EPA/600/R-15/258 | October 2015 | www2.epa.gov/research

United States Environmental Protection Agency

Extraction and Analysis of Lewisite 1, by its Degradation Products, Using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

STANDARD OPERATING PROCEDURE

REVISION 1

Office of Research and Development
National Homeland Security Research Center

DISCLAIMER

The U.S. Environmental Protection Agency through its Office of Research and Development funded and managed the research described here under contract DE-AC52-07NA27344 to U.S. Department of Energy by Lawrence Livermore National Laboratory. It has been subjected to the Agency's review and has been approved for publication. Note that approval does not signify that the contents necessarily reflect the views of the Agency. Mention of trade names, products, or services does not convey official EPA approval, endorsement, or recommendation.

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ACKNOWLEDGMENTS

We would like to acknowledge the following individuals and organization for their contributions towards the development and/or review of this method.

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Office of Research and Development, National Homeland Security Research Center
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Romy Campisano

Lawrence Livermore National Laboratory (LLNL)
Forensic Science Center
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EXTRACTION AND ANALYSIS OF LEWISITE 1, BY ITS DEGRADATION PRODUCTS, USING LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC-MS/MS)

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LIST OF ACRONYMS AND ABBREVIATIONS

ACN	Acetonitrile
AS	Analyte Stock Standard (Solution)
ATL	Analytical Target Level
CAL	Calibration Standard
CAS®	Chemical Abstracts Service
CCV	Continuing Calibration Verification
CID	Collisionally Induced Dissociation
CVAA	Chlorovinyl Arsonous Acid
CVAOA	Chlorovinyl Arsonic Acid
DQO	Data Quality Objective
EPA	U.S. Environmental Protection Agency
ESI	(+) Electrospray Ionization in Positive Mode
GC-MS	Gas Chromatography Coupled with Mass Spectrometry
IDC	Initial Demonstration of Capability
IDL	Instrument Detection Limit
L1	Lewisite 1
LC	Liquid Chromatography
LC-MS/MS	Liquid Chromatography Coupled with Tandem Mass Spectrometry
LFMS	Laboratory Fortified Matrix Spike
LFMSD	Laboratory Fortified Matrix Spike Duplicate
LMB	Laboratory Method Blank
MeOH	Methanol
MDL	Method Detection Limit
MRL	Minimum Reporting Limit
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometer(try)
MS/MS	Tandem Mass Spectrometry
NE	Nebraska Soil
NHSRC	National Homeland Security Research Center
NIST	National Institute of Standards and Technology
OSHA	Occupational Safety and Health Administration
PAA	Phenyl Arsonous Acid
PAOA	Phenyl Arsonic Acid
PTFE	Polytetrafluoroethylene
QC	Quality Control
r^2	Coefficient of Determination
REC	Percent Recovery
RL	Reporting Limit
RPD	Relative Percent Difference
RPM	Revolutions per Minute
SAM	Selected Analytical Methods for Environmental Remediation and Recovery
SD	Standard Deviation (of replicate analyses)
SDS	Safety Data Sheet
SOP	Standard Operating Procedure

S/N	Signal to Noise
SS	Surrogate Standard
SSS	Stock Standard Solution
TOC	Total Organic Carbon
VOA	Volatile Organic Analysis
X	Average Percent Recovery

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1. INTRODUCTION

The U.S. Environmental Protection Agency (EPA) is responsible for developing tools and methodologies that will enable the rapid characterization of indoor and outdoor areas and of water systems following a deliberate/accidental release or a natural disaster. EPA's National Homeland Security Research Center (NHRSC) published *Selected Analytical Methods for Environmental Remediation and Recovery (SAM)* (1), which is a compendium of methods that informs sample collection and analysis during the response to an all-hazards incident. Lewisite is a dangerous vesicant, which can break down into degradation products sufficiently persistent and toxic to be of interest during site remediation after a release. If an incident were to occur, versatile analytical procedures are needed to detect Lewisite (and its degradation products) and to determine the spread and concentration of both in contaminated areas. For the purpose of identifying target analytes described within this procedure, Lewisite refers to Lewisite 1 and was analyzed as Lewisite 1 and its corresponding degradation products. This Standard Operating Procedure (SOP) addresses Lewisite degradation products known as chlorovinyl arsonous acid (CVAA) and chlorovinyl arsonic acid (CVAOA) in soil, wipe extracts, and water. The samples were extracted from the corresponding matrix types and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). LC-MS/MS affords laboratories an enhanced capability to analyze specific environmental matrices for chemical warfare agent degradation products while avoiding complications that may arise from a derivatization process commonly used for gas chromatography-mass spectrometry (GC-MS) analysis. The derivatization process used for GC-MS analysis is more involved and complicated because the derivatized Lewisite product can be problematic when analyzing for Lewisite. For example, GC-MS columns deteriorate rapidly in the presence of Lewisite, making the analysis technique impracticable. LC-MS/MS analysis is a straightforward process where many of the complications associated with GC-MS analysis are resolved. Data corresponding to the analysis of the Lewisite 1 (L1) degradation products by LC-MS/MS are limited in the literature for the matrices investigated. Because Lewisite 1 degradation products are unique, and neither the parent or degradation products exist in the environment, these compounds are unambiguous indicators for Lewisite 1. Preliminary testing to include other Lewisite byproducts, such as Lewisite Oxide, were included but are not presented in this protocol. Further testing is needed to include all degradation products associated with Lewisite, such as Lewisite Oxide, to ensure that sample analyses do not result in false negatives for Lewisite detection.

2. SCOPE AND APPLICATION

This method has been developed to address the analysis of Lewisite 1 degradation products in environmental matrices (water, soil, and wipes) by LC-MS/MS. Because Lewisite 1 hydrolyzes quickly in the environment to CVAA and then oxidizes more slowly to CVAOA, the standard operating procedure that has been developed extracts Lewisite 1 degradation products from the matrices of interest and converts them to CVAOA prior to analysis. The presence of CVAOA and/or CVAA is indicative of Lewisite 1 contamination, as there are no known natural sources for these compounds. Phenyl arsonous acid (PAA), which can be oxidized to phenyl arsonic acid (PAOA), is used as a surrogate to show that both extraction and oxidation processes have been successfully implemented. Lewisite composition typically consists of Lewisite 1, 2, and 3 (90%, 9%, and 1% composition, respectively). Since Lewisite 1 is the major product, only Lewisite 1 was studied. While not tested by this method, a similar analytical strategy should also be applicable to the analysis of Lewisite 2 and Lewisite 3. The following analytes have been determined using this procedure:

Analyte	**CAS Registry Number®**
Lewisite I (L1)	541-25-3
Chlorovinyl arsonous acid (CVAA)	85090-33-1
Chlorovinyl arsonic acid (CVAOA)	64038-44-4

The chemical structures of the analytes and surrogate compounds for this method are presented in Figure 1.

The protocol was developed for the conversion of Lewisite 1 to its degradation products by oxidation in water, wipe, and soil extracts and analyzed by LC-MS/MS. Because the degradation products can be formed only when Lewisite 1 degrades, their presence is consistent with Lewisite 1 being used, thus, indirect detection for Lewisite 1.

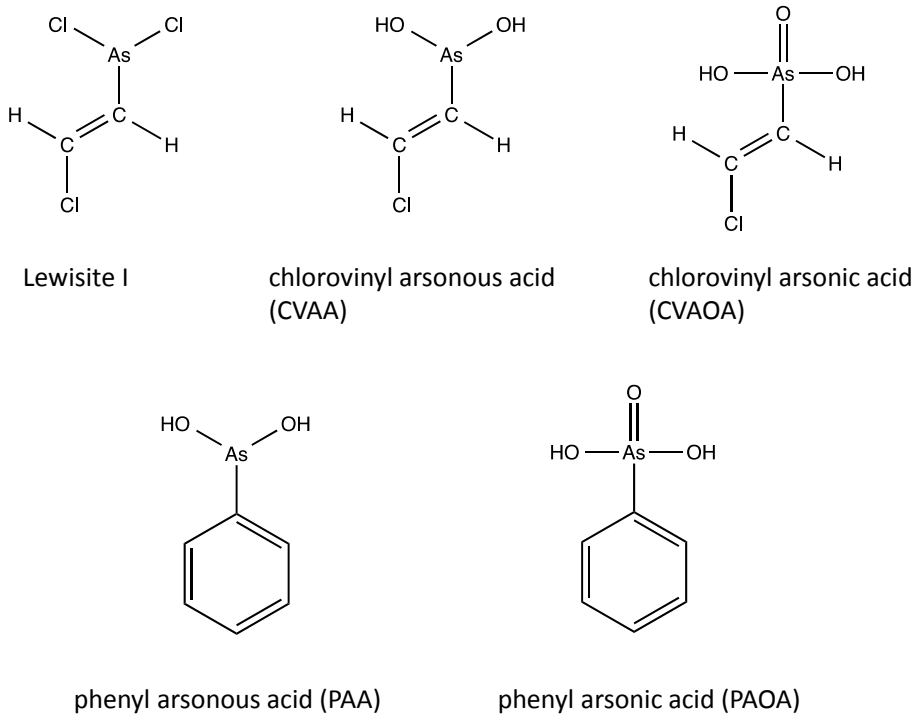

Figure 1. Chemical structures of analytes and surrogates.

2.1. The method detection limit (MDL) metrics are presented using EPA conventions (2-3). The detection limit is defined as the statistically calculated minimum concentration that can be measured with 99% confidence that the reported value is greater than zero (4). The MDL is compound-dependent and reliant on sample preparation, sample matrix, concentration of agent in the samples used for MDL determinations, and instrument performance. MDL studies are performed as an initial demonstration of capability (IDC) and ongoing demonstration of capability to perform the procedure, including changes in instrumentation and operating conditions. These studies evaluate whether the reporting limits (RLs) and calibration standard (CAL) concentrations are appropriate.

2.2. This procedure is intended for use by analysts skilled in the operation of LC-MS/MS instrumentation and the interpretation of the associated data. Due to the inherent complexities of LC-MS/MS analysis, including the need to relate sample characteristics to analytical performance, laboratories should update their initial estimates of performance and should strive to tighten their quality control limits as more experience is gained with this particular procedure.

2.3. METHOD FLEXIBILITY

Many variants of liquid chromatography (LC) and Tandem Mass Spectrometry (MS/MS) technology are currently in operation. In addition, variability exists in the sources of investigated matrices, including wipe materials and composition, soil types, and water sources. This procedure was developed using an Orbitrap LC-MS/MS system, with optimized LC conditions. The procedure has been verified using only the specified equipment and conditions. Other types of LC-MS/MS instrumentation, LC and/or electrospray ionization (ESI) in positive mode MS/MS conditions, sample collection and processing steps, and materials can be used for analysis as long as similar performance is demonstrated, and the quality control measures outlined in this report are implemented.

3. SUMMARY OF METHOD

3.1. The degradation products of Lewisite 1, CVAA and CVAOA, are extracted from soil and wipes using aqueous-based solvents, oxidized to CVAOA using hydrogen peroxide, and detected by LC-MS/MS. It is assumed that any residual Lewisite 1 in samples would be hydrolyzed and oxidized to CVAOA during the extraction process (6). This is an acceptable analytical strategy because there are no known environmental sources for the Lewisite 1 degradation products (i.e., the presence of these compounds is an unambiguous indicator for the presence of Lewisite 1). LC approaches offer the opportunity to detect the Lewisite 1 hydrolysis and oxidation products directly (5, 6), without the added complication of a derivatization step, which is required for gas chromatography-mass spectrometry (GC-MS) analyses (7, 8). The expected rapid hydrolysis of Lewisite 1 in aqueous LC mobile phases precludes the direct detection of Lewisite 1 itself, but species such as CVAA and CVAOA are readily detectable by LC-MS/MS. (See section 13.1.1, for data supporting conversion of Lewisite to its degradation products.) The use of LC-MS/MS provides analytical specificity, by which degradation products of Lewisite 1 can be more easily measured in the presence of interfering compounds. In addition, LC-MS/MS is expected to provide better instrument detection limits (IDLs) (e.g., LC-MS/MS IDL of ~0.01 ng/µL versus an expected GC-MS IDL of ~0.5 ng/µL (7)).

3.2. The target analyte is separated chromatographically and identified by retention time. Comparison of the sample primary multiple reaction monitoring (MRM) transition to the known standard MRM transition from reference spectra under identical LC-MS/MS conditions. Stock standard solutions, made by weighing known masses of analytes/surrogates into known volumes of solvent, are diluted to concentrations appropriate to construct (linear) calibration curves. Then, the concentrations of analytes/surrogates present in the samples are calculated considering the responses of the calibration standards (*i.e.,* the concentration of each analyte is determined by the instrumentation software using external calibration). Surrogate analytes are added to samples to monitor extraction efficiency of the method analytes during the extraction process.

4. DEFINITIONS

4.1. ANALYSIS BATCH – A set of samples analyzed on the same instrument within a 24-hour period and including no more than 20 field samples, beginning and ending with the analysis of the appropriate continuing calibration verification (CCV) standards. Additional CCVs may be required depending on the number of samples (excluding quality control (QC) samples) in the analysis batch and/or the number of field samples.

4.2. CALIBRATION STANDARD (CAL) – A solution prepared from the analyte stock standard solution (AS) and the surrogate/internal standard(s). The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

4.3. COLLISIONALLY INDUCED DISSOCIATION (CID) – The process of converting the translational energy of the precursor ion into internal energy by collisions with neutral gas molecules to bring about dissociation into product ions.

4.4. CONTINUING CALIBRATION VERIFICATION (CCV) – A calibration standard containing the method analytes and surrogate standard(s). The CCV is analyzed periodically to verify the accuracy of the existing calibration for those analytes at or near the mid-level concentrations. Low calibration concentrations can be added, in addition to mid-level concentrations, for further accuracy, but are not required.

4.5. EXTRACTION BATCH – A set of up to twenty field samples (excluding QC samples) extracted together using the same solvents and surrogate(s).

4.6. LABORATORY FORTIFIED MATRIX SPIKE (LFMS) – A field sample to which known quantities of the method analytes are added in the laboratory. The laboratory fortified matrix spike (LFMS) is processed and analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate sample.

4.7. LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (LFMSD) – A laboratory fortified sample matrix duplicate (LFMSD) of the field sample used to prepare the LFMS. The LFMSD is fortified and analyzed identically to the LFMS. The LFMSD is used to assess method precision when the observed concentrations of method analytes are low.

4.8. LABORATORY METHOD BLANK (LMB) – A blank matrix that is treated exactly the same as a sample including exposure to all glassware, equipment, solvents and reagents, and surrogate standards that are used in the analysis batch. The laboratory method blank (LMB) is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

4.9. METHOD DETECTION LIMIT (MDL) – The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero.

4.10. MINIMUM REPORTING LEVEL (MRL) – The minimum concentration that can be reported as a quantitated value for a method analyte in a sample following analysis. This defined concentration can be no lower than the concentration of the lowest calibration standard for that analyte and can be used only if acceptable QC criteria for this standard are met.

4.11. PRECURSOR ION – For the purpose of this method, the precursor ion is the protonated molecule ($[M+H]^+$) or adduct ion of the method analyte. In MS/MS, the precursor ion is mass-selected and fragmented by collisionally induced dissociation (CID) to produce distinctive product ions of lower mass.

4.12. PRODUCT ION – For the purpose of this method, a product ion is one of the fragment ions produced in MS/MS by CID of the precursor ion.

4.13. SAFETY DATA SHEET (SDS) – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

4.14. SURROGATE STANDARD (SS) – A pure chemical(s) added to a standard solution in a known amount(s) and used to measure the relative response of other method analytes that are components of the same solution. The surrogate standard must be a chemical that is structurally similar to the method analytes, has no potential to be present in samples, and is not a method analyte.

4.15. STOCK STANDARD SOLUTION (SSS) – A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

5. INTERFERENCES

Procedural interferences can be caused by contaminants in solvents, reagents, glassware and other apparatus that lead to discrete artifacts or elevated baselines in the selected ion current profiles. All of these materials must routinely be demonstrated to be free from interferences by analyzing Laboratory Method Blanks (LMBs) under the same conditions as the samples. Subtraction of blank values from sample results is not performed.

5.1. All reagents and solvents should be of pesticide grade purity or higher to minimize interference problems. All glassware should be cleaned and demonstrated to be free from interferences.

5.2. Matrix interferences may be caused by contaminants from the sample matrix, sampling devices or storage containers. The extent of matrix interferences will vary considerably from sample source to sample source, depending upon variations in the sample matrix. Matrix interferences and contaminants are likely to be present and may have an effect on the recoveries for the analytical procedure. These interferences lead to elevated baselines and artifacts that may be interpreted as false positives. Wipes were used as received and analyzed to ensure that no interferences were present. Any materials containing interferences with the analytes of interest were not used.

5.3. Matrix effects are known phenomena of electrospray ionization-mass spectrometry (ESI-MS) techniques, especially for coeluting compounds. Managing the unpredictable ionization 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, high background and improper ion ratios.

6. HEALTH AND SAFETY

The toxicity and carcinogenicity of each reagent used in this method have not been precisely defined. However, each chemical compound was treated as a health hazard. Lewisite 1 and its degradation products are vesicants. Exposure to these chemicals should be reduced to the lowest possible level and proper protective equipment should be worn for skin, eyes, etc. Each laboratory is responsible for maintaining an awareness of Occupational Safety and Health Administration (OSHA) regulations regarding the safe handling of chemicals used in this method. A reference file of safety data sheets (SDSs) that address the safe handling of the chemicals should be made available to all personnel involved in the chemical analyses or subject to potential exposure. Additional references are available (9-12).

Personnel shall wear personal protective equipment, which includes nitrile gloves, laboratory coats, and safety glasses with side shields or goggles. Nitrile gloves should be changed frequently, between each operation or after known or suspected contact with hazardous material. All work shall be performed in chemical fume hoods. Sample manipulations should be performed in secondary containment (e.g., phototrays) to allow quick cleanup in the event of a spill. Vial trays should be used to hold vials and minimize the potential for tipping.

Hydrogen peroxide (H_2O_2 (30%)) is a strong oxidizer; it is corrosive, explosive, and can cause severe burns. Contents of a bottle may develop pressure upon prolonged storage. Refrigerated storage is recommended.

Manage wastes from Lewisite extraction and analysis separately from all other laboratory wastes. Pressure may develop in waste containers and require periodic venting. Do not mix wastes with bleach or any material that might react with H_2O_2, if waste excluding Lewisite was used.

7. EQUIPMENT AND SUPPLIES

References to specific brands of equipment and catalog numbers are provided solely as examples and do not constitute an endorsement of the use of such products or suppliers. Glassware, reagents, supplies, equipment, and settings other than those listed in this report may be employed, provided that method performance has been demonstrated and documented for the intended application. Analyses may be performed with any system capable of performing LC-MS/MS as long as performance criteria described within are met. Tested matrix types are listed in Table 1. (All Tables are found at the end of the document.)

7.1 LC-MS/MS INSTRUMENT

7.1.1 LIQUID CHROMATOGRAPHY (LC) SYSTEM - An analytical system complete with a programmable temperature and solvent delivery system (Surveyor LC, ThermoFisher Scientific, Waltham, MA) and all required accessories including syringes, solvent degasser, and autosampler.

7.1.2 ANALYTICAL COLUMN - Atlantis® T3, 100 Å, 3 µm particle size, 2.1 mm x 150 mm (Waters, Milford, MA, Catalog # 186003719; reverse-phase, C18), or equivalent.

7.1.3 TANDEM MASS SPECTROMETER (MS/MS) SYSTEM – A ThermoScientific LTQ Orbitrap (ThermoFisher Scientific, Waltham, MA) mass spectrometer was used in the development of this method. The LC-MS/MS should be tuned and calibrated, as needed, per the vendor's instructions and specifications. A mass spectrometer capable of MRM analysis with the capability to obtain at least 10 scans over a peak with adequate sensitivity is required.

7.1.4 DATA SYSTEM – The LC-MS/MS should be controlled by software that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program. Xcaliber 2.0 SR2 (or similar software) is used for all quantitative analysis for data generated from the LC-MS unit.

7.2 EXTRACTION DEVICE

7.2.1 A shaker table was used for the preparation of soil and wipes samples (Glas-Col® Digital Pulse Mixer, model no. 099ADPM12, Terre Haute, IN; or equivalent). For water samples, a vortex mixer (VWR Mini Vortexer, product. no. 58816-121, or equivalent) was used.

7.3 GLASSWARE AND MISCELLANEOUS SUPPLIES

7.3.1 AUTOSAMPLER VIALS – 2-mL, screw-cap, autosampler vials (Agilent, Santa Clara, CA, product no. 5183-2083), or equivalent.

7.3.2 AUTO PIPETTES – 10.0 mL, 1000 µL, 100 µL and 10 µL ± 1% accuracy.

7.3.3 DESOLVATION GAS – Nitrogen gas generator or equivalent nitrogen gas supply (e.g., nitrogen from liquid nitrogen boil-off) aids in the generation of an aerosol of the ESI liquid spray and should meet or exceed instrument manufacturer's specifications.

7.3.4 COLLISION GAS – Helium, nitrogen, or argon gas used in the collision cell in MS/MS instruments and should meet or exceed instrument manufacturer's specifications.

7.3.5 ANALYTICAL BALANCE – accurate to 0.1 mg; reference weights traceable to Class S or S-1 weights.

7.3.6 THERMOMETER – National Institute of Standards and Technology (NIST)-traceable thermometer.

7.3.7 STANDARD SOLUTION FLASKS – Class A volumetric glassware.

7.3.8 WIPES – 3 in. x 3 in. gauze wipes, 12-ply (Kendall-Curity, product no. 1903), or equivalent. Use as received.

7.3.9 SAMPLE COLLECTION CONTAINERS – 20- or 40-mL volatile organic analysis (VOA) vials with Polytetrafluoroethylene (PTFE) screw caps (Environmental Sampling Supply, product no. 0040-0310-PC, San Leandro, CA), or equivalent.

7.3.10 SAND – purified (JT Baker, Inc., product no. 3382-05; CAS no. 14808-60-7).

7.3.11 VIRGINIA SOIL – (VA soil, obtained from National Exposure Research Laboratory, US EPA, Las Vegas, NV), with composition of 64.5% sand, 28% silt, 7.5% clay, 2.6% Total Organic Carbon (TOC), and pH 4.1 when measured in a 1:1 soil: water mix.

7.3.12 NEBRASKA AGLANDS AP SOIL – (NE soil, obtained from National Exposure Research Laboratory, US EPA, Las Vegas, NV), with composition of 5.1% sand, 57.5% silt, 31.7% clay, 1.9% TOC, and pH 5.5 when measured in a 1:1 soil: water mix.

7.3.13 GEORGIA BT2 SOIL – (GA soil, obtained from National Exposure Research Laboratory, US EPA, Las Vegas, NV), with composition of 46% sand, 22% silt, 32% clay, 0.2% TOC, and pH 5.0 when measured in 1:1 soil: water mix.

7.3.14 GLASS BEADS – glass beads ~ 5 mm diameter (Sigma-Aldrich, product no. 18406) or equivalent. Use as received.

7.3.15 pH INDICATING PAPER – pH range 0-14 (Sigma-Aldrich product no. WHA10362000) or equivalent.

7.3.16 CENTRIFUGE – (Fisher Scientific AccuSpin™ 400 with rotor, product no. 75005195, Fischer Scientific, Pittsburgh, PA) or equivalent.

8 REAGENTS AND STANDARDS

8.1 REAGENTS AND STANDARDS

Laboratories should follow standard QC procedures to determine when the standards should be replaced. Label all standards and verify the correct grade of solvents. Reagent-grade chemicals should be used, unless otherwise indicated. Traceability of standards is established by the manufacturer's specifications provided at time of purchase.

8.1.1 SOLVENTS, REAGENTS and GASES – Water (resistivity of 18.2 MΩ cm at 25 °C, Millipore Milli-Q®, Advantage® A-10 purification system, Millipore, Billerica,

MA) or equivalent, demonstrated to be free of analytes and interferences. Hydrogen peroxide (H_2O_2), 30 wt% in H_2O, Reagent Grade (Sigma-Aldrich, product no. 216763-100mL). Hydrochloric acid (HCl), 10 mmol, diluted from 5N HCl (JT Baker, Baker Analyzed® Reagent, product no. 5618-02) by adding 1.5 mL of 5N HCl to 750 mL laboratory-grade water. Dichloromethane (Fluka brand from Sigma-Aldrich, product no. 34488). Methanol (MeOH), Fluka brand, LC-MS Chromasolv®, ≥99.9% (Sigma-Aldrich product number 34966-4L). Acetonitrile (ACN) (Optima™ LC-MS grade, Fisher Scientific, product number A955-212). Formic acid (Optima™ LC-MS grade, Fisher Scientific, product number A117-50). Nitrogen is used for the generation of aerosol of the ESI liquid spray, and purity should meet instrument manufacturer's specifications. Helium is used as the collision gas in Orbitrap MS/MS applications, and purity should meet instrument manufacturer's specifications. If nitrogen or argon is to be used as a collision gas (*e.g.,* in quadrupole-based tandem mass spectrometry systems), its purity should meet instrument manufacturer's specifications.

8.1.2 MOBILE PHASE A – Solution A (Table 2) consisted of LC-MS grade water with 0.1% formic acid. To prepare 0.5 L, add 0.5 mL of formic acid and dilute to 0.5 L mark with water. This solvent system is prone to some microbial growth and should be replaced regularly.

8.1.3 MOBILE PHASE B – Solution B (Table 2) was comprised of acetonitrile with 0.1% formic acid. To prepare 0.5 L, add 0.5 mL of formic acid and dilute to 0.5 L mark with acetonitrile.

8.1.4 TARGET ANALYTES – Lewisite 1 (L1) was synthesized in-house. The Lewisite purity, generated from the synthesis procedure, was 91% purity (determined by NMR and GC/MS analysis). A Lewisite 1 stock solution (2.03 mg/mL) was first diluted in dichloromethane, fresh from a new bottle, and stored at -20 °C. CVAA was made by diluting 246 µL of 2.03 mg/mL L1 stock solution in dichloromethane to 15.00 mL laboratory-grade water for a 33.3 ng/µL stock solution. LC-MS analysis confirmed that L1 was quantitatively converted to CVAA, and L1 was not present.

8.1.5 SURROGATE ANALYTES – Phenyl arsonous acid (PAA) was made from phenylarsine oxide by hydrolysis. Phenylarsine oxide was obtained from Sigma-Aldrich (product no. 402494, CAS no. 637-03-6; note: current Sigma-Aldrich product no. P3075-1G). Direct dissolution of phenylarsine oxide in water is difficult and requires preparation of an initial concentrated solution in acetonitrile.

8.2 STANDARD SOLUTIONS

Stock standards and all subsequent solutions should be replaced when analyzed solution concentrations deviate more than ± 20% from the prepared concentration. Standards are stored protected from light (amber flasks) and at - 20 °C (± 2 °C). Laboratories should utilize QC practices to determine when standards should be replaced.

8.2.1 SURROGATE STOCK STANDARD SOLUTION (Surrogate SSS)

While phenylarsine oxide was easier to dissolve in dimethylformamide, this solvent was found to co-elute with the analytes of interest, causing ionization suppression effects during analysis by LC-MS/MS. For this reason, initial phenylarsine oxide solutions were made in acetonitrile. A stock solution of 1.00 mg/mL PAA was made in acetonitrile. It was necessary to sonicate this solution for several hours and to let the solution stand in the hood overnight to obtain dissolution of the PAA. Once dissolved, phenylarsine oxide was kept at ~ 4 °C and could be used for at least two months (i.e., no degradation/oxidation was observed over a two-month period). Subsequent dilutions of phenylarsine oxide were made in laboratory grade water (which generated the surrogate compound PAA by hydrolysis) immediately prior to conducting experiments.

8.2.2 ANALYTE STOCK STANDARD SOLUTION (AS)

A 33.3 ng/µL CVAA stock solution was made by diluting 246 µL of 2.03 mg/mL Lewisite stock solution in dichloromethane to 15.00 mL using laboratory-grade water. A 10 ng/µL CVAA stock solution was made by diluting 1.00 mL of 33.3 ng/µL CVAA to 3.33 mL with laboratory-grade water.

8.2.3 CALIBRATION STANDARD SOLUTION (CAL)

8.2.3.1 Initial Calibration Standards

The calibration range of the LC-MS/MS is defined by analyzing standard solutions representing a range of concentrations of the analytes of interest. Six calibration levels were used to generate a linear calibration curve for CVAOA and the correlation coefficient is used to evaluate the quality of the calibration curve (e.g., r^2>0.99 indicates a good fit of the calibration curve with the data points generated by the analysis of the standards). All calibration standards are prepared in a solvent corresponding to the solvent system used for extraction of the relevant matrix (see footnotes of Table 3). The presence of H_2O_2 in the solvent is expected to oxidize all analytes (e.g., only CVAOA and PAOA are present, and no CVAA or PAA remains). To confirm that all analytes were oxidized, CVAA and PAA were monitored but were not detected at tested levels during this investigation. Recommended initial calibration levels are listed in Table 3.

8.2.3.2 Continuing Calibration Verification Standards (CCV)

The continuing calibration verification (CCV) is used to check the continued validity of the initial calibration. The CCV is a mid-range calibration standard and the acceptance criteria is ±35% of the expected value(s) for all analytes. If the CCV does not meet the acceptance criteria, it may be reanalyzed. If after reanalysis the ±35% criteria for the CCV are not met, a new calibration curve must be prepared and used. The CCVs consist of clean solvent that is fortified with a specific concentration of CVAOA and PAOA. A recommended CCV consists of 0.1 ng/µL CVAOA and 0.1 ng/µL PAOA. A CCV check should be performed at a minimum frequency of once every 8 hours, preferably after every 10 field samples.

8.2.3.3 Surrogate Standard

PAA is used as a surrogate standard to provide assurance that the extraction and analysis procedure is in control. PAA is oxidized to PAOA; exhibiting chemical behavior similar to and mass spectral features of CVAA/CVAOA. It is recommended to adjust the concentration of PAA such that its concentration in the sample extract to be analyzed is 0.1 ng/µL (detected as PAOA), assuming 100% recovery. This concentration is comparable to the concentration of the PAOA in recommended CCVs. Alternatively, one could lower the concentration of PAA to demonstrate that a specified low limit of detection is achieved.

9 SAMPLE COLLECTION, PRESERVATION AND STORAGE

The following sections describe procedures for extracting Lewisite 1 degradation products from three matrices. Per the sample holding time study, it is recommended that water and wipe samples be extracted and analyzed within two weeks and that soil samples should be extracted and analyzed as soon as possible after collection (see Section 9.2).

9.1 SAMPLE COLLECTION

9.1.1 VOA vials with PTFE screw caps were used for sample collection for each matrix type. Other containers may be used to collect samples as long as they are tested and verified to ensure they do not contain any interfering compounds.

9.1.2 WATER EXTRACTION

9.1.2.1.1 From the original (VOA) sample container, sample, with a pipette, a drop of water and test its pH with indicating paper. Record the pH. While this method does not require the adjustment of pH to a specific value, pH is recorded as good laboratory practice and to identify samples that may be outside the normal range of pH values associated with natural waters. As this method is applied to more matrices, sample pH might be found to be an important parameter.

9.1.2.2 Measure 500 µL water sample into 2 mL autosampler vial. Spike each sample with 10 µL of 10 ng/µL PAA solution (yielding a 0.1 ng/ µL PAOA in sample extract after dilution). Mix thoroughly. Add 500 µL of 30% H_2O_2 to each sample. Cap and mix solution. Analyze as soon as possible by LC-MS/MS using the conditions listed in Tables 2, 3, and 4.

9.1.2.3 *Preparation of blank sample(s):* Place laboratory-grade water into a VOA vial used to collect the water samples. Treat as a water sample, as described in sections 9.1.2.1 and 9.1.2.2.

9.1.2.4 *Preparation of Matrix Spike/Matrix Spike Duplicate sample(s):* Measure 19.6 mL laboratory-grade water into a VOA vial. Spike with 120 µL of 33.3 µg/mL CVAA solution (which will yield a final extract concentration of 0.1 ng/µL CVAOA). Use the vortex mixer to ensure that the CVAA solution is mixed thoroughly into the water. Treat as a water sample, as described in sections 9.1.2.1 and 9.1.2.2.

9.1.3 SOIL EXTRACTION

9.1.3.1 The sample collection (VOA) container contains 5.00 g of soil/sand. Add 200 µL of a solution containing 10 ng/µL PAA to each sample (which, assuming 100% recovery, will yield an extract concentration of 0.1 ng/µL PAOA). Mix with vortex mixer for approximately 30 seconds to distribute the PAA. Add 5-10 glass beads, new from the bottle, to each sample. Add 10.0 mL 50/50 (v/v) 10 mM HCl /methanol to each sample and cap tightly. Place on shaker table for 30 minutes at 600 revolutions per minute (rpm) (horizontal orientation). Using pH paper, record the approximate pH of the sample extract. Allow the sample extracts to settle by gravity so that 0.5 mL of extract may be collected; it may take ~1 hour for the extracts to settle. If needed, centrifuge the sample extracts for 5 minutes at 500 rpm. (Note: Do not exceed 500 rpm or the vial might break). Transfer 500 µL of sample extract into an autosampler vial. Add 500 µL 30% H_2O_2 to each sample. Cap and mix solution. Analyze as soon as possible by LC-MS/MS using the conditions listed in Tables 2, 3, and 4.

9.1.3.2 *Preparation of the Blank Sample(s):* Weigh 5.00 g of clean sand/soil into a 40-mL VOA vial and cap. Treat as a soil sample described in section 9.1.3.1.

9.1.3.3 *Preparation of the Matrix Spike/Matrix Spike Duplicate Sample(s):* Weigh 5.00 g of clean sand/soil into a 40-mL VOA vial. Spike with CVAA and mix with a vortex mixer for ~30 seconds to distribute the solution. Recommended spike amount is 2 µg CVAA (which, assuming 100% recovery, will yield an extract concentration of 0.1 ng/µL CVAOA). Treat as a soil sample described in section 9.1.3.1.

9.1.4 WIPE EXTRACTION

9.1.4.1 The sample collection (VOA) container contains the wipe samples. Add 400 µL of a solution containing 10 ng/µL PAA (surrogate) to each sample (assuming 100% recovery, final concentration will be 0.1 ng/µL PAOA in the sample extract). Add 20 mL of 10 mM HCl to each vial. Extract for 30 minutes on shaker table (horizontal orientation) at 600 rpm. Transfer 500 µL sample extract to autosampler vial. Add 500 µL 30% H_2O_2 to each

sample. Cap and mix. Analyze as soon as possible by LC-MS/MS using the conditions listed in Tables 2, 3, and 4.

9.1.4.2 *Preparation of the Blank Sample(s):* Place unused wipe into a 40-mL vial with PTFE screw cap. Treat as a wipe sample, as described in section 9.1.4.1.

9.1.4.3 *Preparation of the Matrix Spike/Matrix Spike Duplicate Sample(s):* Place unused wipe into a 40-mL VOA vial. Spike with 4 µg CVAA (which, assuming 100% recovery, will yield an extract concentration of 0.1 ng/µL CVAOA). Treat as a wipe sample, as described in in section 9.1.4.1.

9.2 **SAMPLE STORAGE AND HOLDING TIMES**

9.1.5 Samples should be extracted as soon as possible after collection but water and wipe extract samples must be extracted within 14 days of collection. Soil samples should be extracted immediately. Samples not immediately analyzed from a particular site should be carefully characterized to ensure there is no interaction to cause interferences or degradation of the analytes. Detailed information is described in Section 14.3.

10 QUALITY CONTROL

10.1 Quality control (QC) requirements include the performance of an initial demonstration of capability (IDC) and ongoing QC requirements that must be met to generate data of acceptable quality when preparing and analyzing samples. This section describes the QC parameters, their required frequencies and performance criteria. A Method Detection Limit (MDL) study (Tables 5 and 6, and Section 10.3) were performed to demonstrate laboratory capability. Laboratories are encouraged to institute additional QC practices to meet their specific needs.

10.2 INITIAL DEMONSTRATION OF CAPABILITY

The IDC must be performed successfully prior to the initiation of analysis of field samples. Prior to conducting an IDC, an acceptable initial calibration must be generated as outlined in Section 11.1.

10.2.1 INITIAL DEMONSTRATION OF LOW SYSTEM BACKGROUND

Any time new solvents, reagents, filters and autosampler vials are used, the LMB must be demonstrated to be reasonably free of contamination (i.e., that the criteria are met as stipulated in Section 10.4.1). The LMB is used to ensure that analytes of interest or other interferences are not present in the laboratory environment, the solvent, or the apparatus.

NOTE: Good laboratory practices indicate the use of a blank before and after analyzing a calibration curve for an instrument to ensure that no carryover will occur. If the required criteria are not met and samples were not free of contamination, then the source of the contamination should be identified and eliminated before the performance of any analysis.

10.2.2 MINIMUM REPORTING LEVEL (MRL)

Establish a target concentration for the minimum reporting limit (MRL) based on the intended use of the method. Establish an Initial Calibration (Section 11.1). The lowest calibration (CAL) standard used to establish the initial calibration must be at or below the MRL concentration. If the MRL concentration is too low, ongoing QC requirements may fail repeatedly, and the MRL must be determined again at a higher concentration. The MRL reported in this study is the lowest calibration level.

10.2.3 CONTINUING CALIBRATION VERIFICATION

The CCV is used to check the continued validity of the initial calibration. The CCV is a mid-range calibration standard and the acceptance criterion is ± 35% of the expected value(s) for all analytes. If the CCV does not meet the acceptance criteria, it may be reanalyzed. If after reanalysis the ± 35% criteria for the CCV are not met, a new calibration curve must be made and used. The CCVs consist of clean solvent that is fortified with a specific concentration of CVAOA and PAOA. A recommended CCV consists of 0.1 ng/µL CVAOA and 0.1 ng/µL PAOA. A CCV check should be done at a minimum frequency of once every 8 hours; preferably after every 10 field samples.

10.3 METHOD DETECTION LIMITS

The procedure for the determination of the laboratory detection and quantitation limits for the EPA approach follows 40 CFR Part 136, Appendix B. MDLs represent the minimum concentration at which there is a high degree of statistical confidence that, when the method reports that an analyte is present, that analyte is actually present (i.e., a low risk of false positives).

10.3.1 DETERMINATION OF LABORATORY INSTRUMENT DETECTION LIMITS

The laboratory instrument detection limit (IDL) can be used to establish an estimate of the initial spiking concentration used for determination of the MDL, although other approaches for determining the initial spiking concentration may be used. The laboratory IDL is determined for each analyte as a concentration that produced an average signal-to-noise (S/N) ratio in the range of 3:1 - 5:1 for at least three replicate injections. For example, successively lower concentrations of the analytes are injected until the S/N ratio is in the range of 3:1 – 5:1. Replicates are then injected at that target concentration to ensure that the average S/N of the replicates was within the 3:1 – 5:1 range. Note that since linearity of S/N ratio with increasing or decreasing concentration cannot be assumed, the concentrations determined via this procedure are necessarily approximate.

10.3.2 DETERMINATION OF LABORATORY METHOD DETECTION LIMIT

MDLs represent the optimal detection achieved by a laboratory in a matrix of interest. The analyte spiking solution, containing all target analytes, was added to

the matrix. The 40 CFR Part 136, Appendix B procedure is followed, particularly with regard to spike levels used. Replicate reference matrix samples are spiked at a level between 1-5 times the estimated detection level (e.g., suggested by the IDL procedure in 10.3.1). The resulting MDL must be within 10 times the spike level used, or the MDL determination would be repeated using a more appropriate spike level. Full method sample preparation procedures to prepare and analyze at least seven replicates of the spiked clean matrix of interest are used. Apply the following equation to the analytical results (Student's *t*-factor is dependent on the number of replicates used; the value 3.14 assumes seven replicates):

$$\mathbf{MDL = t_{(n-1,\ 1-\alpha\ =\ 0.99)} \times SD}$$

where

MDL = method detection limit,
$t_{(n-1,1-\alpha\ =\ 0.99)}$ = Student's *t* value for the 99% confidence level with n-1 degrees of freedom (for seven replicate determinations, the Student's t value is 3.143 at a 99% confidence level),
n = number of replicates, and
SD = standard deviation (SD) of replicate analyses.

Data for MDLs are shown in Table 5.

10.4 ONGOING QC REQUIREMENTS

10.4.1 LABORATORY METHOD BLANK

Method blanks are used to determine the background of each particular matrix. An LMB is prepared and analyzed with each extraction batch for confirmation that there are no background contaminants interfering with the identification or quantitation of the target analytes. If there is a contaminant within the retention time window preventing the determination of the target analyte, the source of the contamination should be determined and eliminated before processing samples. The method blanks undergo the same extraction procedure as authentic samples and are spiked with the surrogate standard; however, the method blanks do not contain Lewisite1/CVAA/CVAOA. One method blank is prepared for each set of samples. The maximum number of samples in a set is 20.

10.4.2 CONTINUING CALIBRATION VERIFICATION CHECK

A CCV check should be performed at a minimum frequency of once every 8 hours; preferably after every 10 field samples. CCVs should be within ± 35% of the expected value(s) for all analytes for the data to be considered valid. CCV values should be specified by the sample submitter's Data Quality Objectives (DQOs) or fulfill other QC requirements.

10.4.3 MATRIX SPIKE/LABORATORY FORTIFIED MATRIX SPIKE

A laboratory fortified matrix spike (LFMS) is analyzed to determine that spike

accuracy for a particular sample matrix is not adversely affected by chemical interactions between target analytes and experimental matrices (i.e., wipe materials, sand, etc.). If a variety of sample matrices are analyzed, performance should be established for each matrix or sample type.

10.4.4 When performing sample analyses, it is expected that LFMS and laboratory fortified matrix spike duplicate (LFMSD) samples will be analyzed. LFMS/LFMSDs are representative analyte-free environmental matrices that have been fortified with CVAA and PAA. These samples are taken through the extraction process to show that the method is capable of detecting the analytes of interest in the relevant matrices. LFMS and LFMSD samples should be prepared for each type of matrix. Records are maintained of the target compound spike analyses, and the average percent recovery (X) and the standard deviation (SD) are calculated. Analyte recoveries may exhibit bias for certain matrices. Acceptable recoveries are 50-150% if a low-level concentration near or at the MRL (within a factor of 3) is used. If the recovery does not fall within this range, check with a CCV or prepare a fresh AS solution for analysis. If the recovery of any analyte still falls outside the designated range and the laboratory performance for that analyte is shown to be in control in the CCVs, the recovery is judged to be matrix-biased. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

10.4.5 SURROGATE STANDARD

All samples (CCVs, LMBs, LFMSs, LFMSDs, and CAL standards) are spiked with surrogate standard spiking solution as described in Section 8.2. An average percent recovery of the surrogate compound and the standard deviation of the percent recovery (REC) are calculated and updated regularly.

10.4.6 MATRIX SPIKE DUPLICATE

10.4.6.1 Calculate the relative percent difference (RPD) for the LFMS and LFMSD using the equation:

$$RPD = \frac{|\,LFMS - LFMSD\,|}{(LFMS - LFMSD)/2} \times 100$$

RPDs for duplicate LFMSs should be ≤ 35% for each analyte. Greater variability may be observed when the matrix is fortified at analyte concentrations at or near the MRL (within a factor of two times the MRL concentration). LFMSs at these concentrations must have RPDs that are ≤ 50%. If the RPD of an analyte falls outside the designated range and the laboratory performance for the analyte is shown to be in control in the CCV and in the LMB, the precision is judged matrix-influenced. Report the result for the corresponding analyte in the unfortified sample as "suspect/matrix."

11 INSTRUMENT CALIBRATION AND STANDARDIZATION

All laboratory equipment should be calibrated according to manufacturer's protocols. Demonstration and documentation of acceptable mass spectrometer (MS) tuning and initial calibration is necessary prior to sample analysis. Verification of the tuning of the MS must be repeated each time instrument modification/maintenance is performed and prior to analyte calibration. After initial calibration is successful, a CCV (at the appropriate concentration described in section 10.4.2) should be performed at the beginning and end of each analysis batch.

11.1 INITIAL CALIBRATION FOR ANALYTES

11.1.1 ESI positive mode was used for this method. Optimize the $[M+H]^+$ ion in ESI positive mode for each analyte by infusing an appropriate calibration solution at a flow rate similar to the flow rate used for the LC separation. Adjust MS parameters (voltages, temperatures, gas flows, etc.) until optimal analyte responses are achieved. Optimize the product ion by adjusting the collision energy. Ensure that there are at least 10 scans across the peak for optimal precision. ESI-MS and MS/MS parameters utilized during development of this method are presented in Table 4.

11.1.2 Establish LC operating conditions that will optimize peak resolution and shape. Suggested LC conditions (listed in Table 2) may not be optimal for all LC systems.

11.1.3 The initial calibration contains a six-point curve using the analyte concentrations prepared in section 8.2.3 and shown in Table 3. The lowest calibration curve standard is at the MRL. The calibration curve and all samples should be analyzed in a low to high concentration regimen so carryover is less of a concern in case the LC cleaning cycle does not clean the system adequately between injections. Verify that all analytes have been properly identified and quantified using software programs. Integrate manually, if necessary, in accordance with laboratory quality assurance plans. Depending on the instrument, sensitivity and calibration curve responses may vary. If the polynomial type excludes the point of origin, use a fit weighting of 1/X to give more weighting to the lower concentrations. The coefficient of determination (r^2) of the linear fit should be greater than or equal to 0.98. If one of the calibration standards other than the high or low standard causes the r^2 to be <0.98, this point must be re-injected or a new calibration curve must be analyzed. The r^2 of the quadratic curve should be greater than or equal to 0.99. If one of the calibration standards other than the high or low standards causes the r^2 to be <0.99, follow the same procedure given above for a linear fit. A calibration curve and an instrument blank will be analyzed at the beginning of each batch or daily to ensure instrument stability. When quantitated, each calibration point for each analyte should calculate to be within 70-130% of its true value. The lowest CAL standard should calculate to be within 50-150% of its true value. A new curve will be generated daily. The calibration method is used to quantify all samples. Note that, because of solvent differences, different calibration curves must be made for water samples and soil/wipe samples; see Figure 2. (Remaining Figures are at the end of the SOP.)

11.2 QUANTITATION OF ANALYTES

The quantitation of the target analytes is accomplished with quantitation software as it relates to each specific instrument. For data collected with the Orbitrap, Xcaliber 2.0 SR2 software was used for quantification. Peak areas associated with the MRM transitions for each analyte were compared to those of calibration standards. An external calibration (linear) is used along with monitoring PAA surrogate recoveries. A calibration range of 0.02–0.2 µg/mL is suggested. Refer to Table 4 for the MRM transitions and retention times utilized during the development of this method.

12 ANALYTICAL PROCEDURE

12.1 SAMPLE PREPARATION

12.1.1 Samples were collected and stored as described in Section 9.2. The surrogate (PAA) is added first, then the appropriate solvent is added to the VOA vial.

12.1.2 After extraction, transfer resulting sample extract (via pipette) to a standard, 2-mL autosampler vial.

NOTE: Calibration standards are not filtered. 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.

12.2 SAMPLE ANALYSIS/ANALYTICAL SEQUENCE

12.2.1 Use the same Liquid Chromatography/Mass Spectrometry conditions established per guidance described in Section 11 and summarized in Tables 2, 3, and 4.

12.2.2 Prepare an analytical batch that includes all QC samples and field samples. The first sample to be analyzed is a 10 µL injection of a blank solvent on column followed by the calibration curve.

12.2.3 Update the calibration file and print a calibration report. Review the report for calibration outliers and make area corrections by manual integration, if necessary and appropriate. If corrections have been made, update the calibration file, noting the changes, and regenerate a calibration report. Alternatively, re-analyze "nonconforming" calibration level(s) and repeat the above procedures.

12.2.4 The first sample analyzed after the calibration curve is an additional blank to ensure there is no carryover. If the initial calibration data are acceptable, begin analyzing samples, including QC and blank samples, at their appropriate frequency injecting the same size aliquots (10 µL) under the same conditions used to analyze CAL standards. The ending CCV must have each analyte concentration within 35% of the calculated true concentration or the affected analytes from that run must be qualified as estimates or the samples must be re-analyzed with passing criteria to remove the qualification.

12.2.5 If the absolute amount of a target compound exceeds the working range of the LC-MS system (see Level 6 in CAL standards), the prepared sample is diluted with the appropriate solvent and re-analyzed along with additional samples that may have run after the sample known to exceed the calibration range, because of the possibility of carryover. Care must be taken to ensure that there is no carryover of the analyte that has exceeded the calibration range. If the amount of analyte exceeds the calibration range, a blank sample should be analyzed afterward to demonstrate no carryover will occur.

12.2.6 At the conclusion of the data acquisition, use the same software that is used in the calibration procedure to identify peaks of interest from the predetermined retention time windows. Use the data software to examine the ion abundances of the peaks

in the chromatogram to identify and compare retention times in the sample chromatogram with the retention time of the corresponding analyte peak in an analyte standard.

13 DATA ANALYSIS AND CALCULATIONS

13.1 QUALITATIVE AND QUANTITATIVE ANALYSIS

13.1.1 Complete chromatographic resolution is not needed for accurate and precise measurements of analyte concentrations when using MS/MS. An external calibration is used when monitoring the MRM transitions of each analyte. Quantitation software is utilized to conduct the quantitation of the target analytes and surrogate standard. The MRM transitions of each analyte are used for quantitation and confirmation. The MRM transition serves as a confirmation by isolating the precursor ion, fragmenting the precursor ion to the product ion, and relating the transition to the retention time in the calibration standard. Under these conditions, the elution times of CVAOA and PAOA are approximately 3.7 minutes and 7.0 minutes, respectively. The elution times for CVAA and PAA (the absence of which provides assurances that complete oxidation has occurred) are approximately 6.7 minutes and 12.5 minutes, respectively.

13.1.2 Computer programs used for analysis of data include instrumentation and quantitation software. Manual integration may be necessary for some peak areas if the peak area is not integrated properly (i.e., the integration for the peak is not fully performed by the instrument's software, which will be noticeable by visual inspection of each peak). Inspect all integrated peaks for visible integration errors and manually integrate as necessary to ensure consistent integration of other peaks and/or known calibration peaks. Any manual integration should be carried out by a qualified analyst, noted, and checked against quality control procedures (sections 10 and 11.3).

13.2 Prior to reporting data, the chromatogram should be reviewed for any incorrect peak identifications. The retention time window of the MRM transitions must be within 5% of the retention time of the analyte standard. If this is not true, the calibration curve needs to be re-analyzed to see if there was a shift in retention times during the analysis and the sample needs to be re-injected. If the retention time is still incorrect in the sample, the analyte is referred to as an unknown. If peaks need to be manually adjusted due to incorrect integration by the program, clarification of where professional judgment was used to alter the peaks should be documented during the data reduction and verification process.

14 METHOD PERFORMANCE

14.1 DETECTION LIMITS

14.1.1 Detection limit results for a single laboratory study are presented in Tables 5 and 6. MDLs were compared to analytical target levels (ATLs) to establish the method's ability to detect target analytes at health-based and environmentally relevant levels. ATLs are based on chemical agent health-based standards and guidelines established by U.S. Army Public Health Command (13). U.S. Army

levels were used because civilian health-based guidelines have not been established and are not known to exist. The surface (wipe) ATL was estimated because it was not provided within the U.S. Army document and is based on total arsenic concentration, which may not be an accurate representation for Lewisite (14).

14.2 RECOVERIES AND PRECISION FOR MATRIX TYPES

14.2.1 Section 18 lists recoveries and precision of target analytes for all tested matrices.

14.3 STORAGE STABILITY STUDY

14.3.1 A sample holding time study was initiated to determine the stability of Lewisite (deposited as CVAA) on matrices, including laboratory water, wipes, sand, and Nebraska soil, over a two week period. The samples were spiked on Day 0 and extracted and analyzed immediately or stored at ~ 4 ± 2 °C until being extracted and analyzed on Days 2, 7, or 14. Samples were prepared, using materials and matrices previously described in this procedure. All analyses were performed using the procedures of Sections 9 and 12.

Water samples were prepared by placing 400 mL of laboratory water in a 1-L (one liter), pre-cleaned, amber bottle, and adding 80 µL of 2.03 mg/mL Lewisite in dichloromethane. This solution was shaken vigorously for several minutes and allowed to equilibrate for approximately two hours before placing aliquots of this solution into 20-mL VOA vials (with no headspace) for immediate analysis or storage at 4 ± 2 °C. Three replicate samples in VOA vials were prepared for analysis at each time point. A total of 14 samples, including two extra samples, were prepared for the holding time study. (NOTE: The extra samples were collected as a precautionary step in the event additional samples were needed. For this investigation, the additional samples were not needed and were not analyzed) In addition, four blank samples (laboratory water placed in a 20-mL VOA vial) were prepared on Day 0. These were stored under the same conditions as the spiked samples and a single blank was analyzed on each day that data were collected (i.e. one blank sample was analyzed on Days 0, 2, 7, and 14) following sample preparation procedures (section 9.1.2).

Wipe samples were prepared by placing a single wipe in 40-mL VOA vial. Each wipe was spiked with 120 µL of 33.3 µg/mL CVAA solution. A total of 14 wipe samples were prepared; three replicate samples were analyzed immediately (Day 0) and the remaining samples were stored at 4 ± 2 °C until their extraction and analysis on Days 2, 7, and 14. In addition, four blank samples (clean wipe placed in a 40-mL VOA vial) were prepared on Day 0. These blank samples were stored under the same conditions as the spiked samples, and a single blank was analyzed on each day that data were collected (i.e., one blank sample was analyzed on Days 0, 2, 7, and 14) following sample preparation procedures (section 9.1.4).

Sand/soil samples were prepared by placing 5.00 g of sand/soil in 40-mL VOA vial. Each sand/soil was spiked with 60 µL of 33.3 µg/mL CVAA solution and mixed, using a vortex mixer, for approximately 30 seconds. A total of 14 samples were prepared; three replicate samples were analyzed immediately (Day 0) and the remaining samples were stored at 4 ± 2 °C until their extraction and analysis on

Days 2, 7, and 14. In addition, four blank samples (clean sand/soil placed in a 40-mL VOA vial) were prepared on Day 0. These blank samples were stored under the same conditions as the spiked samples, and a single blank of sand/soil was analyzed on each day that data were collected (i.e., one blank sample was analyzed on Days 0, 2, 7, and 14) following sample preparation procedures (section 9.1.3).

CVAOA concentrations measured for the holding time study are found in Table 7; surrogate information is found in Table 8. Statistical analysis of sample holding time data for CVAOA is presented in Table 9. A statistical software program called "R" (15) was used to determine if differences in CVAOA concentrations as a function of time could be discerned using statistical analyses. Statistical analyses were performed using the data collected by LC-MS/MS, as described in this standard operating procedure.

Dunnett's Test was performed separately for each experimental condition, to compare the CVAOA concentrations measured at each time point (i.e., $t > 0$) with the initial measured CVAOA concentration ($t = 0$). The null hypothesis was that the average CVAOA concentrations at the later times are greater than or equal to the initial CVAOA concentration; the alternative hypothesis was that one or more average CVAOA concentration at a later time is less than the initial CVAOA concentration (a one-sided test). At a significance level (α) of 0.01 (a conservative value of $\alpha = 0.01$ was chosen over the commonly used value of $\alpha = 0.05$ to compensate for the increased rate of statistical false positives resulting from multiple applications of Dunnett's Test), no statistical differences in CVAOA concentrations were observed in water or wipe samples. However, sand and Nebraska soil samples showed statistically significant losses after two days of storage. It is unclear as to whether these decreases are caused by loss of analyte or irreversible binding of the analyte to the soil. Further investigation is needed.

A limited extract holding time study was performed by re-analyzing Day 7 sample extracts nine days after the initial Day 7 analysis (i.e., Day 7 was considered from the initial time for the extract holding study and nine days after the initial Day 7 analysis is considered Day 16). Note that for these experiments, the wipes, sand, and soil were extracted in their storage vials and the resulting sample extracts were not transferred to a new vials prior to being replaced in refrigerated storage for 9 days (i.e., extracts remained in contact with the sample matrices to minimize sample handling). On Day 16 of the study, a 0.5-mL aliquot was removed from each Day 7 analysis vial (still containing the original sample matrix), placed in an autosampler vial to which 0.5 mL of 30% H_2O_2 was added, and analyzed by LC-MS/MS. The results of this analysis are presented in Table 10. The limited data set suggests that wipe extracts and Nebraska soil extracts are relatively stable; sand extracts are not. However, these should be considered preliminary data.

Figures 3 and 4 represent the CVAOA and PAOA responses obtained during calibration; standards were made according to specification in Section 8.2.3, Table 3. Each data point represents one measurement, and all data were collected on the same day. Data suggest that calibration curves are best made in solvents matched to those used for extraction of the relevant sample matrix; however, a single calibration curve could be used for wipes and soils (see also data in Figure 3). For the data shown in Figures 3 and 4, calibration standards for water were diluted in 30% H_2O_2; calibration standards for wipes were diluted in 10 mM HCl and 30%

H_2O_2; and calibration standards for soils were diluted in 10 mM HCl, 25% MeOH, and 30% H_2O_2. Calibration curves were evaluated after each data collection. Data suggest that one calibration curve is sufficient for both wipes and soils, if needed; however, calibration curves should be made as often as necessary for each matrix.

14.4 PROBLEM ANALYTES AND SURFACES

14.4.1 TARGET ANALYTES IN SOIL

Sand and Nebraska soil present challenging matrices for extracting the target analytes. As a result, presence/absence of the target analyte may be best for these particular matrices.

15 POLLUTION PREVENTION

15.1 This method utilizes small volumes of organic solvent and small quantities of pure analytes, thereby minimizing the potential hazards to both analyst and environment.

15.2 For information about pollution prevention that may be applicable to laboratory operations, 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 Street N.W., Washington, D.C., 20036 or on-line at http://www.acs.org/content/dam/acsorg/about/governance/committees/chemicalsafety/publications/less-is-better.pdf (accessed August 15, 2013).

16 WASTE MANAGEMENT

The analytical procedures described within generate relatively small amounts of waste since only small amounts of reagents and solvents are used. Laboratory waste management practices must be conducted consistent with all applicable rules and regulations, and laboratories should protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance with any sewage discharge permits and regulations is required, particularly the hazardous waste identification rules and land disposal restrictions.

16.1 Each laboratory should determine with federal and local officials how to safely dispose of field and QC samples. Waste containers should be properly labeled to identify the contents. Remember to attach the appropriate chemical waste label, date the beginning of collection before using the container and follow all appropriate federal and local waste disposal requirements.

17 REFERENCES

1. U.S. Environmental Protection Agency (EPA), 2012. *Selected Analytical Methods for Environmental Remediation and Recovery* (SAM). EPA/600/R-12/555 July 2012. Cincinnati, Ohio: United States Environmental Protection Agency, Office of Research and Development, National Homeland Security Research Center.

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.

3. Federal Advisory Committee on Detection and Quantitation Approaches and Uses in Clean Water Act Programs. Final Report. (Submitted to U.S. Environmental Protection Agency.) December 28, 2007.

4. Glaser, J.A., D.L. Foerst, G.D. McKee, S.A. Quave, W.L. Budde, "Trace Analyses for Wastewaters." *Environ. Sci. Technol.* 1981, **15,** 1426-1435.

5. Rodin, I., Braun, A., Stavrianidi, A., Shpigun, O. and Rybalchenko, I (2011), "Lewisite Metabolites Detection in Urine by Liquid Chromatography-Tandem Mass Spectrometry", J. Chromatogr. B, 879(32), 3788-3796.

6. Wada, T., Nagasawa, E., Hanaoka, S. (2006), "Simultaneous Determination of Degradation Products Related to Chemical Warfare Agents by High-Performance Liquid Chromatography/Mass Spectrometry," Applied. Organomet. Chem. 20(9), 573-579.

7. Black, R.M., Muir, B. "Derivatisation reactions in the chromatographic analysis of chemical warfare agents and their degradation products," *J. Chromatogra. A*, 1000 (2003) 253–281.

8. Tomes, J.A., Opstad, A.M., Johnsen, B.A. "Determination of organoarsenic warfare agents in sediment samples from Skagerrak by gas chromatography-mass spectrometry,' *Science of the Total Environment*, 356 (2006) 235– 246.

9. *OSHA Safety and Health Standards, General Industry* (29CRF1910). Occupational Safety and Health Administration, OSHA 2206 (Revised, July 1, 2001).

10. *Carcinogens-Working with Carcinogens*, Publication No. 77-206. Atlanta, Georgia: Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute of Occupational Safety and Health, August 1977.

11. *Safety in Academic Chemistry Laboratories*, American Chemical Society Publication, Committee on Chemical Safety, 7th Edition.

12. "Prudent Practices in the Laboratory: Handling and Disposal of Chemicals," National Academies Press (1995), available at http://www.nap.edu (accessed August 15, 2013).

13. U. S. Army Public Health Command (2011) "Chemical Agent Health-Based Standards and Guidelines Summary Table 2: Criteria for Water, Soil, Waste as of July 2011".

14. Contaminants of Potential Concern (COPC) Committee of the World Trade Center Indoor Air Task Force Working Group (2003) "World Trade Center Indoor Environment Assessment: Selecting Contaminants of Potential Concern and Setting Health-Based Benchmarks", available at http://www.epa.gov/wtc/reports/contaminants_of_concern_benchmark_study.pdf, <accessed 3/27/2015>.

15. R Core Team. (2012) "R: A language and environment for statistical computing." R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. URL http://www.R-project.org/.

18 TABLES, FIGURES AND VALIDATION DATA

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Table 1. Materials tested for the Lewisite 1 degradation analysis

Material	Manufacturer/Vendor
Water	(Millipore Milli-Q, Advantage A-10 system, resistivity of 18.2 MΩ.cm at 25 °C), Millipore, Cincinnati, OH
Wipe	3” x 3” 12-ply gauze (Kendall Curity)
Sand, purified	JT Baker, Inc., Phillipsburg, NJ
Virginia Soil (VA soil)	Composition (64.5% sand, 28% silt, 7.5% clay, 2.6% TOC, pH 4.1 in 1:1 soil: water mix)
Nebraska Aglands Ap Soil (NE soil)	Composition (5.1% sand, 57.5% silt, 31.7% clay, 1.9% TOC, pH 5.5 in 1:1 soil: water mix)
Georgia Bt2 soil (GA soil)	Composition (46% sand, 22% silt, 32% clay, 0.2% TOC, pH 5.0 in 1:1 soil: water mix)

Instrumental Conditions

Under these conditions, the elution times of CVAOA and PAOA are approximately 3.7 min and 7.0 min, respectively. The elution times for CVAA and PAA (the absence of which provides assurances that complete oxidation has occurred) are approximately 6.7 min and 12.5 min, respectively.

Table 2. Liquid Chromatography Gradient Conditions*

Time (min)	Flow (µL/min)	% Solution A†	% Solution B††
0	200	5	95
5	200	5	95
15	200	80	20
25	200	80	20
28	200	5	95
38	200	5	95

†A: Acetonitrile (0.1% Formic Acid)
† †B: Water (0.1% Formic Acid)
*Injection volume – 10 µL(recommended)
*Column Temperature: 30 ° C
*Equilibration time: 10 minutes
*Column: 100 Å, 150 mm x 2.1mm, 3µm particle size

Table 3. Recommended Concentrations for LC-MS/MS Calibration Standards

	Concentration of Analyte in Calibration Standard (ng/µL)		Add Together the Following Volumes (µL) to Make Calibration Standard		
Calibration Level	**CVAA**	**PAA**	**10 ng/µL CVAA solution**	**10 ng/µL PAA solution**	**Solvent[a]**
1	0.02	0.02	4	4	2000
2	0.05	0.05	10	10	2000
3	0.08	0.08	16	16	2000
4	0.1	0.1	20	20	2000
5	0.15	0.15	30	30	2000
6	0.2	0.2	40	40	2000

[a] Solvents change depending on matrix: Water, 15% H_2O_2 added; Wipe samples, 5 mM HCl with 15% H_2O_2; Sand/soil, 2.5 mM HCl, 25% MeOH, and 15% H_2O_2.

Mass Spectrometer Conditions

Ionization mode: Positive electrospray
Capillary temperature: 300ºC
Scan segments: The chromatographic run is divided into six scan segments, as summarized in Table 2. This table describes parameters that are specific to the LTQ Orbitrap system used in method development.

Table 4. Scan Segments Associated with Lewisite 1 Degradation Product Analysis by LC-MS/MS (LTQ Orbitrap)

Segment	Retention Time (min)	Scan Events	Type	Comment
1	0.00–3.15	100–750 m/z	full scan	
2	3.15–5.50	100–750 m/z	full scan	$[M+H]^+$ CVAOA (187 m/z→169 m/z)
		187→50–300 m/z	MS/MS[a] (3 sequential scans), CE[b] = 31%	
		187→50–300 m/z		
		187→50–300 m/z		
3	5.50–6.86	100–750 m/z	full scan	$[M-H_2O+H]^+$ CVAA (153 m/z→127 m/z)
		153→50–300 m/z	MS/MS[a] (3 sequential scans), CE[b] = 25%	
		153→50–300 m/z		
		153→50–300 m/z		
4	6.86–9.87	100–750 m/z	full scan	$[M+H]^+$ PAOA (203 m/z→185 m/z)
		203→55–300 m/z	MS/MS[a] (3 sequential scans), CE[b] = 30%	
		203→55–300 m/z		
		203→55–300 m/z		
5	9.87–13.26	100–750 m/z	full scan	$[M-H_2O+H]^+$ PAA (169 m/z→91 m/z)
		169→50–300 m/z	MS/MS[a] (3 sequential scans), CE[b] = 30%	
		169→50–300 m/z		
		169→50–300 m/z		
6	13.26–30.00	100–750 m/z	full scan	

[a] for all MS/MS scans, Activation Q = 0.25, Activation Time = 30 ms, and Isolation Width = 1.4 m/z
[b] Normalized Collision Energy

Method Detection Limit Data

Table 5. Method Detection Limit[a] (MDL) data for CVAOA in Various Matrices

Matrix	Spiked CVAOA Concentration	Measured CVAOA Concentration (avg ± std)[a]	Average Recovery (%)	MDL[b] for CVAOA	Spike: MDL	ATL[d]
Water	0.20 mg/L	0.22 ± 0.01 mg/L	110	0.041 mg/L	4.9	0.027 mg/L[d]
Wipe	3.00 µg	3.72 ± 0.14 µg	124	0.44 µg	8.5	4 µg [e]
Wipe[c, f]	3.00 µg	3.04 ± 0.12 µg	101	0.38 µg	8.1	4 µg [e]
Sand	0.20 µg/g	0.17 ± 0.02 µg/g	85	0.073 µg/g	2.3	0.3 µg/g [d]
Nebraska (NE) Soil	0.20 µg/g	0.22 ± 0.01 µg/g	112	0.032 µg/g	7.1	0.3 µg/g [d]
Virginia (VA) Soil	0.40 µg/g	0.17 ± 0.01 µg/g	43	0.028 µg/g	6.1	0.3 µg/g [d]
Georgia (GA) Soil	0.40 µg/g	0.32 ± 0.02 µg/g	80	0.055 µg/g	5.8	0.3 µg/g [d]

[a] Numbers are the average of seven independent samples plus/minus the standard deviation of the measurements.
[b] Statistically determined MDL = $s \times t_{\alpha=0.01}$, where s is the standard deviation of seven measurements and *t* is the Student *t* value for 6 degrees of freedom (3.143).
[c] Data corrected for small amount of CVAOA in blank for second set of wipe data.
[d] Analyte target level (ATL), derived from U.S. Army Public Health Command (13).
[e] Analyte target level (ATL) was estimated, based on screening level for arsenic at ~ 400 µg/m^2 = 0.04 µg/m^2, which assuming a wipe sample from 100 cm^2, yields 4 µg (14).
[f] Two sets of wipe data were collected at different times.

Table 6. Surrogate (PAOA) Recovery Data Collected During MDL Study (from Samples in Table 5)

Matrix	Spiked PAOA Concentration	Measured PAOA Concentration (avg ± std)[a]	Average Recovery (%)
Water	0.20 mg/L	0.23 ± 0.01 mg/L	110
Wipe	4.0 µg	4.08 ± 0.07 µg	102
Sand	0.40 µg/g	0.35 ± 0.03 µg/g	87
NE Soil	0.40 µg/g	0.34 ± 0.01 µg/g	86
VA Soil	0.40 µg/g	0.11 ± 0.01 µg/g	28
GA Soil	0.40 µg/g	0.30 ± 0.01 µg/g	74

[a] Numbers are the average of seven independent samples plus/minus the standard deviation of the measurements.

Holding Time Study Data

Table 7. Sample Holding Time Data for CVAA Spiked on Various Matrices and Analyzed as CVAOA

			CVAOA Concentrations[a]			
Matrix	**Units**	**Spiked**	**Day 0**	**Day 2**	**Day 7**	**Day 14**
Water	mg/L	0.40	0.41 ± 0.01	0.39 ± 0.01	0.38 ± 0.02	0.40 ± 0.02
Wipe	µg	4.00	5.09 ± 0.12	4.71 ± 0.19	4.85 ± 0.12	4.80 ± 0.20
Sand	mg/kg	0.40	0.39 ± 0.02	0.29 ± 0.02	0.22 ± 0.04	0.16 ± 0.02
NE soil	mg/kg	0.40	0.40 ± 0.02	0.24 ± 0.01	0.12 ± 0.00	0.11 ± 0.01

[a] Numbers are the average of three independent samples plus/minus the standard deviation of the measurements.

Table 8. PAOA Surrogate Data Collected During Sample Holding Time Study

			PAOA Concentrations[a]			
Matrix	**Units**	**Spiked**	**Day 0**	**Day 2**	**Day 7**	**Day 14**
Water	mg/L	0.20	0.20 ± 0.01	0.18 ± 0.00	0.19 ± 0.00	0.20 ± 0.00
Wipe	µg	4.00	4.19 ± 0.10	3.93 ± 0.06	3.95 ± 0.05	4.29 ± 0.09
Sand	mg/kg	0.40	0.38 ± 0.01	0.36 ± 0.01	0.36 ± 0.01	0.65 ± 0.02[b]
NE soil	mg/ kg	0.40	0.35 ± 0.01	0.33 ± 0.00	0.31 ± 0.01	0.57 ± 0.03[b]

[a] Numbers are the average of three independent samples plus/minus the standard deviation of the measurements.

[b] Incorrect spiking at 0.80 mg/kg (used same pipet as for wipes).

Table 9 . Statistical Analysis[a] of Sample Holding Time Study Data for CVAOA (See data in Table 2.)

	Significance Level		
Matrix	$t_2 - t_0$	$t_7 - t_0$	$t_{14} - t_0$
Water	0.107	0.016	0.331
Wipe	0.025	0.105	0.068
Sand	<0.010	<0.010	<0.010
NE Soil	<0.010	<0.010	<0.010

[a] Significance levels (*p* values) for comparison of CVAOA concentrations at time *t* (days) and time 0; $p<0.01$ indicates statistically significant concentration decrease.

Additional Holding Time Data

Table 10. CVAOA Concentration Changes in Sample Extracts; Extracts Were Prepared on Day 7 and Reanalyzed on Day 16 (nine days of storage at 4±2 °C)

	CVAOA Concentrations in Sample Extracts Produced on Day 7 and Reanalyzed on Day 16[a]			
Matrix	**Units**	**Spiked**	**Day 7 (t=0 days)**	**Day 16 (t=9 days)**
Wipe	µg	4.00	4.85 ± 0.12	4.75 ± 0.25
Sand	mg/kg	0.40	0.22 ± 0.04	ND
NE soil	mg/kg	0.40	0.12 ± 0.00	0.10 ± 0.00

[a] Numbers are the average of three independent samples plus/minus the standard deviation of the measurements.

Quality Control Data Collected During MDL Study

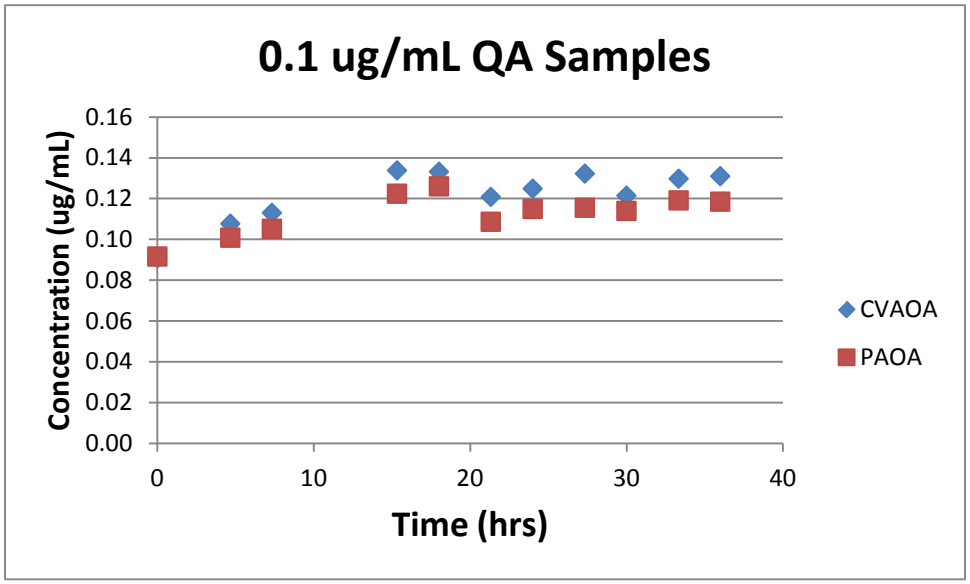


Figure 2. Quality data collected during MDL study.

The above data represent replicate analyses of the same continuing calibration verification (CCV) sample, which contained 0.1 µg/mL each of CVAOA and PAOA in 30% H_2O_2 in laboratory-grade water. These data were collected during the period when data for the wipe, sand, and soil samples were collected (see Tables 3 and 4). These data were collected over the course of a sequence that contained a total of 65 samples run over a period of approximately 40 hours. During this time, all CCVs remained within 35% of their expected values.

Calibration Data for Various Matrices

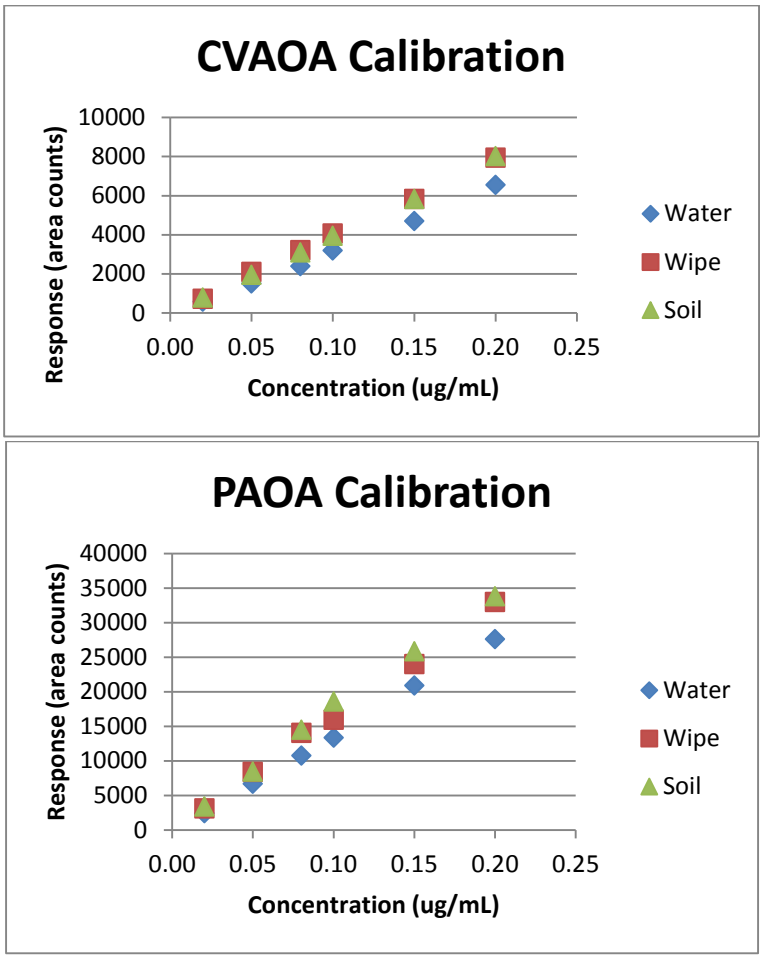
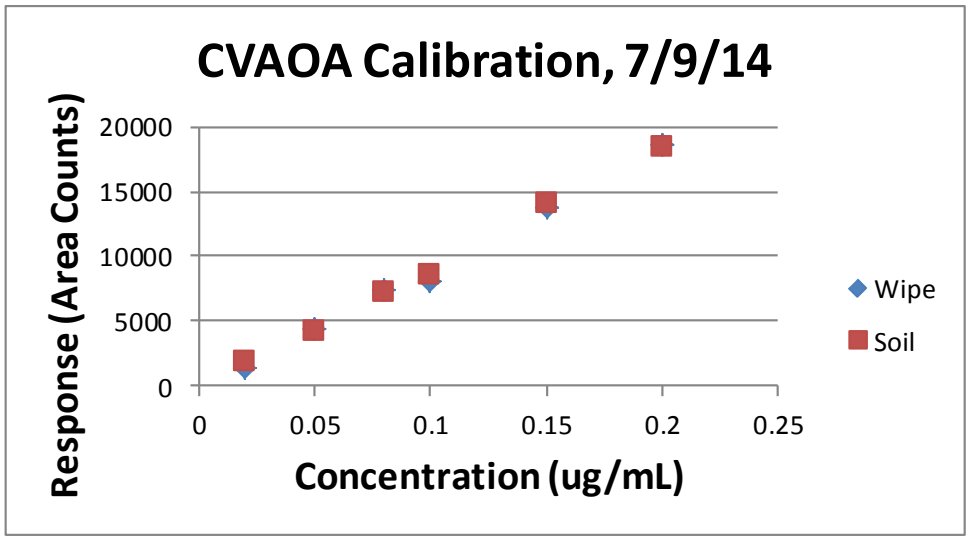


Figure 3. LC-MS/MS response vs. analyte concentration in various solvents. Solvents change depending on matrix: Water, 30% H_2O_2 added; Wipe samples, 10 mM HCl with 30% H_2O_2; Sand/soil, 10 mM HCl, 25% MeOH, and 30% H_2O_2.



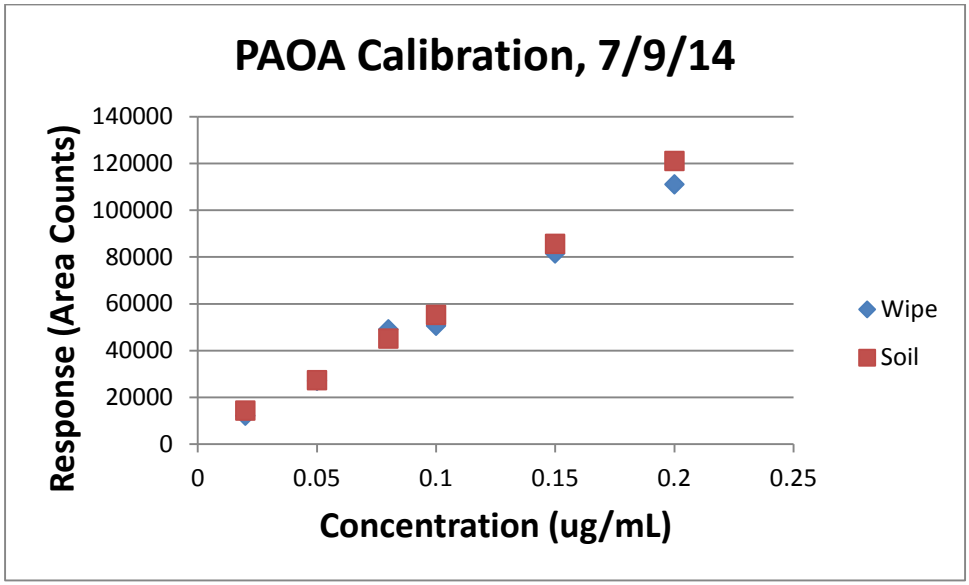


Figure 4. LC-MS/MS response vs. analyte concentration, additional data. Solvents change depending on matrix: Wipe samples, 10 mM HCl with 30% H_2O_2; Sand/soil, 10 mM HCl, 25% MeOH, and 30% H_2O_2.

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