

STANDARD OPERATING PROCEDURE
FOR
OPIOIDS ON SOIL, WATER AND WIPES BY
ALTIS UPLC/MS/MS

PHILIS SOP L-A-310 Rev. 5

Revision Date: 01-23-2024

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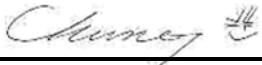
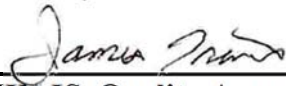
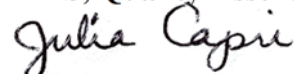
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Revision History

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Opioids on Soil, Water and Wipes by
Altis UPLC/MS/MS
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1.0 Scope and Application

- 1.1 This SOP is for the analysis of Fentanyl and other opiates. This SOP is executed in accordance with the U.S. Environmental Protection Agency and National Environmental Laboratory Accreditation Program (NELAP) requirements.
- 1.2 This procedure covers specific requirements for the determination of Opiate analogs in soil, water, and on wipes 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 sample preparation method was established as a performance-based method to optimize precision, accuracy and operational performance.
- 2.2 This method is used for the analysis of Opiates in water, soil, and wipes.
- 2.3 For the analysis of Opiate analogs, 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 filter unit and the filtrates are analyzed by LC/MS/MS.
- 2.4 The UPLC is run using reverse phase chromatography, and the ions are transferred into the gas phase using electrospray (ES). The mass spectrometer is operated in the positive mode (ES+).
- 2.5 Opiate analogs identified by retention time (within $\pm 5\%$ 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.

- 2.6 The target compounds and the surrogates are identified by retention time and one primary 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 24 hours. 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 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.

- 3.3 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.
- 3.4 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.

- 3.5 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.
- 3.6 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.
- 3.7 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.
- 3.8 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.
- 3.9 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.
- 3.10 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.

- 3.11 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.
- 3.12 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.
- 3.13 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 Filter papers used for wipes should be washed with distilled water and Acetonitrile prior to use.
- 4.4 Syringes and syringe filters are rinsed with 1 – 5 mL of methanol followed by 1 – 5 mL of acetonitrile before use.
- 4.5 All reagents and solvents should be LC/MS or pesticide grade or higher to minimize interference problems.

- 4.6 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 personnel 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 Material Safety Data Sheets (MSDS) for each analyte and reagent used in the mobile laboratory are available to all employees. The MSDS 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 Fentanyl is 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 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.
- 5.9 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.
- 5.10 Pure standard materials and stock standards of these compounds should be handled with suitable protection to skin and eyes. Care should be taken not to breathe the vapors or ingest the materials.
- 5.11 Laboratory personnel are required to be familiar with the general laboratory safety including the location and proper use of safety/emergency equipment.

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.
- 6.3 Microcentrifuge or centrifuge capable of maintaining a speed of 12,000 rpm
- 6.4 Vortexer
- 6.5 Glassware

Graduated cylinders - various sizes

6.6 Syringes

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

6.7 Instrumentation

6.7.1 LC/MS/MS Apparatus

6.7.1.1 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 system with a 50-µL loop for this method.

6.7.1.2 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.

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

6.7.1.4 Data System - TraceFinder software (or similar software) interfaced to the LC/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 unit.

6.8 Other Laboratory Equipment

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

6.9 Supplies

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

6.9.2 Sample Collection Containers: Precleaned glass bottles, vials or jars with polytetrafluoroethylene-lined caps.

- 6.9.3 Small glass vials (8mL are used for storage of sample extracts, calibration standards and stock standards).
- 6.9.4 10 mL vials are used for storage of standards and spiking solutions.
- 6.9.5 40 mL VOA vials
- 6.9.6 15 and 25 mL Falcon Tubes
- 6.9.7 2.1 mL microcentrifuge tubes.
- 6.9.8 Pasture pipettes
- 6.9.9 Wipes: Kendall 3" x 3" type VII gauze sponges.
- 6.9.10 Ottawa Sand, or other clean certified sand matrix
- 6.9.11 3mm glass beads

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 greater 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 (for high protein contamination), 45% Acetonitrile / 45% Isopropyl Alcohol / 10% Acetone (v/v/v)

Or Non Acetone Magic Mix, 45% Acetonitrile / 45% Isopropyl / 10% Methanol (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 is 0.01% Formic Acid + 2.5 mM Ammonium formate in water.

Mobile phase B is 100% Methanol.

7.3.1 Preparation of Mobile Phase A (aqueous)

Mobile phase A consists of 0.01% of formic acid + 2.5mM ammonium formate in water. To prepare 1000 mL, weigh out 157.65 mg of ammonium formate and add to the 1000 mL volumetric flask. Transfer in about 100 mL of water. Add 100uL of formic acid to a 1000-mL volumetric flask. Dilute to the mark with HPLC grade water. Mix and transfer to a HPLC bottle.

7.4 Standards

Standard solutions may be prepared from certified, commercially available solutions or from neat compounds. Compounds used to prepare solutions must be 96% pure or greater and the weight may be used without correction for purity to calculate the concentration of the stock standard. Solution concentrations listed in this section were used to develop this method and are included as an example. Standards for sample fortification should be prepared in the smallest volume that can be accurately measured to minimize the addition of organic solvent to aqueous samples. Prepare all solutions using Class A volumetric glassware. During storage, protect standards from light and keep in a refrigerator at 0 – 6°C. Stock standards are stable for at least one month but should be replaced when analyzed solution concentrations deviate more than $\pm 20\%$ from the prepared concentration.

Table 2 lists some suggested stocks that are available from Sigma-Aldrich (St. Louis, MO, USA) as certified solutions at 100 µg/mL.

7.4.1 Surrogate Stock Standard Solution (Surrogate SSS), 1000 – 2000 µg/mL

Standard stock solutions may be prepared from certified commercially available neat compounds, if available. To prepare a stock from neat materials, obtain the isotopically labeled surrogate, fentanyl-*d*5, and accurately weigh about 0.05 g each on an analytical balance. Transfer the surrogates to individual 25-mL volumetric flasks and dilute each to the mark with acetonitrile in order to achieve concentrations of ca. 2000 µg/mL. The surrogate stock standard solutions are stable for at least a month when stored at 0 – 6°C.

If neat materials are not available, certified, commercially available stocks at 100 µg/mL of the surrogate may be obtained from Sigma-Aldrich (St. Louis, MO, USA) or Cerilliant.

7.4.2 Surrogate Spike Solution – Prepare a surrogate spike solution at a known concentration in 100% MeOH that will be used for spiking samples and blanks. It is recommended that Heroin-D9 be 10 times more concentrated than the other surrogates.

Recommend concentration for surrogate spiking solution, 1000 pg/uL (10,000 pg/uL Heroin-d9) in 100% MeOH

7.4.3 Analyte Stock Standard Solutions, 500-5000 µg/mL

Standard solutions may be prepared from certified, commercially available neat compounds. If analyte stock solutions are made from neat materials, their recommended concentrations should be 500-5000 µg/mL. For example, a standard stock solution of 2000 µg/mL for each compound can be prepared by diluting 0.05 g of the neat material in a 25-mL volumetric flask with methanol. Analyte stock standard solutions are stable for at least a month when stored at 0 - 6°C.

If neat materials are not available, certified, commercially available stocks at 100 µg/mL of each analyte may be obtained from Sigma-Aldrich (St. Louis, MO, USA) or Cerilliant.

7.4.4 Calibration Standard Solutions

Prepare a 1,000 pg/uL (10,000 pg/uL for Heroin and Heroin-d9, 2,000 pg/uL for Remifentanyl, 50,000 pg/uL for Methamphetamine) calibration stock standard in 100% Acetonitrile.

Next, all calibration levels are prepared in a 100% MeOH. Remifentanyl has been shown to be extremely unstable in water and breaks down at an accelerated rate. The addition of methanol helps to stabilize this compound.

Prepare a 100 pg/uL (A) standard in a total volume of 1 mL of 100% MeOH. This can be done with a 1 mL syringe into an autoamplifier via. This will be the base for the serial dilution.

From the base, serially dilute to 50 pg/uL (B) and 25 pg/uL (C) to a final volume of 1 mL in 100% MeOH. with a 1 mL syringe and autosampler vials

Going back to the 100 pg/uL (A) standard serially dilute that to 10, 1.0, 0.1 pg/uL to a final volume of 1 mL in 100% MeOH. with a 1 mL syringe and autosampler vials.

Next, using the 50 pg/uL (B) standard, serially dilute to 5.0, 0.5, 0.05 pg/uL to a final volume of 1 mL in 100% MeOH. with a 1 mL syringe and autosampler vials.

Finally, using the 25 pg/uL (C) standard, serially dilute to 2.5, 0.25, 0.025 pg/uL to a final volume of 1 mL in 100% MeOH. with a 1 mL syringe and autosampler vials.

Reference Table 3 for serial dilution matrix.

Reference Table 4 for example calibration level concentrations.

7.4.5 Continuing calibration verification. 10 uL spike of a 1000 pg/uL into 990 uL of 100% MeOH. From that, take 100 uL and add to 900 uL of MeOH in another autosampler vial for a final concentration of 1.0 pg/uL.

7.4.6 LCS Spiking Solution

It 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:

Recommend concentration for LCS spiking standard is 1,000 pg/uL (10,000 pg/uL Heroin, 2,000 pg/uL Remifentanyl, 50,000 pg/uL for Methamphetamine.)

8.0 Sample Collection, Preservation, 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 MeOH) 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 2.0 mL of methanol. The first wipe is used to wipe the surface in a Z-like pattern horizontally across a defined surface (100 cm²). The second wipe is used to wipe the same surface in a Z-like pattern vertically across a defined surface (100 cm²). 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 samples must be analyzed within 72 hours of collection or as soon as possible. The holding times for wipes has 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 °C 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, the vial septa must be replaced and the vials are stored in a refrigerator in case further analysis was needed. Extracts or diluted samples previously analyzed by LC/MS can be stored up to 28 days in the refrigerator at 0 - 6 °C.

9.0 Quality Control

- 9.1 Quality control (QC) requirements include the Initial Demonstration of Capability, 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), and either a Matrix Spike Duplicate (MSD) or a Field Duplicate Sample. This section details the specific requirements for each QC parameter. The QC criteria discussed in the following sections are summarized in Table 5. 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 12.2.1 through 12.2.4.

- 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 Section 9.6. 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 $\pm 30\%$ of the true value.
- 9.2.3 INITIAL DEMONSTRATION OF PRECISION – Using the same set of replicate data generated for Section 9.2.2, 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 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 and a minimum of seven blanks prepped in three separate batches and analyzed on three separate days. 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 ($\mu\text{g/L}$). 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. This calculation must be performed separately for the spikes and blanks. The larger of the two values will be used.

9.3.1.3 MDL Blank is determined as follows:

- A. If all blanks are non-detect then the MDL blank is not used.
- B. If only some of the blanks have detection, then use the highest value for the MDL blank.
- C. If all blanks have detection then determine the average value and add the MDL determined from the blank results to the average result.
- D. Use the higher of the regular MDL and MDL blank.

9.3.1.4 The Initial MDL should be performed when there is a change of equipment, location of equipment, or a change of procedure.

9.3.2 Ongoing MDL Data Collection

9.3.2.1 Ongoing MDLs are determined by preparing and analyzing two spiked standards at 1-5 times the estimated MDL and two blanks once per quarter 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 initial mdL may be performed annually.

9.3.2.2 At least once per year re-evaluate the MDL by, calculating as above in 9.3.1.2. Use the larger of the spiked determinations and blank determinations for the MDL value.

9.3.3 Ongoing MDL Annual Verification

At least once every thirteen months, re-calculate the MDL spike and MDL blank 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 two times 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 Data Assessment and Acceptance Criteria--Analytical data generated by the quantitation software is reviewed and evaluated by the analyst as follows:
- 9.5.1 Instrument calibration, calibration verifications, SS, other QC measures are evaluated and the results documented on separate forms:
- 9.5.2 For each analyte and surrogate, evaluate the coefficient of determination, R^2 , from the initial calibration curve.
- 9.5.3 Evaluate the % recoveries for all surrogates.
- 9.5.4 Evaluate the % recoveries for the CCV, SCV, LCS, MS, MSD, and evaluate the RPD for the MS/MSD pair.
- 9.5.5 Calibration standards must meet the coefficient of determination criteria and other quality control measures must meet the criteria listed in Table 5.
- 9.5.6 A reported compound that has a retention time outside the established window is considered a false positive response. All false positives are eliminated, and all positively identified target analytes are reported to LIMS.
- 9.5.7 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.
- 9.5.8 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.

- 9.5.9 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.
- 9.5.10 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.
- 9.5.11 Reviewed data are entered into LIMS, hard copies of LIMS report are printed and compared to the original data.
- 9.5.12 All records (electronic or hardcopy) derived from the analytical process are assembled in the analytical data package that consists of:
- 9.5.12.1 LIMS work list
 - 9.5.12.2 QA-017 form signed by the Lead Chemist or peer review
 - 9.5.12.3 Quantitation Report for each Sample and QCS
 - 9.5.12.4 Evaluation reports for CCV and LCS
 - 9.5.12.5 Initial calibration curves generated
 - 9.5.12.6 LIMS report of each sample
- 9.5.13 All electronic data including data packages is stored on a server which is backed up.
- 9.6 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.

- 9.7 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 be 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.
- 9.8 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 5.
- 9.9 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 5. 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.
- 9.10 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 fentanyl and following the analytical method.
- 9.10.1 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.

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

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

Eq. 1

where

A = measured concentration in the fortified sample

B = measured concentration in the unfortified sample, and

C = fortification concentration.

9.10.3 The percent recovery of the matrix spike shall fall within the limits in Table 5. 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.

9.11 MATRIX SPIKE DUPLICATE (MSD) - To check the precision of sample analyses, analyze a sample in duplicate with each batch of 20 or fewer samples. If the same 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.

9.11.1 Calculate the relative percent difference (RPD) between the duplicate values as shown in Eq. 2. Compare the RPD limit in Table 5.

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

Eq. 2

where:

RPD = relative percent difference

MS = matrix spike recovery

MSD = matrix spike duplicate recovery

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

9.12 Corrective Action 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 the 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-018 LC/MS/MS Data Review 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 When tuning and 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 tuning and calibration.
- 9.12.6 See Table 5 for a summary of corrective action taken when QC samples or client sample QC does not meet acceptance criteria
- 9.13 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 may not be acceptable and the analyst does the following:

- 9.13.1 When instrument calibration fails to meet acceptance criteria, the analysis does not start with sample analysis. The problem is investigated and the necessary instrument maintenance is performed, followed by another calibration.

- 9.13.2 If the acceptance criteria for a sample listed in Table 5 of this SOP are not met for MB, CCV, LCS, and the QC samples, then all associated samples must be reanalyzed.
- 9.13.3 If after analysis, any of the criteria for quality control are not met, or the sample is not available for reanalysis, then the analyst must notify the Lead Chemist. The Lead Chemist will implement the corrective action plan.
- 9.13.4 The analyst shall report to the Lead Chemist and indicate of the “QC Summary form” any out-of-control event. Such events include:
 - 9.13.5 Damage to the sampling container.
 - 9.13.6 Holding time exceeded.
 - 9.13.7 Samples do not reflect historical data.
 - 9.13.8 Upward trending or sample results approaching interval warning limits.
 - 9.13.9 Any non-target analyte peak present on the instrument generated chromatogram.

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 eight 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 7.4 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 7 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 the low and/or high point is excluded, minimally a five point (six is recommended) curve is acceptable; however, the reporting range must be modified.
- 10.6 Quadratic 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 $R^2 < 0.98$, this point must be re-injected or a new calibration curve must be generated. If the low and/or high point is excluded, minimally a six point curve is acceptable, but the reporting range must be modified.
- 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.

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 Wipe Samples

- 11.2.1.1 Open the jar, and allow the wipe to dry at room temperature. Allow excess MeOH to evaporate off. The wipe can be damp, but not soaking with MeOH. A dry wipe allows for more accurate volume measurements and studies have shown that the analytes interest are relatively stable up to 48 hrs on a completely dry wipe.
- 11.2.1.2 Place the wipe in a 40 mL VOA vial.
- 11.2.1.3 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).
- 11.2.1.4 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). For a 1000pg/uL surrogate solution, recommended amount is 30 uL. This will give a final concentration result of 1.0 pg/uL on the instrument
- 11.2.1.5 Add 15 mL of methanol and vortex on high for 10 - 20 seconds
- 11.2.1.6 Secure the vial on a shaker table. Extract for 15 minutes at a speed of 1500 - 2000 rpm.
- 11.2.1.7 After extraction, vortex for 5-10 secs before transferring ~1.0 mL of extract into a microcentrifuge tube.
- 11.2.1.8 Microcentrifuge at ~15,000 rpm for 5 minutes
- 11.2.1.9 Carefully transfer supernatant, avoiding solids into an autosampler vial
- 11.2.1.10 Vortex the AS vial for 2-5 seconds before placing in autosampler.
- 11.2.1.11 Final calculations based on 15 mL.

11.2.2 Water

- 11.2.2.1 Transfer 1.0 mL of water using a 1.0 mL syringe into a
 - A. 2.1 mL microcentrifuge tube.
 - B. OR into a 15 mL Falcon tube.

- 11.2.2.2 Spike with a surrogate standard for a final known concentration.
 - A. Example: 1.0 uL of a 1000pg/uL surrogate for an expected concentration of 1.0 pg/Ul.
- 11.2.2.3 Vortex the sample for 5 seconds at 3000 rpm to ensure proper mixing.

NOTE: It has been shown that the lack of vortexing has significant adverse effects on recoveries and precision.
- 11.2.2.4 **Water samples MUST BE extracted and run immediately!** Studies have shown that remifentanil is unstable in water, and a 1.0 pg/uL concentration of remifentanil will completely degrade after 40 hours. (See Figure 2)
- 11.2.2.5 Centrifuge the sample at 12,000 rpm for 5 minutes.
- 11.2.2.6 Carefully transfer the supernatant into an AS vial and cap the vial with the appropriate cap for your system.
- 11.2.2.7 Vortex the AS vial for 5 seconds before placing in autosampler.
- 11.2.3 Soil
 - 11.2.3.1 Weigh out 5g of soil and add to a 40 mL VOA vial. Add 5-10 glass beads.
 - 11.2.3.2 Spike soil with known concentration of surrogate. (Recommended spiking 15 uL of a 1000pg/uL surrogate solution. This will result in a 1.0 pg/uL concentration on instrument.)
 - 11.2.3.3 Add 15.0 mL of MeOH with a graduated cylinder
 - 11.2.3.4 Vortex on high for 10 sec.
 - 11.2.3.5 Shake for 15 minutes at 1500 rpm.
 - 11.2.3.6 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
 - 11.2.3.7 Carefully transfer supernatant into an autosampler vial, avoiding any solids and cap the vial with the appropriate cap for your system.
 - 11.2.3.8 Vortex autosampler vial for 2-5 secs at 3000 rpm prior to placing in autosampler.
 - 11.2.3.9 Final calculation based on 15 mL.

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 8, 9 and 10. 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 (MeOH), 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 50 μ L 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 less than 30% Recovery. Analysis batches following a successful initial calibration may begin with a CCV provided recoveries for each analyte and surrogate is less than 30% D.
- 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 15.1.
- 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

- 12.1.1 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.

- 12.1.2 For quantitative analysis of fentanyl, and the surrogate, the MRM transitions are identified by comparison of the retention times in the sample to those of the standards. External calibration curves are used to calculate the amounts of the target compounds and surrogates. Calculate the concentration in nanograms (ng) for each analyte. If the concentration of the analyte is determined to be above the calibration range, the sample is diluted with reagent methanol to obtain a concentration near the mid-point of the calibration range and reanalyzed.

- 12.2 The surrogates, fentanyl-*d*5, carfentanil-*d*5, and heroin-*d*9, are 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 10.0 of this SOP. The curve is generated by plotting A_x as a function of C_x .

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:

$$\%D = \frac{C_{cal} - C_t}{C_t} \times 100\%$$

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:

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

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

Example MDLs, reporting limits and Precision and Accuracy for Fentanyl analogs on wipes, water, and soils are listed in Table 6. Statistical Precision and Accuracy limits will be determined when adequate data is available.

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 Doyle, Rory M. et.al 2018, Quantitative Analysis of 40 Fentanyl, its Precursors, Analogues and Metabolites in Urine, Oral Fluids and Blood using LC-MS/MS for Forensic Use TP189, Thermoscientific Somerset, NJ, USA, (<https://assets.thermofisher.com/TFS-Assets/CMD/posters/po-65275-lc-fentanyls-msn-asms2018-po65275-en.pdf>)
- 16.2 Code of Federal Regulations, 40 CFR Part 136, Appendix B. Definition and Procedure for the Determination of the Method Detection Limit – Revision 2.0.
- 16.3 PHILIS SOP No. L-D-501, *Manual Integration and Data Integrity*.

17.0 Tables, Figures, and Attachments

Table 1. Analytes Determined

Analyte	CAS#	Formula	Mass (m/z) of Parent Ion	Calibration Range (pg/uL)
Acetyl fentanyl	3258-84-2	C ₂₁ H ₂₆ N ₂ O ₂	(M+H) ⁺ at 323	0.05-10
Alfentanyl	69049-06-5	C ₂₁ H ₃₂ N ₆ O ₃	(M+H) ⁺ at 417	0.05-10
Carfentanyl	61086-44-0	C ₂₄ H ₃₀ N ₂ O ₂	(M+H) ⁺ at 395	0.05-10
Carfentanyl-d ₅	1185158-60-4	C ₂₄ D ₅ H ₂₅ N ₂ O ₂	(M+H) ⁺ at 400	0.05-10
Fentanyl	437-38-7	C ₂₂ H ₂₈ N ₂ O	(M+H) ⁺ at 337	0.05-10
Fentanyl-d ₅	118357-29-2	C ₂₂ D ₅ H ₂₃ N ₂ O	(M+H) ⁺ at 342	0.05-10
Heroin	561-27-3	C ₂₁ H ₂₃ NO ₅	(M+H) ⁺ at 370	0.5-100*
Heroin-d ₉	1338713-49-7	C ₂₁ F ₁₄ D ₉ NO ₅	(M+H) ⁺ at 379	0.5-100
Remifentanyl	132539-07-2	C ₂₀ H ₂₈ N ₂ O ₅	(M+H) ⁺ at 377	0.1-20**
Sulfentanyl	60561-17-3	C ₂₂ H ₃₀ N ₂ O ₂ S	(M+H) ⁺ at 387	0.05-10
Methamphetamine	537-46-2	C ₁₀ H ₁₅ N	(M+H) ⁺ at 151	2.5-500***
Cocaine	50-36-2	C ₁₇ H ₂₁ NO ₄	(M+H) ⁺ at 304	0.05-10

* Heroin is 10 times less sensitive than the other opiates in this list. Recommended calibration range is 0.5-100 pg/uL

** Remifentanyl has a slightly lower response in this system. Recommended calibration range is 0.1-20 pg/uL

*** Methamphetamine is 50 times less sensitive than the other compounds in this list. Recommended calibration range is 2.5 – 500 pg/uL

Table 2. Examples of Commercially-Available Neat Standards

Standard Name	Source/Catalog Number	Analyte Type	Listed Purity
Fentanyl	Cerilliant/F-002-1ML (100 µg/mL)	Target	≥ 99%
Fentanyl-d ₅	CerilliantF-001-1ML (100 µg/mL)	Surrogate	≥ 99%

Table 3. Serial dilution matrix of calibration standards (Example)

Conc. pg/uL				
100	→	50	→	25
↓		↓		↓
10		5		2.5
↓		↓		↓
1.0		0.5		0.25
↓		↓		
0.1		0.05		

Table 4. Preparation of Calibration Standards (Example)

Level	Conc. pg/uL	Conc. Heroin/Heroin- d9 pg/uL	Conc. Remifentanil pg/uL	Conc. Methamphetamine pg/uL
LV 1	0.05	0.5	0.1	2.5
LV 2	0.1	1	0.2	5.0
LV 3	0.25	2.5	0.5	12.5
LV 4	0.5	5	1.0	25
LV 5	1	10	2.0	50
LV 6	2.5	25	5.0	125
LV 7	5	50	10	250
LV 8	10	100	20	500

Table 5. L-A-310 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 $\pm 30\%$ of True Value	Recalibrate if % deviation or drift is not met and compound is an analyte of interest.
Initial Calibration Verification/CCV	Not within $\pm 30\%$ 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 are evaluated every 6 months. Acceptable values are stored in the LIMS system.	If the LCS % recovery 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. Current Acceptance criteria is 30% and is evaluated every 6 months with the values stored in the LIMS.	% recovery same as the LCS. If the RPD value is above the acceptance criteria in the LIMS, then evaluate the system for possible problems. Reprep 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 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.

Table 6. Example QC Acceptance Criteria

OPI by UPLCMSMS		OPI on Wipes 03/15/22			
		MDL	RL	RPD	Control Limits
Compound	CAS No.	Wipe (ug/wipe)	Wipe (ug/wipe)	Wipe (%)	Wipe (% Recovery)
Heroin	561-27-3	0.0072	0.0075	30	50 -150
Remifentanyl	132539-07-2	0.00079	0.0015	30	50 -150
Acetylfentanyl	3258-84-2	0.00033	0.00075	30	50 -150
Fentanyl	437-38-7	0.00071	0.00075	30	50 -150
Carfentanyl	61086-44-0	0.00046	0.00075	30	50 -150
Sulfentanyl	60561-17-3	0.00065	0.00075	30	50 -150
Alfentanyl	69049-06-5	0.00076	0.0015	30	50 -150
OPI by UPLCMSMS		OPI in Water 03/15/22			
		MDL	RL	RPD	Control Limits
Compound	CAS No.	Water (ug/L)	Water (ug/L)	Water (%)	Water (% Recovery)
Heroin	561-27-3	0.656	1.0	30	50 -150
Remifentanyl	132539-07-2	0.158	0.2	30	50 -150
Acetylfentanyl	3258-84-2	0.07	0.1	30	50 -150
Fentanyl	437-38-7	0.047	0.1	30	50 -150
Carfentanyl	61086-44-0	0.060	0.1	30	50 -150
Sulfentanyl	60561-17-3	0.048	0.1	30	50 -150
Alfentanyl	69049-06-5	0.061	0.1	30	50 -150
OPI UPLCMSMS		OPI in Soil 9/3/21			
		MDL	RL	RPD	Control Limits
Compound	CAS No.	Soil (ug/Kg)	SOIL (ug/Kg)	Soil (%)	Soil (% Recovery)
Heroin	561-27-3	2.1	3.0	30	50 -150
Remifentanyl	132539-07-2	0.15	0.30	30	50 -150
Acetylfentanyl	3258-84-2	0.068	0.30	30	50 -150
Fentanyl	437-38-7	0.038	0.30	30	50 -150
Carfentanyl	61086-44-0	0.12	0.30	30	50 -150
Sulfentanyl	60561-17-3	0.059	0.30	30	50 -150
Alfentanyl	69049-06-5	0.12	0.30	30	50 -150

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

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Table 7A. Retention times and SRM transitions for TSQ-Altis

Compound	Retention time (min)
Methamphetamine	5.41
Heroin-d9	6.45
Heroin	6.49
Cocaine	6.58
Remifentanyl	7.14
Acetylfentanyl	7.29
Fentanyl-d5	7.95
Fentanyl	7.97
Carfentanyl	8.26
Carfentanyl-d5	8.28
Sulfentanyl	8.71
Alfentanyl	8.86

Table 7B. SRM transition data for TSQ-Altis

Compound	Start Time (min)	End Time (min)	Precursor (m/z)	Product (m/z)	Collision Energy (V)	Min Dwell Time (ms)	RF Lens (V)	Use Quan Ion
Methamphetamine	5.0	6.5	150.88	91.125	20.12	23.591	31	True
Methamphetamine	5.0	6.5	150.88	119.125	11.36	23.591	31	False
Cocaine	5.0	9.5	304.175	150.0	24.21	23.591	71	False
Cocaine	5.0	9.5	304.175	182.083	18.81	23.591	71	True
Acetylfentanyl	5.0	9.5	323.175	105.125	36.13	23.591	71	False
Acetylfentanyl	5.0	9.5	323.175	188.137	22.73	23.591	71	True
Acetylfentanyl	5.0	9.5	323.175	202.137	22.48	23.591	71	False
Fentanyl	5.0	9.5	337.25	105.196	37.48	23.591	72	False
Fentanyl	5.0	9.5	337.25	188.208	22.94	23.591	72	True
Fentanyl-D5	5.0	9.5	342.27	105.125	36.17	23.591	70	False
Fentanyl-D5	5.0	9.5	342.27	188.208	22.82	23.591	70	True
Heroin	5.0	9.5	370.088	211.125	31.12	23.591	79	False
Heroin	5.0	9.5	370.088	268.054	28.88	23.591	79	True
Heroin	5.0	9.5	370.088	328.155	26.4	23.591	79	False
Remifentanyl	5.0	9.5	377.138	285.125	19.28	23.591	62	False
Remifentanyl	5.0	9.5	377.138	317.137	15.95	23.591	62	True
Remifentanyl	5.0	9.5	377.138	345.125	12.54	23.591	62	False
Heroin-d9	5.0	9.5	379.175	212.125	31.58	23.591	89	False
Heroin-d9	5.0	9.5	379.175	272.226	29.47	23.591	89	True
Heroin-d9	5.0	9.5	379.175	335.208	27.49	23.591	89	False
Sulfentanil	5.0	9.5	387.175	111.125	36.97	23.591	72	False
Sulfentanil	5.0	9.5	387.175	238.125	18.9	23.591	72	True
Sulfentanil	5.0	9.5	387.175	355.208	19.2	23.591	72	False
Carfentanil	5.0	9.5	395.3	246.155	21.47	23.591	71	False
Carfentanil	5.0	9.5	395.3	335.238	18.44	23.591	71	True
Carfentanil	5.0	9.5	395.3	363.208	13.26	23.591	71	False
Carfentanil-d5	5.0	9.5	400.25	246.155	21.72	23.591	72	False
Carfentanil-d5	5.0	9.5	400.25	340.22	19.03	23.591	72	True
Carfentanil-d5	5.0	9.5	400.25	368.167	13.51	23.591	72	False
Alfentanil	5.0	9.5	417.212	197.208	25.3	23.591	75	False
Alfentanil	5.0	9.5	417.212	268.155	17.76	23.591	75	True
Alfentanil	5.0	9.5	417.212	385.238	18.23	23.591	75	False

Table 8. Vanquish UPLC Settings

Vanquish UPLC Settings				
Sample Manager			Column Compartment	
Injection volume (uL)*	1.0		Column: Waters Acquity™ HSS T3, 2.1 mm x 150 mm, 1.8 µm particle size (part # 186003540)	
Draw speed (uL/s)	5.0			
Dispense speed (uL/s)	5.0		Guard Column: Waters VanGuard™ HSS T3 2.1 mm x 5 mm, 1.8 µm particle size (part # 186003967)	
Wash mode	Both			
Wash time (s)	8.0			
Wash speed (uL/s)	50		Column temp: 50 °C Forced Air. Fan, 5	
Puncture offset (um)	100			
Temperature control (C)	20		Preheater Left: 50 °C	
System				
15 min			IF FLOW PATH THROUGH UV-VIS post column cooler: 40 °C, ELSE OFF.	
LC Gradient				
Line No.	Time (min)	Flow Rate (mL/min)	%B	
1	0	Run		
2	0	0.25	10	
3	0.5	0.25	10	
4	6	0.25	60	
5	8	0.25	98	
6	12	0.25	98	
7**	12	0.25	10	
8	New Row			
9	15	STOP RUN		

*Note, Injection volume is defined under the Master Method in TraceFinder software.

**Note, Line 7 is correct. The double 12 mins allow for a vertical drop in the gradient.

Table 9. Vanquish UV-VIS Settings

Vanquish UV-VIS	
Not used for this analysis.	
NOTE: due to the temperature of the column compartment 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 light pipe can shatter.	

Table 10. TSQ-Altis MS/MS Settings

TSQ Altis Parameters			
Global Parameters		Scan Parameters	
Ion Source Properties		SRM Properties	
Method duration (min)	15	Polarity	Positive
Ion source type	H-ESI	Use cycle time	Yes
Spray voltage	Static	Cycle Time (sec)	0.8
Positive ion (V)	500	Use calibrated RF lens	No
Negative ion (V)	2500	Q1 resolution (FWHM)	0.7
Sheath gas (arb)	50	Q3 resolution (FWHM)	0.7
Aux gas (arb)	10	CID gas (mTorr)	1.5
Sweep gas (arb)	1.0	Source fragmentation	0
Ion transfer tube (C)	325	Chromatographic peak width (s)	6
Vaporizer (C)	350	Use chromatographic filter	Yes
		Use Retention time reference	No

TSQ Altis Divert Valve Parameters	
Based on user preferences and analyte retention times.	
1-2: From UPLC system to TSQ	
1-6: From UPLC to waste	
Time (min)	Position
0	1-6
4.9	1-2
9.5	1-6

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Table 11. Wipe Recoveries for Common Surfaces

Wipe % Recovery of Opioid Analogs on Various Surfaces												
	Linoleum		Concrete Smooth		Concrete Porous, rough		Concrete Painted		Metal counter		Desktop	
	%Rec	%RSD	%Rec	%RSD	%Rec	%RSD	%Rec	%RSD	%Rec	%RSD	%Rec	%RSD
Heroin-d9	92.6	46.3	82.3	21.8	73.4	17.9	95.6	28.5	70.5	6.5	73.3	22.0
Fentanyl-d5	86.5	20.4	26.0	5.4	37.5	1.9	83.0	15.3	88.0	9.6	54.5	11.7
Carfentanil-d5	86.0	13.2	54.5	1.3	62.0	0.0	91.5	7.0	97.0	2.9	76.5	10.2
Heroin	77.2	18.7	50.0	4.7	11.8	13.2	88.7	24.5	64.1	12.6	60.7	18.9
Remifentanil	72.3	3.4	46.5	9.1	10.8	36.2	73.8	0.5	72.0	5.9	57.8	12.9
Acetylfentanyl	64.0	4.4	11.5	6.1	3.5	60.6	42.0	0.0	52.5	1.3	28.0	20.2
Fentanyl	74.0	5.7	16.5	12.9	5.0	56.6	52.5	9.4	68.0	4.2	37.5	17.0
Carfentanil	64.0	11.0	32.0	8.8	7.5	47.1	59.0	12.0	69.0	10.2	49.0	11.5
Sulfentanil	77.5	6.4	63.0	2.2	8.5	58.2	82.5	6.0	78.0	7.3	66.0	10.7
Alfentanil	76.0	3.7	70.5	3.0	10.5	47.1	82.5	9.4	81.0	8.7	71.5	8.9

Note: n=3 study, 1 ng/wipe spike

Figure 1. Examples of MRM Chromatograms

Fentanyl and Fentanyl-d5

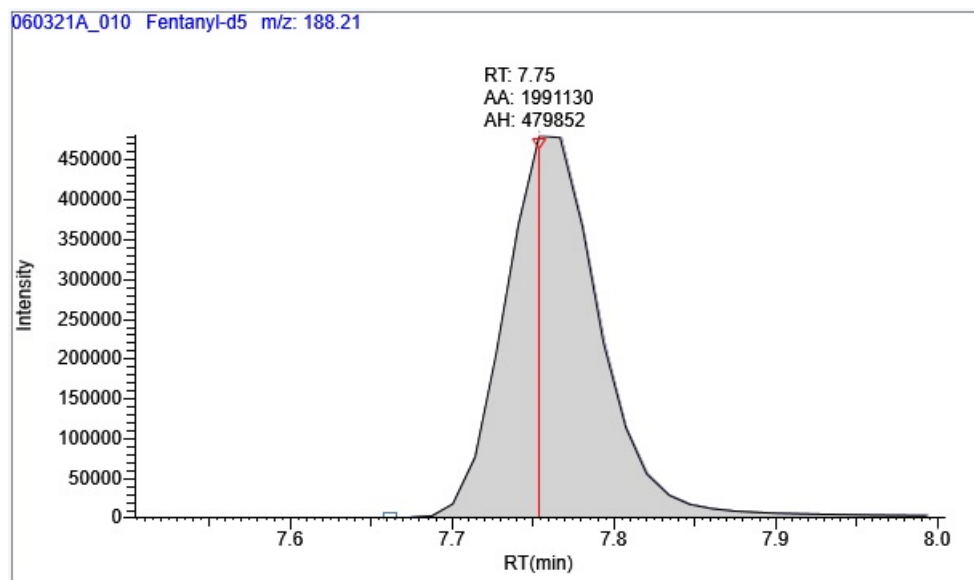
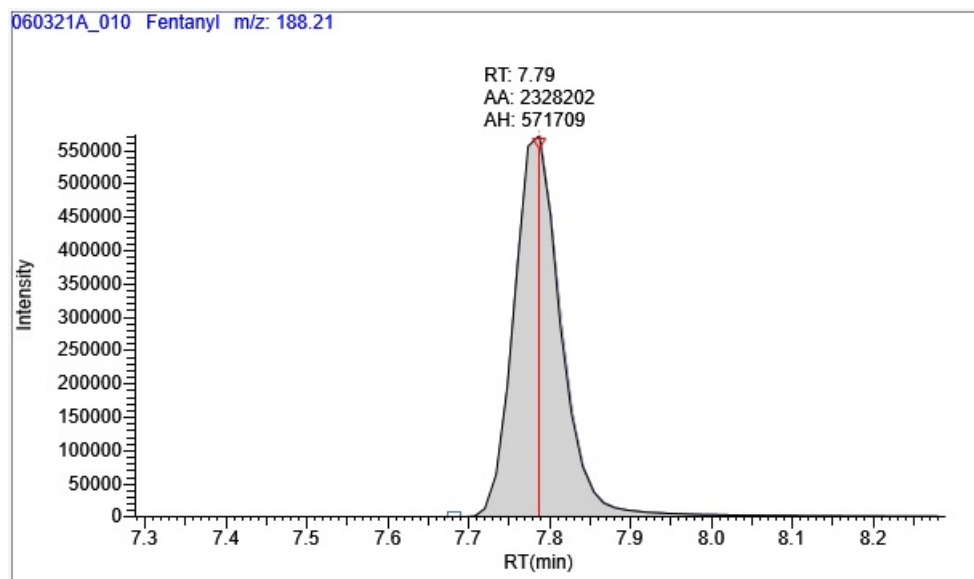
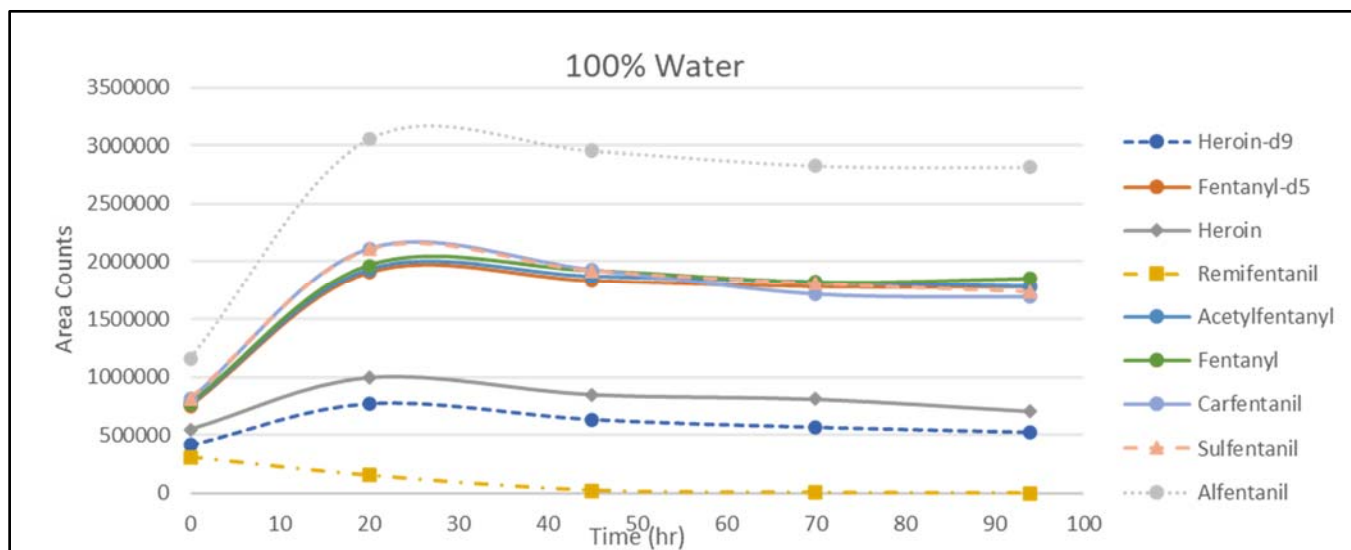


Figure 2. Degradation of opiate analogues in water



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