EPA 600/R-13/224 | September 2013 | www.epa.gov/ord

United States
Environmental Protection
Agency

Surface Analysis of Nerve Agent Degradation Products by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

Office of Research and Development
National Homeland Security Research Center

EPA/600/R-13/224
September 2013

SURFACE ANALYSIS OF NERVE AGENT DEGRADATION PRODUCTS BY LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS)

Sampling and Analytical Method for Wipe Analysis of Surfaces
Revision 1

United States Environmental Protection Agency
National Homeland Security Research Center
26 W. Martin Luther King Jr. Drive
Cincinnati, OH 45268

and

Centers for Disease Control and Prevention
National Institute for Occupational Safety and Health
5555 Ridge Ave
Cincinnati, OH 45213

Last Revised: 12/12

DISCLAIMER

The United States Environmental Protection Agency through its Office of Research and Development (ORD), National Homeland Security Research Center (NHSRC), funded and managed the research described here (IA #DW-75-922440001-0) in collaboration with the National Institute of Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention (CDC), a division of the U.S. Department of Health and Human Services (DHHS). It has been subjected to the Agency's administrative review and approved for publication. The views expressed in this paper do not necessarily reflect the views or policies of the Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Questions concerning this document or its application should be addressed to:

Stuart Willison, Ph.D.
Project Officer
U.S. Environmental Protection Agency
National Homeland Security Research Center
26 W. Martin Luther King Drive, MS NG16 Cincinnati, OH 45268
513-569-7253
Willison.Stuart@epa.gov

Robert Streicher, Ph.D.
Project Officer
National Institute for Occupational Safety and Health Laboratories
Alice Hamilton Laboratory
5555 Ridge Avenue
Cincinnati, OH 45213
513-841-4296
Rps3@cdc.gov

ACKNOWLEDGMENTS

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

United States Environmental Protection Agency (EPA)
Office of Research and Development, National Homeland Security Research Center
Stuart Willison, Project Officer and Method Development
Matthew Magnuson, Technical Reviewer

United States Environmental Protection Agency (EPA)
Office of Emergency Management
Terry Smith, Technical Reviewer

United States Environmental Protection Agency (EPA)
Office of Ground Water and Drinking Water
April Dupre, Technical Reviewer

Centers for Disease Control and Prevention
National Institute for Occupational Safety and Health
Jack Pretty, Laboratory Advisor
Robert Streicher, Project Officer

EXECUTIVE SUMMARY

The sampling and analytical method described herein was developed and tested within the same laboratory to assess the recoveries of nerve agent degradation products from various porous (vinyl tile, painted drywall, wood) and mostly nonporous (laminate, galvanized steel, glass) surfaces. Performance data (method detection limit and precision and accuracy data) are available to demonstrate the fitness-for-purpose regarding the development of a method for nerve agent degradation products in that single laboratory. Samples are collected from surfaces using wipes, the wipes are spiked with a surrogate compound and carried through extraction with distilled water by sonication and filtration steps followed by analysis using liquid chromatography electrospray ionization/tandem mass spectrometry (LC/ESI-MS/MS) by direct injection without derivatization. Detection limit data were generated using wipes on a laminate surface following the procedures of 40 CFR Part 136, Appendix B, as part of EPA's guidelines for determining a method detection limit.

Gauze wipes were selected over other tested wipes (i.e., filter paper, glass fiber filters, nonwoven polyester fiber) because gauze wipes were physically robust during the wiping procedure, contained low background levels, produced no peaks that interfered with the target analytes, and produced the highest percent recoveries of all wipes tested during sample analysis. Percent recoveries were highest for the laminate surface and ranged from 65-87 % for all of the nerve agent degradation products analyzed in ESI negative mode. The resulting equivalent method detection limits obtained from wiping the laminate surface were 0.04 $\mu g/cm^2$ for isopropyl methylphosphonic acid (IMPA), 0.07 $\mu g/cm^2$ for methylphosphonic acid (MPA), 0.05 $\mu g/cm^2$ for ethyl methylphosphonic acid (EMPA), 0.07 $\mu g/cm^2$ for ethyl hydrogen dimethylamidophosphate, sodium salt (EHDMAP) and 0.02 $\mu g/cm^2$ for pinacolyl methylphosphonic acid (PMPA). Diisopropyl methylphosphonate (DIMP) was not recovered unless the surfaces were wiped immediately after spiking due to the volatile nature of this compound. Other complications are presented in the method in section 14.4. Precision and accuracy data were generated from each tested surface fortified with these analytes.

SURFACE ANALYSIS OF NERVE AGENT DEGRADATION PRODUCTS BY LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC-MS/MS)

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ACRONYMS AND ABBREVIATIONS

AS	Analyte Stock Standard (Solution)
CAL	Calibration Standard
CAS®	Chemical Abstracts Service
CCC	Continuing Calibration Check
CDC	Centers for Disease Control and Prevention
CID	Collisionally Induced Dissociation
CWA	Chemical Warfare Agent
DL	Detection Limit
DHHS	U.S. Department of Health and Human Services
DIMP	Diisopropyl Methylphosphonate
DQO	Data Quality Objective
EHDMAP	Ethyl Hydrogen Dimethylamidophosphate, sodium salt
EMPA	Ethyl Methylphosphonic Acid
EPA	U.S. Environmental Protection Agency
ERLN	Environmental Response Laboratory Network
ESI (+)	Electrospray Ionization in Positive Mode
ESI (-)	Electrospray Ionization in Negative Mode
FD	Field Duplicate
IDC	Initial Demonstration of Capability
IDL	Instrument Detection Limit
IMPA	Isopropyl Methylphosphonic Acid
LC	Liquid Chromatography
LC/MS/MS	Liquid Chromatography Coupled with Tandem Mass Spectrometry
LFB	Laboratory Fortified Blank
LFSM	Laboratory Fortified Sample Matrix
LFSMD	Laboratory Fortified Sample Matrix Duplicate
LMB	Laboratory Method Blank
MDL	Method Detection Limit
MPA	Methylphosphonic Acid
MRL	Minimum Reporting Limit
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometer(try)
MSDS	Material Safety Data Sheet
MS/MS	Tandem Mass Spectrometry
NHSRC	National Homeland Security Research Center
NIOSH	National Institute for Occupational Safety and Health
NIST	National Institute of Standards and Technology
ORD	U.S. EPA's Office of Research and Development
OSHA	Occupational Safety and Health Administration
PIR	Prediction Interval of Result
PMPA	Pinacolyl Methylphosphonic Acid
ppb	Parts Per Billion
ppm	Parts Per Million
P&A	Precision and Accuracy
PVDF	Polyvinylidene Fluoride
QC	Quality Control
r^2	Coefficient of determination

REC	Percent Recovery
RL	Reporting Limit
RPD	Relative Percent Difference
RSD	Relative Standard Deviation
RT	Retention Time
SAM	*Selected Analytical Methods for Environmental Restoration Following Homeland Security Events*
SD	Standard Deviation
S/N	Signal to Noise
SS	Surrogate Standard
SSS	Stock Standard Solution
VOA	Volatile Organic Analysis
X	Average Percent Recovery
σ	Standard Deviation

1. INTRODUCTION

1.1. The U.S. Environmental Protection Agency (EPA) is responsible for developing tools and methodologies which will enable the rapid characterization of indoor and outdoor areas and 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)*, formerly referred to as the *Standardized Analytical Methods for Environmental Restoration Following Homeland Security* Events (1), which is a compendium of methods that informs sample collection and analysis during the response to an all-hazards incident. Chemical warfare agents (CWAs) and their degradation products remain a high-priority concern due to the potential for the intentional or unintentional release of these agents. Nerve agents are very dangerous CWAs, which can break down into degradation products sufficiently persistent and toxic to be of interest during site remediation after a release. Accordingly, if an incident were to occur, versatile sampling procedures are needed to detect CWA degradation products from various CWAs and help determine the spread and concentration of these agents and degradation products in contaminated areas. Multiple types of contaminated surfaces from an indoor setting (e.g., walls, posts, windows, floors and furniture) will need to be extensively tested within the contaminated areas. Direct extraction may be a possibility; however, the laboratory procedures can be tedious, complex, and require the destruction of the material being analyzed. Wipe sampling is preferred because it can be performed quickly and easily in a manner less destructive to the tested surface when direct extraction is not feasible.

1.2. After sample collection, selective analysis methods must be implemented to detect and quantify the appropriate agent and/or degradation products in the environmental sample. The appropriate procedure should account for possible contaminants already present within the sample as well as other matrix complications that may arise during analysis to ensure sample integrity and to ensure that the analysis method is applicable to the matrix of interest. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is often the most appropriate and powerful analysis technique for polar nonvolatile compounds. LC-MS/MS affords laboratories an enhanced capability to analyze specific environmental matrices for CWA degradation products while avoiding complications that may arise from derivatization, a step more commonly needed for gas chromatography/mass spectrometry analysis. Although LC-MS analysis methods do exist for nerve agent degradation products from water, currently no known wipe sample collection and analysis protocol for the detection of nerve agent degradation products from contaminated surfaces is documented in the scientific literature.

2. SCOPE AND APPLICATION

2.1. This sampling and analytical procedure was developed and tested in the same laboratory to investigate nerve agent degradation products, which may persist at a contaminated site, via surface wiping followed by analytical characterization. The performance data presented demonstrate the fitness-for-purpose regarding surface analysis in that single laboratory. Surfaces (laminate, glass, galvanized steel, vinyl tile, painted drywall and treated wood) were wiped with cotton gauze wipes, sonicated, extracted with distilled water, and filtered. Samples were analyzed with direct injection electrospray ionization liquid chromatography tandem mass spectrometry (ESI-LC-MS/MS) without derivatization. Detection limit data were generated for all analytes of interest on a laminate surface. Accuracy and precision data were generated from each surface fortified with these analytes. The following analytes have been determined using this procedure:

Analyte	CAS Registry Number®
Diisopropylmethylphosphonate (DIMP)	1445-75-6
Ethyl Hydrogen Dimethylamidophosphate, sodium salt (EHDMAP)	2632-86-2
Ethyl Methylphosphonic acid (EMPA)	1832-53-7
Isopropyl Methylphosphonic acid (IMPA)	1832-54-8
Methylphosphonic acid (MPA)	993-13-5
Pinacolyl Methylphosphonic Acid (PMPA)	616-52-4

2.2. Wipe sampling can be performed quickly and easily when direct extraction is not feasible (*e.g.*, walls, posts, windows, floors and furniture) as wipe sampling can be performed without the destruction of the tested surface. Porous surfaces may have lower recoveries and less precision because the contaminants may sorb into the material. Wipe sampling will recover analyte only from the surface of the analyzed material. It is, therefore, important to understand wipe efficiencies and the materials being wiped. This procedure assesses the recoveries from several porous and nonporous surfaces using wipes.

2.3. 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 and instrument performance. The statistical procedure, utilizing the Laboratory Fortified Sample Matrix samples (LFSM) and LFSM duplicates (LFSMDs), is used to calculate recovery. Precision and accuracy (P&A) 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 concentrations are appropriate.

2.4. 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.

2.5. 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 wipe materials, wipe composition, and compatibility of various wipe materials with some surfaces. This procedure was developed using a triple quadrupole LC-MS/MS, with optimized LC conditions and wipe materials. The procedure has been verified using only the specified equipment and conditions. Other types of LC-MS/MS instrumentation, LC and/or ESI-MS/MS conditions, sample collection and processing steps, and wipe/collection materials can be used for analysis as long as similar performance is demonstrated and the quality control measures outlined in section 10 of this report are implemented.

3. SUMMARY OF METHOD

3.1. Samples are collected from surfaces with wipes and stored at 4 °C (± 2 °C) if samples are not to be analyzed within a 24-hour time period. When the samples are analyzed, samples are spiked with the appropriate surrogate compounds, the appropriate solvent volume is added, the sample solution is sonicated, extracted with a syringe filter unit, then the extract is analyzed directly by LC-MS/MS operated simultaneously in positive and negative electrospray ionization modes, (ESI+) and (ESI-) respectively. Data described in this procedure refer to ESI (-) mode because some complications can occur in ESI (+) mode.

3.2. Each target compound 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 is used to identify analytes. The retention time for the analytes of interest must fall within the retention time window of the standard (within ± 5%). 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 from the wipe and extraction process.

3.3. This procedure utilizes cotton gauze wipes, which were determined to provide the highest recoveries with the least interference for any targeted analyte. Other wipes such as filter paper or glass fiber filters did have comparable recoveries and might be an appropriate alternative but would not be as robust during the wiping procedure for the targeted analytes.

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 check (CCC) standards. Additional CCCs may be required depending on the number of samples (excluding 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 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 precursor ion's translational energy into internal energy by collisions with neutral gas molecules to bring about dissociation into product ions.

4.4. CONTINUING CALIBRATION CHECK (CCC) – A calibration standard containing the method analytes and surrogate standard(s). The CCC 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. DETECTION LIMIT (DL) – The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero.

4.6. EXTRACTION BATCH – A set of up to twenty field samples (excluding quality control [QC] samples) extracted together using the same solvents, surrogate(s), fortifying solutions, and sampling devices.

4.7. FIELD DUPLICATE (FD) – Separate samples collected at the same time and place, under identical circumstances and treated exactly the same as other field samples throughout field and/or laboratory procedures. Analyses of FDs will give a measure of the precision associated with sample collection, preservation, and storage, as well as laboratory procedures.

4.8. LABORATORY FORTIFIED BLANK (LFB) – A blank matrix to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to demonstrate that the methodology is in control and that the laboratory is capable of making accurate and precise measurements.

4.9. LABORATORY FORTIFIED SAMPLE MATRIX (LFSM) – A field sample to which known quantities of the method analytes are added in the laboratory. The LFSM 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.

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

4.11. 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 LMB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

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

4.13. 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.

4.14. 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.

4.15. 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.

4.16. 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.

4.17. 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) (Section 10.4.1) under the same conditions as the samples (5). 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. Wipe 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 positives. Wipes were not pre-cleaned but were analyzed to ensure that there were no interferences present. Any wipe materials containing interferences with the analytes of

interest were not used.

5.3. Matrix effects are known phenomena of ESI-MS techniques, especially for coeluting 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, high background and improper ion ratios.

6. HEALTH AND SAFETY

The toxicity and carcinogenicity of each reagent used in this method have not been defined precisely. However, each chemical compound was treated as a health hazard. 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 MSDSs 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 (6-9).

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. Materials tested for the wipe analysis of nerve agent degradation products are described in Table 1.

7.1 LC-MS/MS APPARATUS

7.1.1 LIQUID CHROMATOGRAPHY (LC) SYSTEM - An analytical system complete with a temperature programmable liquid chromatograph with a solvent mixer (Waters, Milford, MA - Acquity™ or equivalent able to perform the analyses as described) and all required accessories including syringes, solvent degasser, and autosampler.

7.1.2 ANALYTICAL COLUMN - Atlantis® – dC18, 100 mm x 2.1 mm, 3 µm particle size (Waters, Milford, MA, Catalog # 186001299), or equivalent.

7.1.3 TANDEM MASS SPECTROMETER (MS/MS) SYSTEM – An MS/MS instrument (Waters TQD™ or similar instrument) can be used for analysis of the target analytes. 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 – Waters' MassLynx™ 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. Waters' QuanLynx™ (or similar software) is used for all quantitative analysis for data generated from the LC-MS unit.

7.2 EXTRACTION DEVICE

7.2.1 SONICATOR (Fisher Scientific Catalog # 15-335-112) or equivalent.

7.3 GLASSWARE AND MISCELLANEOUS SUPPLIES

7.3.1 AUTOSAMPLER VIALS - Amber 2-mL autosampler vials with pre-slit Teflon®-lined screw tops (Waters Corp., Milford, MA), or equivalent.

7.3.2 DISPOSABLE STERILE SYRINGES - 10.0 mL ± 1% accuracy BD Safety-Lok™ syringes (Catalog No. 14-829-32, Fisher Scientific, Pittsburgh, PA), or equivalent.

7.3.3 AUTO PIPETTES - 10.0 mL, 1000 µL, 100 µL and 10 µL ± 1% accuracy.

7.3.4 DESOLVATION GAS - Nitrogen gas generator or equivalent nitrogen gas supply. Aids in the generation of an aerosol of the ESI liquid spray and should meet or exceed instrument manufacturer's specifications.

7.3.5 COLLISION GAS - Argon gas used in the collision cell in MS/MS instruments and should meet or exceed instrument manufacturer's specifications.

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

7.3.7 National Institute of Standards and Technology (NIST)-traceable thermometer.

7.3.8 STANDARD SOLUTION FLASKS - Class A volumetric glassware

7.3.9 SYRINGE FILTER - Millex® GV Syringe-driven polyvinylidene fluoride (PVDF) 13 mm filter unit , 0.22 µm (Millipore Corporation, Billerica, MA, Catalog # SLGV013NL).

7.3.10 WIPES - Dukal™, 2" x 2" – 12-ply sterile cotton gauze pads, individually packaged (Fisher Scientific, Pittsburgh, PA, Catalog # 17986468).

7.3.11 SAMPLE COLLECTION CONTAINERS - Clean 125 mL Nalgene polypropylene straight-side jars with screw caps (Fisher Scientific, Pittsburgh, PA, Catalog # 11-815-10C), or equivalent.

7.3.12 SAMPLE CONCENTRATION CONTAINERS - Sterile 15 mL conical graduated plastic centrifuge tubes (Fisher Scientific, Pittsburgh, PA, Catalog # 05-538-59A), or equivalent.

8 REAGENTS AND STANDARDS

8.1 REAGENTS AND STANDARDS

When compound purity is assayed to be 98% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Expiration times for

prepared solutions are suggested below, but laboratories should follow standard QC procedures to determine when the standards should be replaced. Label all standards and verify the correct grade of solvents. Traceability of standards is established by the manufacturer's specifications provided at time of purchase.

8.1.1 SOLVENTS, REAGENTS and GASES - Acetonitrile (CAS # 75-05-8), Methanol (CAS # 67-56-1), and LC-MS grade Water (CAS # 7732-18-5), HPLC mass spectrometry pesticide grade or equivalent, demonstrated to be free of analytes and interferences. Formic Acid (Chemical Abstracts Service (CAS) # 64-18-6). Nitrogen is used for the generation of aerosol of the ESI liquid spray, and purity should meet instrument manufacturer's specifications. Argon is used as the collision gas in MS/MS applications, and purity should meet instrument manufacturer's specifications.

8.1.2 MOBILE PHASE A - Solution A consisted of LC-MS grade water and 0.2% of formic acid to prevent microbial growth. To prepare 0.5 L, add 1 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 at least once a week.

8.1.3 MOBILE PHASE B- Solution B was comprised of acetonitrile and 0.2% of formic acid. To prepare 0.5 L, add 1 mL of formic acid and dilute to 0.5 L mark with acetonitrile.

8.1.4 TARGET ANALYTES – MPA (Catalog #: 289868) and EMPA (Catalog #: 112062) were purchased from Sigma-Aldrich (St. Louis , MO). IMPA (Catalog #: ERI-015), DIMP (Catalog #: ERD-083), PMPA (Catalog #: ERP-083), and EHDMAP (Catalog #: ULM-6091-1.2) were purchased from Cerilliant (Round Rock, TX).

8.1.5 SURROGATE ANALYTES - MPA-d_3 (Catalog #: DLM-6196-1.2), PMPA-$^{13}C_6$ (Catalog #: CLM-6620-1.2)and DIMP-d_{14} (Catalog #: ERD-086) were purchased from Cerilliant.

8.2 STANDARD SOLUTIONS

When compound purity is assayed to be at least 98% or greater, the weight can be used without correction to calculate the concentration of the stock standard. 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 4 °C (± 2 °C). Standards are estimated to be stable for at least a month as long as water is not present. Although stability times are suggested, laboratories should utilize QC practices to determine when standards should be replaced.

8.2.1 SURROGATE STOCK STANDARD SOLUTION (Surrogate SSS) (10-1000 µg/mL)

A standard solution may be prepared from certified commercially available methanol solutions or neat compounds. Isotopically-labeled surrogates (MPA-d_3, PMPA-$^{13}C_6$ and DIMP-d_{14}) were purchased as methanol solutions. The surrogate is added to a 10 mL volumetric flask to achieve a concentration of approximately ten times the highest calibration concentration (ten times calibration 7) in solution (i.e., 900 µL of

MPA-d_3 and PMPA-$^{13}C_6$ and 18 µL of DIMP-d_{14} were added to a 10 mL volumetric flask and diluted to the mark with methanol). Surrogate stock standard solutions are stable for at least a month when stored at 4 ºC.

(**NOTE**: Although the listed analytes were used as surrogates in this method, they could also be used as internal standards for quantitation purposes. However, further evaluation would be necessary to ensure that they are viable internal standards and meet QC requirements.)

8.2.2 ANALYTE STOCK STANDARD SOLUTION (AS)

Standard solutions may be prepared from certified commercially available neat compounds. MPA and EMPA were purchased as a neat solid and liquid, respectively. Separate methanol solutions (1000 µg/mL) containing MPA and EMPA were used to make the analyte stock standard solution. DIMP, EHDMAP, IMPA, and PMPA were purchased as methanol solutions. A standard methanol solution with a concentration of 3 µg/mL (ppm) was made in a 25 mL volumetric flask containing DIMP, EMPA, MPA, PMPA, and IMPA (i.e., 18 µL of DIMP, 30 µL of EMPA, 75 µL of MPA, 18 µL of PMPA and 90 µL of IMPA are each added to a 25 mL volumetric flask and diluted to the mark with methanol). EHDMAP is not added to the initial stock standard solution because it is not stable over the suggested stability period when added to the methanol solution. EHDMAP and the surrogate analytes are added to calibration standard solutions only when the solutions are ready for use. The calibration standards and spike solutions are made from the appropriate dilution of this analyte stock standard. The analyte stock standard solution is stable for at least a month when stored at 4 ºC.

8.2.3 CALIBRATION STANDARD SOLUTION (CAL)

Dilution of the 3 µg/mL methanol solution can be used to obtain a 750 ng/mL (ppb) solution in water. A calibration stock standard solution (Level 7) is prepared from the Analyte Stock Standard Solution (AS) and SSS by adding, 2.5 mL of AS, 9 µL of EHDMAP, and 1 mL of the SSS (i.e., 2.5 mL of the AS containing DIMP, EMPA, MPA, PMPA, and IMPA, 9 µL of EHDMAP, and 1 mL of the SSS are added to a 10 mL volumetric flask and diluted to the mark with LC-MS grade water). From Level 7, further dilutions are performed with LC-MS grade water to prepare Levels 6 through 1, as shown in Table 2.

9 SAMPLE COLLECTION, PRESERVATION AND STORAGE

9.1 SAMPLE COLLECTION

9.1.1 Volatile organic analysis (VOA) vials and Nalgene containers were both used for sample collection and both were deemed adequate for use, but Nalgene containers were specifically used in this method. Other vessels may be used as long as they are tested and verified to ensure they do not contain any interfering compounds. As an example for field samples, the field samplers would collect samples with the appropriate water-wetted wipe and place the wipes in a jar with a cap (e.g., 125 mL Nalgene polypropylene straight-sided jar with a polypropylene screw cap) and ship the jar containing the sample to the laboratory. The Nalgene containers did not present contamination problems nor did the results suggest that the analytes of

interest adhere to the jars, so Nalgene containers can be used instead of glass VOA vials used in standard practice.

9.1.2 The wipe is wetted with 1 mL of LC-MS grade water, sufficient to wet the wipe. The surface is wiped in a Z-like pattern horizontally across a defined surface (100 cm^2) (Attachment 19.3), folded, then used to wipe the same surface in a Z-like pattern vertically across a defined surface (100 cm^2). The wipe is placed into a 125 mL Nalgene polypropylene straight-sided jar with a polypropylene screw cap. Surrogates (66.6 µL of the SSS) and LC-MS grade water (5 mL) are added to the jar. Field and/or matrix blanks are needed, according to conventional sampling practices; therefore, one blank sample coupon was analyzed in every sample extraction batch.

9.2 SAMPLE STORAGE AND HOLDING TIMES

9.2.1 Wipe samples should be extracted as soon as possible after collection but must be extracted within 30 days of collection. Samples not immediately analyzed from a particular site should be carefully characterized to ensure there is no interaction with the wipe or a specific surface to cause interferences or degradation of the analytes. An LFSM can be generated for the appropriate time period to verify such an occurrence. Samples can be stored up to 30 days (Table 2) at 4 ºC (± 2 ºC).

10 QUALITY CONTROL

10.1 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 precision and accuracy study (P&A, as shown in section 19.2) as well as a Detection Limit (DL) study (Table 3 and section 19.1) must be performed to demonstrate laboratory capability. Laboratories are encouraged to institute additional QC practices to meet their specific needs.

10.2 INITIAL DEMONSTRATION OF CAPABILITY (IDC)

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 11.2.

10.2.1 INITIAL DEMONSTRATION OF LOW SYSTEM BACKGROUND

Any time a new lot of solvents, reagents, filters and autosampler vials is 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 INITIAL DEMONSTRATION OF PRECISION AND ACCURACY (P&A)

NOTE: Because porosity of the wiped surface will inevitably have an effect on analyte recovery from the surface, accuracy results between calculated values and true values may differ from surface to surface. The precision and accuracy results are based on the wipe used on the laminate (Formica®, Formica Corp., Cincinnati, OH) surface because (1) the laminate surface has been shown to be free of contamination, (2) this surface results in minimal surface interaction between the chemical and the surface, and (3) the laminate is a relatively nonporous surface.

For a P&A, prepare a check standard containing DIMP, EMPA, MPA, PMPA, EHDMAP, and IMPA near or below the midpoint concentration of the calibration range. This check standard should be analyzed with a minimum of four replicates. For this study, four different concentrations are chosen with seven samples each. The check samples are analyzed according to Section 12.

10.2.3 The average percent recovery (X), standard deviations (σ) and the percent relative standard deviation (%RSD) of the recoveries are calculated for each analyte. The % RPD limit of ≤ 30% should be applied to all replicate analyses.

10.2.4 MINIMUM REPORTING LEVEL (MRL)

Establish a target concentration for the MRL based on the intended use of the method. Establish an Initial Calibration (Section 11.2). The lowest 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. The MRL is validated following the procedure below.

10.2.4.1 Fortify, extract, and analyze seven replicate LFBs at the proposed MRL concentration. Calculate the mean measured concentration (*Mean*) and standard deviation for these replicates. Determine the Half Range for the prediction interval of results (HR_{PIR}) using the equation below

$$HR_{PIR} = 3.963s$$

where

s = the standard deviation

3.963 = a constant value for seven replicates (10).

10.2.4.2 Confirm that the upper and lower limits for the Prediction Interval of Result ($PIR = Mean \pm HR_{PIR}$) meet the upper and lower recovery limits as shown below

The Upper PIR Limit must be ≤150% recovery.

$$\frac{Mean + HR_{PIR}}{FortifiedConcentration} \times 100\% \leq 150\%$$

The Lower PIR Limit must be ≥ 50% recovery.

$$\frac{Mean - HR_{PIR}}{FortifiedConcentration} \times 100\% \geq 50\%$$

10.2.5 CALIBRATION VERIFICATION

Mid-level and low-level samples from the calibration curve should be analyzed to confirm the accuracy of the fit of the calibration curve/standards after the end of sample batches.

10.3 METHOD DETECTION LIMITS (MDL)

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 (IDLs)

The laboratory 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 (MDL)

Method Detection Limits (MDLs) represent the optimal detection achieved by a laboratory in a matrix of interest. The analyte spiking solution, containing all six analytes, was added to the surface (section 19.3). The solution on the surface was allowed to completely dry and wiped using a wetted-cotton gauze wipe. Wipe extracts from the laminate coupons are used for the determination of the MDL for surface samples. 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 9.3.1). 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):

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

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 of replicate analyses.
σ = standard deviation of the percent recovery

Data for MDLs are shown in Table 3 and Section 19.1.

10.4 ONGOING QUALITY CONTROL (QC) REQUIREMENTS

10.4.1 LABORATORY FORTIFIED BLANK (LFB)

An LFB is required with each extraction batch to confirm that potential background contaminants are not interfering with 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. LFBs include cotton gauze wipes wetted with water.

10.4.2 LABORATORY METHOD BLANK (LMB)

An LMB is prepared and analyzed with each extraction batch, using LC-MS grade reagent water, 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. LMBs include the extracted wipe used to wipe the surface coupon.

10.4.3 CONTINUING CALIBRATION CHECK (CCC)

CCC standards are analyzed at the beginning and end of each analysis batch. The CCC is analyzed periodically to verify the accuracy of the existing calibration for analytes near the midpoint of the calibration range and/or near the MRL. CCC values should be specified by the sample submitter's Data Quality Objectives (DQOs) or fulfill other QC requirements, such as LFSM acceptance).

10.4.4 LABORATORY FORTIFIED SAMPLE MATRIX (LFSM)

A LFSM 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., coupon/wipe materials). If a variety of sample matrices are analyzed, performance should be established for each surface.

10.4.4.1 Within each analysis batch, an LFSM is prepared and analyzed at a frequency of one sample matrix for every twenty samples. The LFSM is prepared by spiking a sample with the appropriate amount of AS (Section 8.2.2). Select a spiking concentration that is greater than or equal to the matrix background concentration, if known. Records are maintained of the surface target compound spike analyses, and the average percent recovery (X) and the standard deviation of the percent recovery (σ) 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 CCC 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 CCCs, 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 (CCCs, LFBs, LMBs, LFSMs, LFSMDs, FDs, and CAL standards) are spiked with surrogate standard spiking solution as described in Section 8.2.1. 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 FIELD DUPLICATE (FD) OR LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (LFSMD)

Within each analysis batch, a minimum of one FD or LFSMD should be analyzed for every twenty samples. Target compound spike accuracy in the sample matrix is monitored and updated regularly. Duplicates check the precision associated with sample collection, storage and laboratory procedures. Records are maintained of spiked matrix analyses and the average percent recovery (X) and corresponding standard deviation (σ) are calculated. FD/LFSMD samples must be incorporated into the field sampling plan. If the laboratory did not receive FD samples for determination of site-specific P&A, the laboratory will evaluate the site data quality based on the LFSM data, if there is sufficient sample in the site samples to conduct an analysis. FD/LFSMD recovery results will be used for site-specific P&A data. LFSM data are used as FD/LFSMD sample data for this study. RPD values should be $\leq$ 30% for FD/LFSMD samples.

10.4.6.1 Calculate the relative percent difference (RPD) for duplicate measurements (FD_1 and FD_2) using the equation:

$$\text{RPD} = \frac{\left|\text{FD}_1 - \text{FD}_2\right|}{\left(\text{FD}_1 + \text{FD}_2\right)/2} \times 100$$

RPDs for Field Duplicates should be $\leq$ 30% for each analyte. Greater variability may be observed when Field Duplicates have analyte concentrations at or near the MRL (within a factor of two times the MRL concentration). At these concentrations, FDs must have RPDs that are $\leq$ 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 CCC and in the LFB, the precision is judged matrix influenced. Report the result for the corresponding analyte in the unfortified sample as "suspect/matrix."

10.4.6.2 If an LFSMD is analyzed instead of an FD, calculate the RPD for the LFSM and LFSMD using the equation: $$RPD = \frac{|LFSM - LFSMD|}{(LFSM + LFSMD)/2} \times 100$$

RPDs for duplicate LFSMs should be ≤ 30% 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). LFSMs 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 CCC and in the LFB, 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 CCC (at the appropriate concentration described in section 10.4.2) should be performed at the beginning and end of each analysis batch.

11.1 CALIBRATION OF MASS SPECTROMETER

Calibrate the mass scale of the mass spectrometer as prescribed by the manufacturer. The mass calibration file is saved in the mass spectrometer software file folder (MassLynx™ or similar software). The mass calibration solution used in this method is a mixture of NaCsI provided by the manufacturer. Other calibration solutions can also be used per instrument manufacturer's specifications.

11.2 INITIAL CALIBRATION FOR ANALYTES

11.2.1 ESI negative mode is the preferred choice for this method due to the optimal conditions and advantages (e.g., greater peak intensity, few interferences, and lower background) in ESI negative mode over ESI positive mode. However, ESI positive mode may be used if matrix interferences become problematic. The data are presented in both modes in some tables, but for clarification, only ESI negative mode will be discussed in the method.

11.2.2 Optimize the $[M-H]^-$ ion in ESI negative mode for each analyte by infusing an appropriate calibration solution at a flow rate similar to that 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 following the same procedures as for the $[M-H]^-$ ion. 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 Tables 4a and 4b and 5.

11.2.3 Establish LC operating conditions that will optimize peak resolution and shape. Suggested LC conditions (listed in Table 6) may not be optimal for all LC systems.

11.2.4 The initial calibration contains a seven-point curve using the analyte concentrations prepared in section 8.2.3 and shown in Table 7. The lowest calibration curve standard must be 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. At a minimum, a five-point linear or a six-point quadratic calibration curve will be utilized for all analytes. 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. If the low and/or high point is excluded, a six-point curve is acceptable but the calibration range and reporting limits must be modified to reflect this change. 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 (9). 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.

11.3 QUANTITATION OF ANALYTES

The quantitation of the target analytes is accomplished with quantitation software as it relates to each specific instrument (9). An external calibration is used along with monitoring MPA-d_3, PMPA-$^{13}C_6$ and DIMP-d_{14} surrogate recoveries. Refer to Tables 4a and 4b 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. Surrogates (MPA-d_3, PMPA-$^{13}C_6$ and DIMP-d_{14}) are added first, then LC-MS grade water (5 mL) is added to the jar. Sonicate each jar containing the solution for approximately 15 minutes in a water bath at room temperature with no heat required.

12.1.2 After sonication, decant the extraction solvent into a 10 cc lock-tip sterile fitted syringe with a Millex® GV syringe driven filter unit, PVDF filter (0.22 μm), transferring the filtered sample to a sterile 15-mL polypropylene tube (or equivalent).

12.1.3 Transfer (via pipette) to a standard 2 mL sample vial.

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

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 4a, 4b, 5 and 6.

12.2.2 Prepare an analytical batch that includes all QC samples and surface samples. The first sample to be analyzed is a 10 μL injection of a blank (LC-MS grade reagent water) 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 (LC-MS grade reagent water) to ensure there is no carryover (11). 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 CCC must have each analyte concentration within 30% 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 7 in Table 7), the prepared sample is diluted with water 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 standards. 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 (9).

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 PRECISION, ACCURACY AND DETECTION LIMITS

14.1.1 Tables for precision, accuracy and detection limit results for a single laboratory study are presented in Sections 19.1 and 19.2 and Table 3.

14.2 RECOVERIES AND PRECISION FOR OTHER SURFACE TYPES

14.2.1 Section 19.2 lists recoveries and precision of target analytes for a variety of other surfaces.

14.3 WIPE STORAGE STABILITY STUDY

14.3.1 Extract storage was conducted on the laminate surface fortified with the targeted method analytes. Precision and accuracy (n = 4) of the extracts were analyzed on days 0, 2, 3, 7, 14, 21, and 30 days and are reported in Table 2.

14.4 PROBLEM ANALYTES AND SURFACES

14.4.1 TARGET ANALYTES ON UNCLEANED SURFACES

DIMP and some EHDMAP recoveries may be problematic due to the volatility or rapid decomposition of these specific compounds (12). Analysts should be aware that these two specific compounds may not be present within the tested sample matrix and plan accordingly. EHDMAP detection limits in this method are based on samples extracted within the same day. Due to the degradation of EHDMAP, samples analyzed after 24 hours may reflect different results. Furthermore, ESI (+) analysis results are problematic for certain compounds (e.g., IMPA and MPA) due to possible electrospray enhancement/suppression effects, whereas ESI (-) results tend to be more reliable. Both ESI (+) and (-) results are presented. However, detection limits are based on ESI (-) data. Wood surfaces resulted in poor recoveries outside the range of this procedure. As a result, the method should not be used to identify these analytes on a wood surface. Although porosity of the surface is most likely the culprit for low recoveries, further analysis should be performed to determine definitive reasons for poor recoveries from the surface. Direct extraction of the analytes from wood could be used to elucidate whether or not chemical interactions are occurring between target analytes and compounds found in a wood matrix.

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

16.1 The analytical procedures described in this procedure 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.

16.2 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 Restoration Following Homeland Security Events* (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. *Standard Practices for Preparation of Sample Containers and for Preservation of Organic Constituents*, ASTM Annual Book of Standards, Part 31, D3694-78. Philadelphia: American Society for Testing and Materials.

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

7. *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.

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

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

10. Winslow, S.D., Pepich, B.V., Martin, J.J., Hallberg, G.R., Munch, D.J., Frebis, C.P., Hedrick, E.J., Krop, R.A. "Statistical Procedures for Determination and Verification of Minimum Reporting Levels for Drinking Water Methods." *Environ. Sci. Technol.* 2006, **40**, 281-288.

11. Peters, F.T.; Drummer, O.H.; Musshoff, F. "Validation of New Methods", Forensic Science International **2007**, 165 (2): 216-224

12. ASTM Standard D7597 - 09e1, 2009 "Standard Test Method for Determination of Diisopropyl Methylphosphonate, Ethyl Hydrogen Dimethylamidophosphate, Ethyl Methylphosphonic Acid, Isopropyl Methylphosphonic Acid, Methylphosphonic Acid and Pinacolyl Methylphosphonic Acid in Water by Liquid, Chromatography/Tandem Mass Spectrometry" West Conshohocken, PA: ASTM International. 2009, DOI: 10.1520/D7597-09E01 , www.astm.org.

18 TABLES AND VALIDATION DATA

Item *Title*

Table 1. Materials Tested for the Wipe Analysis of Nerve Agent Degradation Products

Material	Manufacturer/Vendor
Glass	Carolina Glass Co./Lowe's
Vinyl Tile	Armstrong/Home Depot
Laminate	Wilsonart® Laminate/Home Depot
Wood (southern pine, pre-treated)	Home Depot
Galvanized steel	McMaster-Carr
Painted Drywall (BEHR® latex paint)	BEHR/Home Depot

Table 2. Holding Time Sample Stability of Nerve Agent Degradation Analytes of Wipe Samples in ESI Negative Mode

ESI (-) Mode														
Concentration	IMPA		MPA		MPA-d_3		EMPA		EHDMAP		PMPA		PMPA-$^{13}C_6$	
ng/mL	175		175		175		70		175		35		175	
Holding Time (days)	Average % Recovery	% RSD	Average % Recovery	% RSD	Average % Recovery	% RSD	Average % Recovery	% RSD	Average % Recovery	% RSD	Average % Recovery	% RSD	Average % Recovery	% RSD
0	87.8	5	86.1	2	78.9	3	74.7	2	106	3	79.9	2	79.5	2
2	72.0	3	70.9	2	72.7	1	65.7	4	24.4	2	74.6	1	71.3	1
3	77.3	2	77.2	2	73.8	4	77.4	4	20.8	3	77.7	1	75.4	1
7	85.7	9	77.8	4	75.6	3	75.1	2	28.2	15	74.4	4	74.4	1
14	75.3	4	72.1	3	71.6	3	68.5	3	29.5	3	74.6	3	74.8	2
21	78.7	2	75.7	3	72.3	4	74.9	1	33.4	3	79.8	3	70.6	2
30	75.3	7	74.4	7	73.5	7	78.7	7	48.8	54	73.5	4	72.6	4

RSD, relative standard deviation

Table 3. Method Parameters for Nerve Agent Degradation Products

LAMINATE			
Analyte	**MDL***		**MRL**
	ng/cm^2 †	**ng/mL**	**ng/mL**
IMPA	0.042	4.2	25
EMPA	0.050	5.0	10
EHDMAP	0.067	6.7	25
MPA	0.065	6.5	25
PMPA	0.017	1.7	5
DIMP	-	-	-

*Final DL Study-8/12. ESI$^-$ ionization mode provided the method detection limit (MDL) and minimum reporting level (MRL) values. See section 19.1 for complete DL data in both ionization modes.

†ng/cm^2 calculation was performed by dividing the concentration spiked onto the surface by the test area of the coupon (100 cm^2).

Table 4a. ESI (+) MRM Ion Transitions, Retention Time (RT) and Variable Mass Spectrometer Parameters

Analyte	Cone voltage	MRM mass transition (parent → product)	Collision energy (eV)	RT* (minutes)
DIMP	22	181.33 → 139.25	7	7.6
IMPA	22	139.29 → 96.80	18	6.6
EMPA	26	125.22 → 96.82	12	4.0
EHDMAP	28	154.29 → 125.82	16	3.2
MPA	45	97.25 → 79.20	15	1.8
DIMP-d_{14}	24	195.45 → 147.20	7	7.6
MPA-d_3	48	100.20 → 82.00	16	1.8

*Retention times should fall within 5% of the given value; otherwise re-analysis may be necessary.

Table 4b. ESI (-) MRM Ion Transitions, Retention Time (RT) and Variable Mass Spectrometer Parameters

Analyte	Cone voltage	MRM mass transition (parent → product)	Collision energy (eV)	RT* (minutes)
IMPA	30	137.18→95.00	18	6.6
EMPA	26	123.10→94.95	12	4.0
EHDMAP	30	152.17→78.92	12	3.2
PMPA	38	179.20→95.00	18	8.7
MPA	45	95.06→78.95	15	1.8
PMPA-$^{13}C_6$	34	185.22→94.99	18	8.7
MPA-d_3	37	98.00→78.80	15	1.8

*Retention times should fall within 5% of the given value; otherwise re-analysis may be necessary.

Table 5. ESI (+) and (-) MS/MS Conditions

MS Parameter	Setting
Capillary Voltage	4.3 kV
Cone Voltage	See Table 4a and b
Extractor	2 Volts
RF Lens	0.2 Volts
Source Temperature	150 °C
Desolvation Temperature	350 °C
Desolvation Gas Flow	600 L/hr
Cone Gas Flow	50 L/hr
Low Mass Resolution 1	14.5
High Mass Resolution 1	14.5
Ion Energy 1	0.5
Entrance Energy	1
Collision Energy	See Table 4a and b
Exit Energy	1
Low Mass Resolution 2	15.0
High Mass resolution 2	15.0
Ion Energy 2	0.5
Multiplier	-560
Gas Cell Pirani Gauge	3.0×10^{-3} Torr
Inter-Channel Delay	0.005 seconds
Inter-Scan Delay	0.005 seconds
Repeats	1
Span	0.1 Daltons
Dwell	0.15 Seconds

Table 6. Liquid Chromatography Gradient Conditions*

Time (min)	Flow (µL/min)	% Solution A†	% Solution B††
0	300	100	0
4	300	100	0
5	300	55	45
9	300	55	45
10	300	40	60
12	300	30	70
13	300	100	0
15	300	100	0

†A: Water (0.2% Formic Acid)
† †B: Acetonitrile (0.2% Formic Acid)
*Injection volume – 10 µL(recommended)
*Column Temperature: 30 ° C
*Autosampler Temperature: 15 °C
*Equilibration time: 2 minutes
*Column:, 100 mm x 2.1mm, 3µm particle size

Table 7. Target Concentrations of Calibration Standards Used During the Development of this Method (ng/mL)

Analyte/Surrogate	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7
DIMP	5	10	20	35	50	100	150
IMPA	25	50	100	175	250	500	750
EMPA	10	20	40	70	100	200	300
EHDMAP	25	50	100	175	250	500	750
PMPA	5	10	20	35	50	100	150
MPA	25	50	100	175	250	500	750
DIMP-d_{14}	5	10	20	35	50	100	150
PMPA-$^{13}C_6$	25	50	100	175	250	500	750
MPA-d_3	25	50	100	175	250	500	750

19 ATTACHMENTS

19.1 Method Detection Limit Data and Calculations

19.2 Precision and Accuracy

19.3 Illustration depicting the wiping pattern on a 100 cm^2 surface

19.1 METHOD DETECTION LIMIT (MDL) DATA AND CALCULATIONS

MDL Data for Seven Replicates for Nerve Agent Degradation Analytes

Analyte	LAMINATE in ESI (+) mode			LAMINATE in ESI (-) mode		
	Concentration 1*			Concentration 1*		
	Average Recovery (ng/mL)	% Recovery	% RSD	Average Recovery (ng/mL)	% Recovery	% RSD
IMPA	54.6	91.1	8	44.7	74.5	3
MPA	65.3	109	6	48.2	80.3	4
EMPA	19.5	81.1	8	20.3	84.5	8
EHDMAP	39.5	65.9	8	39.3	65.4	5
DIMP	ND	ND	-	-	-	-
PMPA	-	-	-	9.9	82.9	5
MPA-d_3	88.8	88.8	6	84.6	84.6	5
DIMP-d_{14}	16.8	84.1	4	-	-	-
PMPA-$^{13}C_6$	-	-	-	87.1	87.1	1
Analyte	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**
IMPA	0.546	91.1	8	0.447	74.5	3
MPA	0.653	109	6	0.482	80.3	4
EMPA	0.195	81.1	8	0.203	84.5	8
EHDMAP	0.395	65.9	8	0.393	65.4	5
DIMP	ND	ND	-	-	-	-
PMPA	-	-	-	0.100	82.9	5
MPA-d_3	0.888	88.8	6	0.846	84.6	5
DIMP-d_{14}	0.168	84.1	4	-	-	-
PMPA-$^{13}C_6$	-	-	-	0.871	87.1	1

*Concentration 1 correlates to the following analyte concentrations: 60 ng/mL for IMPA, MPA, and EHDMAP, 24 ng/mL for EMPA, and 12 ng/mL for DIMP and PMPA. Surrogate recovery concentrations correspond to the following: 100 ng/mL for MPA-d_3 and PMPA-$^{13}C_6$, and 20 ng/mL for DIMP-d_{14}.
†ng/cm^2 calculation was performed by dividing the concentration spiked onto the surface by the test area of the coupon (100 cm^2).

MDL Calculation for Seven Replicates for Nerve Agent Degradation Analytes

LAMINATE in ESI (+) mode			
Analyte	MDL		MRL
	ng/cm^2†	ng/mL	ng/mL
IMPA	0.15	15	25
MPA	0.12	12	25
EMPA	0.047	4.7	10
EHDMAP	0.11	11	25
DIMP	ND	ND	-

MDL, method detection limit; MRL, minimum reporting limit
†ng/cm^2 calculation was performed by dividing the concentration spiked onto the surface by the test area of the coupon (100 cm^2).

LAMINATE in ESI (-) mode			
Analyte	MDL		MRL
	ng/cm^2†	ng/mL	ng/mL
IMPA	0.042	4.2	25
MPA	0.065	6.5	25
EMPA	0.049	4.9	10
EHDMAP	0.067	6.7	25
PMPA	0.017	1.7	5

MDL, method detection limit; MRL, minimum reporting limit
†ng/cm^2 calculation was performed by dividing the concentration spiked onto the surface by the test area of the coupon (100 cm^2).

19.2 PRECISION AND ACCURACY

Concentration levels correspond to the following final concentrations on the surface: Concentration 1 is the same as in Attachment 19.1 (60 ng/mL for IMPA, MPA, and EHDMAP, 24 ng/mL for EMPA, and 12 ng/mL for DIMP and PMPA. Surrogate recovery concentrations correspond to the following for concentrations 1 and 2: 100 ng/mL for MPA-d_3 and PMPA-$^{13}C_6$, and 20 ng/mL for DIMP-d_{14}. Surrogate recovery concentrations correspond to the following for concentrations 3 and 4: 300 ng/mL for MPA-d_3 and PMPA-$^{13}C_6$, and 60 ng/mL for DIMP-d_{14}). Concentration 2 is calibration concentration level 3. Concentrations 3 and 4 are 2.25 and 3 times Concentration 2.

- **Table A. Precision and Accuracy Data for Wipe Analysis of Nerve Agent Degradation Analytes on Laminate Surfaces in ESI (+) Mode**
- **Table B. Precision and Accuracy Data for Wipe Analysis of Nerve Agent Degradation Analytes on Laminate Surfaces in ESI (-) Mode**
- **Table C. Precision and Accuracy Data for Wipe Analysis of Nerve Agent Degradation Analytes on Metal Surfaces in ESI (+) Mode**
- **Table D. Precision and Accuracy Data for Wipe Analysis of Nerve Agent Degradation Analytes on Metal Surfaces in ESI (-) Mode**
- **Table E. Precision and Accuracy Data for Wipe Analysis of Nerve Agent Degradation Analytes on Glass Surfaces in ESI (+) Mode**
- **Table F. Precision and Accuracy Data for Wipe Analysis of Nerve Agent Degradation Analytes on Glass Surfaces in ESI (-) Mode**
- **Table G. Precision and Accuracy Data for Wipe Analysis of Nerve Agent Degradation Analytes on Painted Drywall Surfaces in ESI (+) Mode**
- **Table H. Precision and Accuracy Data for Wipe Analysis of Nerve Agent Degradation Analytes on Painted Drywall Surfaces in ESI (-) Mode**
- **Table I. Precision and Accuracy Data for Wipe Analysis of Nerve Agent Degradation Analytes on Vinyl Tile Surfaces in ESI (+) Mode**
- **Table J. Precision and Accuracy Data for Wipe Analysis of Nerve Agent Degradation Analytes on Vinyl Tile Surfaces in ESI (-) Mode**
- **Table K. Precision and Accuracy Data for Wipe Analysis of Nerve Agent Degradation Analytes on Treated Wood Surfaces in ESI (+) and ESI (-) Mode**

P&A data for wipe analysis of nerve agent degradation analytes on surfaces.

Table A. Precision and Accuracy Data for Wipe Analysis of Nerve Agent Degradation Analytes on Laminate Surfaces in ESI (+) Mode

LAMINATE in ESI (+) mode												
Analyte	Concentration 1			Concentration 2			Concentration 3			Concentration 4		
	Average Recovery (ng/mL)*	% Recovery	% RSD	Average Recovery (ng/mL)*	% Recovery	% RSD	Average Recovery (ng/mL)*	% Recovery	% RSD	Average Recovery (ng/mL)*	% Recovery	% RSD
IMPA	54.6	91.1	8	97.6	97.6	10	219	97.4	6	283	94.2	18
MPA	65.3	109	6	115	115	8	277	123	5	263	87.6	5
EMPA	19.5	81.1	8	40.6	101	7	90.9	101	4	106	88.3	3
EHDMAP	39.5	65.9	8	72.6	72.6	9	127	56.5	6	210	70.1	9
DIMP	ND	-	-	ND	-	-	ND	-	-	ND	-	-
MPA-d_3	88.3	88.3	3	119	119	15	321	107	8	286	95.4	2
DIMP-d_{14}	16.8	84.0	4	18.2	91.2	13	45.3	75.4	8	50.4	84.0	8
Analyte	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**
IMPA	0.546	91.1	8	0.976	97.6	10	2.19	97.4	6	2.83	94.2	18
MPA	0.653	109	6	1.15	115	8	2.77	123	5	2.63	87.6	5
EMPA	0.195	81.1	8	0.406	101	7	0.909	101	4	1.06	88.3	3
EHDMAP	0.395	65.9	8	0.726	72.6	9	1.27	56.5	6	2.10	70.1	9
DIMP	ND	-	-	ND	-	-	ND	-	-	ND	-	-
MPA-d_3	0.883	88.8	3	1.19	119	15	3.21	107	8	2.86	95.4	2
DIMP-d_{14}	0.168	84.0	4	0.182	91.2	13	0.453	75.4	8	0.504	84.0	8

Concentration 1 is for low concentration of analyte on the surface, See attachments 19.1-19.2 for values; Concentration 2 is calibration concentration level 3; Concentrations 3 and 4 are 2.25 and 3 times Concentration 2; RSD is relative standard deviation

* (n = 7 samples at each concentration)

† ng/cm^2 calculation was performed by dividing the concentration spiked onto the surface by the test area of the coupon (100 cm^2).

Table B. Precision and Accuracy Data for Wipe Analysis of Nerve Agent Degradation Analytes on Laminate Surfaces in ESI (-) Mode

LAMINATE in ESI (-) mode												
Analyte	Concentration 1			Concentration 2			Concentration 3			Concentration 4		
	Average Recovery (ng/mL)*	% Recovery	% RSD	Average Recovery (ng/mL)*	% Recovery	% RSD	Average Recovery (ng/mL)*	% Recovery	% RSD	Average Recovery (ng/mL)*	% Recovery	% RSD
IMPA	44.7	74.5	3	69.2	69.2	9	146	64.8	5	234	77.8	1
MPA	48.2	80.3	4	58.3	58.3	6	132	58.5	5	243	80.9	3
EMPA	20.3	84.5	8	38.2	95.4	7	86.5	96.1	4	108	89.9	2
EHDMAP	39.3	65.4	5	71.4	71.4	10	128	56.9	7	217	72.5	5
PMPA	9.90	82.9	5	18.1	90.2	3	41.5	92.2	5	52.3	87.2	3
MPA-d_3	84.6	84.6	5	68.7	68.7	7	170	56.7	3	261	87.0	2
PMPA-$^{13}C_6$	87.1	87.1	1	94.6	94.6	14	245	81.5	4	276	92.1	2
Analyte	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**
IMPA	0.447	74.5	3	0.692	69.2	9	1.46	64.8	5	2.34	77.8	1
MPA	0.482	80.3	4	0.583	58.3	6	1.32	58.5	5	2.43	80.9	3
EMPA	0.203	84.5	8	0.382	95.4	7	0.865	96.1	4	1.08	89.9	2
EHDMAP	0.393	65.4	5	0.714	71.4	10	1.28	56.9	7	2.17	72.5	5
PMPA	0.0990	82.9	5	0.181	90.2	3	0.415	92.2	5	0.523	87.2	3
MPA-d_3	0.846	84.6	5	0.687	68.7	7	1.70	56.7	3	2.61	87.0	2
PMPA-$^{13}C_6$	0.871	87.1	1	0.946	94.6	14	2.45	81.5	4	2.76	92.1	2

Concentration 1 is for low concentration of analyte on the surface, See attachments 19.1-19.2 for values; Concentration 2 is calibration concentration level 3; Concentrations 3 and 4 are 2.25 and 3 times Concentration 2; RSD is relative standard deviation

* (n = 7 samples at each concentration)

† ng/cm^2 calculation was performed by dividing the concentration spiked onto the surface by the test area of the coupon (100 cm^2).

Table C. Precision and Accuracy Data for Wipe Analysis of Nerve Agent Degradation Analytes on Metal Surfaces in ESI (+) Mode

METAL in ESI (+) mode												
Analyte	Concentration 1			Concentration 2			Concentration 3			Concentration 4		
	Average Recovery (ng/mL)*	% Recovery	% RSD	Average Recovery (ng/mL)*	% Recovery	% RSD	Average Recovery (ng/mL)*	% Recovery	% RSD	Average Recovery (ng/mL)*	% Recovery	% RSD
IMPA	65.1	108	20	162	162	17	353	157	16	236	78.7	9
MPA	50.0	83.3	11	86.8	86.8	15	182	80.9	17	213	71.0	13
EMPA	19.6	81.6	12	32.5	81.2	6	74.8	83.1	4	85.7	71.4	7
EHDMAP	16.8	28.0	29	71.5	71.5	14	138	61.4	30	75.9	25.3	55
DIMP	ND	-	-	ND	-	-	ND	-	-	ND	-	-
MPA-d_3	142	142	4	106	106	6	288	96.1	3	210	69.8	5
DIMP-d_{14}	18.1	90.5	6	19.1	95.4	5	55.1	91.8	4	44.2	73.6	5
Analyte	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**
IMPA	0.651	108	20	1.62	162	17	3.53	157	16	2.36	78.7	9
MPA	0.500	83.3	11	0.868	86.8	15	1.82	80.9	17	2.13	71.0	13
EMPA	0.196	81.6	12	0.325	81.2	6	0.748	83.1	4	0.857	71.4	7
EHDMAP	0.168	28.0	29	0.715	71.5	14	1.38	61.4	30	0.759	25.3	55
DIMP	ND	-	-	ND	-	-	ND	-	-	ND	-	-
MPA-d_3	1.42	142	4	1.06	106	6	2.88	96.1	3	2.10	69.8	5
DIMP-d_{14}	0.181	90.5	6	0.191	95.4	5	0.551	91.8	4	0.442	73.6	5

Concentration 1 is for low concentration of analyte on the surface, See attachments 19.1-19.2 for values; Concentration 2 is calibration concentration level 3; Concentrations 3 and 4 are 2.25 and 3 times Concentration 2; RSD is relative standard deviation

* (n = 7 samples at each concentration)

† ng/cm^2 calculation was performed by dividing the concentration spiked onto the surface by the test area of the coupon (100 cm^2).

Table D. Precision and Accuracy Data for Wipe Analysis of Nerve Agent Degradation Analytes on Metal Surfaces in ESI (-) Mode

METAL in ESI (-) mode												
Analyte	Concentration 1			Concentration 2			Concentration 3			Concentration 4		
	Average Recovery (ng/mL)*	% Recovery	% RSD	Average Recovery (ng/mL)*	% Recovery	% RSD	Average Recovery (ng/mL)*	% Recovery	% RSD	Average Recovery (ng/mL)*	% Recovery	% RSD
IMPA	42.7	71.2	8	65.3	65.3	8	127	56.2	2	245	81.8	4
MPA	28.2	47.0	9	58.1	58.1	12	119	52.8	10	172	57.3	12
EMPA	20.0	83.4	8	35.6	89.0	7	76.1	84.6	3	96.8	80.7	4
EHDMAP	13.0	21.7	83	75.6	75.6	16	142	62.9	35	87.8	29.3	48
PMPA	8.80	73.0	6	15.7	78.6	4	35.6	79.2	4	45.8	76.3	5
MPA-d_3	89.0	89.0	11	67.1	67.1	9	185	61.5	7	185	61.8	5
PMPA-$^{13}C_6$	66.9	66.9	3	89.8	89.8	4	266	88.6	3	203	67.6	2
Analyte	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**
IMPA	0.427	71.2	8	0.653	65.3	8	1.27	56.2	2	2.45	81.8	4
MPA	0.282	47.0	9	0.581	58.1	12	1.19	52.8	10	1.72	57.3	12
EMPA	0.200	83.4	8	0.356	89.0	7	0.761	84.6	3	0.968	80.7	4
EHDMAP	0.130	21.7	83	0.756	75.6	16	1.42	62.9	35	0.878	29.3	48
PMPA	0.0880	73.0	6	0.157	78.6	4	0.356	79.2	4	0.458	76.3	5
MPA-d_3	0.890	89.0	11	0.671	67.1	9	1.85	61.5	7	1.85	61.8	5
PMPA-$^{13}C_6$	0.669	66.9	3	0.898	89.8	4	2.66	88.6	3	2.03	67.6	2

Concentration 1 is for low concentration of analyte on the surface, See attachments 19.1-19.2 for values; Concentration 2 is calibration concentration level 3; Concentrations 3 and 4 are 2.25 and 3 times Concentration 2; RSD is relative standard deviation

* (n = 7 samples at each concentration)

† ng/cm^2 calculation was performed by dividing the concentration spiked onto the surface by the test area of the coupon (100 cm^2).

Table E. Precision and Accuracy Data for Wipe Analysis of Nerve Agent Degradation Analytes on Glass Surfaces in ESI (+) Mode

GLASS in ESI (+) mode												
Analyte	Concentration 1			Concentration 2			Concentration 3			Concentration 4		
	Average Recovery (ng/mL)*	% Recovery	% RSD	Average Recovery (ng/mL)*	% Recovery	% RSD	Average Recovery (ng/mL)*	% Recovery	% RSD	Average Recovery (ng/mL)*	% Recovery	% RSD
IMPA	141	234	13	210	210	10	292	129	31	444	148	8
MPA	71.4	119	11	105	105	7	253	112	4	347	116	4
EMPA	25.0	104	5	33.4	83.5	7	84.0	93.3	6	113	94.4	2
EHDMAP	44.1	73.4	6	121	121	4	217	96.5	5	270	90.1	3
DIMP	ND	-	-	ND	-	-	ND	-	-	ND	-	-
MPA-d_3	172	172	6	111	111	10	286	95.3	5	275	91.8	2
DIMP-d_{14}	17.1	85.7	10	17.4	86.8	11	46.6	77.7	8	43.2	72.0	7
Analyte	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**
IMPA	1.41	234	13	2.10	210	10	2.92	129	31	4.44	148	8
MPA	0.714	119	11	1.05	105	7	2.53	112	4	3.47	116	4
EMPA	0.250	104	5	0.334	83.5	7	0.840	93.3	6	1.13	94.4	2
EHDMAP	0.441	73.4	6	1.21	121	4	2.17	96.5	5	2.70	90.1	3
DIMP	ND	-	-	ND	-	-	ND	-	-	ND	-	-
MPA-d_3	1.72	172	6	1.11	111	10	2.86	95.3	5	2.75	91.8	2
DIMP-d_{14}	0.171	85.7	10	0.174	86.8	11	0.466	77.7	8	0.432	72.0	7

Concentration 1 is for low concentration of analyte on the surface, See attachments 19.1-19.2 for values; Concentration 2 is calibration concentration level 3; Concentrations 3 and 4 are 2.25 and 3 times Concentration 2; RSD is relative standard deviation

* (n = 7 samples at each concentration)

† ng/cm^2 calculation was performed by dividing the concentration spiked onto the surface by the test area of the coupon (100 cm^2).

Table F. Precision and Accuracy Data for Wipe Analysis of Nerve Agent Degradation Analytes on Glass Surfaces in ESI (-) Mode

GLASS in ESI (-) mode												
Analyte	Concentration 1			Concentration 2			Concentration 3			Concentration 4		
	Average Recovery (ng/mL)*	% Recovery	% RSD	Average Recovery (ng/mL)*	% Recovery	% RSD	Average Recovery (ng/mL)*	% Recovery	% RSD	Average Recovery (ng/mL)*	% Recovery	% RSD
IMPA	40.4	67.4	7	51.6	51.6	2	131	58.4	9	163	54.3	6
MPA	33.1	55.2	9	65.5	65.5	8	145	64.3	8	185	61.5	7
EMPA	23.1	96.0	9	32.6	81.4	9	83.2	92.4	2	117	97.3	5
EHDMAP	28.2	47.1	18	113	113	12	225	100	23	307	102	6
PMPA	10.7	88.8	3	10.8	54.0	4	39.4	87.5	3	54.8	91.4	2
MPA-d_3	99.2	99.2	2	62.4	62.4	15	169	56.2	8	149	49.7	9
PMPA-$^{13}C_6$	139	139	4	96.3	96.3	7	247	82.3	6	240	79.9	3
Analyte	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**
IMPA	0.404	67.4	7	0.516	51.6	2	1.31	58.4	9	1.63	54.3	6
MPA	0.331	55.2	9	0.655	65.5	8	1.45	64.3	8	1.85	61.5	7
EMPA	0.231	96.0	9	0.326	81.4	9	0.832	92.4	2	1.17	97.3	5
EHDMAP	0.282	47.1	18	1.13	113	12	2.25	100	23	3.07	102	6
PMPA	0.107	88.8	3	0.108	54.0	4	0.394	87.5	3	0.548	91.4	2
MPA-d_3	0.992	99.2	2	0.624	62.4	15	1.69	56.2	8	1.49	49.7	9
PMPA-$^{13}C_6$	1.39	139	4	0.963	96.3	7	2.47	82.3	6	2.40	79.9	3

Concentration 1 is for low concentration of analyte on the surface, See attachments 19.1-19.2 for values; Concentration 2 is calibration concentration level 3; Concentrations 3 and 4 are 2.25 and 3 times Concentration 2; RSD is relative standard deviation

* (n = 7 samples at each concentration)

† ng/cm^2 calculation was performed by dividing the concentration spiked onto the surface by the test area of the coupon (100 cm^2).

Table G. Precision and Accuracy Data for Wipe Analysis of Nerve Agent Degradation Analytes on Painted Drywall Surfaces in ESI (+) Mode

PAINTED DRYWALL in ESI (+) mode												
Analyte	Concentration 1			Concentration 2			Concentration 3			Concentration 4		
	Average Recovery (ng/mL)*	% Recovery	% RSD	Average Recovery (ng/mL)*	% Recovery	% RSD	Average Recovery (ng/mL)*	% Recovery	% RSD	Average Recovery (ng/mL)*	% Recovery	% RSD
IMPA	40.3	67.1	6	67.0	67.0	3	184	81.5	6	236	78.5	3
MPA	59.0	98.3	28	110	110.1	11	223	99.1	5	285	95.0	8
EMPA	18.9	78.7	14	32.5	81.2	7	64.1	71.3	3	78.6	65.5	5
EHDMAP	33.9	56.6	10	50.0	50.0	6	163	72.3	3	231	77.0	2
DIMP	ND	-	-	ND	-	-	ND	-	-	ND	-	-
MPA-d_3	114	114	7	123	123	8	282	94.0	7	299	99.7	8
DIMP-d_{14}	14.6	73.1	13	16.2	81.1	9	45.7	76.1	8	47.5	79.2	9
Analyte	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**
IMPA	0.403	67.1	6	0.670	67.0	3	1.84	81.5	6	2.36	78.5	3
MPA	0.590	98.3	28	1.10	110	11	2.23	99.1	5	2.85	95.0	8
EMPA	0.189	78.7	14	0.325	81.2	7	0.641	71.3	3	0.786	65.5	5
EHDMAP	0.339	56.6	10	0.500	50.0	6	1.63	72.3	3	2.31	77.0	2
DIMP	ND	-	-	ND	-	-	ND	-	-	ND	-	-
MPA-d_3	1.14	114	7	1.23	123	8	2.82	94.0	7	2.99	99.7	8
DIMP-d_{14}	0.146	73.1	13	0.162	81.1	9	0.457	76.1	8	0.475	79.2	9

Concentration 1 is for low concentration of analyte on the surface, See attachments 19.1-19.2 for values; Concentration 2 is calibration concentration level 3; Concentrations 3 and 4 are 2.25 and 3 times Concentration 2; RSD is relative standard deviation

* (n = 7 samples at each concentration)

† ng/cm^2 calculation was performed by dividing the concentration spiked onto the surface by the test area of the coupon (100 cm^2).

Table H. Precision and Accuracy Data for Wipe Analysis of Nerve Agent Degradation Analytes on Painted Drywall Surfaces in ESI (-) Mode

PAINTED DRYWALL in ESI (-) mode												
Analyte	Concentration 1			Concentration 2			Concentration 3			Concentration 4		
	Average Recovery (ng/mL)*	% Recovery	% RSD	Average Recovery (ng/mL)*	% Recovery	% RSD	Average Recovery (ng/mL)*	% Recovery	% RSD	Average Recovery (ng/mL)*	% Recovery	% RSD
IMPA	44.9	74.8	12	59.6	59.6	6	155	68.8	4	216	72.1	4
MPA	22.4	37.4	13	33.2	33.2	16	142	63.1	3	180	30.1	5
EMPA	20.4	85.2	5	28.6	71.5	9	62.3	69.3	3	74.1	61.7	2
EHDMAP	31.5	52.6	8	49.9	49.9	5	155	68.8	3	224	74.7	3
PMPA	13.2	110	6	13.2	65.8	6	33.7	74.9	2	45.4	75.7	2
MPA-d_3	75.6	75.6	6	68.0	68.0	9	205	68.3	4	200	66.6	7
PMPA-$^{13}C_6$	81.6	81.6	4	81.6	81.6	4	255	85.0	3	257	85.8	3
Analyte	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**
IMPA	0.449	74.8	12	0.596	59.6	6	1.55	68.8	4	2.16	72.1	4
MPA	0.224	37.4	13	0.332	33.2	16	1.42	63.1	3	1.80	30.1	5
EMPA	0.204	85.2	5	0.286	71.5	9	0.623	69.3	3	0.741	61.7	2
EHDMAP	0.315	52.6	8	0.499	49.9	5	1.55	68.8	3	2.24	74.7	3
PMPA	0.132	110	6	0.132	65.8	6	0.337	74.9	2	0.454	75.7	2
MPA-d_3	0.756	75.6	6	0.680	68.0	9	2.05	68.3	4	2.00	66.6	7
PMPA-$^{13}C_6$	0.816	81.6	4	0.816	81.6	4	2.55	85.0	3	2.57	85.8	3

Concentration 1 is for low concentration of analyte on the surface, See attachments 19.1-19.2 for values; Concentration 2 is calibration concentration level 3; Concentrations 3 and 4 are 2.25 and 3 times Concentration 2; RSD is relative standard deviation

* (n = 7 samples at each concentration)

† ng/cm^2 calculation was performed by dividing the concentration spiked onto the surface by the test area of the coupon (100 cm^2).

Table I. Precision and Accuracy Data for Wipe Analysis of Nerve Agent Degradation Analytes on Vinyl Tile Surfaces in ESI (+) Mode

VINYL TILE in ESI (+) mode												
Analyte	**Concentration 1**			**Concentration 2**			**Concentration 3**			**Concentration 4**		
	Average Recovery (ng/mL)*	**% Recovery**	**% RSD**	**Average Recovery (ng/mL)***	**% Recovery**	**% RSD**	**Average Recovery (ng/mL)***	**% Recovery**	**% RSD**	**Average Recovery (ng/mL)***	**% Recovery**	**% RSD**
IMPA	57.3	95.5	16	95.0	95.0	26	191	84.8	9	290	96.8	11
MPA	87.5	146	21	108	108	8	136	60.6	13	208	69.4	6
EMPA	22.2	92.6	6	32.8	81.9	13	62.9	69.9	3	111	92.4	5
EHDMAP	42.2	70.4	7	53.6	53.6	7	164	73.0	3	231	76.9	5
DIMP	ND	-	-	ND	-	-	ND	-	-	ND	-	-
MPA-d_3	92.0	92.0	9	94.8	94.8	8	228	76.1	2	250	83.3	2
DIMP-d_{14}	15.0	75.1	8	15.4	76.8	7	52.4	87.4	4	44.7	74.5	10
Analyte	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**
IMPA	0.573	95.5	16	0.950	95.0	26	1.91	84.8	9	2.90	96.8	11
MPA	0.875	146	21	1.08	108	8	1.36	60.6	13	2.08	69.4	6
EMPA	0.222	92.6	6	0.328	81.9	13	0.629	69.9	3	1.11	92.4	5
EHDMAP	0.422	70.4	7	0.536	53.6	7	1.64	73.0	3	2.31	76.9	5
DIMP	ND	-	-	ND	-	-	ND	-	-	ND	-	-
MPA-d_3	0.920	92.0	9	0.948	94.8	8	2.28	76.1	2	2.50	83.3	2
DIMP-d_{14}	0.150	75.1	8	0.154	76.8	7	0.524	87.4	4	0.447	74.5	10

Concentration 1 is for low concentration of analyte on the surface, See attachments 19.1-19.2 for values; Concentration 2 is calibration concentration level 3; Concentrations 3 and 4 are 2.25 and 3 times Concentration 2; RSD is relative standard deviation

* (n = 7 samples at each concentration)

† ng/cm^2 calculation was performed by dividing the concentration spiked onto the surface by the test area of the coupon (100 cm^2).

Table J. Precision and Accuracy Data for Wipe Analysis of Nerve Agent Degradation Analytes on Vinyl Tile Surfaces in ESI (-) Mode

VINYL TILE in ESI (-) mode												
Analyte	Concentration 1			Concentration 2			Concentration 3			Concentration 4		
	Average Recovery (ng/mL)*	% Recovery	% RSD	Average Recovery (ng/mL)*	% Recovery	% RSD	Average Recovery (ng/mL)*	% Recovery	% RSD	Average Recovery (ng/mL)*	% Recovery	% RSD
IMPA	47.0	78.4	12	66.9	66.9	5	161	71.8	2	211	70.4	3
MPA	41.1	68.5	10	69.7	69.7	7	119	52.9	6	165	55.1	4
EMPA	19.0	79.2	11	27.3	68.2	13	65.8	73.2	3	113	94.3	4
EHDMAP	47.9	79.8	10	59.1	59.1	11	168	74.7	3	242	80.6	4
PMPA	8.2	68.5	4	12.2	60.8	6	33.5	74.4	3	43.8	73.0	3
MPA-d_3	86.9	86.9	8	80.6	80.6	11	217	72.3	3	225	74.9	4
PMPA-$^{13}C_6$	81.1	81.1	4	81.8	81.8	6	250.0	83.3	3	266	88.7	3
Analyte	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**
IMPA	0.470	78.4	12	0.669	66.9	5	1.61	71.8	2	2.11	70.4	3
MPA	0.411	68.5	10	0.697	69.7	7	1.19	52.9	6	1.65	55.1	4
EMPA	0.190	79.2	11	0.273	68.2	13	0.658	73.2	3	1.13	94.3	4
EHDMAP	0.479	79.8	10	0.591	59.1	11	1.68	74.7	3	2.42	80.6	4
PMPA	0.0820	68.5	4	0.122	60.8	6	0.335	74.4	3	0.438	73.0	3
MPA-d_3	0.869	86.9	8	0.806	80.6	11	2.17	72.3	3	2.25	74.9	4
PMPA-$^{13}C_6$	0.811	81.1	4	0.818	81.8	6	2.50	83.3	3	2.66	88.7	3

Concentration 1 is for low concentration of analyte on the surface, See attachments 19.1-19.2 for values; Concentration 2 is calibration concentration level 3; Concentrations 3 and 4 are 2.25 and 3 times Concentration 2.

RSD is relative standard deviation

* (n = 7 samples at each concentration)

† ng/cm^2 calculation was performed by dividing the concentration spiked onto the surface by the test area of the coupon (100 cm^2).

Table K. Precision and Accuracy Data for Wipe Analysis of Nerve Agent Degradation Analytes on Treated Wood Surfaces in ESI (+) and ESI (-) Mode

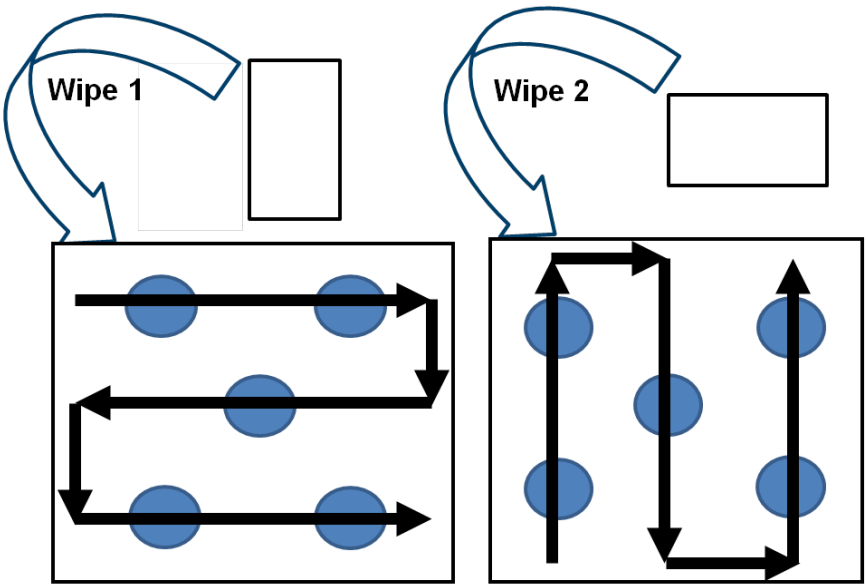
Analyte	TREATED WOOD in ESI (+) mode			TREATED WOOD in ESI (-) mode		
	Concentration 4			Concentration 4		
	Average Recovery (ng/mL)*	% Recovery	% RSD	Average Recovery (ng/mL)*	% Recovery	% RSD
IMPA	20.9	7.00	17	12.3	4.10	9
MPA	5.70	1.90	121	10.7	3.60	16
EMPA	ND	ND	-	ND	ND	-
EHDMAP	8.90	3.00	14	8.1	2.70	21
DIMP	ND	-	-	ND	-	-
PMPA	-	-	-	1.8	3.00	16
MPA-d_3	257	85.6	5	238	79.5	8
DIMP-d_{14}	55.5	92.6	7	-	-	-
PMPA-$^{13}C_6$	-	-	-	277	92.3	3
Analyte	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**	**Average Recovery (ng/cm^2)†**	**% Recovery**	**% RSD**
IMPA	0.209	7.0	17	0.123	4.1	9
MPA	0.570	1.9	121	0.107	3.6	16
EMPA	ND	-	-	ND	-	-
EHDMAP	0.0890	3.0	14	0.0810	2.7	21
DIMP	ND	-	-	ND	-	-
PMPA	-	-	-	0.0180	3.0	16
MPA-d_3	2.57	85.6	5	2.38	79.5	8
DIMP-d_{14}	0.555	92.6	7	-	-	-
PMPA-$^{13}C_6$	-	-	-	2.77	92.3	3

Concentration 1 is for low concentration of analyte on the surface, See attachments 19.1-19.2 for values; Concentration 2 is calibration concentration level 3; Concentrations 3 and 4 are 2.25 and 3 times Concentration 2. RSD is relative standard deviation

* (n = 7 samples at each concentration)

† ng/cm^2 calculation was performed by dividing the concentration spiked onto the surface by the test area of the coupon (100 cm^2).

19.3 Illustration of wiping pattern on 100 cm^2 surface



The analyte spike solution, containing the six analytes of interest, was added to the surface as shown in 19.3, allowed to completely dry (approximately 60-90 minutes depending on droplet size), and wiped using wetted-cotton gauze wipes.

United States
Environmental Protection
Agency

PRESORTED STANDARD
POSTAGE & FEES PAID
EPA
PERMIT NO. G-35

Office of Research and Development (8101R)
Washington, DC 20460

Official Business
Penalty for Private Use
$300