration in samples. Refer to Section 12.2.7 for calculating sample concentration using linear regression techniques.

12.2.6 Calculate the concentration in the sample using the $\overline{RRF}$ and Equations 5 – 8.

EQ. 5 Concentration of Water Sample

$$\text{Concentration (ug/L)} = \frac{(A_x)(I_s)(V_t)(DF)}{(A_{is})(\overline{RRF})(V_o)(V_i)}$$

Where:
A_x = Area of the characteristic ion for the target compound
A_{is} = Area of the characteristic ion for the internal standard
I_s = Amount of internal standard injected in ng
V_o = Volume of water extracted in mL
V_i = Volume of extract injected in µL
V_t = Volume of the extract in µL
(Note: Extraction of water samples does not include concentration, and V_t is equal to the sum of the volumes added for extraction and addition of surrogates, internal standards, and any spiked target compounds.)
$\overline{RRF}$ = Mean Relative Response Factor determined from the initial calibration standard
DF = Dilution Factor. If no dilution is performed, DF = 1.0. The DF for analysis of water samples is defined as:

$$DF = \frac{\text{µL most conc. extract used to make dilution + µL clean solvent}}{\text{µL most conc. extract used to make dilution}}$$

EQ. 6 Concentration of Solid Sample

Note: Equation 6 includes a %moisture (D) factor for those cases when data is to be reported on the basis of dry sample weight. In cases where results are reported in terms of sample weight, this factor is deleted from the equation.]

$$\text{Concentration } \mu g/Kg \text{ (Dry weight basis)} = \frac{(A_x)(I_s)(V_t)(DF)}{(A_{is})(V_i)(\overline{RRF})(W_s)(D)}$$

Where:
A_x, I_s, A_{is}, V_i are as given for water, above.
V_t = Volume of concentrated extract in µL

$$D = \frac{100 - \%\text{Moisture}}{100}$$

W_s = Weight of sample extracted in g
$\overline{RRF}$ = Mean Relative Response Factor determined from the initial calibration standard
DF = Dilution Factor

EQ. 8 Concentration of Wipe Sample

$$\text{Concentration } \mu g/\text{std } cm^2 = \frac{(A_x)(I_s)(V_t)(DF)}{(A_{is})(V_o)(V_i)(\overline{RRF})}$$

Where:
A_x = area response for the compound to be measured, counts
A_{is} = area response for the internal standard, counts
I_s = amount of internal standard, µg
$\overline{RRF}$ = the mean RRF from the most recent initial calibration, dimensionless
Area = area of surface wiped, cm^2
V_t = volume of concentrated extract, µL
V_i = volume of extract injected, µL
DF = dilution factor for the extract. If there was no dilution, DF equals 1. If the sample was diluted, DF is greater than 1.

12.2.7 Calculate the concentration in the sample using linear regression.

Set y = (Peak Area of Target/Peak Area of Internal Standard) and x = (Theoretical Concentration of Target/Theoretical Concentration of Internal Standard).

Plot (Peak Area of Target/Peak Area of Internal Standard [Y-axis]) vs. (Theoretical Concentration of Target/Theoretical Concentration of Internal Standard).

Determine the slope of the line (m) and the y-intercept (b).

Rearrange the line equation to solve for x: x = (y-b)/m.

Multiply x by the concentration of the internal standard to get the concentration of target in extract.

Multiply the concentration of target analyte in the extract by the extract volume and divide by the sample volume to get concentration of target analyte in sample.

12.2.8 Adjusted LOQ Calculations

EQ. 9 Aqueous Adjusted LOQ

$$\text{Adjusted LOQ} = \text{Method LOQ} \times \frac{(V_x)(V_t)(DF)}{(V_o)(V_c)}$$

Where:
V_t, DF, and V_o are as given in Equation 5.
V_x = Method sample volume
V_c = Method concentrated extract volume

EQ. 10 Solid Adjusted LOQ

$$\text{Adjusted LOQ} = \text{Method LOQ} \times \frac{(W_x)(V_t)(DF)}{(W_s)(V_c)(D)}$$

Where:
V_t and DF are as given in Equation 5.
W_s and D are as given in Equation 6.
W_x = Method sample weight
V_c = Method concentrated extract volume

12.2.9 Surrogate Recoveries

Calculate surrogate recoveries for all samples, blanks, and MS/MSDs. Determine if recovery is within limits (Table 4).

Calculate the concentrations of the surrogates using the same equations as used for the target compounds. Calculate the recovery of each surrogate using EQ. 11.

EQ. 11 Percent Recovery

$$\text{Recovery} = \%R = \frac{C_s}{C_n} \times 100$$

Where:
C_s = Measured concentration of the spiked sample aliquot.
C_n = Nominal (theoretical) concentration increase that results from spiking the sample, or the nominal concentration of the spiked aliquot (for LCS).

12.3 Technical Acceptance Criteria for Sample Analysis

12.3.1 The samples must be analyzed on a GC/MS system meeting the instrument performance check, initial calibration, CCV, and blank technical acceptance criteria.

12.3.2 The sample must be extracted and analyzed within the technical holding times.

12.3.3 The sample must have an associated method blank meeting the blank technical acceptance criterion.

12.3.4 The percent recoveries of the surrogates in a sample should be within the recovery limits listed in Table 4. These limits are based on a workgroup consensus and will be updated following method validation. Note: The surrogate recovery requirements do not apply to samples that have been diluted.

12.3.5 The instrumental response (EICP area) for each of the internal standards in the sample must be within the range of 50.0 – 200% of the response of the internal standard in the most recent CCV standard analysis.

12.3.6 The RT shift for each internal standard must be within ± 0.50 minute (30 seconds) between the sample and the most recent CCV standard analysis.

12.3.7 Excluding those ions in the solvent front, no ion may saturate the detector. If a target compound concentration exceeds the upper limit of the initial calibration range, a more dilute aliquot of the sample extract must also be analyzed.

12.4 Corrective Action for Sample Analysis

12.4.1 The sample technical acceptance criteria must be met before data are reported. If the corrective actions described in this section did not solve the problem, all associated sample and blank data must be flagged accordingly.

12.4.2 Corrective action for failure to meet instrument performance checks and initial and continuing calibration verification must be completed before the analysis of samples. If the corrective actions described in Sections 10.2.5 (instrument performance check), 10.3.5 (initial calibration), or 10.4.6 (CCV) did not solve the problem, all associated sample and blank data must be flagged accordingly.

12.4.3 Corrective action for surrogate recoveries in a sample fail to meet the acceptance criteria specified in Section 12.3.4, check calculations, sample preparation logs, surrogate standard spiking solutions, and the instrument operation.

12.4.3.1 If the calculations were incorrect, correct them and verify that the surrogate recoveries meet their acceptance criteria.

12.4.3.2 If the sample preparation logs indicate that the incorrect amount of surrogate standard spiking solution was added to the sample, then re-extract (if possible) and reanalyze the sample after adding the correct amount of surrogate standard spiking solution.

12.4.3.3 If the surrogate standard spiking solution was improperly prepared, concentrated, or degraded, re-prepare the solution, re-extract (if possible), and reanalyze the samples.

12.4.3.4 If the instrument malfunctioned, correct the instrument problem and reanalyze the sample extract. Verify that the surrogate recoveries meet their acceptance criteria.

12.4.3.5 If the instrument malfunction affected the calibrations, recalibrate the instrument before reanalyzing the sample extract.

12.4.3.6 If the above actions do not correct the problem, then the problem may be due to a sample matrix effect. To determine if there was matrix effect, take the following corrective action steps:

12.4.3.7 Re-extract (if possible) and reanalyze the sample. EXCEPTION: If surrogate recoveries in a sample used for a matrix spike/matrix spike duplicate (MS/MSD) were considered unacceptable, then it should be re-extracted/reanalyzed only if surrogate recoveries met the acceptance criteria in <u>both</u> the MS/MSD analyses.

12.4.3.8 If the surrogate recoveries meet acceptance criteria in the re-extracted/reanalyzed sample, then the problem was within the laboratory's control.

12.4.3.9 Submit data from both analyses. Distinguish between the initial analysis and the extraction/reanalysis on all deliverables.

12.4.4 Corrective action for internal standards in a sample that fail to meet their acceptance criteria, check calculations, internal standard solutions, and instrument operation.

12.4.4.1 If the calculations were incorrect, correct them, and verify that the internal standard responses meet their acceptance criteria.

12.4.4.2 If the internal standard solution was improperly prepared, concentrated, or degraded, re-prepare solutions and reanalyze another aliquot of the sample extract (if possible) after adding the correct amount of the freshly prepared internal standard solution.

12.4.4.3 If the instrument malfunctioned, correct the instrument problem and reanalyze the sample extract.

12.4.4.4 If the instrument malfunction affected the calibration, recalibrate the instrument before reanalyzing the sample extract.

12.4.4.5 If the above actions do not correct the problem, then the problem may be due to a sample matrix effect. To determine if there was matrix effect, take the following corrective action steps:

12.4.4.6 Reanalyze the sample extract.

EXCEPTION: If internal standard responses in a sample used for an MS and/or MSD were outside the acceptance windows, then the sample should be reanalyzed only if internal standard compound recoveries met the internal standard acceptance criteria in both the MS/MSD analysis.

12.4.4.7 If the internal standard responses meet acceptance criteria in the reanalyzed sample extract, then the problem was within the laboratory's control.

12.4.4.8 Submit data from both analyses. Distinguish between the initial analysis and the reanalysis on all deliverables.

12.5 Data Assessment and Acceptance Criteria for Quality Control Measures

12.5.1 Analytical data generated by the instrument software are reviewed and evaluated by the analyst as follows:

12.5.2 DFTPP, instrument calibration, calibration verifications, IS/SS, QC measures are evaluated and the results documented on the separate forms:

12.5.2.1 For each 12-hour sequence, a tune evaluation report of DFTPP is generated. A tune is only required if a calibration is performed in that sequence.

12.5.2.2 For each ICAL, an instrument calibration report showing relative and average response factors and percent relative standard deviations is generated.

12.5.2.3 For each CCV, a report showing response factors and percent deviations compared to the associated ICAL is generated.

12.5.2.4 Generate a QA-QC check report for internal standard area counts and percent recoveries for the surrogates.

12.5.2.5 Calculate analyte percent recoveries CCV, LCS, ICV, MS, and RPD for MSD.

12.5.3 All false positives are Q-Deleted, and all positively identified target analytes are reported to LIMS. Include the spectra in the data package for positive results.

12.5.4 Manual integration is applied in cases when the instrument data processing software produces integrated areas that are not valid. The manual adjustments to the chromatographic peak must be performed in a consistent manner for the calibration standards, QC and field samples.

12.5.5 Anytime the analyst alters the instrument generated quantitation report, the hard copies of both reports (original and corrected) must be retained (e.g., manual integration). This may also be documented on pdfs and attached to the final report.

12.5.6 Discrepancies in the analytical run are described in the "QC Summary form" and discussed with the Lead Chemist.

12.5.7 Reviewed data is entered into LIMS, hard copies of LIMS reports are printed and compared to the original data.

12.5.8 All records derived from the analytical process are assembled in the analytical data packages that consist of:

12.5.8.1 LIMS work-order list.

12.5.8.2 Analytical run sheet.

12.5.8.3 "QC Summary Form" signed by the Lead Chemist.

12.5.8.4 DFTPP tune evaluation report.

12.5.8.5 QA-QC check report.

12.5.8.6 Quantitation Report for each Sample and QCS.

12.5.8.7 Evaluation reports for CCV, ICV, LCS, MS, and MSD.

12.5.8.8 Initial calibration form.

12.5.8.9 LIMS report of each sample.

12.5.9 Data packages are placed in files and stored in the PHILIS document storage area.

12.6 Corrective Actions for Out of Control

See the QAPP for the data affected and follow the instructions.

12.7 Contingencies for Handling Out of Control or Unacceptable Data

In cases where quality control measures do not meet acceptance criteria, the quality of the analytical data is not acceptable and the analyst does the following:

12.7.1 When tuning and/or instrument calibration fail to meet acceptance criteria, the analysis does not start. The problem is investigated and the necessary instrument maintenance is performed, followed with reanalysis of DFTPP and/or calibration.

12.7.2 If after analysis, any of the criteria for quality control are not met, or the sample is not available for reanalysis, the analyst must notify the Lead Chemist. The Lead Chemist will implement the corrective action plan.

12.7.3 The analyst shall report to the Lead Chemist and indicate any out control event listed on the "QC Summary form". Such events include:

12.7.3.1 Damage to the sample.

12.7.3.2 Holding time exceeded.

12.7.3.3 Inadequate sample preservation.

12.7.3.4 Sample results exceeds agencies Action Limit

12.7.3.5 Samples do not reflect historical data.

12.7.3.6 Upward trending or sample results approaching internal warning limits.

12.7.3.7 Any non-target analyte peak present on the instrument generated chromatogram.

12.7.3.8 The Lead Chemist will implement the corrective action plan described in the PHILIS corrective action plan document.

13.0 Method Performance

13.1 Laboratory accuracy and precision will be those listed in the single and multiple lab studies from the CWA protocol in the 2013 draft.

13.2 Other specific Quality Assurance Objectives (QAO) may be found in the appropriate statement-of-work or Quality Assurance Project Plan (QAPP) for specific projects.

14.0 Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th St., N.W. Washington, D.C. 20036, (202) 872-4477.

15.0 Waste Management

15.1 The waste produced from this procedure consists of waste collected from the extraction equipment, excess sample, Standards, Methylene Chloride, Acetone, and Methanol.

15.2 Excess reagents are disposed following the SDS instructions.

15.3 Glass pipettes are disposed in the lab scraps waste.

15.4 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operations. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

15.5 For information about pollution prevention that may be applicable to laboratories and research institutions consult *Less is Better: Laboratory Chemical Management for Waste Reduction* available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th St., N.W. Washington, D.C. 20036, http://www.acs.org.

15.6 The Environmental Protection Agency requires that laboratory waste management practices conducted are consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* available from the American Chemical Society.

16.0 References

16.1 U.S. Environmental Protection Agency. Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS). SW-846 Method 8270E. Revision 6. June, 2018.

16.2 U.S. Environmental Protection Agency. Organic Compounds in Water by Micro extraction. SW-846 Method 3511. Revision 0. November 2002.

16.3 U.S. Environmental Protection Agency. Microscale Solvent Extraction. SW-846 Method 3570. Revision 0. November 2002.

16.4 US Environmental Protection Agency. Cleanup. SW-846 Method 3600C. Revision 3. December 1996.

16.5 U.S. Environmental Protection Agency. Silica Gel Cleanup. SW-846 Method 3630C. Revision 3. December 1996.

16.6 U.S. Environmental Protection Agency. Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Ambient Air Using Gas Chromatography/Mass Spectrometry (GC/MS). Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air. Compendium Method TO-13A. January 1999.

16.7 U.S. Environmental Protection Agency, *Standard Analytical Methods for Environmental Restoration Following Homeland Security Events* (SAM) document and information are posted at: http://www.epa.gov/sam/

17.0 Tables, Figures, and Attachments

Table 1. Decafluorotriphenylphosphine (DFTPP) Key Ions and Ion Abundance Recommendations

These recommendations are from "Draft Analytical Protocol for Chemical Warfare Agents using GCMS"

Mass	Ion Abundance Criteria
	Time of Flight (TOF)
51	10.0 – 85.0% of mass 198
68	Less than 5.0% of mass 69
69	Present
70	Less than 2.0% of mass 69
127	10.0 – 80.0% of mass 198
197	Less than 2.0% of mass 198
198	>50% of mass 442
199	5.0 – 9.0% of mass 198
275	10.0 - 60.0% of base ion
365	Greater than 0.5% of mass 198
441	Less than 150% of mass 443
442	Base ion
443	15.0 – 30.0% of mass 442

Note: All ion abundances MUST be normalized to m/z 198.

Table 2. Internal Standards and Surrogates

CWA	Surrogate Compounds	Internal Standards
Fentanyl	Fentanyl-d_5	Chrysene-d_{12}
Acetylfentanil	Fentanyl-d_5	Chrysene-d_{12}
Alfentanil	Fentanyl-d_5	Chrysene-d_{12}
Carfentanil	Fentanyl-d_5	Carfentanil-d5
Heroin	Fentanyl-d_5	Chrysene-d_{12}
Remifentanil	Fentanyl-d_5	Chrysene-d_{12}
Sulfentanil	Fentanyl-d_5	Chrysene-d_{12}

Table 3. Example Retention Times, Relative Retention Times and Characteristic Ions for Target Compounds, Surrogate Compounds, and Internal Standards

Contaminant	Retention Time (sec)	Relative Retention Time	Full Scan	
			Primary Quantitation Ion	Secondary Quantitation Ions
Fentanyl	589.464	49.14	245	189,57,105
Acetylfentanil	453.9	32.557	231	
Alfentanil	496.1	74.782	289	
Carfentanil	482.2	60.855	303	
Heroin	448.0	26.668	369	
Remifentanil	443.1	11.7778	168	
Sulfentanil	472.5	51.114	289	
Carfentanil-d5	481.6	60.255	308	
Fentanyl-d5 (S)	588.882	48.56	151	194,250
Chrysene-d_{12} (IS)	540.319		240	236, 120

Notes:
(S) = Surrogate
(IS) = Internal Standard

Table 4. Example Precision (RPD) and Recovery (%Rec) Limits

Contaminant	%Rec	RPD
Water		
Fentanyl	+/-50%	≤20
Acetylfentanil	+/-50%	≤20
Alfentanil	+/-50%	≤20
Carfentanil	+/-50%	≤20
Heroin	+/-50%	≤20
Remifentanil	+/-50%	≤20
Sulfentanil	+/-50%	≤20
Soil		
Fentanyl	+/-50%	≤20
Acetylfentanil	+/-50%	≤20
Alfentanil	+/-50%	≤20
Carfentanil	+/-50%	≤20
Heroin	+/-50%	≤20
Remifentanil	+/-50%	≤20
Sulfentanil	+/-50%	≤20
Wipes		
Fentanyl	+/-50%	≤20
Acetylfentanil	+/-50%	≤20
Alfentanil	+/-50%	≤20
Carfentanil	+/-50%	≤20
Heroin	+/-50%	≤20
Remifentanil	+/-50%	≤20
Sulfentanil	+/-50%	≤20

Table 5. Example Surrogate Recoveries

Surrogate	%Rec
Water	
Fentanyl-d_5	+/-50%
Soil	
Fentanyl-d_5	+/-50%
Wipes	
Fentanyl-d_5	+/-50%

Table 6. Example Calibration Standard Concentrations (pg on column) used during Laboratory Method Development

GC/MS – Selective Ion Monitoring or TOF								
Analyte	**CAS RN**	**Cal 1**	**Cal 2**	**Cal 3**	**Cal 4**	**Cal 5**	**Cal 6**	**Cal 7**
Fentanyl	437-38-7	20	50	100	5000	1000	2500	5000
Acetylfentanil	3258-84-2	20	50	100	5000	1000	2500	5000
Alfentanil	69049-06-5	20	50	100	500	1000	2500	5000
Carfentanil	59708-52-0	20	50	100	500	1000	2500	5000
Heroin	561-27-3	20	50	100	500	1000	2500	5000
Remifentanil	132539-07-2	20	50	100	500	1000	2500	5000
Sulfentanil	60561-17-3	20	50	100	500	1000	2500	5000
Carfentanil-d5	1185158-60-4	20	50	100	500	1000	2500	5000
Fentanyl-d_5	118357-29-2	20	50	100	500	1000	2500	5000
Chrysens-d_{12}	1719-03-5	20	50	100	500	1000	2500	5000

Notes:
* These surrogates or internal standards are not required for the compounds being addressed by this protocol, but may be used if they are already included in solutions that will be used by the laboratory.
** Programmable injector/ solvent vent only. Data not available for pulsed splitless injection.

Table 7. Example Analyte Method Detection Limits (MDLs) and Reporting Limits (RL)

Method List		**MDL**	**RL**	**MDL**	**RL**	**MDL**	**RL**
Compound	**CAS No.**	**100 mL Water Sep Funnels ug/L**	**Water ug/L**	**Soil ug/Kg**	**Soil ug/Kg**	**Wipes ug/Wipe**	**Wipes ug/Wipe**
Fentanyl	437-38-7	0.277	1.0	0.73	2.0	0.0118	0.030
Acetylfentanil	3258-84-2	0.36	1.0	0.6	2.0	0.0106	0.030
Alfentanil	69049-06-5	0.35	1.0	0.567	2.0	0.0109	0.030
Carfentanil	59708-52-0	0.289	1.0	0.55	2.0	0.011	0.030
Heroin	561-27-3	0.802	2.0	0.726	2.0	0.0153	0.050
Remifentanil	132539-07-2	0.333	1.0	0.657	2.0	0.0125	0.030
Sulfentanil	60561-17-3	0.327	1.0	0.64	2.0	0.0103	0.030

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