STANDARD OPERATING PROCEDURE

FOR

FGA ANALYSIS BY UPLC/MS/MS

PHILIS SOP L-A-311 Rev. 3

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PREPARED BY

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Office of Emergency Management
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Revision History

Revision	Name	Date	Description of Change
0	James Garcia James Travis	05/20/2021	SOP Development
1	James Travis	08/27/2021	Revision
2	James Travis James Garcia	09/23/2021	Revision
3	Tom Antony Courtney Armani Marshall Nisbeth	06/13/2024	Revision

SOP REVISION FORM

SOP Name:	**Analysis of FGAs by UPLC/MS/MS**		
Purpose: *(Review or Revise)*	*SOP #:*	*Rev. #:* *(Being Reviewed or Revised)*	*Origination / Release Date:*
Revision	*SOP No. L-A-311*	2	09/27/2021
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New SOP Revision Date:	**07/08/2024**	**New SOP Revision #:** *(If Applicable)*	**3**

For *Revision* : Summary of Revisions (specify sections)

Table of Contents	SOP rearranged to fit EPA's 17 section format, meaning several sections were combined or removed due to redundancy.
Table 1	Removed T2CP, added Fentanyl-d5 and VX
2.2	Updated Filter type
2.4	Replaced T2CP with Fentanyl-d5 and clarified required MRM transitions
3.2	Defined Instrument Calibration Standards
5.5	Updated MSDS to SDS and added VX to FGA warning
5.8	Corrected the type of hazard that exists with this method
5.9-5.11	Eliminated for being duplicate sections of previously covered material
6.2	Removed incorrect language
6.4.1.4	Corrected the analytical system used
6.6.4	Added alternative option for storing standards/spiking solutions
6.6.8	Removed outdated and unused filter system
7.4	Replaced T2CP with Fentanyl-d5, removed Table 2
Tables 2 – 8	Updated numbering of tables 2 through 8 due to deletion of original Table 2
7.4.1	Updated surrogate prep for Fentanyl-d5
7.4.3	Updated calibration solution prep to pg/uL and prepped in methanol with larger calibration range
Table 2	Updated to reflect changed in 7.4.3
7.4.4	Replaced 1000 fg/uL with 1 ng/uL. Added specifications about Water and Soil LCS spiking solutions
8.2	Included soil and water, fixed grammar, and corrected instrument
9.1	Included "when possible" in reference to MS, MSD, and FSD
9.1	Table reference number was changed from 5 to 4 to reflect updates
9.2	Corrected section reference to "sections 9.2.1 through 9.3.1.3"
9.2.4	Corrected instrument to LC/MS/MS

9.3.1.1	Removed “a minimum of seven blanks prepped in three separate batches
9.3.1.2	Removed language about blank MDL determination
9.3.1.3	Removed, blank MDL calcs are not required
9.1.3.4	Specified that change of location refers to location within the laboratory
9.3.2	Removed subsections and merged annual MDL calculations requirements.
9.3.3	Removed language about blank MDL determination, added “re-calculate the MDL spike as above in 9.3.2”
9.7-9.8; 9.9.3; 9.10.1; 9.11.1.4;	Changed table number 5 to 4 to reflect update
9.11, 9.12	Added these bullets to the QA section due to the consolidation of the document to fit the EPA format.
9.12.5-6	Updated table 5 to table 4 to reflect changes to table numbering
10.2	Changed to nine calibration standards, fixed table reference
10.2	Updated table 4 to table 3 to reflect changes to table numbering
10.3	Updated table 6 to table 5 to reflect changes to table numbering
10.5	Allowed for multiple calibration points to be disabled and forbid points from being disabled when bracketed by enabled points
10.6	Changed requirement for quadratic R^2 from .98 to .99. Allowed for multiple calibration points to be disabled and forbid points from being disabled when bracketed by enabled points
10.8	Added analyst discretion clause about ion ratio acceptability
11.0	Updated soil, water and wipe procedures to reflect current methods
11.2.1.3	Changed “concentration” to “amount”
11.2.1.8	Added section about vortexing sample during extraction
11.2.1.9	Changed “transfer” to “decant” and “filtrate” to “liquid”
11.2.1.10	Added section about vortexing sample prior to loading on the instrument
11.2.2.1	Removed capacity requirement of airtight syringe
11.2.2.3	Changed “syringe” to “vial”
11.2.2.4	Added section about filtering sample through a syringe filter
11.2.2.5	Changed methanol amount to 50uL
11.2.2.6	Added instructions to vortex AS vial
11.2.3.9	Changed “transfer” to “decant” and “filtrate” to “liquid”
11.0 – NOTE	Removed brand stipulation
11.3.1	Changed Tables 8, 9 and 10 to Tables 7, 8 and 9
11.3.3	Changed 50 uL injection to 1 uL. Clarified CCV recovery criteria
11.4	Changed “See Section 15.1” to “See Section 12.1”
12.1	Merged subsections and eliminated duplicate language
12.2	Replaced T2CP with Fentanyl-d5
13.0	Removed reference to table 5, the table was removed from the SOP. No longer relevant.
20.0	Completely eliminated “Contingencies for handling out of control or unacceptable data” as it was entirely replication of previously stated material.
Table 4	Added new section for matrix spike and matrix spike dup

Table 5	Added Fentanyl-d5, VX, removed T2CP, updated RTs
Table 6	Changed injection volume from 10 uL to 1 uL, updated LC Gradient to current, updated temperature settings, and updated system time
Table 7	Added “possibly”
Table 8	Changed method duration, cycle time, and chromatographic peak width
Figure 2	Added VX, updated values to current MDL

For *Review*: Comments

Standard Operating Procedure
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TABLES AND FIGURES

Standard Operating Procedure
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1.0 Scope and Application, and Components to be Analyzed

This SOP is for the analysis of Fourth Generation Agents (FGA) and VX. This SOP is executed in accordance with the U.S. Environmental Protection Agency and National Environmental Laboratory Accreditation Program (NELAP) requirements. This method was developed using the Thermo Scientific TSQ ALTIS LC/MSMS.

PHILIS utilizes this method for the determination of the concentrations of the analytes listed in Table 1.

Method detection limits are determined using the procedure outlined in 40 CFR Part 136, Appendix B, Revision 2.0 (Method Update Rule).

This procedure covers specific requirements for the determination of FGAs and VX using ultra performance liquid chromatography (UPLC) and detected with tandem mass spectrometry (MS/MS) using electrospray ionization (ESI). This method prescribes separation using reverse phase chromatography followed by detection using multiple reaction monitoring (MRM) spectrometry. The compounds shown in Table 1 are listed in the order of their retention times and are qualitatively and quantitatively determined by this method.

2.0 Summary of Method

2.1 This FGA sample preparation method was established as a performance-based method to optimize precision, accuracy and operational performance.

2.2 Samples are shipped to the lab between 0°C and 6°C and analyzed as soon as possible after collection. To prepare for analysis, samples are spiked with surrogate, and then diluted or extracted using the appropriate sample preparation method. The diluted samples or the extracts are filtered using a syringe-driven PTFE membrane filter unit and the filtrates are analyzed directly by LC/MS/MS.

2.3 The UPLC is run with reverse phase chromatography, and the ions are transferred into the gas phase using electrospray (ES). The mass spectrometer is operated in the positive mode (ESI+).

2.4 FGA's are identified by retention time (within ± 5% of a standard) and by a quantitation Multiple Reaction Monitoring (MRM) transition. MRM is a non-scanning mass spectrometric technique, performed on tandem mass spec instruments in which collision-

induced dissociation is used as a means to increase selectivity. The target analytes and surrogate are quantitated using an external calibration procedure. The target compounds and the surrogate Fentanyl-d5 are identified by retention time and at least one MRM transition. The target analytes and surrogates are quantitated using the MRM transitions utilizing an external calibration.

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. 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.

All batches require one MB, LCS, and MS/MSD pair or MS and Sample Duplicate when possible.

3.2 Instrument Calibration Standards (ICS): A solution prepared from the primary dilution standard solution or stock standard solutions, internal standards and surrogate analytes. The ICS solutions are used to calibrate the instrument response with respect to analyte concentration.

3.3 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.

3.4 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.

3.5 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.

3.6 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.

3.7 Method Detection Limit (MDL): The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. It is determined by analyzing seven or more replicates of a spiked analyte free matrix and the resulting statistical calculation, in accordance with 40 CFR 136, Appendix B, Revision 2.0.

3.8 Multiple Reaction Monitoring (MRM): Multiple reaction monitoring (also known as Selective Reaction Monitoring or SRM) is a highly specific and sensitive mass spectrometry technique that can selectively quantitate compounds within complex mixtures. The MRM technique is performed on triple quadrupole (MS/MS) instruments by setting the first quadrupole (Q1) at a specific mass to select a precursor (parent) ion, which can be isolated and fragmented to deliver a unique product (daughter) ion. The third quadrupole (Q3) is set at another specific mass to allow the passage of the product (daughter) ion, which can then be quantitated. The specific pairs of m/z values associated to the precursor and product ions selected are referred to as "transitions" and effectively constitute mass spectrometric assays that allow you to identify and quantitate a specific compound. Parallel acquisitions of multiple precursor/product (parent/daughter) ion transitions are completed during a chromatographic run. These transitions are measured within the same analysis on the chromatographic time scale by rapidly toggling between the different precursor/product pairs. Typically, the triple quadrupole instrument cycles through a series of transitions and records the signal of each transition as a function of the elution time. The method allows for additional selectivity by monitoring the chromatographic coelution of multiple transitions for a given analyte.

3.9 Primary Dilution Standard (PDS): A solution of one or several analytes prepared in the laboratory from SSS and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.10 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.

3.11 Required Detection Limit (RDL): Detection limits established by a client or regulatory authority for analytes of concern. The laboratory MDL values must be equal or lower than the RDL. This is also known as the CRQL, the contract-required quantitation limit.

3.12 Second Source Calibration Verification (SCV): A solution prepared from a source that is different from the calibration standards. The SCV is immediately following the ICS, and is used to verify calibration standard accuracy.

3.13 Stock Standard Solution (SSS): A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased as certified from a reputable commercial source.

3.14 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.

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

4.0 Interferences

4.1 Method interferences may be caused by contaminants in wipes, solvents, reagents, glassware, and other apparatus producing discrete artifacts or elevated baselines. All of these materials are demonstrated to be free from interferences by analyzing method blanks (MB) under the same conditions as samples. Subtraction of blank values from sample results is not performed.

4.2 All glassware and containers should be washed in hot water with detergent followed by distilled water. Glassware must subsequently be cleaned with methanol or acetone.

4.3 Syringes are rinsed with 1 – 5 mL of methanol followed by 1 – 5 mL of acetonitrile before use.

4.4 All reagents and solvents should be LC/MS or pesticide grade or higher to minimize interference problems.

4.5 Matrix effects are well known phenomena of ESI-MS techniques, especially for co-eluting compounds. Managing the unpredictable suppression and enhancement caused by these effects is recognized as an integral part of the performance and verification of an ESI-MS procedure. The data presented in this procedure were designed to demonstrate that the procedure is capable of functioning with realistic samples. Each analyst is encouraged to observe appropriate precautions and follow the described QC

procedures to help minimize the influence of ESI-MS matrix effects on the data reported. Matrix effects include ion suppression/enhancement and high backgrounds.

5.0 Safety

Laboratory personal are required to be familiar with the general laboratory safety plan including the location and proper use of safety/emergency equipment

5.1 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 all samples.

5.2 Specific Safety Concerns or Requirements

Eye protection that satisfies ANSI Z87.1, laboratory coat, and disposable nitrile or Silver-Shield 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.

5.3 Each chemical and sample should be treated as a potential health hazard. Exposure should be reduced to the lowest possible level. Procedures involving primary standards and sample preparation must be performed in a fume hood.

5.4 Extraction solvents such as acetone, hexane and especially methylene chloride have appreciable vapor pressure that requires proper venting if using a separatory funnel. After a few manual shakes, hold the funnel upside down, open the stopcock and position the funnel to be directed in the hood and away from the individual(s) to release buildup of solvent pressure, repeat as necessary.

5.5 Safety Data Sheets (SDS) for each analyte and reagent used in the mobile laboratory are available to all employees. The SDS and the PHILIS Chemical Hazard Summary Sheet must be read and understood by the analyst prior to initial use of a chemical.

WARNING: Precautions must be used even for the simplest procedures involving these agents. If FGAs/VX are suspected, laboratory personnel must be thoroughly trained in appropriate safety procedures prior to using this method.

5.6 The toxicity and/or carcinogenicity of the common reagents and analytes used in this method have been defined; however, each chemical and sample should be treated as a potential health hazard. Exposure should be reduced to the lowest possible level. Procedures involving primary standards and sample preparation should be performed in a fume hood.

5.7 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.

5.8 Exposure to FGA/VX 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.

6.0 Equipment and Supplies

6.1 Sampling and sample preparation equipment for wipe samples

6.2 Shaker table, VWR model DMS-2500 High Speed Micro Plate Shaker, catalog number 13500-890, or equivalent.

6.2.1 Graduated cylinders of various sizes

6.2.2 40 mL pre-cleaned VOA bottles fitted with Teflon™-lined screw caps.

6.2.3 Disposable Pasteur pipettes

6.3 Disposable plastic syringes with Luer lock fitting.

Gas-tight glass syringes - various sizes from 10 µL to 1000 µL.

6.4 Instrumentation

6.4.1 LC/MS/MS Apparatus

6.4.1.1 UPLC system (LC) - A complete LC system is needed to analyze samples. Any system that is capable of performing at the flows, pressures, controlled temperatures, sample volumes, and requirements of the SOP may be used. The system includes HPLC bottles for mobile phases and wash solvents (various sizes, e.g., 500 or 1000 mL), a binary pumping unit and temperature-controlled compartments for the samples and the chromatographic column. PHILIS uses the ThermoFisher Vanquish UPLC binary pumping system with a 50-µL loop for this method.

6.4.1.2 Analytical Column - Waters Acquity™ HSS T3 C18 column, 2.1 mm x 150 mm, 1.8 µm particle size (part # 186003540) and corresponding guard or pre-column. Any equivalent pair of a guard and analytical column that achieves adequate resolution may be used. The retention time and order of elution may change depending on the type of column used.

6.4.1.3 Tandem Mass Spectrometer (MS/MS) - an MS/MS system capable of MRM analysis. Any system that is capable of performing the requirements. PHILIS uses the ThermoFisher TSQ Altis System.

6.4.1.4 Data System - TraceFinder software (or similar software) interfaced to the LC/MS/MS that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program. TraceFinder (or similar software) is used for all quantitation for data generated from the LC/MS/MS unit.

6.5 Other Laboratory Equipment

Analytical balance capable of reading to ±0.0001g with certified reference weights.

6.6 Supplies

6.6.1 Autosampler vials – amber or polypropylene vials for LC autosampler, 1 – 2 mL.

6.6.2 Sample collection containers: Precleaned glass bottles, vials or jars with polytetrafluoroethylene-lined caps.

6.6.3 Small glass vials (1 mL or 2 mL) are used for storage of sample extracts, calibration standards and stock standards.

6.6.4 10 mL or small glass vials are used for storage of standards and spiking solutions.

6.6.5 Wipes: Kendall 3” x 3” type VII gauze sponges that were precleaned in MeOH or IPA.

6.6.6 Clean Ottawa sand or equivalent.

6.6.7 3mm glass beads, or equivalent

6.6.8 Syringe-driven filter: 13mm 0.20um PTFE, Luer lock inlet VWR Cat#28145-491, or equivalent

7.0 Reagents and Standards

7.1 Reagents

7.1.1 Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.1.2 Argon- UHP or greater used for collision gas and should meet or exceed instrument manufacturer's specifications.

7.1.3 Nitrogen- UHP or better, used for desolvation and nebulization and should meet or exceed instrument manufacturer's specifications.

7.1.4 Water- ASTM Type I or equivalent. It must be demonstrated that this water does not contain contaminants at concentrations sufficient to interfere with analysis. LC/MS grade water (Fisher Optima W6-4, or equivalent) may also be used.

7.1.5 Acetonitrile (ACN) (CAS# 75-05-8) LC/MS grade or better.

7.1.6 Methanol (CAS# 67-56-1)- LC/MS grade or better

7.1.7 Isopropyl Alcohol (IPA) (CAS# 67-63-0) Pesticide grade or better

7.1.8 Acetone (CAS# 1567-89-1) Pesticide grade or better

7.1.9 Formic Acid (CAS# 64-18-6) - LC/MS grade or better.

7.1.10 Ammonium formate (CAS#540-69-2) >98%

7.2 Wash Solutions

7.2.1 Needle washing solution.

Magic Mix: 45% Acetonitrile / 45% Isopropyl Alcohol / 10% Acetone (v/v/v)

7.2.2 Seal wash solution:

75% Isopropyl Alcohol / 25% Water / 0.1% Formic Acid (v/v/v)

7.3 Mobile Phases

Mobile phase A: premixed 0.1% Formic acid in water,

Mobile phase B: premixed 0.1% Formic acid in acetonitrile

7.4 Standards

Standard solutions of the agents are obtainable through Lawrence Livermore National Laboratories. They arrive in flame sealed ampoules at a concentration of 10 ug/mL. Due to the nature of the agents, they must be handled in accordance with PHILIS CWA handling procedures.

Fentanyl-d5 can be obtained commercially through Cerillant (100 ug/mL, Cat No. F-001)

7.4.1 Surrogate Stock Standard Solution (Surrogate SS),

Prepare Fentanyl-d5 at 100 pg/uL in MeOH.

7.4.2 Analyte Stock Standard Solutions, 10 ug/mL

Standard solutions can be obtained through Lawrence Livermore National Laboratories. They arrive in flame sealed ampoules at a concentration of 10 ug/mL.

7.4.3 Calibration Standard Solutions

Calibration standards are prepared via serial dilution. Prepare 100 pg/uL standards separately of the FGAs, VX and Fentanyl-d5 surrogate.

Prepare 1 mL of a 10 pg/uL standard in optima methanol combining the three standards. This can be done with a 1mL syringe into an autoampler vial. This will be the base for the serial dilution.

From the base, serially dilute to 5 pg/uL(B) and 2.5 pg/uL(C) to a final volume of 1 mL in optima methanol with a 1 mL syringe and autosampler vials.

Going back to the 10 pg/uL(A) standard serially dilute that to 1, 0.1 pg/uL to a final volume of 1mL in optima methanol with a 1 mL syringe and autosampler vials.

Next, using the 5 pg/uL(B) standard, serially dilute to 0.5, 0.05 pg/uL to a final volume of 1mL in optima methanol with a 1 mL syringe and autosampler vials.

Using the 2.5 pg/uL (C) standard, serially dilute to 0.25, 0.025 pg/uL to a final volume of 1mL in optima methanol with a 1 mL syringe and autosampler vials.

7.4.4 LCS Spiking Solution

The LCS spiking solution may be prepared from the primary source (i.e., the stocks used to prepare the calibration standards) or from a secondary source, if available. The preparation and the concentration of the LCS spiking solution depend on the specific analyte and the extraction method. The following guidelines are for the preparation of the LCS spiking solution for each matrix:

Wipes and Soil: prepare an LCS spiking solution in optima methanol containing 1 ng/uL of the target compounds by diluting aliquots of the analyte stocks (Section 10.4.3).

Water: prepare an LCS spiking solution in optima methanol containing 100 pg/uL of the target compounds by diluting aliquots of the analyte stocks (Section 10.4.3).

8.0 Sample Collection, Preservation, Shipment and Storage

8.1 Sample Collection

8.1.1 The exact choice of sampling vessel and procedure is not critical for the analysis and can be adjusted to meet project needs as long as the different materials have been tested and show no presence or interferences of the target analytes.

8.1.2 As an example for wipe samples, the field sampling team collects samples using an appropriate wetted wipe (methanol). The wipe sample is placed in a jar with a sealed cap for shipment to the laboratory, (e.g., VOA vial or glass jar with a Teflon™-lined screw cap).

8.1.3 Wipe samples are collected by using precleaned (in IPA) Kendall 3"x 3" type VII gauze sponges. The required analyte spike solution containing the analytes of interest is added to the surface, allowed to dry, and wiped with each wipe separately. Two wipes are separately wetted with approximately 300 µL of methanol. The first wipe is used to wipe the surface in a Z-like pattern horizontally across a defined surface (100 cm^2). The second wipe is used to wipe the same surface in a Z-like pattern vertically across a defined surface (100 cm^2). Wipes are placed in individual 40-mL VOA vials. Field and/or matrix blanks are needed, according to conventional sampling practices.

8.1.4 Sample preservatives are not used in this method.

8.2 Sample Storage and Holding Times

Wipe, soil, and water samples must be analyzed within 72 hours of collection or as soon as possible. The holding times have not been determined, but should be analyzed as soon as possible, since the target analytes are subject to rapid breakdown. At the laboratory, samples can be stored in a refrigerator at 0 - 6 ºC until requested for analysis. Samples from a particular site should be carefully characterized to ensure that there is no interaction with the wipe or specific surface to cause interferences or degradation of the analytes after 24 hours. After injection in the LC/MS/MS, the vial septa must be replaced and the vials are stored in a refrigerator in case further analysis is needed. Extracts or diluted samples previously analyzed by LC/MS/MS can be stored up to 28 days in the refrigerator at 0 - 6 ºC.

9.0 Quality Control and Acceptance Criteria

9.1 Quality control (QC) requirements include the Initial Demonstration of Capability (IDC), the determination/verification of the detection limit, and subsequent analysis in each analysis batch of a Method Blank (MB), Continuing Calibration Verification Standards (CCV), a Laboratory Control Sample (LCS), a Matrix Spike (MS) when possible, and either a Matrix Spike Duplicate (MSD) or a Field Duplicate Sample (FDS) when possible. This section details the specific requirements for each QC parameter. The QC criteria discussed in the following sections are summarized in Table 4. These criteria are considered the minimum acceptable QC criteria.

9.2 INITIAL DEMONSTRATION OF CAPABILITY (IDC) – Requirements for the initial demonstration of capability include a method blank, precision and accuracy samples, and an mdl determination which are described in the following sections 9.2.1 through 9.3.1.3.

9.2.1 INITIAL DEMONSTRATION OF LOW SYSTEM BACKGROUND – Before any field samples are analyzed, and any time a new set of reagents is used, it must be demonstrated that a method blank does not contain analytes of interest above the reporting limit and there are no other peaks that will interfere with the determination of the analytes of interest.

9.2.2 INITIAL DEMONSTRATION OF ACCURACY – Prior to the analysis of the IDC samples, verify calibration accuracy with the preparation and analysis of a mid-level CCV as defined in Section12.6. If the analyte recovery is not within 30% of the true value, the accuracy of the method is unacceptable. The source of the problem must be identified and corrected. After the accuracy of the calibration has been verified, prepare and analyze a minimum of four replicate LCSs fortified at 500ng/L, or near the mid-range of the initial calibration curve, according to the procedure described in Section 10. The average recovery of the replicate values must be within ± 30% of the true value.

9.2.3 INITIAL DEMONSTRATION OF PRECISION – Using the same set of replicate data generated for Section 12.2.2, calculate the standard deviation and percent relative standard deviation of the replicate recoveries. The relative standard deviation (%RSD) of the results of the replicate analyses must be less than 20%.

9.2.4 METHOD MODIFICATIONS – This is a performance based method. The analyst is permitted to optimize LC/MS/MS instrument conditions. The analyst is also allowed to choose an alternate surrogate standard with approval of the Quality Assurance Manager. Each time such method modifications are made, the analyst must document the changes and repeat the procedures of the IDC.

9.3 MDL Procedure

MDLs and RLs are established by analyzing a minimum of seven replicates of a standard at or near the estimated MDL. Tabulation of results and MDL calculations are performed by the method in 40 CFR, Part 136, Method Update Rule Revision 2.

9.3.1 Initial MDLs

9.3.1.1 Initial MDLs are established by analyzing a minimum of seven replicates of the low-level calibration standard. The MDL should be spiked 1 to 5 times the estimated MDL. Extract and analyze the MDL standards and blanks with the same procedure as regular samples.

9.3.1.2 For each compound, calculate the mean and standard deviation of the replicates in micrograms per liter (µg/L). Then calculate the MDL by multiplying the standard deviation by the Student's t value. The one-sided (single-tailed) Student's t values at the 99% confidence levels are used (e.g., t = 3.143 at the 99% confidence level for n = 7). MDL results are stored in Element each time they are calculated.

9.3.1.3 The Initial MDL should be performed when there is a change of equipment, change of location of equipment within the laboratory, or a change of procedure.

9.3.2 Ongoing MDL Data Collection

Ongoing MDL's are determined by preparing and analyzing spiked standards at 1-5 times the estimated MDL and blanks annually for a minimum of seven determinations. The blanks and spikes may be analyzed in the same prep batch, but is not required. If the instruments are being used regularly, the MDL spikes may be added to the routine batches and the regular blanks used. All blanks analyzed during the evaluation period should be used. If client samples are not received on a regular basis, an ongoing mdl verification may be performed annually.

9.3.3 Ongoing MDL Annual Verification

At least once every thirteen months, re-calculate the MDL spike as above in 9.3.2 from the collected spiked samples and method blank results.

9.3.4 Include data generated within the last twenty four months, but only data with the same spiking level. Only documented instances of gross failures (instrument malfunctions, mislabeled samples, cracked vials, etc.) may be excluded from the calculations.

9.4 Reporting Level (RL) – The RL is the threshold concentration of an analyte that a laboratory can expect to accurately quantitate in an unknown sample. The RL cannot be established at an analyte concentration that is less than either the Method Detection Limit or a concentration which would yield a response less than a signal-to-noise (S/N) ratio of three. Depending upon the study's data quality objectives it may be set at a higher concentration. **Although the lowest calibration standard must be at or below the RL, the RL must never be established at a concentration lower than the lowest calibration standard**.

9.5 METHOD BLANK (MB) – An MB is required with each analysis batch of samples to determine any background system contamination. If within the retention time window of any analyte, the MB produces a peak that would prevent the determination of that analyte, determine the source of contamination and eliminate the interference before processing samples. Background contamination must be reduced to an acceptable level before proceeding. Background from method analytes or contaminants that interfere with the measurement of method analytes must be below the MDL. If the target analytes are detected in the MB at concentrations equal to or greater than this level, then all data for the problem analyte(s) must be considered invalid for all samples in the analysis batch. Method blanks for each matrix are prepared as follows:

Wipe MB: Spike a known amount of Surrogate Spiking Solution (Section 10.4.2) on to one clean wipe, keeping in mind a final extraction volume of 15 ml and place in a 40-mL VOA vial.

9.6 CONTINUING CALIBRATION VERIFICATION (CCV) – A CCV is prepared in the same manner as the initial calibration solutions in Table 3 LV5. It is analyzed during an analysis batch at a required frequency to confirm that the instrument meets initial calibration criteria. If an ICAL started the sequence, the beginning CCV may be eliminated. The CCV must be analyzed at the beginning and end of each batch of 20 samples or within 24 hours after the initial calibration curve was generated. The results from the CCV must have a percent deviation of less than 30% from the calculated concentration of the target analytes and surrogates. If the results are not within criteria, the problem must be corrected and either all samples in the batch must be re-analyzed against a new calibration curve or the affected results must qualified as estimated with an indication that they do not fall within the performance criteria of the test method. If the analyst inspects the vial containing the end CCV and notices that the sample evaporation affecting the concentration, a new end CCV may be made and analyzed. If this new end CCV has a percent deviation of less than 30% from the calculated concentration for the target analytes and surrogates, the results may be reported unqualified.

9.7 LABORATORY CONTROL SAMPLE (LCS) – To ensure that the instrument is in control, analyze an LCS that is prepared with the target compounds at a concentration near the mid-point of the calibration curve (Section 10.4.6). The LCS is analyzed with each batch of 20 samples or less. The results from the LCS must fall within the limits in Table 4.

9.8 SURROGATE RECOVERY – The surrogate standard is spiked into all samples, method blanks, LCSs, and MS/MSDs prior to sample analysis. It is also added to the calibration and check standards. The surrogates are a means of assessing method performance. The results obtained for a surrogate recovery must fall within the limits of Table 4. If the limits are not met, the sample must be reanalyzed, and if still outside of limits, then the affected results must be qualified with an indication that they do not fall within the performance criteria of the test method.

9.9 MATRIX SPIKE (MS) – To check for interferences in the specific matrix being tested, perform a MS on at least one sample from each batch of 20 or fewer samples by spiking the samples with a known concentration of FGAs and following the analytical method.

9.9.1 If the spiked concentration plus the background concentration exceeds that of the highest calibration standard, the sample must be diluted to a level near the midpoint of the calibration curve. The MS/MSD should be at the same dilution as the original sample.

9.9.2 Calculate the percent recovery of the matrix spike (P) using Eq 1:

$$P = \left[\frac{(A - B)}{C} \right] x\,100$$

Eq. 1

where

A = measured concentration in the fortified sample

B = measured concentration in the unfortified sample, and

C = fortification concentration.

9.9.3 The percent recovery of the matrix spike shall fall within the limits in Table 4. If the percent recovery is not within these limits, a matrix interference may be present in the selected sample. Under these circumstances, one of the following remedies must be employed: the matrix interference must be removed, all samples in a batch must be analyzed by a test method not affected by the matrix interference, or the sample results must be qualified with an indication that they do fall within the performance criteria of the test method.

9.10 MATRIX SPIKE DUPPLICATE (MSD) - To check the precision of sample analyses, analyze a sample in duplicate with each batch of 20 or fewer samples. If the sample contains the analyte at a level greater than 5 times the detection limit of the method, the sample and duplicate may be analyzed unspiked; otherwise, an MSD should be used.

9.10.1 Calculate the relative percent difference (RPD) between the duplicate values as shown in Eq. 2. Compare the RPD limit in Table 4.

$$RPD = \left[\frac{|MS - MSD|}{(MS + MSD)/2} \right] 100$$

Eq. 2

where:

RPD = relative percent difference

MS = matrix spike recovery

MSD = matrix spike duplicate recovery

9.10.2 If the result exceeds the precision limit, the batch must be re-analyzed or the result associated with that sample must be qualified with an indication that they do not fall within the performance criteria of the test method.

9.11 Data Assessment and Acceptance Criteria for Quality Control Measures

Analytical data generated by the quantitation software is reviewed and evaluated by the analyst as follows:

9.11.1 Instrument calibration, calibration verifications, SS, other QC measures are evaluated and the results documented on separate forms:

9.11.1.1 For each analyte and surrogate, evaluate the coefficient of determination, R^2, from the initial calibration curve.

9.11.1.2 Evaluate the % recoveries for all surrogates.

9.11.1.3 Evaluate the % recoveries for the CCV, SCV, LCS, MS, MSD, and evaluate the RPD for the MS/MSD pair.

9.11.1.4 Calibration standards must meet the coefficient of determination criteria and other quality control measures must meet the criteria listed in Table 4.

9.11.2 Field sample assessment

9.11.2.1 A reported compound that has a retention time outside the established window is considered a false positive response.

9.11.2.2 A reported compound that has no confirming ions, confirming ion retention times that do not align with its own, or out of control confirming ion ratios (subject to analyst discretion as mentioned in 13.8) is considered a false positive response.

9.11.2.3 A reported compound that has a signal to noise ratio of <3 or a confirming ion signal to noise ratio of <2 is considered a false positive response.

9.11.2.4 A reported compound that has an unacceptable peak shape is considered a false positive response. An unacceptable peak shape may indicate a need for instrument maintenance or re-injection of the sample and further analysis.

9.11.2.5 All false positives are eliminated, and all positively identified target analytes are reported to LIMS.

9.11.3 Manual integration is ONLY applied in cases when the instrument data processing software produces integrated areas that are not valid. Manual adjustments to the chromatographic peak must be performed in a consistent manner for the calibration standards, QC and field samples. Refer to PHILIS SOP L-D-501.

9.11.4 Chromatograms of all field samples are examined to identify additional peaks that are not included in the integration report, which were not identified as target analytes. If such peaks are present, the Lead Chemist should be notified immediately in that case.

9.11.5 Anytime the analyst alters the instrument generated quantitation report, the hard copies of both reports (original and analyst's corrected) must be retained (e.g., manual integration). The analyst should seek to minimize manual integrations by proper instrument maintenance, retention time updates, setting integration parameters, etc.

9.11.6 Discrepancies or anomalies in the analytical run are described in the QA-020B form, discussed with the Lead Chemist, and documented in the case narrative.

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

9.11.8 All records (electronic or hardcopy) derived from the analytical process are assembled in the analytical data package that consists of:

9.11.8.1 LIMS work list

9.11.8.2 QA-020B form signed by the Lead Chemist or peer review

9.11.8.3 Quantitation Report for each Sample and QCS

9.11.8.4 Evaluation reports for CCV and LCS

9.11.8.5 Initial calibration curves generated

9.11.8.6 LIMS report of each sample

9.11.9 All electronic data including data packages is stored on a server which is backed up.

9.12 Corrective Actions for Out of Control

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:

9.12.1 When tuning or instrument calibration fails to meet acceptance criteria, the analysis does not start. The problem is investigated and the necessary instrument maintenance is performed, followed with tuning and calibration.

9.12.2 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.

9.12.3 The analyst shall report to the Lead Chemist and indicate on the QA-020B form, any out-of-control event. Such events include:

9.12.3.1 Damage to the sample.

9.12.3.2 Holding time exceeded.

9.12.3.3 Inadequate sample preservation.

9.12.3.4 Sample results exceeds the Agency's action limit

9.12.3.5 Samples do not reflect historical data.

9.12.3.6 Upward trending or sample results approaching interval warning limits.

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

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

9.12.5 If the acceptance criteria for a sample listed in Table 4 of this SOP are not met for MB, CCV, LCS, and the QC samples, then all associated samples must be reanalyzed.

9.12.6 See Table 4 for a summary of corrective action taken when QC samples or client sample QC does not meet acceptance criteria.

10.0 Calibration and Standardization

10.1 The mass spectrometer must be calibrated per manufacturer specifications prior to each analysis batch. In order to obtain accurate analytical values through this test method within the confidence limits, the following procedure must be followed when performing the test method.

10.2 To calibrate the instrument, analyze nine calibration standards containing the target analytes and the surrogate(s) prior to the analysis as shown in Table 3. Prepare the calibration solutions as described in Section 10.4 of this SOP.

10.3 Inject each standard and obtain chromatographic data. An external calibration method is used to monitor the primary MRM transitions of each analyte. For each analyte, the area under its primary MRM transition peak is utilized to conduct quantitation. The mass assignments are given in Table 5 and will vary depending on the instrument tuning conditions and mass axis calibrations.

10.4 The quantitation method is set to an external calibration using the peak areas as a function of concentration in pg/uL. Concentrations may be calculated using the quantitation software to generate linear or quadratic calibration curves. Forcing the calibration curve through zero is prohibited.

10.5 Linear calibration may be used if the coefficient of determination, R^2, is >0.98 for the analyte (Section 12.3). The point of the origin is excluded and a fit weighing of 1/x is used in order to give more emphasis to the lower concentrations. If one of the calibration standards other than the high or the low point causes the R^2<0.98, then this point must be re-injected or a new calibration curve generated. If low and/or high points are excluded, minimally a five point (six is recommended) curve is acceptable; however, the reporting range must be modified. No calibration point shall be excluded when bracketed by included points (Example: Level 3 will not be excluded when Levels 2 and 4 are included) except in the case of a known issue such as preparation with incorrect materials, broken autosampler vial, or a faulty injection.

10.6 Quadratic calibration may be used if the coefficient of determination, R^2, is >0.99 for the analyte (Section 12.3). The point of the origin is excluded and a fit weighing of 1/x is used in order to give more emphasis to the lower concentrations. If one of the calibration standards other than the high or the low point causes the R^2<0.99, this point must be re-injected or a new calibration curve must be generated. If low and/or high points are excluded, minimally a six point curve is acceptable, but the reporting range must be modified. No calibration point shall be excluded when bracketed by included points (Example: Level 3 will not be excluded when Levels 2 and 4 are included) except in the

case of a known issue such as preparation with incorrect materials, broken autosampler vial, or a faulty injection.

10.7 The retention time window of the peaks of the MRM transitions must be within 5% of the retention time of the analyte in the most recent CCV or the middle point of the associated initial calibration. If the peak is outside of the retention time window then reanalyze the calibration curve to determine if there was a shift in retention time during the analysis, and then the sample needs to be re-injected. If the retention time is still incorrect in the sample, then refer to the analyte as an unknown.

10.8 MRM Ion ratios and acceptance criteria are determined by TraceFinder software for MRM's that have more than one transition. Analyst discretion can be applied to ion ratios, especially with low level detections.

11.0 Procedure

11.1 Preliminary Sample Preparation

11.1.1 Samples are collected and stored as described in Section 11. Remove samples from storage.

11.1.2 Verify that the samples have been logged into LIMS, and are within holding time. If the sample exceeds holding time, notify the Lead Chemist and follow the corrective action plan.

11.1.3 Batch up to 20 environmental samples for extraction.

11.2 Sample Preparation/Extraction.

11.2.1 Soil Samples

11.2.1.1 Prepare an extraction solvent of 5.0% MeOH in water (v/v).

11.2.1.2 Weigh 5.0 grams of soil into a 40 mL VOA vial.

11.2.1.3 Spike with a known amount of a known concentration surrogate.

11.2.1.4 Add 5-10 glass beads.

11.2.1.5 Add 5.0 mL of 5.0% MeOH in water (v/v)

11.2.1.6 Vortex for 30 seconds on high

11.2.1.7 Shake for 15 minutes at 1,500 rpm for 15 minutes

11.2.1.8 After extraction, vortex for 5-10 secs before transferring ~1.0 mL of extract into a microcentrifuge tube. Centrifuge at 15,000 rpm for 5 minutes.

11.2.1.9 Decant a small amount of liquid to an AS vial for direct injection.

11.2.1.10 Vortex AS vial for 2-5 seconds at 3000 rpm before loading on instrument.

11.2.2 Water Samples

11.2.2.1 Draw up 950 uL of water sample into an airtight glass syringe. Transfer into an autosampler vial.

11.2.2.2 Spike water sample with a known amount of a known concentration surrogate

11.2.2.3 Invert vial a couple of times.

11.2.2.4 Draw up entire sample into syringe. Fit the syringe with a 13mm, 0.2um PTFE syringe filter. Filter sample and collect into a second auto sampler vial.

11.2.2.5 Add 50 uL of 100% MeOH to the autosampler vial. If the final volume exceeds 1.0 mL record and adjust calculations accordingly.

11.2.2.6 Vortex AS vial for 2-5 seconds at 3000 rpm before loading on instrument.

11.2.3 Wipe Samples

11.2.3.1 Prepare an extraction solvent of 5.0% MeOH in water (v/v)

11.2.3.2 Place the wipe in a 40 mL VOA vial.

11.2.3.3 Spike the sample with a known concentration (keeping in mind, a final volume of 15 mL) of the Surrogate Spike Solution (Section 10.4.2).

11.2.3.4 Spike each P&A wipe sample with a known concentration (keeping in mind a final volume of 15 mL) of the LCS Spiking Solution for Wipe Samples (Section 10.4.6).

11.2.3.5 Add 15 mL of the extraction solvent, 5.0% MeOH in water (v/v).

11.2.3.6 Vortex for 30 seconds on high

11.2.3.7 Shake for 15 minutes at 1,500 rpm for 15 minutes

11.2.3.8 After extraction, vortex for 5-10 secs before transferring ~1.0 mL of extract into a microcentrifuge tube. Centrifuge at 15,000 rpm for 5 minutes.

11.2.3.9 Decant a small amount of liquid to an AS vial for direct injection.

11.2.3.10 Vortex AS vial for 2-5 seconds at 3000 rpm before loading on instrument.

NOTE: Calibration standards are not filtered through the syringe-driven filter units since no particulates should be present. The filters used in this study were not shown to affect analyte concentrations. If alternate filtering is incorporated, the filters should be subjected to QC requirements to ensure they do not introduce interferences or retain the target analytes.

11.3 Sample Analysis and Calibration Procedure

11.3.1 Analysis is performed using the LC/MS/MS instrument programmed according to the parameters described in Tables 6, 7 and 8. All samples must be analyzed using the same mass spectrometric conditions.

11.3.2 A typical sequence will start with one or two solvent blanks (ACN), the ICAL or a CCV standard, an instrument blank, the QC from the batch, the samples, and finally an ending CCV. If the samples being analyzed are suspicious or possibly high in non-target analytes, running solvent blanks at the end of the sequence will help maintain the quality of your instrument.

11.3.3 Once the calibration curve meets acceptance criteria, the analysis of samples may begin. Inject 1 µL of the blank, extracts or QC samples using the sample injection technique as used for the standards. The order of analysis after the calibration is method blank (MB), laboratory control sample (LCS), sample(s), duplicate(s), matrix spike sample(s) followed by a closing continuing calibration verification sample (CCV). For this method, the CCV is equivalent to the Level 5 concentration of the initial calibration. All analysis batches must finish with a closing CCV, and compound recovery in the closing CCV must be within 30% of the true value. Analysis batches following a successful initial calibration may begin with a CCV provided recoveries for each analyte and surrogate is within 30% of the true value.

11.3.4 The data system will determine the concentration of each analyte in the extract using calculations in Section 15. Quantitation is based on the curves generated from the initial calibration, not the continuing calibration verification.

11.3.5 Identified compounds are reviewed for proper integration. Manual integrations are performed if necessary and are documented by the analyst and reviewed for QC approval. 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 the reason for the manual integration.

11.4 Identification of Analytes: See Section 12.1.

11.5 Dilutions

11.5.1 If the response for any analyte exceeds the current calibration range, a dilution of the extract is prepared and analyzed. An appropriate dilution should be in the upper half of the calibration range.

11.5.2 If the surrogates are diluted to a level where accurate quantitation is not possible then surrogates should be reported as diluted out.

11.5.3 Reporting Dilutions: The least dilute sample 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 Identification of Analytes

For quantitative analysis of FGAs and their surrogate, the MRM transitions are identified by comparison of the retention times in the sample to those of the standards. The analyte is identified by the retention time being within 5% of the retention time of that analyte in the most recent CCV or midpoint of the calibration curve.

12.2 The surrogate, Fentanyl-d5, is used to monitor the performance of all of the analytes in this method. If the surrogate recovery does not meet the quality control criteria of this method, the data is qualified for the appropriate analyte.

12.3 The concentration of each analyte is calculated using a multipoint linear or quadratic regression curve established in Section 13.0 of this SOP. The curve is generated by plotting Ax as a function of Cx.

where:

A_x is the area of the peak of the quantitation ion selected for MRM transition

C_x is the concentration of the analyte

12.3.1 Calculating the sample concentration based on linear regression:

$$C_x = \frac{A_x - b}{m}$$

where:

C_x is the concentration of the analyte

A_x is the area of the quantitation MRM transition

m is the slope

b is the y-intercept

12.3.2 Calculating the sample concentration based on quadratic regression:

$$C_x = \frac{-b \pm \sqrt{b^2 - 4a(c - A_x)}}{2a}$$

where:

C_x is the concentration of the analyte

A_x is the area of the quantitation MRM transition

a is the coefficient of the quadratic term

b is the coefficient of the linear term

c is the constant term

12.4 Percent deviation calculation for the CCV is performed using the following equation:

$$\%D = \frac{C_{cal} - C_t}{C_t} x100\%$$

where:

C_{cal} is the calculated concentration

C_t is the theoretical spiked concentration

12.5 Percent recovery for MS and LCS are performed using the following equation:

$$\%R = \left[\frac{\left(C_{spk} - C_x\right)}{C_t}\right]100$$

where:

C_{spk} is the concentration of the analyte in the spiked sample

C_x is the concentration of the analyte in the reference (parent) sample; (C_x = 0 for LCS.)

C_t is the theoretical spike concentration.

13.0 Method Performance

MDLs, RLs, and acceptance criteria are presented in Figure 2.

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 operations. The EPA has established a preferred hierarchy of option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address waste generation. When wastes cannot be feasible reduced at the source, recycling is 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, http://www.acs.org.

15.0 Waste Management

15.1 Laboratory waste should be kept to a minimum. Since these wastes are different than most laboratory wastes, they should be disposed of per the PHILIS Health and Safety Plan, the site disposal waste plan, and in conjunction with the PHILIS Health and Safety Officer.

15.2 The EPA requires that laboratory waste management practices be conducted 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 at the address listed in section 17.2.

16.0 References

16.1 Dryer, Mark; Koester, Carolyn. *LC/MS/MS Methods and Information for NTAs and Phosphonic Acids*. Lawrence Livermore National Laboratories. September 2020

16.2 Code of Federal Regulations, 40 CFR Part 136, Appendix B. Definition and Procedure for the Determination of the Method Detection Limit – Revision 1.11.

16.3 PHILIS SOP No. L-D-501, *Manual Integration and Data Integrity.*

17.0 Tables, Diagrams, Flowcharts and Validation Data

Table 1. Analytes Determined

Analyte	CAS#	Formula	Mass (m/z) of Parent Ion	Calibration Range (pg/uL)
VX	159939-87-4	C11H26NO2PS	[268.37]+	0.025-10.0
A230	2387496-12-8	C7H16FN2OP	[195.2]+	0.025-10.0
Fentanyl-d5	118357-29-2	C22H23D5N2O	[342.5]+	0.025-10.0
A232	2387496-04-8	C7H16FN2O2P	[211.2]+	0.025-10.0
A234	2387496-06-0	C8H18FN2O2P	[225.21]+	0.025-10.0

Table 2. Serial dilution matrix of calibration standards (Example)

Conc. pg/uL				
10 (A)	→	5 (B)	→	2.5 (C)
↓		↓		↓
1		0.5		0.25
↓		↓		↓
0.1		0.05		0.025

Table 3. Preparation of Calibration Standards (Example)

Level	Conc. of FGA pg/uL	Conc. of VX pg/uL	Conc. of Fentanyl-d5 pg/uL
LV 1	0.025	0.025	0.025
LV 2	0.05	0.05	0.05
LV 3	0.1	0.1	0.1
LV 4	0.25	0.25	0.25
LV 5	0.5	0.5	0.5
LV 6	1.0	1.0	1.0
LV 7	2.5	2.5	2.5
LV8	5.0	5.0	5.0
LV9	10.0	10.0	10.0

Table 4. L-A-311 Method QC Criteria

Item	Measure	Action
Initial Calibration (ICAL)	Coefficient of determination, R^2.	Evaluate points in the curve for use of linear or quadratic regression (R^2 must be ≥0.98 for linear regression, or R^2 must be > 0.99 for quadratic regressions). Also evaluate upper and lower points for removal. Criteria still not met, recalibrate if compound is an analyte of interest.
ICAL Low Point Eval. for compounds using linear or quadratic regression	Not within ±30% of True Value	Recalibrate if % deviation or drift is not met and compound is an analyte of interest.
Initial Calibration Verification/CCV	Not within ±30% of true value for deviation or drift.	Recalibrate if % deviation is not met and the compound is an analyte of interest.
Method Blank	Analyte(s) at or above reporting limit.	If the associated samples are non-detect, no action is required. If the analyte(s) is/are detected in the sample, flag with a "b" or reanalyze. If the analyte level in the sample is 10 times greater than the blank contamination, the results are not affected. Locate the source of the contamination.
Laboratory Control Spike (LCS)	% recovery. Laboratory acceptance criteria (accuracy) are evaluated every 6 months. Acceptable values are stored in the LIMS system.	If the LCS % recovery(accuracy) is high and the sample is non-detect, no action is required. If the LCS is high and the samples have detects, reanalyze the sample. If the LCS is low, the samples should be reanalyzed.
Laboratory Control Spike Duplicate (LCSD)	Same criteria as the LCS with the addition of RPD (precision). Current Acceptance criteria is 20% and is evaluated every 6 months with the values stored in the LIMS.	% recovery(accuracy) same as the LCS. If the RPD (precision) value is above the acceptance criteria in the LIMS, then evaluate the system for possible problems. Reprepare and reanalyze samples as necessary and if possible.
Surrogate(S)	% recovery. Laboratory acceptance criteria are evaluated every 6 months. Acceptable values are stored in LIMS.	If the % recovery (accuracy) is outside laboratory acceptance criteria on a QC sample, evaluate the system. Surrogate recalibration may be necessary. If the % recovery is on a client sample, reprep and reanalyze if possible. If the % recovery is within criteria, report the sample within limits. If % recovery is outside criteria and is confirmed, then there is a matrix effect. Flag the results as estimated and report the initial result.
Matrix Spike and Matrix Spike Duplicate	% recovery. Laboratory acceptance criteria (accuracy) are evaluated every 6 months. Acceptable values are stored in the LIMS system. Same criteria as the MS with the addition of RPD (precision). Current Acceptance criteria is 20% and is evaluated every 6 months with the values stored in the LIMS.	If the LCS % recovery(accuracy) is high and the sample is non-detect, no action is required. If the LCS is high and the samples have detects, reanalyze the sample. If the LCS is low, the samples should be reanalyzed. % recovery(accuracy) same as the LCS. If the RPD (precision) value is above the acceptance criteria in the LIMS, then evaluate the system for possible problems. Reprepare and reanalyze samples as necessary and if possible.

*Guidance value only, subject to change based on QC charting.

Table 5. Retention Times, and SRM transitions for TSQ-Altis

Analyte		MRM Mass Transition m/z	Collision Energy (V)	RF Lens	Retention Time (min)
VX	Primary	268.175 -> 128.137	17.59	Use Calibrated RF Lens	6.60
	Conf.	268.175 -> 86.208	26.44		
	Conf.	268.175 -> 44.054	35.2		
A230	Primary	195.2 -> 122.04	15.99	Use Calibrated RF Lens	8.14
	Conf.	195.2 -> 81.04	26.4		
	Conf.	195.2 -> 74.12	14.18		
A232	Primary	211.2 -> 137.9	17.13	Use Calibrated RF Lens	8.47
	Conf.	211.2 -> 74.04	14.06		
	Conf.	211.2 -> 97.06	30.02		
A234	Primary	225.21 -> 197.2	13.72	Use Calibrated RF Lens	9.04
	Conf.	225.21 -> 124.04	17.85		
	Conf.	225.21 -> 74.02	20.5		
Fentanyl-d5	Primary	342.12 -> 188.215	23.32	Use Calibrated RF Lens	8.37
	Conf.	342.12 -> 105.054	37.6		
	Conf.	342.12 -> 221.208	22.73		

Table 6. Vanquish UPLC Settings

Vanquish UPLC Settings		
Sample Manager		Column Compartment
Injection volume (uL)*	1.0	Waters Acquity™ HSS T3 column, 2.1 mm x 150 mm, 1.8 µm particle size (part # 186003540) Column temp: 40C Forced Air. Fan, 5 Preheater Left: 40C IF FLOW PATH THROUGH UV-VIS post column cooler: 40C, ELSE OFF.
Draw speed (uL/s)	5.0	
Dispense speed (uL/s)	5.0	
Wash mode	Both	
Wash time (s)	4.0	
Wash speed (uL/s)	50	
Puncture offset (um)	100	
Temperature control (C)	15	
System		
17 min		

LC Gradient			
Line No.	Time (min)	Flow Rate (mL/min)	%B
1	0	Run	
2	0	0.250	1
3	2	0.250	1
4	9.5	0.250	75
5	11.5	0.250	95
6	16	0.250	95
7**	16	0.250	1
8	New Row		
9	17	STOP RUN	

*Note, Injection volume is defined under the Master Method in TraceFinder software.
**Note, Line 7 is correct. The double 16 mins allow for a vertical drop in the gradient.

Table 7. Vanquish UV-VIS Settings

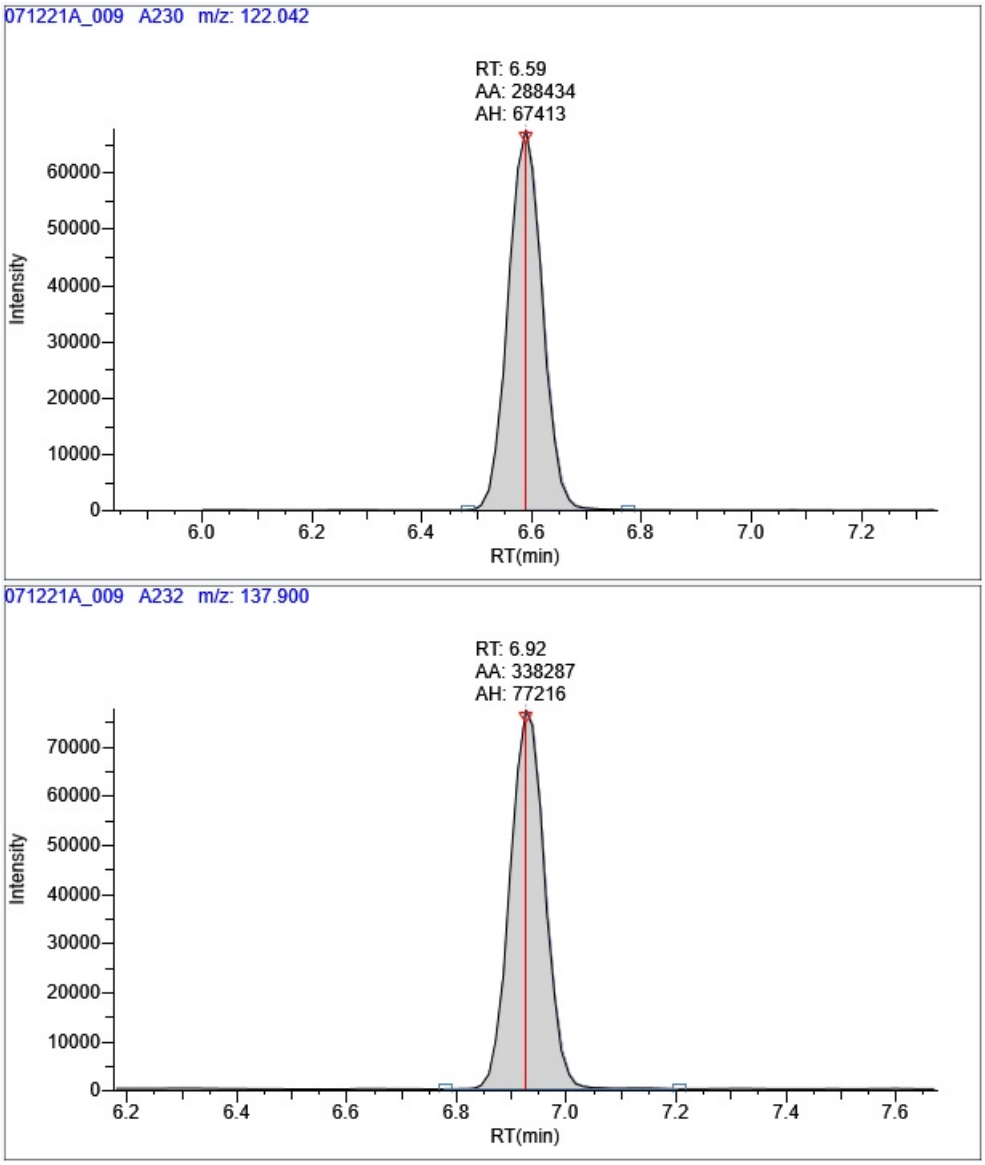
Vanquish UV-VIS
Not used for this analysis. NOTE: due to the temperature of the column compartment possibly exceeding 40C, if the flow path of the UPLC passes through the UV-VIS, the post column cooler MUST be turned ON and set to 40C or the crystal cell can split.

Table 8. TSQ-Altis MS/MS Settings

TSQ Altis Parameters			
Global Parameters		Scan Parameters	
Ion Source Properties		SRM Properties	
Method duration (min)	17	Polarity	Positive
Ion source type	H-ESI	Use cycle time	Yes
Spray voltage	Static	Cycle Time (sec)	0.6
Positive ion (V)	3700	Use calibrated RF lens	Yes
Negative ion (V)	2500	Q1 resolution (FWHM)	0.7
Sheath gas (arb)	35	Q3 resolution (FWHM)	0.7
Aux gas (arb)	15	CID gas (mTorr)	1.5
Sweep gas (arb)	1.0	Source fragmentation	0
Ion transfer tube (C)	350	Chromatographic peak width (s)	10
Vaporizer (C)	300	Use chromatographic filter	Yes
		Use Retention time reference	No

TSQ Altis Divert Valve Parameters	
Based on performance and analyte retention times.	
1-2 : From UPLC to ALTIS 1-6 : From UPLC to waste	
Time (min)	Position
0	1-6
5.9	1-2
12.1	1-6

Figure 1. Examples of MRM Chromatograms



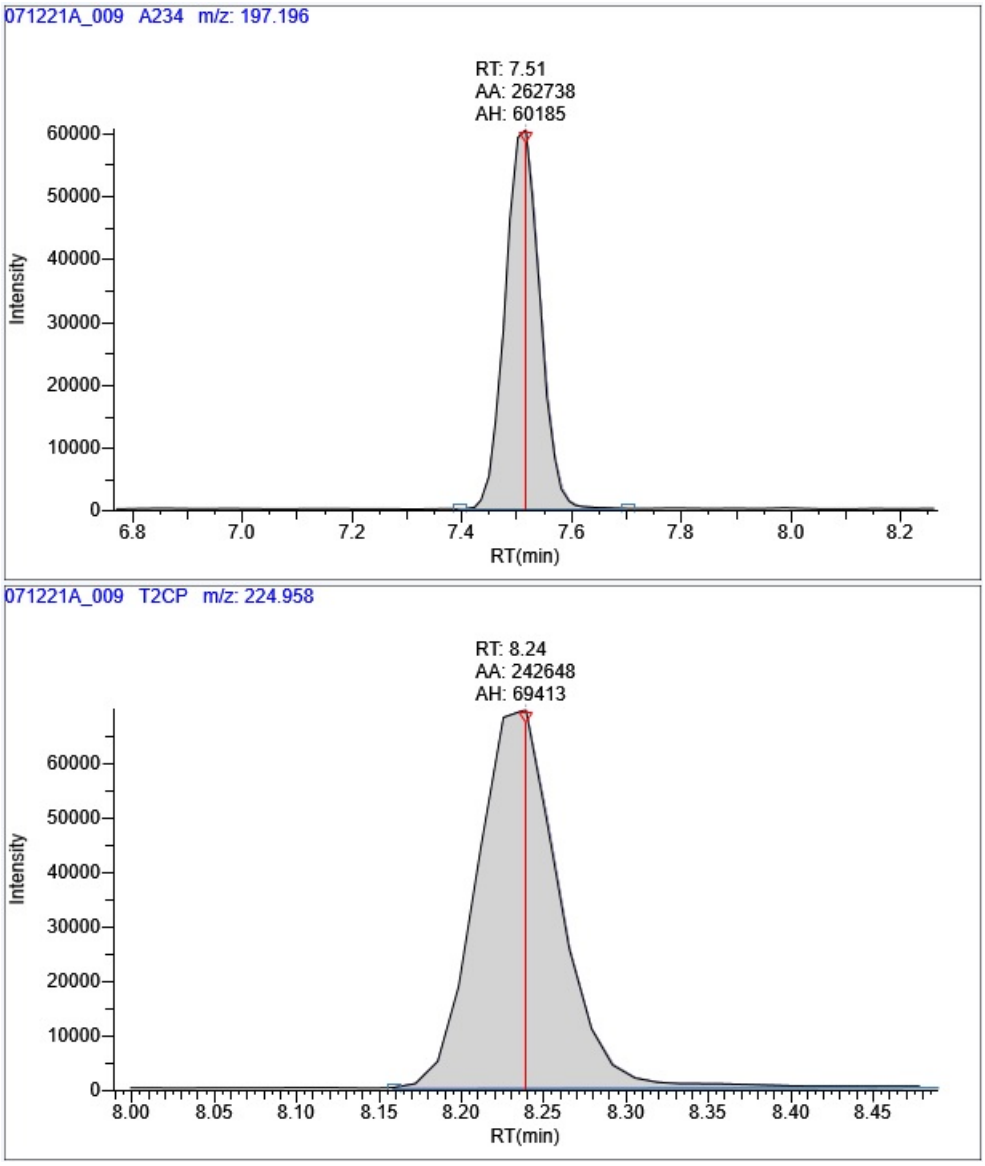

071221A_009 A234 m/z: 197.196
RT: 7.51
AA: 262738
AH: 60185
Intensity
RT(min)
071221A_009 T2CP m/z: 224.958
RT: 8.24
AA: 242648
AH: 69413
Intensity
RT(min)

Figure 2. Analyte MDL, RL, and Acceptance Criteria

FGA/VX by UPLCMSMS		FGA/VX Wipe 06/05/24			
		MDL	RL	RPD	Control Limits
Compound	CAS No.	Wipe (ug/wipe)	Wipe (ug/wipe)	Wipe (%)	Wipe (% Recovery)
A-230	2387496-12-8	0.001125	0.0025	30	50-150
A-232	2387496-04-8	0.0012735	0.0025	30	50-150
A-234	2387496-06-0	0.001287	0.0025	30	50-150
VX	159939-87-4	0.00011175	0.00025	30	50-150
FGA/VX by UPLCMSMS		FGA/VX Water 06/11/24			
		MDL	RL	RPD	Control Limits
Compound	CAS No.	Water (ug/L)	Water (ug/L)	Water (%)	Water (% Recovery)
A-230	2387496-12-8	0.0951	0.2	30	50-150
A-232	2387496-04-8	0.0881	0.2	30	50-150
A-234	2387496-06-0	0.0564	0.2	30	50-150
VX	159939-87-4	0.0284	0.1	30	50-150
FGA/VX by UPLCMSMS		FGA/VX Soil 06/04/24			
		MDL	RL	RPD	Control Limits
Compound	CAS No.	Soil (ug/Kg)	SOIL (ug/Kg)	Soil (%)	Soil (% Recovery)
A-230	2387496-12-8	0.102	0.2	30	50-150
A-232	2387496-04-8	0.068	0.2	30	50-150
A-234	2387496-06-0	0.0591	0.2	30	50-150
VX	159939-87-4	0.0423	0.1	30	50-150

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