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Surface Analysis Using Wipes for the Determination of Nitrogen Mustard Degradation Products by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

SAMPLING AND ANALYTICAL PROCEDURE FOR ANALYSIS OF SURFACES USING WIPES REVISION 2

Office of Research and Development
National Homeland Security Research Center

Centers for Disease Control and Prevention
National Institute for Occupational Safety and Health

EPA/600/R-11/143

SURFACE ANALYSIS USING WIPES FOR THE DETERMINATION OF NITROGEN MUSTARD DEGRADATION PRODUCTS BY LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS)

Sampling and Analytical Procedure for Analysis of Surfaces Using Wipes
Revision 2

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DISCLAIMER

The information in this document is a single-laboratory-developed sampling and analytical procedure (SAP) that has been funded wholly or in part by the U.S. Environmental Protection Agency (EPA) and in collaboration with the National Homeland Security Research Center, part of EPA's Office of Research and Development, and the National Institute of Occupational Safety and Health (NIOSH), a division of the U.S. Department of Health and Human Services (DHHS), under IA #DW-75-922440001-0. The method development and document preparation were supported under contract number EP08C000010. This document has been subjected to the Agency's review and has been approved for publication. Note that approval does not signify that the contents necessarily reflect the views of the Agency. NIOSH and EPA do not endorse the purchase or sale of any commercial products or services.

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FOREWORD

The National Homeland Security Research Center (NHSRC), part of U.S. EPA's Office of Research and Development (ORD), is focused on developing and delivering scientifically sound, reliable, and responsive products. These products are designed to address homeland security information gaps and research needs that support the Agency's mission of protecting public health and the environment. A portion of NHSRC's research is directed at decontamination of indoor surfaces, outdoor areas, and water infrastructure. This research is conducted as part of EPA's response to chemical, biological, and radiological (CBR) contamination incidents. NHSRC has been charged with delivering tools and methodologies (e.g. sampling and analytical methods, sample collection protocols) that enable the rapid characterization of indoor and outdoor areas, and water systems following terrorist attacks, and more broadly, natural and manmade disasters.

The *Selected Analytical Methods for Environmental Remediation and Recovery (SAM)* document is a compendium of methods that informs sample collection and analysis during the response to an incident. SAM can be used by public and private laboratories which are analyzing a large number of samples associated with chemical, biological, or radiological contamination. Even though some of the analytes in SAM already have existing analytical methods, others are in need of improvements that enhance analytical capability and meet more rigorous performance criteria. Furthermore, not all of the analytical methods listed in the SAM document address all possible matrices (e.g., water, soil, air, glass) encountered in sample collection following an incident. The analytical methods in SAM have been verified in a single laboratory, but most still need to undergo multi-laboratory validation with respect to a specific contaminant in association with a specific matrix.

The single-laboratory-developed Sampling and Analytical Procedure, described herein, demonstrates the procedure for analysis of nitrogen mustard degradation products on surfaces using wipes by liquid chromatography/tandem mass spectrometry (LC/MS/MS). A companion study report (*Companion Document for Surface Analysis Using Wipes for the Determination of Nitrogen Mustard Degradation Products by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS))*, describes the development efforts, a synopsis of the supporting data collected, and scientific justifications for the decisions made. NHSRC welcomes your comments as we move one step closer to achieving our homeland security mission, and our overall mission of protecting human health and the environment.

Jon Herrmann,
Director National Homeland Security Research Center

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EXECUTIVE SUMMARY

Wipe sampling can be performed quickly and easily when direct extraction is not always possible (*e.g.*, walls, floors and furniture). As a result, wipe sampling is preferred for analysis without destruction of the tested surface. However, wipe sampling can remove analytes only from the surface of a material, which could result in lower recoveries and produce less reliable quantitative data from porous surfaces. It is therefore important to understand wipe efficiencies and the materials being wiped. This procedure assesses the recoveries from various porous and nonporous surfaces to determine the presence of nitrogen mustard degradation products. Wipes were analyzed using 100% methanol extraction by sonication, filtration, and concentration followed by analysis by liquid chromatography electrospray ionization/tandem mass spectrometry (LC/ESI-MS/MS) by direct injection without derivatization. Data generated from a formica surface resulted in detection limits of 0.12 $\mu g/cm^2$ for TEA, 0.06 $\mu g/cm^2$ for EDEA, 0.07 $\mu g/cm^2$ for MDEA, and 0.04 $\mu g/cm^2$ for DEA. Accuracy and precision data were generated from each tested surface fortified with these analytes, then qualitatively and quantitatively determined.

SURFACE ANALYSIS USING WIPES FOR THE DETERMINATION OF NITROGEN MUSTARD DEGRADATION PRODUCTS BY LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS)

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LIST OF ACRONYMS AND ABBREVIATIONS

ACN	Acetonitrile
AS	Analyte Standard
CAL	Calibration Standard
CAS	Chemical Abstracts Service
CBR	Chemical, Biological and/or Radiological
CCC	Continuing Calibration Check
CID	Collisionally Induced Dissociation
CV	Calibration Verification
CWA	Chemical Warfare Agent
DEA	Diethanolamine
DHHS	U.S. Department of Health and Human Services
DL	Detection limit
EDEA	*N*-Ethyldiethanolamine
EPA	U.S. Environmental Protection Agency
ERLN	Environmental Response Laboratory Network
ESI	Electrospray Ionization
IDC	Initial Demonstration of Capability
IDL	Instrument Detection Limit
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
LRB	Laboratory Reagent Blank
MDEA	*N*-Methyldiethanolamine
MDL	Method Detection Limit
MRL	Minimum Reporting Limit
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometry
MSDS	Material Safety Data Sheet
MS/MS	Tandem Mass Spectrometry
MSP	Method Specific Parameter
NH_4OAc	Ammonium Acetate
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
PPB	Parts per Billion
PPM	Parts per Million
P&A	Precision and Accuracy
PVDF	Polyvinylidene Fluoride
QA	Quality Assurance
QC	Quality Control
QL	Quantitation Limit
QMP	Quality Management Plan
REC	Percent Recovery
RL	Reporting Limit

RSD	Relative Standard Deviation
RT	Retention Time
RTS	Retention Time Shift
SAM	*Standardized Analytical Methods for Environmental Restoration Following Homeland Security Events*
SAP	Sampling and Analytical Procedure
SD	Standard Deviation
S/N	Signal to Noise
SS	Surrogate Standard
SSS	Stock Standard Solution
TC	Target Compound
TEA	Triethanolamine
UPLC	Ultra-Performance Liquid Chromatography
X	Average Percent Recovery

1. SCOPE AND APPLICATION

1.1. This procedure covers the determination of nitrogen mustard degradation products on surfaces using wipes. Surfaces were wiped and wipes were analyzed using 100% methanol extraction by sonication, filtration, and concentration followed by analysis by liquid chromatography electrospray ionization/tandem mass spectrometry (LC/ESI-MS/MS) by direct injection without derivatization. Detection limit data were generated for all analytes of interest on surfaces. Accuracy and precision data were generated from each surface fortified with these analytes, then qualitatively and quantitatively determined. The following analytes were determined using this procedure:

Analyte	CAS Registry Number
Triethanolamine (TEA)	102-71-6
N-Ethyldiethanolamine (EDEA)	139-87-7
N-Methyldiethanolamine (MDEA)	105-59-9
Diethanolamine (DEA)	111-42-2

1.2. Wipe sampling can be performed quickly and easily when direct extraction is not always possible (*e.g.*, walls, floors and furniture). As a result, wipe sampling is preferred for analysis without destruction of the tested surface. However, wipe sampling can remove analyte only from the surface of a material, which could result in lower recoveries and produce less reliable quantitative data from porous surfaces. It is therefore important to understand wipe efficiencies and the materials being wiped. This procedure assesses the recoveries from various porous and nonporous surfaces using wipes.

1.3. Detection limit (DL) metrics were presented using EPA conventions[1-3]. The detection limit was defined as the statistically calculated minimum concentration that can be measured with 99% confidence that the reported value is greater than zero. The statistical procedure, utilizing the Laboratory Fortified Sample Matrix samples (LFSM) and their duplicates, will be used to calculate uncertainty. Precision and accuracy (P&A) studies should be performed as an initial demonstration of capability (IDC) and after any modifications to the procedure, including changes in instrumentation and operating conditions. These studies will evaluate whether the reporting limits and calibration standard concentrations are appropriate.

1.4. The Minimum Reporting Level (MRL) is the lowest analyte concentration that meets data quality objectives that are developed based on the intended use of this sampling and analytical procedure (SAP). The MRL is the lowest true concentration for which the future recovery is predicted (between 50 and 150% recovery) and is listed as the lowest calibration level (Level 1).

1.5. This method was intended for use by analysts skilled in the operation of LC/MS/MS instruments 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.

1.6. 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 conditions, and wipe/collection materials can be used for analysis as long as similar performance is demonstrated and the quality control measures can be observed.

2. SUMMARY OF METHOD

2.1. Samples are collected from surfaces with wipes and stored at 4 °C (± 2 °C) for samples not immediately analyzed within a 24-hour time period. When the samples are ready to be analyzed, samples are spiked with a surrogate compound, the appropriate solvent is added, the sample solution is sonicated, the solution is extracted with a syringe filter unit, then the extract is concentrated and analyzed directly by LC/MS/MS operated in the positive electrospray ionization (ESI+) mode.

2.2. Each target compound was separated and identified by retention time and by comparing the sample primary multiple reaction monitoring (MRM) transition to the known standard MRM transition from reference spectra under identical LC/MS/MS conditions. The retention time for the analytes of interest must fall within the retention time of the standard (within ± 5%). The concentration of each analyte is determined by the instrumentation software using external calibration.

2.3. The detection limit (DL) and quantitation limit (QL) for these compounds were calculated using an EPA approach and are listed in Table 1 and Attachment 18.1. The precision and accuracy (P&A) quality control acceptance criteria are shown in Attachment 18.2. Stability studies suggest samples can be stored up to 28 days (Table 2) at 4 ºC (± 2 ºC). The concentrations of the calibration standards are listed in Table 3 and the retention times, mass transitions, and mass spectrometer parameters are listed in Table 4. The gradient conditions and ESI-MS/MS conditions are listed in Tables 5 and 6, respectively. This SAP was tested on several wipes in previous studies to establish that filter paper provided the highest recoveries with the least interference for any targeted analytes. Analytes spiked onto surfaces were wiped and the recoveries from both porous and nonporous surfaces were reported.

The overall performance of the filter paper, in terms of analyte recovery and fewest interferences, suggested that the filter paper was an appropriate wipe for recovering the analytes from a surface while analyzing the described nitrogen mustard degradation products. Other wipes such as cotton gauze would require a pre-cleaning step due to the presence of interferences to the targeted analytes.

3. DEFINITIONS

3.1 ANALYSIS BATCH – A set of samples analyzed on the same instrument, not exceeding 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 in the analysis batch and/or the number of field samples.

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

3.3 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.

3.4 CONTINUING CALIBRATION CHECK (CCC) – A calibration standard containing the method analytes and surrogate standard. The CCC is analyzed periodically to verify the accuracy of the existing calibration for those analytes at or near the mid-level concentrations.

3.5 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.

3.6 SURROGATE STANDARD (SS) – A pure chemical 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. This method uses a deuterated analyte.

3.8 LABORATORY FORTIFIED BLANK (LFB) – A volume of solvent or other 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.

3.9 LABORATORY FORTIFIED SAMPLE MATRIX (LFSM) – A preserved 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 and the measured values in the LFSM must be corrected for background concentrations.

3.10 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 occurrence of method analytes is low.

3.11 LABORATORY REAGENT BLANK (LRB) – An aliquot of solvent or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents and reagents and surrogate standards that are used in the analysis batch. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.12 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.

3.13 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 quality control (QC) criteria for this standard are met. A procedure for verifying a laboratory's MRL is provided in Section 9.2.4.

3.14 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.

3.15 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.

3.16 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.

4. INTERFERENCES

Procedural interferences may 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 LRBs (Section 9.4.1) under the same conditions as the samples.[4] Subtraction of blank values from sample results is not performed.

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

4.2 Matrix interferences may be caused by contaminants from the sample, 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.

4.3 Matrix effects are well known phenomena of ESI-MS techniques, especially for co-eluting compounds. Managing the unpredictable suppression and enhancement caused by these effects is recognized as an integral part of the performance and verification of an ESI-MS procedure. The data presented in this procedure were designed to demonstrate that the procedure is capable of functioning with realistic samples. Each analyst is encouraged to observe appropriate precautions and follow the described QC procedures to help minimize the influence of ESI-MS matrix effects on the data reported. Matrix effects include ion suppression/enhancement, high background and improper ion ratios.

5. 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 safe handling of chemicals used in this method. A reference file of material safety data sheets (MSDSs) that address the safe handling of the chemicals should be made available to all personnel involved in the chemical analyses. Additional references are available.[5-7]

6. EQUIPMENT AND SUPPLIES

References to specific brands of equipment and catalog numbers were provided solely as examples and do not constitute an endorsement of the use of such products or suppliers.

6.1 LC/MS/MS APPARATUS

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

6.1.2 ANALYTICAL COLUMN - Waters – Atlantis™ HILIC Silica, 100 mm x 2.1 mm, 3 µm particle size, or equivalent.

6.1.3 TANDEM MASS SPECTROMETER (MS/MS) SYSTEM – A 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.

6.1.4 DATA SYSTEM - 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. QuanLynx™ (or similar software) is used for all quantitation for data generated from the LC/MS unit.

6.2 NITROGEN EVAPORATOR

6.2.1 A nitrogen evaporation device, such as the N-Evap 24 - port device (Organomation Associates, Inc.) equipped with a water bath that can be maintained at 50 °C for final analyte concentration (< 10 mL volume). Evaporation times are expected to increase without this feature. Other nitrogen devices, which do not have a temperature control feature, may be used as long as QC criteria can still be reached.

6.3 EXTRACTION DEVICE

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

6.4 GLASSWARE AND MISCELLANEOUS SUPPLIES

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

6.4.2 DISPOSABLE STERILE SYRINGES - 10.0 mL ± 1% accuracy (Fisher Scientific, Pittsburgh, PA), or equivalent.

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

6.4.4 DESOLVATION GAS - Ultra Pure 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.

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

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

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

6.4.8 STANDARD SOLUTION FLASKS - Class A volumetric glassware

6.4.9 SYRINGE FILTER - Millex® GV Syringe-driven filter unit (PVDF) 0.22 µm (Millipore Corporation, Catalog # SLGV013NL).

6.4.10 WIPES - Whatman 42 ashless, 55 mm filter paper (Fisher Scientific, Pittsburgh, PA, Catalog # 09-845A).

6.4.11 SAMPLE COLLECTION CONTAINERS - Clean Nalgene containers with screw cap (Fisher Scientific, Pittsburgh, PA, Catalog # 11-815-10C), or equivalent.

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

7. REAGENTS AND STANDARDS

7.1 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 of 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.

7.1.1 SOLVENTS - Acetonitrile (CAS # 75-05-8), Methanol (CAS # 67-56-1), and Water (CAS # 7732-18-5), HPLC mass spectrometry pesticide grade or equivalent, demonstrated to be free of analytes and interferences.

7.1.2 AMMONIUM ACETATE (CAS # 631-61-8, ACS Reagent Grade or equivalent demonstrated to be free of analytes and interferences.)

7.1.3 ACETIC ACID (CAS # 64-19-7, Concentrated, ACS Reagent Grade or equivalent demonstrated to be free of analytes and interferences.)

7.1.4 MOBILE PHASE A - Solution A consisted of 95% of 25 mM ammonium acetate at pH 4.2, and 5% of acetonitrile to prevent microbial growth. To prepare 1 L, add 1.93 g of ammonium acetate to water, adjust to pH 4.2 with acetic acid and dilute to 1 L mark. Add 950 mL of the 25 mM ammonium acetate at pH 4.2 solution to a 1L container. Add 50 mL of acetonitrile. This solvent system is still prone to some microbial growth and should be replaced once a week.

7.1.5 MOBILE PHASE B- Solution B was comprised of 95% acetonitrile and 5% 25 mM ammonium acetate. To prepare 1 L, add 1.93 g of ammonium acetate to 1 L of water. Add 950 mL of acetonitrile to a 1L container. Add 50 mL of the 25 mM ammonium acetate solution.

7.1.6 TARGET ANALYTES - Triethanolamine (CAS # 102-71- 6), *N*-ethyldiethanolamine (CAS # 139-87-7), *N*-methyldiethanolamine (CAS # 105-59-9) and diethanolamine (CAS # 111-42-2).

7.1.7 SURROGATE ANALYTE- Bis(2-hydroxyethyl)-d_8-amine (Diethanolamine-d_8) (CAS # 103691-51-6**)**

7.2 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 vials) and at 4 °C (± 2 °C). Standards were estimated to be stable for at least a month. Although stability times are suggested, laboratories should utilize QC

practices to determine when standards should be replaced.

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

A standard solution may be prepared from a certified commercially available neat compound. Isotopically-labeled surrogate, diethanolamine-d_8 (CAS # 103691-51-6), was obtained from CDN Isotopes. The surrogate was added to a 50 mL volumetric flask in order to achieve a concentration of 1000 µg/mL in solution (i.e., 50 mg or 45.8 µL of diethanolamine-d_8 was added to a 50 mL volumetric flask and diluted to mark with methanol). Further dilutions of the 1000 µg/mL concentration were used to obtain 100 and 10 µg/mL solutions in methanol. Surrogate stock standard solutions were stable for at least a month when stored at 4 ºC.

(**NOTE**: Although diethanolamine-d_8 was used as a surrogate in this SAP, diethanolamine-d_8 could be used as an internal standard for diethanolamine for quantitation purposes. However, further evaluation would be necessary to ensure that diethanolamine-d_8 is a viable internal standard and meets QC requirements.)

7.2.2 ANALYTE STOCK STANDARD SOLUTION (AS)

Standard solutions may be prepared from certified, commercially available neat compounds. All neat compounds are viscous liquids at room temperature. Neat materials of triethanolamine and diethanolamine were obtained from Chem Service (West Chester, PA). *N*-ethyldiethanolamine and *N*-methyldiethanolamine were obtained from Aldrich as neat materials. A standard solution concentration of 1000 µg/mL for each compound was obtained in 50 mL volumetric flasks (*e.g.*, 44.4 µL of TEA, 45.87 µL of DEA, 49.31 µL of EDEA and 48.08 µL of MDEA were each added to separate 50 mL volumetric flasks and diluted to the mark with methanol) . Further dilutions of the 1000 µg/mL concentration can be used to obtain 100 and 10 µg/mL solutions in methanol. The calibration standards were made from appropriate dilution concentration of these stock standards.

(**NOTE:** All spiking solutions should be within ten times the DL).

7.2.3 CALIBRATION STANDARD SOLUTION (CAL)

A calibration stock standard solution (Level 7) was prepared from the Analyte Standard (AS) solution concentrations, containing, triethanolamine, *N*-ethyldiethanolamine, *N*-methyldiethanolamine, diethanolamine and the surrogate diethanolamine-d_8 in methanol (i.e., 250 µL of TEA, DEA, EDEA, MDEA and DEA-d_8 of a 100 µg/mL solution was added to a 50 mL volumetric flask and diluted to mark with methanol). From Level 7, further dilutions were performed to prepare Levels 6 through 1 as shown in Table 3. All concentrated stock standard solutions were made in methanol.

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 SAMPLE COLLECTION

8.1.1 The exact choice of sampling vessel and procedure is not critical for the analysis and can be adjusted to meet the needs of the situation as long as the different materials have been tested and show no presence of the target analytes. As an example for field samples, the field samplers would collect samples with the appropriate wetted wipe (methanol) 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.

8.1.2 Wipe samples were collected using Whatman 42 ashless 55 mm circle filter paper. The required analyte spike solution containing the four analytes of interest was added to the surface, allowed to dry, and wiped with each wipe separately. Two wipes were separately wetted with approximately 300 µL of methanol. The first wipe is used to wipe the surface in a Z-like pattern horizontally across a defined surface (100 cm^2) (Figure 18.3). The second wipe is used to wipe the same surface in a Z-like pattern vertically across a defined surface (100 cm^2). Then both wipes are placed into a 125 mL Nalgene polypropylene straight-sided jar with a polypropylene screw cap. Surrogate (DEA-d_8) and methanol solvent (10 mL) are added to the jar. Because the wipe can lie flat on the bottom of the jar, the solvent fully immerses the wipes. Field and/or matrix blanks are needed, according to conventional sampling practices.

8.2 SAMPLE STORAGE AND HOLDING TIMES

8.2.1 Samples should be analyzed as soon as possible. All samples were refrigerated at 4 ºC (± 2 ºC) from the time of collection until analysis unless the samples were analyzed within a 24-hour time period. At the laboratory, samples were stored in the refrigerator at 4 ºC (± 2 ºC) until requested for analysis. Samples should be analyzed within 48 hours of collection or as soon as possible. Samples from a particular site should be carefully characterized to ensure that there is no interaction with the wipe or specific surface to cause interferences or degradation of the analytes after 48 hours. After injection in the LC/MS, the vial septa were replaced and the vials were stored in a refrigerator in case further analysis was needed. Samples can be stored up to 28 days (Table 2) in the refrigerator at 4 ºC (± 2 ºC).

9. QUALITY CONTROL

9.1 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 18.2 Attachment) as well as a detection limit study (DL, as shown in Table 1 and Section 18.1 Attachments) must be performed to demonstrate laboratory capability or whenever a major modification is made to this SAP. Laboratories are encouraged to institute additional QC practices to meet their specific needs.

9.2 INITIAL DEMONSTRATION OF CAPABILITY (IDC)

The IDC must be successfully performed prior to the analysis of field samples. Prior to conducting an IDC, an acceptable Initial Calibration must be generated as outlined in Section 10.2.

9.2.1 INITIAL DEMONSTRATION OF LOW SYSTEM BACKGROUND

Any time a new lot of solvents, reagents and autosampler vials is used, the laboratory reagent blank (LRB) must be demonstrated to be reasonably free of contamination and that criteria are met in Section 9.4.1. The LRB was used to ensure that analytes of interest or other interferences were not present in the laboratory environment, the solvent, or the apparatus.

NOTE: Good laboratory practices indicate the use of a blank during calibration of instrumentation to ensure that no carryover occurs between samples. If the required criteria were 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.

9.2.2 INITIAL DEMONSTRATION OF PRECISION AND ACCURACY (P&A)

NOTE: *Because porosity 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 Formica® (Formica, Cincinnati, OH) surface because the Formica surface has been shown to be mostly free of contamination and is a relatively nonporous surface.*

For a precision and accuracy study (P&A), prepare a check standard containing triethanolamine, *N*-ethyldiethanolamine, *N*-methyldiethanolamine, diethanolamine and diethanolamine-d_8, 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 were chosen with seven samples each. The check samples were analyzed according to Section 11.

9.2.3 The average percent recovery (X), standard deviations (σ) and the percent relative standard deviation (%RSD) of the recoveries were calculated for each analyte. The % RSD value of $\leq 25\%$ should be applied to all analytes.

9.2.4 MINIMUM REPORTING LEVEL (MRL)

Establish a target concentration for the MRL based on the intended use of the method. Establish an Initial Calibration (Section 10.2). 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. The MRL is reported in this study as the lowest calibration level.

9.2.5 Calibration verification (CCV)

A mid-level sample 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.

9.3 DETECTION AND QUANTITATION LIMITS (DL and QL)

The procedure for the determination of the laboratory detection and quantitation limits for the EPA approach follows 40 CFR Part 136 Appendix B as described in the Environmental Response Laboratory Network (ERLN). Detection limits (DLs) 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). Quantitation limits (QLs) represent the smallest detectable concentration of analyte greater than the detection limit, where the precision and bias achieve program objectives. The DL and QL were determined for each target analyte.

9.3.1 Determination of laboratory instrument detection limits (IDLs)

Laboratory instrument detection limits (IDLs) were determined for each instrument used for analyses. Although the determination of the laboratory IDL is not an EPA requirement, the laboratory IDL can be used to establish an estimate of the initial spiking concentration used for determination of the DL. The laboratory IDL was 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 were injected until the S/N ratio was in the range of 3:1 – 5:1. Replicates were 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:** S/N ratios must be demonstrated; linearity of S/N ratio with increasing or decreasing concentration cannot be assumed.)

9.3.2 Determination of laboratory MDL

DLs represent the optimal detection achieved by a laboratory in a matrix of interest. Formica coupons were used for the determination of the MDL for surface samples. The 40 CFR Part 136, Appendix B procedure was followed, particularly with regard to spike levels used. Replicate reference matrix samples were spiked at a level between 1-5 times the estimated detection level (e.g., the IDL, 3 times the standard deviation of replicate instrument measurements of the analyte in desired solvent, or the region where there is a break in the slope at the low end of the standard curve). The resulting DL must be within 10 times the spike level used, or the DL 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 were 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):

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

where

MDL = method detection limit

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

Data for DLs are shown in Table 1 and Attachment 18.1.

9.4 ONGOING QC REQUIREMENTS

9.4.1 LABORATORY REAGENT BLANK (LRB)

A reagent blank was prepared and analyzed with each analysis batch, using methanol, for confirmation that there were no background contaminants interfering with the identification or quantitation of the target analytes. If there was 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.

9.4.2 CONTINUING CALIBRATION CHECK (CCC)

CCC standards (near the midpoint of the calibration range) are analyzed at the beginning of each analysis batch, after every twenty field samples, and at the end of the analysis batch.

9.4.3 LABORATORY FORTIFIED SAMPLE MATRIX (LFSM)

A LFSM is analyzed to determine that spike accuracy for a sample matrix is not adversely affected. If a variety of sample matrices is analyzed, performance should be established for each surface.

9.4.3.1 Within each analysis batch, a 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 analyte AS (Section 7.2.2). 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 is used. If the accuracy does not fall within this range, check with a CCC or prepare a fresh AS solution for analysis.

9.4.4 SURROGATE STANDARD

All samples were spiked with surrogate standard spiking solution as described in Section 7.2.1. An average percent recovery of the surrogate compound and the standard deviation of the percent recovery were calculated and updated regularly.

9.4.5 MATRIX SPIKE (MS) OR LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (LFSMD)

Within each analysis batch, a minimum of one MS or LFSMD should be analyzed. 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. MS/LFSMD samples must be incorporated into the field sampling plan. If the laboratory did not receive MS samples for determination of site-specific precision and accuracy (P&A), the laboratory will evaluate the site data quality based on the Laboratory Fortified Sample Matrix (LFSM) data, if there is sufficient sample in the site samples to conduct an analysis. MS/LFSMD recovery results will be used for site-specific precision and accuracy (P&A) data. LFSM data were used as MS/LFSMD sample data for this study. RSD values should be $\leq$ 30% for samples.

9.4.6 METHOD MODIFICATION QC REQUIREMENTS

The analyst may modify the separation technique, LC column, mobile phase composition, LC conditions and MS conditions so as long as all QC and ongoing QC criteria are met. It is the laboratory's responsibility to review the results when method modifications are implemented. If repeated failure occurs, the modification must be abandoned.

10. 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.[8] Verification for 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 should be performed at the beginning and end of each analysis batch.

10.1 CALIBRATION OF MASS SPECTROMETER

Mass calibration of the mass spectrometer (Waters Acquity™ or equivalent) is performed monthly or when mass shifts of more than 0.5 daltons are noticed by the analyst. The mass calibration file is saved in the mass spectrometer software file folder (MassLynx™ or similar software). The mass calibration solution used is a mixture of NaCsI provided by the manufacturer. Other calibration solutions can also be used per instrument manufacturer's specifications. The detailed procedure for mass calibration of the mass spectrometer can be found in the software instruction manual provided by the manufacturer.

10.2 INITIAL CALIBRATION FOR ANALYTES

10.1.1 Optimize the $[M+H]^+$ ion for each analyte by infusing a 500 ng/mL methanol solution directly into the MS at a flow rate of 0.3 mL/min. The MS parameters

(voltages, temperatures, gas flows, etc.) are varied until optimal analyte responses are achieved. Optimize the product ion by following the same procedures 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 are presented in Tables 4 and 6.

10.1.2 Establish LC operating conditions that will optimize peak resolution and shape.[9] Suggested LC conditions (listed in Table 5) may not be optimal for all LC systems.

10.1.3 The initial calibration contains a seven-point curve using the analyte concentrations prepared in section 7.2.3 and are shown in Table 3. The lowest calibration curve standard must be at the MRL. 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. The coefficient of determination (r^2) of the linear fit should be greater than or equal to 0.98. The coefficient of determination (r^2) of the quadratic curve should be greater than or equal to 0.99. A calibration curve and an instrument blank will be analyzed at the beginning of each batch or daily to ensure instrument stability.[10] 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 saved and used to quantify all samples.

10.3 QUANTITATION OF ANALYTES

The quantitation of the target analytes is accomplished with quantitation software as it relates to each specific instrument (QuanLynx™ or similar software).[11] An external calibration is used along with monitoring diethanolamine-d_8 surrogate recovery. Refer to Table 4 for the MRM transitions and retention times.

11. ANALYTICAL PROCEDURE

11.1 SAMPLE PREPARATION

11.1.1 Samples were collected and stored as described in Section 8. Surrogate (DEA-d_8) and methanol solvent (10 mL) were added to the jar. Sonicate each jar containing the methanol solution for approximately 15 minutes in a water bath at room temperature with no heat required.

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

11.1.3 Place the 15-mL polypropylene tube on the nitrogen evaporator and set the temperature of the water bath to 50 °C (± 5 °C).

11.1.4 Concentrate sample in the 15 mL polypropylene tube to < 2 mL using the N-Evap (Thomson Instrument Co., Clear Brook, VA) concentrator.

11.1.5 Dilute the sample to 2 mL (± 5% accuracy) final volume using methanol. (**NOTE:** More suitable glassware with better accuracy may be used to ensure an exact 2 mL final volume.)

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

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

11.2 SAMPLE ANALYSIS/ANALYTICAL SEQUENCE

11.2.1 Establish Liquid Chromatography/Mass Spectrometry conditions as per guidance described in Section 10 and summarized in Tables 4, 5 and 6.

11.2.2 Prepare a sequence that includes all QC samples and surface samples. The first sample to be analyzed is a 5 µL injection on column of a blank (methanol).

11.2.3 The calibration standards, Levels 1 through 7, are analyzed next. 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 as necessary. Print quantitation reports for the calibration standards.

11.2.4 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 and regenerate a calibration report. Alternatively, re-analyze "nonconforming" calibration level(s) and repeat the above procedures.

11.2.5 The first sample analyzed after the calibration curve is a blank to ensure there is no carryover.[9] If the initial calibration data are acceptable, begin analyzing samples, including QC and blank samples, at their appropriate frequency injecting the same size aliquots (5 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.

11.2.6 EXCEEDING THE CALIBRATION RANGE: If the absolute amount of a target compound exceeds the working range of the LC/MS system (see Level 7 in Table 3), the prepared sample is diluted with methanol and re-analyzed. 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 afterwards to demonstrate no carryover will occur.

11.2.7 At the conclusion of the data acquisition, use the same software that was used in the

calibration procedure to identify peaks of interest from the retention time windows. Use the data software to examine the ion abundances of the peaks in the chromatogram and compare retention times with the retention time of the corresponding peak in an analyte standard.

11.2.8 All qualitative and quantitative measurements are performed as described in Sections 9.2 and 9.3. When not being analyzed, samples are stored in the refrigerator at 4 ºC (± 2 ºC) and protected from light in screw cap top vials equipped with Teflon-lined septa.

12. DATA ANALYSIS AND CALCULATIONS

12.1 QUALITATIVE AND QUANTITATIVE ANALYSIS

12.1.1 An external calibration is used when monitoring the MRM transitions of each analyte. Quantitation software (such as QuanLynx™) is utilized to conduct the quantitation of the target analytes and surrogate standard. The MRM transitions of each analyte are used for quantitation and confirmation. The MRM transition serves as a confirmation by isolating the precursor ion, fragmenting the precursor ion to the product ion, and relating the transition to the retention time in the calibration standard.[11]

12.1.2 Computer programs used for analysis of data include instrumentation and quantitation software (*e.g.,* MassLynx™ with QuanLynx™). The manufacturer's quantitation software manual should be consulted to ensure the proper use of the software. The quantitation method is set as an external calibration using the peak areas in ng/mL as long as the analyst is consistent. 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 match the integration of other peaks and/or known calibration peaks. Any manual integration should be carried out by a qualified analyst and checked against quality control procedures (sections 9 and 10.3).

12.1.3 If the polynomial type is linear and excludes the point of origin, use a fit weighting of 1/X in order to give more weighting to the lower concentrations. The retention time window of the MRM transitions must be within 5% of the retention time of the analyte in a mid-range calibration 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. The coefficient of determination, r^2, should be > 0.98 for each analyte. If one of the calibration standards other than the high or low standard causes the curve 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.

12.1.4 If the polynomial type is quadratic, the point of origin is excluded and a fit weighting of 1/X is used in order to give more weighting to the lower concentrations. The

retention time window of the MRM transitions must be within 5% of the retention time of the analyte in a mid-range calibration 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. The coefficient of determination, r^2, should be > 0.99 for each analyte. If one of the calibration standards other than the high or low standard causes the curve to be <0.99, this point must be re-injected or a new calibration curve should be analyzed. If the low or high point is excluded, a six-point curve is acceptable using a quadratic fit. An initial seven-point curve over the calibration range is suggested in the event the low and/or high point must be excluded to obtain a coefficient of determination > 0.99. In this event, the calibration range and detection limits must be modified to reflect this change.

12.2 Prior to reporting data, the chromatogram should be reviewed for any incorrect peak identification. 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.

12.3 All data packages will be verified by a qualified analyst to ensure incorrect peak identifications or poor integrations were properly identified. The qualified analyst will sign off on the narrative and checklist.

13. METHOD PERFORMANCE

13.1 PRECISION, ACCURACY AND DETECTION LIMITS

13.1.1 Tables for precision, accuracy and detection limit results for a single laboratory study are presented in Sections 18.1 and 18.2 and Table 1.

13.2 RECOVERIES AND PRECISION FOR OTHER SURFACE TYPES

13.2.1 Section 18.2 lists recoveries and precision of target analytes for a variety of other surfaces.

13.3 PROBLEM ANALYTES AND SURFACES

13.3.1 TARGET ANALYTES ON PRE-CLEANED AND UNCLEANED SURFACES

Target analytes were spiked on surfaces and the wipe samples were tested for differences between pre-cleaned surfaces, using methanol, and wipe samples from uncleaned surfaces (used as received). When surfaces are cleaned prior to analysis, noticeable differences in TEA and DEA recoveries may occur due to a pre-existing presence/contamination of surfaces with these specific two compounds (matrix blanks will confirm the presence of TEA and DEA). Potential matrix effects are also indicated, suggesting laboratories should seek to understand matrix effects occurring in specific samples through thoughtful choice of MS materials. Although pre-cleaning surfaces would provide a more accurate analysis of the true recovery of TEA and DEA, it is not practical in a real scenario. Analysts should be aware that these two specific compounds may already be present within the tested sample matrix

and plan accordingly. Wood and painted drywall surfaces resulted in poor recoveries outside the range of this procedure. As a result, the SAP should not be used to identify these analytes in relation to these specific surfaces. Although porosity of the surface is most likely the culprit for low recoveries, further analysis should be performed to determine definitive reasoning of poor recoveries from the surface.

14. POLLUTION PREVENTION

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

14.2 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://membership.acs.org/c/ccs/pub_9.htm (accessed November 2009).

15. WASTE MANAGEMENT

15.1 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 that laboratories protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions.

15.2 Each laboratory should determine with 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 and date the beginning of collection before using the container.

16. REFERENCES

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

2. Federal Advisory Committee on Detection and Quantitation Approaches and Uses in Clean Water Act Programs. Final Report. December 28, 2007.

3. Lloyd Currie. "Quantitative Determination: Application to Radiochemistry." 1968. *Anal.Chem* 40(3):586-593L.

4. *Standard Practices for Preparation of Sample Containers and for Preservation of Organic Constituents*, American Society for Testing and Materials, Philadelphia. ASTM Annual Book of Standards, Part 31, D3694-78.

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

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

7. *Safety in Academic Chemistry Laboratories*, American Chemical Society Publication, Committee on Chemical Safety, 7th Edition. Information on obtaining a copy is available at http://membership.acs.org/C/CCS/pub_3.htm (accessed November, 2009). Also available by request at OSS@acs.org.

8. Eichelberger, J.W.; Harris, L.E.; Budde, W.L. "Reference Compound to Calibrate Ion Abundance Measurement in Gas Chromatography-Mass Spectrometry Systems" *Analytical Chemistry* **1975,** 47(7): 995-1000. http://pubs.acs.org/doi/abs/10.1021/ac60357a018.

9. McNair, N.M.; Bonelli, E.J. *Basic Chromatography*; Consolidated Printing: Berkeley, CA, 1969; p. 52.

10. Burke, J.A. "Gas Chromatography for Pesticide Residue Analysis; Some Practical Aspects", *J. Assoc. Off. Anal. Chem.* **1965,** 48:1037-1058.

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

17. TABLES AND VALIDATION DATA

Item	*Title*	*Number of Pages*	*Revision Number*	*Date Revised*
Table 1	Method Parameters	1	1	3/2011
Table 2	Holding Time Study for Nitrogen Mustard Degradation Analytes	1	1	3/2011
Table 3	Concentration of Calibration Standards	1	1	3/2011
Table 4	MRM Retention Times and MRM Ions and Variable Mass Spectrometer Parameters	1	1	3/2011
Table 5	Gradient Conditions for Liquid Chromatography	1	1	3/2011
Table 6	ESI^+-MS/MS Conditions	1	1	3/2011
Table 7	Materials Tested for the Wipe Analysis of Nitrogen Mustard Degradation Products	1	1	3/2011
Table 8	List of Consumable Materials Used During Sampling	1	1	3/2011

Table 1. Method Parameters

FORMICA					
Analyte	DL*		LOQ		MRL
	ng/cm²†	ng/mL	ng/cm²†	ng/mL	ng/mL
TEA	0.12	12.32	1.23	123.2	10
EDEA	0.06	6.25	0.63	62.6	10
MDEA	0.07	6.85	0.69	68.5	10
DEA	0.04	4.37	0.44	43.7	10

*Last DL Study- March 2011.
†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 2. Holding Time Sample Stability of Nitrogen Mustard Degradation Analytes

Concentration 50 ng/mL (n = 5)										
	TEA		EDEA		MDEA		DEA		DEA-d_8	
Holding Time (days)	Average % Recovery	% RSD	Average % Recovery	% RSD	Average % Recovery	% RSD	Average % Recovery	% RSD	Average % Recovery	% RSD
0	92.98	6	97.56	7	94.52	6	93.47	5	96.32	7
7	93.57	6	85.15	9	82.36	8	85.06	7	89.75	9
14	74.93	10	87.06	6	82.38	8	82.11	6	85.77	11
21	75.30	7	83.84	10	82.97	10	77.84	10	83.51	11
28	78.35	5	87.77	5	86.60	5	74.16	12	90.08	9

Table 3. Concentrations of Calibration Standards (ng/mL)

Analyte/Surrogate	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7
Triethanolamine	10	25	50	100	250	350	500
N-Ethyldiethanolamine	10	25	50	100	250	350	500
N-Methyldiethanolamine	10	25	50	100	250	350	500
Diethanolamine	10	25	50	100	250	350	500
Diethanolamine-d_8	10	25	50	100	250	350	500

Table 4. MRM Ion Transitions, Retention Time and Variable Mass Spectrometer Parameters

Analyte	Cone voltage	MRM mass transition (parent → product)	Collision energy (eV)	RT* (minutes)
Triethanolamine	30	150.09 → 132.10	12	9.7
N-Ethyldiethanolamine	30	134.02 → 116.10	14	11.1
N-Methyldiethanolamine	30	120.03 → 102.00	12	13.0
Diethanolamine	30	106.00 → 88.10	12	12.1
Diethanolamine-d_8 (Surrogate)	30	114.20 → 96.22	12	12.2

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

Table 5. Gradient Conditions for Liquid Chromatography

Time (min)	Flow (µL/min)	% Solution A†	% Solution B††
0	300	90	10
1	300	90	10
2	300	87	13
12	300	87	13
16	300	85	15
17	300	70	30
18	300	90	10
21	300	90	10

†A: 95% - ACN / 5% - 25mM NH_4OAC
††B: 95% - 25mM NH_4OAC (pH 4.22) / 5% - ACN
Injection volume - 5µL (recommended)
*Column Temperature: 30 °C
*Autosampler Temperature: 15 °C
*Equilibration time: 3 minutes
*Column: Atlantis™ HILIC silica, 100mm x 2.1mm, 3µm particle size

Table 6. ESI+-MS/MS Conditions

MS Parameter (ESI+)	Setting
Capillary Voltage	1.0 kV
Cone Voltage	See Table 4
Extractor	2 Volts
RF Lens	0.2 Volts
Source Temperature	150 °C
Desolvation Temperature	300 °C
Desolvation Gas Flow	800 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 4
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	0.3 Seconds

Table 7. Materials Tested for the Wipe Analysis of Nitrogen Mustard Degradation Products

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

Table 8. List of Consumable Materials Used During Sampling

Material	Vendor
Whatman 42 ashless circle filters, 55 mm	Fisher Scientific (Pittsburgh, PA)
125 mL Nalgene polypropylene straight-side jars with screw caps	Fisher Scientific (Pittsburgh, PA)
10 mL BD safety-lok syringes	Fisher Scientific (Pittsburgh, PA)
Corning 15 mL graduated plastic centrifuge tubes	Fisher Scientific (Pittsburgh, PA)
Millipore 13 mm Millex filter, 0.22 µm PVDF	Fisher Scientific (Pittsburgh, PA)
Waters 1.8 mL amber glass vials with pre-slit silicone PTFE screw cap	Waters Corp. (Milford, MA)

18. ATTACHMENTS

18.1 Detection and Quantitation Limits

18.2 Precision and Accuracy

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

18.1 DL AND QL CALCULATIONS

DL Calculations for Seven Replicates for Nitrogen Mustard Degradation Analytes

FORMICA									
	TEA			EDEA			MDEA		
Average Spike Concentration (ng/mL) (n=7)	Average Recovery ng/mL	% Recovery	% RSD	Average Recovery ng/mL	% Recovery	% RSD	Average Recovery ng/mL	% Recovery	% RSD
50.00	54.45	108.9	7	32.13	64.27	6	36.92	73.84	6
Formica Blank	26.30			0			0		
Average Spike Concentration (ng/cm^2) (n=7)	Average Recovery (ng/cm^2)	% Recovery	% RSD	Average Recovery (ng/cm^2)	% Recovery	% RSD	Average Recovery (ng/cm^2)	% Recovery	% RSD
0.50	0.54	108.9	7	0.32	64.27	6	0.37	73.84	6
Formica Blank	0.26			0			0		

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

FORMICA						
	DEA			DEA-d_8		
Average Spike Concentration (ng/mL) (n=7)	Average Recovery (ng/mL)	% Recovery	% RSD	Average Recovery (ng/mL)	% Recovery	% RSD
50.00	41.05	82.09	3	33.02	66.05	3
Formica Blank	5.42			33.49	66.98	
Average Spike Concentration (ng/cm^2) (n=7)	Average Recovery (ng/cm^2)	% Recovery	% RSD	Average Recovery (ng/cm^2)	% Recovery	% RSD
0.50	0.41	82.09	3	0.33	66.05	3
Formica Blank	0.05			0.33	66.98	

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

18.2 PRECISION AND ACCURACY

Precision and Accuracy (P&A) data for wipe analysis of nitrogen mustard degradation analytes on surfaces. (n = 7 samples at each concentration)

FORMICA									
	TEA			EDEA			MDEA		
Average Spike Concentration (ng/mL) (n=7)	Average Recovery (ng/mL)	% Recovery	% RSD	Average Recovery (ng/mL)	% Recovery	% RSD	Average Recovery (ng/mL)	% Recovery	% RSD
50	75.32	150.63*	8	37.11	74.23	17	39.49	78.98	7
75	71.12	94.83	8	31.27	41.69	8	46.76	62.34	11
100	81.11	81.11	6	65.46	65.46	4	66.82	66.82	5
150	123.96	82.64	10	91.24	60.82	14	95.14	63.43	9
Average Formica Blank	23.41	-	-	0	-	-	0	-	-
Average Spike Concentration (ng/cm^2)† (n=7)	Average Recovery (ng/cm^2)	% Recovery	% RSD	Average Recovery (ng/cm^2)	% Recovery	% RSD	Average Recovery (ng/cm^2)	% Recovery	% RSD
0.50	0.75	150.63*	8	0.37	74.23	17	0.40	78.98	7
0.75	0.71	94.83	8	0.32	41.69	8	0.47	62.34	11
1.00	0.81	81.11	6	0.66	65.46	4	0.67	66.82	5
1.50	1.24	82.64	10	0.91	60.82	14	0.95	63.43	9
Average Formica Blank	0.23	-	-	0	-	-	0	-	-

*TEA recoveries >150% are consistent with TEA being a native species to this material, as evidenced by blank coupon samples. TEA and DEA are not present in sovent blank or wipe blank samples, but are detected at low levels on the material blank sample, suggesting that TEA and DEA exist for this material.†ng/cm^2 calculation was performed by dividing the concentration spiked onto the surface by the test area of the coupon (100 cm^2).

FORMICA						
	DEA			DEA-d_8		
Average Spike Concentration (ng/mL) (n=7)	Average Recovery (ng/mL)	% Recovery	% RSD	Average Recovery (ng/mL)	% Recovery	% RSD
50	56.97	113.93	25	37.49	74.97	6
75	49.13	65.51	6	41.23	54.97	3
100	68.47	68.47	7	62.29	62.29	5
150	99.18	66.12	10	93.05	62.03	12
Average Formica Blank	2.41	-	-	27.00	54.01	-
Average Spike Concentration (ng/cm^2)† (n=7)	Average Recovery (ng/cm^2)	% Recovery	% RSD	Average Recovery (ng/cm^2)	% Recovery	% RSD
0.50	0.57	113.93	25	0.37	74.97	6
0.75	0.49	65.51	6	0.41	54.97	3
1.00	0.69	68.47	7	0.62	62.29	5
1.50	0.99	66.12	10	0.93	62.03	12
Average Formica Blank	0.02	-	-	0.27	54.01	-

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

METAL									
	TEA			EDEA			MDEA		
Average Spike Concentration (ng/mL) (n=7)	Average Recovery (ng/mL)	% Recovery	% RSD	Average Recovery (ng/mL)	% Recovery	% RSD	Average Recovery (ng/mL)	% Recovery	% RSD
50	147.19	294.37*	10	42.30	84.60	7	42.76	85.52	6
75	145.46	193.95*	18	58.43	77.91	8	59.01	78.68	6
100	172.36	172.36*	13	72.25	72.25	9	70.18	70.18	6
150	174.86	116.57	9	91.17	60.78	14	71.68	47.79	14
Average Metal Blank	117.57	-	-	0	-	-	0	-	-

*TEA recoveries >150% are consistent with TEA being a native species to this material, as evidenced by blank coupon samples and discussed in section 13.3. TEA and DEA are not present in sovent blank or wipe blank samples, but are detected at low levels on the material blank sample, suggesting that TEA and DEA exist for this material.

METAL						
	DEA			$DEA\text{-}d_8$		
Average Spike Concentration (ng/mL) (n=7)	Average Recovery (ng/mL)	% Recovery	% RSD	Average Recovery (ng/mL)	% Recovery	% RSD
50	64.41	128.81	5	45.32	90.64	10
75	80.65	107.54	5	69.33	92.44	6
100	85.62	85.62	5	72.87	72.87	4
150	97.95	65.30	4	96.35	64.23	3
Average Metal Blank	16.28	-	-	50.25	100.50	-

GLASS									
	TEA			EDEA			MDEA		
Average Spike Concentration (ng/mL) (n=7)	Average Recovery (ng/mL)	% Recovery	% RSD	Average Recovery (ng/mL)	% Recovery	% RSD	Average Recovery (ng/mL)	% Recovery	% RSD
50	219.24	438.48*	6	25.93	51.87	7	30.28	60.56	6
75	256.12	341.50*	10	49.72	66.29	5	56.65	75.53	4
100	256.18	256.18*	12	52.56	52.56	14	55.44	55.44	14
150	260.51	173.67*	8	78.22	52.14	12	83.81	55.87	10
Average Glass Blank	202.57	-	-	0	-	-	0	-	-

*TEA recoveries >150% are consistent with TEA being a native species to this material, as evidenced by blank coupon samples and discussed in section 13.3. TEA and DEA are not present in sovent blank or wipe blank samples, but are detected at low levels on the material blank sample, suggesting that TEA and DEA exist for this material.

GLASS						
	DEA			DEA-d_8		
Average Spike Concentration (ng/mL) (n=7)	Average Recovery (ng/mL)	% Recovery	% RSD	Average Recovery (ng/mL)	% Recovery	% RSD
50	57.51	115.01	14	29.68	59.37	11
75	91.64	122.19	4	60.67	80.9	5
100	77.2	77.2	19	54.96	54.96	21
150	119.25	79.5	8	91.52	61.02	11
Average Glass Blank	18.83	-	-	52.47	104.94	-

VINYL TILE									
	TEA			EDEA			MDEA		
Average Spike Concentration (ng/mL) (n=7)	Average Recovery (ng/mL)	% Recovery	% RSD	Average Recovery (ng/mL)	% Recovery	% RSD	Average Recovery (ng/mL)	% Recovery	% RSD
100	29.95	29.95	25	29.34	29.34	11	29.1	29.1	9
150	137.26	91.51	7	13.77	9.18	11	15.95	10.63	7
200	142.59	71.3	7	52.11	26.06	8	63.96	31.98	7
300	224.48	74.83	10	86.98	28.99	15	75.42	27.79	16
Average Vinyl Blank	17.6	-	-	0	-	-	0	-	-

VINYL TILE						
	DEA			$DEA\text{-}d_8$		
Average Spike Concentration (ng/mL) (n=7)	Average Recovery (ng/mL)	% Recovery	% RSD	Average Recovery (ng/mL)	% Recovery	% RSD
100	36.09	36.09	13	27.5	27.5	19
150	31.91	21.27	19	17.53	11.69	4
200	70.5	35.25	12	46.07	23.04	6
300	78.61	26.2	10	59.86	19.95	14
Average Vinyl Blank	7.00	-	-	53.5	107	-

WOOD*									
	TEA			EDEA			MDEA		
Average Spike Concentration (ng/mL) (n=7)	Average Recovery (ng/mL)	% Recovery	% RSD	Average Recovery (ng/mL)	% Recovery	% RSD	Average Recovery (ng/mL)	% Recovery	% RSD
500	100.24	20.05	16	9.69	1.94	28	15.8	3.16	30
Average Wood Blank	73.17	-	-	0	-	-	0	-	-

WOOD*						
	DEA			$DEA\text{-}d_8$		
Average Spike Concentration (ng/mL) (n=7)	Average Recovery (ng/mL)	% Recovery	% RSD	Average Recovery (ng/mL)	% Recovery	% RSD
500	36.94	7.39	22	10.39	2.08	32
Average Wood Blank	18.88	-	-	57.25	114.5	-

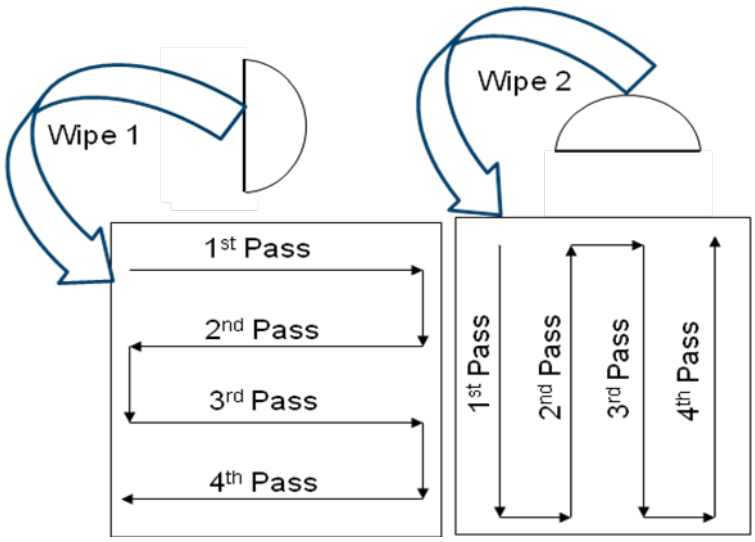
*Recoveries of all target analytes from this surface are below the acceptable range provided in this SAP. As a result, the SAP should not be used to identify these analytes in relation to this specific surface. Although porosity of the surface is most likely the culprit for low recoveries, further analysis should be performed to determine definitive reasoning of poor recoveries from the surface.

PAINTED DRYWALL*									
	TEA			EDEA			MDEA		
Average Spike Concentration (ng/mL) (n=7)	Average Recovery (ng/mL)	% Recovery	% RSD	Average Recovery (ng/mL)	% Recovery	% RSD	Average Recovery (ng/mL)	% Recovery	% RSD
500	109.63	21.93	10	74.97	14.99	17	84.91	16.98	18
Average Drywall Blank	72.79	-	-	0	-	-	0	-	-

PAINTED DRYWALL*						
	DEA			DEA-d_8		
Average Spike Concentration (ng/mL) (n=7)	Average Recovery (ng/mL)	% Recovery	% RSD	Average Recovery (ng/mL)	% Recovery	% RSD
500	87.02	17.4	18	56.57	11.31	20
Average Drywall Blank	23.11	-	-	39.76	79.52	-

*Recoveries of all target analytes from this surface are below the acceptable range provided in this SAP. As a result, the SAP should not be used to identify these analytes in relation to this specific surface. Although porosity of the surface is most likely the culprit for low recoveries, further analysis should be performed to determine definitive reasoning of poor recoveries from the surface.

18.3 Illustration of wiping pattern on 100 cm^2 surface



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