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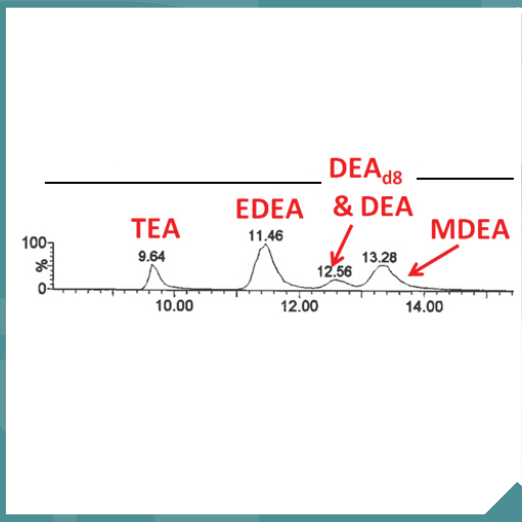
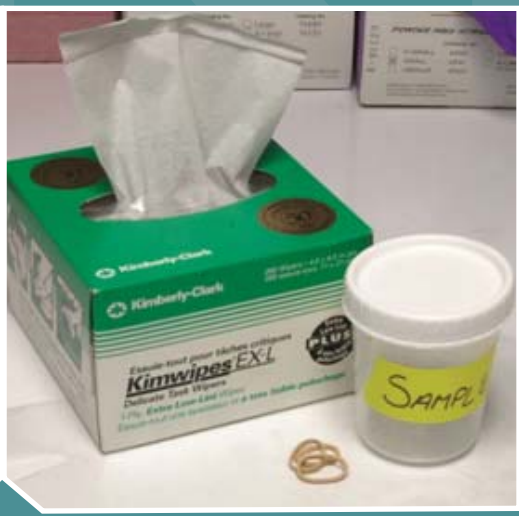
United States
Environmental Protection
Agency

Technical Report for Surface Analysis of Nitrogen Mustard Degradation Products by Liquid Chromatography/ Tandem Mass Spectrometry (LC/MS/MS)





Office of Research and Development
National Homeland Security Research Center

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Technical Report for Surface Analysis of Nitrogen Mustard Degradation Products by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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United States Environmental Protection Agency
National Homeland Security Research Center
Cincinnati, OH 45268

and

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

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Disclaimer

The information in this technical document is comprised of multiple experiments with intent to develop a single-laboratory-developed sampling and analytical procedure (SAP) for the surface analysis, using wipes, of nitrogen mustard degradation products using LC-MS/MS (EPA 600/R-11/143). The research has been funded wholly or in part by the U.S. Environmental Protection Agency (EPA), Office of Research and Development (ORD), National Homeland Security Research Center (NHSRC) and in collaboration with 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 and EP10C000016. This document has been subjected to the Agency's review and has been approved for publication. Note that approval does not signify the content necessarily reflects 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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EXECUTIVE SUMMARY

The U.S. Environmental Protection Agency (EPA) is charged with developing tools and methodologies that enable the rapid characterization of indoor and outdoor areas and water systems following terrorist attacks or natural or manmade disasters. Chemical warfare agents (CWAs) and their degradation products remain a high-priority concern due to the presence of primary warfare agents in the U.S. chemical warfare agent inventory and some CWA degradation products can be as toxic as the parent compounds. Nitrogen mustard agents are vesicant CWAs which can break down into environmentally persistent degradation products. Sample stability studies suggest nitrogen mustard degradation products can persist in the environment for several weeks, and probably much longer depending on the associated environmental conditions. If an incident were to occur within an indoor setting, versatile sampling procedures are needed to detect CWA degradation products from various CWAs, including nitrogen mustard, from multiple types of contaminated surfaces (*e.g.*, walls, floors and furniture).

Several different wipes were tested, but only the filter paper wipe was considered viable straight out of the box. Filter paper wipes were selected over other wipes (including cotton gauze and non-woven polyester fibers) because they did not contain peaks that interfered with the target analytes, resulted in the highest percent recoveries and the lowest background levels during sample analysis. For nitrogen mustard and its degradates, cotton gauze would be an inappropriate choice unless the gauze is pre-cleaned and treated prior to use, a time-consuming and potentially costly approach, due to the contamination of TEA and DEA within the wipe. Sampling kits provided to samplers in the field, equipped with pre-packaged cotton gauze, would need to be tested to ensure that targeted analytes are not present, whereas no pretreatment is needed for filter paper.

Selective analysis methods must be employed to detect the appropriate degradation products from the environmental sample. The described sampling and analysis procedure employs the use of LC/MS because of its versatility, which will aid laboratories with the enhanced capability and capacity to analyze certain environmental matrices for polar CWA degradation products. Gas chromatography-mass spectrometry analysis requires an extra derivatization step, which is often problematic. Although LC-MS analytical methods currently exist for nitrogen mustard degradation products in water, there are no known wipe sampling collection and analysis protocol for the detection of nitrogen mustard degradation products from contaminated surfaces.

This report describes experimental details for the research method development and application, by a single laboratory, to assess the recoveries of nitrogen mustard degradation products from porous (vinyl tile, painted drywall, wood) and nonporous (laminate, galvanized steel, glass) surfaces. Performance data (method detection limit and precision and accuracy) are available to demonstrate the fitness-for-purpose towards developing a protocol for nitrogen mustard degradation products in that single laboratory. Analysis of blank samples revealed the presence of TEA and DEA on all tested surfaces, most notably in metal, glass, painted drywall and wood. This was expected given the common commercialization of TEA and DEA in industrial applications (*e.g.*, metal working fluids, soaps, foaming agents, cleaning agents, etc.). Samples are collected from spiked surfaces with wipes and carried through methanol extraction by sonication, filtration, and concentration 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 from the application of wipes to a laminate surface, following 40 CFR Part 136, Appendix B, as part of EPA's guidelines for determining a method detection limit. Percent recoveries for the laminate surface were 66-109% for all targeted nitrogen mustard degradation products. The resulting method detection limits obtained from the wipes were 0.12 ng/cm^2 for triethanolamine (TEA), 0.06 ng/cm^2 for *N*-ethyldiethanolamine (EDEA), 0.07 ng/cm^2 for *N*-methyldiethanolamine (MDEA), and 0.04 ng/cm^2 for diethanolamine (DEA).

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List of Acronyms and Abbreviations

ACN	Acetonitrile
CAS	Chemical Abstract Service
CWA	Chemical Warfare Agent
DHHS	U.S. Department of Health and Human Services
DEA	Diethanolamine
DL	Detection Limit
EDEA	*N*-Ethyldiethanolamine
EPA	U.S. Environmental Protection Agency
ESI	Electrospray Ionization
HILIC	Hydrophobic Interaction Liquid Chromatography
HPLC	High performance liquid chromatography
IDL	Instrument Detection Limit
LC	Liquid Chromatography
LC-MS/MS	Liquid Chromatography Coupled with Tandem Mass Spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantitation
MDEA	*N*-Methyldiethanolamine
MDL	Method Detection Limit
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometry
MSDS	Material Safety Data Sheets
MS/MS	Tandem Mass Spectrometry
NH_4OAc	Ammonium Acetate
NHSRC	National Homeland Security Research Center
NIOSH	National Institute for Occupational Safety and Health
NMAM	NIOSH Manual of Analytical Methods
ORD	Office of Research and Development
PPB	Parts per billion
PPE	Personal Protection Equipment
PPM	Parts per million
P&A	Precision and Accuracy
PVDF	Polyvinylidene Fluoride
PTFE	Polytetrafluoroethylene
QAPP	Quality Assurance Project Plan
QA	Quality Assurance
QC	Quality Control
R^2	Correlation Coefficient
RSD	Relative Standard Deviation
RT	Retention Time
RTS	Retention Time Shift
SAM	Selected Analytical Methods for Environmental Remediation and Recovery
SAP	Sampling and Analytical Procedure
SD	Standard Deviation
S/N	Signal to Noise
SOP	Standard Operating Procedure
TEA	Triethanolamine
TQD	Triple Quadrupole Detector
UPLC	Ultra-Performance Liquid Chromatography

1.0 Introduction

After the terrorist attacks of September 11, 2001, and the anthrax attacks in the fall of 2001, reports prepared by the Environmental Protection Agency (EPA) identified several research gaps that needed to be addressed to improve the country's preparedness in the event of a terrorist attack. A critical area identified was the need for a list of standardized analytical methods to be used by all laboratories when analyzing samples from a homeland security incident. 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 incident. *SAM* can be used by public and private laboratories that are analyzing a large number of samples associated with chemical, biological, biotoxin or radiological contamination in environmental matrices. 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, while some methods are standardized for selected chemicals in specific matrices, not all of the analytical methods listed in the *SAM* document address all possible matrices (e.g., water, soil, air, surfaces) encountered in sample collection following an incident. Some of the analytical methods in *SAM* have been verified in a single laboratory, but most still need to undergo verification with respect to a specific contaminant in association with a specific matrix.

Contamination by Chemical Warfare Agents (CWAs) and their degradation products remain an environmental concern due to their persistence, toxicity and presence of primary warfare agents in the U.S. CW agent inventory [2] and the possible threat of the use of these agents in a homeland security-type incident. While compiling methods for *SAM*, CWAs and their degradation products were selected for inclusion based on environmental persistence and toxicity. Many of the CWA degradation products are in need of more appropriate methods that will enhance sampling and analysis capability to improve lab capacity by using better analysis techniques, such as liquid chromatography-mass spectrometry (LC-MS), an appropriate and powerful technique for polar CWA degradation product analysis. Such degradation products are not analyzed well using GC-MS without a derivatization step, a tedious and time-consuming process when throughput of numerous samples is critical. For instance, derivatization typically does not result in complete transformation of the target analytes with respect to their analysis products, especially when water is present, resulting in detection limit complications. Establishing a collaborative effort with additional technical expertise and research capabilities in analytical methods to that of the U.S. EPA seemed appropriate for CWA degradation products involving specific matrices. The National Institute for Occupational Safety and Health (NIOSH) was chosen because of their capabilities in analytical methods for several chemicals using High Performance Liquid Chromatography (HPLC), which can be found in the NIOSH Manual of Analytical Methods (NMAM) [3]. NIOSH has used LC/MS to measure chemotherapeutic drugs, isocyanates, and components of metal-working fluids, all polar analytes. Furthermore, Ultra-Performance Liquid Chromatography (UPLC) is also becoming a powerful tool in the field of analytical chemistry for its enhanced ability for rapid throughput of samples. Because of the power and versatility of LC-MS, application to CWA degradation products may provide laboratories with improved capability to analyze certain environmental matrices after an incident.

The information within this technical document is comprised of multiple experiments resulting in the successful efforts, as well as complications, that may arise when working with nitrogen mustard degradation products on surfaces associated with the single-laboratory-developed sampling and analysis procedure (SAP) using LC-MS/MS (EPA 600/R-11/143). Experimental details described wthin will help fill data gaps related to the need for wipe sampling and analysis during or after an incident involving CWAs and their degradation products. Wipe sampling is the preferred collection method because there is less destruction of the tested surface, and wipe sampling can be performed quickly and easily when direct extraction is not always possible. The purpose of the SAP was to develop a method for the detection and recovery of CWA degradation products, specifically ethanolamine-based nitrogen mustard degradation products, from various porous (vinyl tile, painted drywall, wood) and mostly nonporous (laminate, galvanized steel, glass) surfaces using wipes with proper quality assurance objectives set forth in the Quality Assurance Project Plan (QAPP). Filter paper wipes were selected over other wipes (including cotton gauze, glass fiber filter and non-woven polyester fiber wipes) because the filter paper wipes did not produce chromatographic peaks that interfered with the target analytes, resulting in the highest percent recoveries and lowest background levels for the filter paper wipes during sample analysis. Research investigating the various wipes was described in report EPA 600/R-11/143 [5]. The method detection limits obtained from the filter paper wipes with a laminate surface were 0.12 ng/cm^2 for triethanolamine (TEA), 0.06 ng/cm^2 for *N*-ethyldiethanolamine

(EDEA), 0.07 ng/cm^2 for *N*-methyldiethanolamine (MDEA), and 0.04 ng/cm^2 for diethanolamine (DEA). Precision and accuracy data were generated from each tested surface fortified with these analytes. Various parameters, including the selection of surface materials, instrumental factors, limits of detection and quantitation and recoveries of the analytes from actual surfaces, were investigated and used to demonstrate the fitness-for-purpose of the method for nitrogen mustard degradation products in a single laboratory.

2.0 Determination of Analytes by LC/MS/MS

Four compounds known to be degradation products of nitrogen mustard CWAs are triethanolamine (TEA), *N*-ethyldiethanolamine (EDEA), *N*-methyldiethanolamine (MDEA) and diethanolamine (DEA). Neat standards of each compound (TEA, DEA, EDEA, MDEA and DEA-d_8) were used to prepare methanol solutions containing all analytes. Approximate 1000 parts per million (ppm) stock standard solutions containing each individual compound were prepared (*e.g.*, 44.4 µL TEA, 45.87 µL DEA, 49.31 µL EDEA, 48.08 µL MDEA and 45.87 µL DEA- d_8 in 50 mL of methanol), diluted to make 10 ppm solutions and each 10 ppm stock solution was utilized to develop a calibration solution containing all of the target analytes at an approximate concentration of 500 ng/mL (Level 7) (*e.g.,* 1.25 mL of each 10 ppm stock solution added to a 25 mL flask and diluted to the mark with methanol). The remaining concentration levels 1 through 6 were prepared from the level 7 solution, with all approximate concentrations in parts per billion (ppb) exhibited in Table 1. All spiking and calibration solutions were stored in amber volumetric flasks at 4 °C (± 2 °C). Holding time study data on the stability of the solutions can be found in Section 5.3. A calibration curve was generated from analyte concentration levels 1-7 and was qualitatively and quantitatively determined by LC-MS/MS in a low to high order to ensure that no carryover would occur.

Table 1. Suggested target calibration levels for nitrogen mustard degradation analytes (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

After the calibration curve has passed all quality control verifications described in the SAP and Section 4.2, sample collection and processing procedures were followed as described in Section 4.1. For example, solutions of appropriate concentration were used to spike a surface. The sample was allowed to dry, collected using a filter wipe, extracted, filtered, concentrated and analyzed to determine the presence of nitrogen mustard degradation products. Direct injection without derivatization, using liquid chromatography/tandem mass spectrometry (LC-MS/MS) provided complete analysis of each sample. Diethanolamine-d_8 (DEA-d_8) served as the surrogate standard for this project because its properties are similar to all of the analytes of interest and the deuterated compound is unlikely to be present in environmental samples. Although certain LC-MS effects such as ESI suppression/enhancement are not taken into account, the deuterated compound still satisfies the quality control provisions, *i.e.,* when added at a known concentration to the sample prior to processing, the deuterated compound provides a measure of the overall efficiency of the recovery. The analyzed data suggest that the surrogate is an appropriate choice at this time. The instrument's software served as a valuable resource tool for providing accurate recovery results. At the time, it was more advantageous to use the deuterated compound as a surrogate for the sampling process rather than the internal standard. All qualitative and quantitative control parameters were monitored to ensure all that quality assurance protocols were met.

2.1 Mobile Phase Composition and Gradient Conditions

Alternative mobile phase compositions for possible use with the target analytes were tested, including ammonium acetate and ammonium formate buffers. Although ammonium formate did produce a lower background signal than the acetate constituent, the ammonium formate solution produced chromatographic peaks for the four compounds of interest that overlapped chromatographically. As a result, the ammonium acetate was chosen over the formate. Different concentrations of ammonium acetate (10 mM, 15 mM and 25 mM) were also investigated. Sensitivity of each analyte peak increased as ammonium acetate concentrations were increased; however, higher molar concentrations of the buffer may result in retention time shifts and/or result in blockage of the column or guard column. The highest concentration of 25 mM ammonium acetate was chosen for sensitivity reasons, but if any of the problems mentioned above occur, lower concentrations should be used.

In addition to the use of ammonium acetate mobile phase for the analysis of DEA, TEA, MDEA, EDEA and DEA-d_8, acetonitrile was also used to produce a binary mixture of the two solutions under gradient conditions. For this experiment, the instrument was equipped with a binary solvent system; however, in some cases, instruments can be outfitted with ternary solvent systems for performing gradient elutions and the ternary system may be used, if applicable. Due to the manufacturer's suggestion for operation of the Hydrophobic Interaction Liquid Chromatography (HILIC) column, the ammonium acetate solution was buffered to below pH 5 with acetic acid. Deterioration of the column, working outside this range, would otherwise occur quicker than anticipated. Other HILIC columns may have a wider pH range tolerance capable of using the solvents described herein without the need for buffering, which may be worth using, when applicable.

The binary solvent system for the proposed gradient consisted of two different solutions (A and B). Solution A consisted of 25 mM ammonium acetate at pH 4.2 (buffered with glacial acetic acid) and 5% acetonitrile added to prevent microbial growth (A: 95% 25 mM ammonium acetate at pH 4.2 and 5% acetonitrile). Solution B consisted of 95% acetonitrile and 5% 25 mM ammonium acetate to achieve the overall approximate composition of 25 mM for ammonium acetate. Gradient conditions are displayed in Table 2. Preliminary studies using Ultra-performance liquid chromatography (UPLC) technology can shorten run times to less than 10 minutes when throughput becomes an issue using a specialty HILIC UPLC column and LC unit capable of handling higher pressures. Since most laboratories are not equipped with an LC unit capable of handling such pressures, the data are not included in this study report.

Table 2. Liquid chromatography gradient conditions and parameters

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

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

2.2 Mass Spectrometer Parameters

Optimal transitions for cone voltages and collision energies were determined by two different techniques. The first technique involved manually tuning the instrument for each of the five analytes, resulting in proper cone voltages with the strongest signal intensity for each analyte. The strongest signal intensity was defined as having the greatest signal

intensity with uniform width over a span of 1 Dalton at half the peak height. Daughter ions were chosen based on the collision energy that provided the greatest signal intensity of the intended daughter peak relative to the parent ion peak.

The second technique used the MassLynx™ software specific to the Waters TQD instrument (AutoTune Wizard™) and required only the input of the original masses for each of the analytes of interest. The instrument software was capable of accurately determining the proper cone voltages resulting in the strongest signal intensities for each analyte as well as the appropriate collision energies for each daughter ion. Automatic tuning performed by the instrument's software was the preferred tuning method because it was quick and efficient and found the same MRM mass transitions as the manual tuning technique. If an incorrect or undesired daughter ion was chosen by the program during the automatic tuning process, the program was re-run until the correct daughter ion was found. Manual tuning can also be performed, as described above. Mass transitions and variable mass spectrometer parameters (Table 3 and 4) were selected by the automatic tuning software for the analysis of the nitrogen mustard degradation analytes. The process by which all the analytes can be tuned simultaneously is quick, requiring only a couple of minutes, making the simultaneous tuning technique the preferred method. The use of the instrument capabilities to tune properly to the target analytes will save time, an efficiency that can be crucial when large quantities of samples need to be analyzed and quick throughput is necessary.

Table 3. MRM ion transitions and variable mass spectrometer parameters for each analyte

Analyte	Cone voltage	MRM mass transition (parent → daughter)	Collision energy (eV)
Triethanolamine	30	150.09 → 132.10	12
N-Ethyldiethanolamine	30	134.02 → 116.10	14
N-Methyldiethanolamine	30	120.03 → 102.00	12
Diethanolamine	30	106.00 → 88.10	12
Diethanolamine-d_8 (Surrogate)	30	114.20 → 96.22	12

Table 4. ESI $^+$ MS/MS operating conditions

MS Parameter (ESI+)	Setting
Capillary Voltage	1.0 kV
Cone Voltage	See Table 3
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 eV
Entrance Energy	1
Collision Energy	See Table 3
Exit Energy	1
Low Mass Resolution 2	15.0
High Mass resolution 2	15.0
Ion Energy 2	0.5 eV
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

2.3 Establishing an Instrument Detection Limit

The determination of the capability of an instrument to detect target analytes at very low levels is important and can be accomplished by establishing the instrument's detection limit (IDL). Successive decreases in solution concentration starting with a known concentration containing all five compounds followed by analysis at each concentration were used to observe signal:noise (S/N) ratios at each concentration. A signal:noise ratio of at least 3:1 was achieved with low level concentrations to ascertain the IDL with a Waters Acquity™ and tandem quadrupole detector (TQD), for liquid chromatography and mass spectrometric analysis, respectively. Table 5 and Figure 1 depict the IDLs obtained for the five nitrogen mustard degradation compounds using a Waters Acquity and TQD system.

Table 5. Retention times (RTs), approximate instrument detection limit (IDL) concentrations and signal:noise (S:N) ratios of nitrogen mustard degradation analytes

Analyte of Interest	RT* (minutes)	IDL (ng/mL)	Signal: Noise Ratio (S:N)
Triethanolamine (TEA)	9.7	1.0	8.36
N-Ethyldiethanolamine (EDEA)	11.1	1.0	5.68
N-Methyldiethanolamine (MDEA)	13.0	1.0	5.77
Diethanolamine (DEA)	12.1	1.0	4.07
Diethanolamine-d_8 (DEA-d_8)	12.2	1.0	5.49

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

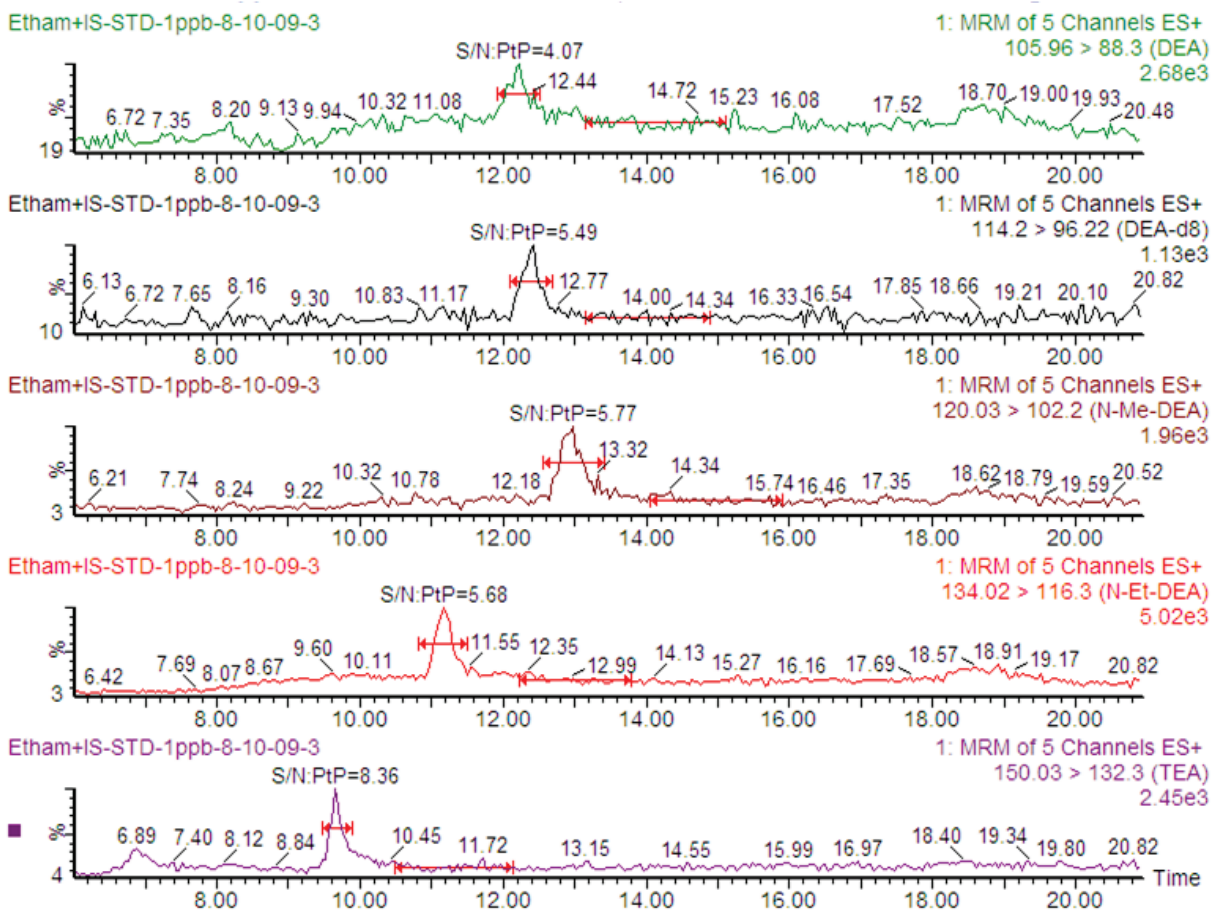

Figure 1. Chromatogram depicting the IDLs for nitrogen mustard degradation compounds (DEA, surrogate DEA-d_8, MDEA, EDEA, and TEA, respectively from top to bottom) in methanol. (Analyte names and MRM transitions are listed on the top right of each chromatogram)

3.0 Selection of Wipe Materials

In general, wipes are comprised of different materials from various manufacturers. Furthermore, wipe sampling kits can be assembled and are intended to contain pre-cleaned materials. Although wipe sampling kits were not tested in this experiment, the sterile wipes that were tested in this study were found to contain contaminants that produced chromatographic interferences with the analytes of interest. Therefore investigation of pre-cleaned kits should be explored to ensure that no interferences or contamination is present. In an effort to understand wipe sampling methods, analytes were tested on several wipe materials to determine wipe efficacy assessing two different factors, recovery and possible interferences with target analytes. The analysis of different types of wipes evaluated which wipe would have the least amount of interference with the compounds of interest and provide the lowest background signal for a blank extract from a wipe in methanol solution. Wipes tested in this experiment were both common wipes, typically used in wipe methods, and not-so-common wipes. Common wipes included sterile cotton gauze pads (Certi-Gauze Pad™), Ghost™ wipes (used for metals analysis), and an Alphawipe® (a continuous synthetic polyester, low particle, high absorptivity wipe). Alphawipes are laundered and packaged in a clean room. Less common wipes used for experimentation were Millipore™ nitrocellulose fiber filters, Whatman® glass microfiber filter paper, Reeve Angel glass microfiber filter paper, Whatman® filter paper, and a nonwoven polyester fiber cloth from National Nonwovens Textile (thick absorbent cloth). The nitrocellulose filter deteriorated in methanol resulting in a cloudy solution and clogged filters and was not used for the remainder of the experiment. Ghost™ wipes were also dropped due to complications from the extraction procedure and low recoveries of the target analytes.

Performance results from each wipe material spiked with nitrogen mustard degradation products are shown in Table 6 and Figure 2. Cotton gauze and non-woven polyester fiber cloth wipes exhibited high levels of TEA and DEA contamination present in blank and known spiked low concentrations of the target analytes. Recoveries of the target analytes from

Alphawipes were low and exhibited a peak possible interfering with TEA (Figure 2). Both glass fiber and filter paper wipes did not contain any interference peaks associated with the target analytes and appeared to be free of any contaminants that would affect the background of a blank sample. Glass fiber filter wipes were not as robust as filter paper wipes, disintegrating upon wiping any surface. Filter paper was the preferred wipe to use on all surfaces for this study because of the lack of necessity for a pre-cleaning step because of interferences and contaminants and good recoveries of the targeted analytes.

Table 6. Average recoveries from three different approximate spike concentrations tested on different wipe materials

COTTON GAUZE								
	TEA		EDEA		MDEA		DEA	
Spike Concentration (ng/mL)	Recovered (ng/mL)	% Recovery	Recovered (ng/mL)	% Recovery	Recovered (ng/mL)	% Recovery	Recovered (ng/mL)	% Recovery
50	74	148	14	28	15	30	217	433
25	64	254	7	27	9	37	173	691
10	68	682	4	35	5	49	168	1680
NON-WOVEN POLYESTER FIBER CLOTH								
	TEA		EDEA		MDEA		DEA	
Spike Concentration (ng/mL)	Recovered (ng/mL)	% Recovery	Recovered (ng/mL)	% Recovery	Recovered (ng/mL)	% Recovery	Recovered (ng/mL)	% Recovery
50	484	968	41	82	38	75	97	193
25	548	2190	27	107	25	99	42	168
10	508	5080	13	126	12	120	37	373
ALPHAWIPE™								
	TEA		EDEA		MDEA		DEA	
Spike Concentration (ng/mL)	Recovered (ng/mL)	% Recovery	Recovered (ng/mL)	% Recovery	Recovered (ng/mL)	% Recovery	Recovered (ng/mL)	% Recovery
50	11	23	11	22	11	21	9	18
25	5	19	5	21	5	20	4	16
10	0.9	9	2	18	2	17	1	10
WHATMAN GLASS FIBER								
	TEA		EDEA		MDEA		DEA	
Spike Concentration (ng/mL)	Recovered (ng/mL)	% Recovery	Recovered (ng/mL)	% Recovery	Recovered (ng/mL)	% Recovery	Recovered (ng/mL)	% Recovery
50	8	16	53	106	28	56	18	36
25	3	14	24	97	11	45	6	24
10	0.1	1	9	86	3	34	2	19
WHATMAN FILTER								
	TEA		EDEA		MDEA		DEA	
Spike Concentration (ng/mL)	Recovered (ng/mL)	% Recovery	Recovered (ng/mL)	% Recovery	Recovered (ng/mL)	% Recovery	Recovered (ng/mL)	% Recovery
50	41	82	50	101	42	83	38	77
25	20	78	26	103	22	89	20	80
10	7	74	9	87	8	76	6	62

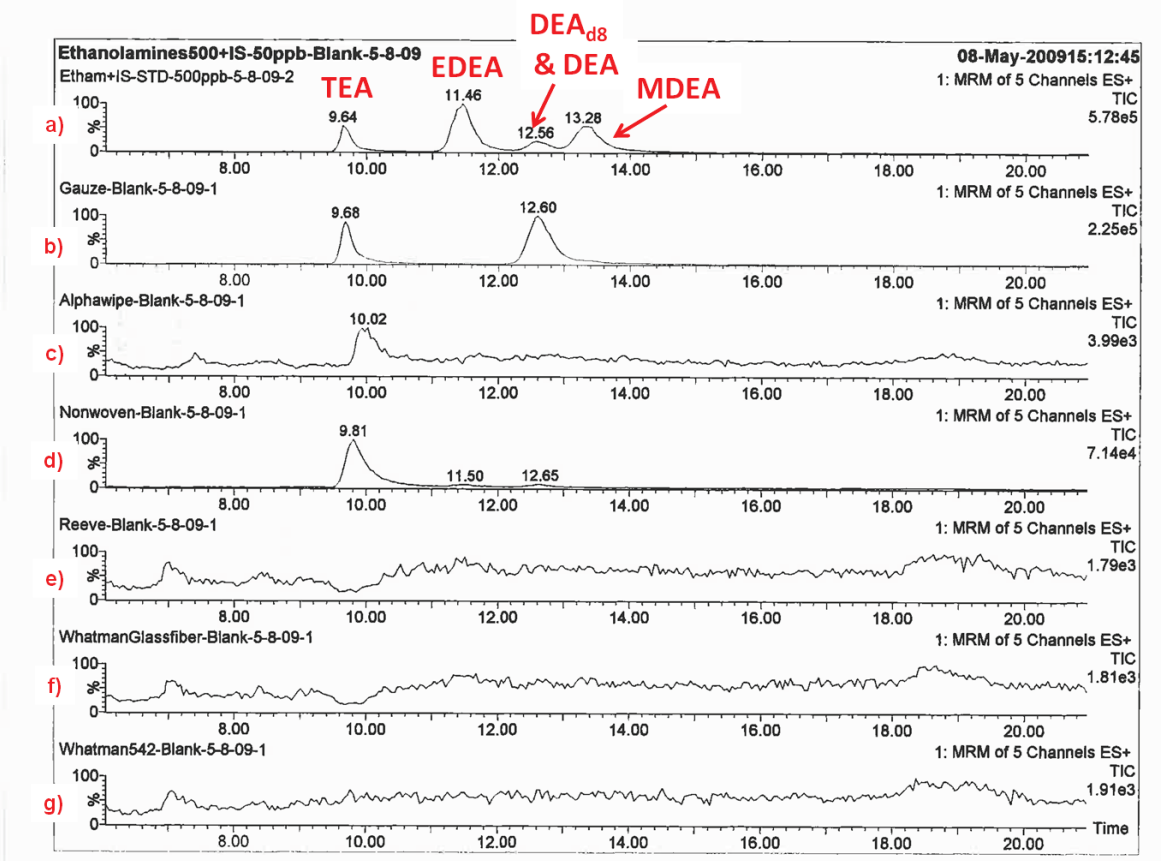

Figure 2. MRM chromatograms exhibiting contaminants within wipe materials with the addition of only methanol to the wipe. Sample a) is strictly for comparison. a) Nitrogen mustard degradation products stock standard at 500 ng/mL, b) Cotton gauze blank in methanol, c) Alphawipe® blank in methanol, d) Nonwoven polyester fiber wipe blank in methanol, e) Reeve® glass fiber wipe blank in methanol, f) Whatman® glass fiber wipe blank in methanol, g) Whatman® filter paper blank in methanol.

4.0 Determination of the Recovery of Analytes from Surface Materials

A variety of surface materials, both porous and nonporous, were tested to emulate what would possibly be encountered at common urban settings. Porous surfaces (vinyl tile, wood, painted drywall) and nonporous surfaces (glass, steel, laminate) and are listed in Table 7. All materials could be obtained from commercial suppliers and/or manufacturers. Other materials such as carpet and concrete were part of the tested materials but were subsequently dropped from the experiment because they did not produce recoveries above the analytical protocol detection limit. Further investigation with the described porous materials is needed to determine if recovery from such a surface is possible. A pre-determined area of 100 cm^2 (10 cm x 10 cm) was selected for surface wiping. Analytes were spiked onto each surface to be tested and then wiped with the chosen filter paper wipe to assess wipe efficacy and recovery. This approach was selected to mimic an actual field sampling event. Sample collection, extraction and processing procedures using the filter paper are discussed in the subsequent sections.

Table 7. Surface materials tested for the wipe analysis of nitrogen mustard degradation products

Surface Material	Manufacturer/Vendor
Glass	Carolina Glass Co./Lowe's
Vinyl Tile	Armstrong/Home Depot
Formica	Wilsonart® Laminate/Home Depot
Pretreated Pine Wood (2" x 4" pine)	Home Depot
Galvanized steel	McMaster-Carr
Painted Drywall (paint & primer in one, single coat, acrylic)	BEHR/Home Depot

4.1 Sample Collection/Extraction/Concentration Procedure

All wipe sampling and collection materials used during the sampling and processing of samples were tested to ensure that none of the target analyte species were native components of any of the sampling materials used in the method and that no significant loss (>10%) of analyte species occurred. Materials used in sampling are listed in Table 8. Polypropylene sampling containers were used over conventional glassware because preliminary studies suggest target analytes may have a propensity to adhere to the glassware affecting recoveries. If different sampling materials than those described within this report are used, it will be necessary to test the alternate materials to ensure they do not contain any of the targeted analytes of concern or result in significant losses.

Table 8. List of consumable materials used during sampling

Sampling and Collection Material	Manufacturer	Vendor
Whatman 42 ashless circle filters, 55 mm	GE Healthcare Life Sciences (Piscataway, NJ)	Fisher Scientific (Pittsburgh, PA)
125 mL Nalgene polypropylene straight-side jars with screw caps	Nalge Nunc International (Rochester, NY)	Fisher Scientific (Pittsburgh, PA)
10 mL BD safety-lok syringes	Becton, Dickinson and Company (Franklin Lakes, NJ)	Fisher Scientific (Pittsburgh, PA)
Corning 15 mL graduated plastic centrifuge tubes	Corning Incorporated (Corning, NY)	Fisher Scientific (Pittsburgh, PA)
Millipore 13 mm Millex filter, 0.22 µm PVDF	EMD Millipore (Billerica, MA)	Fisher Scientific (Pittsburgh, PA)
Waters 1.8 mL amber glass vials with pre-slit silicone polytetrafluoroethylene (PTFE) screw cap	Waters Corp. (Milford, MA)	Waters Corp. (Milford, MA)

4.1.1 Sample Collection

Sample coupon sizes of the various surface materials (*i.e.,* glass, stainless steel/metal, formica, vinyl, and wood) were cut to provide a 10 cm x 10 cm (100 cm^2) template. Each coupon was spiked with the appropriate concentration of a solution containing DEA, TEA, MDEA and EDEA in a pattern comprised of five equivalent spots (Figure 3). The solution was allowed to dry on the surface for approximately 5-10 minutes to ensure complete solvent evaporation. Two separate wipes were used to wipe the surface, with each wipe wetted with 300 µL of methanol (sufficient to wet the entire wipe). Coupons were wiped in a Z-wipe pattern (Figure 4), with the first wipe used in a horizontal Z-wipe pattern and placed in a 125 mL Nalgene® polypropylene straight-sided jar with a polypropylene screw cap. The second wipe was used in a

vertical Z-wipe pattern, placed in the same jar and capped.

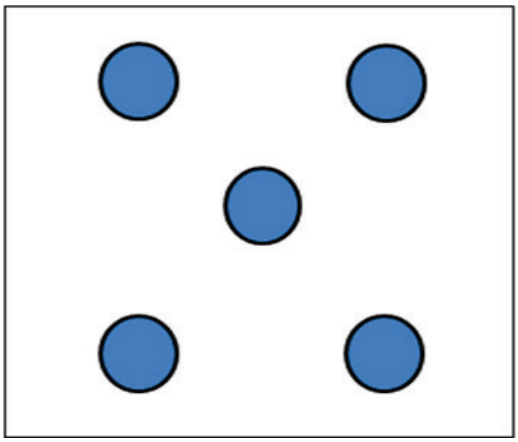

Figure 3. Illustration depicting the spiking pattern on a 100 cm^2 surface coupon.

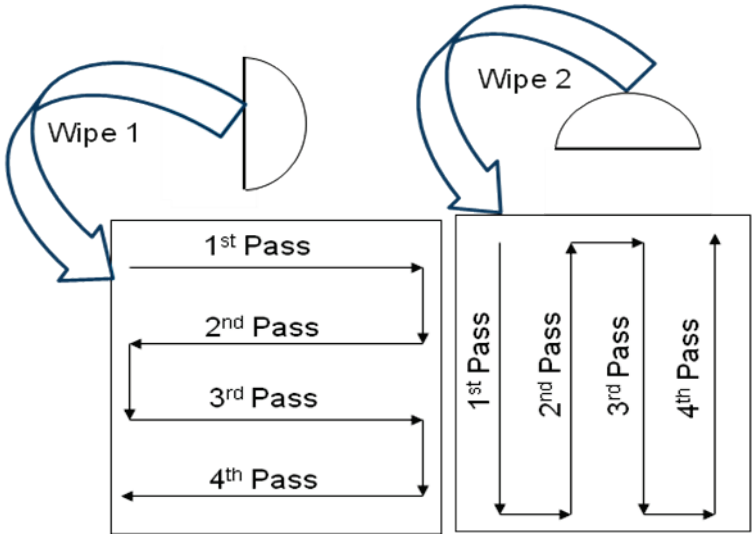

Figure 4. Illustration of wiping pattern on 100 cm^2 surface.

4.1.2 Extraction

When the samples were ready to be analyzed, the deuterated surrogate, DEA-d_8, was added to the jar and, after 5 minutes, approximately 10 mL of methanol was added to the jar. The jar was subsequently capped. The solvent volume fully immerses the wipes as they lie flat on the bottom of the jar. The jar was sonicated for 10-15 minutes in a water sonication bath at room temperature. Sonication studies (section 5.4) suggest optimal extraction time periods were approximately 10-15 minutes. Following sonication, the resulting solution was drawn into a 10 mL disposable Luer-lok syringe with a lock tip, fitted with a 0.22 µm polyvinylidene fluoride (PVDF) filter and filtered into a sterile 15 mL tube. Solvent recovery was approximately 80-90% of the original 10 mL solution.

4.1.3 Concentration

The solution was concentrated using a N_2-evaporation apparatus with a temperature-controlled water bath, maintained at 45-50 °C for final analyte concentration (< 10 mL volume). Solutions were concentrated to below 2 mL, then diluted with methanol to the 2 mL mark. The accuracy of the volume added to the tubes was comparable to that of an calibrated pipette. Other collection vials/tubes with more accurate markings may be desired if lower detection is necessary. The solution was lightly shaken, added to a 2 mL amber sample vial and analyzed by LC-MS/MS. Any remaining solution was stored in a refrigerator at or below 4 ºC (±2 °C).

4.2 Identification and Quantitation

Nitrogen mustard degradation compounds were analyzed at different concentrations, within the range of the calibration curve (10-500 ng/mL). Some sample concentrations were different from the actual values of the curve to demonstrate accuracy over various points of the calibration curve. (**NOTE:** All chosen concentrations were within the calibration range to ensure analyte response linearity.) Even with a linear response function (where the R^2 value is above 0.99), the concentrations that were not identical to the calibration levels fell within the linear range with little to no bias illustrating how well the wipe analysis concentrations fit along the curve. Qualitatively, a positive identification of an analyte molecule required the retention time window of the MRM transition to be within 5% of the retention time of the analyte standard. Analysis of seven replicate samples at each concentration demonstrates the precision of the method at various concentrations.

Quantitative analysis of samples was accomplished by the performance of a linear regression of the peak areas for each nitrogen mustard degradation product in the seven calibration curve standards. The instrument software, QuanLynx™, was used to generate a polynomial calibration curve, which provided a mathematical relationship between peak area and analyte concentration, along with an accompanying correlation coefficient, R^2. If the polynomial type is linear or quadratic and excludes the point of origin, a fit weighting of 1/X was used to more heavily weight lower concentrations values, associated with low detection limits, over the higher concentration values. The SAP quality assurance/quality control (QA/QC) guidelines stipulate a minimum R^2 value of 0.98 for linear fits and 0.99 for quadratic fits. All of the calibration curves for the nitrogen mustard degradation SAP in this study had a correlation coefficient (R^2) value of greater than 0.99 for a linear fit to ensure quality performance. Although no points needed to be excluded, if a calibration standard, other than the high or low standard, causes the curve's correlation coefficient to fall below the stipulated minimum of 0.98 or 0.99, the standard should be re-injected and a new calibration curve must be generated and analyzed. An external calibration was used to monitor the MRM transitions of each analyte. Quanlynx™ software was utilized for the quantitation of the target analytes and external standard. The MRM transitions of each analyte were used for quantitation and confirmation by isolating the parent ion, fragmenting the parent ion to the daughter ion and relating the transition to the retention time in the calibration standard.

4.3 Waste Handling and Prevention

A waste container for all compatible chemicals used in these studies was dated and labeled prior to the addition of any waste. The waste container's label should be an accurate log of the container's chemical contents as well as the approximate amount of each species added to the container and was maintained throughout its use.

5.0 Results and Discussion

5.1 Detection Limit Determination

MDLs and LOQs were determined using EPA and NIOSH conventions. For simplicity and to avoid confusion, only EPA detection and quantitation limits were displayed in the SAP, but both are available in this report. The EPA-preferred protocol utilized guidelines in 40 CFR Part 136, Appendix B – Definition and Procedure for the Determination of the Method Detection Limit – Revision 1.11[5]. NIOSH guidelines require the use of Standard Operating Procedure (SOP) 018 and 504 for chemical analysis [6, 7].

5.1.1 EPA MDL

Calculation of the method detection limit (MDL) for the nitrogen mustard degradation compounds was performed according to guidelines in 40 CFR Part 136, Appendix B – Definition and Procedure for the Determination of the Method Detection Limit (Table 9). Protocol required the resulting MDL to be within ten times the spike level. Subsequent lower concentration levels for a mixture containing all analytes of interest were spiked onto each coupon surface, carried through the extraction/concentration process (Section 4.1) and analyzed until a S/N ratio of at least 3:1 was obtained. MDL levels were selected by evaluating three separate low concentration level samples in order to determine the lowest concentration level to be reproducibly recovered (< 20% RSD). Once the S/N ratio of approximately 3:1 was achieved, samples were subjected to full method preparation, extraction and analytical procedures for at least seven replicates for the matrix of interest. The MDL was determined by using the standard deviation (σ) of the seven replicates multiplied by the Student's t-factor for seven replicate samples (Student's t-factor is dependent on the number of replicates used; 3.143 assumes seven replicates) as shown in Table 9. The MDL was calculated using the formula below and the resulting data are displayed in Table 10 and Appendix A. Subsequent LOQ determination was calculated by multiplying the standard deviation of the MDL results by a factor of ten.

Table 9. Student's t-statistic value as it relates to the number of replicate samples

Number of Replicates (n)	Student's t-statistic
7	3.143

$$MDL = 3.143 \times SD \quad [5]$$

n = number of replicates = 7
3.143 = Student's t-value for (n-1) = 6 degrees of freedom at 99% confidence
SD = standard deviation of replicate analyses.
*** NOTE:** The value used for the Student's t-statistic will change if the number of samples analyzed or the confidence level is altered.

Unspiked coupons were wiped and analyzed for the presence of nitrogen mustard degradation analytes that may be native to the surface materials as well as interferences. In each case, coupons were spiked with only the surrogate and not with the targeted analytes, and were taken through the same sampling and analysis procedure. Data from the analysis of unspiked (blank) coupons are shown in Table 12 as part of the precision and accuracy (P&A) data. Large relative standard deviations suggest the native analyte contamination/interference content of the coupon surfaces was not uniform. Furthermore, background subtraction for blank analyte levels was not performed. Analysis of blank samples revealed the presence of TEA and DEA (and/or interferences) on all tested surfaces, most notably in metal, glass, painted drywall and wood. This observation was not too surprising given the commonality and commercialization of TEA and DEA in industrial applications (*e.g.*, metal working fluids, soaps, foaming agents, cleaning agents, etc.). Although the data were affected by the contamination at lower concentrations and detection limit levels, higher concentrations (still below levels of concern) will be less likely to contribute to error. For this reason, the standard deviation is believed to contribute to higher MDLs and LOQs than what the SAP was capable of and can be found if the tested surfaces were cleaned prior to examination. Pre-cleaning surfaces does not, however, mimic a real-world scenario. Therefore all surfaces were used as received and the data represent an actual collection of analytes from an uncleaned surface with the knowledge that very low levels will show the presence of TEA and DEA and should be noted. Recovery levels of EDEA and MDEA were not affected by the contamination levels of TEA and DEA, suggesting that the SAP can be used for EDEA and MDEA as written. Further investigation of TEA and DEA is still needed to verify that the method can be used for its fitness-for-purpose.

Table 10. EPA calculation for MDL and LOQ in ng/cm^2 and ng/mL for nitrogen mustard degradation analytes on a laminate surface. Concentration levels (ng/mL) were divided by the surface area (100 cm^2) to achieve (ng/cm^2) results

Analyte	LAMINATE			
	MDL		LOQ	
	ng/cm^2	ng/mL	ng/cm^2	ng/mL
TEA	0.12	12.3	0.39	39.2
EDEA	0.06	6.3	0.20	19.9
MDEA	0.07	6.9	0.22	21.8
DEA	0.04	4.4	0.14	13.9

5.1.2 NIOSH MDL

NIOSH defined its detection limit as the mass of an analyte which gives a mean signal three times ($3\sigma_b$) above the mean blank signal, where σ_b is the standard deviation of the blank signal. The LOQ was defined as the mass corresponding to the mean blank signal + $10\sigma_b$ (i.e., ± 30% uncertainty, which would be 3.33 x MDL) or the mass above which recovery is ≥ 75%.

Four or more low-level concentrations were spiked onto the sampling media to cover a range from the expected MDL to no greater than ten times the anticipated MDL. Of the concentrations being used for a low-level experiment, 1-2 should be at or below the expected Limit of Detection (LOD), 1-2 at or near the mid-range, and 2-3 should be at or near the mid-range up to 10 x MDL. These samples were extracted and analyzed under the same conditions as would be encountered for field samples. A graph was compiled of the responses of the concentrations vs. the mass (or concentration) used. A linear regression equation was obtained and the standard error of the regression (s_y) was calculated (as explained in SOP 018). The MDL was determined as $3s_y$/slope. The LOQ for this study was reported as 3.33 x MDL due to the fact that some recoveries were below 75%, whereas other recoveries for the compounds were all above 75% even at the lowest concentrations. Contamination of the surfaces from the pre-existing presence of the analytes made it difficult to determine an appropriate MDL and LOQ using either EPA or NIOSH technique. As with the EPA determination, blank subtraction of the analytes was not performed in this experiment. The data are displayed in Table 11.

Painted Drywall and Wood MDL values were not calculated using the NIOSH determination because only the highest concentration level was used within the calibration range, and recoveries from that spike level were still low for all targeted analytes (1-20%).

Table 11. NIOSH calculation for MDL and LOQ determination for nitrogen mustard degradation analytes on surfaces.

LAMINATE							
TEA		EDEA		MDEA		DEA	
Spike (ng/mL)	Recovered (ng/mL)	Spike (ng/mL)	Recovered (ng/mL)	Spike (ng/mL)	Recovered (ng/mL)	Spike (ng/mL)	Recovered (ng/mL)
50	75	50	37	50	40	50	57
75	71	75	31	75	47	75	49
100	81	100	65	100	67	100	69
150	124	150	91	150	95	150	99
s_y	12.25	s_y	11.61	s_y	9.15	s_y	10.48
Slope	0.52	Slope	0.61	Slope	0.25	Slope	0.47
Slope RSD	0.31	Slope RSD	0.26	Slope RSD	0.49	Slope RSD	0.29
	ng/mL		ng/mL		ng/mL		ng/mL
LOD	71	LOD	57	LOD	110	LOD	67
LOQ	235	LOQ	190	LOQ	366	LOQ	223
	ng/cm^2		ng/cm^2		ng/cm^2		ng/cm^2
LOD	0.71	LOD	0.57	LOD	1.10	LOD	0.67
LOQ	2.35	LOQ	1.90	LOQ	3.66	LOQ	2.23

METAL							
TEA		EDEA		MDEA		DEA	
Spike (ng/mL)	Recovered (ng/mL)	Spike (ng/mL)	Recovered (ng/mL)	Spike (ng/mL)	Recovered (ng/mL)	Spike (ng/mL)	Recovered (ng/mL)
50	147	50	42	50	43	50	64
75	145	75	78	75	59	75	81
100	172	100	72	100	70	100	86
150	175	150	91	150	72	150	98
s_y	9.79	s_y	13.01	s_y	7.97	s_y	4.52
Slope	0.32	Slope	0.41	Slope	0.27	Slope	0.31
Slope RSD	0.41	Slope RSD	0.42	Slope RSD	0.39	Slope RSD	0.20
	ng/mL		ng/mL		ng/mL		ng/mL
LOD	92	LOD	94	LOD	89	LOD	44
LOQ	306	LOQ	314	LOQ	295	LOQ	146

GLASS							
TEA		EDEA		MDEA		DEA	
Spike (ng/mL)	Recovered (ng/mL)	Spike (ng/mL)	Recovered (ng/mL)	Spike (ng/mL)	Recovered (ng/mL)	Spike (ng/mL)	Recovered (ng/mL)
50	219	50	26	50	30	50	58
75	256	75	50	75	57	75	92
100	256	100	53	100	55	100	77
150	261	150	78	150	84	150	119
s_y	15.54	s_y	6.19	s_y	7.97	s_y	14.63
Slope	0.34	Slope	0.49	Slope	0.49	Slope	0.54
Slope RSD	0.62	Slope RSD	0.17	Slope RSD	0.22	Slope RSD	0.37
	ng/mL		ng/mL		ng/mL		ng/mL
LOD	137	LOD	38	LOD	49	LOD	81
LOQ	457	LOQ	126	LOQ	163	LOQ	271

VINYL TILE							
TEA		EDEA		MDEA		DEA	
Spike (ng/mL)	Recovered (ng/mL)	Spike (ng/mL)	Recovered (ng/mL)	Spike (ng/mL)	Recovered (ng/mL)	Spike (ng/mL)	Recovered (ng/mL)
100	30	100	29	100	29	100	36.
150	137	150	14	150	16	150	32
200	143	200	52	200	64	200	71
300	224	300	87	300	75	300	79
s_y	32.6	s_y	16.74	s_y	18.07	s_y	13.56
Slope	0.88	Slope	0.34	Slope	0.28	Slope	0.25
Slope RSD	0.25	Slope RSD	0.34	Slope RSD	0.44	Slope RSD	0.37
	ng/mL		ng/mL		ng/mL		ng/mL
LOD	111	LOD	148	LOD	194	LOD	163
LOQ	370	LOQ	492	LOQ	645	LOQ	542

5.2 Precision and Accuracy Determination

Initial demonstration of a laboratory's capability to generate data of acceptable quality will be possible through the performance of a precision and accuracy (P&A) study. P&A sample data sets were collected at four concentration levels using a standard containing triethanolamine, *N*-ethyldiethanolamine, *N*-methyldiethanolamine and diethanolamine. Solutions were spiked onto the coupons at various concentrations, ranging from at or below the midpoint concentration in the calibration curve (with the exception of porous surfaces such as wood and painted drywall), generally different from those chosen for calibration standards. All chosen concentration levels must fall within levels 1 – 7 of the calibration standards listed in Table 1 to ensure analyte response linearity. One blank sample was added to each of the four sample sets to determine the presence of native species within the selected materials. As discussed in section 4.1, unspiked coupons were wiped and analyzed for the presence of nitrogen mustard degradation analytes that may be native to the substrate materials. Data from the analysis of unspiked (blank) coupons are shown in Table 12. As in section 5.1, TEA and DEA were present in every substrate material. Large relative standard deviations indicated that the native analyte content of the coupon surfaces was not uniform. Although background subtraction for blank analyte levels was not performed, such an analysis could be carried out for TEA and/or DEA recoveries. As referenced in Section 5.1.1, recovery levels of EDEA and MDEA were not affected by the contamination levels of TEA and DEA, suggesting that the SAP can be used for EDEA and MDEA as written. Seven replicates were used for this study for added statistical value. The average recoveries and standard deviations were calculated as described in Section 5.1 and are displayed in Table 12.

Nonporous surfaces should yield higher recoveries of the analytes. Porous materials are expected to result in lower recoveries due to analyte permeation into the material, requiring direct extraction of the entire test coupon as the better alternative for increased analyte recoveries. Alternative solvents used to extract target analytes from porous surfaces may result in improved recoveries; however, this experiment was not tested at this time. Due to the low recoveries on porous materials (painted drywall and wood) a concentration (500 ng/mL) higher than the midpoint of the calibration curve was used for P&A studies. Formica, metal and glass all provide acceptable recoveries at the 50 ng/mL level. Vinyl tile recoveries were not as high due to the porosity of the surface. As expected, painted drywall and wood produced the lowest recoveries and further testing would be needed to obtain better recoveries for these substrates, most likely direct extraction or a better sampling technique, including alternative solvents or wipes, for porous surfaces is needed.

Table 12. Precision &Accuracy (P&A) data for wipe analysis of nitrogen mustard degradation analytes on surfaces.

LAMINATE									
	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	151	8	37	74	17	40	79	6
75	71	95	8	31	72	8	47	62	11
100	81	81	6	66	66	4	67	67	5
150	124	83	10	91	61	14	95	63	9
Average Formica Blank	23	-	-	0	-	-	0	-	-

LAMINATE						
	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	114	25	38	75	6
75	49	66	6	41	55	3
100	69	69	7	62	62	5
150	99	66	10	93	62	12
Average Formica Blank	2	-	-	27	54	-

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	294	10	42	85	7	43	86	6
75	146	194	18	58	78	7	59	79	6
100	172	172	13	72	72	9	70	70	6
150	175	117	9	91	61	14	72	48	14
Average Metal Blank	118	-	-	0	-	-	0	-	-

METAL						
	DEA			DEA-d_8		
Average Spike Concentration (ng/mL) (n=7)	Average Recovery (ng/mL)	% Recovery	% RSD	Average Recovery (ng/mL)	% Recovery	% RSD
50	64	129	5	45	91	10
75	81	108	5	69	92	6
100	86	86	5	73	73	4
150	98	65	4	96	64	3
Average Metal Blank	16	-	-	50	100	-

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	439	6	26	52	7	30	61	6
75	256	342	10	50	66	5	57	76	4
100	256	256	12	53	53	14	55	55	14
150	261	174	8	78	52	12	84	56	10
Average Glass Blank	203	-	-	0	-	-	0	-	-

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	58	115	14	30	59	11
75	92	122	4	61	81	5
100	77	77	19	55	55	21
150	119	80	8	92	61	11
Average Glass Blank	19	-	-	53	105	-

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	30	30	25	29	29	11	29	29	9
150	137	92	7	14	9	11	16	11	7
200	143	71	7	52	26	8	64	32	7
300	225	75	10	87	29	15	75	28	16
Average Vinyl Blank	18	-	-	0	-	-	0	-	-

VINYL TILE						
	DEA			$DEA-d_8$		
Average Spike Concentration (ng/mL) (n=7)	Average Recovery (ng/mL)	% Recovery	% RSD	Average Recovery (ng/mL)	% Recovery	% RSD
100	36	36	13	28	28	19
150	32	21	19	18	12	4
200	71	35	12	46	23	6
300	79	26	10	60	20	14
Average Vinyl Blank	7	-	-	54	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	20	16	10	2	28	16	3	30
Average Wood Blank	73	-	-	0	-	-	0	-	-

WOOD						
	DEA			$DEA-d_8$		
Average Spike Concentration (ng/mL) (n=7)	Average Recovery (ng/mL)	% Recovery	% RSD	Average Recovery (ng/mL)	% Recovery	% RSD
500	37	7	22	10	2	32
Average Wood Blank	19	-	-	57	115	-

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	110	22	10	75	15	17	85	17	18
Average Drywall Blank	73	-	-	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	17	18	57	11	20
Average Drywall Blank	23	-	-	40	80	-

5.3 Holding Time Study

An evaluation of a holding time study provided a measurement of the stability of the wipe medium with the nitrogen mustard degradation analytes. Holding time studies consisted of five sets of eight samples: three blank wipe samples (spiked with neither analyte nor surrogate) and five analyte-spiked wipe samples. In blank samples, each wipe was wetted with 400 µL of methanol and placed into the sample jars. Samples were spiked with analyte to achieve a final analyte concentration of 50 ng/mL, using a 500 ng/mL spiking standard, with equal volume of the spiking standard added to each wipe of the sample. Each sample vial contained two filter papers. Following wetting or spiking, each sample set was analyzed after its designated holding time period of 0, 7, 14, 21, or 28 days. Each set of samples was stored at 4 °C (±2 °C) until processed and analyzed and the data are displayed in Table 13.

Determination of the variance between data sets being statistically different from the variance at time 0 data set was accomplished by performing the f-test. F-test results suggested no significant differences in the variances for each analyte at the different time frames. A paired t-test compared the mean recovery of each analyte at each holding time to its corresponding mean recovery at time 0 with 95% confidence. T-test results indicated no significant differences between the mean recovery values of any analyte to its counterpart at time 0. Analysis of results from these two tests suggests that both sample and sampling media were stable for time periods up to and including 28 days. Corresponding statistical data are presented in Appendix B.

Table 13. Holding time sample stability of wipes spiked with 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	93	6	98	7	95	6	93	5	96	7
7	94	6	85	9	82	8	85	7	90	9
14	75	10	87	6	82	8	82	6	86	11
21	75	7	84	10	83	10	78	10	84	11
28	78	5	88	5	87	5	74	12	90	9

5.4 Sonication Study

To decrease sample processing time in the event of a national security incident, variable sonication times involving spiked wipes were investigated to determine the optimal recovery of nitrogen mustard degradation products. The initial proposal of a 25 minute sonication time for the extraction of ethanolamine-based analytes from wipes (either direct-wipe spiking or after wiping spiked surfaces) was proposed. Data were collected at 25 minutes to serve as a control as the existing protocol calls for 25 minutes of sonication time. Data sets consisting of three samples, containing two Whatman 42 filters per sample, were spiked to achieve a final analyte concentration of 50 ng/mL, with equal volumes of the spiking standard added to each wipe of the sample. Sonication times of 5, 10, 15, and 25 minutes were analyzed to determine if comparable analyte recovery could be achieved with shorter sonication times.

Data presented in Table 14 indicated that a ten minute sonication time was sufficient for quantitative extraction of the desired analytes from the wipes. The recovery of the analytes at all sonication times was nearly quantitative, and five minute sonication time periods may be sufficient for analysis. Additional replicates for added statistical power would be needed to assess the effect of sonication time on analyte recovery from spiked wipes more effectively. A data set evaluating samples not subjected to sonication could also provide valuable input. Unless larger (or multiple) sonication baths are accessible or smaller sample vials are used, the number of samples available to simultaneously process was a limiting factor in sample turnaround time. As a result, shorter sonication times, larger shaker tables, alternative sampling vials, or all of the above may be suitable replacements for the materials used in this study.

Table 14. Analyte recovery from analyte spiked wipes at various sonication intervals.

Concentration 50 ng/mL (n = 3)										
	TEA		EDEA		MDEA		DEA		DEA-d_8	
Sonication time (min)	Average % Recovery	% RSD	Average % Recovery	% RSD	Average % Recovery	% RSD	Average % Recovery	% RSD	Average % Recovery	% RSD
5	89	7	92	10	91	9	94	13	97	9
10	100	5	108	3	104	8	111	5	120	3
15	85	5	94	3	98	2	96	2	105	3
25	94	6	107	8	103	7	111	9	116	8

6.0 Conclusion

A procedure for recovering nitrogen mustard degradation analytes from surfaces through wiping was developed and characterized on laminate, metal/stainless steel, glass, vinyl, painted drywall and wood. Several different wipes were tested, but only one wipe was considered viable straight out of the box when analysis of these analytes is being performed. Cotton gauze wipes were individually packaged and considered sterile, meaning they had been cleaned during the manufacturing process. Not only did cotton gauze wipes tested in this specific experiment contain very high levels of TEA and DEA, other cotton gauze wipes would also contain similarly high levels. The most preferred wipe used during sample analysis is typically cotton gauze. However, in this case it would not be appropriate to use a cotton gauze wipe unless further pre-cleaning and treatment has occurred. Furthermore, sampling kits provided to samplers in the field are equipped with pre-packaged wipes. Even if the wipes are pre-cleaned, testing on wipe materials is needed to ensure that targeted analytes were not present. For nitrogen mustard and its degradates, cotton gauze would need to be properly treated prior to use to remove all contaminations and interferences, a time-consuming and potentially costly approach, whereas no pretreatment is needed for filter paper. As a result, the preferred wipes used for the SAP were the Whatman® filter papers. Although glass fiber wipes are an alternative to the preferred wipe, they may not fare well when the rigors of wiping a surface are encountered. Whatman® filter paper wipes not only produce little to no interferences and low background blank levels, the overall recoveries using Whatman® filter paper wipes were reasonably high as well.

In addition to testing different wipes, alternative solvent systems were also explored. Both ammonium formate and ammonium acetate were found to be suitable for the analysis of the ethanolamine-based nitrogen mustard degradation compounds. If ammonium formate were to be used, full scale workup and procedures are needed in order to determine if this solvent system produces similar results. Ammonium acetate was chosen for this particular experiment because of the chromatographic separation of the targeted analytes. Such separation would allow for preliminary and possibly future analyses of the compounds on different instrumentation, such as an ion trap mass spectrometer. Various concentrations were also explored to observe the effect of eluent concentration. Although 25 mM ammonium acetate concentration was chosen for this study, such a high concentration of buffer might result in retention time shifts and clogging of the analytical column. The highest concentration of 25 mM ammonium acetate was chosen for sensitivity reasons, but if any of the problems mentioned above occur, caution should be noted and levels can be adjusted accordingly if such issues occur.

Recovery of the tested analytes from commonly encountered surfaces through wipe sampling was possible at low levels. Holding time studies indicated that the analytes and sampling media are stable for at least 28 days, but the target analytes are expected to persist much longer. Wipe sampling was a viable means of screening all tested surfaces (except painted drywall and wood) for the presence of degradation analytes. However, the capacity of wipe sampling for quantitation from surfaces was unclear due to incomplete recovery and sometimes poor reproducibility because of the presence of native TEA and DEA on surfaces. Qualitatively, the data could be used to determine the presence of EDEA and MDEA on all surfaces where recoveries were above the detection limit. Higher concentrations and/or a larger calibration curve range or

direct extraction may be necessary for analysis on more porous surfaces. For more porous surfaces, a better sampling procedure needs to be developed to convey the presence of such targeted analytes adequately.

All surfaces exhibited some type of TEA and DEA contamination at different levels. Surfaces with the highest TEA and DEA contamination were glass and metal, not surprising since most metal working fluids and cleaners are known to contain TEA and/or DEA. For this reason, the standard deviation is believed to contribute to higher MDLs and LOQs than what the SAP truly indicates. Lower MDLs and LOQs could be found if the tested surfaces were cleaned prior to examination. However, such a process would not be indicative of a real-world scenario. All surfaces were therefore used as received and the data represent an actual collection of analytes from an uncleaned surface with the knowledge that very low levels will show the presence of TEA and DEA and should be noted. Analytical investigation of blank samples detected no presence of EDEA and MDEA on any of the surfaces, suggesting the SAP will work well for those specific compounds at low levels. Only for analyses at low levels would the presence of TEA and DEA be an issue, possibly during site characterization for samples collected following decontamination, degradation rates, etc. However, since these specific compounds are common in real world applications and are not considered to be as toxic, higher MDL and LOQ levels are suggested (10-100-fold current MDLs used within the corresponding SAP) to prevent problems with recovery and standard deviation.

While the performance demonstration may be compatible for EDEA and MDEA, it is possible that further experimentation and alteration of experimental parameters may lead to better recoveries and lower detection limits, if needed, especially for TEA and DEA. Similarly, selection of alternate columns with wider pH ranges may lead to a simplified elution gradient. As instrumentation continues to improve, so can the analytical detection limits for any targeted compound. UPLC-MS/MS is an example of faster run times and potentially better results. If any of the experimental conditions are changed, the laboratory should rigorously establish the performance of the analytical approach operated under the modified conditions. Further studies will also be needed to test other nonporous surfaces to determine how well this particular wipe can perform on any surface that may need to be tested during a remediation event. The data serve as a proof-of-concept for the use of Whatman® filter paper in sampling procedures for the analysis of nitrogen mustard degradation products on most surfaces. Filter paper wipes appear to be an easier alternative for the sampling and analysis of nitrogen mustard degradation products than the commonly used cotton gauze wipe.

7.0 References

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

6. SOP 018: *Standard Operating Procedures for Industrial Hygiene Sampling and Chemical Analysis*, NIOSH/DPSE Quality Assurance Manual, July, 1994.

7. SOP 504: *Limits of Detection and Quantitation*, CEMB Laboratory Services, February, 2004.

Appendix A

Table A-1. Reagents and CAS numbers

CHEMICAL NAME	CAS #
Acetonitrile, ACN, LC/MS grade	75-05-8
Water, H_2O, LC/MS grade	7732-18-5
Methanol, MeOH, LC/MS grade	67-56-1
Ammonium acetate, NH_4OAc	631-61-8
Glacial acetic acid, HOAc	64-19-7
Diethanolamine, DEA	111-42-2
Triethanolamine, TEA	102-71-6
N-Methyldiethanolamine, MDEA	105-59-9
N-Ethyldiethanolamine, EDEA	139-87-7
bis(2-Hydroxyethyl)-d_8-amine (Diethanolamine-d_8), DEA-d_8	103691-51-6

Equipment

Waters Acquity TQD LC/MS/MS system, or equivalent
Fisher Scientific FS 140 H ultrasonic cleaner, or equivalent
OA-Sys N-evap 111 nitrogen evaporator, or equivalent
Mettler AE 240 analytical balance, or equivalent

Supplies

Automated Pipettes (100 µL, 1000 µL, and 10 mL)
Pipette tips (100 µL, 1000 µL, and 10 mL)
Class A volumetric flasks (250 mL, 500 mL, and 1000mL)
Class A amber glass volumetric flasks (10 mL, 25 mL, and 50mL)
10 cm x 10 cm pre-cut coupons (glass, metal/stainless steel, formica, vinyl, wood)
Whatman 42 ashless circle filters, 55 mm
10 mL BD safety-lok syringes
Millipore 13 mm Millex filter, 0.22 µm PVDF
15 mL graduated polypropylene centrifuge tubes
Waters 1.8 mL amber glass vials with pre-slit silicone PTFE screw cap

Special safety precautions

Experimenters should be familiar with MSDS sheets for all solvents and reagent chemicals used. Nitrile gloves, safety glasses and other PPE should be worn when working in the laboratory.

Procedure

1.	Spike coupons with standard solution, using 5 spots per coupon. Let dry completely.
2.	Wipe coupons with two Whatman 42 55 mm filters: a. Wet wipe with 300 µL MeOH. b. 1st wipe: wipe horizontally in a "Z-pattern". c. 2nd wipe: wipe vertically in a "Z-pattern". d. After wiping, place wipes flat in 125 mL Nalgene jar. e. Add approx. 10 mL of MeOH to each jar to completely cover wipes.
3.	Sonicate samples in jars for approximately 10 minutes.
4.	Withdraw as much of the solution as possible with a 10 mL disposable Luer-Lok syringe.
5.	Filter sample through a 13 mm 0.22 µm PVDF filter into a 15 mL graduated polypropylene centrifuge tube.
6.	Concentrate dilute sample down to ≤ 2 mL in a warm water bath using N_2 (being careful not to evaporate to complete dryness).
7.	Adjust sample volume to 2 mL, if necessary, with MeOH.
8.	Add sample into an amber glass autosampler vial and place into autosampler, documenting each sample's position in the autosampler tray.
9.	Inject a 5 µL aliquot of sample into the LC-MS/MS instrument and analyze.

Table A-2. Detection limit (DL) results for (n=7) samples for wiping the surface of coupons with a 100 cm^2 area

LAMINATE									
	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	54	109	7	32	64	6	37	74	6
Formica Blank	26			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	109	7	0.32	64	6	0.37	74	6
Formica Blank	0.26			0			0		

LAMINATE						
	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	41	82	3	33	66	3
Formica Blank	5			34	67	
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	3	0.33	66	3
Formica Blank	0.05			0.33	67	

Appendix B: Statistical Data and Calculations for Holding Time Studies

The f-test was administered to determine if independent populations have significantly different variances. Variations would have an effect for when pooling the standard deviations and determining the number of degrees of freedom when conducting a t-test. The f-value is calculated using the equation shown below:

$$F = \frac{s_1^2}{s_2^2}$$

s_1 and s_2 represent the standard deviations of the two pools of data being compared and s_1 is the larger of the two standard deviations (i.e., F > 1 by definition).

The calculated F-value was compared to the critical F-value at the 95% confidence level where the data sets for s_1 and s_2 have $(n_1 - 1)$ and $(n_2 - 1)$ degrees of freedom, respectively. Since 5 replicates were analyzed for all holding times, the number of degrees of freedom for all data sets was 4. The F-test data is shown in Table B-1. The data indicated no significant differences in the variances existed for any analyte at time 0 when compared to its counterpart. Consequently, the pooled standard deviation, s_{pooled}, was calculated using the formula below:

$$s_{pooled} = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{(n_1 + n_2 - 2)}}$$

n_1 = number of samples in data set 1
n_2 = number of samples in data set 2
s_1 = standard deviation of data set 1
s_2 = standard deviation of data set 2

S_{pooled}, along with the mean recoveries for each analyte at time t (t = 7, 14, 21, or 28 days) and time 0 are used to calculate a t-value to compare the two means with a paired t-test at 95% confidence using the formula below:

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{s_{pooled}\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}, \text{ with } n_1 + n_2 - 2 \text{ degrees of freedom}$$

$\bar{x}_1$ = mean value of data set 1
$\bar{x}_2$ = mean value of data set 2
S_{pooled} = pooled standard deviation
n_1 = number of samples in data set 1
n_2 = number of samples in data set 2
degrees of freedom = $n_1 + n_2 - 2 = 8$

The t-test data is shown in Table B-2. T-test data exhibited no significant difference in the mean for any analyte at any holding time when compared to its counterpart at its initial holding time at day 0. Since there was no significant difference in the means, all analytes at every holding time tested showed recoveries that are within 10% of their counterparts at 0 days holding time. Therefore, all analytes studies were stable for a period of at least 28 days.

Table B-1. F-test analysis for nitrogen mustard degradation analytes

TEA F-test				
	Standard Deviation	Calculated F-value	Critical F-value	Significant?
Day 0	5.20			
Day 7	5.64	1.18	6.39	no
Day 0	5.20			
Day 14	7.71	2.20	6.39	no
Day 0	5.20			
Day 21	5.20	1.00	6.39	no
Day 0	5.20			
Day 28	3.88	1.80	6.39	no
EDEA F-test				
	Standard Deviation	Calculated F-value	Critical F-value	Significant?
Day 0	6.48			
Day 7	7.65	1.39	6.39	no
Day 0	6.48			
Day 14	5.16	1.58	6.39	no
Day 0	6.48			
Day 21	8.79	1.84	6.39	no
Day 0	6.48			
Day 28	4.54	2.03	6.39	no
MDEA F-test				
	Standard Deviation	Calculated F-value	Critical F-value	Significant?
Day 0	5.82			
Day 7	6.34	1.19	6.39	no
Day 0	5.82			
Day 14	6.74	1.34	6.39	no
Day 0	5.82			
Day 21	8.65	2.21	6.39	no
Day 0	5.82			
Day 28	4.64	1.57	6.39	no
DEA F-test				
	Standard Deviation	Calculated F-value	Critical F-value	Significant?
Day 0	4.31			
Day 7	5.89	1.87	6.39	no
Day 0	4.31			
Day 14	5.16	1.43	6.39	no
Day 0	4.31			
Day 21	7.59	3.11	6.39	no
Day 0	4.31			
Day 28	9.20	4.57	6.39	no
DEA-d8 F-test				
	Standard Deviation	Calculated F-value	Critical F-value	Significant?
Day 0	6.29			
Day 7	7.72	1.51	6.39	no
Day 0	6.29			
Day 14	9.24	2.16	6.39	no
Day 0	6.29			
Day 21	9.15	2.12	6.39	no
Day 0	6.29			
Day 28	8.27	1.73	6.39	no

Table B-2. T-test analysis for nitrogen mustard degradation analytes

TEA t-tests, 95% CI, DoF = 8

	$\bar{x}$	s	s_{pooled}	Calculated t-value	Critical t-value	Significant?
Day 0	92.98	5.20				
Day 7	93.57	5.64	5.42	0.07	2.306	no
Day 0	92.98	5.20				
Day 14	74.93	7.71	6.58	1.74	2.306	no
Day 0	92.98	5.20				
Day 21	75.30	5.20	5.20	2.15	2.306	no
Day 0	92.98	5.20				
Day 28	78.35	3.88	4.59	2.02	2.306	no
EDEA t-tests, 95% CI, DoF = 8						
	$\bar{x}$	s	s_{pooled}	Calculated t-value	Critical t-value	Significant?
Day 0	97.56	6.48				
Day 7	85.15	7.65	7.09	1.11	2.306	no
Day 0	97.56	6.48				
Day 14	87.06	5.16	5.85	1.13	2.306	no
Day 0	97.56	6.48				
Day 21	83.84	8.79	7.72	1.12	2.306	no
Day 0	97.56	6.48				
Day 28	87.77	4.54	5.59	1.11	2.306	no
MDEA t-tests, 95% CI, DoF = 8						
	$\bar{x}$	s	s_{pooled}	Calculated t-value	Critical t-value	Significant?
Day 0	94.52	5.82				
Day 7	82.36	6.34	6.09	1.26	2.306	no
Day 0	94.52	5.82				
Day 14	82.38	6.74	6.30	1.22	2.306	no
Day 0	94.52	5.82				
Day 21	82.97	8.65	7.37	0.99	2.306	no
Day 0	94.52	5.82				
Day 28	86.60	4.64	5.26	0.95	2.306	no
DEA t-tests, 95% CI, DoF = 8						
	$\bar{x}$	s	s_{pooled}	Calculated t-value	Critical t-value	Significant?
Day 0	93.47	4.31				
Day 7	85.06	5.89	5.16	1.03	2.306	no
Day 0	93.47	4.31				
Day 14	82.11	5.16	4.75	1.51	2.306	no
Day 0	93.47	4.31				
Day 21	77.84	7.59	6.17	1.60	2.306	no
Day 0	93.47	4.31				
Day 28	74.16	9.20	7.18	1.70	2.306	no
DEA-d8 t-tests, 95% CI, DoF = 8						
	$\bar{x}$	s	s_{pooled}	Calculated t-value	Critical t-value	Significant?
Day 0	96.32	6.29				
Day 7	89.75	7.72	7.04	0.59	2.306	no
Day 0	96.32	6.29				
Day 14	85.77	9.24	7.90	0.84	2.306	no
Day 0	96.32	6.29				
Day 21	83.51	9.15	7.85	1.03	2.306	no
Day 0	96.32	6.29				
Day 28	90.08	8.27	7.35	0.54	2.306	no

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