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LOW-LEVEL ANALYTICAL METHODOLOGY UPDATES TO SUPPORT DECONTAMINANT PERFORMANCE EVALUATIONS

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Project CA06DEC407 was a DTRA-funded effort designed to address the challenges associated with quantifying low-level residual agent to support decontaminant contact- and vapor-test evaluations. The program had three main objectives that were successfully accomplished. The primary program objective was to develop improved analytical methods for the confident detection of low levels of agents VX, HD, and GD at published requirement levels. The lowest requirements used at the time of this program to establish the required detection limits were the JPID program 2003 and the JSSED program 2005 requirement documents. An analytical method is an electronic file containing the settings the analytical equipment uses during operation. These settings are optimized for the specific objective (i.e., low-level VX). Six methods were developed to address low-level VX, HD, and GD analysis for extract sample and vapor sorbent tube analysis. The second program objective was to establish methods for the detection of common agent byproducts that could form during testing. Several methods were developed to address the common VX, HD, and GD decontaminant reaction byproducts. The third program objective was to make the new methods available to establish uniformity in procedures across testing locations. The project produced 15 methods that are documented in an appendix.

15. SUBJECT TERMS

GC/MS	Contact Test	Vapor Test	VX	Contact Hazard
LC/MS/MS	Residual Agent	Decontaminant	Panel Testing	Vapor Hazard
Decontamination Efficacy				

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PREFACE

The work described in this report was authorized under DTRA JSTO Project No. CA06DEC407. The work was started in March 2006 and completed in December 2007.

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The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. Manufacturer names and model numbers are provided for completeness.

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LOW-LEVEL ANALYTICAL METHODOLOGY UPDATES TO SUPPORT DECONTAMINANT PERFORMANCE EVALUATIONS

1. INTRODUCTION

Deeontaminant testing evaluates the ability of a deeontamination proeess to remove a eontaminant from a material. For ehemieal warfare agent, deeontamination materials must be cleaned to low-risk levels speeified in requirements doeuments. The ability to measure hazards relies on the analytieal methodology used to analyze the samples generated during deeontamination testing. This report diseusses the development of the analytical methodology to eonfidently report whether the deeontamination requirement has been met. The demands plaeed on the analytieal methodology (low-level detection requirements) were determined by the sample generation and treatment, and the speeified low-risk level requirements.

If eontamination oeeurs, the risk to personnel and equipment must be redueed to eomplete the mission. There are four levels of deeontamination: (1) immediate, (2) operational, (3) thorough, and (4) elearanee.[1] Eaeh level has spceifie objeetives and specifications for the amount of agent that may remain on the material.

- Immediate deeontamination oeeurs within 15 min of contamination, with emphasis on saving lives and limiting the sprcad of eontamination.
- Operational deeontamination is performed to sustain operations by reducing the spread of eontamination and providing temporary relief from long-term mission-oriented proteetive posture (MOPP). FM 3-11.5 reeommends the initiation of this deeontamination within 1 h for Chemieal Agent Resistant Coating (CARC) surfaees and within 6 h for non-CARC surfaees. Thorough deeontamination is the signifieant reduetion in eontamination level for long-term MOPP reduetion.
- Thorough decontamination is initiated when the mission allows. However, the field manual does recommend short times as many decontamination techniques beeome less effeetive over greater time.
- Clearanee deeontamination is the reduction in eontamination to a level that allows for unrestrieted use, transportation, and disposal.

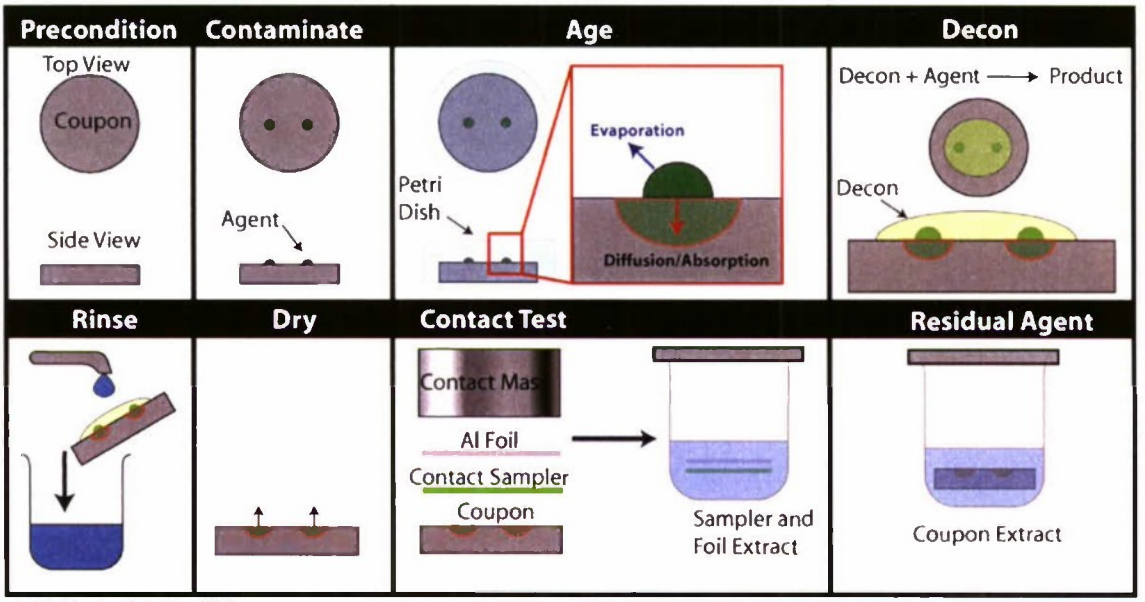
Deeontamination methods inelude neutralization, physieal removal, and weathering. *Neutralization* is the reaetion of the eontaminant and the deeontaminant material to produee a reaetion produet that is of lower toxieity eompared with the original eontaminant. *Physical removal* is the reloeation of the eontamination from the surfaee of interest to a surfaee of lesser importance. *Weathering* ineludes proeesses sueh as evaporation. Most deeontaminants and deeontamination proeesses use a eombination of neutralization and physieal removal. Deeontaminant development and testing typieally foeuses on thorough deeontamination to develop deeontaminants that ean signifieantly reduee eontamination in a short period of time.

1.1 Chemical Decontaminant Testing

TOP 8-2-061, Chemical Biologieal Deeontaminant Testing, is the collection of test proeedures eommonly used to evaluate deeontaminant performanee.[2] TOP 8-2-061 eontains proeedures for evaluating kineties; deeontaminant endpoint; eontaet and vapor hazards; and material, detector, and

proteetive equipment eompatibility. The two tests of interest for the improved analytieal methods were the eontaet and vapor tests.

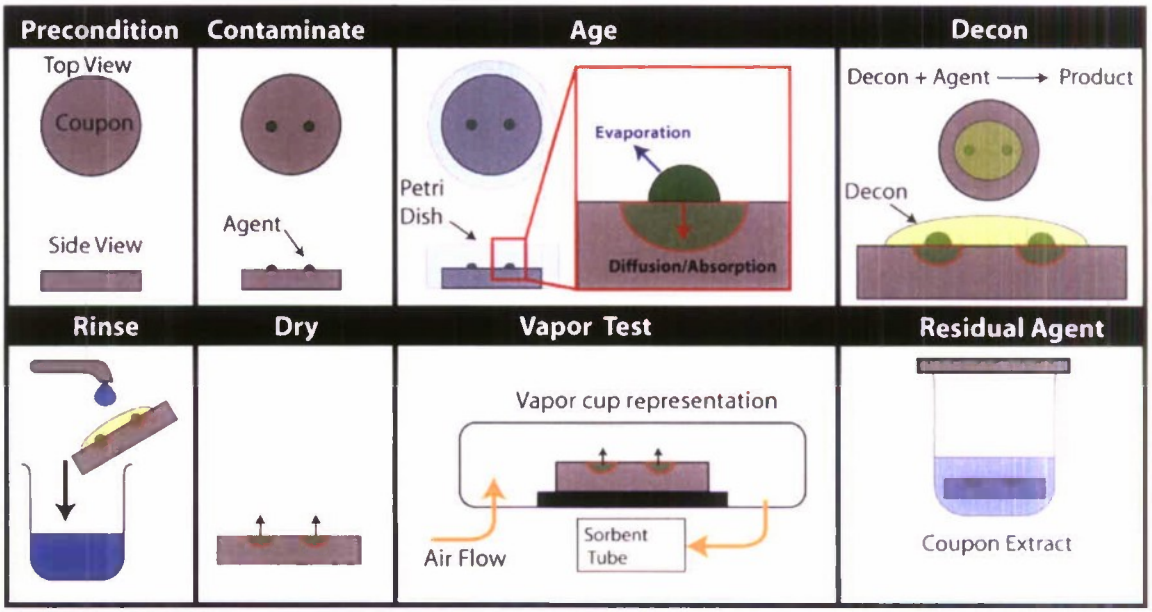
The *contact test* is the measure of the eontaminant present after the deeontamination proeess that eould pose a hazard through transfer to skin or other surfaees. A eontaet sampler is used in this test as a surrogate for human skin. The eontaet sampler is used to eolleet the agent from the eoupon surfaee. A eontaet test event is ealled a *touch*. A toueh is eharaeterized by the eontaet area, eontaet pressure, eontaet time duration, and skin eondition (wet versus dry). The eontaet sampler is extraeted and the eontamination eolleeted during a toueh is quantified using the analytieal methods and reported as a mass. The eontaet test simulates the interaetion of skin with the surfaee of interest. A single toueh does not indieate the total residual agent in the eoupon after deeontamination; but rather, the mass of eontaminant that eould be bioavailable by toueh or available for eontaet transfer. This mass is used to ealeulate the eontaet hazard, whieh is eompared against the requirement doeument values. For eompleteness, the material of interest is extraeted in solvent and analyzed using the analytieal methods to determine the residual agent. The laboratory-seale Deeontamination Performanee Evaluation eontaet test process is shown in Figure 1 using a standard 2 in. disk eoupon.



sketch Mantooth-Lalain 2007

Figure 1. Representation of the panel contact test process.

The vapor test is the measure of the vaporous emission rate of the eontaminant from the test material after the deeontamination process that eould pose a hazard to unprotected personnel. Samples are plaeed in vapor ehambers and air is passed over the sample and eolleeted onto a solid sorbent tube. The agent eolleeted on the tube is thermally desorbed and analyzed using the analytieal methods. This mass is used to ealeulate the ehamber vapor eoneentration and emission rates, whieh enable the ealeulation of a vapor eoneentration that ean be eompared to the requirement doeument values. Additionally, the material of interest (e.g., eoupon) ean be extraeted in solvent and analyzed using the analytieal methods to determine the residual agent. The laboratory-seale deeontamination performanee evaluation vapor test proeess is shown in Figure 2, using a standard 2 in. disk eoupon.



sketch Mantooth-Lalain 2007

Figure 2. Representation of the panel vapor test process.

1.2 Decontaminant Performance Requirements

To evaluate decontaminant performance, the data from the contact and vapor tests are typically compared to another value. The comparison can be a reference decontaminant or field detector response. For decontaminants with potential application to an acquisition program, test data are typically compared to the program requirements document values for decontamination efficacy. The Joint Requirements Office (JRO) for Chemical, Biological, Radiological, and Nuclear (CBRN) Defense is responsible for the planning, coordination, and oversight of joint CBRN defense operational requirements.[3] The JRO mission includes the preparation of Joint CBRN Defense Requirements Documents. These documents are based on the needs of the user's mission to determine the allowable risk to unprotected personnel. Several requirements documents exist for decontamination evaluations. The two documents of interest to this research program were for the Joint Platform Interior Decontamination (JPID) program and the Joint Service Sensitive Equipment Decontamination (JSSED) program. These two programs contain the lowest level requirements for the decontamination programs at the time this report was generated. The JPID and JSSED program requirement documents contain system performance requirements for thorough chemical decontamination efficacy.[4-9] The performance requirements are specified for post-decontamination vapor (offgas) and contact exposure levels. These levels are further divided into a threshold level and a lower objective level. Before this project, many of the existing analytical methods could not detect to the lowest requirement levels, preventing the ability to identify whether a candidate technology met a requirement and was fit for an acquisition program.

The JSSED program objective was to "provide a first-ever capability to decontaminate chemical and biological warfare agents and toxins from sensitive electronic, avionics, and electro-optic equipment."[6] The JSSED program required that the decontaminant be compatible with, and not degrade sensitive materials or equipment. In addition, decontaminated equipment will retain tactical mission capability. The JPID system is to "provide the capability to decontaminate CB warfare agents within interiors of aircraft, vehicles, ships, and buildings (to include avionics, electrical, electronic, and environmental systems equipment) and the associated cargo without damaging surfaces or sensitive equipment within the platforms."[7]

The data generated during testing was compared to the requirement document specifications to determine if the decontaminant performance met the specifications. The data and comparisons were used to make decisions regarding technology selections and risk assessments. The data must be accurate, with sufficient confidence that these decisions and assessments can be made. The acceptable contamination levels for thorough decontamination, stated in the requirement document, are extremely low. In addition, the frequent updates to these documents have, in some cases, resulted in significant reductions to the acceptable levels, sometimes changing by two orders of magnitude.[4, 6] These low levels and changing requirements place additional demands on analytical methodology supporting decontaminant testing. The low levels require the development of methodology that has been optimized for the sample types generated from decontaminant testing. The methodology needs to be robust and produce accurate results. The evaluation of several candidate technologies for the JSSED program in 2006 identified the need for focused low-level agent analytical methods, optimized to support decontaminant evaluations. In some cases, the failure of a technology to be evaluated successfully was due, not to the ability of the technology to clean the surface, but rather to the method detection limits that were significantly higher than the requirement levels.

1.3 Low-Level Methodology Program

BA06DEC407 was a DTRA-funded effort, designed to address the challenges associated with quantifying low-level residual agent to support decontaminant contact and vapor test evaluations. The program had three main objectives:

- The primary program objective was to develop improved analytical methods to enable confident detection of low levels of the chemical agents, VX, HD, and GD, at published requirement levels.
    - The lowest requirements at the time of this program, used to establish the required detection limits, were the JPID program 2003 and the JSSED program 2005 requirement documents.
    - An analytical method is an electronic file containing the settings the analytical equipment uses during operation. These settings were optimized for the specific objective (i.e., low-level VX).
    - The analytical equipment is the hardware that uses the analytical methods.
- The second program objective was to establish methods for the detection of common agent byproducts that could form during decontaminant testing.
- The third program objective was to make the new methods available to establish uniformity in test procedures across testing locations.

Low-level detection of chemical agents has been the goal of many programs over the years. However, the term "low-level detection" is a gross generalization that is meaningless without specific detection ranges and sample matrices. For a specific program these parameters include the goals of the program, the types of samples, matrices, and collection processes. There is no magic bullet that works for all detection ranges and all samples in all matrices. No single method can accomplish all these factors, thus many unique methods were established to meet these needs as they arose. All low-level detection methods have the same primary objective: to utilize program-specific guidance and/or requirements to establish and detect to target values. However, the approach used must be optimized and aligned with program objectives. The target detection limits of the analytical methodology are

determined by the combination of how the sample was generated and treated, and the specified low-risk level requirements.

The need for multiple methods to meet different and specific program objectives is not unique to decontamination sample analysis. The EPA publication SW-846[10] is a collection of over 200 documents, including specific methods to be used as guidance when evaluating materials to the standards set forth by the Resource Conservation and Recovery Act (RCRA) for the sampling and analysis of wastes. In the SW-846 publication, the EPA offers guidance on many aspects of analyte detection and waste characterization, from choosing the best procedure to specific methods for the characterization of wastes. However, the EPA emphasizes that the information contained within SW-846, albeit comprehensive, is optional guidance only. Method 8000b in the SW-846 publication, titled "Determinative Chromatographic Separations", details chromatographic guidance for all SW-846 methods, including chromatographic performance, retention time (RT) determination, interference discussion, calibrations and quality control (QC) information, and maintenance issues, among others. The document concedes that there are many ways to analyze samples, and that specific methods are required. The methods required depend on the factors involved with the overall analysis of the sample, including sample collection, matrix, sample type, chromatography platform, detector used, etc.

As a specific example, one of the most prominent, well-established programs utilizing low-level chemical agent detection is the U.S. Army Chemical Materials Agency (CMA) Chemical Demilitarization Program.[11] Although this program has multiple facets, including the destruction of the chemical agent stockpiles through incineration or alternative technologies, the focus of the low-level detection was borne out of the overall goal of destroying the stockpiles in a manner that is low risk for the worker, the public, and the environment.[12] Each Chemical Demilitarization Facility (CDF) utilizes a support laboratory to ensure/verify that the agent is destroyed completely and that the safety goal is achieved.

While CDF laboratories and decontamination performance evaluations generate liquid samples as a result of the decontamination of chemical warfare agents, there are significant differences in the sample matrix, sample treatment, chromatography requirements, sensitivity requirements, and use of the analytical results. These fundamental differences underscore the need for different and specific methods to meet the objectives of the respective program. One type of sample evaluated at the CDF laboratories is liquid waste stream samples. Most methods for liquid samples were developed exclusively for the alternative technologies sites generating hydrolysate matrix (sodium hydroxide/agent) as the result of the neutralization process. The purpose of analyzing the hydrolysate is to verify that agent is below the site-specific detection requirements, as established by the Army and/or the state in which the site resides, in order to clear the hydrolysate as waste. These agent-specific and site-specific methods take into consideration the matrix effects and interferences that are specific to hydrolysate samples. Since analysis is performed primarily for the purpose of clearing waste, no liquid CDF laboratory methods were developed for use in evaluating decontamination effectiveness for a surface of interest (the objective of decontamination performance evaluation). The CDF methods are specific to the analysis of waste streams (which is often aqueous hydrolysate) from large volumes of waste (potentially hundreds of liters) and are compared to requirements for waste disposal.

Liquid samples generated in decontamination performance evaluation used organic liquids to extract analyte from test materials (e.g., contact sampler or material/coupon) to generate samples. The test sample matrix included residual agent, potentially neutralized agent byproducts, potentially remaining decontaminant (that could be caustic, organic, oxidative, etc.), and other compounds that may be extracted from the test material (e.g., plasticizers or pigments from the contact sampler or material/coupon being extracted) using a specific extraction solvent (e.g., isopropyl alcohol [IPA] for VX, acetonitrile for GD, or chloroform for HD). The analytical method was specifically developed to chromatographically separate the sample matrix and quantify the analyte of interest. In the case of contact sampler extractions, the results were then compared with toxicological data relating to the

percutaneous absorption of agent, which resulted from a touch, to determine the potential contact hazard. Comparison of CDF waste stream samples and decontamination performance evaluation samples showed that even though both samples corresponded to liquid extracts regarding the decontamination of chemical warfare agents, the contents of the liquid extract would be significantly different, requiring different analytical techniques to chromatographically separate and quantitate the analyte of interest. Further, the objective of the method to clear waste streams, versus the identification of contact hazards, may have corresponded to instrument sensitivities (i.e., detection limits) that were vastly different. As a result, specific methods had to be developed to meet the respective program objectives.

CDF laboratories also utilize vapor-sampling methods to monitor the air in and around the agent destruction process. These methods detect agent collected from vapor samples and were developed, based on target masses determined from the chemical agent monitoring levels established[13] and later refined in 2004[14] by the Army. Since these methods were utilized to detect and quantify exposure levels, samples were collected over the typical times that the worker, or public, may be exposed. The General Population Limit (GPL) is one of the most stringent airborne exposure limits (AELs) utilized by CMA. GPL is defined by CMA as "the maximum concentration (of chemical agent) to which the general population may be exposed 24 h per day, seven days a week, for a 70 year lifetime."[15] The objective of this CMA method was to detect vapor concentrations sampled in actual environments for long sampling times (e.g., 12 h). This is not the objective of decontaminant vapor testing.

Decontaminant vapor testing methodology and data analysis is described in detail in the chemical decontamination performance evaluation source document.[16] Briefly, the objective of the vapor test is to measure the vapor emission rate of a material after decontamination. The calculation of the vapor emission rate is accomplished by measuring the chamber's vapor concentration as a function of time. This vapor concentration does NOT correspond to the vapor concentration to which unprotected personnel would be exposed. The chamber's vapor concentration is a function of the volume of the chamber, the airflow rate through the chamber, the surface area of the vapor source, and the emission rate of the vapor source. The emission rate changes as a function of time. To characterize this change, multiple vapor samples must be acquired for the period of interest (e.g., 6–12 h). This requires a sampling time shorter than the time used for environmental monitoring and, thus, needs to resolve and confidently measure agent mass (in nanograms) on a sample tube. Once the emission rate for a sample has been determined, the vapor concentration for a specified scenario can be calculated and compared to a requirement. For example, the emission source could be scaled to an expected scenario (e.g., a painted surface scaled to the surface area of a tank) that would be placed in a specified volume (e.g., a garage). The resulting vapor concentration detected by environmental monitoring could then be calculated. Because of the different sampling, data treatment, and objectives, monitoring the environment and the dynamic vapor chamber will require specific analytical methods.

The JPID and JSSED ORDs serve specific objectives: to provide the ability to decontaminate the interiors of vehicles and facilities or pieces of sensitive equipment with a lessened potential for damage or degradation. Low-level agent methods are needed to assure the low risk occupation of decontaminated spaces and the accurate performance of equipment after decontamination. The vapor methods developed must be performed with analytical equipment capable of monitoring the air within a given enclosure or around a specific material. Target masses are determined from the AELs established within the JPID and JSSED ORDs. The liquid methods must successfully detect agent with possible unreacted decontaminant or interferences resulting from the material of interest.

Method validation requires the determination of the Limit of Detection (LOD), Limit of Quantitation (LOQ), and Uncertainty of Measurement for all methods, liquid, or vapor. Because many requirements for low-level detection of decontamination evaluation had not been met, it was imperative to develop new low-level methods that focused solely on target detection levels set forth by the JPID and JSSED ORDs. These ORDS contain the lowest target values for decontamination contact and vapor hazards published at the time of the Low-Level program.

1.4 Analytical Method Development and Documentation

As part of the FY06 program, a market search was conducted to determine the analytical tools capable of meeting the requirements for decontamination performance evaluation testing. These tools had to determine the chemical decontamination efficacy per the contact and vapor tests required in TOP 8-2-061. The systems selected were evaluated on the ability to meet the specific analytical challenges for the JPID and JSSED programs for chemical warfare agents HD, VX, and GD. In addition, the chosen tools had to have sufficient sensitivity and breadth to meet anticipated requirements such as GPL monitoring and analysis of toxic industrial chemicals, toxic industrial materials, or other chemicals of interest. The equipment chosen was capable of significantly lower detection limits than the current requirements. This enhanced ability will enable modification of methods as requirements change, without additional large capital investment costs.

A gas chromatograph with mass spectrometer (GC/MS) system and a liquid chromatograph with tandem mass spectrometers (LC/MS/MS) system were acquired for the Low-Level program. The GC/MS was best suited for the vapor sample analyses and the residual HD and GD methods. The GC/MS system was an Agilent 6890 GC with an Agilent 5975 Inert Mass Selective Detector (MSD), using the Agilent MSD ChemStation instrument control software. Liquid and vapor sample introduction into the GC was performed using a Gerstel MultiPurpose Sampler (MPS) for liquid samples or a Markes Thermal Desorption System (TDS) for vapor samples. The Gerstel system components included the Cooled Injection System (CIS) inlet with peltier cooling option, the MPS2 - MultiPurpose Sampler for standard large volume liquid and headspace injection, and optional equipment such as the Twister Thermal Desorption Unit (TDU). The Markes system was comprised of the ULTRA Autosampler and Unity System for thermal desorption of sorbent tubes. The LC/MS/MS system was best suited for ultra low sensitivity such as residual VX measurements and for many of the agent byproduct analyses. The LC/MS/MS system was comprised of an Agilent 1200 LC with autosampler connected to an Applied Biosystems API5000 high performance triple quadrupole MS.

GC and LC systems are often complementary tools. In many cases a given analyte can be analyzed on one system, but not on the other. The differences between the tools are related to the physical mechanisms used to perform the chromatography or to detect the analyte. Gas chromatography relies on the ability to volatilize an analyte in order to perform chromatography. Some samples do not volatilize or the compounds may degrade in the injection port where volatilization occurs and, therefore, they are not well suited for analysis by GC. Liquid chromatography injects a liquid sample into the chromatographic column and does not rely on volatilizing an analyte. However, the MS detector used for LC requires that the analyte molecule be ionized in the liquid mobile phase for analysis. Nonpolar compounds, such as HD, are not easily ionized by the methods used in LC/MS/MS techniques. As a result, the nature and properties of a given analyte often determine the most appropriate instrument/technique required to detect the analyte.

Each agent requires a specific analytical method. The development of an agent method starts with the identification of a target detection limit. This detection limit is based on the requirement and the experimental method used to generate the sample. The description of this process is detailed in this report for each method used. In the case of liquid extraction samples, the selection of the extraction solvent is carefully chosen so that the solvent solubilizes the analyte of interest, and provides good chromatography on the analytical hardware and good extraction efficiency from the samples. The combination of the analyte of interest, solvent system, and required sensitivity determines the analytical hardware that will be used (e.g., GC versus LC). Analytical method parameters are then established and refined to provide the optimized chromatography and sensitivity to quantitatively detect the analyte of interest.

Chemical agent byproduct identification may require unique methods or may be performed as part of an agent method. The byproducts of interest are chosen for identification based upon

the most commonly produced byproducts from agent degradation. Byproduct identification screening methods are developed in the same manner as the agent methods. Agent methods developed with a byproduct identification component were verified by analyzing the byproducts and byproduct-agent mixtures to demonstrate separation and impact on agent quantitation. Quantitation of the byproducts was not an objective of this program.

The methods are documented individually using a standardized format containing all pertinent information. Three of the standard fields are Analyte Concentration Range, Apparatus, and Method Parameters. The Analyte Concentration Range section provides an overview of the method target, calibration range, calibration curve-fitting model and weighting, limit of detection (LOD), limit of quantification (LOQ), solvent, and quantitation ion(s). The methods are identified as quantitative or qualitative. The byproduct methods as presented here are qualitatively aligned with the program objective for byproduct identification; qualitative methods only confirm the presence of an analyte, not its concentration. All qualitative methods in this document can be quantitative, if a second set of calibration standards using the byproduct are prepared and analyzed. For quantitative methods, the LOD and LOQ are calculated based on the laboratory evaluation of the final method. The LOD and LOQ should be calculated by each laboratory using these methods, as these values are a function of instrument configuration and sensitivity. Section 2 details the analytical equipment and standard preparation tools. The appendix provides the complete listing of instrumentation settings for the method.

The tests were performed from March 2006 to December 2007 in the Decontamination Sciences Branch laboratories at the Edgewood Chemical Biological Center, APG, MD. The results for the method development studies and final methods are presented in this report.

1.5 Summary of Conclusions

The purpose of the Low-Level program was to develop low-level residual agent methods and agent byproduct identification methods, and to provide these methods to the decontaminant testing community. The summary of conclusions is provided in the following list.

- Program objectives: Three main objectives were successfully completed under this program. This report details the method development and final method evaluation. The complete package of methodology fulfilling the program requirements is provided as Appendix A.

- Low-level chemical agent and byproduct identification methods for extract samples: Table 1 contains a summary listing of the agent, byproduct, concentration range, and corresponding analytical method for analysis.

Table 1. Liquid extract sample analytical methodology list.

Compound	Range (ng/mL)	Method	Report Section
VX	0.05–10	LCE VX_ULL.dam	5.4.1.1
EA2192	0.5–10	LCE VX_ULL.dam	5.4.1.1
VX	1–750	LCE VX_LL.dam	5.4.1.2
EA2192	10–750	LCE VX_LL.dam	5.4.1.2
EMPA	5.0–500	LCE EMPA.dam	5.4.1.3
VX	250–2000	GCE VX_DEANS.M	5.4.1.4
HD	2–2000	GCE HD_DEANS.M	6.4.1.1
H-sulfone	10–2000	GCE H-Sulfone_DEANS.M	6.4.1.2
TDG	25,000–100,000	GCE TDG_DEANS.M	6.4.1.3
H-sulfoxide	10–500	LCE H-Sulfoxide.dam	6.4.1.4
GD	2–2000	GCE GD_DEANS.M	7.4.1.1
GD-acid	5,000–500,000	GCE GD-ACID_DEANS.M	7.4.1.2

- Low-level chemical agent methods for vapor samples: Table 2 contains a summary listing of the agent, concentration range, and corresponding analytical method for analysis.

- Low-level VX method for extract samples: Based on the method development and validation work performed and documented herein, the ultra-low-level (ULL) VX extraction method (LCE VX_ULL.dam) detected and quantified VX in IPA from 0.05 to 10 ng/mL using LC/MS/MS. This exceeded the research target concentration of 0.45 ng/mL established from the JPID03 Operational Requirements Document (ORD) requirement of 0.005 mg/m^2.

- Low-level HD method for extract samples: Based on the method development and validation work performed and documented herein, the HD extraction method (GCE HD_DEANS.M) detected and quantified HD in chloroform from 2 to 2000 ng/mL using a GC/MS. This result exceeded the research target concentration of 4.5 ng/mL established from the JPID03 ORD requirement of 0.05 mg/m^2.

- Low-level GD method for extract samples: Based on the method development and validation work performed and documented herein, the GD extraction method (GCE GD_DEANS.M) detected and quantified GD in acetonitrile from 2 to 2000 ng/mL using a GC/MS. This result exceeded the research target concentration of 4.5 ng/mL established from the JPID03 ORD requirement of 0.05 mg/m^2.

- Low-level VX method for vapor samples: The research target was established as the lowest mass on column that can be confidently measured, allowing reasonable sample throughput in support of decontaminant performance evaluations. The low-level VX vapor method (GCV VXLL-DEANS.M) detected and quantified VX as the G-analog with a mass-on-column range of 0.5–100 ng using a GC/MS.

- Low-level HD method for vapor samples: The research target was established as the lowest mass on column that can be confidently measured, allowing reasonable sample throughput in support of decontaminant performance evaluations. The low-level HD vapor method (GCV HDLL-DEANS.M) detected and quantified HD with a mass-on-column range of 0.5–100 ng using a GC/MS.

- Low-level GD method for vapor samples: The research target was established as the lowest mass on column that can be confidently measured, allowing reasonable sample throughput in support of decontaminant performance evaluations. The low-level GD vapor method (GCV GDLL-DEANS.M) detected and quantified GD with a mass-on-column range of 0.5–100 ng using a GC/MS.

- Formal documentation: The third program objective was to make the new methods available to establish uniformity in test procedures across testing locations. This was achieved by preparing formal method documentation for each method containing method description and instrument operating parameters. (Appendix A)

    - The method parameters set forth in this document were established on the instrument via the instrument software, and were specific to the instruments described in this document. These parameters may be used on similar or other GC/MS and/or LC/MS/MS platforms, and are comprehensive enough to be an excellent guide for establishing low-level methods. Methods transferred to instruments, set up as described, should obtain similar results. However, if parameters were applied to instrumentation differently than described, the method may not produce the same results. Method checkout must be performed to include analyzing solvent blanks, chemical agent standards, and byproduct standards in order to determine the instrument-specific performance and method optimization.

Table 2. Vapor sample analytical methodology list.

Compound	Range (ng)	Method	Report Section
VX (G analog)	0.5–100	GCV VXLL-DEANS.M	8.4.2.1
VX (G analog)	50–1500	GCV VXHL-DEANS.M	8.4.2.2
HD	0.5–100	GCV HDLL-DEANS.M	9.4.1.1
HD	50–2500	GCV HDHD-DEANS.M	9.4.1.2
GD	0.5–100	GCV GDLL-DEANS.M	10.3.1.1
GD	50–2500	GCV GDHL-DEANS.M	10.3.1.2

    - As instrument technologies improve and software platforms are updated, certain terminology and instrument configurations may change. The method parameters detailed in Appendix A are major method instrument parameters that should be available in future updates of the software platforms, even if software aesthetics and specific terminology have changed from the issuance of these methods.

- Byproduct methodology: All byproduct methods developed were intended for screening purposes only. Based upon the byproduct analyte RT and expected mass spectrum, a qualification may be made to identify an unknown peak as a specific byproduct. No quantitation of byproduct analytes was intended with these methods. Future work may determine the ability of the methods to quantify byproducts through the generation of calibration curves that span the appropriate working ranges, using standards diluted with high purity solvents. Future method capabilities to quantify byproducts will be evaluated statistically and reported accordingly.

1.6 Analytical Chromatography and Method Development

1.6.1 Overview of Analytical Instrumentation/Chromatography

Often, experimentation results in samples composed of complex mixtures of compounds. When identifying and/or quantifying one or more compounds within the mixture, analytical instrumentation can be employed. Analytical instrumentation facilitates the separation of the individual compounds, or analytes, within the sample via chromatography. Analytical chromatography instrumentation basically consists of the following: a means of sample introduction, a platform for analyte separation (e.g., chromatography), and a detector (e.g, MS) to identify and quantify the analytes. The results from analyzing a sample on a chromatographic system are viewed in the form of a chromatogram, which is a graphical representation of the analytes that have been separated.

Sample Introduction: Samples collected and introduced into the chromatograph consist of either liquid extraction samples or vapor samples. Each type of sample is introduced into the system for separation and analyte detection in a unique manner with specialized sample introduction instrumentation specific to sample type.

1.6.2 Chromatography

Chromatography is the separation of a complex mixture into individual components, based upon interactions with a mobile phase and a stationary phase.

- The *mobile phase* is the element of chromatography that moves the sample through the system (e.g., carrier gas).

- The *stationary phase* is the element of chromatography that interacts with the sample components to cause separation (e.g., material inside the analytical column).

The Low-Level program utilizes two types of chromatography: liquid and gas chromatography.

- Liquid chromatography uses a liquid mobile phase. This mobile phase, consisting of mixtures of solvents, chemicals, and high purity water, must be suitable to the liquid samples containing the analytes of interest.

- Gas chromatography uses an inert gas mobile phase of helium, hydrogen, or nitrogen. The methods developed for this program utilize helium as the mobile phase. Gas chromatography requires samples to be in the gaseous state for analysis. Samples may be collected and introduced into a GC as vapor or as liquid. However, liquid samples must be volatilized to the gaseous state before moving through the system.

Regardless of whether LC- or GC-based instrumentation is used, the primary purpose of the mobile phase is to move the sample through the chromatographic system. The stationary phase is contained within an analytical column, which is where separation occurs as the sample is swept across the stationary phase by the mobile phase. Separation, or partitioning, of the sample components is based on the interactions of the sample with the stationary phase. These interactions occur due to the physical and chemical properties of the analytes. For example, each component has unique properties such as molecular weight, boiling point, vapor pressure, and polarity, among others. These factors affect the way that the analytes interact with the stationary phase. The amount of time it takes an analyte to move through the analytical system from sample introduction to detection is known as the retention time (RT). Some analytes may have many interactions with the stationary phase (i.e., strong attraction), and thus, take longer to move through the analytical column, generating a longer RT. Other analytes may have very few interactions, depending on their physical and chemical properties and, therefore, will have less retention, moving through or eluting from the column much faster. If all analytical parameters remain the same, (oven temperature, column stationary phase, column length, etc.) each individual analyte will have the same RT every cycle. Compounds similar in physical and chemical properties will have similar RTs, while compounds that have very different properties will have very different RTs. Figure 3 contains a simple representation of chromatography.

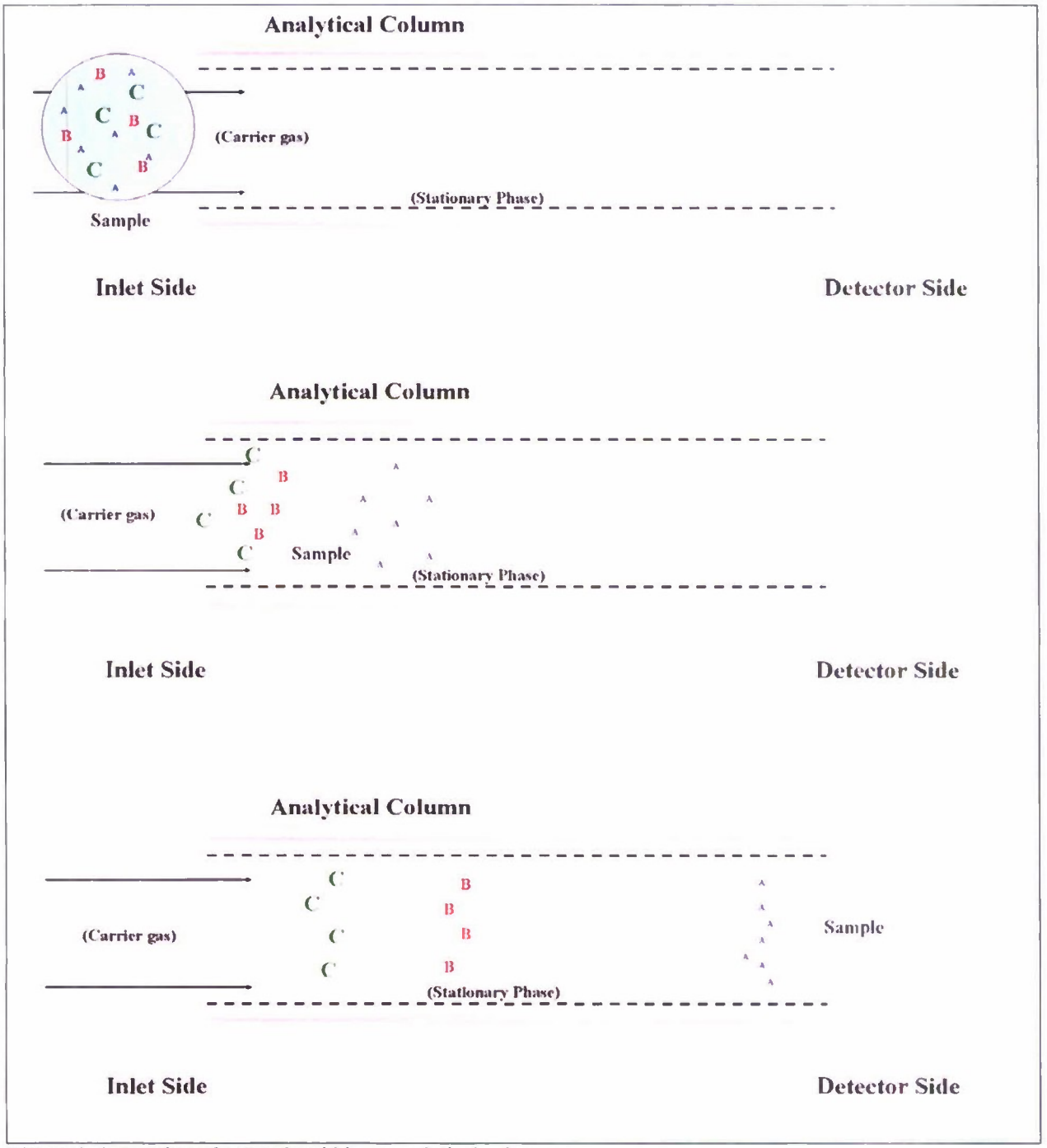


Figure 3. Separation of a sample within an analytical column.

A complex mixture (i.e., sample) to be analyzed, typically contains one or more analytes of interest to be identified and quantified. After separation, the partitioned sample components will elute into a detector for quantitation. The detector response is proportional to the amount of analyte eluting from the detector. Analytical results are represented by a chromatogram that shows RT versus detector response. The chromatogram will contain peaks representing the detector response for each analyte as it exits the analytical column and enters the detector. Figure 4 contains a simple representation of a chromatogram in relation to the analytes eluting from an analytical column.

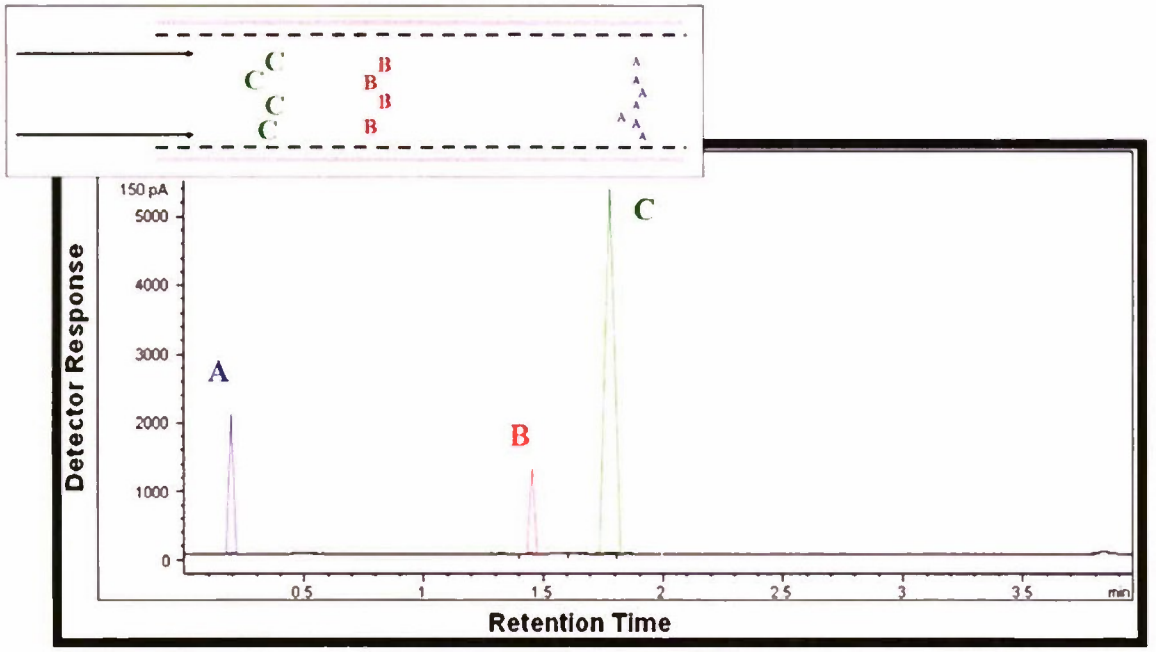


Figure 4. Chromatogram showing RT versus detector response.

When there is no analyte in the sample, the detector response represents the *baseline*. A baseline detector response is established when the mobile phase only is moving through the system and into the detector. As analyte elutes from the column into the detector, the detector response will begin an upward slope away from the baseline. The maximum amount of analyte in the detector is represented as the apex of a peak on a chromatogram. At the apex, RT is determined, the amount of analyte decreases as the compound fully elutes from the column, and the detector response returns to baseline. This process will continue for each analyte separated in a sample.

Detector response is most often evaluated as the area under the peak, as opposed to the height of the peak. The area under the peak on the chromatogram can be determined through integration. An algorithm, built into the instrument software, distinguishes detector response as either baseline or an analyte peak. If the instrument software identifies a deviation from baseline, whereby the detector signal increases enough to cause a positive slope, it will mark the beginning of the peak. The software will mark the end of the peak as the point where the downward slope of the decreasing detector signal returns to baseline. Because the baseline may be offset from zero, a line along the baseline will be drawn and the area under the peak will be mathematically determined by the software. This peak is said to have been *integrated* and a detector response is reported. Examples of an integrated and a non-integrated peak on a chromatogram are shown in Figure 5 and Figure 6, respectively. Note that the integrated peak has been colored yellow, representing the area under the peak, or detector response. Also note that detector response would now be available for this peak.

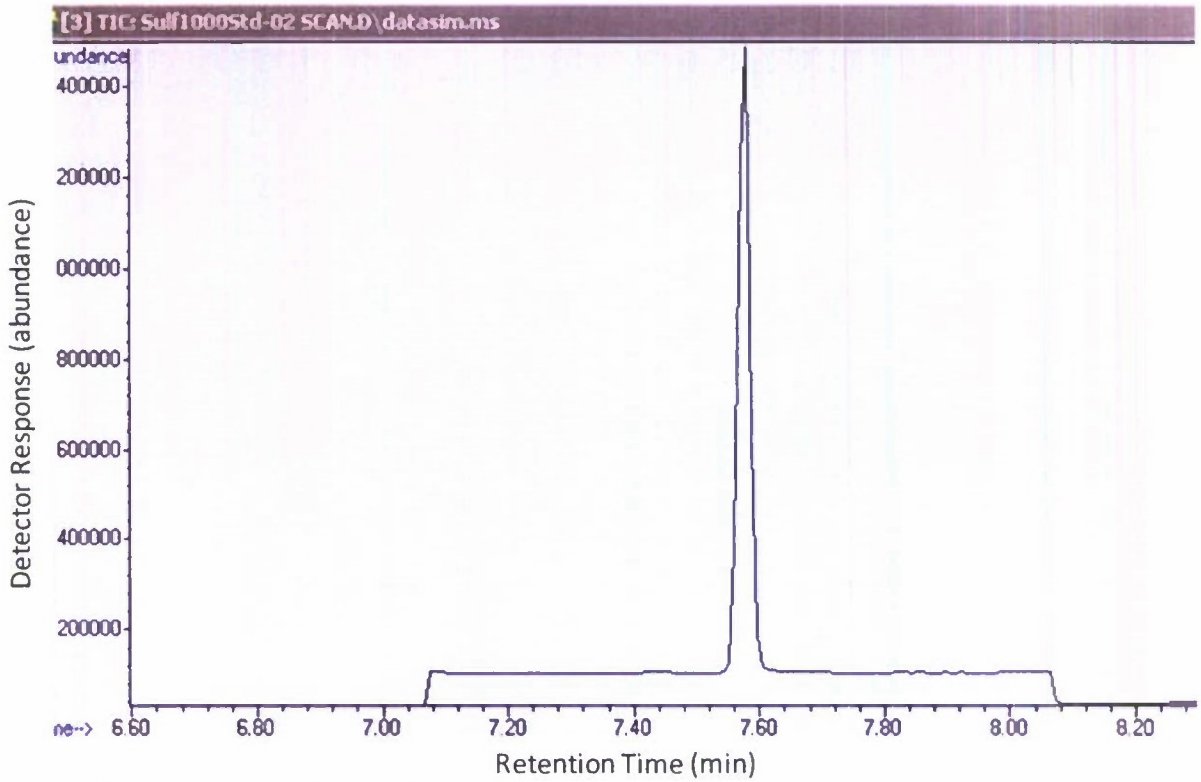


Figure 5. Chromatogram showing a non-integrated peak.

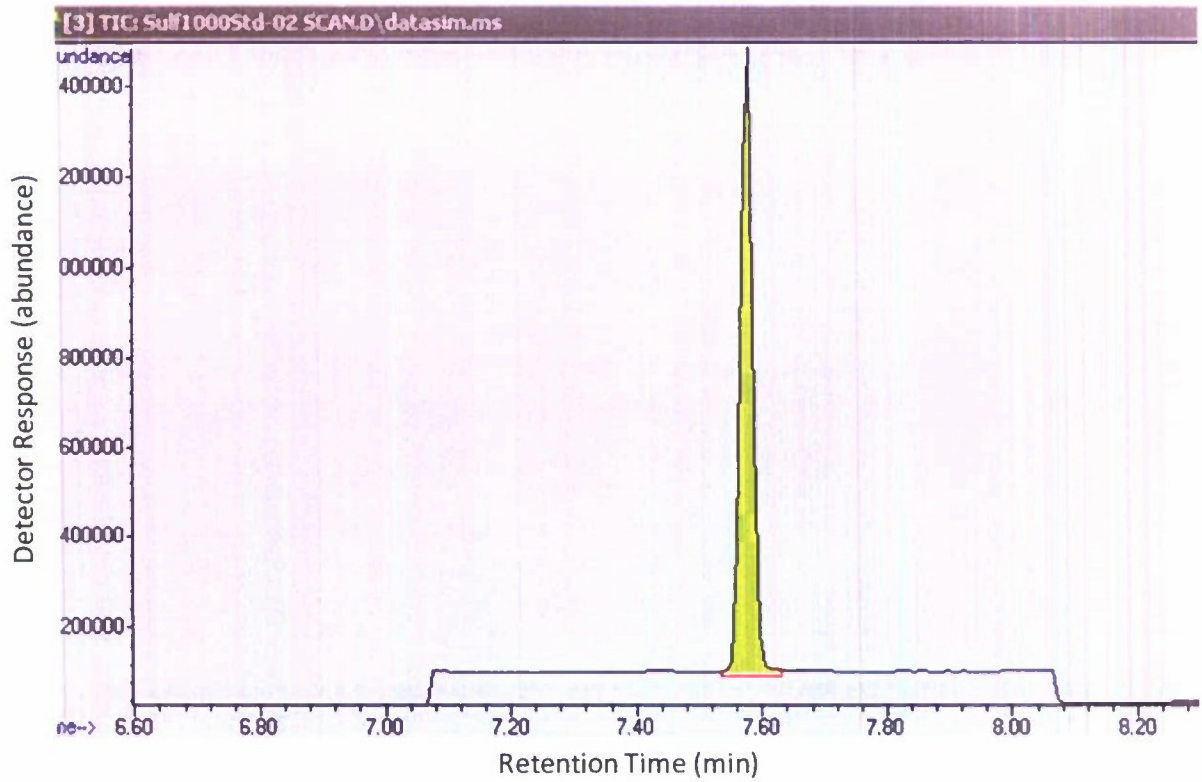


Figure 6. Chromatogram showing an integrated peak.

Once a chromatogram is obtained, the peak shape of the analyte of interest can be evaluated to determine the quality of the chromatography. Two factors that are evaluated when determining the overall quality of chromatography are efficiency and resolution. *Efficiency* is the ability of the chromatographic system to produce sharp peaks on a chromatogram. *Resolution* is the ability of the system to separate adjacent peaks on a chromatogram. Efficiency and resolution are peak characteristics that can be altered by changing the parameters associated with the chromatographic system. However, they tend to be inversely related, i.e., working to improve resolution may adversely affect efficiency and vice versa. Factors affecting chromatography (efficiency, resolution, and RT) include stationary phase material, analytical column length, flow rate of the mobile phase, solvent gradient in LC, oven temperature in GC, etc.

When reviewing a chromatogram, it is imperative that the correct analyte peak be identified. Many times external factors can result in additional peaks on the chromatogram. These external factors include, but are not limited to:

- Presence of solvent
- Additional analytes from the sample collection process (i.e., dirty sample matrix)
- Low-purity solvents and/or standards
- Not cleaning the syringe from injection to injection
- Worn inlet liners, o-rings, or other sample introduction components
- An aging or damaged analytical column
- Dirty detector
- Other factors related to general instrument and/or sample analysis performance.

The presence of additional peaks on a chromatogram is typically not an issue for concern as long as the peak from the analyte of interest can be correctly identified with no interference from the peak(s) from the additional compound(s). An *interferent* is any compound that affects the identification and quantitation of the analyte peak. Unwanted analytes may interfere with the analyte of interest by co-eluting with and masking the analyte peak, enhancing or diminishing the analyte peak causing erroneous detection, altering the analyte peak within the sample, preventing its detection, or causing false positive/negatives, among others. All efforts should be made to mitigate known or found interferents. For those interferences that cannot be mitigated, one or more chromatography parameters must be altered. One such parameter may be to change the type of analytical column (stationary phase, length, etc.).

Detectors: A multitude of detectors are available for detecting analytes of all compositions. This program was based upon the detection, identification, and quantitation of chemical agent and agent byproduct analytes of interest. The primary purpose of a detector was to detect and quantify the analytes of interest as they elute from the analytical column. Three types of detectors that were most suitable for detecting chemical agent were a flame ionization detector (FID), a flame photometric detector (FPD), and an MS.

An FID is a nearly universal detector that will elicit a detector response for any compound containing ionizable carbon-hydrogen bonds. This detector ionizes analyte C-H bonds in a high temperature flame. The resulting ion is attracted to an oppositely charged component called a *collector*. The resulting impact creates a signal that is processed and reported as detector response. The detector response of the FID is proportional, not only to the amount of analyte being detected, but also to

the number of C-H bonds available for ionization. An FID is an excellent all-around detector, but does not have the sensitivity necessary for this program. The FID is only appropriate for use with the GC and not for the LC.

An FPD, as a detector on a GC, is a very selective detector for chemical agent due to its principles of operation. An FPD is very selective for phosphorus- or sulfur-containing compounds. As analytes elute from an analytical column, they are burned in a low-temperature, hydrogen-rich flame that excites the analyte molecules, causing them to emit photons of light as they return to the ground state. The FPD contains either a phosphorus or sulfur wavelength filter that filters out all photons of light except for those emitted at, or very near, the wavelengths of 525 nm for phosphorus or 394 nm for sulfur. As these photons pass through the filter, they enter a photomultiplier tube (PMT) where they pass through a series of dynodes, resulting in an amplified signal reported as a detector response. The detector response is proportional to the amount of analyte eluting into the detector from the analytical column. The FPD is an excellent detector for chemical agents because the nerve agents contain a phosphorus atom, while the blister agent HD contains a sulfur atom. This very selective detector will not detect all the byproducts, and it does not have the sensitivity to meet the detection limit requirements set forth by the program. Also, an FPD is not available or suitable for use with the LC.

An MS can be used as a near universal detector or it can be used as a highly selective detector. There are many types of MS detectors such as quadrupole MS, ion trap MS, and time-of-flight (TOF) MS, among others. The mass spectrometers used for this program are quadrupole, or mass filter, "mass specs". The MS can ionize and/or fragment sample molecules into specific, charged species (ions) that can be selectively filtered and detected. Utilizing the MS, known analytes of interest can be very selectively detected, based on the most abundant ions expected from the fragmentation process. This ability gives excellent selectivity and increased sensitivity over other more traditional detectors such as FID or FPD. The MS is also fully capable as a quantitation detector, identifying and quantifying peaks, based on chromatographic RT and detector response. Additionally, an MS can be used in conjunction with both GC and LC platforms. The sensitivity of the MS as a detector allows for lower detection limit requirements to be met. For these reasons, the detection and quantitation of analytes for this program is accomplished utilizing an MS.

1.6.3 Overview of Mass Spectrometry

An MS is a detector that is part of the overall chromatographic system. As a detector, the MS detects and quantifies analytes of interest as they elute from the analytical column. The additional information obtained when molecules are fragmented into specific, charged species is referred to as *mass spectral data*. Along with RT of the analyte, the spectral information can be used to identify unknown compounds and/or qualify known compounds by confirming or denying the identity of a peak. This identification can be based upon analyte make-up, evident in the relative abundances of the ions on the mass spectrum. In mass spectrometry, a chromatogram still represents the chromatography and response of the analytes eluting from the analytical column at specific RTs versus detector response. Due to the detection of ions, an MS chromatogram is referred to as an *ion chromatogram*, which can either be a total ion chromatogram (TIC) or an extracted ion chromatogram (XIC). A TIC shows the total detector response of all ions that are being detected, while the XIC shows the detector response of specific ions being detected. TIC and XIC examples are shown in Figure 7 and Figure 8, respectively.

Each type of ion chromatogram also contains the spectral data that can be accessed and viewed in the form of a mass spectrum, showing the resulting ions versus detector response. Mass spectral data is available for any time on the chromatogram. Note that the best mass spectrum, representing a particular analyte, would be acquired at the apex of an analyte peak on the ion chromatogram. A simple example of a mass spectrum is provided in Figure 9.

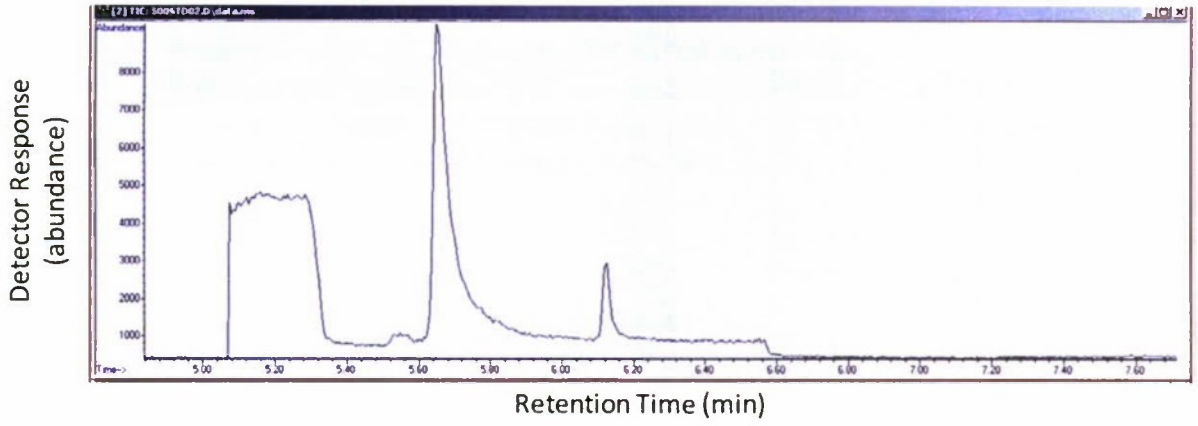


Figure 7. An example of a total ion chromatogram collected by a GC/MS.

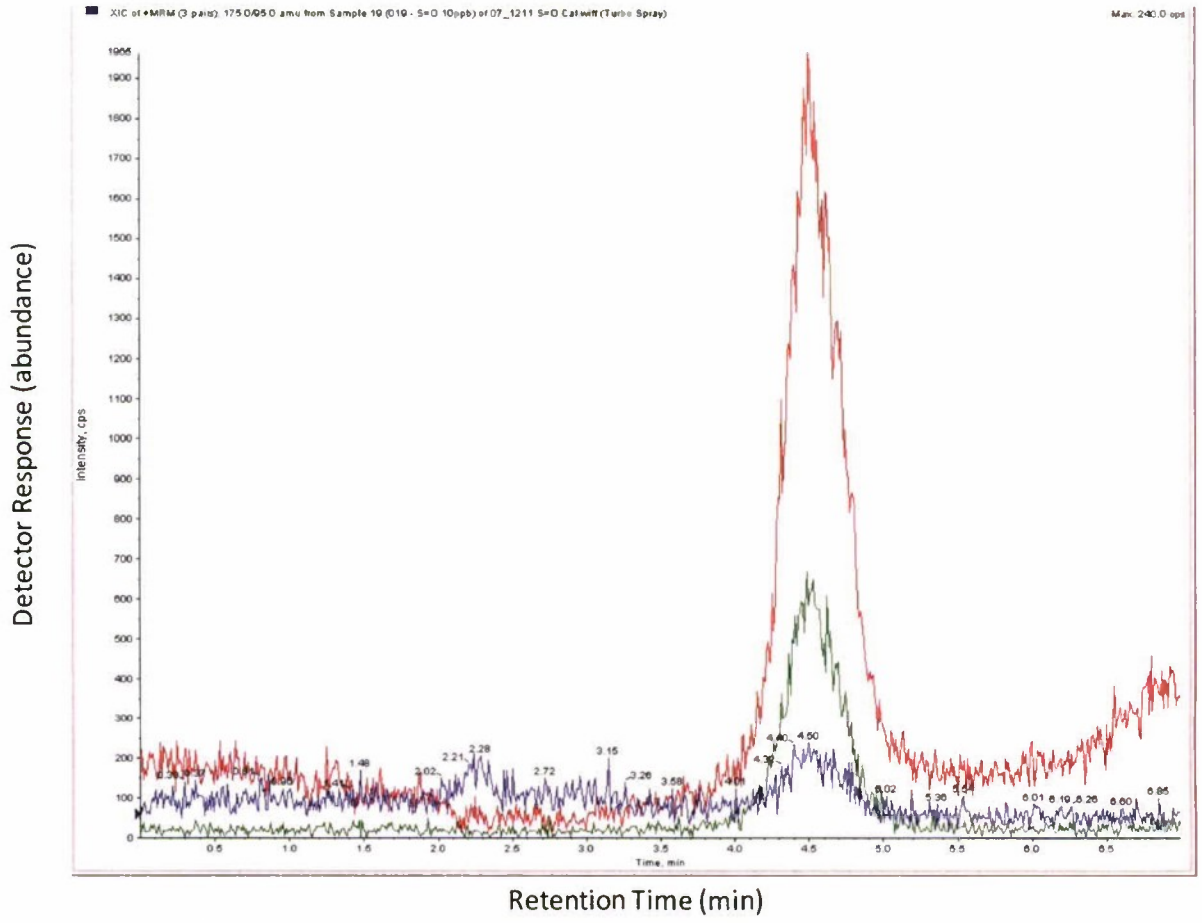


Figure 8. An example of an extracted ion chromatogram collected by an LC/MS/MS.

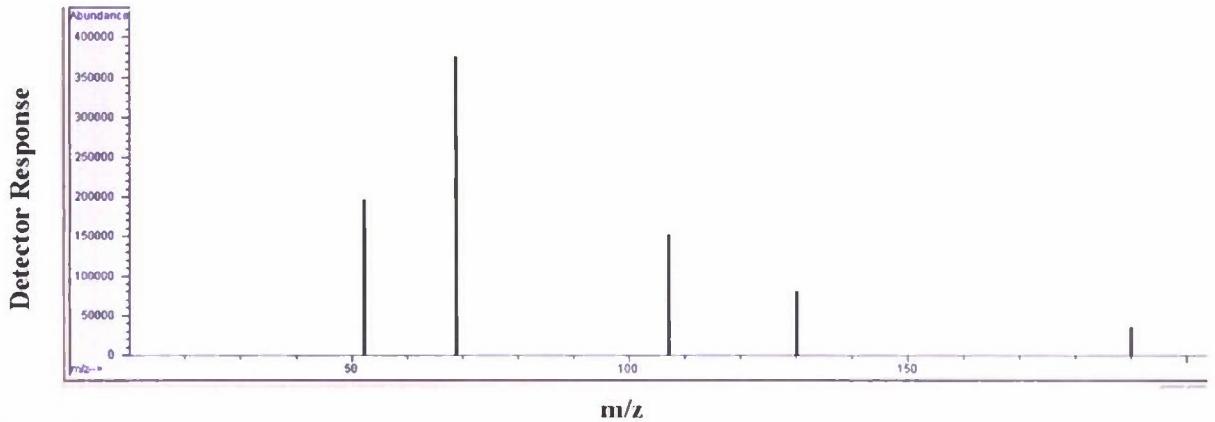


Figure 9. An example mass spectrum.

Although the technology and terminology of the MS used for the GC/MS system differs from that of the LC/MS/MS system, the principle of operation is very similar. The MS operates under high vacuum. The high vacuum is created with multiple vacuum pumps, one of which is typically a turbo molecular pump. The purpose of the high vacuum system is to create a long mean free path in the analyzer of the detector so that charged particles may move through freely, without impacting the molecules present in atmosphere. This greatly increases the sensitivity of the instrument. However, poor vacuum, possibly caused by ambient air leaking into the system, can significantly degrade the detector sensitivity. The high vacuum must be established before sample analysis can occur. Once the MS is ready to analyze, analyte molecules enter an ion source where they come into contact with an ionizing force, which creates the charged particles (i.e., ions) of the molecule. During ionization, some analyte molecules remain intact and ionize without fragmenting into smaller ions; these are called *molecular ions*. Other molecules will fragment into smaller pieces of the analyte. The resulting ion fragments are referred to by their associated mass-to-charge (m/z) ratio. The *m/z ratio* refers to the mass (m) in atomic mass units (amu) divided by the charge (z) associated with the ion fragment. A fragment that has one electron removed during the ionization process would have a charge of positive one (+1). In this case, the m/z would refer to the molecular weight of the resulting fragment, allowing for the makeup of the fragment to be identified. Fragmentation occurs consistently and reproducibly, resulting in very similar spectral data from analysis to analysis of a given analyte.

The MS used for this program utilized different techniques to ionize sample molecules. For example, in GC/MS, the MS, which is called an MSD by the manufacturer, is capable of ionizing sample molecules either by chemical ionization (CI) or by electron impact ionization (EI), depending on the type of ion source installed. The MSD is most often used in EI mode. Therefore, EI was used for the method development associated with the Low-Level program. In EI, the analyte molecules are ionized when contacting a beam of electrons passing from one filament to another in the ionization chamber of the ion source. Normally, the EI mode ionization creates m/z ratios with a charge of +1. This allows for virtually all resulting m/z ions to be evaluated based on molecular weight. This type of ionization is the most common. EI provides one of the major benefits of utilizing an MS: the capability to compare an acquired spectrum to a library of mass spectral data. Because the EI mode creates ions and ion fragments reproducibly and consistently, many thousands of mass spectra have been compiled into searchable libraries that are commercially available. Mass spectral libraries contain entries showing the expected mass spectrum for an analyte of interest. Utilizing these libraries allows the search for and comparison of found spectral data to expected spectral data. Library searching can be performed as probability-based matching (PBM), which is a comparison of a collected mass spectrum to the most likely library match. Searching can also be performed as parametric retrieval, which allows a library entry mass spectrum to be called upon by compound name, Chemical Abstracts Service (CAS) number, molecular weight, or some other known compound parameter. Mass spectral data, acquired in EI mode, are the only widely

available data in searchable libraries. Note that libraries are not readily available for spectral data acquired in the CI mode, due to the differing spectra acquired for the same analyte, depending on type of reaction gas used and the charges of ions formed.

The LC/MS/MS has multiple ion sources available, allowing for different types of ionization. The ion source used for the Low-Level program was able to perform atmospheric pressure chemical ionization (APCI) or electrospray ionization (ESI), depending on the type of source probe installed. The Low-Level program used the probe capable of ESI. In ESI, an ion spray voltage creates charged droplets of solvent molecules that create ion fragments of the sample molecules as they evaporate. The LC/MS/MS creates ions and ion fragments that are both positively (+) charged and negatively (-) charged. The LC/MS/MS was set up to analyze in either positive or negative ion mode, depending on the positive or negative charge of the ions that are to be evaluated. The charges applied to the analyzer facilitated the detection of specifically charged ions.

After ionization of the analyte molecules, the ions have to be processed and counted. Both gas and liquid chromatography-based MS technologies chosen for this program were quadrupole-based systems. A quadrupole consists of four diametrically opposed rods, arranged in a hyperbolic geometry. As alternating and direct currents are applied in different ratios, ion fragments of specific m/z ratios are allowed to pass through the quadrupole into the ion detection component of the detector. This process is known as *mass filtering* and a quadrupole is commonly referred to as a mass filter. The MS can scan for all ions in a given mass range, or it can be set to be much more selective and filter for selected ions. For example, in GC/MS, a single quadrupole instrument, there are two modes of data acquisition—Scan mode and Selected Ion Monitoring (SIM) mode. The Scan mode of acquisition will filter and detect every ion within a specified mass range. Examples of mass spectra acquired in the Scan mode and SIM modes of acquisition are shown in Figure 10 and Figure 11, respectively. The SIM mode of acquisition allowed the selection of a few ions; in these examples m/z ions of 72, 114, 127, and 167. Evaluating these few ions allows more time to filter each ion and allow it to pass through the quadrupole into the detector component.

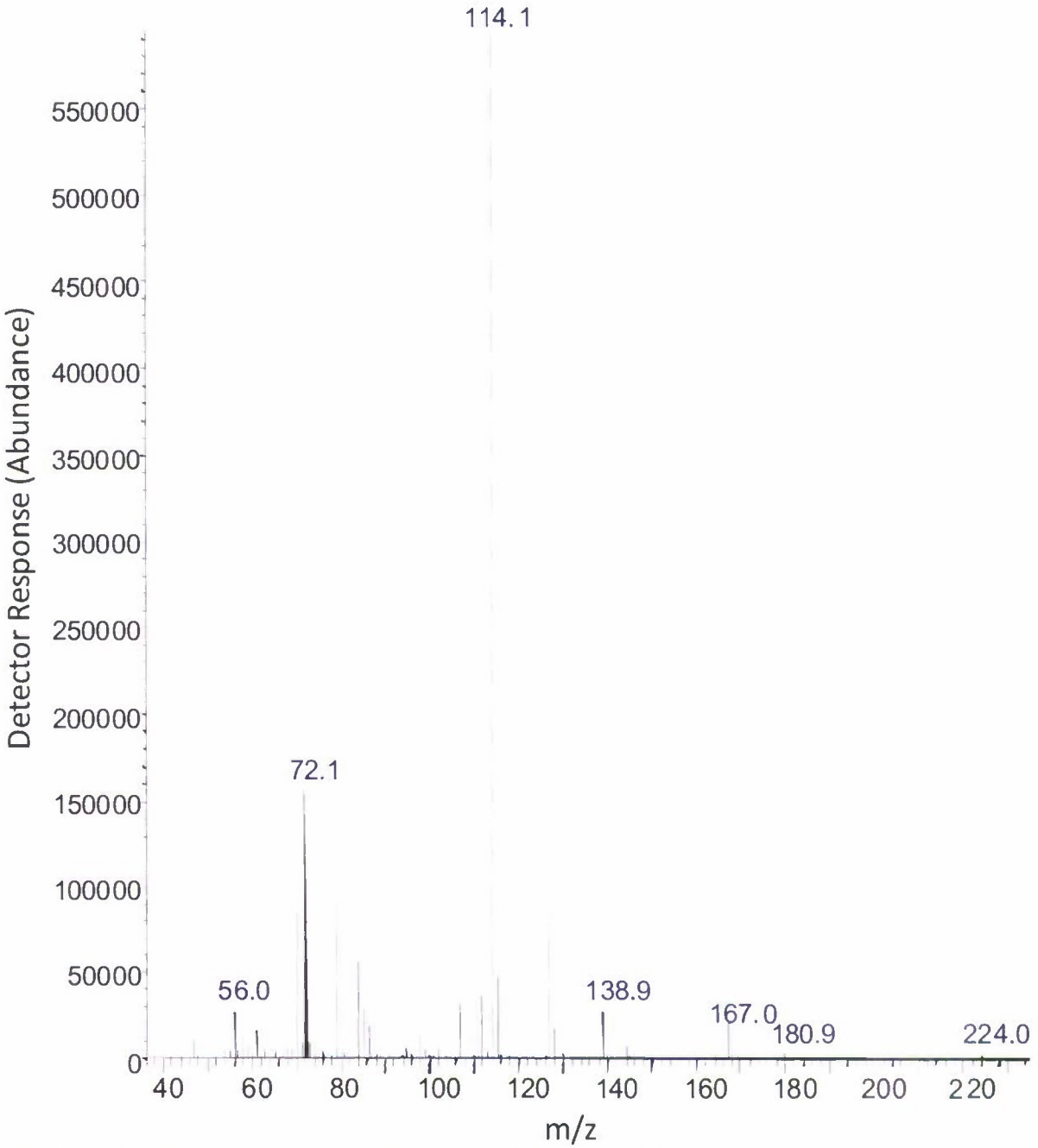


Figure 10. GC/MS mass spectrum acquired in the Scan mode of acquisition.

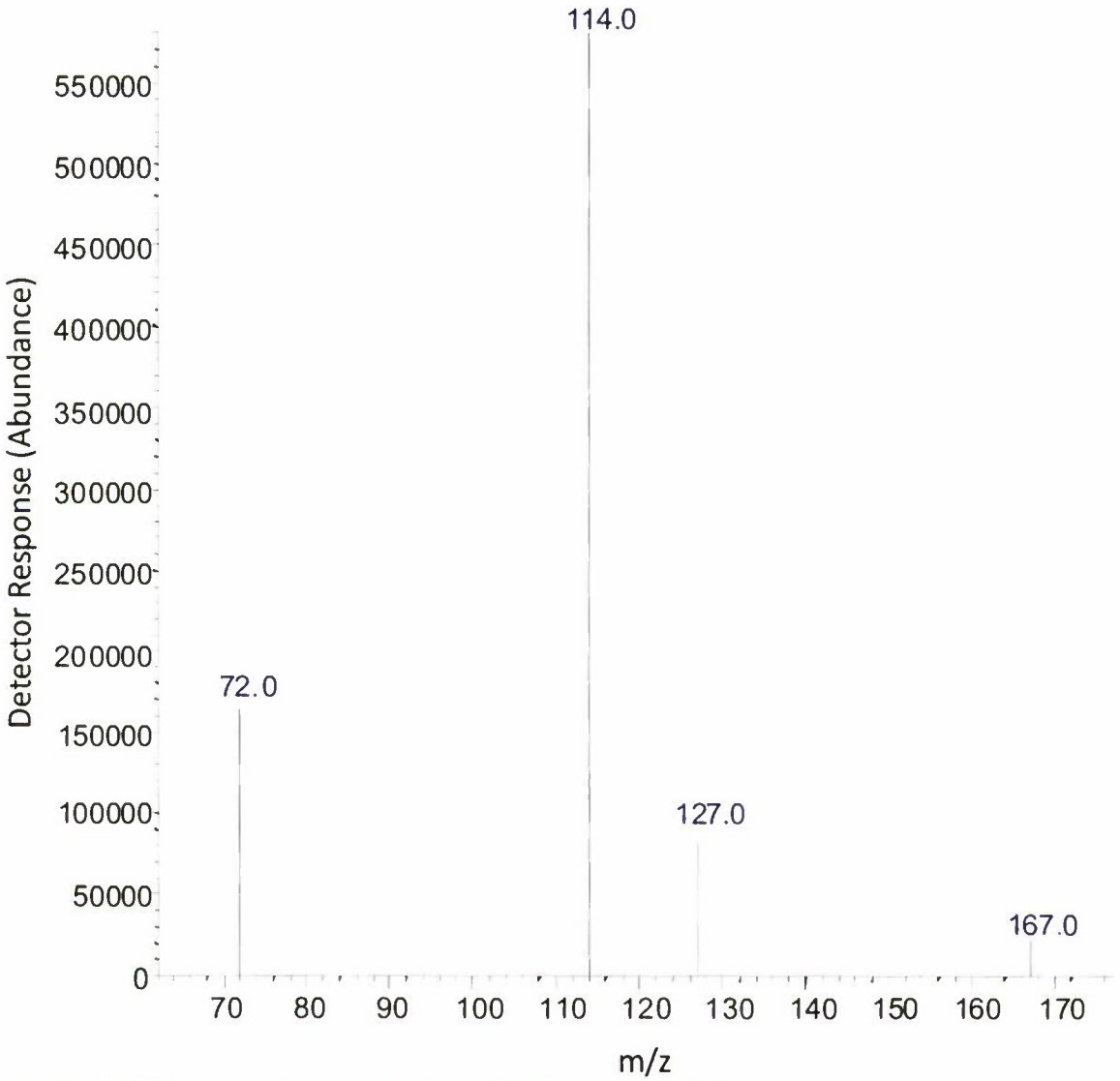


Figure 11. GC/MS mass spectrum acquired in the SIM mode of acquisition.

The LC/MS/MS is a triple quadrupole (QqQ) instrument that has the ability to perform mass filtering twice on a given analyte. This instrumentation is referred to as MS/MS and allows for evaluations via multiple reaction monitoring (MRM). MRM on a triple quadrupole instrument is the MS filtering ions of certain masses (parent ions) in a quadrupole (Q1), the further fragmentation of these ions in a collision cell (Q2) via collision-induced dissociation (CID) to create daughter ions, and then filtering these daughter ions with another quadrupole (Q3). The LC/MS/MS capabilities provide for the ultimate in selectivity. Due to the fact that there are so many ways that analytes can be detected with this instrument to produce spectral data (ESI, APCI, scanning, MRM, positive ion mode, negative ion mode, etc.), there are no widely accepted libraries available for LC/MS/MS spectra, such as there are for the EI GC/MS. It is also important to note that the LC/MS/MS spectra cannot be compared to GC/MS libraries. Therefore, parent/daughter ions were chosen and MS/MS spectra were evaluated, based upon literature and/or instrument optimization. An example of an LC/MS/MS mass spectrum showing MRM parent/daughter ion pairs is provided in Figure 12.

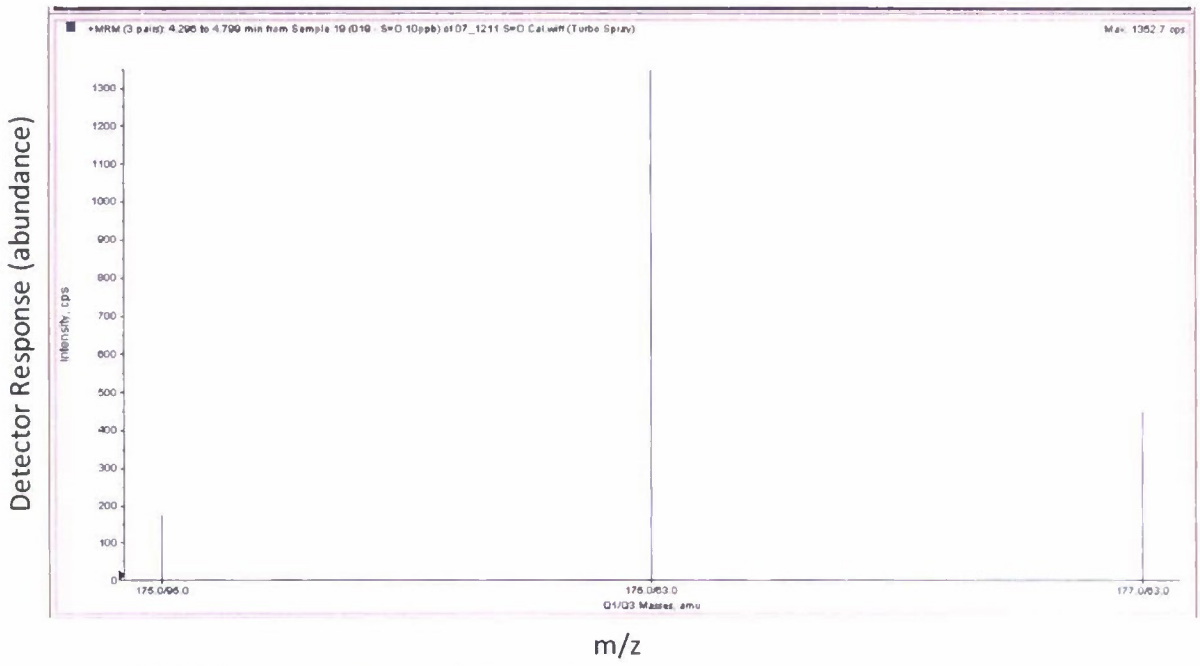


Figure 12. LC/MS/MS mass spectrum acquired by MRM.

An MS collects data in scans, or cycles, depending on the mode of acquisition. A scan/cycle is one pass through the range of ions to be detected by the instrument whether evaluating a range of ions (Scan mode) or selected ions (SIM mode). Each scan collected by the MS contains mass spectral information. The summed total of the scans throughout an analytical evaluation makes up the total ion chromatogram. The number of scans/cycles collected can be adjusted to establish a balance between the amount of mass spectral information collected in one scan and the total number of scans collected. As an example, the parameter for controlling this in the SIM mode of acquisition on a GC/MS is called Dwell time. *Dwell time* is a parameter set for each ion of interest in the SIM mode. The higher the Dwell time setting, the longer the MSD will collect abundance for that ion. Higher Dwell times will lower the number of times the MSD can scan through all the ions selected in the SIM mode. If it takes too long to complete one scan, and not enough scans are collected across a peak due to these higher Dwell times, important mass spectral information may be lost. It is imperative to ensure that no mass spectral information is lost; therefore, adjusting the method Dwell times to increase the number of cycles per second will ensure that the best possible mass spectral information is available. This information is typically collected at the highest concentration of analyte in the detector and represented by the apex of the peak on the TIC.

The ability to obtain mass spectral information, allowing for correct identification of analytes through mass spectral interpretation is one of the most important features of the MS. The phenomenon of isotopes can also greatly aid in identifying molecular structures of analytes. Isotopes are naturally occurring variations of elemental atoms in nature. Each element has a specific number of protons and neutrons in the nucleus of an atom that makes up its molecular weight in amu. The number of protons and, therefore, electrons are what differentiate the atom of one element from an atom of another element. Some atoms of an element carry additional neutrons in the nucleus. Since the number of protons has not changed, the atom is still the same elemental material; however, the additional neutrons carry mass and affect the overall molecular weight of the atom. These atoms are called *isotopes*. In some cases, the naturally occurring isotope(s) of an element have been quantified and, when present on a mass spectrum, can be identified and used as a tool to determine the molecular makeup of a particular ion. An example regularly seen in chemical agent samples is the isotope of chlorine (Cl). The elemental form of

Cl weighs ~35 amu and is represented as ^{35}Cl. However, in nature, about 25% of Cl atoms contain two extra neutrons in the nucleus, causing the molecular weight of the Cl isotope to be 37 amu, represented as ^{37}Cl. The two extra neutrons are very evident on the mass spectrum of the HD molecule, which contains two Cl atoms (Figure 13).

When examining the mass spectrum of HD, the molecular ion m/z 158 is present, as is another ion at m/z 160. Also, fragments appear at m/z 109 and 111. The ions at m/z 160 and 111 are the same fragments as m/z 158 and 109, respectively, but contain the ^{37}Cl isotope with +2 amu. The presence of the isotope peak helps identify Cl as at least one of the components of the ion fragment. An example of the mass spectrum of HD, showing the isotope peak is shown in Figure 14. An example of the chemical structure of the m/z 109 and 111 fragments is provided in Figure 15.

In review, the capability of an MS to ionize analyte molecules and create ions that can be filtered, detected, and represented on an ion chromatogram/mass spectrum with excellent sensitivity are the attributes that make the MS the most desirable detector for the Low-Level program. These attributes, along with mass spectral interpretation, allow the quantitation and identification of analytes.

$$Cl - CH_2 - CH_2 - S - CH_2 - CH_2 - Cl$$

Figure 13. Chemical structure of HD.

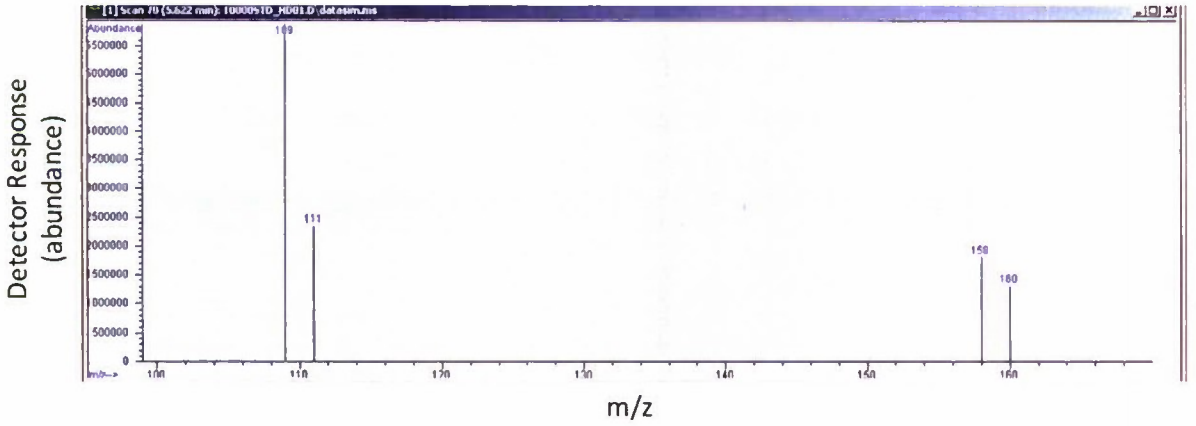


Figure 14. Mass spectrum of HD showing peaks containing the isotope of Cl.

$^{35}Cl - CH_2 - CH_2 - S - CH_2^+$ m/z 109 ion

$^{37}Cl - CH_2 - CH_2 - S - CH_2^+$ m/z 111 ion

Figure 15. Chemical structure of the HD ion fragment m/z 109 and 111.

1.6.4 Overview of an Analytical Method and Method Development

Analytical instrumentation provides the means to take a complex mixture, separate all of the components of the mixture then identify and quantify certain analytes of interest within the mixture. The sample analysis is only part of the overarching analytical method. The method consists of sample collection and/or preparation, as well as analytical instrument parameters.

Sample collection and/or preparation, as part of contact and vapor decontamination testing, generates the complex mixtures (i.e., samples) for analysis. These samples must be analyzed and the resulting data reported with confidence. To accomplish this, the parameters of the analytical instrument must allow good chromatography of the analytes of interest so that they may be detected and quantified without interference from other analytes or instrument issues. Method development is the selection and balance of the right solvent system, chromatographic settings (temperatures, flows, columns), and detector settings. The appropriate settings are needed to chromatographically resolve and separate an analyte from other compounds, and provide the capability to quantitatively characterize the analyte to the desired detection limit.

(1) Instrument Selection: The analytes of interest for this program included the chemical agents VX, HD, and GD, along with select hydrolysis and oxidation byproducts of each. Instrument method development began with a decision regarding the type of instrument to use (GC/MS vs. LC/MS/MS). The technology used to conduct method development was chosen based on literature review, previous work with the analytes, and screening of the analytes on the instrumentation. An example for the selection process follows: HD, which is a very non-polar compound, could not be detected using LC/MS/MS due to the challenges of LC/MS/MS ESI creating ions from non-polar compounds; therefore, all HD analysis was performed on a GC/MS. Conversely, the H-sulfoxide was easily detectable using the LC/MS/MS, but showed poor chromatographic peak performance on the GC/MS; therefore, method development was performed with the LC/MS/MS.

(2) Parameter Setup: Once an instrument was identified, generic instrument parameters were set up based on information available. Information may be available through literature review, previous experience, mass spectrum library searching, etc. Instrument parameters included all facets of sample analysis—from choosing the appropriate sample introduction amount to ensuring that the MS was optimized to detect the expected analyte ions.

(3) Standard Preparation: With this generic setup, standards were prepared (analyte in pure solvent) and introduced into the system for analysis. The solvent used for each analyte must be stringent enough to extract the analyte into solution during the decontamination test. It must also be appropriate for use on the instrument. For example, water would make a very poor solvent for use on the GC/MS due to its low volatility. Using high purity standards helps ensure that the peak associated with the analyte of interest on the chromatogram has been correctly identified. It is not uncommon to see additional peaks on the chromatogram when analyzing samples. These peaks can be attributed to instrument contamination, analytical column stationary phase, standard impurities, and/or interferents from the sample collection/preparation efforts.

(4) Baseline Acceptance Testing: After correctly identifying the analyte peak, it is imperative to perform baseline acceptance testing. Baseline acceptance can be performed using high purity solvent. A detector response should only occur if an analyte or impurity is detected. High purity solvent should not elicit a detector response beyond the first few moments of the analytical run, if at all, depending on instrument configuration. When no analyte is in the system, the baseline at the analyte RT should be flat. Any baseline deviation at the analyte RT could cause analysis issues and needs to be addressed prior to method validation.

Finally, once these steps have been completed, analyte optimization can commence. Analyte optimization consists of adjussting the parameters for ultimate sensitivity, while maintaining good chromatography (efficiency and resolution). Specific method development highlights are discussed for each analyte of interest in later sections. Specific method parameters for each analytical method are provided in Appendix A.

1.7 Capturing Methodology Improvements

The improvements are not only captured in this technical report, but also under a larger methodology improvements document, the Chemical Decontaminant Performance Evaluation Testing 2007 Source Document.[16] The Source Document is the product of project BA06DEC414 for improved test methodology. The primary objective of the BA06DEC414 project was to improve the rigor of existing test methods from TOP 8-2-061, Chemical Biological Decontaminant Testing,[2] for the generation of defensible and comparable decontamination efficacy data for the quantitative determination of post-decontamination contact and vapor hazards and residual agent. Program BA06DEC414 focused on updating the panel contact and vapor test for the determination of contact and vapor hazard, respectively. Execution of these improved methods yields higher fidelity data presented in appropriate context. The data generated from these updated methods enhances all components of the decontaminant life cycle, including research and development, science and technology, testing and evaluation, and developmental and operational testing activities, technology readiness assessments to determine technology readiness level, technology comparisons, risk assessments, and milestone decisions.

The 2007 Source Document organization utilizes a textbook chapter and section structure. Each chapter is focused on a specific topic such as the contact test method. Each chapter is divided into sections for each task supporting that topic. The foundation of the test method chapter is a basic research procedure outline including reagents, materials, procedures, calculations, and reporting. The basic foundation is augmented by incorporating the elements required by ISO 17025 and ASTM methods such as procedure summary, terminology, reporting criteria, quality assurance (QA), QC, and test acceptance criteria. The format of the Source Document facilitates method insertion into performing laboratory quality systems. Each test section carries relevant terminology, references, calculations, and QA/QC requirements so that each chapter subsection can be used as an independent method. The methods generated in the Low-Level program are documented in an appendix that will be added to future Source Document releases. In addition, lessons learned and improvements from the Low-Level program will be documented in future Source Document releases as chromatographic analysis guidance methods for the contact and vapor test chapters.

2. MATERIALS AND EXPERIMENTAL DESIGN

2.1 Information and Use of Chemical Agents, Byproducts, and Simulants

This testing used chemical agents, agent byproducts, and simulants. All chemical agents used were CASARM grade, with purities on record, obtained from either NMR or GC/MS analyses. The materials used in this test program including lot, purity, and source are provided in Table 3 and Table 4. Chemical agents are used only in properly certified surety facilities, capable of handling such materials safely. The test personnel were trained and approved to work with chemical agents. Program personnel adhered to security, health, and safety requirements of the U.S. Army Research, Development, and Engineering Command (RDECOM) including, but not limited to, good laboratory safety practices, using safety office approved methods, and wearing proper personal protective equipment (PPE). This report and methods do not claim to address all of the safety concerns associated with chemical decontaminant testing, as the requirements may vary based on facility, state, and other regulatory requirements. It is the responsibility of the user of this method to establish appropriate environmental, health, and safety practices for the use of this method, and handling of generated wastes, in compliance with applicable

regulations prior to use. Users of this method should conduct testing in appropriate facilities and follow proper laboratory practices including the use of appropriate PPE and Material Safety Data Sheets (MSDSs).

Table 3. Chemical agent listing.

Contaminant	Grade	Lot	Purity %
GD	CASARM	GD-U-2323-CTF-N	98.8
HD	CASARM	HD-U-5032-CTF-N	98.5
VX	CASARM HP	VX-U-5055-CTF-N	89.5

Table 4. Chemical agent byproducts.

Name	CAS No.	Source	Product No.
Pinacolyl methylphosphonate	616-52-4	Sigma-Aldrich	386588-1G
2,2'-Thiodiethanol	111-48-8	Sigma-Aldrich	166782-100G
Ethyl methylphosphonate	1832-53-7	Sigma-Aldrich	386561-1G
Divinyl sulfone	77-77-0	Sigma-Aldrich	V3700-5g
Bis(2-chloroethyl)sulfone	471-03-4	Sigma-Aldrich	S741930-1EA
S-(2-diisopropylaminoethyl) methylphosphonothioic acid	73207-98-4	ECBC	N/A
Bis(2-chloroethyl)sulfoxide	5819-08-9	ECBC	N/A

2.2 Analytical Equipment

Analytical methods were developed on instrumentation specific to, and capable of, achieving the goals of the Low-Level program. The instrument-specific method parameters described in this report were established using the instrument software. These parameters may be used on similar or other GC/MS and/or LC/MS/MS platforms, and are comprehensive enough to be an excellent guide for establishing low-level methods. Methods transferred to instruments, which are set up as described, should obtain similar results. However, the application of different parameters to the instrumentation may not produce the same results. All instrumentation (similar or otherwise) may have different detection capabilities; these method parameters are guidelines. Once set up, all instrumentation requires a thorough checkout in accordance with manufacturer recommendations. Instrument-specific method parameters may need to be adjusted. For example, the GC auxiliary pressure source may be Aux 3, 4, or 5 on an Agilent 6890 GC or Aux 1, 2, or 3 on an Agilent 7890 GC. Additionally, method checkout must be performed to include analyzing solvent blanks and pure chemical agent and byproduct standards. This is done to determine instrument-specific parameters such as baseline acceptance, analyte retention times, and optimized Deans Switch heart-cut times.

2.2.1 GC/MS System for Liquid Sample Analyses

The liquid extraction samples were analyzed on an Agilent 6890 GC, equipped with a 5975 MSD. The GC was outfitted with an Agilent Deans Switch, allowing for flow switching during analysis so that only a specific portion of the sample effluent, known to include the analyte of interest, was directed to the MSD. All other flow was directed to an FPD. This action extended the life of the MSD by minimizing exposure of the detector to unwanted sample components. Sample introduction was performed using a GERSTEL MPS2 and a Gerstel Cooled Injection System (CIS4) inlet with the peltier

cooling option. The instrument operation and data analysis were performed with the Agilent Technologies MSD ChemStation software package (v. D.02.00.275). The Gerstel sample introduction equipment is controlled by the Gerstel Master software that is integrated into the Agilent MSD ChemStation software. This instrument and all the ancillary equipment required for operation were collectively referred to as GCE. Instrument capabilities were dependent upon the analyte(s) of interest and the method-specific instrument parameters associated with the analyte(s).

When using the GCE system, the Gerstel MPS2 syringe requires cleaning between samples to prevent sample cross-contamination and to extend the life of the syringe. Analyzing large batches of samples can introduce contamination from dirty samples into the syringe. This may cause the inadvertent contamination of other samples, solvent blanks, or standards as the instrument sequentially moves through the sample queue. Wash steps programmed into the method parameters will clean the syringe before and after each injection to ensure that the syringe is clean and free of contamination. Cleaning the syringe between samples ensures that the only analytes introduced into the chromatographic system are those contained in each discreet sample. Cross-contamination of samples, due to a dirty syringe, could have a serious impact on the analytical data. For example, if a solvent blank was unknowingly cross-contaminated with chemical agent from a standard or other sample, an agent peak may be detected upon analysis. In this scenario, the detected agent peak in the solvent blank may falsely indicate carryover and potentially prompt unnecessary corrective action.

The Gerstel software allows for multiple wash steps using two solvent wash vials. Note that the solvent(s) selected must be stringent enough to clean the syringe. For this program, the solvents hexane and methanol were used to clean the syringe. Typically, one solvent wash was performed for each solvent prior to sample introduction. Two solvent washes were performed for each solvent post sample injection. Note that, prior to sample introduction, the syringe may also be washed or rinsed with the sample to be injected. However, it was observed during method development that rinsing with the sample was unnecessary and only created additional waste-handling issues. Thorough cleaning will also extend the life of the syringe. As contamination builds in the barrel of the syringe, the plunger of the syringe will slide less freely and the syringe may fail. It was also observed during method development that certain solvents, such as chloroform and hexane, tended to dry out the barrel of the syringe, also causing the syringe to fail. Here again, solvent selection is important; a secondary solvent wash of methanol will help keep the barrel of the syringe lubricated. It was suggested that hexane and methanol be used as the wash solvents.

2.2.2 LC/MS/MS for Liquid Sample Analyses

The liquid extraction samples were analyzed on an Applied Biosystems API5000 Triple-Quadrupole MS, equipped with the TurboV Ion Source. Sample introduction and chromatography were performed with an Agilent 1200 series LC, which included the Agilent Binary Pump, Degasser, Thermal Column Compartment (TCC), High Performance Automatic Liquid Sampler (ALS), and the ALS Thermostat. Sample effluent was directed from the LC directly to the TurboV ion source of the API5000 MS. The instrument operation and data analysis were performed with the Applied Biosystems Analyst software package (v. 1.4.2). This instrument and all the ancillary equipment required for operation were collectively referred to as LCE. Instrument capabilities were dependent upon the analyte(s) of interest and the method-specific instrument parameters associated with the analyte(s).

2.2.3 GC/MS System for Vapor Sorbent Tube Analyses

With a special type of sample introduction, the GC/MS system was used for the analysis of collected vapor samples.

The vapor samples were analyzed on an Agilent 6890 GC equipped with a 5975 MSD. The GC was outfitted with an Agilent Deans Switch, allowing flow switching during analysis so that only

a small portion of the sample effluent, including the analyte of interest, was directed to the MSD. All other flow was directed to an FPD. Vapor sample introduction was performed using a Markes TDS, which includes the Markes Ultra 100-tube Autosampler, the Unity Thermal Desorber, and a Sample Mass Flow Controller. A sample was introduced into the GC/MS system via a sorbent bed desorption under higher temperature and flow. The sorbents used were Tenax-TA and Chromosorb-106. The instrument operation and data analysis were performed with the Agilent Technologies MSD ChemStation software package (v. D.03.00.611). This instrument and all the ancillary equipment required for operation were collectively referred to as GCV. Instrument capabilities were dependent upon the analyte(s) of interest and the method-specific instrument parameters associated with the analyte(s).

There are several considerations when operating the GCV system. One special consideration was that the Markes Unity software, which controls the Markes TDS, is a standalone software platform and is not integrated into the GC/MS software. Although the system will not operate without both software packages running in tandem, method parameters are available and adjusted on both packages.

Another special consideration of the GCV was that control of any necessary sample splitting is achieved with the Markes TDS. In traditional GC, liquid samples are injected into a liquid inlet. Often, the liquid inlet has split/splitless capabilities. When a traditional inlet is set to the splitless mode, an injected sample is vaporized and introduced onto the analytical column. However, it is not uncommon to have very concentrated samples that would pose problems during analysis, such as overloading the column, causing poor chromatography, overwhelming the detector, and causing major analyte carryover issues, among others. When samples of high concentration are analyzed, a traditional GC inlet, set to the split mode, allows a specific portion of the sample to be carried away (i.e., split) from the rest of the sample and vented from the instrument. This split portion will not enter the analytical column and prevents the system from having the problems associated with a splitless injection of a high concentration sample. Traditional GC inlet parameters are set within the instrument software.

The Markes TDS offers vapor sample introduction into the GC via installation through a modified GC split/splitless inlet; therefore, the ability of the GC to offer a split option is removed. High mass vapor tubes that would cause chromatographic or detection issues may still require the removal of a proportional amount of sample prior to analysis. The vapor sample split is accomplished in the Markes TDS utilizing parameter-controlled internal sample pathways and desired split amount flow rates (measured in mL/min). The unwanted split portion of the sample is directed through a split pathway, exits the system, and is routed through a Teflon line to appropriate engineering controls. The Markes TDS allows for splitting the sample three ways:

- Splitting during transfer from the sample collection tube to the cold trap
- Splitting during transfer of the sample from the cold trap to the analytical column
- Splitting the sample at both locations (for very high mass samples)

The method parameters controlling the desired split flow rates, adjustable from 5 to 100 mL/min, are set in the Markes Unity TDS software.

2.2.4 Analytical Instrument Software Platforms

Analytical instrument control and sample data analysis are performed using the instrument manufacturer's provided software package. As instrument technologies improve and software platforms are updated, certain terminology and instrument configurations may change. The methods and the parameters presented in this report should be viewed as a starting point for analyzing the analytes of interest. The detailed major method instrument parameters should be available in future updates of the

software platforms, even if software aesthetics and specific terminology have changed from the issuance of these methods.

Multiple software platforms were used for method development. The Agilent GC/MS systems for extraction and vapor sample analysis (GCE and GCV) use the Agilent MSD ChemStation Software with Enhanced Data Analysis. The GCE system, with the Gerstel MPS2 sample introduction system, uses the Gerstel Master proprietary software package, which is integrated into the Agilent MSD ChemStation. The GCV system, with the Markes TDS sample introduction uses the Markes TDS proprietary software package, in addition to the Agilent MSD ChemStation software. The Applied Biosystems LCE system uses the Applied Biosystems Analyst software package. Note that the LCE system Agilent Technologies LC is controlled by the Applied Biosystems Analyst software. A summary of the platforms and software version is provided in Table 5.

Table 5. Method development instrumentation platforms and software versions.

Instrument	Description	Software	Version
GCE	Agilent Technologies 6890N GC/5975 inert XL MSD	MSD ChemStation	D.02.00.275
	Gerstel MPS2/CIS4	Master	1.86.17.8
GCV	Agilent Technologies 6890N GC/5975B inert XL EI/CI MSD	MSD ChemStation	D.03.00.611
	Markes Thermal Desorption System	Unity: Thermal Desorption System Control Software	2.2.0
LCE	API5000 MS	Analyst	1.4.2
	Agilent Technologies 1200 Series LC		

2.2.5 Deans Switch/MSD Heart-cut

As described previously, the GC/MS is a very selective and very sensitive detector used for identification and quantitation of the program analytes of interest. When actively analyzing, the MSD applies potentials to many charged components to allow analyte ionization/fragmentation, mass filtering, and ion detection. These charged components contain critical surfaces that must remain ultra-clean to maintain the overall sensitivity of the detector. During sample analysis, there is a high likelihood that these critical surfaces will come into contact with unnecessary compound molecules and ion fragments, resulting in decreased sensitivity over time. This decreased sensitivity necessitates MSD maintenance and results in instrument downtime. It is important to note that the MSD ChemStation software includes certain instrument parameters to preserve the life of some of the charged components (for example, solvent delay); however, when analyzing hundreds of samples per week, additional measures are required. The methods developed for this program utilize an Agilent Technologies Microfluidics Deans Switch, which allows for sample flow to switch, or *heart-cut*, during sample analysis.

The Deans Switch is a multicomponent system that facilitates extremely low dead volume heart cutting by switching flow to a second detector during a desired time frame. *Dead volume*, caused by flow switching, is when a part of the GC sample pathway has no mobile phase carrier gas in it so that as a sample moves into this area, this volume is filled by the mobile phase and sample before the sample continues through the flow pathway. Dead volume in a GC system can seriously impact chromatographic performance; therefore, low dead volume is very important to maintain within a GC system. The Deans Switch consists of a manifold for the column connections, multiple sample flow pathways, auxiliary mobile phase (compressed helium gas) pressure sources, and a solvent vapor exit (SVE) valve to allow flow switching. The Deans Switch manifold has three connections. The analytical

column is connected from the inlet to the manifold. From here, two deactivated transfer lines extend to two detectors. The primary detector, the FPD, receives all sample flow when the valve is in the "off" position. At a very specific time, the valve can be switched to the "on" position so that the secondary transfer line receives the heart-cut sample flow, allowing the analyte of interest to enter the MSD. This heart cutting preserves the MSD from all unwanted compounds, except those that may closely elute to the analyte of interest. The heart-cut time is determined during method development. By analyzing the analyte with the flow continuously switched to the MSD as the second detector, the time required for a given analyte to reach the detector is determined. Once this RT is determined, the desired time frame encompassing the analyte RT is selected and set in the method parameters. These method parameters will actuate the SVE valve on and then off again, causing the heart-cut around the RT. All effluent outside of this time frame is sent to the FPD rather than the MSD.

Note that when viewing a TIC, it is not uncommon to see a stair-step increase in the baseline. Prior to the stair-step, the MSD analyzer is only receiving clean carrier gas directly from the auxiliary pressure source. When the valve actuates and the sample flow from the analytical column enters the MSD, the baseline will often appear to step up. Once the valve switches to the off position, the baseline will appear to step-down again. This may also be seen when altering selected ions within a run. Figure 16 contains an example of both.

To accomplish successful heart-cutting, pressures must be balanced using the Agilent Deans Switch Calculator. Entering method-operating parameters (primary and secondary column dimensions, temperature, flows, etc.) into the calculator provides a deactivated restrictor length for use between the Deans Switch and the primary detector (FPD). Figure 17 contains an example of the Deans Switch Calculator.

In conclusion, the Deans Switch maintains an ultraclean environment in the MSD, which improves sensitivity and reduces downtime for source maintenance. Furthermore, cutting away all undesired portions of the analytical run from the MSD extends the life of the filament and reduces interferences in detection over a period of time by preventing a buildup of effluent on the source and other MSD parts.

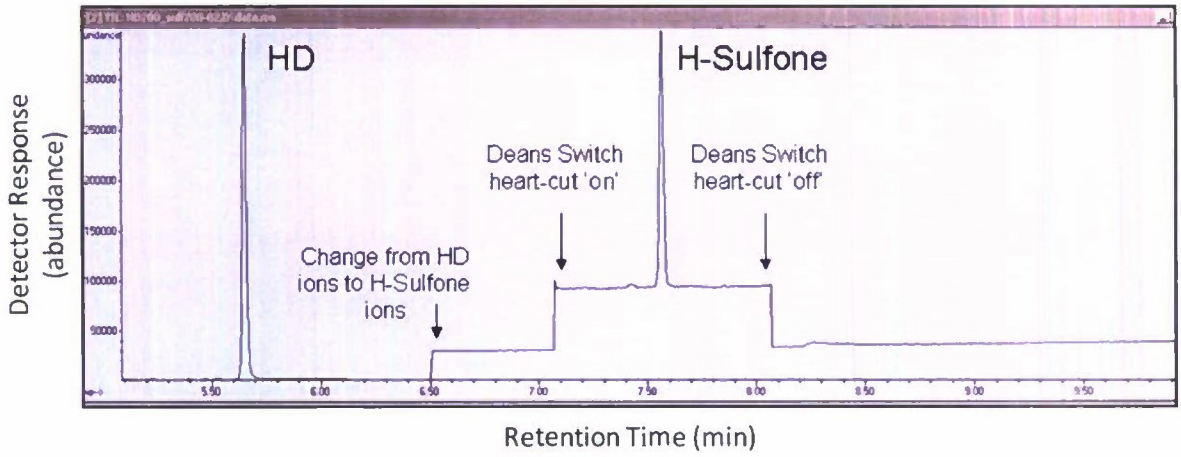


Figure 16. TIC containing HD and H-sulfone peaks. Note the stair-step phenomenon caused by the Deans Switch SVE valve and/or altering selected ions from HD to H-sulfone.

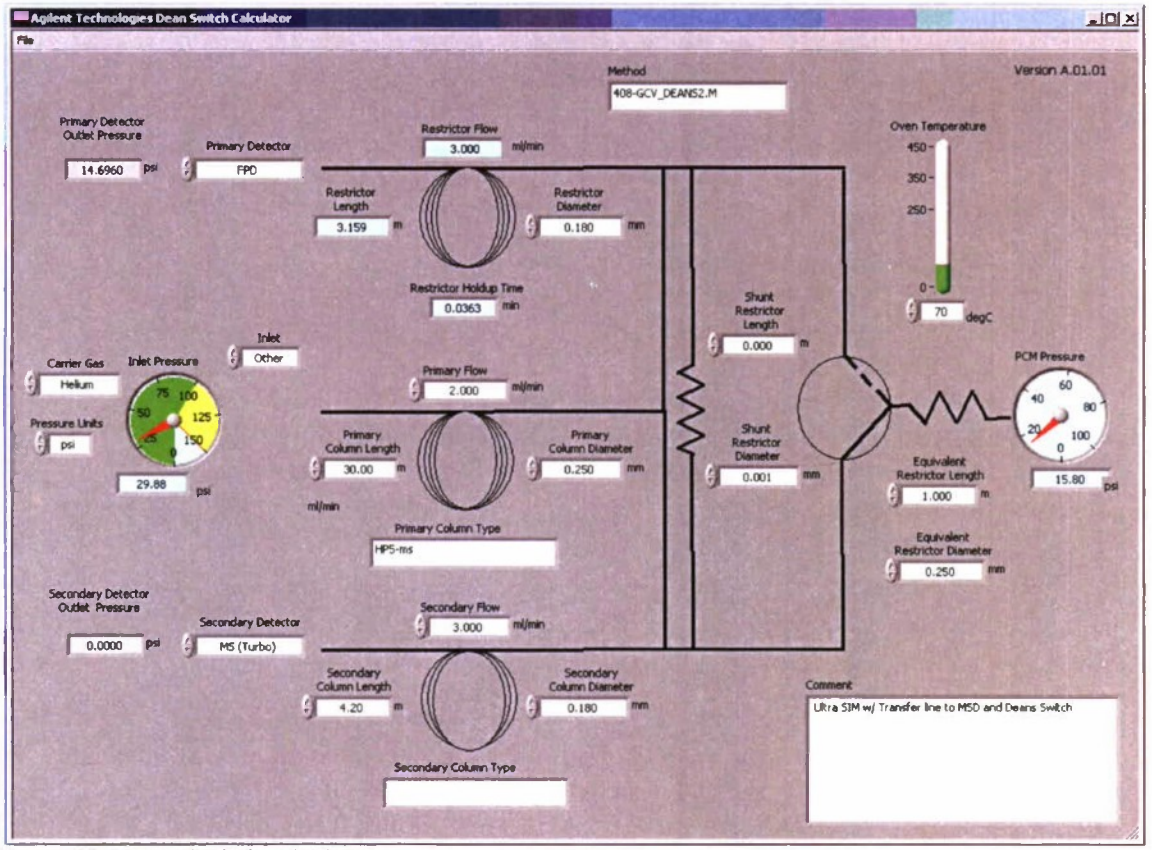


Figure 17. Deans Switch calculator.

2.3 Analytical Standard Preparation

In addition to the instrumentation parameters required to generate a quantitative method, the accurate preparation of standards used for instrument calibration is vital to the production of an accurate calibration curve, and thus, the accuracy of the reported sample concentrations. Each analytical method described in this report provides the concentrations used to generate a calibration curve. The guidance provided below will help produce these standards as accurately as possible.

There are many subtle details that lead to the accuracy of a standard, ranging from tool selection, operator technique, and training, to glassware cleaning practices. For example, because of the materials involved (chemical warfare agents), special glassware cleaning protocols may be implemented. To ensure that glassware is fully cleaned (i.e., decontaminated) between uses, glassware is typically submerged in bleach for 24 h, followed by a thorough cleaning with soap and warm water. If the post-bleach rinse is not thorough, residual bleach in the glassware could decontaminate the standard and provide a negative bias to the standard. Further, the glassware should be dry before usage; however, Class A glassware should never be dried in an oven as the heating process may alter the volumetric accuracy/calibration of the flask. Air-displacement pipettes are often highly inaccurate for viscous or highly volatile materials, such as VX or GD; therefore, the use of positive displacement pipettes is recommended to minimize these errors.

One of the fundamental assumptions in the accurate preparation of the standards is that the agent must be of a specified purity and density. This method uses the volumetric delivery of the agent to the first standard. The mass delivered is calculated by multiplying the density of the agent times the volume delivered. The density of a liquid can change with temperature, sometimes significantly. In most cases, chemical agents are stored at cold temperatures to minimize vapor pressure and degradation. If the

liquid agent is not at the temperature that corresponds to the specified density, bias can be introduced into the concentration of the standards and thus into all test results. For this reason, the agent should be allowed sufficient time to equilibrate to room temperature before use.

The following outline provides guidance using the preparation of a VX standard as an example. The sample dilution scheme is shown in Table 6. Volumetric flasks used in this case are V_{Final} = 10.00 mL.

1. Identify target concentrations needed for specific method (e.g., Appendix A methods).

   a. Determine the corrected density of neat agent using the density and the purity. Use the corrected density for all calculations.

   $$Density_{Corrected} = Density_{Standard} \cdot Purity/100\% \qquad \text{Equation 1}$$

   b. Identify volume (V_{final}) of Class A volumetric glassware (typically 10 mL) to be used for standards preparation.

2. For each of the standards, calculate the volume of higher concentration solutions needed to make each of the lower concentration solutions. $V_{Initial}$ corresponds to the volume (in mL) of the high concentration standard delivered to the volumetric flask. When calculating the $Conc_{Final}$, the $Conc_{Initial}$ used should be the 'actual concentration' and not the target concentration. For neat, use $Conc_{Initial} = Density_{Corrected}$. For all other solution preparations, use

   $$Conc_{Final} = Conc_{Initial} \cdot V_{Initial}/V_{Final} \qquad \text{Equation 2}$$

3. Identify the standard with the appropriate concentration that can be diluted to the target concentration using an initial volume between 50 µL and 1000 µL. This is an iterative process where multiple considerations must be weighed.

   a. Using the target concentration as a guide, estimate the appropriate amount of a higher concentration solution needed to attain the target concentration in V_{final}. This is an iterative process, but the goal should be to approach the target concentration without attempting to deliver an unreasonable volume. For example, Standard 1 is prepared using 1000 µL of Standard A. To precisely deliver the target concentration, 1007.38 µL of Standard A should be delivered into a 10.00 mL V_{final}. Since a pipette is not available in this range, a volume of 1000 µL is used to attain an actual concentration of 992,700 ng/mL. This variation does not impact accurate concentration assignments because standard concentrations are reported using actual concentrations rather than target concentrations.

   b. A second consideration for the selection of the dilution volume is the accuracy with which $V_{Initial}$ can be delivered. Typically, pipette accuracy decreases as the selected volume approaches the lower limit of the tool. For example, the Gilson M1000 pipette has a range of 100–1000 µL. The relative systematic error at 100 µL is 3% vs. 0.8% at 1000 µL; thus it is advisable to identify the pipette tools available and to select $V_{Initial}$

such that it is in the mid- to full-scale range of the pipette. If multiple pipettes, spanning various delivery volumes are available, the pipette that provides the highest accuracy should be used.

4. Use dilution scheme prepared in step 3 to make standards.

   a. Pre-fill the volumetric flask with ~8 mL of appropriate solvent and cap.

   b. Using a clean pipette tip for each solution, add the calculated volume of solution to the volumetric flask. Use the pipette manufacturer's recommendations for setting the pipette delivery volume. For example, the specifications for Gilson Microman Positive Displacement Pipettes indicate that when decreasing the setpoint volume; ensure that the setting is not overshot. When increasing the volume, pass the value by one-third of a turn and slowly back down to the mark without overshooting. This will ensure the most accurate delivery (i.e., that the actual setpoint volume is delivered).

   c. Fill volumetric flask to the line with solvent. Cap the flask, invert three times.

   d. Transfer solution to GC vials. Cap GC vials.

5. Report standard concentrations using actual concentrations.

Table 6. Example calibration standard set.

Standard Name	Target Conc. (ng/mL)	Std Solution	Volume Std Sln (mL)	Flask Vol (mL)	Actual Conc. (ng/mL)
StdA*	10,000,000	Neat	0.110	10	9,926,714
Std1	1,000,000	StdA	1.000	10	992,671
Std2	100,000	StdA	0.100	10	99,267
Std3	10,000	Std2	1.000	10	9927
Std4	2,000	Std2	0.200	10	1985
Std5	1,000	Std2	0.100	10	993
Std6	750	Std2	0.076	10	754
Std7	500	Std2	0.051	10	506
Std8	250	Std3	0.250	10	248
Std9	100	Std3	0.100	10	99
Std10	50	Std3	0.050	10	50
Std11	25	Std7	0.500	10	25
Std12	10	Std7	0.200	10	10
Std13	5.00	Std7	0.100	10	5
Std14	2.50	Std9	0.250	10	2.48
Std15	1.00	Std9	0.100	10	0.99
Std16	0.50	Std9	0.050	10	0.50
Std17	0.20	Std12	0.200	10	0.20
Std18	0.10	Std12	0.100	10	0.10
Std19	0.05	Std12	0.050	10	0.05

*The red shading represents the first standard prepared from neat agent material. Follow-on serial dilutions begin from this standard. Gray shading is for ease of reading.

Each chemical agent analyte of interest is diluted in a different type of solvent. Note that there are many solvents that may be appropriate for use with chemical agent analytes. For example, hexane is a solvent commonly used when diluting HD. Solvents are chosen for method development based on compatibility with the agent, the ability of the solvent to extract the agent from other liquid and solid materials, and the performance of the agent in the solvent upon analysis. Table 7 lists the agents and solvents used for this program.

Table 7. Chemical agent and solvent pairs.

Agent	Solvent
VX	Isopropanol (IPA) (CAS # 67-63-0)
HD	Chloroform (CAS # 67-66-3)
GD	Acetonitrile (CAS # 75-05-8)

2.4 Analytical Calibration Curve

A calibration curve (or calibration model) describes the relationship between analyte standards of known concentrations (ng/mL) or mass (ng) and their corresponding detector responses. After the calibration standards have been analyzed, the calibration can be described by a calibration model (a mathematical equation). The calibration process determines the coefficient values (e.g., a, b, c, *and* m) for the model. Typical calibration models include linear, quadratic, and Power Law. Using the calibration model, the concentration of a sample (x) is determined by the detector response (y). Table 8 lists the calibration models and their solutions. The coefficients for a calibration model are determined by a statistical regression using a technique such as linear least squares. The statistical regression may be executed by the instrument control software (e.g., Applied Biosystems Analyst, ChemStation) or may be executed by external statistical software (e.g., Excel, Sigma Plot, Matlab). In some cases, weighting may be implemented in the regression to provide higher confidence in a specific concentration range. For example, a 1/x (i.e., 1/concentration) weighting will result in higher analytical accuracy (i.e., confidence) at low concentrations and may or may not influence accuracy at higher concentrations in the calibration model. If the objective of the measurement is to compare data to a requirement, it may be optimal to weight a calibration curve near the corresponding requirement concentration. For the methods described in this report, each method describes whether weighting was applied to the calibration. An example of the application of weighting is described in Section 3.1.

Table 8. Equations and solutions for various calibration models.

Calibration Model	Fit Equation	Solution
Linear	$y = mx + b$	$x = \frac{y - b}{m}$
Quadratic	$y = ax^2 + bx + c$	$x = \frac{-b + \sqrt{b^2 - 4a(c - y)}}{2a}$
Power Law	$y = ax^b$	$x = \left(\frac{y}{a}\right)^{1/b}$

Once a regression has been performed to determine the coefficients for the calibration model, the "goodness-of-fit" for the model should be evaluated to judge if the model is a good representation of the detector response. One of the most commonly used criteria is the *coefficient of determination* (r^2), which is the square of the *correlation coefficient* (r, also known as the Pearson product moment correlation coefficient). A correlation coefficient of 1.0 indicates a positive correlation of the calibration levels. A coefficient of determination (r^2) less than 1.0 indicates a lack of correlation, or more specifically, the fraction of the total squared error that is explained by the model. Caution should be used with r^2 interpretation in that this value does not indicate if the correct calibration model was selected, nor does it indicate the accuracy of the model (it only represents correlation of the data to the model). In addition to the r^2 criteria, it is also recommended that the relative standard deviation (RSD) for each standard be less than 20% RSD, as discussed in Section 3.1.

2.5 QC Samples

Even with the acquisition of an acceptable calibration curve, there is the possibility that the instrument calibration could drift or change during the analysis of samples. The best way to prevent these issues is to keep the instrument well maintained; however, even the best-maintained instrument may drift during an analysis. To ensure confidence that accurate results are generated, QC samples should be analyzed during a run. The order in which the standards, analytical samples, and QC samples are run is referred to as the sample sequence (or sample queue). Most often, the instrument control software calls this order a *sequence*. Logic should be applied in the organization of the sample queue, because effects such as carryover, interferents, or instrument drift could invalidate large sets of data. The following guidance discusses each type of QC sample, what the sample indicates, how the sample is used, and finally how the QC samples should be integrated into the sample queue.

QC samples are used to ensure confidence in the reported analytical value. QC samples enable trend analysis, determination of bias in sample analysis, detection of instrument drift and carryover, and provide information on the error in the reported analytical value. QC samples include initial calibration verification (ICV), continuing calibration verification (CCV), and blank samples. If the QC samples do not meet performance criteria, this indicates a possible problem that must be corrected to ensure confidence in the analytical data.

ICVs are used to verify that a standard of known concentration is accurately determined by the instrument and calibration model. Although not required, an ICV should be a standard that was **not** used to generate the calibration curve. The accuracy of the ICV sample can be calculated using a relative percent deviation (RPD) as defined by Equation 3.

$$RPD_{ICV} = \frac{C_{Calc} - C_{known}}{\left(\frac{C_{Calc} + C_{known}}{2}\right)} \times 100\% \qquad \text{Equation 3}$$

where

C_{Calc} = concentration/mass calculated from the calibration model
C_{known} = known concentration/mass of sample

Acceptance criteria for the ICV should be established. Typical values would be in the range of ±15–30% RPD. An ICV with an RPD within the acceptance criteria indicates a successful calibration; failure to meet the ICV acceptance criteria indicates low confidence in the calibration curve, and thus, low confidence in the data produced from the calibration. ICV samples are typically run shortly after the standards in the queue, discussed later in this document.

The sensitivity of an analytical instrument ean change over time for various reasons including buildup of analyte, presence of interferents from samples, variations in room temperature, or the need to perform maintenanee, such as changing an injeetion port septum, eonditioning, or replaeing the eolumn. To confirm that the pcrformanee of the system does not ehange (i.e., the ealibration changed or drifted) during a sequenee, use of CCV samples is required. A CCV sample is often a rerun of a standard as a confirmation that the measured eoneentration has not ehanged, and thus, the instrument is performing consistently. This can be accomplished by caleulating the RPD of the caleulated concentration of the CCV sample (C_{Calc}) to the known eoneentration (C_{known}) as shown in Equation 4.

$$RPD_{CCV} = \frac{C_{Calc} - C_{known}}{\left(\frac{C_{Calc} + C_{known}}{2}\right)} \times 100\% \qquad \text{Equation 4}$$

Acceptance eriteria are established to determine if a CCV indieates an acceptanee or failure to hold calibration. Typically, a value of greater than ±30% RPD is used as failure criteria for a MSD. The variation in eoneentration/mass of CCV samples is a result of errors from both systematie and random sourees. The 30% performanee criterion is a eommonly aeeepted standard. If a failure is detected, eorrective actions must be taken to return the instrument to calibration (e.g., instrument maintenanee, rerun standards). Further samples aequired before and after a failed CCV are suspect and will need to be rerun, as discussed later.

It is recommended that more than one concentration be tested as a CCV. The selection of whieh eoneentrations to use is dependent on the objeetive of the experiment. Overall, the CCV eoneentration should correspond to the eoneentrations expeeted from the test. It is expeeted that most deeontamination tests will be attempting to meet a requirement; thus, ineluding a CCV near the requirement concentration and one at a mid- to high-end of ealibration is advisable. Other government organizations, sueh as the FDA, reeommend three values corresponding to approximately three times the LOQ, mid-range, and high-range eoneentrations for CCVs.[16]

The last type of QC sample diseussed here is the use of blanks. A blank sample is a sample eontaining only the extract solvent (or uncontaminated vapor tube). A blank should not elieit a deteetor response at the expeeted RT of the analyte of interest. Blanks are an effective means of evaluating baseline performanee and eonfirming there is no analyte earryover in the system. Analyte carryover is the detection of an analyte in one sample that was the result of the analysis of a previous sample. The result of analyte carryover is a positive bias in a sample, present mostly in low eoneentration/mass samples. Part of the method development discussed in this report includes efforts to minimize carryover such as determining the number and type of solvent rinses performed on the injeetion syringe between samples. When running a solvent blank, a deteetor response noted at the expeeted RT ean be indicative of earryover of high eoneentration/mass samples, interferences, a dirty system, or other problem that may affect the identifieation and quantitation of the analyte of interest. Blank samples are included in the sample queue at regular intervals to eheck for carryover, as discussed later. The reported eoneentration must be below the LOD for the analysis method in order for the blank or solvent-only sample to meet the acceptance criteria. This eriterion was established to ensure that any earryover would not appreeiably affeet the results of the sample analyzed next in the sequence.

The acquisition of a successful calibration model indicates an instrument that is ealibrated under ideal eonditions. As samples are analyzed over a period of time (analytical sequences can last from 1 to 36+ h and inelude 1–100+ samples), many things ean happen to the analytical system that could change the instrument sensitivity and performanee, as previously diseussed. For this reason, QC samples

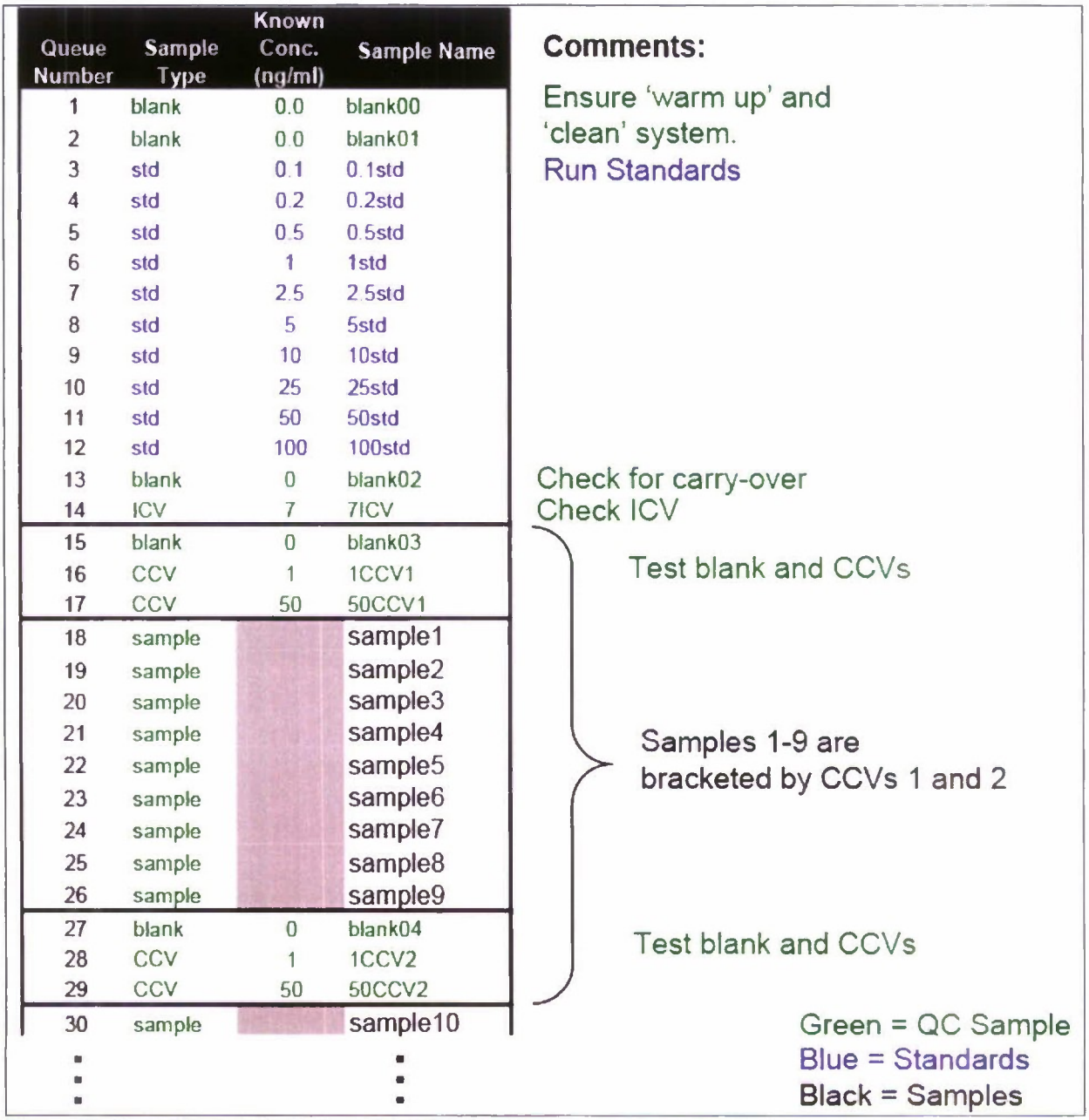
must occur at regular intervals during the sample queue to confirm that the analytical instrument is performing within specifications.

In addition to the QC samples, the order that the samples are run should be considered. In decontamination testing, some samples may have concentrations that are significantly different (e.g., the contact-test for a bare metal versus an elastomer). If there is any carryover in a system, a very high concentration sample followed by a low concentration sample could yield a false high concentration (i.e., positive bias) in the low concentration sample. For this reason, it is advisable to use the best judgment in the organization of samples to prevent this situation. In addition to the sample concentration, some samples are "cleaner" than others. For example, the contact test involves the extraction of a contact sampler that has been rinsed in acetone to remove impurities. This extraction is relatively clean in that the only compounds in the extract solution are likely to be the agent and possibly some agent reaction byproducts. In comparison, residual agent extractions are, in some cases, not "clean" in that the extraction process may have removed other compounds from the coupon (e.g., plasticizers extracted from elastomers like silicone). It is possible that some interferents extracted from the coupons may alter the performance of the analytical instrumentation (e.g., interferents coating the column), which could be detected by failing CCVs or positive blanks. To minimize rerunning large numbers of samples, it is advisable to put samples that are "dirty" or likely to contain impurities together at the end of a queue. If the interferents are significant enough, sample cleanup procedures (e.g., solid phase extraction) may be needed to accurately analyze the samples and prevent damaging the analytical instrumentation (e.g., destroying the column). In summary, running the "cleanest" samples first and ordering the samples from anticipated lowest to highest concentration are the best practices.

In most cases, a queue is initiated with a series of blanks, followed by the standards, another blank, an ICV, a CCV block then samples, with iterations of CCV blocks and samples until the queue is complete, terminating with a CCV block. Figure 18 provides a demonstrative sample queue. The first blank ensures that the system is warmed up and flushed out, while the second blank can be used to verify the system is clean and there is no carryover from the previous runs. The standards are then run from lowest to highest concentration (minimizing the effect of any potential carryover). The blank run after the highest standard is primarily used to detect any carryover in the system. Because this blank follows the highest concentration anticipated to be run in this sequence, if carryover is occurring, it will be reflected in this sample. The ICV sample is run after the blanks and standard to confirm that an acceptable calibration was acquired.

After the ICV, the following sample begins a CCV block. In this example a CCV block consists of a blank, a low-level CCV (1 ng/mL), and a mid-level CCV (50 ng/mL). After a CCV block, a set of samples is run, followed by another CCV block. The CCVs surrounding the samples are said to bracket the samples. For a set of samples to be accepted, both bracketing CCV blocks must pass. In the event of a QC sample failing to meet an acceptance criteria (e.g., blank detecting carryover or CCV greater than 30% RPD), the samples preceding and following the CCV block would be suspect and should be rerun. It is not possible to identify when the instrument was failing to meet specifications, thus all samples between the last passing QC sample and the failing sample should be rerun. In addition to this guidance, other documents, such as Chapter 9 of the U.S. Army Engineering Manual EM 200-1-10, can be used to accept or reject data.[17]

The frequency of CCV blocks is a balance of the instrument run time and confidence in instrument performance. For example, given the frequency of nine samples per CCV block, the failure of one CCV block would dictate that 18 samples are rerun (the samples before and after the failing CCV block). Selecting a large number of samples between CCV blocks results in a shorter total queue run-time; however, a CCV block failure results in a significant number of samples to rerun. Conversely, running a CCV block between every sample is also not practical. Typical intervals for CCV blocks are 5–20 samples. If particular samples are expected to cause instrumentation problems or extra confidence is desired, CCV blocks can be run more often.



Queue Number	Sample Type	Known Conc. (ng/ml)	Sample Name
1	blank	0.0	blank00
2	blank	0.0	blank01
3	std	0.1	0.1std
4	std	0.2	0.2std
5	std	0.5	0.5std
6	std	1	1std
7	std	2.5	2.5std
8	std	5	5std
9	std	10	10std
10	std	25	25std
11	std	50	50std
12	std	100	100std
13	blank	0	blank02
14	ICV	7	7ICV
15	blank	0	blank03
16	CCV	1	1CCV1
17	CCV	50	50CCV1
18	sample		sample1
19	sample		sample2
20	sample		sample3
21	sample		sample4
22	sample		sample5
23	sample		sample6
24	sample		sample7
25	sample		sample8
26	sample		sample9
27	blank	0	blank04
28	CCV	1	1CCV2
29	CCV	50	50CCV2
30	sample		sample10
⋮			⋮

Figure 18. Example analytical queue with QC samples.

2.6 Solid Sorbent Tube Procedures

2.6.1 Solid Sorbent Sampling Overview

The collection of a vapor sample requires sampling air. When collecting air samples to determine the concentration of an analyte, there must be a means to selectively trap and concentrate the analyte. This process must be able to collect a representative sample containing analytes that, upon analysis, can be identified and quantified with confidence. Solid sorbent sampling allows the selective collection of analytes from a vapor stream in a reproducible manner resulting in the confident identification and quantitation of the collected sample.

Solid sorbent sampling is based upon a sorbent material having an affinity for a particular analyte of interest. This affinity is rooted in physical and chemical properties such as polarity of the analyte and the sorbent bed. For example, a polar sorbent material will have a higher affinity for a polar analyte than a non-polar analyte, due to the electrical interactions that the two like materials will have with one another. There are many benefits of solid sorbent sampling as a means of vapor sample collection.

- The first benefit is that the sorbent material is packed into a sorbent tube allowing air to pass across the material in which the analytes are trapped. In this configuration, sample collection parameters such as airflow rate and sampling time can be adjusted to allow sampling of a particular amount of air. Knowing the volume of air that was sampled, along with a found amount determined upon analysis, will allow the quantitation of airborne concentration of the analyte. Control of these parameters will also allow specific expected masses to be collected when a target airborne concentration is known.

- Another benefit of solid sorbent sampling is the retention of compounds adsorbed on the sorbent material without chemical reaction of the sorbent with the analyte. It is imperative that the sample collected remain representative of the vapor stream sampled. Additionally, it is crucial that the collected compounds are able to be removed, or desorbed, from the sorbent material. This is often accomplished with *thermal desorption*, heat directly applied to the sorbent tube and flow of inert compressed gas through the tube. Note that ambient air may be used in lieu of inert gas, but this can cause increased chromatographic noise upon analysis due to oxidation of the sorbent material.

- One final benefit is that the sorbent material, upon desorption and subsequent cooling, is re-usable—once desorbed, the tube is ready to be conditioned and placed back into circulation for future sampling. Ultimately, sorbent material will wear out after repeated sampling and desorption cycles. In this case, the sorbent tube may be repacked with fresh sorbent material.

This Low-Level program utilizes a custom-made vapor manifold to collect samples on Markes TDS tubes treated with SilcoSteel coating to reduce analyte adsorption on the tube surface (this effect can be significant, especially for low-level detection of analyte). Thermal desorption of these tubes is performed on a Markes TDS, in accordance with manufacturer recommendations. The typical life cycle of a tube entails manufacture, receipt and initial conditioning, spiking or sampling, desorption/analysis, tube conditioning, and conditioning verification of the tubes. Once a tube has been verified as clean, the life cycle repeats at the tube spiking or sampling step and continues until the sorbent material is no longer effective for sampling, at which time the tube will be taken out of circulation. The tube may be repacked with fresh sorbent and reused.

Typical solid sorbents for chemical agent vapor sample collection include Tenax-TA and Chromosorb 106. Tenax-TA was used for initial chemical agent method development because it is an excellent all around sorbent material with an affinity for all the chemical agents used for this program. Tenax-TA is a 2, 6-diphenyl-p-phenylene oxide porous polymer. Specific information regarding Tenax-TA is available in the MSDS. Additionally, Chromosorb 106 was used for VX vapor-sampling methods after the initial method development with Tenax TA. Follow-on method development with Chromosorb 106 was performed based on the breakthrough potential of the VX sampling molecule, G-analog, through Tenax TA. Chromosorb 106 is a polyaromatic porous polymer and was chosen based on its use as a sorbent material for VX vapor sampling in many programs, including the CMA Chemical Demilitarization Program. Specific information regarding Chromosorb 106 is available in the MSDS.

2.6.2 New Tube Receipt

Upon delivery of sorbent tubes, an inventory and visual inspection are performed to ensure that the tubes ordered are accounted for, all tubes have no obvious defects, no sorbent is leaking, the correct type of sorbent is used, and that the tubes have SilcoSteel coating. After inspection, a two-step initial conditioning process is performed using the Markes TC-20 in accordance with the manufacturer recommendations for the sorbent material. For example, Tenax-TA tubes are heated to 320 °C for 120 min with high purity nitrogen (N_2) at a flow rate of 60 mL/min using 20 psi. A second conditioning step is performed at 335 °C for 60 min. This initial conditioning is performed to remove any contaminants from the sorbent material that may be present from the tube manufacturing and shipment process. The initial conditioning also helps to establish a baseline low enough for collection and detection of small amounts of agent at the ORD levels.

2.6.3 Tube Spiking

Tube spiking is the process of making analytical calibration samples for vapor analysis. Tube spiking is accomplished by using a microliter (µL) syringe to introduce a specified volume of liquid chemical agent standard, prepared in high purity solvent, to the sorbent material contained within a sorbent tube. All tube spiking was performed on conditioned and verified-clean tubes. The tube had a flow rate to pull the standard onto the sorbent bed and to aspirate away the solvent in which the standard was delivered. Tube spiking and sample collections were always performed on the same end of the tube. Sample analysis desorbed the sample from the same end used for sample collection or spiking.

The spiking for this program was performed on a custom-made spiking manifold. The spiking manifold consisted of seven independently controlled vacuum ports. Each port had a metering valve controlling the flow rate through the tube, and a fitting to hold the tube in a vertical position. The entire manifold had a vacuum supplied by a carbon vane type vacuum pump. Flow rate (mL/min) for each tube to be spiked was adjusted to the method-specific parameter for the given analyte. Note that the flow rate was not a critical parameter to determine expected mass during the spiking process. However, if the flow rate was too high or too low, the expected mass could be affected. Tubes were typically spiked with a 5 µL volume of standard, using a 10 µL syringe. The spiked tube was then allowed to remain on the manifold, under vacuum, to aspirate for 3 min (method specific). As observed with vacuum flow rates, aspiration times affected the expected mass of analyte introduced. Shortly after aspiration, the tubes were capped with DiffLok caps. DiffLok caps are specific to Markes tubes and are manufactured to help prevent tube contamination when exposed to ambient conditions.

2.6.4 Tube Analysis

A vapor tube to be analyzed will undergo thermal desorption to release the analyte from the sorbent material. The Markes TDS had multiple pathways that were directed by multiple solenoid valves, controlled by the software. The sample tube was loaded into the autosampler, lifted into an oven, flow and heat were applied, and the sample was desorbed and directed to the cold trap. The cold trap was used to focus the sample by adsorbing the sample onto another sorbent tube. The focusing step allowed analyte, which may have migrated through the sorbent bed of the collection tube during sample collection, to be desorbed and re-adsorbed onto another low-temperature sorbent, which focused the sample into a tight band. Upon desorption of the cold trap, the focused sample was introduced onto the head of the analytical column all at once. GCV method-specific parameters are provided in Appendix A. The Markes TDS operator manuals show detailed flow pathway diagrams.

2.6.5 Tube Conditioning

Per the manufacturer, solid sorbent tubes are typically reusable for about 100 heating cycles. Because they are reused, one challenge was to ensure that reused tubes are clean before being placed back into circulation. Commercial hardware is available to condition and clean tubes between uses. Tube conditioners typically heat the tubes to a specified temperature (usually above the desorption temperature for most analytes but below the breakdown temperature of the sorbent) and nitrogen or air is purged through the tube to desorb any residual analytes. A Markes TC-20 was used to condition the tubes prior to reuse. The TC-20 was capable of conditioning 20 tubes at one time. If the sorbent material retained any agent from the previous sampling/desorption cycle, a positive bias in the results of the next sample would be induced. The retention of analyte on a tube after analysis is called *carryover*. To prevent carryover from interfering with the next sample/analysis cycle, the tubes were conditioned, or cleaned. To verify that the tubes had been cleaned, a representative sampling of the total number of conditioned tubes was re-analyzed and verified as blank.

2.6.6 Chromatographic Confirmation of Conditioning

To ensure that the sorbent tubes were clean enough to be used to generate instrument calibration curves or for sample collection, the tubes were checked after going through the TC-20 cleaning program. Checking each sorbent tube individually would be labor intensive and adversely affect test-sample analysis throughput. The American National Standards Institute Z1.4, Sampling Procedures and Tables for Inspection by Attributes,[18] provides sampling plans that can provide a high level of confidence that batches of sorbent tubes are clean and ready for use, without requiring the checking of each individual tube. The sampling plans in the standard were designed so that the more "defects or items failing the acceptance criteria" a batch or lot contains, the more likely that it will be rejected. In this case, if a batch of sorbent tubes fails to meet the acceptance criteria, the batch of sorbent tubes will undergo a second cleaning in the TC-20.

To use the ANSI Z1.4 standard, a "batch or lot" size is defined, an inspection level is chosen, and an Acceptable Quality Level (AQL) is selected. An AQL is the percentage of defective/failing items in a lot that the user is willing to tolerate most of the time. Since a single TC-20 will condition up to 20 sorbent tubes, the batch size was set at 20. The ANSI Z1.4 standard has three general inspection levels designated I, II, and III. General inspection level II is the inspection level recommended for use by the standard. General inspection levels I and III are used when less or more tolerance is necessary. The combination of a batch size of 20 and a general inspection level of II recommends sampling plans found in Table C of the standard. After reviewing Table C for individual plans, an AQL of 2.5 was selected for a single-sampling plan (meaning that the batch would be sampled just once). Selecting an AQL of 2.5 means that for most of the batches of sorbent tubes accepted as clean, at most only 2.5% (or 0.5 sorbent tubes) of the sorbent tubes in the batch could fail the acceptance criteria. This sampling plan required five sorbent tubes to be sampled at random for analysis once the TC-20 cleaning procedure was complete. According to the accept/reject numbers in the plan, if one tube failed the acceptance criteria, the batch of sorbent tubes failed and would be cleaned and inspected again. If all five sorbent tubes meet the acceptance criteria, then the batch is ready for reuse. An example tube analysis is shown in Figure 19.

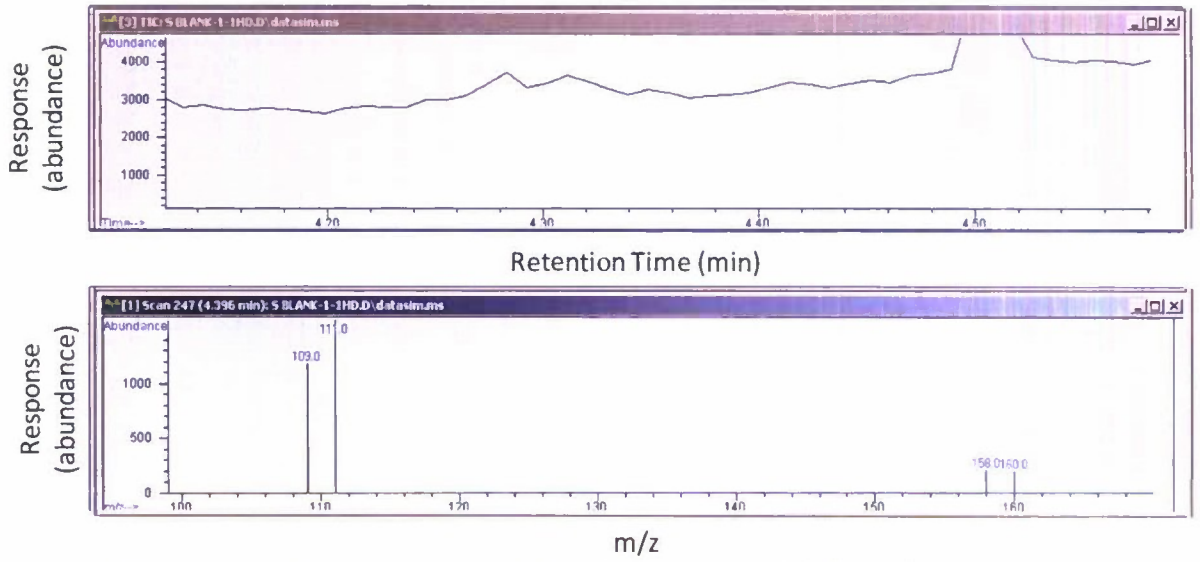


Figure 19. GC/MS TIC and mass spectrum of a sorbent tube chromatographically verifying cleanliness post-conditioning for HD using method GCV HDLL-DEANS.M.

2.7 Test Materials

Decontaminant panel testing evaluated the ability of a decontaminant to reduce the hazard on a surface of interest. Test materials typically included materials representative of decorative, structural, and functional materials common to building construction, sensitive equipment, and vehicles. The test materials evaluated encompassed a variety of material properties, compositions, and porosities. Small panels (coupons) were cut from parent stock material. The test coupons were 2 in. circular disks with a surface area of 3.14 in^2 (20.27 cm^2, 0.002027 m^2). All coupons were inspected for obvious defects and only acceptable coupons were used. All coupons in inventory were tracked using the Decontamination Sciences coupon inventory system. The inventory tracked the lot, date of preparation, original stock material information, and coupon preparation method. Sufficient coupons were acquired for testing to prevent lot changes during testing. Coupons of each lot were maintained for lot-to-lot comparisons, if needed for future work. Uniformity was ensured by obtaining a large enough quantity of material that multiple test samples could be obtained with uniform characteristics (e.g., test coupons were all cut from the interior rather than the edge of a large piece of material). All coupons were stored in zip-tight bags in containers to prevent/limit contact with foreign substances until the coupons were needed for testing. The test materials used to evaluate the methods included: green CARC on aluminum 7075 coupons (lot: 07-AC-O01); aluminum 7075 (lot: 07-AL-L01); borosilicate glass (lot: 07-GL-B01); silicone MQ/VNQ/PMQ/PVMQ (lot: 07-SI-A01); Viton FKM (lot: 07-VI-A01); and stainless steel 316L (lot: 07-NS-D01).

The contact test samples evaluated included solvent extraction of contact sampler and several coupon materials. The contact sampler was medical-grade dental dam latex, product number 1013751, lot number 280C1, manufactured by Henry Schein, Inc.

2.8 Method Evaluation and Data Quality

Data quality refers to the accuracy, precision, and confidence in the data produced by a laboratory. Data are of high quality "if they are fit for their intended uses in operations, decision making, and planning. Method evaluation is used to ensure that the data produced are "fit for use."

As methods were developed, the error or uncertainties in the measurements were calculated to determine the accuracy of the data being produced. The method validation tests determine the robustness, ruggedness, and suitability of the method. Validation tests were used to isolate any improvements made to the method prior to routine use.

The data were reported as accurately and precisely as possible. The analysts were trained, and their accuracy and proficiency verified before they performed testing or sample analysis operations in the laboratory.

The data validation process ensured the completeness and consistency that made the data appropriate for a specific use. The data were produced and collected by the analysts onto data collection forms and laboratory notebooks. Laboratory audits of the data and laboratory forms were a part of this process. Laboratory audits were also used to identify opportunities for improvement.

Tracking laboratory performance allowed the analytical methods and other laboratory operations to be kept under control by tracking the QC measures used to monitor operations.

2.8.1 Method Validation

Method validation is the formal and systematic evaluation of an analytical method. The objective of method validation was to demonstrate that the procedure, when used correctly, produced results that were fit for their intended use. Method validation examined the linearity, range, detection limits, specificity, precision, and accuracy of an analytical method to ensure that the results produced by the analytical method were fit for their intended use.

- *Linearity* is the ability of the method to obtain test results that are directly, or by well-defined mathematical transformation, proportional to analyte concentration within a defined range.

- *Range* is the interval between the upper and lower levels of analyte (inclusive) that have been confirmed with precision, accuracy, and linearity, using the method as written.

- *Accuracy* is the measurement of the exactness of an analytical method; the closeness in agreement between the conventional true value (or an accepted reference value) and the value found.

- *Precision* is the measurement of agreement among individual test results—when an analytical method is used repeatedly for multiple samplings of a homogeneous sample—and the method operating during a short time interval under the same conditions.

- *Specificity* ensures the identity of the analyte of interest.

- The *LOD* is the lowest concentration of an analyte in a sample that can be detected, but not quantitated.

- The *LOQ* is the lowest concentration of an analyte in a sample that can be determined (quantitated) with acceptable precision and accuracy.

Validated methods provided assurance that a measurement was reliable. Every method developed within this program was validated.

2.8.2 LOD and LOQ

LOD: The LOD is defined as the lowest concentration of an analyte in a sample that can be detected, but not quantified.

There are multiple ways to determine the LOD of an analytical method. It can be determined from a signal-to-noise ratio (S/N), usually 3:1 or it can be calculated at levels approximating the LOD, according to one of the following formulas:

- LOD = 3 × SD, where SD is the standard deviation of the reported concentration of replicate samples at the estimated LOD concentration. The estimated LOD value is the lowest standard concentration needed to satisfy the detection requirement.[19]

- LOD = 3 (SD ÷ S), where S is the slope of the calibration curve and SD is the standard deviation of the response, based upon the standard deviation of blank samples, residual standard deviation of multiple regression lines, or standard deviation of the y-intercepts of the regression lines.[20]

The use of the calibration curve to determine the LOD requires the calibration curve to fit a linear model. Not all of the analytical methods discussed in this report fit a linear calibration model. Therefore, the LOD reported in the methods was calculated using LOD = 3 × SD, where SD is the standard deviation of replicate samples at the estimated LOD concentration. The LOD was calculated for each method and is reported in the appropriate method section. Figure 20 shows an example of calculating the LOD.

	Replicate Sample Analysis Results
	0.32
	0.78
	0.45
	0.21
	0.5
	0.65
	0.47
	0.21
Standard Deviation	0.20
LOD	0.60

Figure 20. LOD calculation example for sample concentration 0.5 ng/mL.

LOQ: The LOQ is defined as the lowest concentration of an analyte in a sample that can be determined (quantitated) with acceptable precision and accuracy under the stated operational conditions of the method.

The LOQ is reported as the concentration of the analyte with the precision and accuracy of the measurement and can be determined multiple ways. The LOQ can be determined as a signal-to-noise ratio (S/N), usually 10:1 or it can be calculated at levels approximating the LOQ according to one of the following formulas:

- LOQ = 10 × SD, where SD is the standard deviation of the reported concentration of replicate analysis of samples at the estimated LOD concentration. The preliminary estimated LOD is the lowest calibration standard needed to satisfy the method requirement for detection.[19]

- LOQ = 10 (SD ÷ S), where S is the slope of the calibration curve and SD is based upon the standard deviation of the blank, the residual standard deviation of the regression lines, or the standard deviation of the y-intercepts of the regression lines.[20]

The use of the calibration curve to determine the LOD requires the calibration curve to fit a linear model. Since not all of the analytical methods discussed in this report fit a linear calibration model, and a uniform approach to calculating this value for all methods was needed, the LOQ reported in the methods was calculated using LOQ = 10 × SD, where SD is the standard deviation of replicate samples at the estimated LOD concentration. The LOQ was calculated for each method and is reported in the respective method section of this report. Figure 21 shows an example of calculating the LOQ.

	Replicate Sample Analysis Results
	0.32
	0.78
	0.45
	0.21
	0.5
	0.65
	0.47
	0.21
Standard Deviation	0.20
LOQ	2.01

Figure 21. LOQ calculation example for sample concentration 0.5 ng/mL.

2.8.3 Uncertainty of Measurement

All measurements are subject to error. Some errors are due to random effects, such as short-term variations in temperature or relative humidity, and variations in the performance of the measurer. Other errors are due to systematic effects such as bias and drifts in instrument calibration, which can be corrected. The uncertainty takes into account these random and systematic effects and is a quantitative indication of the quality of the result, allowing assessment of its reliability. Often, a result is compared with a limiting value, defined in a specification or regulation. Knowing the uncertainty of the measured result shows whether the result is well within the acceptable limits or just barely makes it.

Measurement uncertainty provides a realistic comparison of results from different laboratories, within a laboratory, or with reference values given in specifications or standards. Measurement uncertainty provides a means to establish that the test procedure, including metrological characteristics of the equipment used, will allow valid measurements and results to be obtained. Measurement uncertainty provides the ability to identify the effects that contribute the most error, in order to improve methods and procedures. The uncertainty in measurement was calculated for each method and is reported in the appropriate method sections. The explanation of the uncertainty calculations is available in the GUM reports.

3. METHOD UTILIZATION

3.1 Calibration Curve Fitting

One of the many important factors in quantitative analysis is the accuracy of a calibration curve. The accuracy of the reported result is dependent on the accuracy of all procedures used in the preparation of a calibration curve from making the standards to the regression of the detector response to generate a calibration curve. Verification that an accurate calibration curve has been acquired requires a statistical analysis of the results. A single universal indicator/value for detecting the right curve or a good calibration has been an elusive goal—the evaluation of a calibration model requires several types of analysis to confirm an acceptable calibration. While the evaluation of a correlation coefficient (r) or coefficient of determination (r^2) is a common method to evaluate a calibration curve, it is not a full description of the system. The coefficient of determination (r^2) indicates a correlation between the data and the calibration and does not indicate accuracy or lack of fit. To demonstrate how r^2 can be misleading and provide guidance on how to evaluate a calibration model, a demonstration using VX on a LC/MS/MS system is illustrated. The following demonstration illustrates the impact of weighting. The same principles apply to the selection of the calibration model (i.e., which equation to use for the calibration curve).

Figure 22 shows the data collected from an LC/MS/MS for a set of VX standards. Using Matlab, a linear regression was applied to the data and calibration coefficients were determined. The r^2 value for this fit was 0.9993, indicating excellent correlation. There are several misleading aspects to Figure 22. The very high r^2 value implies a good fit and that the calibration curve is acceptable for use. Visual inspection of the curve indicates that the line goes through all of the data points, also implying a good fit. However, the dynamic range of the detector response and concentration each cover three orders of magnitude. As a result of the large dynamic range and the use of a linear graph scale, the lowest four standards represent 40% of the data and are graphed on only 0.01% of the graph area. As a result, the low concentration standards cannot be visually resolved for inspection.

VX Conc. (ng/mL)	Detector Response (counts)
0.101	6,320
0.203	11,900
0.496	26,800
0.993	52,300
2.480	125,000
5.060	254,000
10.125	549,000
25.313	1,250,000
49.630	2,600,000
99.267	4,920,000

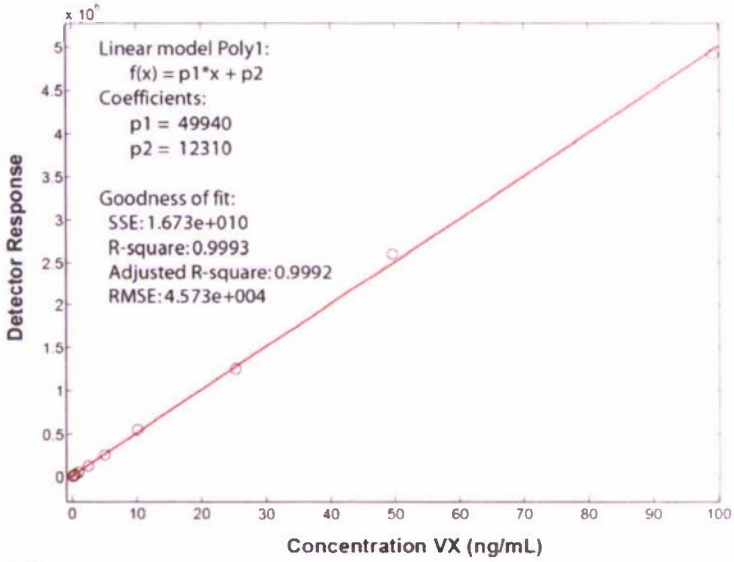


Figure 22. VX calibration curve with linear plot.

To compensate for the compression of the x and y axes when using a linear scale, the same data should be plotted on a log-log scale, as seen in Figure 23. From this graph it is immediately apparent that the calibration model does not pass through the low concentration standards. The deviation

of the calibration model from the standards would impart a substantial bias to the results. The r^2 value for this fit was 0.9993, even with a poor fit at low concentrations. The compression of the linear scale graph did not enable this deviation to be readily observed.

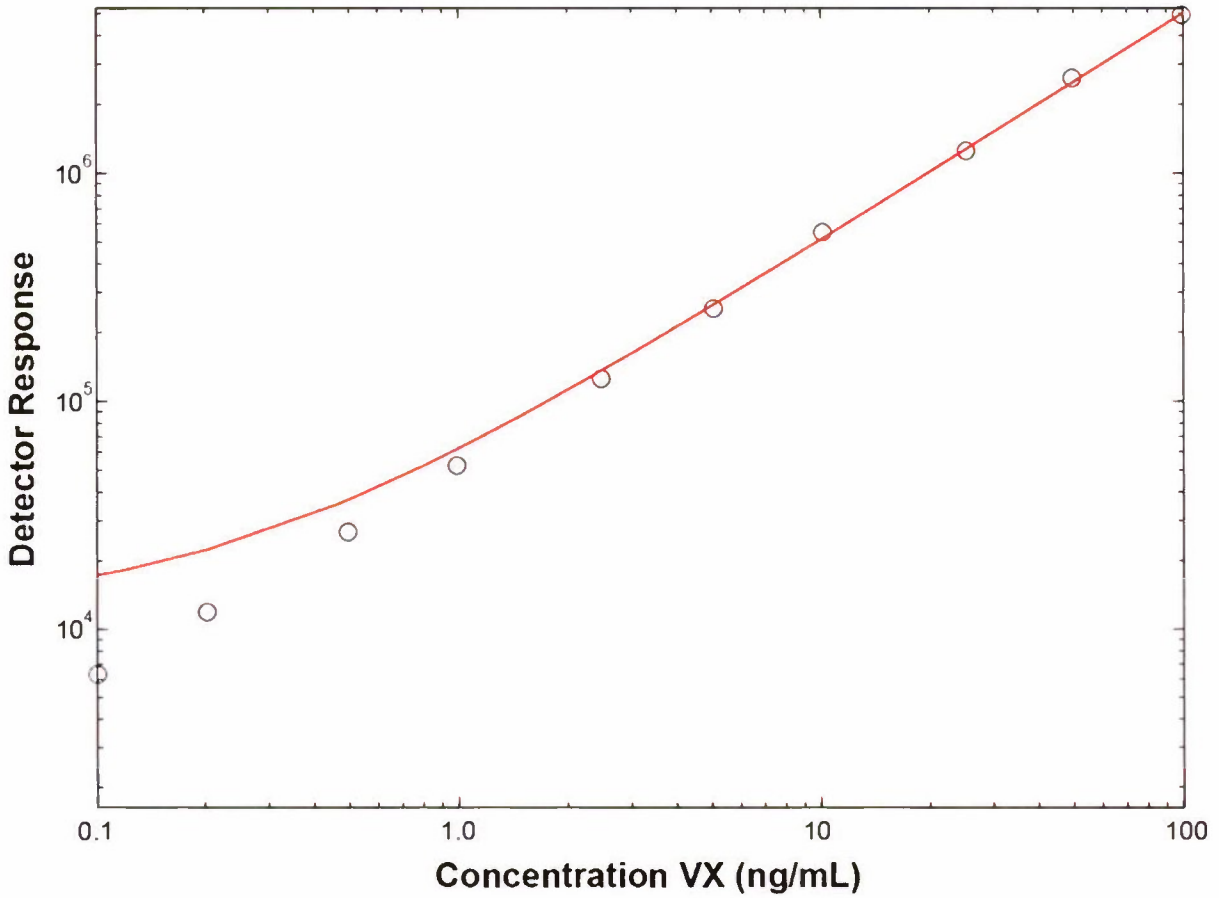


Figure 23. Log-log scale VX calibration model.

This leads to the question of how much error is present in the calibration. There are several methods that can be applied to assess the error in the calibration model, including residual analysis, which is presented in the goodness-of-fit parameters in Figure 22 as the sum of the square of the errors (SSE) and root mean square error (RMSE). The SSE and RMSE values are calculated from the difference in the detector response to the calibration model response (y value) at the tested concentrations. For both SSE and RMSE, a smaller value represents a better fit. However, these values are not normalized and a good fit for one instrument could produce an SSE value orders of magnitude different from another instrument. RMSE and SSE are excellent indicators for comparison of different models for the same data, where the smaller value indicates the better fit.

Another method to analyze error in the calibration model is to calculate the concentration (x) of a standard, based on its detector response (y) from the calibration model, and compare it to the known concentration. For example, applying the calibration model to the response (549,000) of the 10.125 ng/mL results in a concentration of 10.747 ng/mL as shown in Equation 5.

$$x = \frac{y-b}{m} = \frac{549000-12310}{49940} = 10.747 \text{ ng/mL} \qquad \text{Equation 5}$$

The error of the calibration model to the known concentration (C_{known}) can be calculated using two methods, RPD, and recovery. The RPD can be calculated as shown in Equation 6.

$$RPD = \frac{C_{model} - C_{known}}{\left(\frac{C_{model} + C_{known}}{2}\right)} \times 100\% = \frac{10.747 - 10.125}{\left(\frac{10.747 + 10.125}{2}\right)} \times 100\% = 5.96\% \qquad \text{Equation 6}$$

An RPD closer to zero represents a better fit. Because this form of RPD does not use an absolute value in the numerator, the value indicates a negative or positive bias to the value. The above value indicates a slight (5.96%) positive bias (i.e., the model returns a higher concentration than the "known" value). The second method uses the concept of recovery[21] as defined by Equation 7.

$$\text{recovery} = \frac{C_{model}}{C_{Known}} \times 100\% = \frac{10.747}{10.125} \times 100\% = 106.1\% \qquad \text{Equation 7}$$

A recovery closer to 100% indicates a better fit. Similar to the RPD, the recovery value of 106.2% indicates that the model introduces a slight positive bias to the data. Both the recovery and RPD methods supply a normalized result where common acceptance criteria can be established. Standard good analytical practices use CCVs to ensure that instrument calibration is maintained throughout the run sequence; pass/fail criteria for the CCVs on a MS is usually ±30% RPD. Because RPD is already being calculated for the CCVs, it is recommended here that RPD be used to characterize the error in the calibration model. The recommended acceptance criteria for a calibration curve should be equal to or more stringent than the criteria for CCVs. Other agencies, such as the FDA, have suggested that all standards above the LOQ should have a RPD less than ±15%, and that standards at the LOQ should have an RPD less than ±20% RPD.[21]

To continue the example of the VX calibration curve, Table 9 illustrates the concentration calculated from the calibration model and the RPD for each standard. The RPD values for the lower concentrations clearly indicate a poor fit, in addition to the calibration model returning negative values at lower concentrations. If this calibration model had been accepted for use based on the r^2 value alone, low concentration samples could have underestimated the real hazard by a factor of two or more (assuming negative values were rejected). Ideally, if the appropriate calibration model was selected, the RPD values should be equally (and randomly) distributed about zero. A trend or curvature in a plot of concentration vs. RPD indicates that a different calibration model or weighting should be used.

Table 9. RPD values for a linear regression with VX standards on LCE.

VX Concentration (ng/mL)	Detector Response (counts)	Calc. Conc. (ng/mL)	RPD (%)
0.101	6320	-0.120	-2332.61%
0.203	11,900	-0.008	-216.86%
0.496	26,800	0.290	-52.37%
0.993	52,300	0.801	-21.43%
2.480	125,000	2.257	-9.44%
5.060	254,000	4.840	-4.45%
10.125	549,000	10.747	5.96%
25.313	1,250,000	24.784	-2.11%
49.630	2,600,000	51.816	4.31%
99.267	4,920,000	98.272	-1.01%

The RPD analysis indicated that a direct linear regression on the data does not produce an acceptable calibration model. The next issue was how to identify the appropriate calibration model. In this case, the reason for the poor fit in the direct linear regression was related to the heteroscedasticity of the data.[22] Heteroscedastic data are characterized by a system where the absolute error (e.g., standard deviation) of a response varies with the abscissa (e.g., concentration). For heteroscedastic data, the standard deviation of the detector response for multiple analyses of a low-level standard is smaller than that for a high-level standard. However, the RSD, the standard deviation divided by the mean, for heteroscedastic data is typically consistent across concentrations. This is typical for most chromatographic detectors. If the data are homoscedastic, the standard deviations of the responses are independent of concentration.

Weighting of a calibration curve is appropriate if the data are heteroscedastic because the higher concentration standards are dominating the regression analysis. By applying a weighting to lower concentration standards, such as a 1/concentration weighting, the contributions of the low concentration standards can be balanced with the higher concentration standards. Table 10 shows the impact of weighting on the calculated concentration and RPD for four weightings. The $1/x^n$ notation corresponds to $1/\text{concentration}^n$ for this data, n=0 corresponds to no weighting, n=0.5 corresponds to 1/square root(x). An additional term is introduced in the goodness-of-fit parameters, which is the sum of the absolute value of the RPD for all standards. Much like SSE, this value is not normalized, and a smaller number indicates a better fit. The sum of the absolute value of the RPD is a gauge for the performance of the fit across all data points. It is not optimal to select a calibration model based on the RPD for a particular standard, but rather the model that best represents all standards. For example, if the weighting was selected by only the lowest level standard (0.1 ng/mL), the $1/x^2$ weighting provides the lower RPD. However, for the 0.2 ng/mL standard, the 1/x weighting provides smaller RPD. It is better to look at the RPD of the system, which is best represented by the sum of the absolute value of the RPDs. Note that the r^2 value changes by only 0.04% from the $1/x^2$ weighting to $1/x^0$ weighting, yet the accuracy of the calibration model is significantly different. (The more accurate fits have the lower r^2 values.)

Based on the results in Table 10 and Figure 24, the 1/x and $1/x^2$ weighted regressions provided the best fit. At this point, the weighting that provided the smallest sum of absolute value of RPDs was recommended[22]; in this case the $1/x^2$ weighting was chosen. Caution should be used when applying tests such as an F-test (or a Mandel Fitting Test) because these tests assume equal weighting for all data points and will likely indicate the unweighted model as the better fit.

Overall, the calibration should be reported by providing the known concentrations, the detector responses, and the RPD for each standard, in addition to the calibration model, weighting, r^2, and coefficients. Acceptance of a calibration curve, based on r^2 alone, does not imply accuracy. The RPD for every standard should meet an acceptance criterion (e.g., RPD less than 15%). Providing all of this information demonstrates the accuracy of the calibration and enables reprocessing of the data, if an inappropriate model was selected.

Table 10. Regression of the VX calibration using a linear calibration model with different weighting.

VX Known Conc. (ng/mL)	Detector Response (counts)	$1/x^0$ (none)	$1/x^{0.5}$	$1/x^1$	$1/x^2$
		Calc. Conc. (ng/mL)/ [RPD %]	Calc. Conc. (ng/mL)/ [RPD %]	Calc. Conc. (ng/mL)/ [RPD %]	Calc. Conc. (ng/mL)/ [RPD %]
0.101	6320	-0.120 / [2332]	0.065 / [-43.70]	0.094 / [-6.95]	0.099 / [-1.54]
0.203	11,900	-0.008 / [-217]	0.176 / [-14.35]	0.205 / [0.84]	0.209 / [2.74]
0.496	26,800	0.290 / [-52.37]	0.472 / [-4.89]	0.500 / [0.76]	0.500 / [0.85]
0.993	52,300	0.801 / [-21.43]	0.980 / [-1.34]	1.005 / [1.17]	0.999 / [0.63]
2.480	125,000	2.26 / [-9.44]	2.43 / [-2.18]	2.44 / [-1.45]	2.42 / [-2.37]
5.060	254,000	4.84 / [-4.45]	4.99 / [-1.32]	5.00 / [-1.22]	4.95 / [-2.27]
10.125	549,000	10.75 / [5.96]	10.86 / [7.05]	10.84 / [6.82]	10.72 / [5.70]
25.313	1,250,000	24.78 / [-2.11]	24.82 / [-1.99]	24.72 / [-2.36]	24.44 / [-3.52]
49.630	2,600,000	51.82 / [4.31]	51.68 / [4.05]	51.45 / [3.61]	50.86 / [2.44]
99.267	4,920,000	98.3 / [-1.01]	97.85 / [-1.44]	97.40 / [-1.90]	96.26 / [-3.08]
Regression	**Slope** $\left(\frac{counts}{ng/mL}\right)$	49,940	50,250	50,500	51,100
	Intercept (counts)	12,310	3065	1562	1238
	Weight	$1/x^0$ (none)	$1/x^{0.5}$	$1/x^1$	$1/x^2$
Goodness-of-fit	r^2 (unitless)	0.99928	0.99927	0.9991	0.9989
	SSE (counts)	1.67E+10	2.60E+09	4.30E+08	2.18E+07
	RMSE (counts)	45,727	18,031	7331	1650
	$\sum\lvert RPD\rvert$ (%)	2650	82.29	27.09	25.15

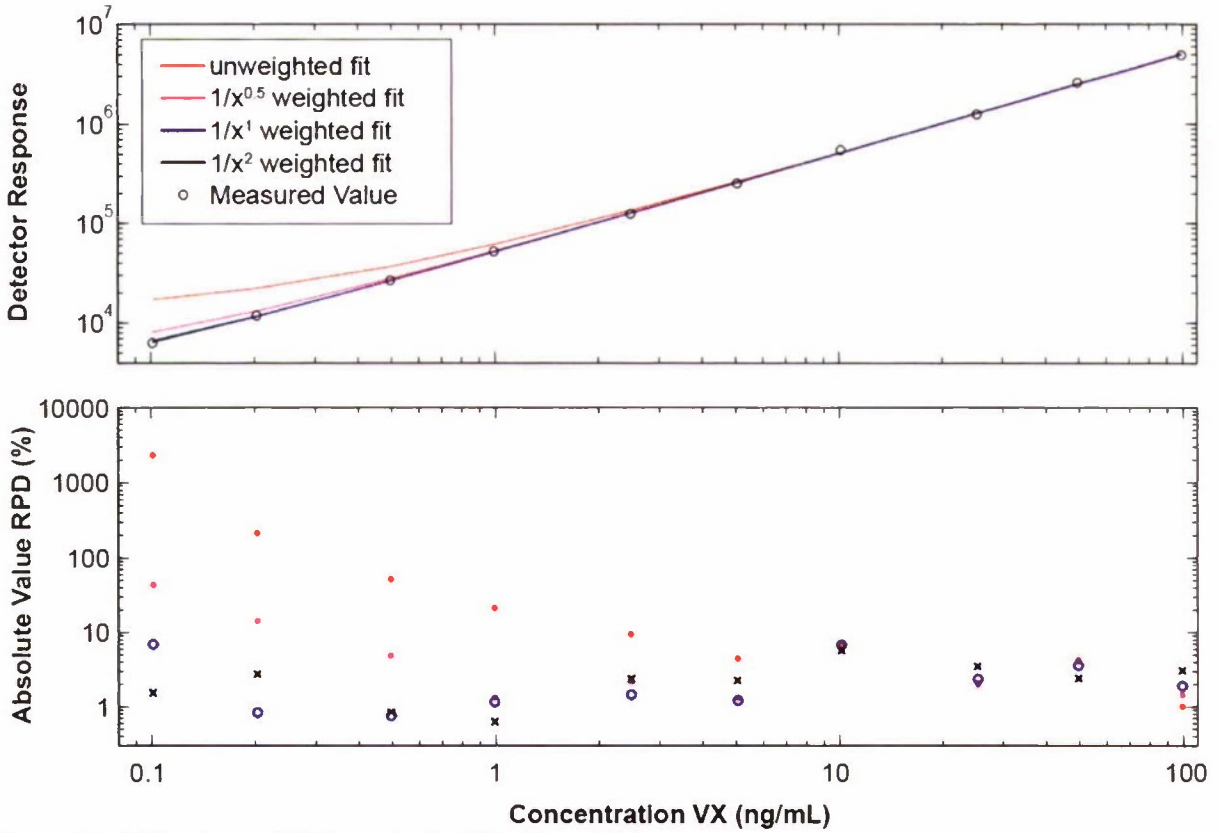


Figure 24. Calibration and RPD graphs for VX with weighting.

3.2 Sample Queues

The software for each analytical instrument has the ability to analyze samples individually or in sequences. Analyzing samples individually requires an operator to input sample information and start the analysis for each sample. Analyzing samples in a sequence allows a group of samples to be analyzed automatically and sequentially until complete. To analyze a sequence, an operator may input the sample information for all samples in the order they are to be analyzed, designate a destination directory on the software computer, and begin the analysis. Once the sequence has begun, the collection of sample data is automatic and will run to completion in the order that the samples were entered. The sequence of samples to be analyzed, ordered, and in line, is also referred to as a *sample queue*. The ability of the instrument software to accept and analyze sample queues is extremely beneficial due to the typically large number of samples to be evaluated. Often, a sample queue may take hours to complete. Note that the sample queue will pause if there are any software errors or hardware failures.

3.3 Data Analysis

Data analysis consists of any action involving the evaluation of the data associated with an analytical run. The software platforms associated with the analytical instrumentation used for this program have many features associated with data analysis. Many of these features have specific applications and are not used in typical data analysis. For example, the Agilent MSD ChemStation has an entire data analysis configuration dedicated to environmental samples and data reporting. The Low-Level program uses the Enhanced Data Analysis option as opposed to the EnviroQuant Data Analysis.

Typical data analysis activities include: loading data files for evaluation, reviewing mass spectral data, performing library searches, and building calibration databases. All these activities are performed in accordance with manufacturer recommendations and/or local operating procedures. Some additional data analysis tools may be utilized to ensure that the analyte of interest is correctly identified and properly quantified. These tools include, but are not limited to, evaluating mass spectral peak relative abundances and ensuring proper peak integration.

The primary ability of an MS is the ability to consistently and reproducibly create ions from sample molecules for detection. A mass spectrum will contain ions of certain m/z ratios. Because the technology allows the ionization process to be consistent and reproducible, the resulting mass spectrum for a given compound should be very similar from one analysis to another. The response of each ion on a mass spectrum is compared to one of the ions to establish relative abundance. Simply, if ion m/z B has a response of 50 abundance counts, and is compared to ion m/z A that has 100 abundance counts, ion m/z B will have a relative abundance of 50% of ion m/z A. When examining a data file, most often ions on a mass spectrum will be compared to the largest ion, called the *base peak*; however, this may not always be the case. The relative abundances of the ions on a mass spectrum for a given compound become its identifiable fingerprint. As previously discussed, this is what makes GC/MS data collected in the EI mode so valuable, because the data can be compared to thousands of entries in mass spectral libraries.

The Agilent MSD ChemStation data analysis allows these relative abundances to be established within the calibration database. Each sample compared to the calibration database will be identified by RT and by the ions present in the mass spectrum. A quantifiable result, called Q-value, will evaluate the quality match of relative abundances on the found mass spectrum to the expected relative abundances of the analyte of interest in the calibration database. The Q-value, along with RT, can be a useful quality tool for determining that the found peak is the analyte of interest. For this program, Q-value has no threshold limits associated. For example, if a Q-value of 65 is reported, this may not be enough information to decide whether or not the found peak is the analyte of interest. Other factors must be considered, such as RT and found concentration. Lower concentrations may not accurately represent the ions in the desired relative abundances. A very high Q-value (greater than 90) represents an extremely close match between relative abundances on a found mass spectrum to the expected relative abundances in the calibration database. Conversely, a very low Q-value (less than 10) represents a poor match. Figure 25 shows a TIC and mass spectrum of a GD standard, analyzed and compared to a GD calibration database. This standard showed a reported Q-value of 100, indicating a perfect match with the ion relative abundances listed in the calibration table.

Figure 26 shows a TIC and mass spectrum of an unknown peak that eluted close enough to the GD RT to produce a found concentration. This peak showed a reported Q-value of 11, indicating a poor match with the ion relative abundances listed in the calibration table. The RT of 3.9 min does not match the expected GD RT of 3.8 min.

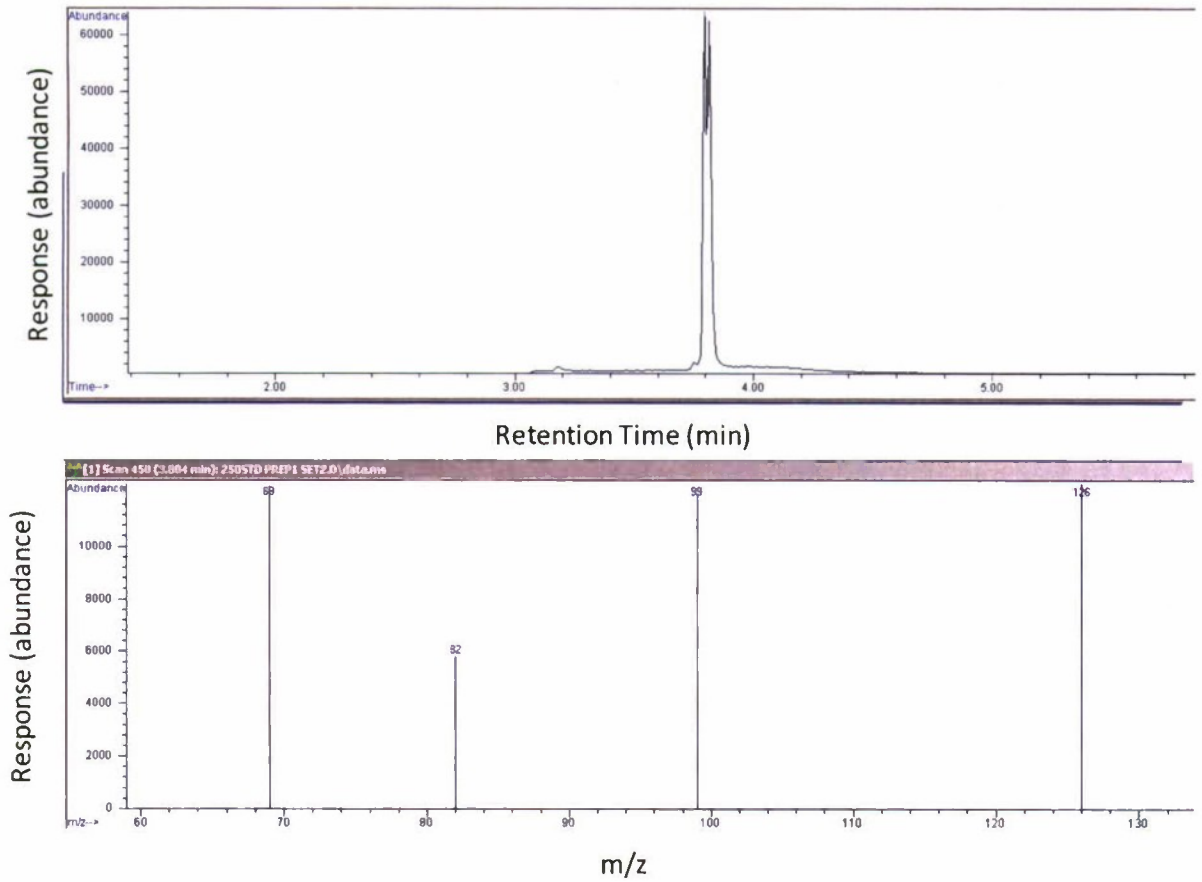


Figure 25. GD Sample (RT 3.8 min) showing a Q-value of 100.

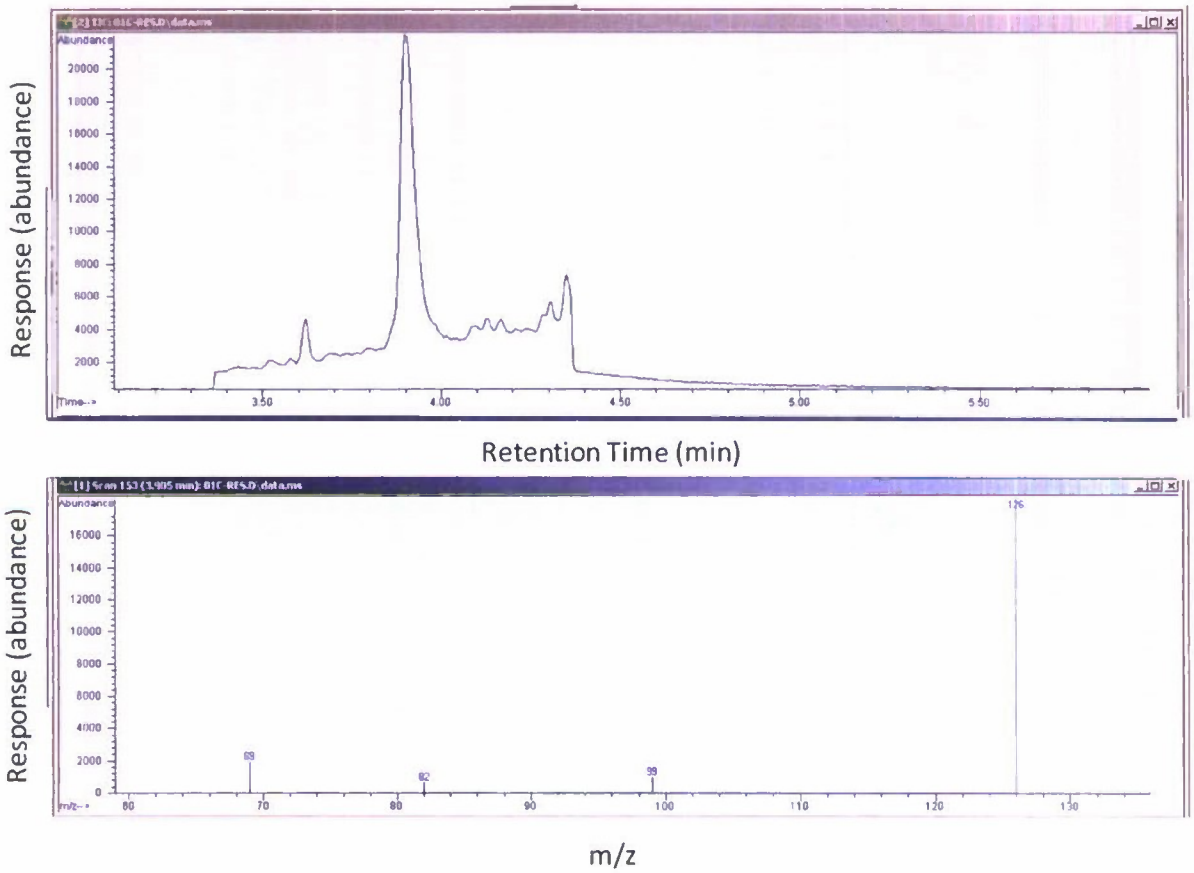


Figure 26. Unknown sample (RT 3.9 min) showing a Q-value of 11 when compared to the GD calibration database.

The instrument software quantifies found concentrations based on integration of the peak on a chromatogram. The data analysis portion of the software platforms used contains very powerful integrators that will seek out almost any signal deviation from baseline and report a detector response and found concentration when compared to the calibration database. This can be a challenge when performing data analysis. Occasionally, a stretch of rising baseline may be integrated and reported as a peak with an associated concentration. Upon review, the analyst must make a determination on whether or not the 'peak' is truly due to analyte or if it is, in fact, a baseline. The instrument software contains integration parameters that may be adjusted to help distinguish between analyte and baseline. Integration parameters are instrument-specific, not method-specific, and may be adjusted on an instrument-to-instrument basis, depending on, among other things, baseline performance. The integration parameters default to very lenient peak settings so that many peaks on the chromatogram will integrate.

One option an analyst has, if baseline is continually integrated, is to adjust integration parameters. It is recommended that only trained analysts adjust integration parameters, and that this adjustment is performed in accordance with manufacturer recommendations. An example of an integration setting that may need adjustment is slope sensitivity. Integration of a peak on a chromatogram should begin as the detector signal deviates from baseline to a higher amount, as analyte elutes from the column into the detector. Very low slope sensitivity means that the deviation away from baseline does not have to be severe for the software to mark the beginning of a peak for integration. Many times, if this setting is too low, normal rise and fall of baseline will integrate, potentially causing a found concentration within the analyte of interest RT window. Increasing the slope sensitivity will necessitate a more severe deviation from baseline for integration to begin. One goal of good chromatography and, therefore, method development, is to adjust method parameters to produce efficient or sharp peaks on a

chromatogram. Sometimes a peak may be present on the chromatogram that is passed over by the software integrator; i.e., the peak is not integrated or is poorly integrated. The software has the ability to deal with poor integration by allowing the analyst to manually integrate peak on a chromatogram. Again, this feature is only recommended for use by trained analysts. Manual integration allows the analyst to determine the beginning and ending points of a peak, and to draw an integration line between them, resulting in a calculated detector response. Manual integration may be required for many reasons. For example, if a peak of interest is outside the expected RT window, the peak shape is less than desirable, or the software integrator misinterprets the beginning or ending point of the peak.

4. CHEMICAL AGENTS AND BYPRODUCTS

This section provides a short description of the properties, reactions, and mass-spectral characteristics of the chemical agents and byproducts evaluated in this program. The most common byproducts formed through decontamination hydrolysis or oxidation reaction were selected for this study.

4.1 VX Agent and Byproduct Properties and Reactions

By definition, nerve agents are organophosphorus compounds that inhibit the enzyme acetylcholinesterase (AChE). This enzyme is essential in normal body function to remove the neurotransmitter acetylcholine (ACh) from its receptor sites. With AChE inhibited, ACh remains at the receptor sites, causing the common symptoms of nerve agent exposure such as over stimulation of the muscles and over secretion of glands and mucous membranes, among others. The ultimate fate of nerve agent exposure is death.

The chemical agent VX is, by comparison, the most toxic of the nerve agents. VX is also known by the chemical name O-ethyl S-(2-diisopropylaminoethyl) methylphosphonothioate and has a CAS number of 50782-69-9. VX has a molecular weight of 267 amu and a molecular formula of $C_{14}H_{26}NO_2PS$. The VX molecule differs from other nerve agents in that it contains a sulfur component. VX is an odorless, amber-colored liquid that is oily in appearance and is not readily soluble in water. VX is a very non-volatile compound and, therefore, does not evaporate readily. The solubility and volatility of VX causes it to be the most persistent of the nerve agents. Due to its low volatility and high persistency, the main route of exposure is by contact and subsequent absorption through the skin. VX acts very quickly and death can occur within 15 min after exposure to a lethal dose.[23] Due to the toxicity of VX, published airborne exposure limits and method detection limit requirements are very low, posing challenges for analytical instrument method development. These challenges are discussed in the method development and method capabilities sections of this report. The VX chemical structure and properties are provided in Figure 27 and Table 11, respectively.

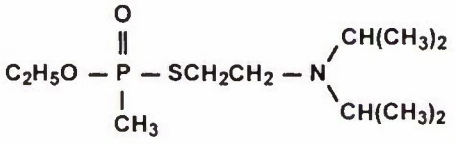


Figure 27. Chemical structure of VX.

VX is relatively stable at ambient temperatures and does not readily undergo hydrolysis due to its low water solubility. For example, at temperatures less than 9.4 °C, VX is miscible in water.[23] However, if VX is in the presence of water at room temperature, over time it will undergo hydrolysis to form byproducts. The way VX hydrolyzes and, therefore, the byproducts that are formed depend on the

pH of the reaction. These reactions have been well characterized by Small,[24] among others. It is important to note that certain VX hydrolysis byproducts still retain toxic properties. The two main byproducts of VX hydrolysis, and the byproducts of interest for this program, are EA2192 and ethyl methylphosphonic acid (EMPA). EA2192 is also known by the chemical name S-(2-diisopropylaminoethyl) methylphosphonothioic acid (CAS number 73-207-98-4) and has the molecular formula $C_9H_{22}NO_2PS$ with a molecular weight of 239 amu. EMPA (CAS number 1832-53-7) has a molecular formula of $C_3H_9O_3P$ and a molecular weight of 124 amu. The chemical structures for EA2192 and EMPA are provided in Figure 28 and Figure 29, respectively. The byproduct properties are provided in Table 11. This section highlights the chemical reactions that form these products. The ability to analyze and detect these byproducts is discussed in the method capabilities section of this report.

Table 11. VX and byproduct properties*.

Compound	VX	S-(2-Diisopropyl-aminoethyl) methyl-phosphonothioic acid	Ethyl Methylphosphonate
Synonym(s)	VX	EA2192	EMPA, Ethyl methylphosphonic acid
Product of	N/A	VX	VX
Reaction	N/A	Hydrolysis	Hydrolysis
CAS Number	50782-69-9	73-207-98-4	1832-53-7
Formula	$C_{11}H_{26}NO_2PS$	$C_9H_{22}NO_2PS$	$C_3H_9O_3P$
MW	267.37 amu	239 amu	124.08 amu
Physical State	Liquid	Solid	Liquid
Color	Colorless when pure	White	
Boiling Point	298 °C	Unknown	
Freezing Point	Below -51 °C		
Melting Point	Between -39 to -60 °C	138.0–140.0 °C	
LD50	0.143 mg/kg	0.63 mg/kg	
LCt50 (respiratory)	10 mg-min/m³		
LCt50 (percutaneous)	150 mg-min/m³		
Density, liquid	1.0083 g/mL @ 25 °C	Unknown	1.172 g/mL @ 25 °C
Vapor Pressure	8.78×10^{-4} torr @ 25 °C	ND	3.6×10^{-4} torr @ 25 °C
Volatility	10.5 mg/m³ @ 25 °C	0–100 mg/m³ @ 25 °C(Low)	N/A
Vapor Density (air=1)	9.2 (calculated)	Unknown	N/A
Water Solubility	5 g/L @ 21.5 °C	Infinitely Soluble	1.8×10^{5} g/L @ 21.5 °C
Present in Ambient Conditions (Y/N)	Y	N	
Present in Desert Conditions (Y/N)	Y	N	

*The shaded cells indicate unknown or unavailable information.

During decontamination testing it is imperative to screen for EA2192 because, as a potential byproduct of decontamination, EA2192 is a toxic material. EA2192 will result from the hydrolysis of VX when the pH of the reaction is between 7 and 10 (Figure 30).

EMPA is a common byproduct of the hydrolysis of VX when the pH of the hydrolysis reaction is less than 6 or greater than 10 (Figure 31). EMPA is formed from the phosphonic acid of VX and does not retain the toxicity of the VX molecule.

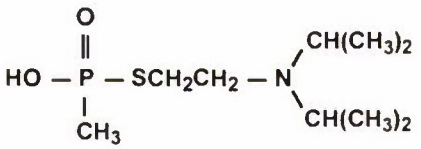


Figure 28. Chemical structure of EA2192.

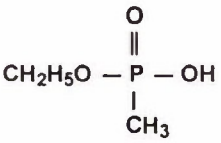


Figure 29. Chemical structure of EMPA.

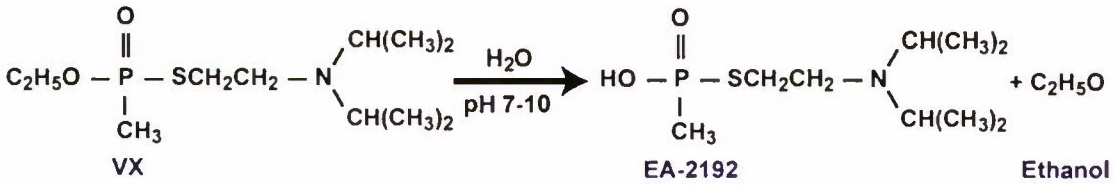


Figure 30. Hydrolysis of VX at pH 7–10.

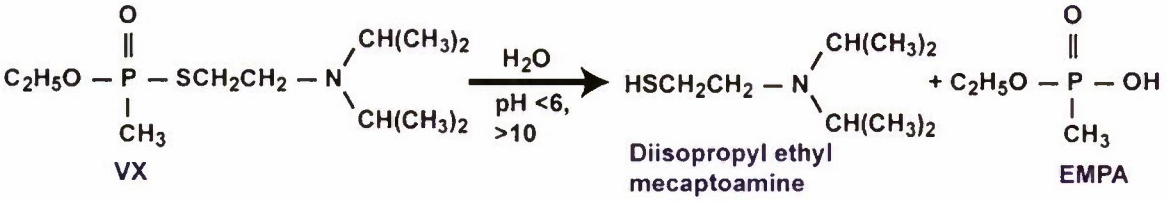


Figure 31. Hydrolysis of VX at pH less than 6, greater than 10.

4.2 HD Agent and Byproduct Properties and Reactions

Another class of toxic chemical agent is that of vesicants, which are more commonly called blister agents. The toxicity mechanism for a blister agent, in general, is very different from that of nerve agents. In review, nerve agents attack and inhibit a specific enzyme, causing an imbalance in the normal body functions of a neurotransmitter, leading to death. Blister agents tend to absorb into and attack the tissues of the body, causing severe inflammation, blistering, and often severe, irreversible damage. The effects of blister agent exposure are typically delayed. The time to onset of symptoms and the severity of symptoms are proportional to the amount and time of exposure. Blister agents pose a significant risk to internal exposure through inhalation and can be lethal in large doses.[23]

The blister agent mustard, also known as sulfur mustard, is abbreviated as HD, and has a chemical name of bis (2-chloroethyl) sulfide. Synonyms of HD include 2,2'-dichloroethyl sulfide and 1,1'-thiobis (2-chloroethane).[23] HD has the molecular formula $C_4H_8Cl_2S$, a molecular weight of 158 amu, and its CAS number is 505-60-2. The HD chemical structure and properties are provided in Figure 32 and Table 12, respectively.

$$Cl - CH_2 - CH_2 - S - CH_2 - CH_2 - Cl$$

Figure 32. Chemical structure of HD.

Table 12. HD and byproduct properties*.

Compound	Bis(2-chloroethyl sulfide)	2,2'-Thiodiethanol	Bis(2-chloroethyl) sulfone	Bis(2-chloroethyl) sulfoxide	2,2'-Sulfonyl-diethanol	Divinyl sulfone	2-Chloroethyl vinyl sulfone
Synonym(s)	HD, Mustard Gas, Sulfur	Thiodiglycol (TDG)	Mustard Sulfone	Mustard Sulfoxide			
Product of	N/A	HD	HD	HD	Sulfone	Sulfone	Sulfone
Reaction	N/A	Hydrolysis	Oxidation	Oxidation	Hydrolysis	Dechlorination	Dechlorination
CAS #	505-60-2	111-48-8	471-03-4	5819-08-9	2580-77-0	77-77-0	7327-58-4
Formula	$C_4H_8Cl_2S$	$C_4H_{10}O_2S$	$C_4H_8Cl_2O_2S$	$C_4H_8Cl_2O_2S$	$C_4H_{10}O_4S$	$C_4H_6O_2S$	$C_4H_7ClO_2S$
MW	158 amu	122.19 amu	191.07 amu	174 amu	154.18 amu	118.15 amu	
Physical State	Oily liquid	Liquid	Solid		Liquid	Liquid	
Color	Pale yellow to amber	Colorless to pale yellow				Clear to very faint yellow	
Boiling Point	218 °C	164–166 °C @ 20 mmHg	N/A		N/A	234 °C	
Freezing Point	14.45 °C	-18 to -10 °C					
Melting Point	14.45 °C	-16 °C	N/A		N/A	-26.0 °C	
LD 50	3.3 mg/kg	6610 mg/kg	50 mg/kg	150 mg/kg			
LCt50 (respiratory)	1,000 mg-min/m^3			1430 mg-min/m^3			
LCt50 (percutaneous	10,000mg-min/m^3						
Density, liquid	1.2685 g/mL at 25 °C	1.1824 g/mL	N/A		1.236 g/mL	1.177 g/mL	
Vapor Pressure	0.106 mmHg @ 25 °C	0.00002 mmHg @ 20 or 25 °C	0.96 mmHg @ 20 or 25 °C	0.65 mmHg @ 20 or 25 °C	23.5 mmHg @ 20 °C	0.09 mmHg @ 20 or 25 °C	0.023 mmHg @ 20 or 25 °C
Volatility	906 @ 25 °C	N/A	N/A			N/A	
Vapor Density (air=1)	5.5 (calculated)	4.22	N/A			N/A	
Water Solubility	0.68 g/L @ 20 °C	Miscible	11 g/L	93 g/L	5.5 g/L	140 g/L	78 g/L
Present in Ambient Conditions (Y/N)	Y	N					
Present in Desert Conditions (Y/N)	Y	N					

*The shaded cells indicate unknown or unavailable information.

HD is rather persistent at lower temperatures due to its low water solubility and its relatively high freezing point of 14.45 °C.[23] HD will volatilize in warmer climates, and is less persistent than VX, although it poses an increased airborne risk due to its volatility.

One of the major contributors to the formation of byproducts from HD is the chemical reaction hydrolysis. Hydrolysis occurs when HD is in the presence of water and suitable conditions persist.[25] Although HD is rather insoluble in water, it does hydrolyze readily when in solution. As HD undergoes hydrolysis, intermediate products are formed; however, the ultimate byproduct of the hydrolysis of HD is thiodiglycol (TDG). TDG (CAS number 111-48-8) is also known by the synonyms 2,2'-thiodiethanol and bis(2-hydroxyethyl)sulfide. Thiodiglycol has the molecular formula $C_4H_{10}O_2S$ with a molecular weight of 122 amu. The chemical structure of TDG and the hydrolysis reaction of HD, whereby TDG is formed, are shown in Figure 33 and Figure 34, respectively.

Other byproducts are formed via oxidation reactions, such as those associated with the decontamination of HD by supertropical bleach (STB). The two mustard oxidation byproducts of interest are mustard sulfoxide and mustard sulfone (Figure 35). Mustard sulfoxide (CAS number 5819-08-9) is also known by the chemical name bis(2-chloroethyl) sulfoxide and has the molecular formula $C_4H_8Cl_2OS$ with a molecular weight of 174 amu. Mustard sulfoxide, as the first step in the oxidation of HD, is typically non-toxic, whereas the product of the second step of the oxidation of HD, mustard sulfone, retains some of the toxic properties of HD. Mustard sulfone (CAS number 471-03-4) is also known by the chemical name bis(2-chloroethyl) sulfone and has the molecular formula $C_4H_8Cl_2O_2S$ with a molecular weight of 191 amu. Oxidation of HD is typically stepwise, whereby the sulfoxide is formed first and then the sulfone is formed; however, under certain conditions, the sulfone may be formed directly.[24] Table 12 lists the properties of the HD byproducts.

It is worth noting that additional byproducts are formed from the mustard sulfone. Two of these byproducts, 2-chloroethyl vinyl sulfone and divinyl sulfone, may be formed by way of elimination reactions, or dechlorination, of the mustard sulfone (Figure 36). These additional byproducts were not evaluated for this program.

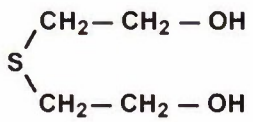


Figure 33. Chemical structure of thiodiglycol (TDG).

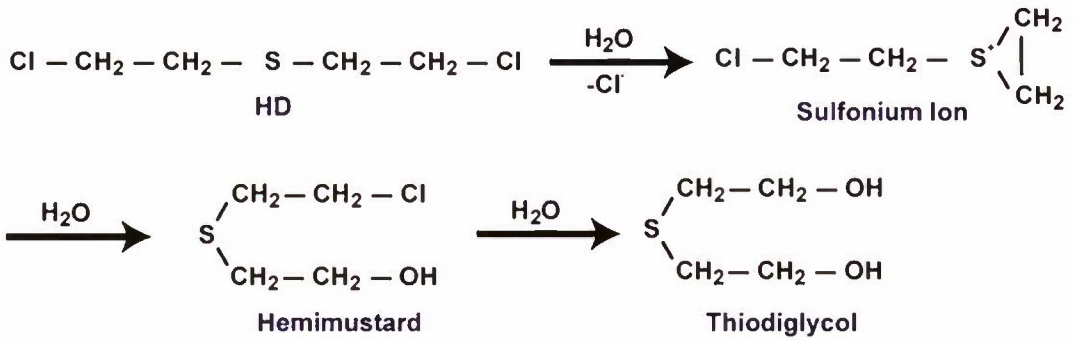


Figure 34. The formation of TDG via hydrolysis of HD.

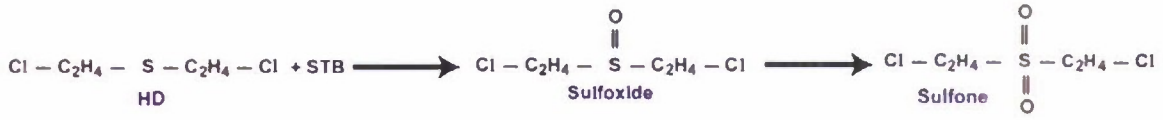


Figure 35. Mustard oxidation reaction byproducts.

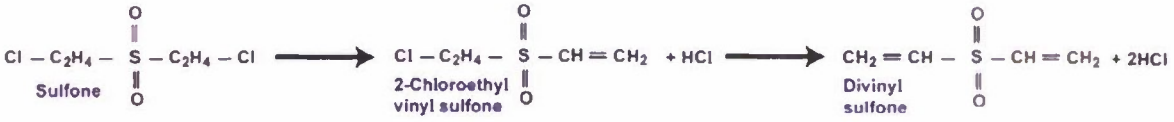


Figure 36. Sulfone elimination reactions.

4.3 GD Agent and Byproduct Properties and Reactions

The chemical agent GD is a G-series nerve agent. Soman, another name for GD, is the most toxic of the G-series nerve agents. It is also known by the chemical name pinacolyl methylphosphonofluoridate and has a CAS number of 96-64-0. GD has a molecular weight of 182 amu and a molecular formula of $C_7H_{16}FO_2P$. The GD chemical structure and properties are provided in Figure 37 and Table 13, respectively.

GD is colorless in both liquid and vapor forms. The volatility of GD is much greater than that of VX and poses a significantly greater vapor hazard. The persistency of GD is not as great as that of VX; therefore, it may be found in thickened form called thickened-GD (TGD), which is GD with thickening additives to increase persistency. GD is less stable and less soluble in water than other G-series agents.[25] As with all nerve agents, GD does not readily hydrolyze; however, over time it will slowly hydrolyze to form the byproduct pinacolyl methylphosphonic acid, also known as GD-acid. The chemical structure of GD-acid and the hydrolysis reaction of GD from which GD-acid is formed are shown in Figure 38. The GD-acid molecule is very similar to the GD molecule and is formed via the loss/replacement of the fluoride atom with hydroxide (-OH). GD-acid is also known by the synonym pinacolyl methylphosphonate and has the molecular formula $C_7H_{17}O_3P$ with a molecular weight of 180 amu. Table 13 lists the properties of the byproduct GD-acid. GD-acid will slowly undergo further hydrolysis to methylphosphonic acid (MPA); however, MPA was not evaluated for this program.

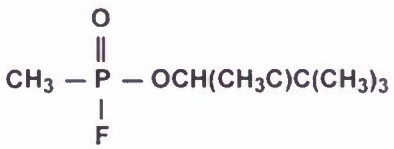


Figure 37. Chemical structure of GD.

Table 13. GD and byproduct properties*.

Compound	GD	Pinacolyl Methyl-phosphonate
Synonym(s)	GD	GD-acid
Product of	N/A	GD
Reaction	N/A	Hydrolysis
CAS Number	96-64-0	616-52-4
Formula	$C_7H_{16}FO_2P$	$C_7H_{17}O_3P$
MW	182.178 amu	180.19 amu
Physical State	Liquid	Liquid
Color	Colorless when pure	
Boiling Point	198 °C	96.0–106.0 °C @ 8 mmHg
Freezing Point	-42 °C	N/A
Melting Point	-42 °C	N/A
LD50	5 mg/kg	
LCt50 (respiratory)	35 mg-min/m³	
LCt50 (percutaneous)	3000 mg-min/m³	
Density, liquid	1.0252 g/mL @ 25 °C	1.032 g/mL @ 25 °C
Vapor Pressure	0.401 torr @ 25 °C	N/A
Volatility	3930 mmHg @ 25 °C	N/A
Vapor Density (air=1)	6.3 mg/m³ (calculated)	N/A
Water Solubility	21 g/L @ 20 °C	N/A
Present in Ambient Conditions (Y/N)	Y	
Present in Desert Conditions (Y/N)	Y	

*The shaded cells indicate unknown or unavailable information.

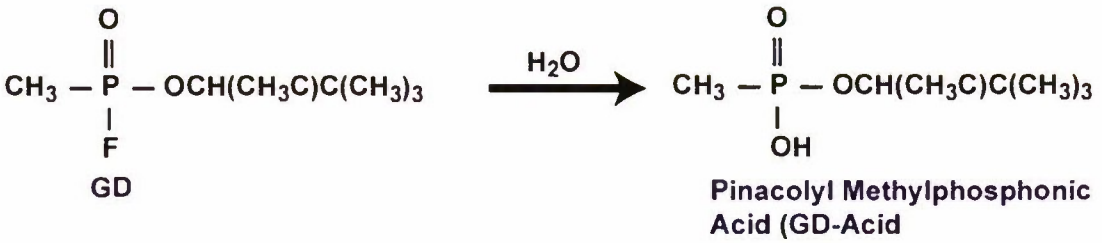


Figure 38. Hydrolysis of GD to form GD-acid.

5. ANALYSIS OF VX EXTRACTS

5.1 Decontamination Efficacy Requirement Documents

The method detection limit target was determined using the pertinent panel test parameters and the published requirement document levels. The calculations were based on a 20 mL extraction volume and a 0.00202 m^2 contaminated surface area (equivalent to a 2 in. diameter circular contamination area). The requirement document surface concentration levels for threshold (T) and objective (O) of primary interest at the time of this program are listed in Table 14. The JPID 2003 objective values are reported using only zeroes, which make scientific evaluations and calculations difficult. For each non-specific requirement value, a number 5 was added to the next decimal point to enable measurement and calculation. For example "0.00" is shown as "0.005". The contaminant mass on coupon was calculated using the contamination area as surface concentration × coupon area. The nominal extract concentration was calculated by dividing the mass on coupon by the extraction volume. The high end of the target detection range was set using a value that was 10% lower than the nominal extraction concentration. The low end of the target detection range was set at an order of magnitude below the high-end value. The lowest range among the group of requirement levels was selected as the analytical method target detection range. The range selected for the analysis of decontamination testing for VX extract samples is shaded in Table 14.

Table 14. Decontamination testing detection targets for VX extract samples.

Requirement Document	Surface Conc. (mg/m^2)	Mass on Coupon (ng)	Nominal Extract Conc. (ng/mL)	Target Detection Range (ng/mL)*		
JPID 05 T	0.040	80.8	4.04	3.64	to	0.364
JPID 05 O	0.005	10.1	0.51	**0.45**	**to**	**0.045**
JSSED 03 O	0.780	1,575.6	78.78	70.90	to	7.090
JSSED 04 T	0.780	1,575.6	78.78	70.90	to	7.090
JSSED 04 O	0.500	1,010.0	50.50	45.45	to	4.545

*The values shaded in green represent the selected largel detection range for low-level method development.

5.2 Initial Method Development

One of the most common tools for analyzing volatile organic extraction samples is the GC/MS. All initial method development was performed on the GCE GC/MS. To review, GCE consists of an Agilent model 6890 GC equipped with a 5975 MSD. An Agilent Technologies Deans Switch was configured on this instrument and used as a flow-switching apparatus to send all non-analyte effluent away from the MSD and toward a FPD. This technique was used to preserve the MSD from potential liquid extraction sample contamination. Liquid extraction samples were introduced by injection, using a Gerstel MPS2 and a Gerstel CIS4 inlet installed on the GC. As method development began on the GCE, the low-level detection requirements for VX posed an initial challenge. The most stringent detection target was provided by the JPID03 ORD, which, after applying the program experimental parameters (e.g., contact sampler extraction efficiency of ~90%), yielded a target detection limit of 0.45 ng/mL. This low target concentration, as the detection limit target, was especially challenging given the injection volume limitations of a typical GC system. The Gerstel MPS2 and CIS4 inlet provides the ability to adjust parameters to allow large volume injections for more mass of analyte on column. Large volume injections of VX solution was a consideration for meeting the detection limit requirement.

One of the major benefits of utilizing an MS in the more typical EI ionization mode is the ability to compare an acquired spectrum to a library of entries showing the expected mass spectrum for an analyte of interest. Library searching can be performed as PBM, which is a comparison of a collected mass spectrum to the most likely library match. Searching can also be performed as parametric retrieval, which allows a library entry mass spectrum to be called upon by compound name, CAS number, molecular weight, or some other known compound parameter. Initially, a NIST 2.0 library search was conducted using the Agilent MSD ChemStation software Enhanced Data Analysis parametric retrieval. The search was performed for VX by CAS number (50782-69-9) to obtain the NIST database entry giving the mass spectrum and major ions for this analyte. The NIST library entry number is 226161 and the 10 largest peaks for VX, per the NIST library are detailed in Table 15.

Table 15. VX and byproduct mass spectra characteristics.

Compound	VX	S-(2-diisopropylaminoethyl) methylphosphonothioic acid	Ethyl methyl phosphonate
Synonym(s)	VX	EA2192	EMPA, Ethyl methylphosphonic acid
NIST No.	226161	273473	273563
ID No.	68951	68915	54530
DB	mainlib	mainlib	mainlib
Peak 1	114 (999)	114 (999)	97 (999)
Peak 2	72 (210)	72 (278)	79 (784)
Peak 3	127 (130)	30 (202)	80 (267)
Peak 4	70 (90)	43 (127)	47 (223)
Peak 5	30 (80)	70 (99)	81 (202)
Peak 6	79 (80)	41 (98)	65 (193)
Peak 7	115 (80)	59 (90)	125 (188)
Peak 8	43 (70)	86 (89)	27 (166)
Peak 9	84 (60)	115 (79)	29 (166)
Peak 10	167 (50)	71 (59)	45 (118)

After examining the mass spectrum for VX, the MSD was set up to simultaneously analyze in the Scan and SIM modes of acquisition. Based on the ion abundances from the expected mass spectrum, the ions selected for the SIM mode were m/z 114 (base peak), 127, 167, and 72. Note that the molecular ion, m/z 267, was not present on the mass spectrum due to how the molecule ionizes in the MS.

Initial method parameters were selected for the GC based on the properties of the analyte and solvent. All standards were prepared in-house using high purity solvent and a standard preparation dilution scheme to prepare initial working standards. Solvent blanks were analyzed before and after the VX standard to ensure that there were no baseline issues that might interfere with the analyte of interest. Initial injections (1 µL) were made with solvent blanks and a higher concentration VX standard (10,000 ng/mL). To provide an intense signal to the detector for higher confidence in PBM, a higher concentration of standard was injected. In addition to enabling the identification of the correct peak via library matching, it allowed the determination of retention time and evaluation of the peak shape on the TIC. Evaluating peak shape (efficiency and resolution) allowed the method to be tuned for optimal chromatography. Typically, peaks on the TIC can be identified by the mass spectrum, which contains selected ions in certain relative abundances. As lower concentrations of the standard were evaluated to

determine detection limit capabilities in comparison to the ORDs target detection limit, it was observed that selected ion relative abundances deviated from the ideal.

Peak RT is a function of molecular identity, and is not a function of analyte concentration. By initially identifying the correct peak via library matching and noting the RT, identification of the peak was possible at much lower concentrations. When the ions of interest were not in their ideal relative abundances at these lower concentrations, analyte peaks could be identified by RT. After analysis of the initial samples, the peak was successfully identified by PBM upon matching the mass spectrum from the Scan mode of acquisition with the VX entry in the NIST 2.0 library. The PBM of a 10,000 ng/mL VX standard, prepared in high purity IPA, identified the analyte peak at 7.6 min as VX with a 71.2% match quality. The next highest match for the analyte peak only gave a 19.0% match quality. The analyses were repeated in triplicate, with similar results. From this finding, a conclusion was reached that the analyte peak was correctly identified as VX. The mass spectrum for VX, in the SIM mode of acquisition, also showed the ions in the expected relative abundances. It is also important to note that the solvent blank did not show a chromatographic peak at the RT of the peak identified as VX. An example VX standard, analyzed on the MSD in both the Scan and SIM modes of acquisition, with their corresponding mass spectum, are shown in Figure 39 and Figure 40, respectively. A solvent blank chromatogram with mass spectrum is shown in Figure 41.

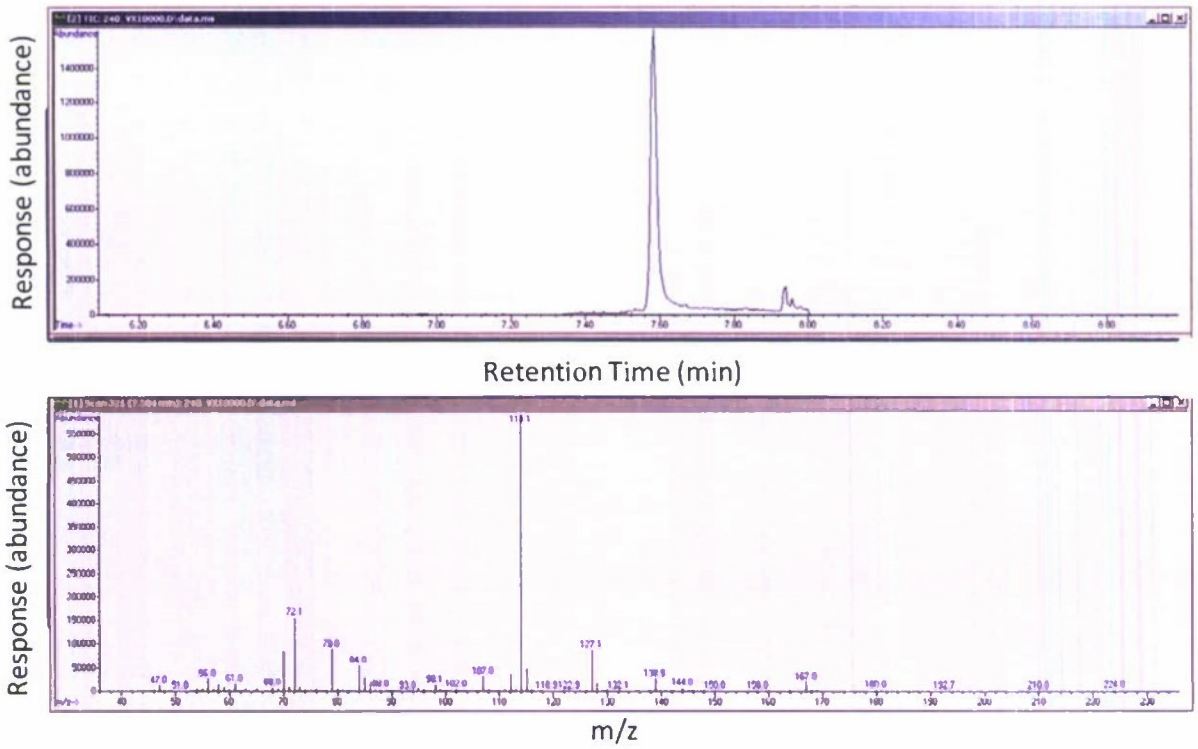


Figure 39. GC/MS SCAN TIC and mass spectrum of 10,000 ng/mL VX on GCE (VX RT = 7.6 min).

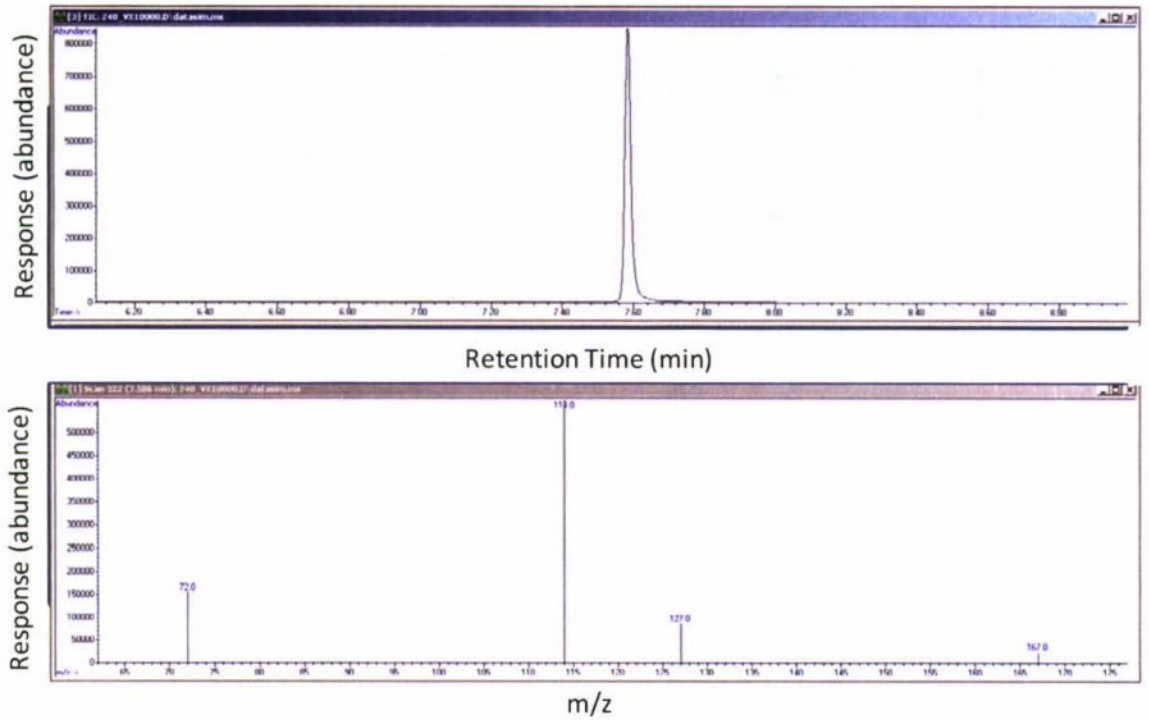


Figure 40. GC/MS SIM TIC and mass spectrum of 10,000 ng/mL VX on GCE (VX RT = 7.6 min).

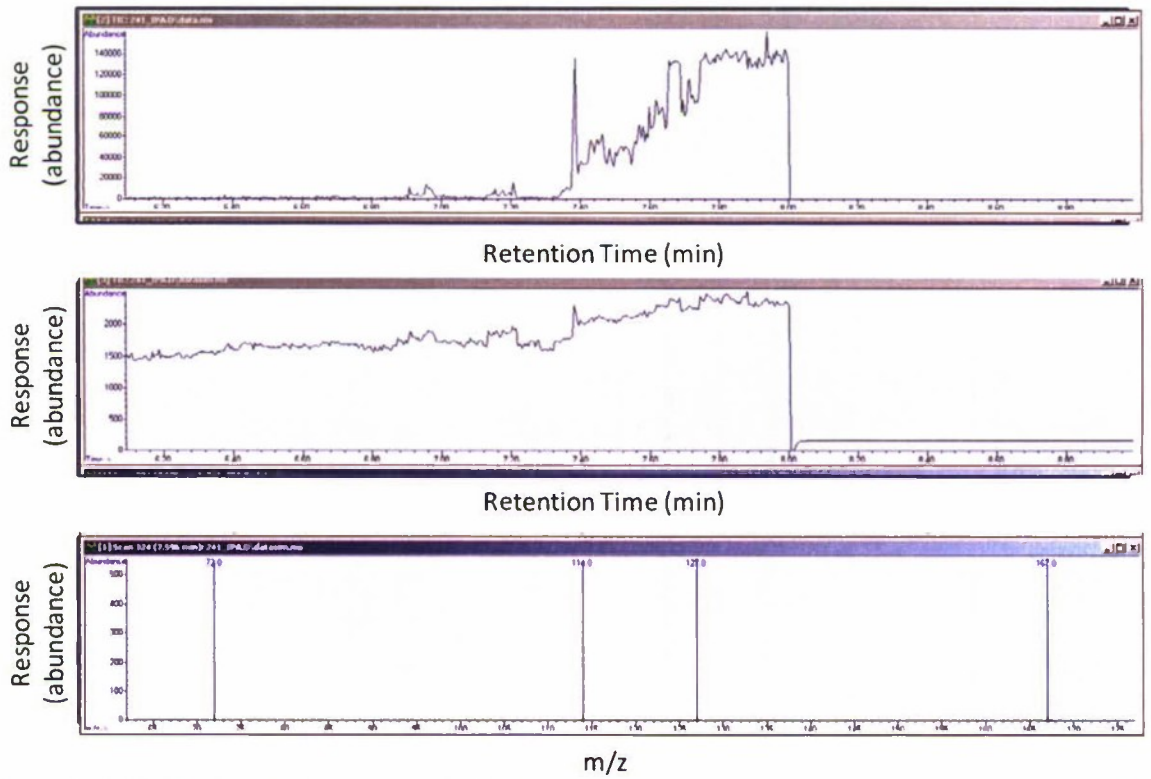


Figure 41. GC/MS SCAN and SIM analysis of an IPA solvent blank, showing no peak at RT 7.6 min and SIM spectrum ions at low, absolute, and incorrect relative abundances.

Additional injections were made at decreasing concentrations in an attempt to identify the observed method detection limit. The observed lower detection limit was determined by the presence of a peak at the expected RT, capable of being integrated, that maintained an acceptable signal to noise ratio (SNR). *Signal-to-noise (S/N)* is the comparison of the analyte peak response to a representative area of baseline to determine if the strength of the analyte response is greater than a representative area of the baseline or background. It is generally accepted that a SNR of > 3:1 is suitable.

The observed VX lower detection limit on the GCE was ~100 ng/mL during method development. The upper observed detection limit was not established due to the ability of the GC/MS to detect VX at concentrations significantly higher than the program requirements. The lower detection limit could have been improved with follow-on method development; however, given the JPID03 ORD detection requirements versus the initial GCE instrument lower detection capabilities observed, it was determined that efforts for the low-level method development for VX should be focused in another direction. Thus, VX method development was transitioned to an LC/MS/MS.

The LC/MS/MS VX work was performed on the LCE instrument; an Applied Biosystems API5000 Triple-Quadrupole Mass Spectrometer with a TurboV ion source. Sample introduction and chromatography were performed using an Agilent Technologies 1200 Series LC.

The LCE, as a triple-quadrupole instrument, had the ability to perform product ion evaluations via MRM. Initial method parameters were set up, and a VX standard prepared in IPA (1,000 ng/mL) was infused via a syringe pump directly into the MS for parameter optimization. Infusion experiments are used to evaluate how the analyte ionizes and fragments in scan and product ion modes. There is no widely accepted library available for LC/MS/MS spectra, such as the NIST 2.0 library for the GC/MS. (GC/MS ionization and fragmentation follows different mechanisms [i.e., rules] than the LC/MS/MS, thus the LC/MS/MS spectra cannot be compared to GC/MS libraries.) Therefore, parent/daughter ions were chosen, and the product ion spectral data was evaluated, based upon literature and/or instrument optimization. Method development on the LC/MS/MS typically follows the same pathway to optimize the instrument for a given analyte. Deviations may be made depending on previous experiences, literature review, and known information regarding a given analyte.

The following is a general description of the method development process for the analytes of interest on the LC/MS/MS. Initially, for VX, a standard was infused directly into the MS via a syringe pump, while the instrument scanned for a specific mass range through quadrupole number one (Q1). As expected, an ion at m/z 268 was present representing VX $[M+H]^+$.[26] From there, the parameters were adjusted to allow the ion at m/z 268 exiting Q1 to be further fragmented in the collision cell (Q2) region via collision-induced dissociation (CID) with nitrogen, while quadrupole number three (Q3) scanned for the product ions of m/z 268. Based upon the results from the Q3 product ion scan, the parameters were adjusted to optimize the MS/MS fragmentation into consistent and reproducible relationships.

For VX, product ion fragments appeared for m/z 268/128, 268/167, and 268/139, among others. Once these product ion relationships were discovered, verified, and established, an MS/MS method was set up to allow MRM, whereby these product ions were created, selectively filtered, and detected. Once optimized, the LC was configured and flow injection analysis (FIA) was evaluated via injections through the LC to evaluate peak RT, efficiency, resolution, and instrument detection limits. The VX RT on LCE was 4.0 min. Figure 42 shows an example chromatogram of VX.

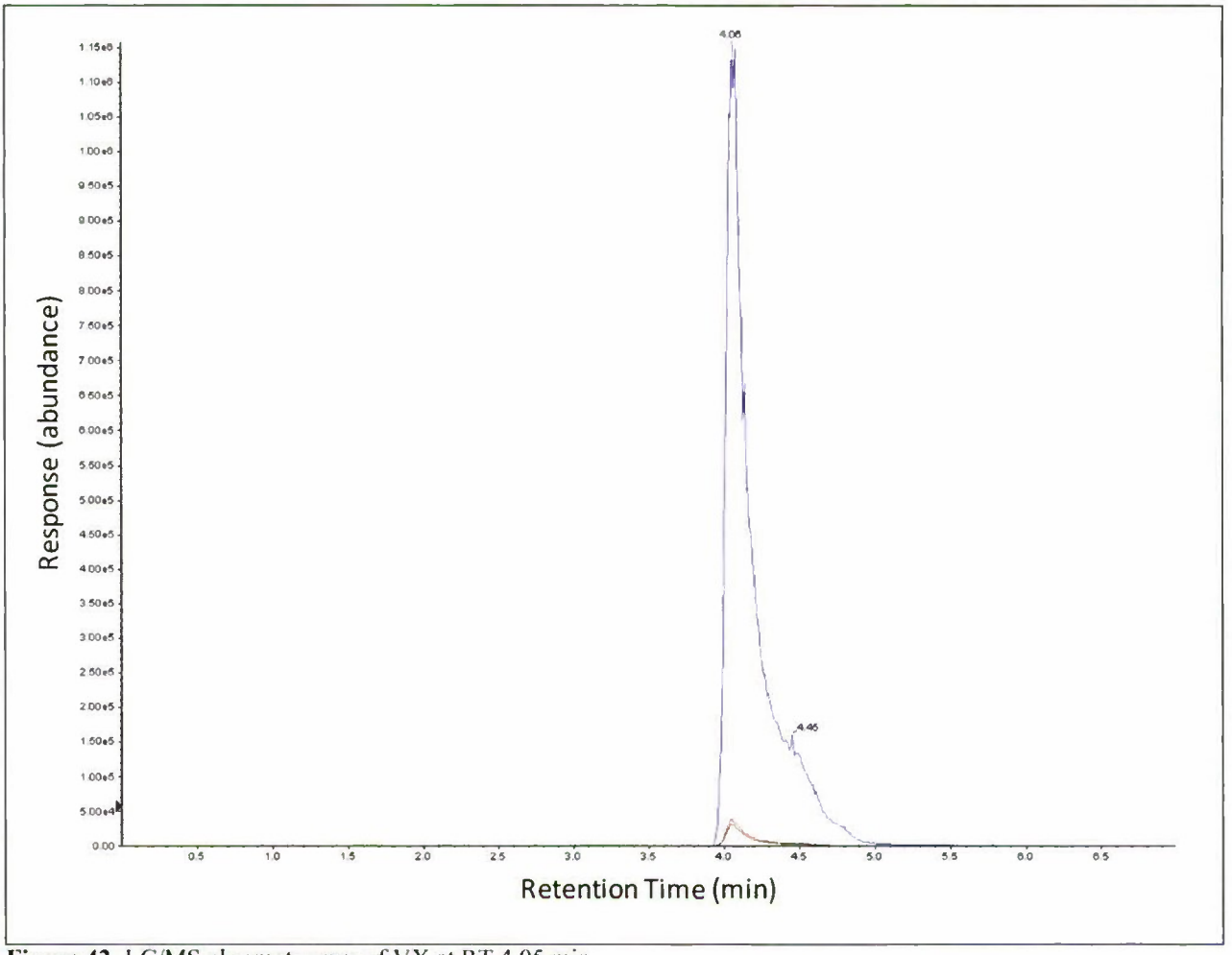


Figure 42. LC/MS chromatogram of VX at RT 4.05 min.

The sensitivity of this instrument at this point was very promising. Injections were made with solutions spanning a wide concentration range in an attempt to identify the observed upper and lower method detection limits. Initial standards evaluations showed that the LCE instrument had a working calibration range of ~10–1,000 ng/mL. It was noted, however, that the detector began to saturate, or maximize, at the upper end of this range; therefore, the observed upper detection limit was established at 750 ng/mL. The initial lower detection limit of 10 ng/mL was not low enough to meet the detection limit requirements of the program. Continuing method development on LCE lowered the observed detection limit to 0.05 ng/mL by adjusting instrument parameters; however, this increase in sensitivity significantly affected the upper detection limit point of detector saturation.

When preparing the liquid extraction samples throughout the contact testing, calculations based on experience and educated guesses are made to estimate concentrations of the samples to be analyzed on the analytical instrumentation. The ability to detect a wide concentration range was beneficial to maximize the effectiveness of testing, limit the number of samples to be run multiple times, and/or limit the need to dilute samples after the testing had occurred. This wide concentration range may span several orders of magnitude and, therefore, a number of calibration standards are required for confidence. Given the sensitivity of the LCE, and the very low detection requirement presented by the JPID03 ORD, it was not possible to achieve such a large concentration range in one method. This was due to potential carryover of the larger concentrations interfering with the lower level samples, quality checks, and solvent blanks. It was determined that two methods would be required for VX on the LCE:

(1) an ultra low-level (ULL) method that exceeded the JPID03 ORD requirements and (2) a wider concentration range, low-level method that allowed samples of higher concentrations to be analyzed and served as a complement to the ULL method capabilities.

5.2.1 Initial Method Capabilities

With observed detection limits established that met the program requirements, initial capabilities of the VX methodology were assessed. Initial method evaluation established a calculated LOD and LOQ as well as a calibration curve fit and weighting.

5.2.1.1 Method LCE VX_ULL.dam — Initial

The primary purpose of the LCE VX_ULL.dam method was to detect and quantify ultra low-level concentrations of the chemical agent VX in liquid extraction samples. This method was developed to exceed the low-level JPID03 ORD requirements. Based on the method development, the ULL VX extraction method (LCE VX_ULL.dam) detected and quantified VX in IPA from 0.05 to 10 ng/mL using LC/MS/MS. The results of the extraction method exceeded the research target concentration of 0.45 ng/mL, established from the JPID03 ORD requirement of 0.005 mg/m^2. No significant method interferents were identified during method development. Sample throughput was approximately seven samples per hour.

The LCE VX_ULL.dam method was appropriate for liquid samples extracted with the solvent IPA (C_3H_8O; CAS number 67-63-0) and containing the chemical agent VX. This method utilized eight calibration standards at VX concentrations of ~0.05, 0.1, 0.2, 0.5, 1.0, 2.5, 5, and 10 ng/mL. The method use, detection limit, and quantitation limit were based on using the full standard set. The standards were prepared in IPA. The mobile phase was a 50:50 mixture of the aqueous and organic phases. The organic phase consisted of 95% acetonitrile, 5% deionized water, 0.1% formic acid, and 5 mM ammonium acetate. The aqueous phase consisted of 95% deionized water, 5% acetonitrile, 0.1% formic acid, and 5 mM ammonium acetate. The method was conducted in an isocratic configuration.

The initial method performance for VX_ULL was:

- Quantitative Method
- Calibration Range: 0.05–10 ng/mL VX
- Calibration Model: Weighted linear regression
- Calibration Weighting: 1/standard concentration
- LOD: 0.013 ng/mL
- LOQ: 0.039 ng/mL
- Sample Solvent: IPA
- Quantitation Ion Pair: VX 268.1/128

Calibration standards were prepared and analyzed multiple times for calibration consistency and detection limit determination. Additionally, the high concentration standard of the set was analyzed in triplicate, followed by solvent blanks to determine method carryover. The carryover from the high-level calibration standard had no affect on the method or the ability of the method to detect

and quantify the low end of the calibration range. The calibration range of 0.05–10 ng/mL yielded a linear response. This initial method used a weighted linear regression calibration model. The weighting applied was 1/standard concentration (1/x). The application of 1/x weighting ensured accuracy at the lower levels of the calibration curve, possibly sacrificing the accuracy at higher concentrations. Accuracy at or below requirement levels was ideal to instill confidence that a requirement had been met.

The LCE VX_ULL.dam method had a calculated LOD of 0.013 ng/mL and an LOQ of 0.039 ng/mL. The precision of the method was determined by analysis of the multiple CCV samples analyzed. There were 16 replicate analyses of the 0.05 ng/mL standard, which produced a standard deviation of 0.0044 and an RSD of 9.65%. This indicated very good reproducibility at the lowest standard in the calibration curve. For the other standards, six replicates were analyzed, producing standard deviations ranging from 0.0065 to 0.37 and RSDs ranging from 1.91 to 8.5%.

Based on the method development and validation work performed and documented herein, the ULL VX extraction method (LCE VX_ULL.dam) detected and quantified VX in IPA from 0.05 to 10 ng/mL. This result exceeded the research target concentration of 0.45 ng/mL established from the JPID03 ORD requirement of 0.005 mg/m^2.

5.2.1.2 Method LCE VX_LL.dam — Initial

The primary purpose of the LCE VX_LL.dam method was to detect and quantify low-level concentrations of the chemical agent VX in liquid extraction samples. This method was developed as a complementary method to the ULL method (LCE VX_ULL.dam), to allow VX decontamination testing at slightly higher concentrations and with a larger concentration range than used for the ULL method. The LCE VX_LL.dam method detected VX solution concentrations from 10 to 750 ng/mL. No significant method interferents were identified during method development. Sample throughput was approximately seven samples per hour.

The LCE VX_LL.dam method was appropriate for liquid samples extracted with the solvent IPA (C_3H_8O; CAS number 67-63-0) and containing the chemical agent VX. This method utilized seven calibration standards at VX concentrations of ~10, 25, 50, 100, 250, 500, and 750 ng/mL. The method use, detection limit, and quantitation limit were based on using the full standard set. The standards were prepared in IPA. The mobile phase was a 50:50 mixture of the aqueous and organic phases. The organic phase consisted of 95% acetonitrile, 5% deionized water, 0.1% formic acid, and 5 mM ammonium acetate. The aqueous phase consisted of 95% deionized water, 5% acetonitrile, 0.1% formic acid, and 5 mM ammonium acetate. The method was conducted in an isocratic configuration.

The initial method performance for VX_LL was:

- Quantitative Method
- Calibration Range: 10–750 ng/mL VX
- Calibration Model: Quadratic calibration model
- Calibration Weighting: None
- LOD: 1.41 ng/mL
- LOQ: 4.27 ng/mL
- Sample Solvent: IPA

- Quantitation Ion: VX 268.1/128

Calibration standards were prepared and analyzed multiple times for calibration consistency and detection limit determination. Additionally, the high standard of the set was analyzed in triplicate, followed by solvent blanks to determine method carryover. The carryover from the high-level calibration standard was negligible, and had no affect on the method or the ability of the method to detect and quantify the low end of the calibration range. The calibration range of 10–750 ng/mL yielded a quadratic response. This initial method used a quadratic calibration model with no weighting.

The LCE VX_LL.dam method had an LOD of 1.41 ng/mL and an LOQ of 4.27 ng/mL. The precision of the method was determined by analysis of the multiple CCV samples analyzed. Fifteen replicate analyses of the 10 ng/mL standard produced a standard deviation of 0.43 and an RSD of 4.32%, which indicated very good reproducibility at the lowest standard in the calibration curve. For the other standard concentrations, five replicates were analyzed producing standard deviations ranging from 0.53 to 33.77 and RSDs ranging from 4.25 to 5.46%.

5.2.1.3 Method GCE VX_DEANS.M — Initial

The primary purpose of the GCE VX_DEANS.M method was to detect and quantify the chemical agent VX in liquid extraction samples. This method was developed to complement the LCE VX methods and analyze extraction samples with higher expected concentrations than those allowed by the LCE methods. This method detected VX solution concentrations from 250 to 2000 ng/mL. No significant method interferents were identified during method development. Sample throughput was approximately six samples per hour.

The GCE VX_DEANS.M method was appropriate for liquid samples containing the chemical agent VX and extracted with the solvent IPA (C_3H_8O; CAS number 67-63-0). This method utilized five calibration standards at concentrations of ~250, 500, 750, 1000, and 2000 ng/mL VX. The method use, detection limit, and quantitation limit were based on using the full standard set. The standards were prepared in IPA.

The initial method performance for VX was:

- Quantitative Method
- Calibration Range: 250–2000 ng/mL VX
- Calibration Model: Quadratic calibration model
- Calibration Weighting: None
- LOD: 32.01 ng/mL
- LOQ: 97.00 ng/mL
- Sample Solvent: IPA
- Quantitation Ion: m/z 114

Three sets of calibration standards were prepared. Each calibration preparation set was analyzed two times for calibration consistency and detection limit determination. The calibration range of 250–2000 ng/mL yielded a quadratic response. This initial method used a quadratic calibration model with no weighting.

The GCE VX_DEANS.M method had an LOD of 32.01 ng/mL and an LOQ of 97.00 ng/mL. The precision of the method was determined by analysis of the multiple CCV samples analyzed. Six replicate analyses of the 250 ng/mL standard produced a standard deviation of 9.70 and an RSD of 3.43%, which indicated very good reproducibility at the lowest standard in the calibration curve. For the other standard concentrations, six replicates were analyzed, producing standard deviations ranging from 1.08 to 14.10 and RSDs ranging from 0.05 to 2.99%.

5.3 VX Byproduct Method Development

The second overall program objective was to establish methods for the detection of common agent byproducts that could form during decontaminant testing. The purpose of these methods was to screen for common byproducts of chemical agents for the confident detection and identification of the byproduct analyte, when present. These methods are qualitative methods and serve as complementary methods to the fully quantitative agent methods.

Two of the more common byproducts for the chemical agent VX, formed via hydrolysis, are EA2192 and EMPA.

Method development and optimization of EA2192 occurred in much the same way as VX on the LC/MS/MS. Method development of the analytes VX and EA2192 was performed in parallel. Following the method development paradigm of VX, EA2192 standards were prepared in IPA to known concentration from a stock standard. An EA2192 standard (1,000 ng/mL) was infused into the MS via syringe pump while the instrument scanned for a specific mass range through quadrupole number one (Q1). As expected, an ion at m/z 240 was present representing EA2192 $[M+H]^+$.[26] The parameters were adjusted to allow the ion at m/z 240 exiting Q1 to be further fragmented in Q2, while quadrupole number three (Q3) scanned for the product ions of m/z 240. Based upon the results from the Q3 product ion scan, the parameters were adjusted to optimize the product ion fragmentation into consistent and reproducible relationships. The MRM parameters observed for EA2192 were m/z 240.1/128, 240.1/162 and 240.1/139.

After optimization on the MS, FIA was performed through the LC using the VX LC method parameters. It was observed that the RT of EA2192 on LCE was 3.3 min; an example chromatogram of EA2192 is shown in Figure 43.

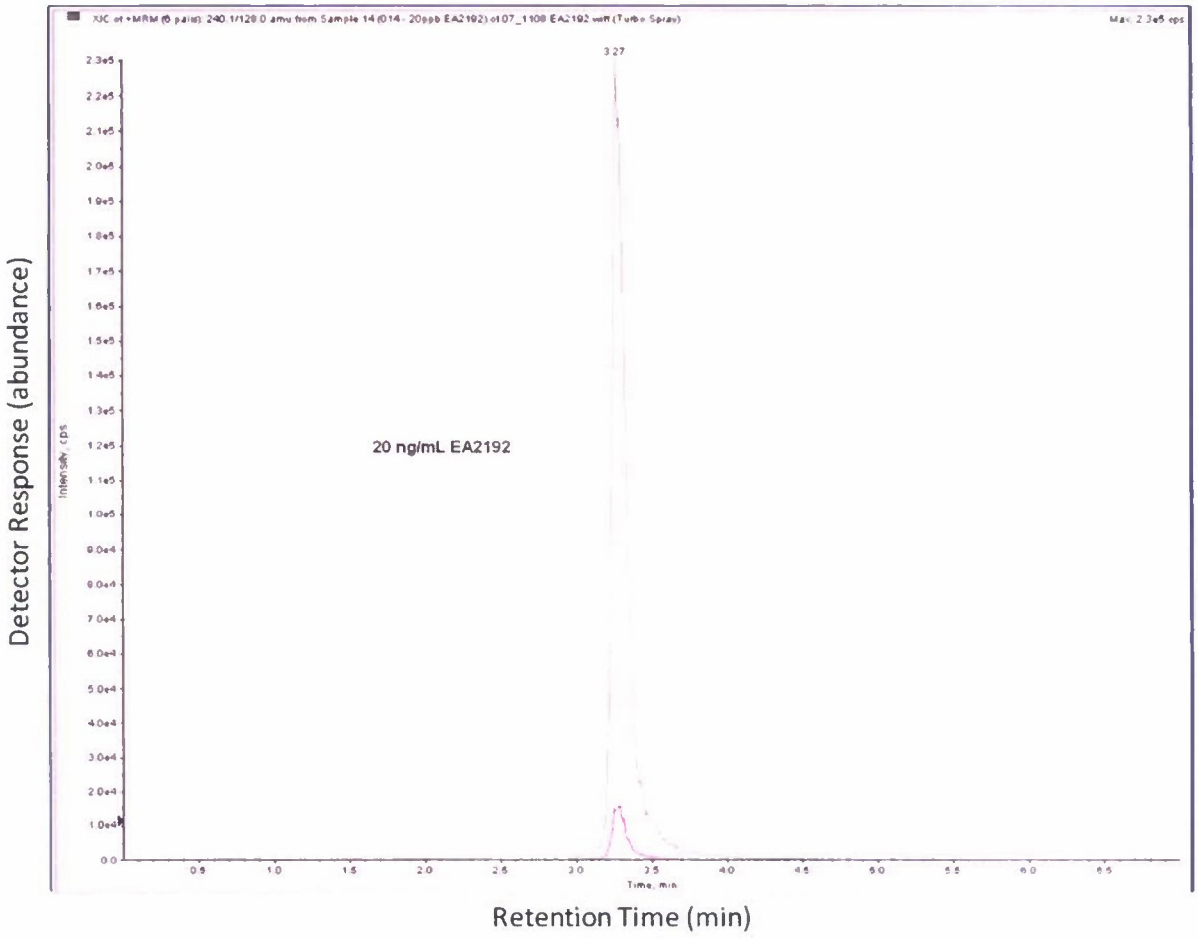


Figure 43. XIC of 20 ng/mL EA2192/IPA (RT=3.3 min).

The overall observed method detection limits for EA2192 were ~1-1,000 ng/mL. Future work may determine the ability of these methods to quantify EA2192 through generation of calibration curves spanning an appropriate working range using standards diluted in high purity solvent. Future method capabilities to quantify EA2192 will be determined statistically and reported accordingly.

Another method consideration for the LCE was the ability to screen for the VX hydrolysis byproduct EMPA. Optimization of EMPA began in positive ion mode with a Q1 scan for ion m/z 125 $[M+H]^+$.[26] However, continued method development showed that EMPA was better analyzed with a method in the negative ion mode of acquisition (ion m/z 123 $[M-H]^-$) for increased sensitivity and detection capabilities. EMPA standards were prepared, MS optimization occurred, and an MRM method (123/95, 123/79, 123/77) was established to allow the screening of this analyte. The EMPA RT on LCE in negative ion mode was 3.3 min. An example chromatogram of 20 ng/mL EMPA/IPA is shown in Figure 44.

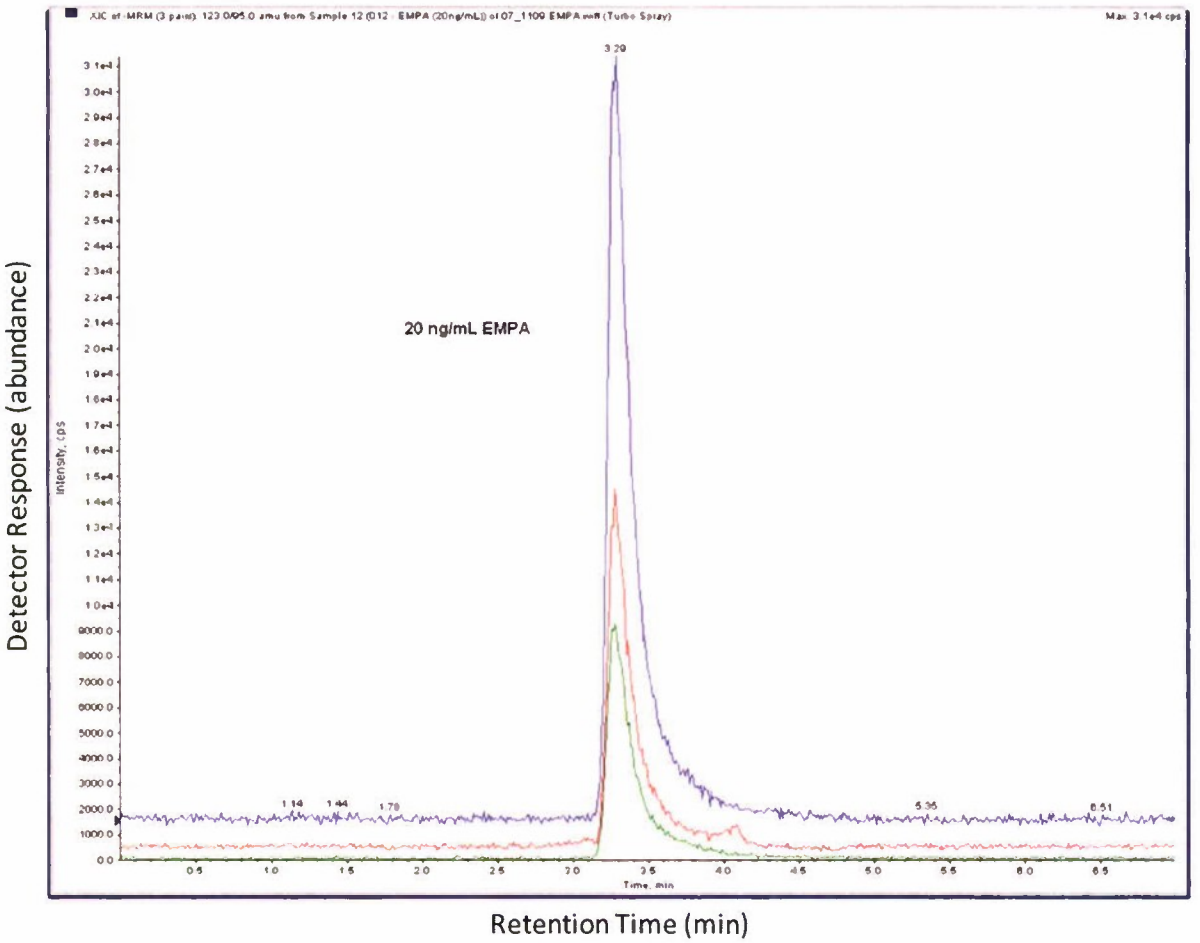


Figure 44. LC analysis of 20 ng/mL of EMPA (RT 3.3 min) using method LCE EMPA.dam - XIC.

The observed method detection limits for EMPA were ~1-1,000 ng/mL. Future work may determine the ability of the method to quantify EMPA through the generation of calibration curves, using standards diluted in high purity solvents, and spanning an appropriate working range. Future method capabilities to quantify EMPA will be determined statistically and reported accordingly.

Additional byproduct method development may be performed for all agents upon the acquisition of stock standard material. Some additional byproduct work for VX has been completed. When presented with a sample suspected of containing VX and potential VX byproducts, generic method parameters were established on GCE and the sample was analyzed to identify VX and VX byproducts via PBM of the mass spectral data acquired in the Scan mode. From the analysis of this sample, VX was identified at RT 7.3 min with a 72.9% NIST library search match quality. VX-Pyro (CAS number 32288-17-8, diethyl dimethyl pyrophosphonate, MW = 230 amu) was identified at RTs 5.6 and 5.8 min with a match quality of 98.6% and 98.5%, respectively. Additionally, VX-disulfide (CAS number 65332-44-7, bis(2-diisopropylaminoethyl) disulfide, MW = 320 amu) was identified at RT 8.7 min with a 59.6% match quality. VX-Pyro (NIST Library Entry number 226278) and VX-disulfide (NIST Library Entry number 226233) are known VX byproducts.[25] See Figure 45 for the TIC and Mass Spectra for these compounds. Note that in the absence of a quality standard material of known concentration, no follow-on method development was done.

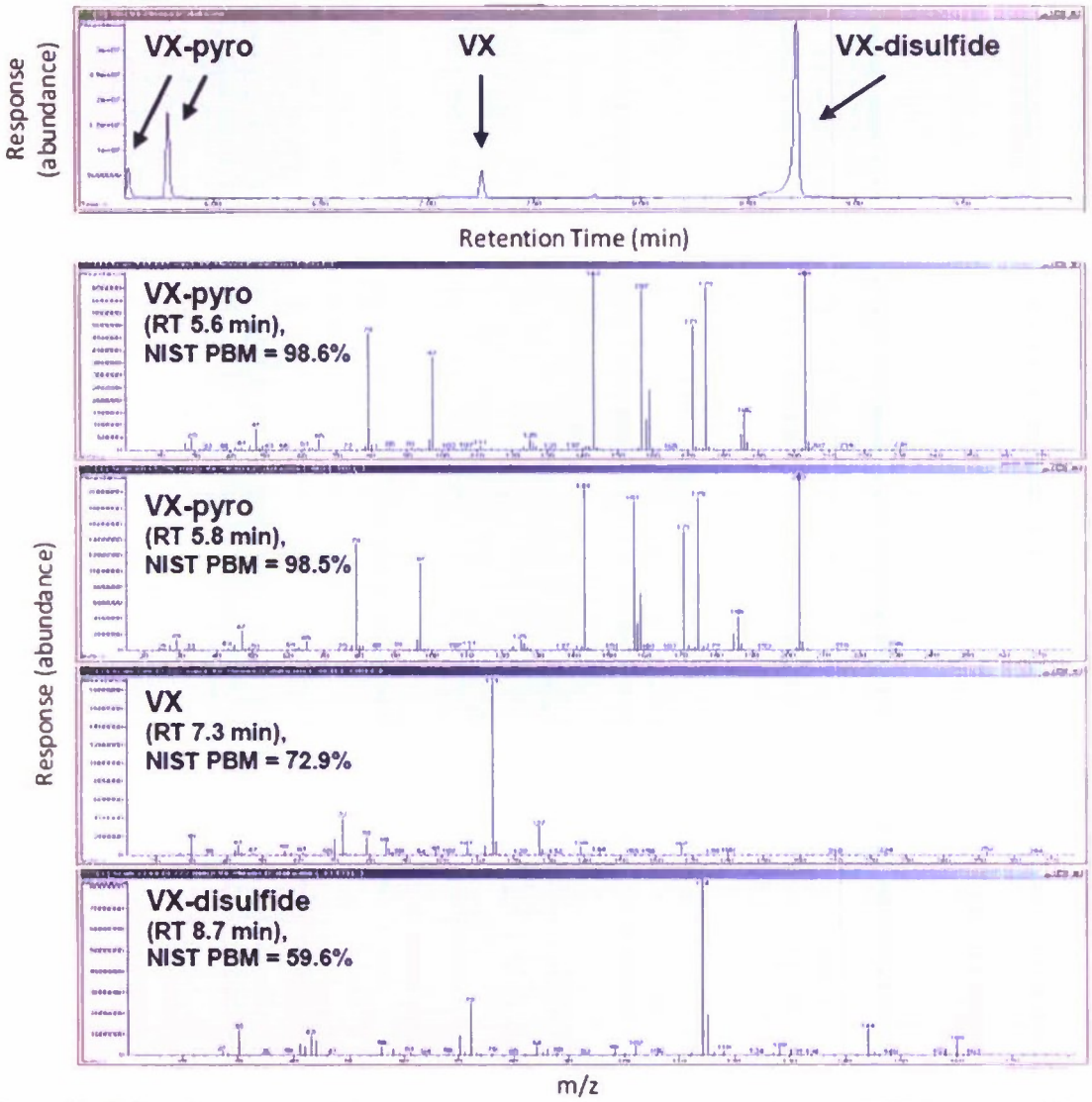


Figure 45. TIC and mass spectral data of additional VX byproducts identified by NIST library matching.

5.4 Final Method Refinement

As with all aspects of a quality system that undergoes continuous improvement, method development and validation continues to ensure that the methods used are the best, most robust methods, giving the greatest possible precision and accuracy available for the technology. The VX low-level methodology was refined after initial method development to offer more reliable analysis, an improved concentration range, better detection, and more confident data analysis.

The most notable refinement for the VX_ULL and VX_LL methods was the inclusion of the MRM transitions for EA2192. It was observed during byproduct method development that EA2192 detection was very similar to detection of VX. Given these similarities, and the fact that both analytes were detected by ESI in the positive ion mode, the methods were combined whereby the EA2192 MRM transitions were incorporated into the existing VX_ULL and VX_LL methods. Working with the VX_LL method, initial injections were made to evaluate the ability of the method to chromatographically resolve and detect both analytes. Method refinement was performed to ensure chromatographic resolution while

maintaining MS sensitivity. Method parameters were evaluated and refined, and consisted of adjusting method parameters and configuration to maintain detection capabilities and to allow good chromatographic separation and baseline resolution of the two analytes of interest. The most notable changes to the method during this refinement period included:

- Column type—a column with different dimensions (longer with more stationary phase) was chosen for chromatographic separation and to ensure good baseline resolution of the analytes of interest.

- LC flow rate—the flow rate of the mobile phase was adjusted after changing the analytical column to help achieve the chromatographic separation and to ensure good baseline resolution between the separated analytes.

- LC mobile phase composition—the organic solvent portion of the mobile phase was changed from acetonitrile to IPA to give better chromatographic performance (i.e., improved peak efficiency and reduced peak tailing).

Verification was also performed to ensure that there were no issues quantifying VX in the presence of EA2192. An example chromatogram demonstrating resolution of EA2192 (RT = 3.3 min) and VX (RT=4.0 min), as well as the ability of the method to detect varying concentrations of VX in the presence of EA2192, is shown in Figure 46.

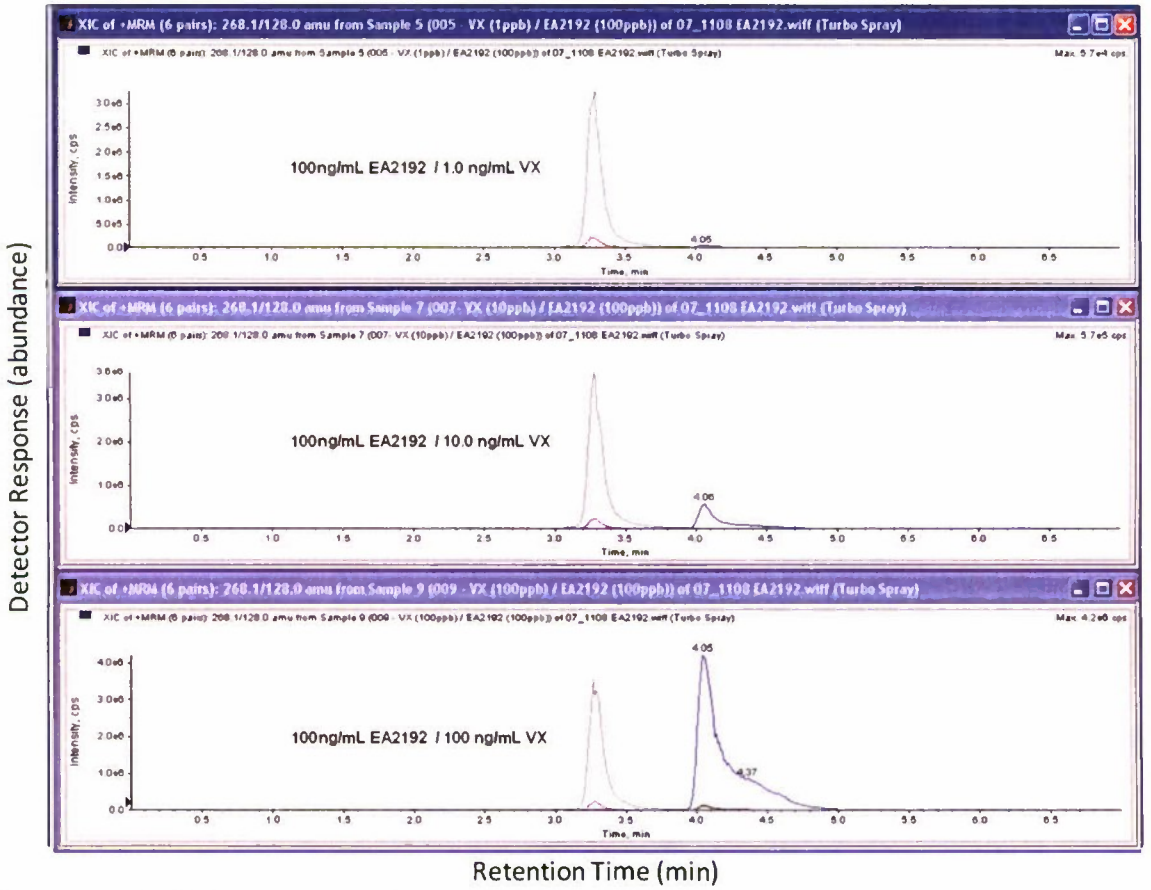


Figure 46. Method refinement example chromatogram showing VX (RT 4.0 min) and EA2192 (RT 3.3 min) at varying concentrations using method LCE VX_LL.dam.

Further method refinement included expanding the calibration range for VX_LL and adjusting the calibration curve weighting for both methods to $1/x^2$. For the VX_LL method, additional standards were added to the low end of the calibration range to give a larger dynamic calibration concentration range and to provide overlap with the VX_ULL method. The new calibration concentration range for VX_LL was 1–750 ng/mL. Both the VX_LL and the VX_ULL method calibration curve weights were $1/x^2$ to provide added confidence at the low end of the calibration curve. This was especially important for the VX_ULL method, which exceeded the program detection limit requirement of 0.45 ng/mL.

After completion of the method refinement, final method parameters were established. Appendix A contains the formal method write-up, the final method parameters, and a summary of the method capabilities.

5.4.1 Final Method Capabilities

After refinement was complete, the final method capabilities were examined. The final method capabilities were then used to determine the most appropriate methodology for decontamination test sample analysis.

5.4.1.1 Method LCE VX_ULL.dam — Final

Method Purpose and Performance Overview: The primary purpose of the LCE VX_ULL.dam method is to detect and quantify ultra-low concentrations of the chemical agent VX in liquid extraction samples. The secondary purpose of this method is to screen liquid extraction samples for EA2192, a hydrolysis byproduct of VX. This method was developed to exceed the low-level JPID03 ORD requirements. Based on the method development and validation work performed and documented herein, the LCE VX_ULL.dam extraction method detects and quantifies VX in IPA from 0.05 to 10 ng/mL, using an LC/MS/MS. The results of the extraction method exceeded the research target concentration of 0.45 ng/mL, established from the JPID03 ORD requirement of 0.005 mg/m^2. Additionally, this method can detect EA2192. No significant method interferents were identified during method development, validation, or subsequent interference testing. Sample throughput is approximately seven samples per hour.

Method Summary and Operational Details: The LCE VX_ULL.dam method is appropriate for liquid samples extracted with the solvent IPA (C_3H_8O; CAS number 67-63-0) and containing the chemical agent VX and/or the VX byproduct EA2192. The presence of EA2192 does not interfere with the ability of this method to detect and quantify VX. This method utilizes eight calibration standards at VX concentrations of ~0.05, 0.1, 0.2, 0.5, 1.0, 2.5, 5, and 10 ng/mL. The method use, detection limit, and quantitation limit are based on using the full standard set. The calibration standards are prepared in IPA. The analytical column is an Agilent Technologies Zorbax SB-C18 (4.6 mm inside diameter [ID] x 75 mm length [L], 3.5 µm particle size [part number 866953-902]). The mobile phase is a 50:50 mixture of the aqueous and organic phases. The organic phase consists of 95% IPA, 5% deionized water, 0.1% formic acid, and 5 mM ammonium acetate. The aqueous phase consists of 95% deionized water, 5% IPA, 0.1% formic acid, and 5 mM ammonium acetate. The method is conducted in an isocratic configuration.

The method performance for VX_ULL is:

- Quantitative Method
- Calibration Range: 0.05 to 10 ng/mL
- CCV levels: 0, 0.5, 2.5 ng/mL

- Calibration Model: Weighted quadratic regression
- Calibration Weighting: $1/x^2$
- LOD: 0.002 ng/mL
- LOQ: 0.006 ng/mL
- Sample Solvent: IPA
- Quantitation Ion Pair: VX 268.1/128

The method performance for EA2192 is as follows:

- Qualitative Method
- Sample Solvent: IPA
- Qualitative Ion Pair: EA2192 240.1/128

LOD and Quantitation and Measurement Uncertainty: Calibration standards were prepared and analyzed multiple times for calibration consistency and detection limit determination. The final method evaluation pulled calibration and CCV performance data sets from decontamination performance testing queues. The high concentration standard of the calibration set was followed by solvent blanks to determine method carryover. The carryover from the high-level calibration standard has no affect on the method or the ability of the method to detect and quantify the low end of the calibration range. The calibration range of 0.05–10 ng/mL appeared to show a linear response; however, it was determined that a quadratic fit provided the best results. This method used a weighted quadratic regression calibration model with an applied weighting of $1/x^2$. The application of $1/x^2$ weighting ensures accuracy at the lower levels of the calibration curve. Accuracy at the upper end of the calibration concentration range was maintained with the $1/x^2$ weighting. Accuracy at or below requirement levels was ideal to instill confidence that a requirement had been met. A demonstration of the LCE VX_ULL.dam calibration performance can be seen in Figure 47.

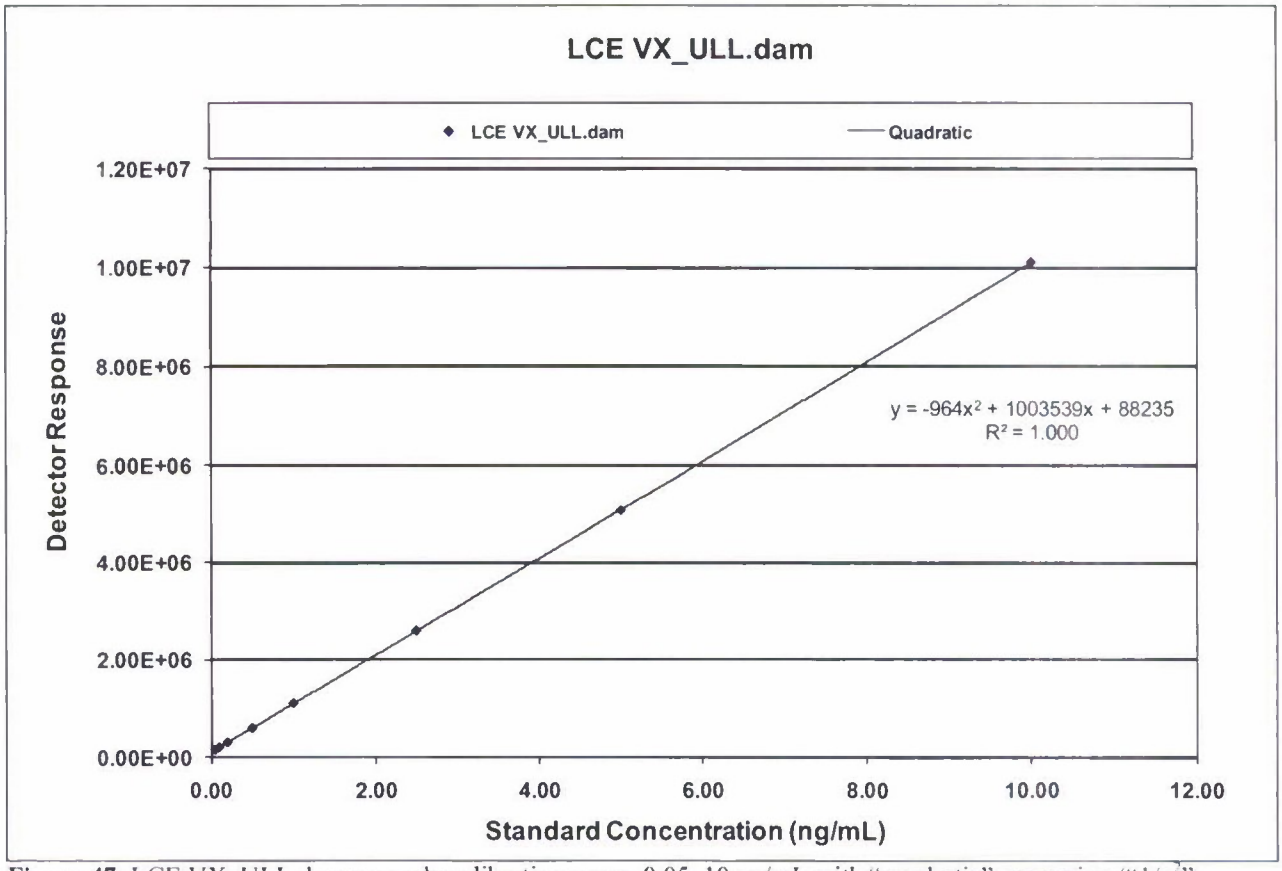


Figure 47. LCE VX_ULL.dam example calibration curve, 0.05–10 ng/mL with "quadratic" regression ("$1/x^2$" weighting).

The validation of the LCE VX_ULL.dam method yielded a calculated LOD of 0.002 ng/mL and an LOQ of 0.006 ng/mL. The precision of the method was determined by analysis of CCV samples analyzed at multiple levels, from multiple queues, over multiple days. There were 51 replicate analyses of the 0.5 ng/mL CCV, which produced an average reported concentration of 0.49 ng/mL, with a standard deviation of 0.03 and an RSD of 5.96%. Additionally, there were 51 replicate analyses of the 2.5 ng/mL CCV, which produced an average reported concentration of 2.48 ng/mL, with a standard deviation of 0.10 and an RSD of 4.18%. This indicated excellent reproducibility at the CCV levels within the calibration curve. Furthermore, there were eight replicate analyses of the 0.05 ng/mL low-level standard, which produced a standard deviation of 0.0006 and an RSD of 1.26%. This indicated excellent reproducibility at the lowest standard in the calibration curve. For the other standards, replicates were analyzed, producing standard deviations ranging from 0.0006 to 0.1566, and RSDs ranging from 0.82 to 4.83%, indicating excellent overall method performance.

The accuracy of the method is demonstrated in Table 16 as uncertainty in the measurements at each standard in the calibration curve, with 95% confidence. For a standard or sample, containing 0.5 ng/mL of analyte, the analytical result reported was 0.5 ± 0.02 ng/mL.

Table 16. List of VX_ULL.dam standard concentrations, their associated confidence interval, and calculated confidence bounds.

Standard Concentration (ng/mL)	95% Confidence Interval (ng/mL)	Lower Confidence Interval (ng/mL)	Upper Confidence Interval (ng/mL)
0.05	0.002	0.048	0.052
0.1	0.003	0.098	0.105
0.2	0.007	0.196	0.210
0.5	0.017	0.479	0.513
1.0	0.034	0.964	1.032
2.5	0.083	2.41	2.57
5.0	0.170	4.80	5.14
10	0.420	9.65	10.49

Based on the method development and validation work performed and documented herein, the VX extraction method (LCE VX_ULL.dam) detected and quantified VX in IPA from 0.05 to 10 ng/mL. This result exceeded the research target concentration of 0.45 ng/mL established from the JPID03 ORD requirement of 0.005 mg/m2.

5.4.1.2 Method LCE VX_LL.dam — Final

Method Purpose and Performance Overview: The primary purpose of the LCE VX_LL.dam method is to detect and quantify low-level concentrations of the chemical agent VX in liquid extraction samples. The secondary purpose of this method is to screen liquid extraction samples for EA2192, a hydrolysis byproduct of VX. This method was developed as a complementary method to the LCE VX_ULL.dam method, to allow VX decontamination testing at higher concentrations and within a larger concentration range than those used for the ULL method. The LCE VX_LL.dam method detected VX solution concentrations from 1 to 750 ng/mL. Additionally, this method detects EA2192. No significant method interferents were identified during method development, validation, or subsequent interference testing. Sample throughput was approximately seven samples per hour.

Method Summary and Operational Details: The LCE VX_LL.dam method is appropriate for liquid samples extracted with the solvent IPA (C_3H_8O; CAS number 67-63-0) and containing the chemical agent VX and/or the VX byproduct EA2192. The presence of EA2192 does not interfere with the ability of this method to detect and quantify VX. This method utilizes ten calibration standards at VX concentrations of ~1, 2.5, 5, 10, 25, 50, 100, 250, 500, and 750 ng/mL. The method use, detection limit, and quantitation limit are based on using the full standard set. The standards are prepared in IPA. The analytical column is an Agilent Technologies Zorbax SB-C18 (4.6 mm inside diameter [ID] x 75 mm length [L], 3.5 µm particle size [part number 866953-902]). The mobile phase is a 50:50 mixture of the aqueous and organic phases. The organic phase consists of 95% IPA, 5% deionized water, 0.1% formic acid, and 5 mM ammonium acetate. The aqueous phase consists of 95% deionized water, 5% IPA, 0.1% formic acid, and 5 mM ammonium acetate. The method is conducted in an isocratic configuration.

The method performance for VX_LL is as follows:

- Quantitative Method
- Calibration Range: 1–750 ng/mL
- CCV Levels: 0, 5, 50, 250 ng/mL

- Calibration Model: Weighted quadratic calibration model
- Calibration Weighting: $1/x^2$
- LOD: 0.17 ng/mL
- LOQ: 0.34 ng/mL
- Sample Solvent: IPA
- Quantitation Ion: VX 268.1/128

The method performance for EA2192 is as follows:

- Qualitative Method
- Sample Solvent: IPA
- Qualitative Ion: EA2192 240.1/128

LOD and Quantitation and Measurement Uncertainty: Calibration standards were prepared and analyzed multiple times for calibration consistency and detection limit determination. The final method evaluation pulled calibration and CCV performance data sets from decontamination performance testing queues. The high concentration standard of the calibration set was followed by solvent blanks to determine method carryover. The carryover from the high-level calibration standard has no affect on the method or the ability of the method to detect and quantify the low end of the calibration range. The calibration range of 1–750 ng/mL yielded a quadratic response. This method used a weighted quadratic calibration model with an applied weighting of $1/x^2$. The application of $1/x^2$ weighting ensures accuracy at the lower levels of the calibration curve. Accuracy at the upper end of the calibration concentration range was maintained with the $1/x^2$ weighting. A demonstration of the LCE VX_LL.dam calibration performance can be seen in Figure 48.

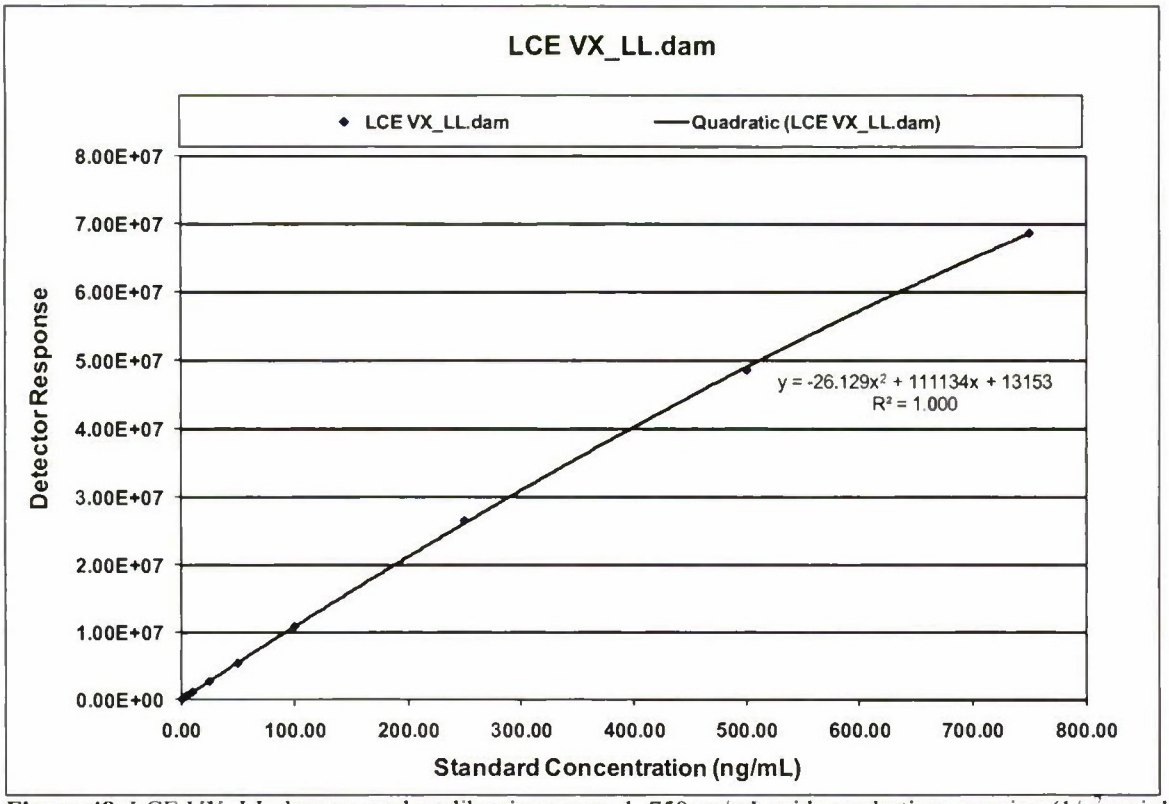


Figure 48. LCE VX_LL.dam example calibration curve, 1–750 ng/mL with quadratic regression ($1/x^2$ weighting).

The LCE VX_LL.dam method yielded a calculated LOD of 0.17 ng/mL and an LOQ of 0.34 ng/mL. The precision of the method was determined by analysis of CCV samples analyzed at multiple levels, from multiple queues, over multiple days. There were 52 replicate analyses of the 50 ng/mL CCV, which produced an average reported concentration of 52 ng/mL with a standard deviation of 1.73 and an RSD of 3.32%. Additionally, there were 52 replicate analyses of the 250 ng/mL CCV, which produced an average reported concentration of 256 ng/mL with a standard deviation of 11.18 and an RSD of 4.37%. This indicated very good reproducibility at the CCV levels within the calibration curve. Furthermore, there were six replicate analyses of the 1 ng/mL low-level standard, which produced a standard deviation of 0.01 and an RSD of 1.03%. This indicated very good reproducibility at the lowest standard in the calibration curve. For the other standards, replicates were analyzed, producing standard deviations ranging from 0.01 to 62.51 and RSDs ranging from 0.25 to 8.66% indicating excellent, overall method performance.

The accuracy of the method is demonstrated in Table 17 as uncertainty in the measurements at each standard in the calibration curve with 95% confidence. For a standard or sample, containing 5 ng/mL of analyte, the analytical result reported was 5 ± 0.02 ng/mL.

Table 17. List of VX_LL.dam standard concentrations, their associated confidence interval, and calculated confidence bounds.

Standard Concentration (ng/mL)	95% Confidence Interval (ng/mL)	Lower Confidence Interval (ng/mL)	Upper Confidence Interval (ng/mL)
1	0.09	0.91	1.09
2.5	0.07	2.43	2.57
5	0.02	4.98	5.02
10	0.07	9.93	10.07
25	0.37	24.63	25.37
50	0.85	49.15	50.85
100	1.80	98.20	101.80
250	4.63	245.37	254.63
501	10.01	490.99	511.01
751	17.26	733.74	768.26

5.4.1.3 Method LCE EMPA.dam — Final

Method Purpose and Performance Overview: The purpose of the LCE EMPA.dam method is to detect EMPA. EMPA is a hydrolysis byproduct of the chemical agent VX. This method was optimized to support the analysis of liquid extracts following the chemical agent decontamination performance evaluation testing. This testing was performed for contact sampler and coupon extracts for a 2 in. diameter test surface area, which were extracted in no greater than 20 mL of extraction solvent. No significant method interferents were identified during method development. Sample throughput was approximately seven samples per hour.

Method Summary and Operational Details: The approximate concentration range for this method is from 5 to 500 ng/mL. This method is appropriate for liquid samples extracted with the solvent IPA (C_3H_8O; CAS number 67-63-0) and suspected to contain the VX byproduct EMPA.

The method performance for EMPA is as follows:

- Qualitative Method
- Sample Solvent: IPA
- Quantitation Ion: 123/95

5.4.1.4 Method GCE VX_DEANS.M — Final

Method Purpose and Performance Overview: The primary purpose of the GCE VX_DEANS.M method is to detect and quantify the chemical agent VX in liquid extraction samples. This method complements the LCE VX methods with the ability to analyze extraction samples with higher expected concentrations than those allowed by the LCE methods. This method detects VX solution concentrations from 250 to 2000 ng/mL. No significant method interferents were identified. Sample throughput is approximately six samples per hour.

Method Summary and Operational Details: The GCE VX_DEANS.M method is appropriate for liquid samples containing the chemical agent VX and extracted with the solvent IPA (C_3H_8O; CAS number 67-63-0). This method utilizes five calibration standards at concentrations of

~250, 500, 750, 1000, and 2000 ng/mL VX. The method use, detection limit, and quantitation limit are based on using the full standard set. The standards are prepared in IPA.

The method performance for VX is as follows:

- Quantitative Method
- CCV Levels: 0, 500, 500 ng/mL
- Calibration Range: 250–2000 ng/mL VX
- Calibration Model: Weighted quadratic calibration model
- Calibration Weighting: $1/x^2$
- LOD: 38.8 ng/mL
- LOQ: 39.6 ng/mL
- Sample Solvent: IPA
- Quantitation Ion: 114

LOD and Quantitation and Measurement Uncertainty: Three sets of calibration standards were prepared. Each calibration preparation set was analyzed two times for calibration consistency and detection limit determination. The calibration range of 250–2000 ng/mL yielded a quadratic response. This method used a quadratic calibration model with no weighting. A demonstration of the GCE VX_DEANS.M calibration curve is shown in Figure 49.

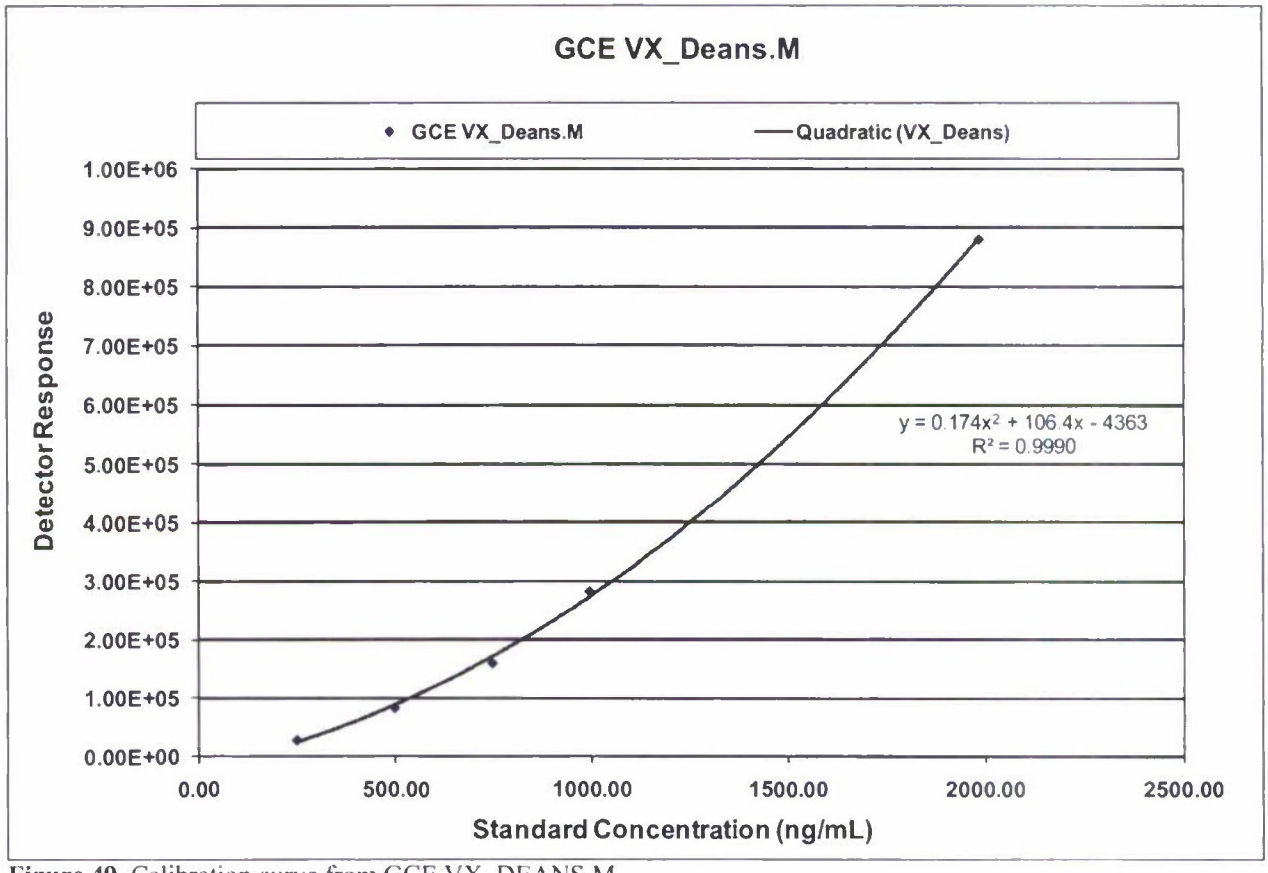


Figure 49. Calibration curve from GCE VX_DEANS.M.

The validation of the GCE VX_DEANS.M method yielded a calculated LOD of 38.9 ng/mL and an LOQ of 39.6 ng/mL. The precision of the method was determined by analysis of multiple CCV samples. Six replicate analyses of the 250 ng/mL standard produced an average reported concentration of 253.7 ng/mL with a standard deviation of 3.01 and an RSD of 1.19%, which indicated very good reproducibility at the lowest standard in the calibration curve. For the other standard concentrations, six replicates were analyzed, producing standard deviations ranging from 1.08 to 14.10 and RSDs ranging from 0.05 to 2.99% indicating very good overall method performance.

The accuracy of the method is demonstrated in Table 18 as uncertainty in the measurements at each standard in the calibration curve with 95% confidence. For a standard or sample, containing 500 ng/mL of analyte, the analytical result reported was 500 ± 87 ng/mL.

Table 18. List of VX_LL.dam standard concentrations, their associated confidence interval and calculated confidence bounds.

Standard Concentration (ng/mL)	95% Confidence Interval (ng/mL)	Lower Confidence Interval (ng/mL)	Upper Confidence Interval (ng/mL)
248	68.49	179.51	316.49
496	87.03	408.97	583.03
745	110.00	635.00	855.00
993	129.95	863.05	1122.95
1985	233.46	1751.54	2218.46

5.5 Method Demonstration for Decontaminant Performance Testing

The method demonstration is an example of method performance from the analysis of samples generated by chemical agent decontaminant testing.

5.5.1 LCE VX_ULL.dam Method Demonstration

The LCE VX_ULL.dam method was used to analyze extraction efficiency samples from polycarbonate coupon materials that were spiked with a known amount of the chemical agent VX and extracted with high purity IPA. This set of extraction efficiency samples were collected as part of test number EE9V13. The objective of EE9V13 was to show the extraction efficiency of the solvent IPA for VX. This test examined the ability of IPA to extract known amounts of the chemical agent VX from 2 in. coupons of polycarbonate material. Dose-confirmation or *tool* samples were collected by delivering agent directly into IPA solution. The purpose of the dose-confirmation samples was to normalize the known amount of agent being delivered by the tool. Tool samples were spiked, using the agent delivery tool, directly into solution in replicates of five for each concentration to be examined. These samples, upon analysis, allowed the determination of the actual known amount of agent delivered on the test materials.

The materials were spiked with the known amounts of VX and subsequently extracted with 20 mL of IPA. The expected concentration of the solution was determined from the known amount of agent (ng) delivered, divided by the amount of extraction solvent (mL). This expected concentration assumed 100% extraction efficiency. Upon analysis, the reported concentration was converted to a mass recovered (ng). The mass recovered was compared to the mass delivered to determine the calculated extraction efficiency. This test was performed in accordance with the extraction efficiency testing guidance set forth in the 2007 Source Document.[16]

Upon test completion, the samples were aliquoted into LC autosampler septa vials and analyzed on the LCE LC/MS/MS. The expected concentrations of the test samples spanned both LCE VX method ranges. Samples with expected concentrations of 0.10, 0.25, 1.65, and 5 ng/mL were analyzed on the LCE VX_ULL.dam method having a calibration concentration range of 0.05–10 ng/mL.

Method parameters were established on LCE in accordance with Appendix A. Prior to analysis of test samples, the instrument was calibrated using VX calibration standards prepared in high purity solvent. The LCE VX_ULL.dam calibration curve for this test had a correlation coefficient (r) value of 1.000. The calibration curve was established with a weighted quadratic regression curve fit ($1/x^2$). The calibration curve from this test is shown Figure 50. A summary of the method performance with calculated LOD and LOQ is shown in Table 19.

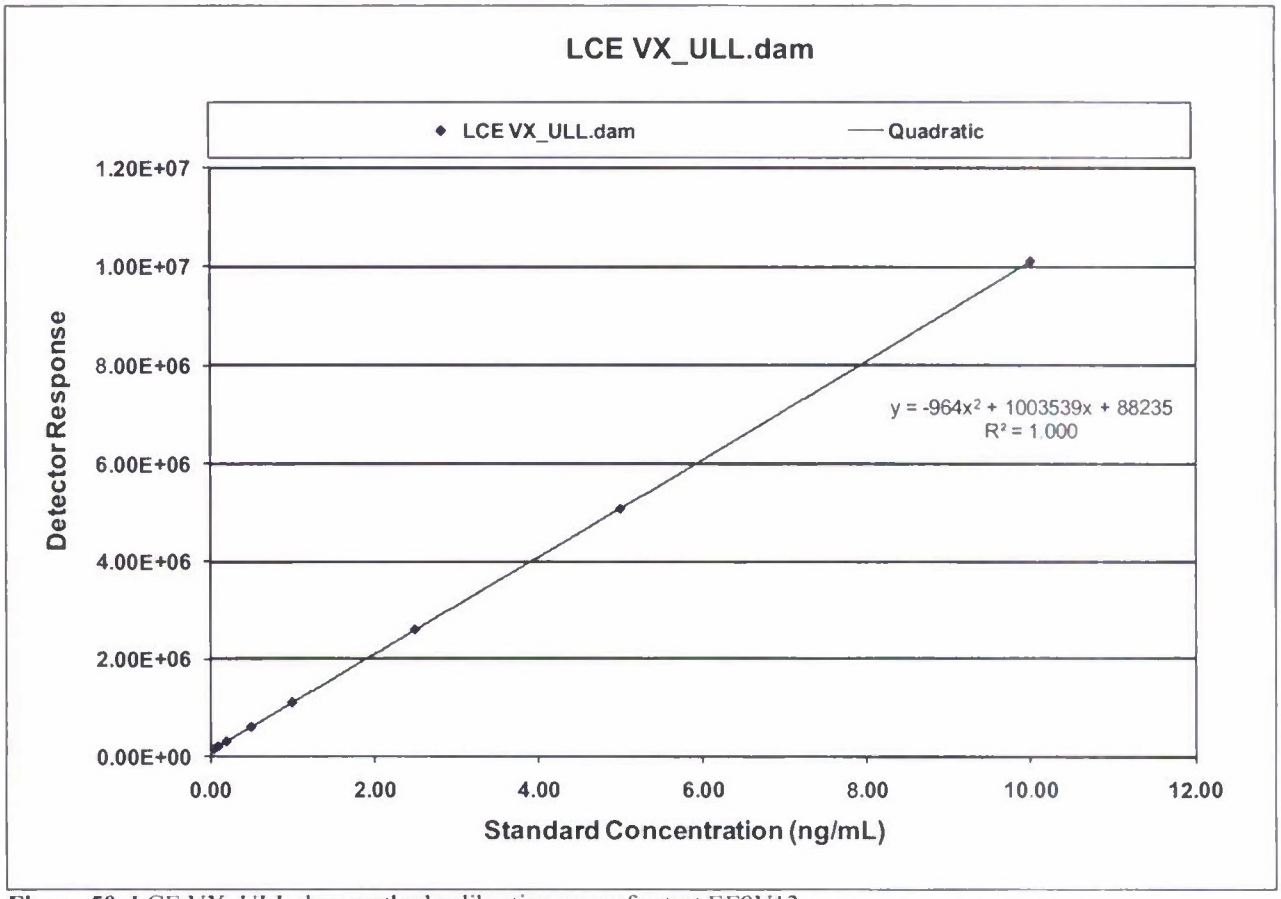


Figure 50. LCE VX_ULL.dam method calibration curve for test EE9V13.

Table 19. LCE VX_ULL.dam method summary for test EE9V13.

Method	LCE VX_ULL.dam
Test ID	EE9V13
Platform	LCE (LC/MS/MS)
Sample Type	Liquid
Analyte(s)	VX
LOD	0.002 ng/mL
LOQ	0.006 ng/mL
Data Acquisition	ESI MS/MS – MRM
Quantitation Ion	268>128 m/z

CCV samples were analyzed every tenth sample, and were found to be within ±30% of their expected concentrations. Solvent blanks were performed throughout the test for continuing baseline acceptance. Analysis of the blank samples showed no carryover or interferences. The sample queue, with analytical data, for the extraction efficiency samples tested with the LCE VX_ULL.dam method is shown in Figure 51. All samples were successfully analyzed using this LCE VX_ULL method.

Sample Name	Response	Reported Conc (ng/mL)
EE9V13_blankA	4.17E+04	BD
EE9V13_blankB	4.49E+04	BD
EE9V13_0.05std01	1.60E+05	0.05
EE9V13_0.1std01	2.12E+05	0.10
EE9V13_0.2std01	3.14E+05	0.20
EE9V13_0.5std01	6.07E+05	0.50
EE9V13_1std01	1.11E+06	1.00
EE9V13_2.5std01	2.60E+06	2.49
EE9V13_5std01	5.07E+06	4.97
EE9V13_10std01	1.01E+07	10.05
EE9V13_blankC	1.30E+04	BD
EE9V13_blank01	9.20E+03	BD
EE9V13_0.5ccv01	5.83E+05	0.47
EE9V13_2.5ccv01	2.56E+06	2.45
EE9V13_16.5kT-1	1.52E+06	1.41
EE9V13_16.5kT-2	2.03E+06	1.92
EE9V13_16.5kT-3	1.52E+06	1.41
EE9V13_16.5kT-4	1.51E+06	1.40
EE9V13_16.5kT-5	1.54E+06	1.43
EE9V13_1kT-1	1.56E+05	0.05
EE9V13_1kT-2	1.18E+05	0.01
EE9V13_1kT-3	1.11E+05	BD
EE9V13_1kT-4	1.04E+05	BD
EE9V13_1kT-5	1.06E+05	BD
EE9V13_blank02	9.47E+03	BD
EE9V13_0.5ccv02	5.83E+05	0.47
EE9V13_2.5ccv02	2.56E+06	2.45
EE9V13_2.5kT-1	2.90E+05	0.18
EE9V13_2.5kT-2	3.05E+05	0.19
EE9V13_2.5kT-3	3.11E+05	0.20
EE9V13_2.5kT-4	2.99E+05	0.19
EE9V13_2.5kT-5	2.93E+05	0.18
EE9V13_50kT-1	5.35E+06	5.25
EE9V13_50kT-2	5.68E+06	5.58
EE9V13_50kT-3	5.64E+06	5.54
EE9V13_50kT-4	5.57E+06	5.47
EE9V13_50kT-5	5.56E+06	5.46
EE9V13_blank03	1.24E+04	BD
EE9V13_0.5ccv03	5.97E+05	0.49
EE9V13_2.5ccv03	2.56E+06	2.45

Sample Name	Response	Reported Conc (ng/mL)
EE9V13_5kT-1	1.89E+06	1.78
EE9V13_5kT-2	5.96E+05	0.48
EE9V13_5kT-3	5.97E+05	0.49
EE9V13_5kT-4	5.79E+05	0.47
EE9V13_5kT-5	5.77E+05	0.47
EE9V13_01PC-RE5	1.07E+05	BD
EE9V13_02PC-RE5	1.08E+05	BD
EE9V13_03PC-RE5	1.11E+05	BD
EE9V13_04PC-RE5	9.99E+04	BD
EE9V13_05PC-RE5	1.00E+05	BD
EE9V13_blank04	6.56E+03	BD
EE9V13_0.5ccv04	5.92E+05	0.48
EE9V13_2.5ccv04	2.56E+06	2.45
EE9V13_06PC-RE5	2.84E+05	0.17
EE9V13_07PC-RE5	2.91E+05	0.18
EE9V13_08PC-RE5	2.95E+05	0.18
EE9V13_09PC-RE5	2.77E+05	0.17
EE9V13_10PC-RE5	2.70E+05	0.16
EE9V13_11PC-RES	5.58E+05	0.45
EE9V13_12PC-RE5	5.54E+05	0.44
EE9V13_13PC-RE5	5.56E+05	0.44
EE9V13_14PC-RES	5.52E+05	0.44
EE9V13_15PC-RE5	5.39E+05	0.43
EE9V13_blank05	7.64E+03	BD
EE9V13_0.5ccv05	5.79E+05	0.47
EE9V13_2.5ccv05	2.51E+06	2.40
EE9V13_16PC-RE5	1.44E+06	1.33
EE9V13_17PC-RE5	1.47E+06	1.36
EE9V13_18PC-RE5	1.41E+06	1.30
EE9V13_19PC-RE5	1.46E+06	1.35
EE9V13_20PC-RE5	1.39E+06	1.28
EE9V13_21PC-RES	5.50E+06	5.40
EE9V13_22PC-RE5	5.38E+06	5.28
EE9V13_23PC-RE5	5.28E+06	5.18
EE9V13_24PC-RE5	5.23E+06	5.13
EE9V13_25PC-RE5	5.38E+06	5.28
EE9V13_blank06	1.09E+04	BD
EE9V13_0.5ccv06	5.79E+05	0.47
EE9V13_2.5ccv06	2.53E+06	2.42

BD = Below Detect (LOD)

BQ = Below Quant (LOQ)

Figure 51. VX_ULL example performance data from test EE9V13.

5.5.2 LCE VX_LL.dam Method Demonstration

The LCE VX_LL.dam method was used to analyze decontamination performance testing samples from EPDM and tire rubber coupon materials that were spiked with a known amount of the chemical agent VX, treated with bleach decontaminant, and extracted with high purity IPA (C_3H_8O; CAS number 67-63-0). This set of samples was collected as part of test number POUVX02. One objective of POUVX02 was to evaluate the performance of bleach decontaminant on EPDM and tire rubber coupons. Dose-confirmation or *Tool* samples were collected by delivering agent directly into solution. The purpose of the dose-confirmation samples was to normalize the known amount of agent being delivered by the tool. Tool samples were spiked, using the agent delivery tool, directly into solution in replicates of five. These samples, upon analysis, allowed the determination of the actual known amount of agent delivered on the test materials.

The materials were spiked with VX at the contamination density required by the test, treated with decontaminant and subsequently extracted with 20 mL of IPA. The expected concentration of the solution was determined from the known amount of agent delivered (ng) and divided by the amount of extraction solvent (mL). Upon analysis, the found concentration was converted to a mass recovered (ng). The mass recovered was compared to the mass delivered to determine the calculated remaining agent. This test was performed in accordance with the panel testing guidance set forth in the 2007 Source Document.[16]

Upon test completion, the samples were aliquoted into septa vials, diluted as needed for the calibration range and set up on the LCE for analysis. The expected concentrations of the test samples fell within the calibration range (1–750 ng/mL) and were analyzed on the LCE VX_LL.dam method.

Method parameters were established on LCE in accordance with Appendix A. Prior to analysis of test samples, the instrument was calibrated using VX calibration standards prepared in high purity solvent. The LCE VX_LL.dam calibration curve for this test had a correlation coefficient (r) value of 1.000. The calibration curve was established with a weighted quadratic regression curve fit ($1/x^2$). The calibration curve from this test is shown Figure 52. A summary of the method performance with calculated LOD and LOQ is shown in Table 20.

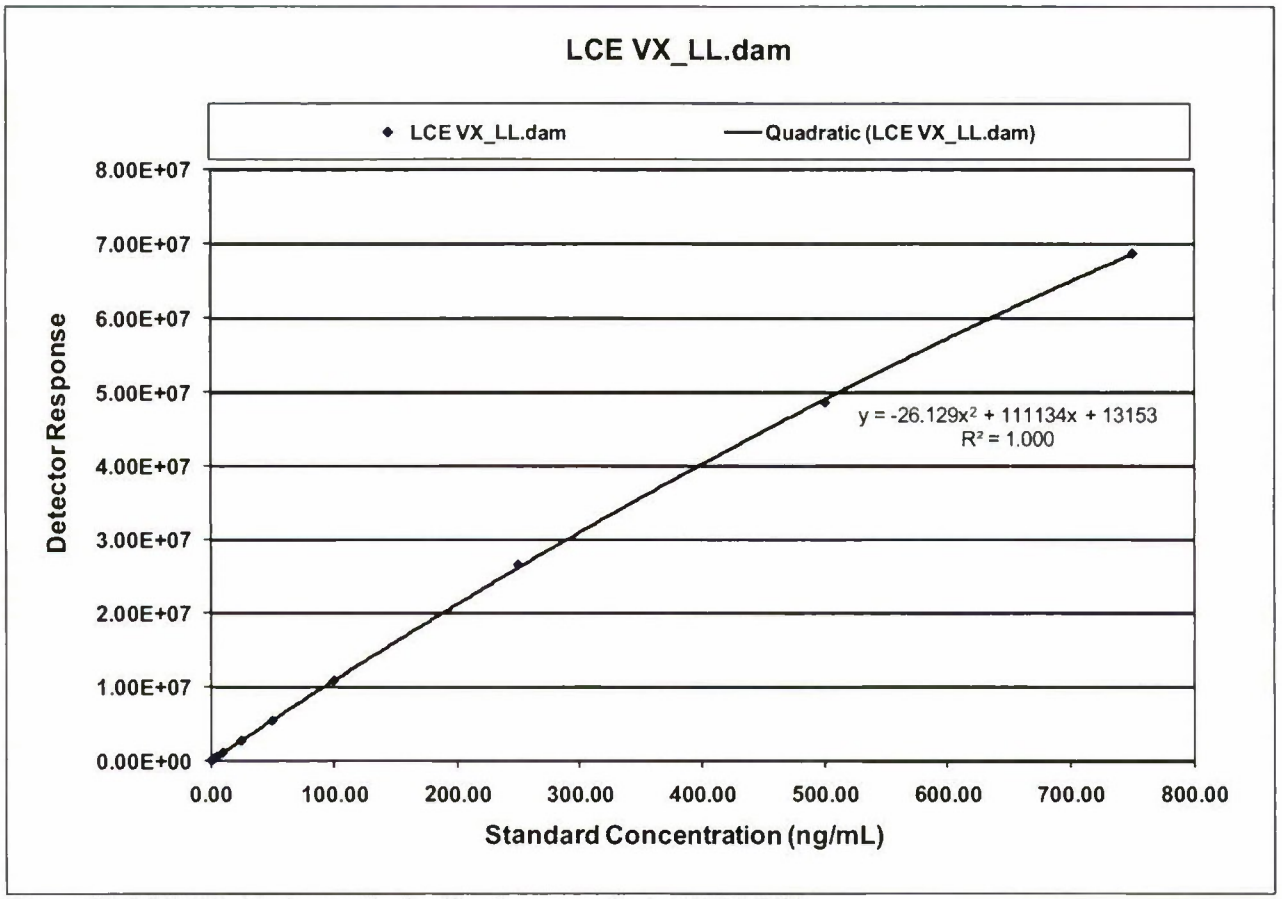


Figure 52. LCE VX_LL.dam method calibration curve for test POUVX02.

Table 20. LCE VX_LL.dam method summary for test POUVX02.

Method	LCE VX_LL.dam
Test ID	POUVX02
Platform	LCE (LC/MS/MS)
Sample Type	Liquid
Analyte(s)	VX
LOD	0.042 ng/mL
LOQ	0.188 ng/mL
Data Acquisition	ESI MS/MS – MRM
Quantitation Ion	268>128 m/z

Initial and continuing calibration verifications were performed every ninth sample, and were found to be within 30% of their expected concentrations. Solvent blanks were performed throughout the test for continuing baseline acceptance. Analysis of blank samples showed little carryover and no interferences.

Figure 53 provides the sample queue, with analytical data, for the performance testing samples analyzed with the LCE VX_LL.dam method. All samples were successfully analyzed using this LCE VX_LL method.

Sample Name	Response	Reported Conc (ng/mL)
POUVX02_blankA	4.51E+04	BQ
POUVX02_blankB	2.63E+04	BQ
POUVX02_1std01	1.23E+05	0.99
POUVX02_2.5std01	3.01E+05	2.59
POUVX02_5std01	5.61E+05	4.93
POUVX02_10std01	1.12E+06	9.97
POUVX02_25std01	2.75E+06	24.74
POUVX02_50std01	5.43E+06	49.26
POUVX02_100std01	1.09E+07	100.22
POUVX02_250std01	2.66E+07	254.28
POUVX02_501std01	4.87E+07	495.96
POUVX02_751std01	6.88E+07	752.77
POUVX02_blankC	1.16E+05	0.93
POUVX02_blank01	7.21E+04	0.53
POUVX02_50ccv01	5.58E+06	50.64
POUVX02_250ccv01	2.68E+07	256.33
POUVX02_1tool-1	2.14E+07	201.87
POUVX02_1tool-2	2.21E+07	208.85
POUVX02_1tool-3	2.10E+07	197.90
POUVX02_1tool-4	2.20E+07	207.85
POUVX02_1tool-5	2.10E+07	197.90
POUVX02_01EPDM-RES	6.61E+06	60.15
POUVX02_02EPDM-RES	6.17E+06	56.08
POUVX02_03EPDM-RES	4.73E+06	42.83
POUVX02_04EPDM-RES	5.86E+06	53.22
POUVX02_blank02	2.74E+04	BQ
POUVX02_50ccv02	5.55E+06	50.36
POUVX02_250ccv02	2.69E+07	257.35

Sample Name	Response	Reported Conc (ng/mL)
POUVX02_05EPDM-RES	5.93E+06	53.86
POUVX02_06EPDM-RES	4.64E+06	42.00
POUVX02_07EPDM-RES	2.13E+07	200.88
POUVX02_08EPDM-RES	2.19E+07	206.85
POUVX02_09EPDM-RES	2.25E+07	212.84
POUVX02_10EPDM-RES	2.11E+07	198.90
POUVX02_11EPDM-RES	2.34E+07	221.86
POUVX02_12EPDM-RES	2.23E+07	210.84
POUVX02_13TR-RES	2.74E+07	262.48
POUVX02_blank03	9.19E+04	0.71
POUVX02_50ccv03	5.76E+06	52.30
POUVX02_250ccv03	2.78E+07	266.59
POUVX02_14TR-RES	2.14E+07	201.87
POUVX02_15TR-RES	1.82E+07	170.33
POUVX02_16TR-RES	1.83E+07	171.31
POUVX02_17TR-RES	2.07E+07	194.93
POUVX02_18TR-RES	1.86E+07	174.24
POUVX02_19TR-RES	7.12E+06	64.87
POUVX02_20TR-RES	4.93E+06	44.66
POUVX02_21TR-RES	7.19E+06	65.52
POUVX02_22TR-RES	4.35E+06	39.34
POUVX02_blank04	3.74E+04	0.22
POUVX02_50ccv04	5.77E+06	52.39
POUVX02_250ccv04	2.72E+07	260.43

BD = Below Detect (LOD)

BQ = Below Quant (LOQ)

Figure 53. VX_LL example performance data from test.

6. ANALYSIS OF HD EXTRACTS

6.1 Decontamination Efficacy Requirement Documents

The method detection limit target was determined using the pertinent panel test parameters and the published requirement document levels. The calculations were based on a 20 mL extraction volume and a 0.00202 m^2 contaminated surface area (equivalent to a 2 in. diameter circular contamination area). The requirement document surface concentration levels for threshold (T) and objective (O) of primary interest at the time of this program are listed in Table 21. The JPID 2003 objective values are reported using only zeroes, which make scientific evaluations and calculations difficult. For each non-specific requirement value, a number 5 was added to the next decimal point to enable measurement and calculation. For example, "0.0" is shown as "0.05". The contaminant mass on coupon was calculated using the surface concentration × coupon area. The nominal extract concentration was calculated by dividing the mass on coupon by the extraction volume. The high end of the target detection range was set using a value that was 10% lower than the nominal extraction concentration. The low end of the target detection range was set at an order of magnitude below the high-end value. The

lowest range among the group of requirement levels was selected as the analytical method target detection range. The range selected for the analysis of decontamination testing for HD extract samples is shaded in Table 21.

Table 21. Decontamination testing detection targets for HD extract samples.

Requirement Document	Surface Conc. (mg/m^2)	Mass on Coupon (ng)	Nominal Extract Conc. (ng/mL)	Target Detection Range (ng/mL)*		
JPID 05 T[a]	3.00	6,060.0	303.0	272.7	to	27.27
JPID 05 O[a]	0.05	101.0	5.1	**4.5**	to	**0.45**
JSSED 03 O[a]	100.00	202,000.0	10,100.0	9,090	to	909
JSSED 04 T[b]	100.00	202,000.0	10,100.0	9,090	to	909
JSSED 04 O[b]	0.50	1,010.0	50.5	45.5	to	4.55

*The values shaded in green represent the selected target detection range for low-level method development.

6.2 Initial Method Development

Method development work for HD was performed on the instrument designated GCE as previously described. As method development began on GCE, the low-level detection requirements for HD were evaluated. The most stringent detection target was provided by the JPID03 ORD, which, after applying the program experimental parameters, yielded a target detection limit of 4.5 ng/mL. Initially, an NIST 2.0 library search was performed using the Agilent ChemStation software Enhanced Data Analysis. The search was performed for HD by CAS number (505-60-2) to obtain the NIST database entry giving the mass spectrum and major ions for this analyte. The NIST library entry number is 289463 and the 10 largest peaks for HD, per the NIST library, are detailed in Table 22.

Table 22. HD and byproduct mass spectra characteristics.

Compound	HD	Thiodiglycol (TDG)	Mustard Sulfone	Mustard Sulfoxide	2,2'-Sulfonyl-diethanol	Divinyl Sulfone
NIST No.	289463	229583	289499	273377	69985	229642
ID No.	65664	25714	75	25966	14537	35418
DB	mainlib	mainlib	mainlib	mainlib	mainlib	mainlib
Peak 1	109 (999)	61 (999)	27 (999)	63 (999)	45 (999)	75 (999)
Peak 2	111 (383)	45 (535)	63 (982)	27 (894)	27 (656)	27 (755)
Peak 3	63 (347)	104 (336)	28 (463)	76 (407)	93 (455)	66 (294)
Peak 4	27 (252)	91 (330)	65 (330)	65 (274)	43 (265)	47 (175)
Peak 5	158 (238)	47 (267)	26 (98)	47 (121)	29 (262)	59 (173)
Peak 6	160 (162)	60 (187)	62 (84)	59 (118)	63 (203)	43 (171)
Peak 7	47 (158)	31 (172)	92 (69)	45 (111)	41 (201)	69 (136)
Peak 8	45 (132)	46 (163)	64 (44)	26 (108)	66 (166)	76 (135)
Peak 9	65 (112)	27 (157)	127 (37)	112 (97)	31 (165)	26 (124)
Peak 10	59 (95)	44 (149)	128 (33)	83 (80)	65 (123)	60 (114)

After examining the mass spectrum for HD, the MSD was set up to simultaneously analyze in the SCAN and SIM modes of acquisition. Based on the ion relative abundances from the expected mass spectrum, the ions selected for the SIM mode were m/z 109 (base peak), 111, 158, and 160. Note that the ion at m/z 158 was the molecular ion, and represents the entire HD molecule (158 amu).

As with VX, initial method parameters were selected for the GC based on the properties of the analyte and sovent. All standards were prepared using a standard preparation dilution scheme similar to that described in Section 2.3. Initial injections were made with solvent blanks and a higher concentration HD standard (10,000 ng/mL). Solvent blanks were analyzed before and after the HD standard to ensure that there were no baseline issues that might interfere with the analyte of interest. The goals of injecting a higher concentration standard were to identify the correct peak via library matching, to determine RT, and to evaluate peak shape on the TIC. Evaluating peak shape (efficiency and resolution) allowed the method parameters to be altered for optimal chromatography. Typically, peaks on the TIC can be identified by the mass spectrum, which contains selected ions in certain relative abundances. As lower concentrations of the standard were evaluated to determine detection limit capabilities in comparison with the ORDs target detection limit, selected ion relative abundances were observed to deviate from the ideal. By initially identifying the correct peak via library matching, and noting the RT, identification of the peak was possible at much lower concentrations. When the ions of interest were not in their ideal relative abundances at these lower concentrations, analyte peaks could be identified by RT.

After analysis of the initial samples, the peak was successfully identified by PBM of the mass spectrum from the SCAN mode of acquisition with the HD entry in the NIST 2.0 library. PBM of a 10,000 ng/mL HD standard in high purity chloroform identified the analyte peak at 5.6 min as HD with a 97.6% match quality. The next highest match for the analyte peak only gave a 0.97% match quality. The analysis was repeated in triplicate with similar results, giving the conclusion that the analyte peak was correctly identified as HD. The mass spectrum for the HD in SIM mode of acquisition also showed the ions in the expected relative abundances. Note that the solvent blank did not show a chromatographic peak at the RT of the peak identified as HD. An example of the HD standard analyzed in the SIM and SCAN modes of acquisition on the MSD is provided in Figure 54. An example solvent blank chromatogram is provided in Figure 55.

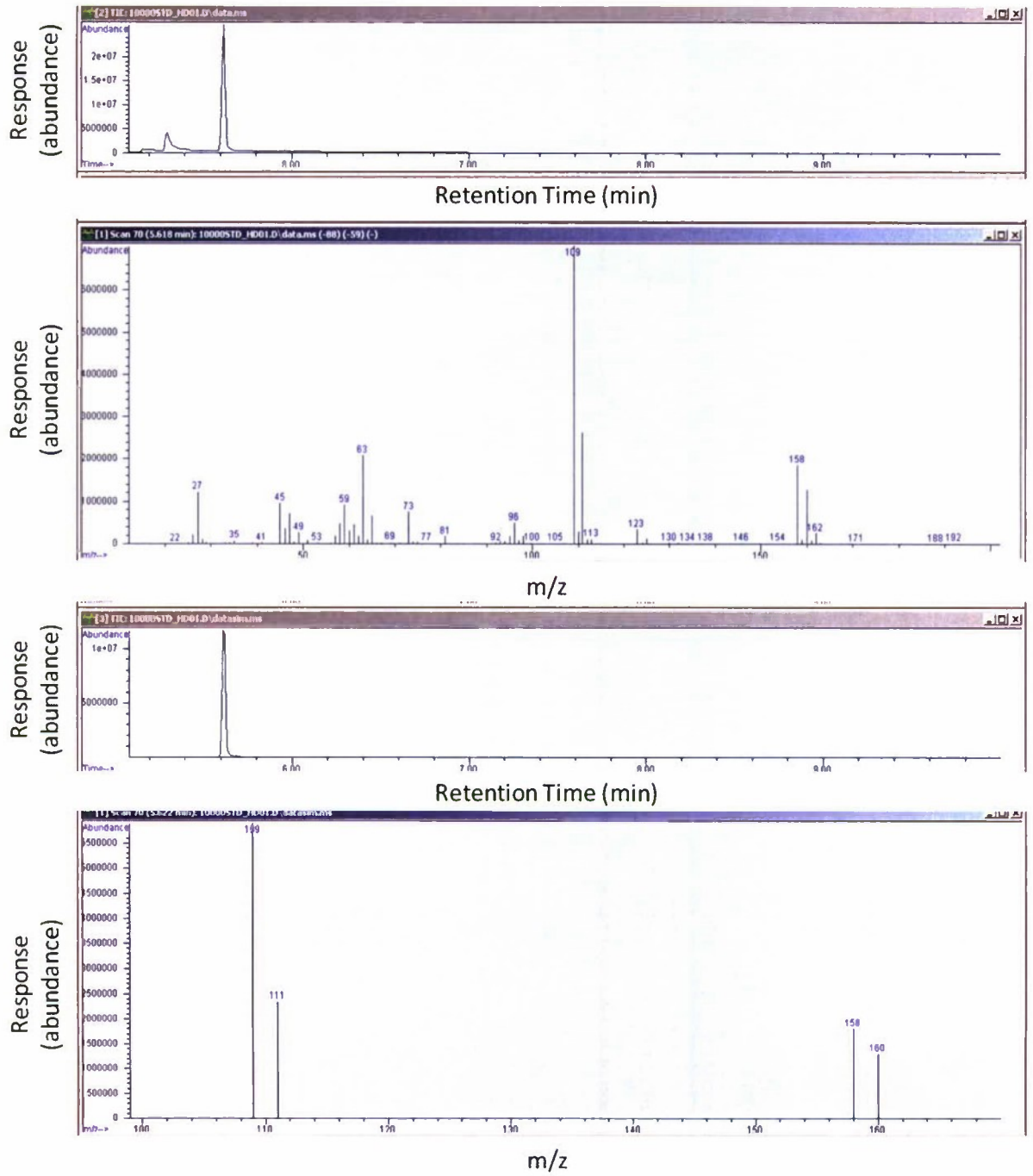


Figure 54. GC/MS scan and SIM analysis of 10,000 ng/mL HD on GCE.

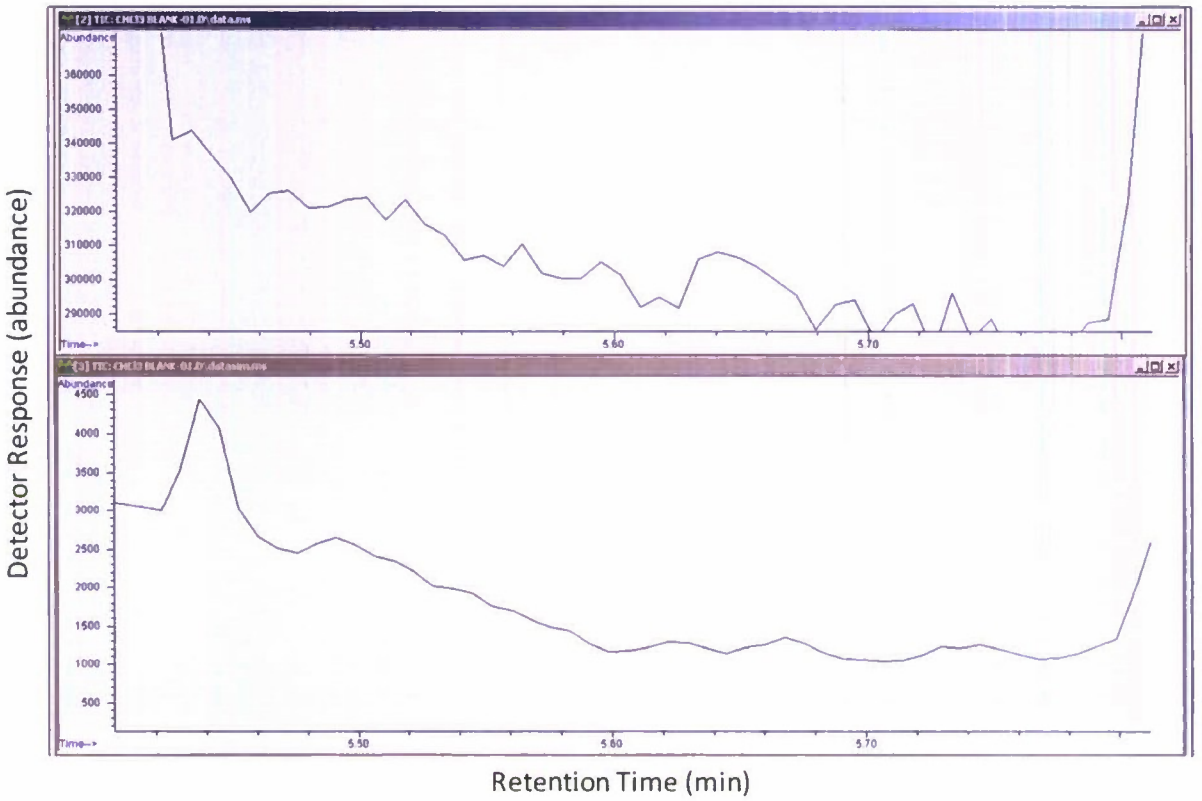


Figure 55. GC/MS scan and SIM analysis of a chloroform solvent blank on GCE zoomed at HD RT of 5.62 min.

Additional injections were made at decreasing concentrations in an attempt to identify the observed method detection limits. The detection limit capabilities for HD on the GCE were observed to be ~2 ng/mL while maintaining an acceptable SNR>3:1. The upper observed detection limit was not established due to the ability of the GC/MS to detect HD at concentrations significantly higher than the program requirements. A target method concentration range, spanning four orders of magnitude, was established at ~2–2000 ng/mL. This target range exceeds the low-level JPID03 ORD detection requirement (4.5 ng/mL) and provides a flexible method concentration range for sample analysis. When preparing liquid extraction samples throughout the contact testing, calculations were made to estimate the expected sample concentrations to be analyzed on the analytical instrumentation. It is beneficial to have the ability to detect a wide concentration range to maximize the effectiveness of testing, limit the number of samples to be run multiple times, and/or limit the need to dilute samples after the testing has occurred. This wide concentration range may span several orders of magnitude, and, therefore, a large number of calibration standards are required for confidence. Based on the method concentrations, it was initially decided that two methods would be required for HD on the GCE. There would be no difference in method parameters on the instrument; however, the method would have a dual purpose. The first would be as an ultra low-level (ULL) method that would exceed the detection limit requirements of the program. The second would be as a low-level (LL) method with a larger concentration range that would serve as a complement to the ULL method capabilities.

6.2.1 Initial Method Capabilities

With observed detection limits established, which exceeded the program requirements, initial capabilities of the HD methodology were assessed. Initial capabilities established a calculated LOD and LOQ as well as calibration curve fit and weighting.

6.2.1.1 Method GCE HD_DEANS.M (ULL) — Initial

The primary purpose of the GCE HD_DEANS.M (ULL) method was to detect and quantify ULL concentrations of the chemical agent HD in liquid extraction samples. This method was developed to exceed the low-level JPID03 ORD requirements. The JPID ORD for HD is 0.05 mg/m^2. Given the research parameters outlined in the Low-Level program, this value translates to a research target concentration of 4.5 ng/mL. The overall GCE HD_DEANS.M method will be used as either a ULL method with a concentration range spanning 2–50 ng/mL or as a LL method with a concentration range spanning 25–2000 ng/mL. No significant method interferents were identified during method development. Sample throughput was approximately four samples per hour.

The GCE HD_DEANS.M (ULL) method was appropriate for liquid samples containing the chemical agent HD and extracted with the solvent chloroform ($CHCl_3$; CAS number 67-66-3). The overall method utilized 13 calibration standards at concentrations of ~2, 5, 10, 25, 50, 100, 150, 200, 250, 500, 750, 1000, and 2000 ng/mL HD; however, the ULL method utilized five calibration standards at concentrations of ~2, 5, 10, 25 and 50 ng/mL. The method use, detection limit, and quantitation limit were based on using the five calibration standards for the ULL calibration range. The standards were prepared in chloroform.

The initial method performance for HD-ULL was as follows:

- Quantitative Method
- Calibration Range: ~2–50 ng/mL HD
- Calibration Model: Linear regression calibration model
- Calibration Weighting: None
- LOD: 1.50 ng
- LOQ: 4.56 ng
- Sample Solvent: Chloroform
- Quantitation Ion: 111

Calibration standards were prepared and analyzed eight times for calibration consistency and detection limit determination. The calibration range of 2–50 ng/mL yielded a linear response; therefore, this method used a linear regression calibration model. Accuracy at or below requirement levels was ideal to instill confidence that a requirement had been met.

The GCE HD_DEANS.M (ULL) method had an LOD of 1.50 ng/mL and an LOQ of 4.56 ng/mL. The precision of the method is determined by analysis of the multiple CCV samples analyzed. There were eight replicate analyses of the 2 ng/mL standard, which produced a standard deviation of 0.5 and an RSD of 23.07%. These values indicate acceptable reproducibility at the lowest standard in the calibration curve. For the other standards, eight replicates were analyzed, producing standard deviations ranging from 0.46–1.23 and RSDs ranging from 1.03 to 12.64%. The calibration curves were reproducible—the %RSD of the slope and y-intercept for eight calibration curves was 14.39% and 53.23%, respectively, and the correlation coefficients ranged from 0.9974 to 0.9995.

Based on the method development and validation work performed and documented herein, the GCE HD_DEANS.M (ULL) extraction method detected and quantified HD in chloroform

from 2 to 50 ng/mL. This result exceeded the research target concentration of 4.5 ng/mL established from the JPID03 ORD requirement of 0.05 mg/m2.

6.2.1.2 Method GCE HD_DEANS.M (LL) — Initial

The primary purpose of the GCE HD_DEANS.M (LL) method was to detect and quantify LL concentrations of the chemical agent HD in liquid extraction samples. This method was developed to complement the ULL calibration range, which exceeds the JPID03 ORD requirements. The overall GCE HD_DEANS.M method was used as either a ULL method with a concentration range spanning 2–50 ng/mL or as a LL method with a concentration range spanning 25–2000 ng/mL. No significant method interferents were identified during method development. Sample throughput was approximately four samples per hour.

The GCE HD_DEANS.M (LL) method was appropriate for liquid samples containing the chemical agent HD and extracted with the solvent chloroform ($CHCl_3$; CAS number 67-66-3). The overall method utilized thirteen calibration standards at concentrations of ~2, 5, 10, 25, 50, 100, 150, 200, 250, 500, 750, 1000, and 2000 ng/mL HD; however the LL method utilized ten calibration standards at concentrations of ~25, 50, 100, 150, 200, 250, 500, 750, 1000, and 2000 ng/mL HD. The method use, detection limit, and quantitation limit were based on using the ten calibration standards for the LL calibration range. The standards were prepared in chloroform.

The initial method performance for HD-LL was as follows:

- Quantitative Method
- Calibration Range: ~25–2000 ng/mL HD
- Calibration Model: Quadratic calibration model
- Calibration Weighting: None
- LOD: 4.62 ng
- LOQ: 14.00 ng
- Sample Solvent: Chloroform
- Quantitation Ion: 111

Calibration standards were prepared and analyzed eight times for calibration consistency and detection limit determination. The calibration range of 25–2000 ng/mL showed a quadratic response; therefore, the LL method used a quadratic calibration model with no weighting. This method had an LOD of 4.62 ng/mL and an LOQ of 14.00 ng/mL. The precision of the method is determined by analysis of the multiple CCV samples analyzed. Eight replicate analyses of the 25 ng standard produced a standard deviation of 1.40 and an RSD of 5.51%. For the other standards, eight replicates were analyzed producing standard deviations ranging from 1.90 to 14.49 and RSDs ranging from 0.10 to 8.04%. The calibration correlation coefficients ranged from 0.9994 to 0.9999.

6.3 HD Byproduct Method Development

The second overall program objective was to establish methods for the detection of common agent byproducts that could form during decontaminant testing. The purpose of these methods is to screen for common byproducts of chemical agents and confidently detect and identify the byproduct

analyte, when present. These methods are qualitative methods and serve as complementary methods to the fully quantitative agent methods.

Following the HD method development on the GCE, method development work began on the byproducts of HD. As previously discussed, there were three major byproducts of interest for this program. The first was TDG, the primary hydrolysis product of HD. Next, were mustard sulfoxide (H-sulfoxide) and mustard sulfone (H-sulfone), the two oxidation products of HD. The method development process for these byproducts followed the same general procedures as previously outlined for HD. The goal of method development for these analytes was to screen extraction samples for the presence of these byproducts. Therefore, it was important to be able to evaluate these analytes when diluted in chloroform, which was the same solvent as the HD extraction samples. All of these analytes had a mass spectrum available through parametric retrieval library searching, associated with the Agilent MSD ChemStation software. The ten largest ion peaks associated with these compounds are listed in Table 22. Standards were prepared by diluting them to the appropriate working concentration from stock standards. TDG was diluted from neat material. H-sulfoxide and H-sulfone were diluted from stock standards obtained from Dr. Phil Smith (SAIC) at ECBC. All three byproducts were analyzed separately on the GCE using generic methods configured with the appropriate SIM/SCAN parameters for the analyte of interest. Data analysis showed that TDG and H-sulfone were present as peaks that could be identified successfully by PBM. The H-sulfoxide peak was present on the resulting chromatogram; however, it did not give acceptable results by PBM. H-sulfone and TDG were further examined with the GCE, while the H-sulfoxide method development turned to the LCE.

H-sulfone looked very promising on the GCE system with very generic method parameters. One of the most desirable goals for byproduct detection and identification was to be able to screen for the byproduct using the same method as the chemical agent of interest. A major limiting factor when analyzing these byproducts was the detection capabilities of the GCE instrument. H-sulfone, upon initial identification and subsequent method development, appeared to have a detection concentration range very similar to that of HD. Given this observation, the GCE HD_DEANS.M method was adjusted to SCAN for H-sulfone. After the peak was identified, and the RT was noted (7.6 min), the method was set up to screen for H-sulfone in the SIM mode of acquisition. Additional injections were made at decreasing concentrations in an attempt to identify the observed method detection limits. The detection limit capabilities for H-sulfone on the GCE were observed to be ~10 ng/mL. H-sulfone performed very well in this method. The observed RT, along with the concentration range, made it a logical decision to develop a method that could screen for both HD and H-sulfone at the same time. The SIM parameters and timing events were adjusted, and standards were prepared that contained both analytes. The concentration of H-sulfone was held constant while the concentration of HD varied from 50 to 200 ng/mL. An example chromatogram with HD (RT 5.6 min) and H-sulfone (RT 7.6 min), analyzed within the same method, is shown in Figure 56.

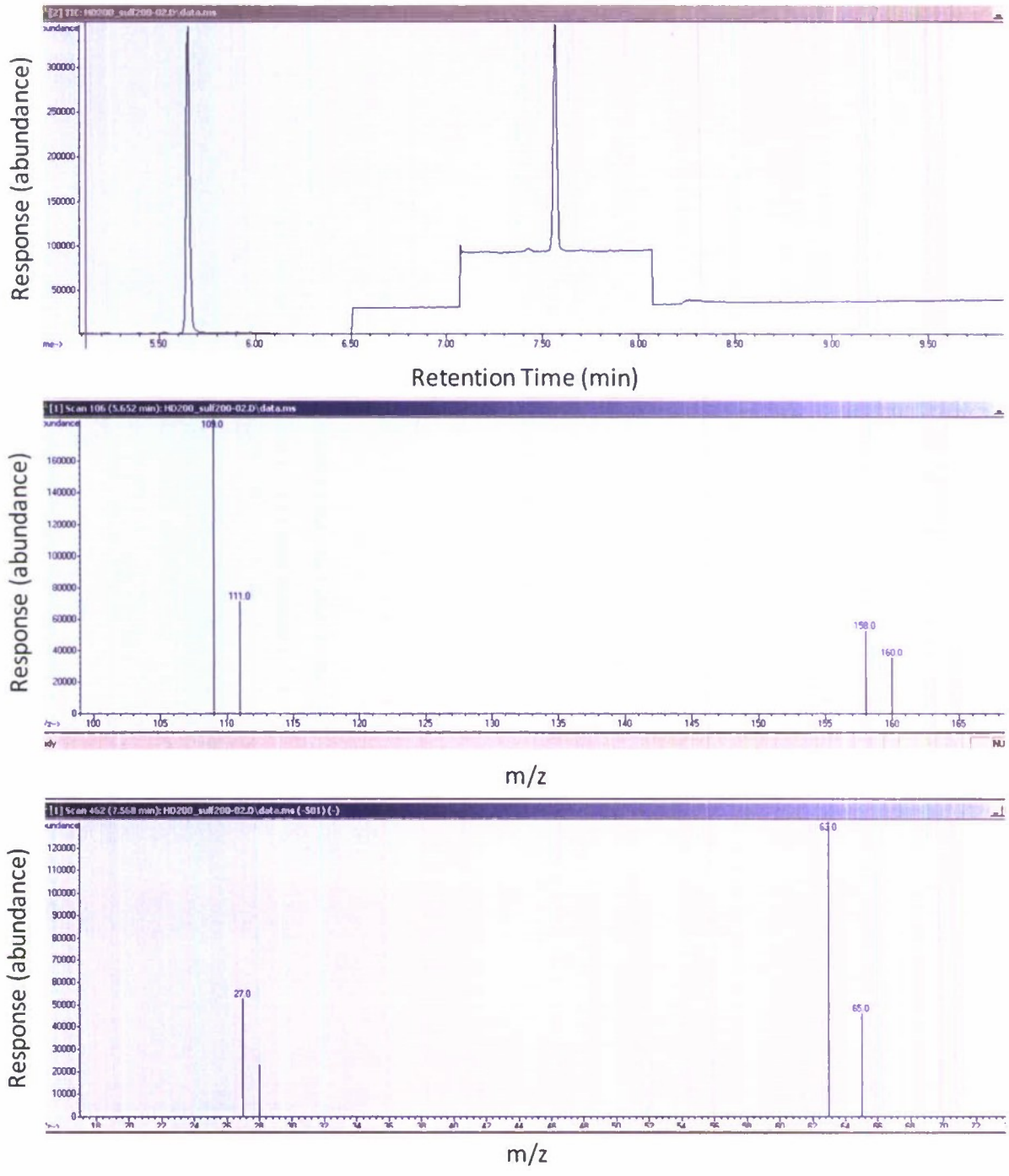


Figure 56. GC/MS analysis of HD (RT 5.6 min) and H-sulfone (RT 7.6 min) on GCE.

Because the purpose of the method was to screen for the H-sulfone as an addition to examining HD, it was important to determine if the detection and quantitation of the HD peak was affected by the presence of H-sulfone. These standards were run multiple times and, based on the results; the presence of H-sulfone did not affect the quantitation of HD. In summary, the GCE method allowed screening and identification of HD and H-sulfone as individual analytes, or when present in the same sample. Initial work has shown that H-sulfone does not affect the quantitation of HD; however, at this

time the method is a screening method only. Future work may determine the actual ability of the method to quantify HD in the presence of H-sulfone. HD analyzed at varying concentrations in the presence of H-sulfone is shown in Figure 57.

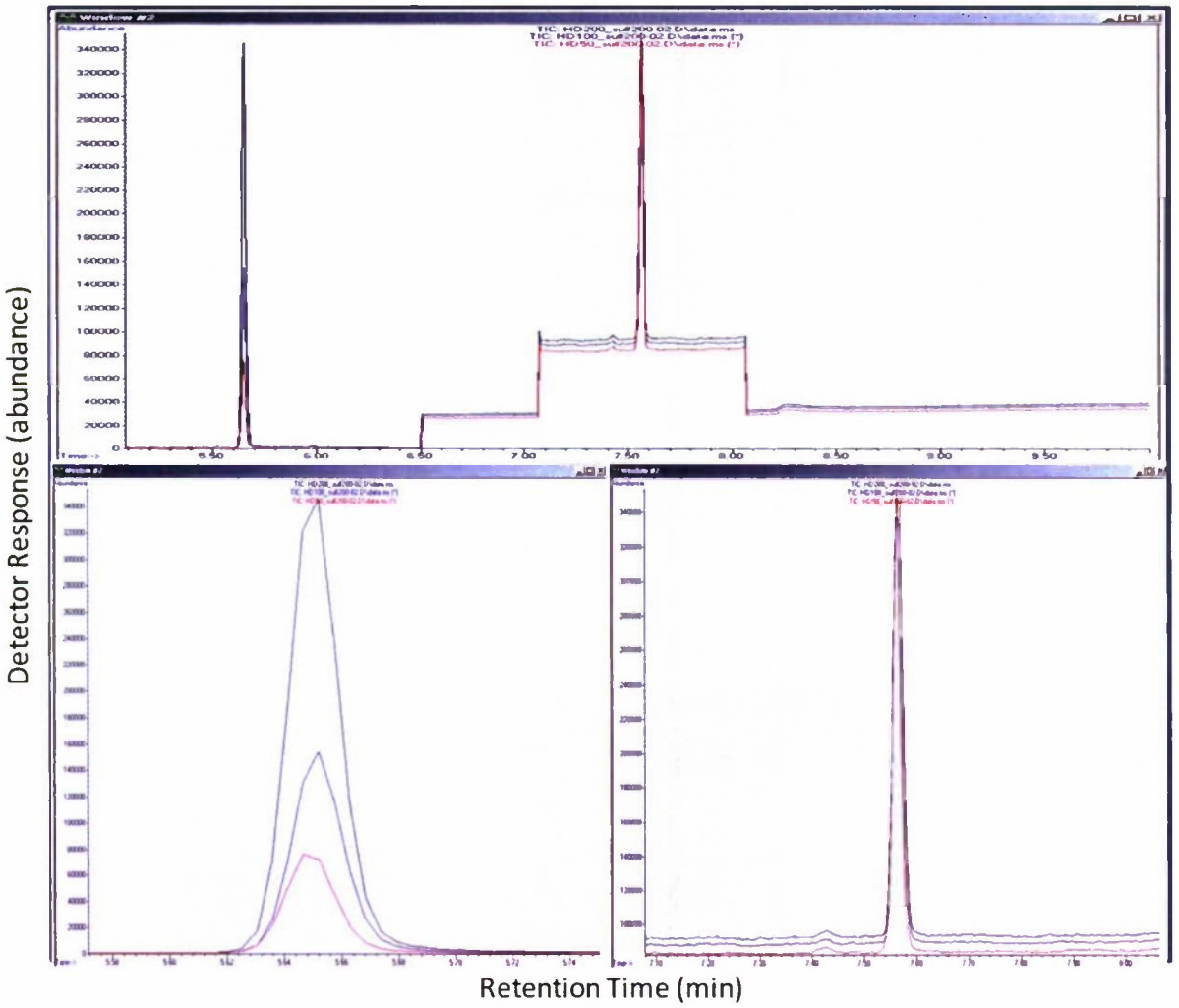


Figure 57. Overlays of three TICs from analyses containing HD (50–200 ng/mL) and H-sulfone (200 ng/mL) with zooms of the analyte peaks from each run.

Future work may determine the ability of the method to quantify H-sulfone through the generation of calibration curves. These curves would span an appropriate working range, using standards diluted in high purity solvent. Future method capabilities to quantify H-sulfone will be determined statistically and reported accordingly.

The TDG on the GCE with the generic method parameters showed a less than desirable chromatographic peak. Method parameters were adjusted for better chromatography, but it is important to note that, in comparison with HD, very high concentrations were required for TDG to elicit a peak. Once TDG was identified and the RT was determined (4.4 min), the method was updated to analyze in the SIM mode of acquisition. Additional injections were made at decreasing concentrations in an attempt to identify the observed method detection limits. The detection limit capability for TDG on the GCE system was observed to be ~25,000 ng/mL. The detection limitations of TDG on the GCE, as well as the method

parameters required for good peak shape, do not make TDG a good candidate to be screened in the presence of HD; therefore, TDG remained a stand-alone analyte in this method. An example chromatogram showing TDG (RT 4.4 min) analyzed on GCE is shown in Figure 58.

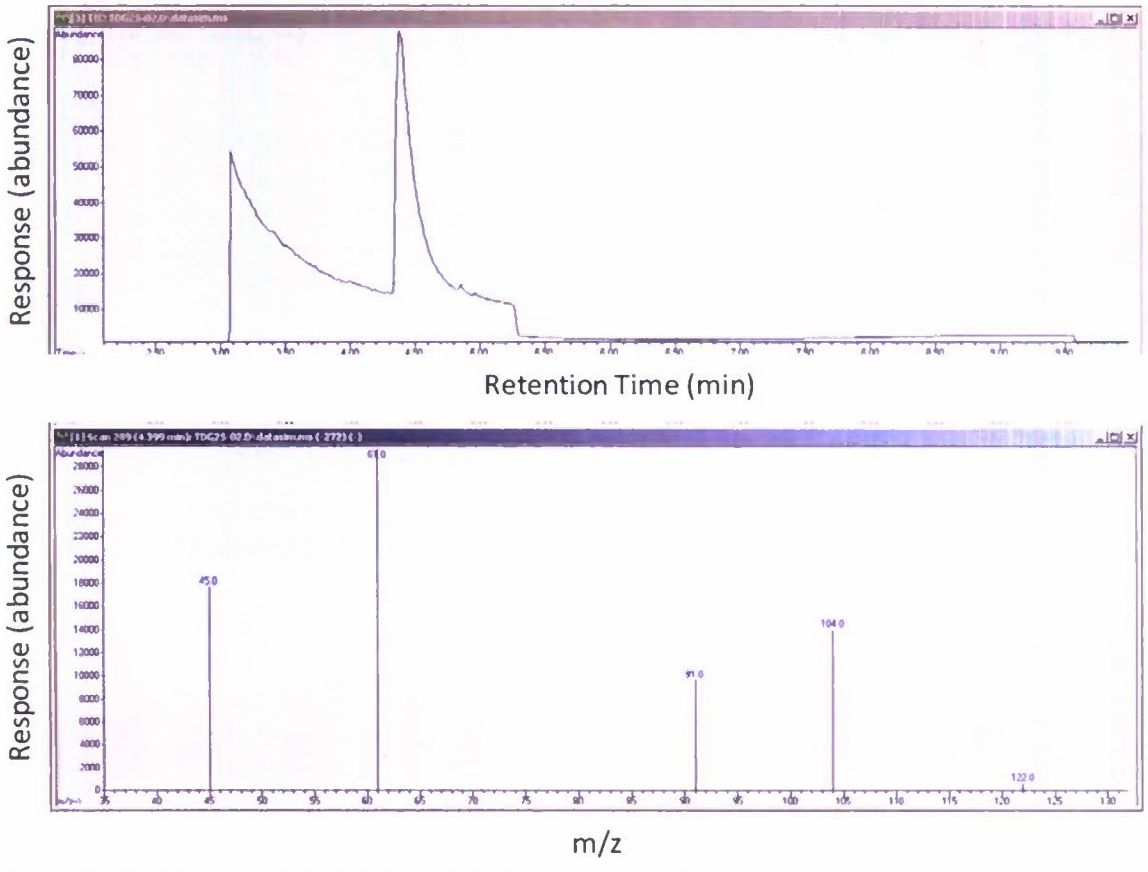


Figure 58. 25,000 ng/mL TDG (RT 4.4 min) TIC and mass spectrum.

Given the detection limits observed for this method, GCE may not be the most suitable instrument for screening TDG. Follow-on method development of TDG may be directed to LCE. Future work may determine the ability of the method to quantify TDG through the generation of calibration curves. The curves need to span an appropriate working range, using standards diluted in high purity solvent. Future method capabilities to quantify TDG will be determined statistically and reported accordingly.

H-sulfoxide method development and optimization was performed on LCE and commenced in much the same manner as previously described for the other LC/MS/MS analytes in this report. An ion at m/z 175, representing $[M+H]^+$ was present, which allowed an MRM method to be set up to detect the MS/MS ions 175/63, 175/95, and 177/63. After setting up the method and identifying the analyte RT via injection through the LC, additional injections were made at decreasing concentrations in an attempt to identify the observed method detection limits. The RT for H-sulfoxide on LCE was 4.5 min. The observed lower detection limit capability for H-sulfoxide on the LCE was ~10 ng/mL. The purpose of this method was to screen for and identify H-sulfoxide; therefore, no calibration range was determined. An example H-sulfoxide chromatogram from LCE is shown in Figure 59.

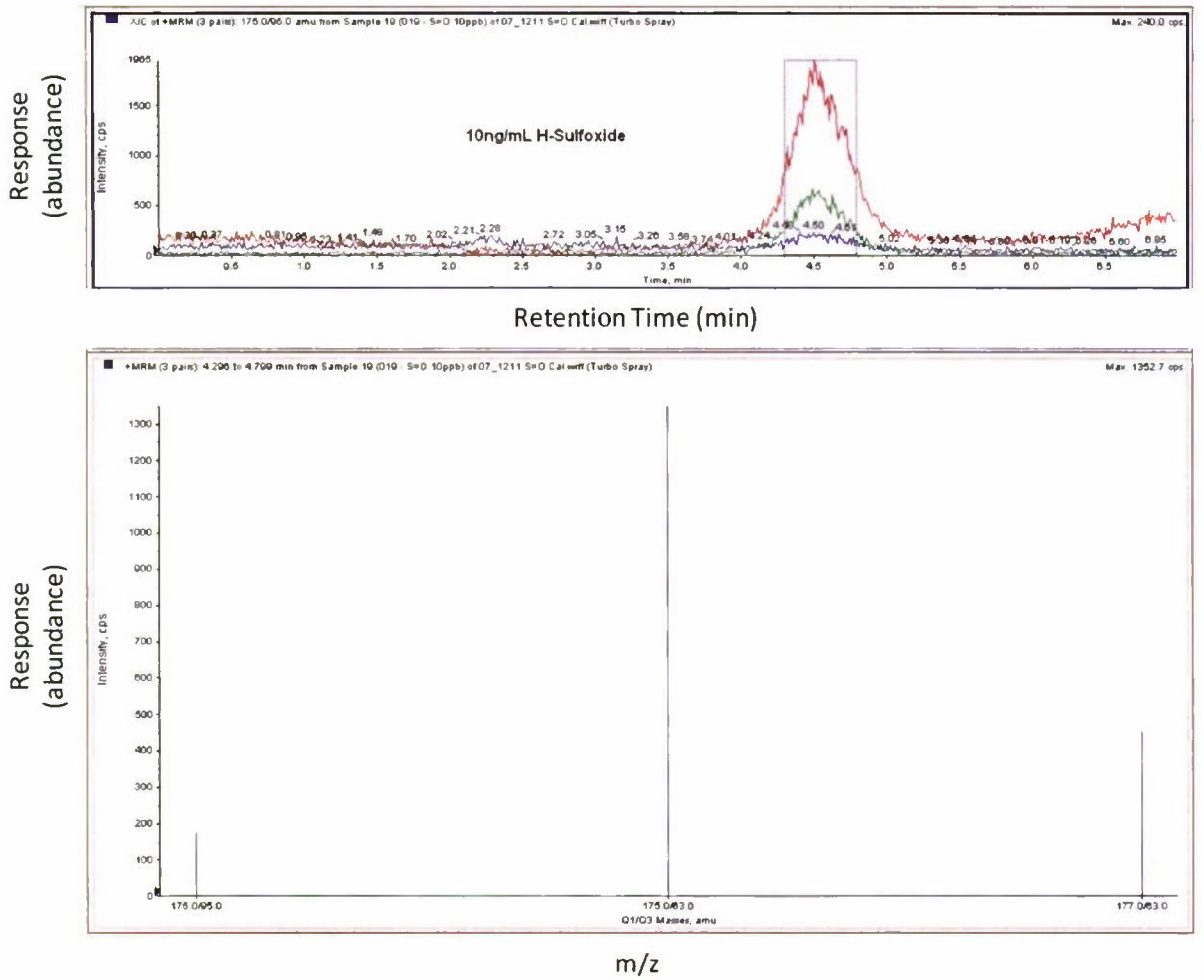


Figure 59. 10 ng/mL H-sulfoxide (RT 4.5 min) XIC with mass spectrum.

Future work may determine the ability of the method to quantify H-sulfoxide through generation of calibration curves. The curves need to span an appropriate working range using standards diluted in high purity solvent, in accordance with Section 2.3. Future method capabilities to quantify H-sulfoxide will be determined statistically and reported accordingly. Appendix A contains the formal method write-up. Further discussion of the capabilities of this method follows in Section 6.2.1.

6.4 Final Method Refinement

As with all aspects of a quality system that undergoes continuous improvement, method refinement and validation continues to ensure that the methods used are the best, most robust methods giving the greatest possible precision and accuracy available for the technology. The HD low-level methodology was refined after initial method development to offer more reliable analysis, an improved concentration range, better detection, and more confident data analysis.

After the initial method capabilities check of the HD_DEANS.M GCE methodology, slight method refinement occurred to improve the performance of the method across the entire concentration range of 2–2000 ng/mL. The goal in refinement of the HD_DEANS.M method was to establish method parameters that would allow the routine use of the method with the entire concentration range. This would eliminate the need to have two concentration ranges for the same method. As

developed, there was no difference in instrument method parameters for HD_ULL versus HD_LL. The only difference in the method was the calibration range utilized. Refining the method enabled the use of the HD_DEANS.M method across the entire calibration range. This was accomplished by refining the chromatographic parameters for improved performance at the low end of the calibration range, and by implementing curve weighting to the quadratic calibration model for increased confidence at the low end of the calibration range. The chromatographic parameters were refined to reduce the amount of baseline and to improve the SNR of the 2 ng/mL calibration standard. Baseline/background was improved by reducing the amount of chloroform solvent (and therefore matrix) that was entering the analytical column. This was accomplished by optimizing the solvent vent and splitless time parameters of the Gerstel CIS4 inlet.

After completion of the method refinement, final method parameters were established. Appendix A contains the formal method write-up, the final method parameters, and a summary of the method capabilities.

6.4.1 Final Method Capabilities

After refinement was complete, the final method capabilities were examined. The final method capabilities were then used to determine the most appropriate methodology for decontamination test sample analysis.

6.4.1.1 Method GCE HD_DEANS.M — Final

Method Purpose and Performance Overview: The primary purpose of the GCE HD_DEANS.M method is to detect and quantify the chemical agent HD in liquid extraction samples. This method was developed to exceed the low-level JPID03 ORD requirements. The JPID ORD for HD is 0.05 mg/m^2. Given the research parameters outlined in the Low-Level program, this value translates to a research target concentration of 4.5 ng/mL. No significant method interferents were identified during method development or subsequent interference testing. Sample throughput is approximately four samples per hour.

Method Summary and Operational Details: The GCE HD_DEANS.M method is appropriate for liquid samples containing the chemical agent HD and extracted with the solvent chloroform ($CHCl_3$; CAS number 67-66-3). This method utilizes 13 calibration standards at concentrations of ~2, 5, 10, 25, 50, 100, 150, 200, 250, 500, 750, 1000, and 2000 ng/mL HD. The method use, detection limit, and quantitation limit are based on using the full standard set. The standards are prepared in chloroform.

The final method performance for GCE HD_DEANS.M is as follows:

- Quantitative Method
- Calibration Range: 2–2,000 ng/mL HD
- CCV Levels: 0, 10, 150, 750 ng/mL
- Calibration Model: Weighted quadratic calibration model
- Calibration Weighting: $1/x^2$
- LOD: 0.37 ng/mL
- LOQ: 1.23 ng/mL

- Sample Solvent: Chloroform
- Quantitation Ion: 111

LOD and Quantitation and Measurement Uncertainty: Calibration standards were prepared and analyzed seven times for calibration consistency and detection limit determination. The final method evaluation pulled calibration and CCV performance data sets from decontamination performance testing queues. The high concentration standard of the calibration set was followed by solvent blanks to determine method carryover. Slight carryover was observed following the high concentration calibration standard; however, the carryover from the high-level calibration standard has no affect on the method or the ability of the method to detect and quantify the low end of the calibration range. The calibration range of 2–2000 ng/mL yielded a quadratic calibration response. This method used a weighted quadratic calibration model ($1/x^2$). Accuracy at the upper end of the calibration concentration range was maintained with the $1/x^2$ weighting. Accuracy at or below requirement levels was ideal to instill confidence that a requirement had been met. A demonstration of the GCE HD_DEANS.M calibration performance can be seen in Figure 60.

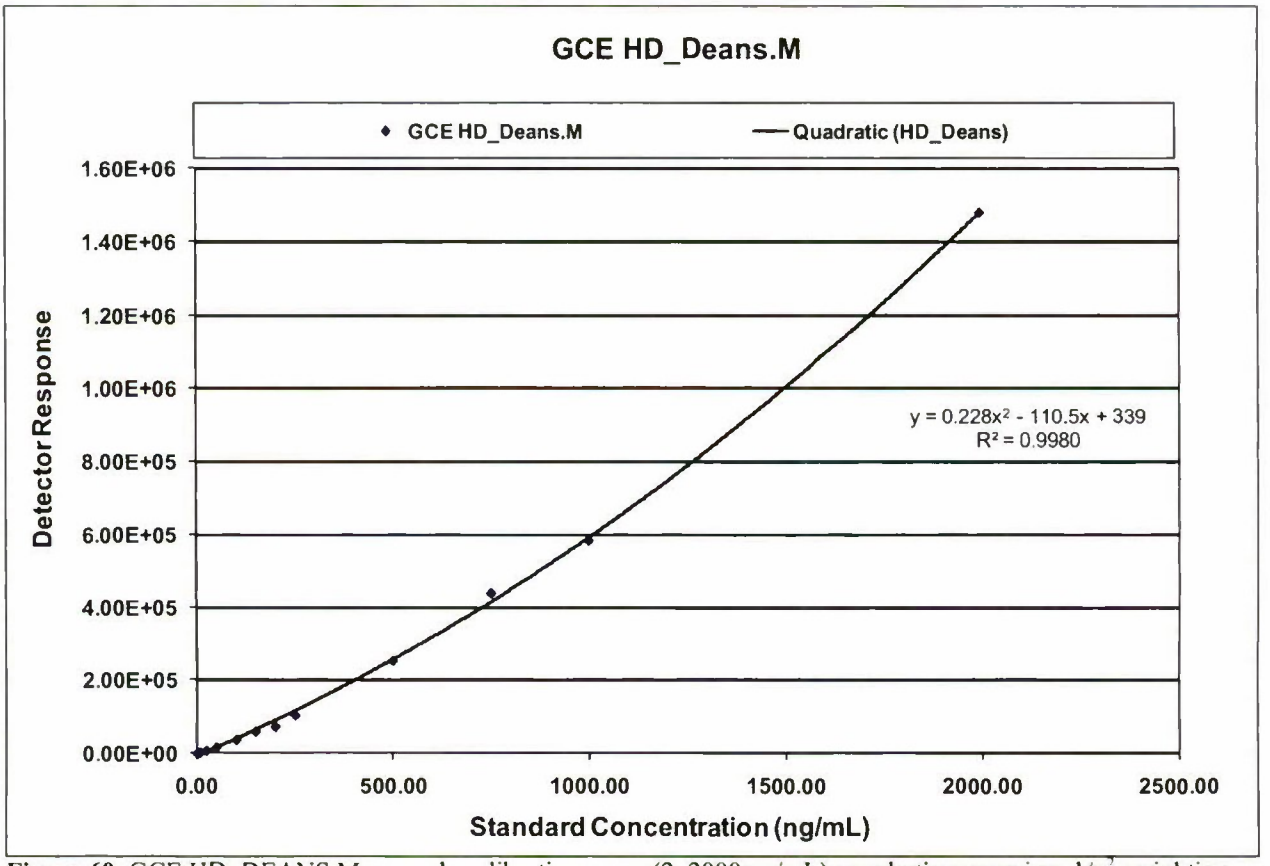


Figure 60. GCE HD_DEANS.M example calibration curve (2–2000 ng/mL); quadratic regression, $1/x^2$ weighting.

The GCE HD_DEANS.M method yielded a calculated LOD of 0.37 ng/mL and an LOQ of 1.23 ng/mL. The precision of the method was determined by analysis of CCV samples analyzed at multiple levels, from multiple queues over multiple days. There were 67 analyses of the 10 ng/mL CCV, which produced an average reported concentration of 8.9 ng/mL with a standard deviation of 1.08 and an RSD of 12.18%. There were 67 analyses of the 150 ng/mL CCV, which produced an average reported

concentration of 148 ng/mL with a standard deviation of 17.56 and an RSD of 11.87%. Finally, there were 67 analyses of the 750 ng/mL CCV, which produced an average reported concentration of 797 ng/mL with a standard deviation of 67.40 and an RSD of 8.46%. This indicated good reproducibility at the CCV levels within the calibration curve. Furthermore, there were seven replicate analyses of the 2 ng/mL standard, which produced a standard deviation of 0.12 and an RSD of 5.57%. This indicated very good reproducibility at the lowest standard in the calibration curve and a marked improvement compared to the initial method capabilities of the HD ULL method. For the other standards, multiple replicates were analyzed, producing standard deviations ranging from 0.38 to 25.73 and RSDs ranging from 0.81 to 13.16%, indicating very good overall method performance.

The accuracy of the method is demonstrated in Table 23 as uncertainty in the measurements at each standard in the calibration curve with 95% confidence. For a standard or sample, containing 10 ng/mL of analyte, the analytical result reported was 10 ± 2.06 ng/mL.

Table 23. List of HD_DEANS.M standard concentrations, their associated confidence interval, and calculated confidence bounds.

Standard Concentration (ng/mL)	95% Confidence Interval (ng/mL)	Lower Confidence Interval (ng/mL)	Upper Confidence Interval (ng/mL)
2	0.78	1.22	2.78
5	1.21	3.79	6.21
10	2.06	7.94	12.06
25	4.69	20.31	29.69
50	9.05	40.95	59.05
100	17.64	82.36	117.64
150	26.07	123.93	176.07
200	34.36	165.64	234.36
250	42.51	207.49	292.51
499	81.92	417.08	580.92
749	120.96	628.04	869.96
998	161.02	836.98	1159.02
1996	352.01	1643.99	2348.01

Based on the method development and validation work performed and documented herein, the HD extraction method (GCE HD_DEANS.M) detected and quantified HD in chloroform from 2 to 2,000 ng/mL. This result exceeded the research target concentration of 4.5 ng/mL established from the JPID03 ORD requirement of 0.05 mg/m2.

6.4.1.2 Method GCE H-Sulfone_DEANS.M — Final

Method Purpose and Performance Overview: The primary purpose of the GCE H-sulfone_DEANS.M method is to detect and identify the chemical agent HD and H-sulfone, the oxidation byproduct of HD, in liquid extraction samples. This method was developed to screen samples and identify the analytes of interest when present independently or within the same sample. This method detected and identified HD solution concentrations from ~2 to 2000 ng/mL. This method can identify H-sulfone solution concentrations from ~10 to 2000 ng/mL. No significant method interferents were identified during method development or subsequent interference testing. Sample throughput is approximately four samples per hour.

Method Summary and Operational Details: The GCE H-sulfone_DEANS.M method is appropriate for liquid samples containing the chemical agent HD and/or the HD byproduct H-sulfone and extracted with the solvent chloroform ($CHCl_3$; CAS number 67-66-3). This is a byproduct screening method and does not have an associated calibration range. This method allows screening and identification of HD and H-sulfone as individual analytes or when present in the same sample. Testing showed that detection of HD was not affected by the presence of H-sulfone.

The method performance for H-Sulfone is as follows:

- Qualitative Method
- Sample Solvent: Chloroform
- Quantitation Ion: 63

6.4.1.3 Method GCE TDG_DEANS.M — Final

Method Purpose and Performance Overview: The primary purpose of the GCE TDG_DEANS.M method is to detect and identify the hydrolysis byproduct of HD, TDG, in liquid extraction samples. This method was developed to screen samples and identify the analyte of interest. This method detected and identified TDG solution concentrations from ~25,000 to 100,000 ng/mL. Given the detection limits observed for this method, GC analysis may not be the most suitable platform for screening TDG. Due to the large concentrations required, this method is not appropriate for analyzing HD and TDG simultaneously. No significant method interferents were identified. Sample throughput was approximately five samples per hour.

Method Summary and Operational Details: The GCE TDG_DEANS.M is a byproduct screening method and does not have an associated calibration range. This method is appropriate for liquid samples containing the HD byproduct TDG and extracted with the solvent chloroform ($CHCl_3$; CAS number 67-66-3).

The method performance for TDG is as follows:

- Qualitative Method
- Sample Solvent: Chloroform
- Quantitation Ion: 61

6.4.1.4 Method LCE H-Sulfoxide.dam — Final

Method Purpose and Performance Overview: The purpose of the LCE H-Sulfoxide.dam method is to detect H-sulfoxide. H-sulfoxide is an oxidation byproduct of the chemical agent HD. This method was developed to screen samples and identify the analyte of interest. The approximate concentration range for this method is from 10 to 500 ng/mL. No significant method interferents were identified. Sample throughput is approximately seven samples per hour.

Method Summary and Operational Details: The LCE H-Sulfoxide.dam method is a byproduct screening method and does not have an associated calibration range. This method was appropriate for liquid samples extracted with the solvent chloroform ($CHCl_3$; CAS number 67-66-3) and suspected to contain the HD byproduct H-sulfoxide. The method performance for H-sulfoxide is as follows:

- Qualitative Method
- Sample Solvent: Chloroform
- Quantitation Ion: 175/63

6.5 Method Demonstration for Decontaminant Performance Testing

The method demonstration is an example of method performance from the analysis of samples generated by chemical agent decontaminant testing.

6.5.1 GCE HD_DEANS.M Method Demonstration

The HD_DEANS.M method was used to analyze decontamination performance testing samples from silicone and CARC coupon materials that were spiked with neat agent and decontaminated using 5% bleach solution, among others, and extracted with high purity chloroform ($CHCl_3$; CAS number 67-66-3). This set of samples was collected as part of test number POUH05. Dose-confirmation or *Tool* samples were collected by delivering agent directly into solution. The purpose of the dose-confirmation samples was to normalize the known amount of agent being delivered by the tool. Tool samples were spiked, using the agent delivery tool, directly into solution in replicates of five for each concentration. These samples, upon analysis, allowed the determination of the actual known amount of agent delivered on the test materials.

The materials were spiked with the known amounts of HD, treated with decontaminant and subsequently extracted with 20 mL of chloroform. The expected concentration of the solution was determined from the known amount of agent (ng) delivered, previous test data with the decontaminant and test materials, and divided by the amount of extraction solvent (mL). Upon analysis, the found concentration was converted to a mass recovered (ng). The mass recovered was compared to the mass delivered to determine the calculated remaining agent. This test was performed in accordance with the panel testing guidance set forth in the Source Document.[16]

Upon test completion, the samples were aliquoted into GC autosampler septa vials, diluted as needed for the calibration range, and set up on the GCE for analysis. The expected concentrations of the test samples fell within the calibration range (2–2,000 ng/mL) and were analyzed with the method GCE HD_DEANS.M.

Method parameters were established on GCE in accordance with Appendix A. Prior to analysis of test samples, the instrument was calibrated using HD calibration standards prepared in high purity solvent. The GCE HD calibration curve for this test had a correlation coefficient value of 0.9980. The calibration curve was established with a weighted quadratic curve fit ($1/x^2$). The calibration curve for this test is shown in Figure 61. A summary of the method performance with calculated LOD and LOQ is shown in Table 24.

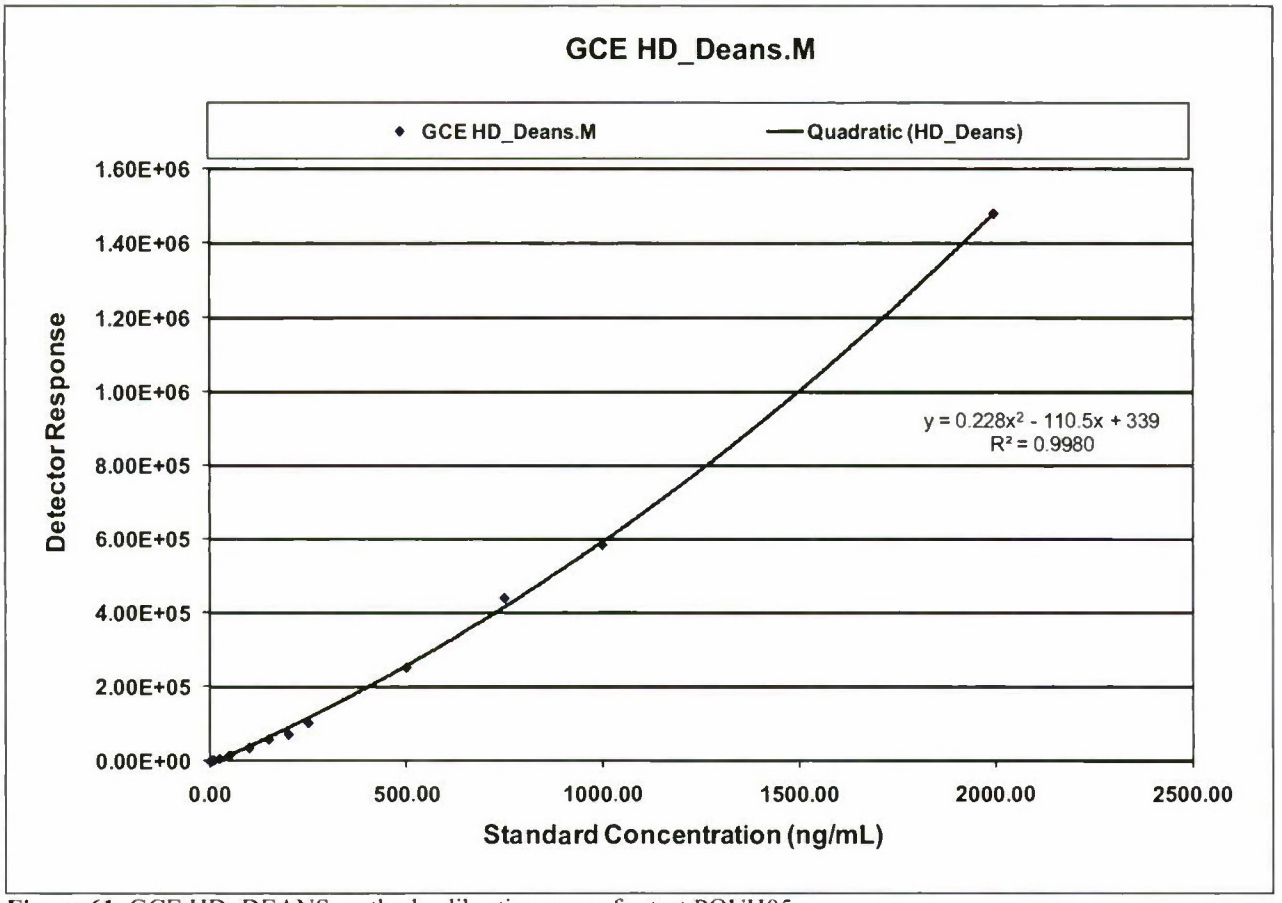


Figure 61. GCE HD_DEANS method calibration curve for test POUH05.

Table 24. GCE HD_DEANS.M method summary for test POUH05.

Method	GCE HD_DEANS.M
Test ID	POUH05
Platform	GCE (GC/MS)
Sample Type	Liquid
Analyte(s)	HD
LOD	0.82 ng/mL
LOQ	1.97 ng/mL
Data Acquisition	EI SIM
Quantitation Ion	111 m/z

CCV samples were analyzed every ninth sample, and were found to be within ±30% of their expected concentrations. Solvent blanks were performed throughout the test for continuing baseline acceptance. Analysis of the blank samples showed no carryover and no interferences. The sample queue, with analytical data, for the performance testing samples analyzed with the GCE HD_DEANS.M method is shown in Figure 62. All samples were successfully analyzed using this GCE HD_DEANS method.

Sample Name	Q Value	Response	Reported Conc (ng/mL)
POUH05_blankA	88	18	BD
POUH05_blankB	35	30	BD
POUH05_2std01	81	661	2.20
POUH05_5std01	88	1342	4.20
POUH05_10std01	95	2907	8.77
POUH05_25std01	97	7560	22.22
POUH05_50std01	95	16179	46.52
POUH05_100std01	98	36869	102.00
POUH05_150std01	99	59178	158.00
POUH05_200std01	96	72738	190.40
POUH05_250std01	98	103900	260.90
POUH05_499std01	99	252939	545.78
POUH05_749std01	99	440445	832.62
POUH05_998std01	99	584624	1,021.75
POUH05_1996std01	99	1479054	1,909.00
POUH05_blankC	1	27	BD
POUH05_blank01	1	232	BD
POUH05_10ccv01	93	2783	8.41
POUH05_150ccv01	99	58789	157.06
POUH05_749ccv01	99	446112	840.48
POUH05_1tool-1	97	134457	325.52
POUH05_1tool-2	98	125500	306.99
POUH05_1tool-3	98	138916	334.62
POUH05_1tool-4	99	134802	326.23
POUH05_1tool-5	98	122809	301.36
POUH05_015-RE5	99	302991	628.23
POUH05_025-RE5	98	205689	462.78
POUH05_035-RE5	99	447699	842.68
POUH05_045-RE5	98	167363	390.99
POUH05_blank02	1	203	BD
POUH05_10ccv02	92	3127	9.42
POUH05_150ccv02	97	54381	146.26
POUH05_749ccv02	99	438708	830.20
POUH05_055-RE5	98	255241	549.69
POUH05_065-RE5	99	185420	425.36
POUH05_075-RE5	99	340466	686.86
POUH05_085-RE5	98	246757	535.23
POUH05_095-RE5	99	302732	627.82
POUH05_105-RE5	98	252491	545.02
POUH05_115-RE5	98	240343	524.18
POUH05_12S-RE5	99	248073	537.48
POUH05_135-RE5	99	223920	495.46
POUH05_blank03	1	226	BD
POUH05_10ccv03	98	2864	8.65
POUH05_150ccv03	97	56454	151.35
POUH05_749ccv03	99	432310	821.27

Sample Name	Q Value	Response	Reported Conc (ng/mL)
POUH05_145-RE5	99	184861	424.31
POUH05_155-RE5	99	301544	625.92
POUH05_165-RE5	99	417334	800.15
POUH05_175-RE5	99	57397	153.66
POUH05_185-RE5	99	390693	761.85
POUH05_195-RE5	98	213900	477.61
POUH05_205-RE5	99	590674	1,029.24
POUH05_215-RE5	99	265812	567.48
POUH05_225-RE5	99	332636	674.81
POUH05_blank04	1	21	BD
POUH05_10ccv04	96	2779	8.40
POUH05_150ccv04	97	61909	164.62
POUH05_749ccv04	99	444965	838.89
POUH05_235-RE5	99	379087	744.86
POUH05_24S-RE5	98	244181	530.80
POUH05_255-RE5	98	256334	551.54
POUH05_265-RE5	99	165717	387.81
POUH05_275-RE5	99	565720	998.12
POUH05_285-RE5	99	323693	660.92
POUH05_295-RE5	98	235879	516.44
POUH05_30S-RE5	99	381244	748.03
POUH05_315-RE5	99	313483	644.90
POUH05_blank05	1	28	BD
POUH05_10ccv05	95	3327	10.00
POUH05_150ccv05	98	58309	155.89
POUH05_749ccv05	99	389512	760.13
POUH05_325-RE5	98	177488	410.39
POUH05_335-RE5	99	352066	704.52
POUH05_345-RE5	99	238166	520.41
POUH05_355-RE5	99	354828	708.70
POUH05_365-RE5	98	234173	513.47
POUH05_37S-RE5	99	229302	504.95
POUH05_385-RE5	99	284274	597.99
POUH05_395-RE5	99	227895	502.47
POUH05_405-RE5	97	112731	279.99
POUH05_blank06	15	32	BD
POUH05_10ccv06	92	3106	9.35
POUH05_150ccv06	98	56318	151.02
POUH05_749ccv06	99	430821	819.18

BD = Below Detect (LOD)

BQ = Below Quant (LOQ)

Figure 62. GCE HD_DEANS example data.

7. ANALYSIS OF GD EXTRACTS

7.1 Decontamination Efficacy Requirement Documents

The method detection limit target was determined using the pertinent panel test parameters and the published requirement document levels. The calculations were based on a 20 mL extraction volume and a 0.00202 m^2 contaminated surface area (equivalent to a 2 in. diameter circular contamination area). The requirement document surface concentration levels for threshold (T) and objective (O) of primary interest at the time of this program are listed in Table 25. The JPID 2003 objective values are reported using only zeroes, which make scientific evaluations and calculations difficult. For each non-specific requirement value, a number 5 was added to the next decimal point to enable measurement and calculation. For example "0.0" is shown as "0.05". The contaminant mass on coupon was calculated using the surface concentration × coupon area. The nominal extract concentration was calculated by dividing the mass on coupon by the extraction volume. The high end of the target detection range was set using a value that was 10% lower than the nominal extraction concentration. The low end of the target detection range was set at an order of magnitude below the high-end value. The lowest range among the group of requirement levels was selected as the analytical method target detection range. The range selected for the analysis of decontamination testing for GD extract samples is shaded in Table 25.

Table 25. Decontamination testing detection targets for GD extract samples.

Requirement Document	Surface Conc. (mg/m^2)	Mass on Coupon (ng)	Nominal Extract Conc. (ng/mL)	Target Detection Range (ng/mL)*		
JPID 05 T[f]	1.70	3,434.0	171.7	154.5	to	15.45
JPID 05 O[f]	0.05	101.0	5.1	**4.5**	to	**0.45**
JSSED 03 O[4]	16.70	33,734.0	1,686.7	1,518	to	151.80
JSSED 04 T[b]	16.70	33,734.0	1,686.7	1,518	to	151.80
JSSED 04 O[b]	0.50	1,010.0	50.5	45.5	to	4.55

*The values shaded in green represent the selected target detection range for low-level method development.

7.2 Initial Method Development

Method development work for GD was performed on the instrument designated GCE as previously described. As method development began on GCE, the low-level detection requirements for GD were evaluated. The most stringent detection target was provided by the JPID03 ORD, which, after applying the program experimental parameters, yielded a target detection limit of 4.5 ng/mL. Initially, a NIST 2.0 library search was performed using the Agilent ChemStation software Enhanced Data Analysis. The search was performed for GD by CAS number (96-64-0) to obtain the NIST database entry giving the mass spectrum and major ions for this analyte. The NIST library entry number is 226124 and the 10 largest peaks for GD, per the NIST library are detailed in Table 26.

Table 26. GD and byproduct mass spectra characteristics.

Compound	GD	PMPA	MPA	Pinacolyl alcohol
NIST No.	226124	273479	226497	1163
ID No.	78999	54739	39294	21133
DB	mainlib	mainlib	mainlib	mainlib
Peak 1	126 (999)	97 (999)	81 (999)	57 (999)
Peak 2	99 (850)	124 (618)	18 (799)	45 (750)
Peak 3	82 (500)	80 (550)	96 (749)	56 (625)
Peak 4	69 (490)	41 (407)	31 (499)	41 (387)
Peak 5	41 (370)	79 (231)	78 (349)	87 (287)
Peak 6	57 (210)	29 (166)	15 (199)	29 (175)
Peak 7	43 (150)	69 (159)	48 (199)	69 (175)
Peak 8	83 (140)	39 (156)	63 (189)	27 (112)
Peak 9	84 (100)	43 (150)	65 (169)	39 (112)
Peak 10	85 (90)	57 (150)	79 (99)	43 (87)

After examining the mass spectrum for GD, the MSD was set up to simultaneously analyze in the SCAN and SIM modes of acquisition. Based on the ion relative abundances from the expected mass spectrum (Table 26), the ions selected for the SIM mode were m/z 126 (base peak), 99, and 82. Note that the molecular ion, m/z 182, was not present on the GC/MS mass spectrum.

As with VX, initial method parameters were selected for the GC based on the properties of the analyte and sovent. All standards were prepared using high purity solvent, following a standard preparation dilution scheme. Initial injections were made with solvent blanks and a higher concentration GD standard (10,000 ng/mL). Solvent blanks were analyzed before and after the GD standard to ensure that there were no baseline issues that might interfere with the analyte of interest. The goals of injecting a higher concentration standard were to identify the correct peak via library matching, to determine RT, and to evaluate peak shape on the TIC. Evaluating peak shape (efficiency and resolution) allowed the method to be altered for optimal chromatography. Note that GD exists as stereoisomers,[27] and therefore, on a chromatogram, appears as a doublet peak. *Stereoisomers* are molecules that are made up of the same chemical formula, have the same chemical bonds, and are, therefore, the same molecule. However, the shape of the molecule, or overall geometry, is different due to positioning of the atoms that make up the components of the molecule. An example chromatogram of GD is shown in Figure 63. The representative doublet peak indicates the presence of the isomeric GD.

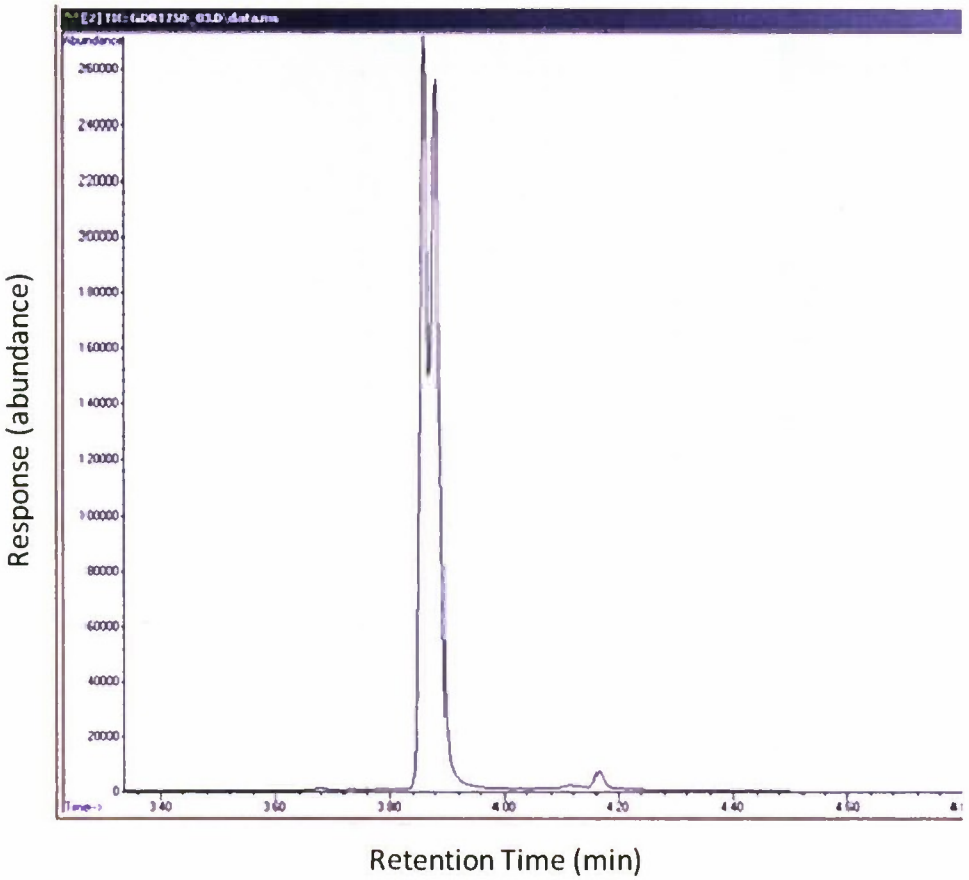


Figure 63. Chromatogram of GD, analyzed on GCE, showing the common doublet peak.

The agent GD, appearing as a doublet peak on a chromatogram, did not affect identification or quantitation within the final method. Typically, peaks on the TIC can be identified by the mass spectrum, which contains selected ions in certain relative abundances. As lower concentrations of the standard were evaluated to determine detection limit capabilities in comparison with the ORDs target detection limit, selected ion relative abundanees were observed to deviate from the ideal. By initially identifying the correct peak via library matching and noting the RT, identification of the peak was possible at much lower concentrations. When the ions of interest were not in their ideal relative abundances at these lower concentrations, analyte peaks could be identified by RT.

After analysis of the initial samples, the peak was sueeessfully identified by PBM of the mass spectrum from the SCAN mode of acquisition with the GD entry in the NIST 2.0 library. PBM of a 10,000 ng/mL GD standard in high purity acetonitrile identified the analyte peak at 3.8 min as GD with a 96.2% match quality. The next highest match for the analyte peak only gave a 2.88% match quality. The analysis was repeated in triplicate with similar results, giving the conelusion that the analyte peak was correctly identified as GD. The mass spectrum for the GD in SIM mode of acquisition also showed the ions in the expeeted relative abundanees. Note that the solvent blank did not show a chromatographie peak at the RT of the peak identified as GD. An example of the GD standard, analyzed in the SIM and SCAN modes of aequisition on the MSD, is provided in Figure 64. An example solvent blank chromatogram is provided in Figure 65.

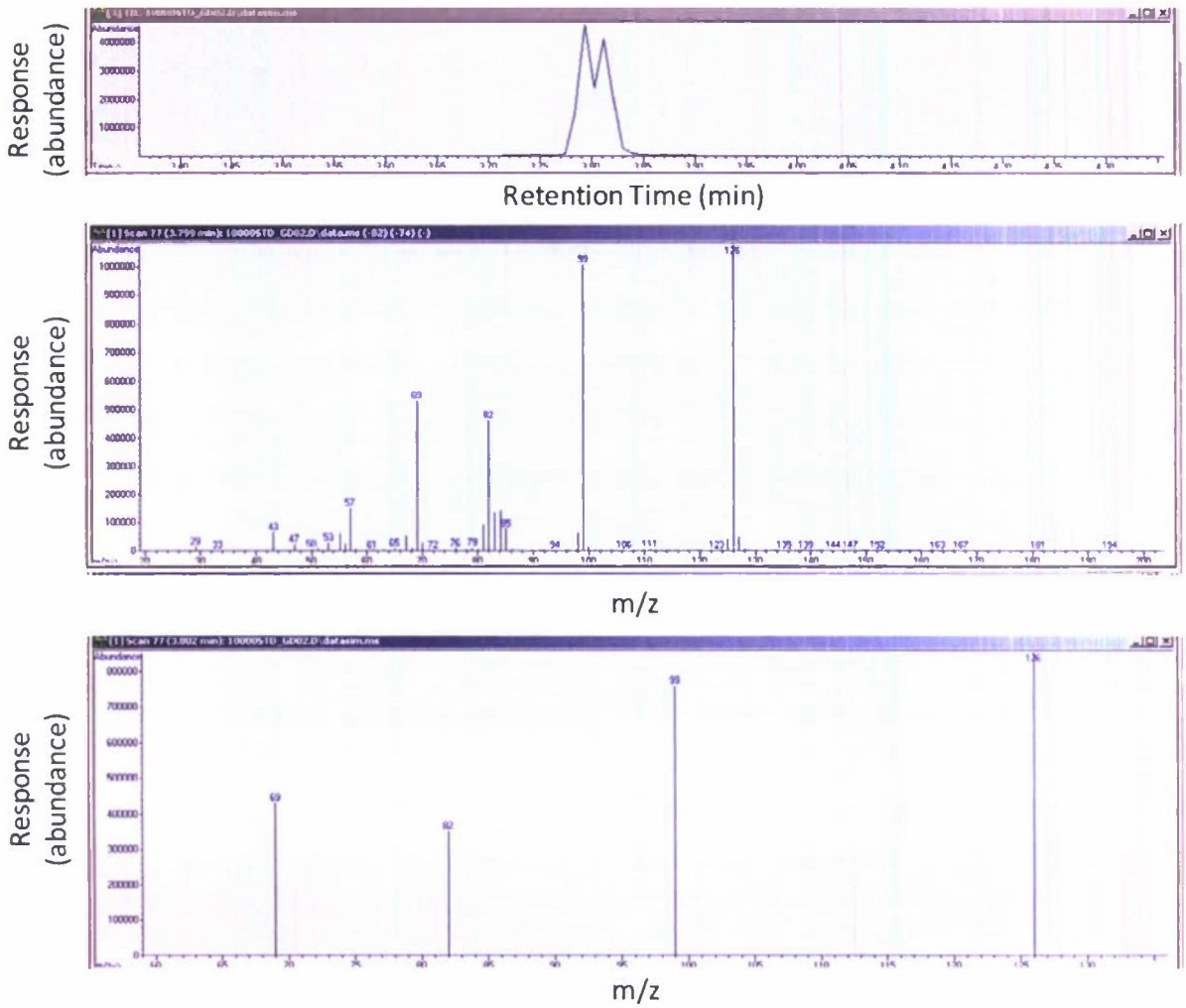


Figure 64. GC/MS SCAN and SIM analysis of 10,000 ng/mL GD on GCE.

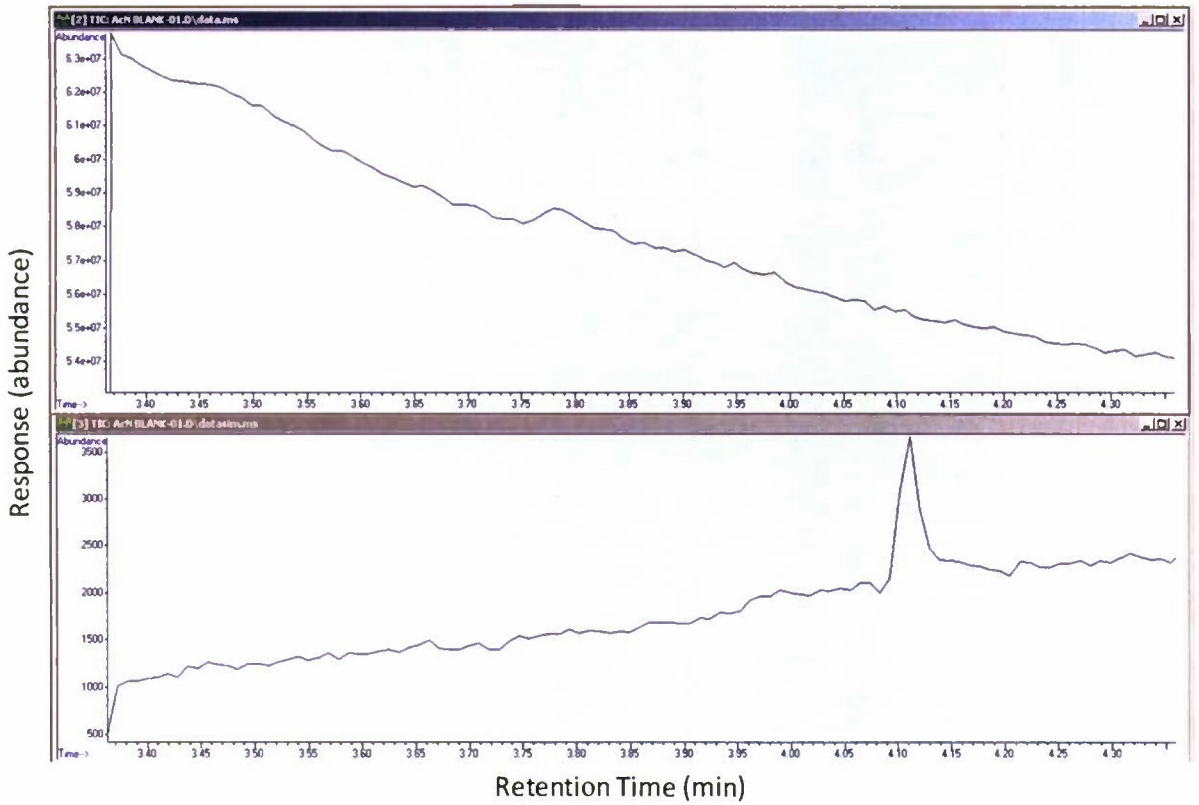


Figure 65. GC/MS SCAN and SIM analysis of an acetonitrile solvent blank on GCE zoomed at GD RT of 3.80 min.

Additional injections were made at decreasing concentrations in an attempt to identify the observed method detection limits. The detection limit capabilities for GD on the GCE were observed to be ~2.5 ng/mL while maintaining an acceptable SNR>3:1. The upper observed detection limit was not established due to the ability of the GC/MS to detect GD at concentrations significantly higher than the program requirements. A target method concentration range, spanning four orders of magnitude, was established at ~2.5–2000 ng/mL. This target range exceeds the low-level JPID03 ORD detection requirement of 4.5 ng/mL, while providing a flexible method concentration range for sample analysis. When preparing liquid extraction samples throughout the contact testing, calculations were made to estimate the expected sample concentrations to be analyzed on the analytical instrumentation. It is beneficial to have the ability to detect a wide concentration range to maximize the effectiveness of testing, limit the number of samples to be run multiple times, and/or limit the need to dilute samples after the testing has occurred. This wide concentration range may span several orders of magnitude, and, therefore, a large number of calibration standards are required for confidence. Based on the method concentration, it was initially decided that two methods would be required for GD on the GCE. There would be no difference in method parameters on the instrument; however, the method would have a dual purpose. The first would be as an ultra low-level (ULL) method that would exceed the detection limit requirements of the program. The second would be a low-level (LL) method with a larger concentration range that would serve as a complement to the ULL method capabilities.

7.2.1 Initial Method Capabilities

With observed detection limits established that exceeded the program requirements, initial capabilities of the GD methodology were assessed. Initial capabilities established a calculated LOD and LOQ as well as calibration curve fit and weighting.

7.2.1.1 Method GCE GD_DEANS.M (ULL) — Initial

The primary purpose of the GCE GD_DEANS.M (ULL) method was to detect and quantify ultra-low levels of the chemical agent GD in liquid extraction samples. This method was developed to meet the low-level JPID03 ORD requirements. The JPID ORD for GD is 0.05 mg/m^2. Given the research parameters outlined in the Low-Level program, this value translates to a research target concentration of 4.5 ng/mL. Based on the method capabilities, this method had two intended uses. For confidence across the entire concentration range, the method was used as a ULL method with a concentration range spanning 2.5–50 ng/mL and as a LL method with a concentration range spanning 50–2000 ng/mL. No significant method interferents were identified during method development. Sample throughput was approximately six samples per hour.

The GCE GD_DEANS.M (ULL) method was appropriate for liquid samples containing the chemical agent GD and extracted with the solvent acetonitrile (CH_3CN; CAS number 75-05-8). The overall method utilized eleven calibration standards at concentrations of ~2.5, 5, 10, 25, 50, 100, 250, 500, 750, 1000, and 2000 ng/mL GD; however, the ULL method utilized five calibration standards at concentrations of ~2.5, 5, 10, 25, and 50 ng/mL. The method use, detection limit, and quantitation limit were based on using the five calibration standards for the ULL calibration range. The solvents were prepared in acetonitrile.

The initial method performance for GD-ULL is as follows:

- Quantitative Method
- Calibration Range: ~2.5–50 ng/mL GD
- Calibration Model: Linear regression calibration model
- Calibration Weighting: None
- LOD: 1.52 ng/mL
- LOQ: 4.61 ng/mL
- Sample Solvent: Acetonitrile
- Quantitation Ion: 126

Three standard sets were prepared and each was analyzed twice, producing six calibration curves for analysis. The low-level method used a linear regression calibration model with no weighting. This method had an LOD of 1.52 ng/mL and an LOQ of 4.61 ng/mL. The precision of the method was determined by analysis of the multiple CCV samples analyzed. Six replicate analyses of the 2.5 ng/mL standard produced a standard deviation of 2.03 and an RSD of 64.88%. For the other standards, six replicates were analyzed, producing standard deviations ranging from 0.96 to 3.21 and RSDs ranging from 3.12 to 18.06%. The calibration curves were reproducible—the %RSD of the slope for six calibration curves was 7.72% and the correlation coefficients ranged from 0.9744 to 9988.

Based on the method development and validation work performed and documented herein, the ULL GD extraction method (GCE GD_DEANS.M [ULL]) detected and quantified GD in acetonitrile from 2.5 to 50 ng/mL. This exceeded the research target concentration of 4.5 ng/mL established from the JPID03 ORD requirement of 0.05 mg/m^2.

7.2.1.2 Method GCE GD_DEANS.M (LL) — Initial

The primary purpose of the GCE GD_DEANS.M (LL) method was to detect and quantify LL concentrations of the chemical agent GD in liquid extraction samples. This method was developed to complement the ULL calibration range, which exceeds the JPID03 ORD requirements. The overall GCE GD_DEANS.M method will be used as a ULL method with a concentration range spanning 2–50 ng/mL and as a LL method with a concentration range spanning 50–2000 ng/mL. No significant method interferents were identified during method development. Sample throughput was approximately six samples per hour.

The GCE GD_DEANS.M (LL) method was appropriate for liquid samples containing the chemical agent GD and extracted with the solvent acetonitrile (CH_3CN; CAS number 75-05-8). The overall method utilized eleven calibration standards at concentrations of ~2.5, 5, 10, 25, 50, 100, 250, 500, 750, 1000, and 2000 ng/mL GD; however, the LL method utilized seven standards at concentrations of ~50, 100, 250, 500, 750, 1000, and 2000 ng/mL GD. The method use, detection limit, and quantitation limit were based on using the full standard set. The solvents were prepared in acetonitrile.

The method performance for GD is as follows:

- Quantitative Method
- Calibration Range: ~50–2000 ng/mL GD
- Calibration Model: Quadratic calibration model
- Calibration Weighting: None
- LOD: 27.13 ng/mL
- LOQ: 82.22 ng/mL
- Sample Solvent: Acetonitrile
- Quantitation Ion: 126

The LL method used a quadratic calibration model with no weighting. This method had an LOD of 27.13 ng/mL and an LOQ of 82.22 ng/mL. The precision of the method was determined by analysis of the multiple CCV samples analyzed. Six replicate analyses of the 50 ng/mL standard produced a standard deviation of 8.54 and an RSD of 15.02%. For the other standards, six replicates were analyzed producing standard deviations ranging from 1.53 to 32.49 and RSDs ranging from 0.08 to 17.31%. The calibration curves were reproducible—the %RSD of the slope for six calibration curves was 8.77% and the correlation coefficients ranged from 0.9977 to 1.00.

7.3 GD Byproduct Method Development

The second overall program objective was to establish methods for the detection of common agent byproducts that could form during decontaminant testing. The purpose of these methods was to screen for common byproducts of chemical agents for the confident detection and identification of the byproduct analyte, when present. These methods are qualitative methods and serve as complementary methods to the fully quantitative agent methods.

Following the GD method development on the GCE, method development work turned to the primary hydrolysis byproduct of GD; pinacolyl methyl phosphonic acid (PMPA) or GD-acid. The

goal of this method was to screen extraction samples for the presence of GD-acid; therefore, it was important to evaluate this analyte when diluted in the same solvent as the GD extraction samples, acetonitrile. This analyte had a mass spectrum available through parametric retrieval library searching associated with the Agilent MSD ChemStation software. Table 26 details the ten largest ion peaks associated with GD-acid. Standards were diluted to an appropriate working concentration from stock material obtained from Sigma-Aldrich.

GD-acid on the GCE with the generic method parameters showed a less than desirable chromatographic peak. Method parameters were adjusted for better chromatography, but in comparison with GD, very high concentrations were required to elicit a peak for GD-acid. Once GD-acid was identified and the RT was determined (5.9 min), the method was updated to analyze in the SIM mode of acquisition. An example chromatogram is shown in Figure 66. Additional injections were made at decreasing concentrations in an attempt to identify the observed method detection limits. The detection limit capability for GD-acid on the GCE was observed at ~5,000 ng/mL. The detection limitations of GD-acid on the GCE, as well as the method parameters required for good peak shape, do not make it a good candidate to be screened in the presence of GD; therefore, GD-acid will remain a stand-alone analyte in this method. Future method development for screening GD-acid will be performed on the LCE (LC/MS/MS).

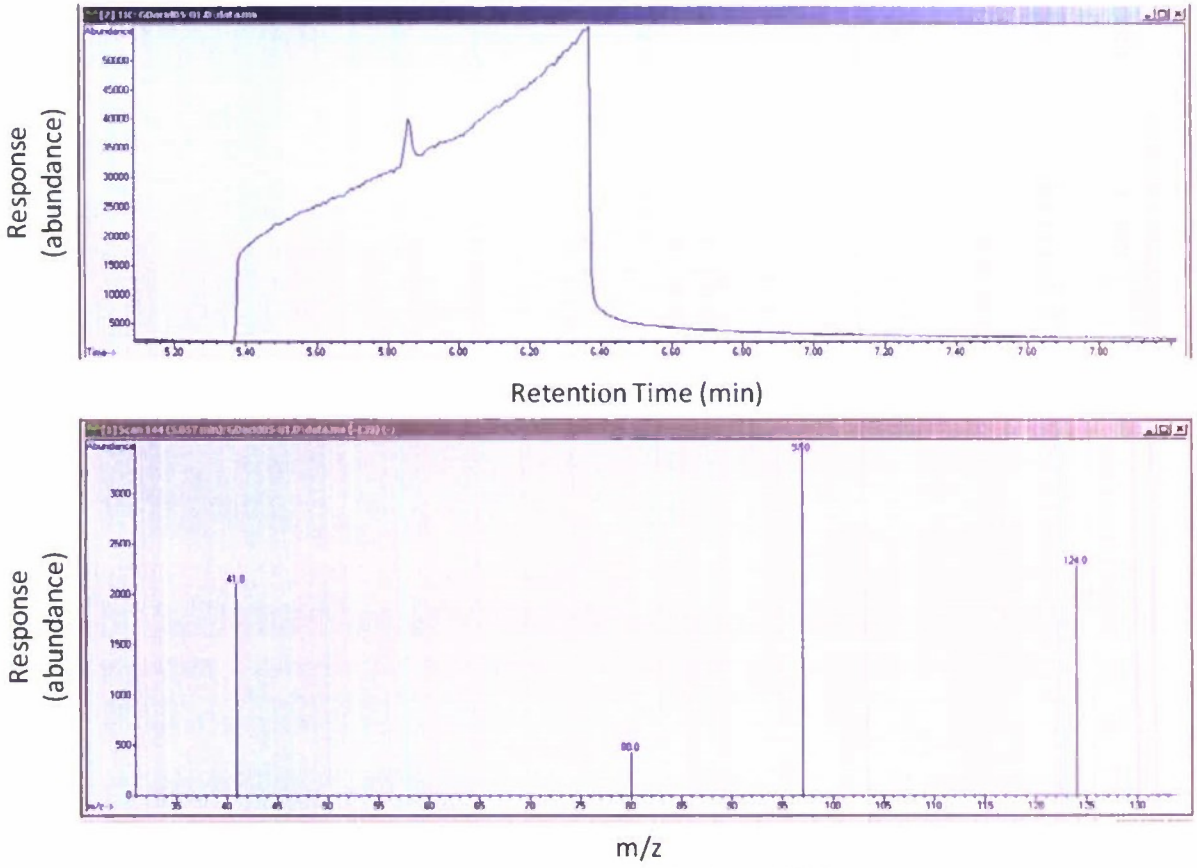


Figure 66. GC/MS analysis of 5,000 ng/mL of GD-acid (RT 5.9 min) on GCE.

Future work may determine the ability of the method to quantify GD-acid through the generation of calibration curves spanning an appropriate working range, using standards diluted in high

purity solvent. Future method capabilities to quantify GD-aeid will be determined statistieally and reported accordingly.

7.4 Final Method Refinement

As with all aspects of a quality system that undergoes continuous improvement, method refinement and validation continues to ensure that the methods used are the best, most robust methods giving the greatest possible precision and accuracy available for the teehnology. The GD low-level methodology was refined after initial method development to offer more reliable analysis, an improved concentration range, better detection, and more confident data analysis.

After the initial method capabilities check of the GD_DEANS.M GCE methodology, slight method refinement oeeurred to improve the performance of the method aeross the entire eoneentration range of 2.5–2,000 ng/mL. The goal of method refinement of the GD_DEANS.M method was to establish method parameters that would allow the routine use of the method with the entire concentration range. This would eliminate the need to have two concentration ranges for the same method. There were no differences in instrument method parameters for GD_ULL versus GD_LL; the only difference in the method was the ealibration range utilized. Refining the method enabled the use of the GD_DEANS.M method aeross the entire ealibration range. This was accomplished by refining the chromatographic parameters for improved performance at the low end of the calibration range and by implementing curve weighting to an overall quadratie ealibration model. During method refinement, the concentration range was adjusted to more closely mimie the ealibration range of the HD methodology. To accomplish this, the lowest calibration standard was changed from 2.5 to 2 ng/mL. Additional calibration levels were added at 150 and 200 ng/mL for an entire set of 13 ealibration standards.

After completion of the method refinement, final method parameters were established. Appendix A contains the formal method write-up, the final method parameters, and a summary of the method eapabilities.

7.4.1 Final Method Capabilities

After refinement was eomplete, the final method eapabilities were examined. The final method capabilities were then used to determine the most appropriate methodology for deeontamination test sample analysis.

7.4.1.1 Method GCE GD_DEANS.M — Final

Method Purpose and Performance Overview: The primary purpose of the GCE GD_DEANS.M method is to detect and quantify the chemieal agent GD in liquid extraction samples. This method was developed to exceed the low-level JPID03 ORD requirements. The JPID ORD for GD is 0.05 mg/m^2. Given the researeh parameters outlined in the Low-Level program, this value translates to a researeh target concentration of 4.5 ng/mL. No significant method interferents were identified during method development, validation, or subsequent interferenee testing. Sample throughput is approximately six samples per hour.

Method Summary and Operational Details: The GCE GD_DEANS.M method is appropriate for liquid samples containing the chemieal agent GD and extracted with the solvent acetonitrile (CH_3CN; CAS number 75-05-8). This method utilizes 13 calibration standards at concentrations of ~2, 5, 10, 25, 50, 100, 150, 200, 250, 500, 750, 1000, and 2000 ng/mL GD. The method use, detection limit, and quantitation limit were based on using the full standard set. The standards were prepared in acetonitrile.

The final method performance for GD is as follows:

- Quantitative Method
- Calibration Range: ~2–2,000 ng/mL GD
- CCV Levels: 0, 10, 150, 750 ng/mL
- Calibration Model: Weighted quadratic calibration model
- Calibration Weighting: $1/x^2$
- LOD: 0.11ng/mL
- LOQ: 0.38 ng/mL
- Sample Solvent: Acetonitrile
- Quantitation Ion: 126

LOD and Quantitation and Measurement Uncertainty: Calibration standards were prepared and analyzed six times for calibration consistency and detection limit determination. The final method evaluation pulled calibration and CCV performance data sets from decontamination performance testing queues. The high concentration standard of the calibration set was followed by solvent blanks to determine method carryover. Slight carryover was observed following the high concentration calibration standard; however, the carryover from the high-level calibration standard has no affect on the method or the ability of the method to detect and quantify the low end of the calibration range. The calibration range of 2–2000 ng/mL yielded a quadratic calibration response. This method used a weighted quadratic calibration model ($1/x^2$). Accuracy at the upper end of the calibration concentration range was maintained with the $1/x^2$ weighting. Accuracy at or below requirement levels was ideal to instill confidence that a requirement had been met. A demonstration of the GCE GD_DEANS.M calibration performance can be seen in Figure 67.

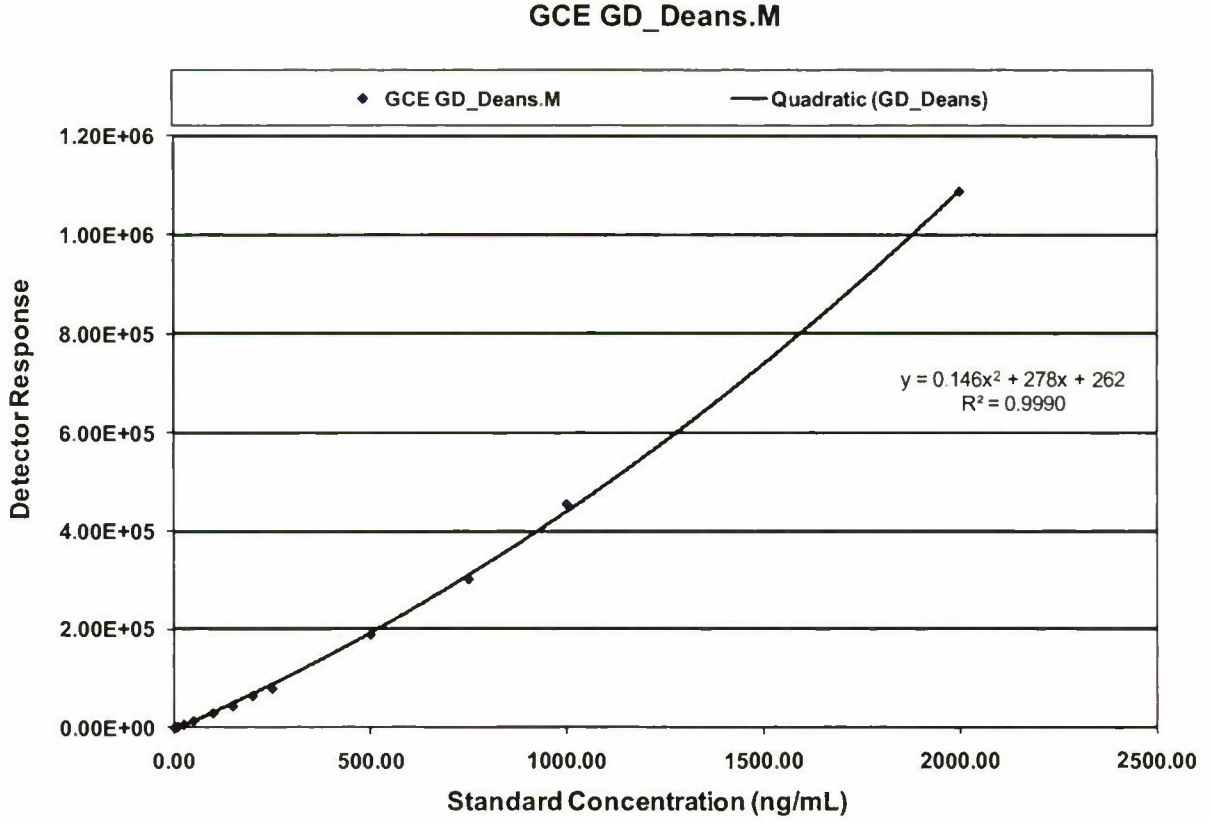


Figure 67. GCE GD_DEANS.M example calibration curve (2–2000 ng/mL); quadratic regression, $1/x^2$ weighting.

The GCE GD_DEANS.M method yeilded a calculated LOD of 0.11 ng/mL and an LOQ of 0.38 ng/mL. The precision of the method was determined by analysis of CCV samples analyzed at multiple levels, from multiple queues over multiple days. There were 48 analyses of the 10 ng/mL CCV, which produced an average reported concentration of 11.0 ng/mL with a standard deviation of 2.08 and an RSD of 12.18%. There were 48 analyses of the 150 ng/mL CCV, which produced an average reported concentration of 165 ng/mL with a standard deviation of 20.9 and an RSD of 12.61%. Finally, there were 48 analyses of the 750 ng/mL CCV, which produced an average reported concentration of 813 ng/mL with a standard deviation of 93.7 and an RSD of 11.5%. This indicated good reproducibility at the CCV levels within the calibration curve. Furthermore, there were six replicate analyses of the 2 ng/mL standard, which produced a standard deviation of 0.04 and an RSD of 1.82%. This indicated very good reproducibility at the lowest standard in the calibration curve and a marked improvement compared to the initial method capabilities of the GD ULL method. For the other standards, multiple replicates were analyzed, producing standard deviations ranging from 0.23 to 55.6 and RSDs ranging from 0.92 to 6.46%, indicating very good overall method performance.

The accuracy of the method is demonstrated in Table 27 as uncertainty in the measurements at each standard in the calibration curve with 95% confidence. For a standard or sample, containing 10 ng/mL of analyte, the analytical result reported was 10 ± 0.2 ng/mL.

Table 27. List of standard concentrations and the associated confidence intervals.

Standard Concentration (ng/mL)	95% Confidence Interval (ng/mL)	Lower Confidence Interval (ng/mL)	Upper Confidence Interval (ng/mL)
2	0.65	1.35	2.65
5	0.36	4.64	5.36
10	0.20	9.80	10.20
25	1.92	23.08	26.92
50	4.79	45.21	54.79
100	10.47	89.53	110.47
150	16.07	133.93	166.07
200	21.60	178.40	221.60
250	27.08	222.92	277.08
500	53.99	446.01	553.99
750	81.09	668.91	831.09
1000	109.55	890.45	1109.55
2000	251.16	1748.84	2251.16

Based on the method development and validation work performed and documented herein, the GD extraction method (GCE GD_DEANS.M) detected and quantified GD in acetonitrile from 2 to 2,000 ng/mL. This result exceeded the research target concentration of 4.5 ng/mL established from the JPID03 ORD requirement of 0.05 mg/m^2.

7.4.1.2 Method GCE GD-acid_DEANS.M — Final

Method Purpose and Performance Overview: The purpose of the GCE GD-acid_DEANS.M method is to detect and identify the hydrolysis byproduct of GD, GD-acid, in liquid extraction samples. This method was optimized to support the analysis of liquid extracts following the chemical agent decontamination performance evaluation testing for contact sampler and coupon extracts using a 2 in. diameter test surface area, extracted in no greater than 20 mL of extraction solvent. This method detects and identifies GD-acid solution concentrations from ~5,000 to 100,000 ng/mL. NOTE: Due to the large concentrations required, this method is not appropriate for analyzing GD and GD-acid simultaneously. No significant method interferents were identified. Sample throughput was approximately six samples per hour.

Method Summary and Operational Details: The GCE GD-acid_DEANS.M method is a byproduct screening method and does not have an associated calibration range. This method is appropriate for liquid samples extracted with the solvent acetonitrile and containing the byproduct GD-acid. The method performance for GD-acid is as follows:

- Qualitative Method
- Sample Solvent: Acetonitrile
- Quantitation Ion: 97

7.5 Method Demonstration for Decontaminant Performance Testing

The method demonstration is an example of method performance from the analysis of samples generated by chemical agent decontaminant testing.

7.5.1 GCE GD_DEANS.M Method Demonstration

The GD_DEANS.M method was used to analyze decontamination performance testing samples from coupon materials including stainless steel, CARC, and glass. The coupons were spiked with neat agent, decontaminated, and extracted with high purity acetonitrile (CH_3CN; CAS number 75-05-8). This set of samples was collected as part of test number SGDC01. One objective of SGDC01 was to perform contact testing of these materials with the test. Dose-confirmation or *Tool* samples were collected by delivering agent directly into solution. The purpose of the dose-confirmation samples was to normalize the known amount of agent being delivered by the tool. Tool samples were spiked, using the agent delivery tool, directly into solution in replicates of five for each concentration. These samples, upon analysis, allowed the determination of the actual known amount of agent delivered on the test materials.

The materials were spiked with the known amounts of GD, treated with decontaminant and subsequently extracted with 20 mL of acetonitrile. The expected concentration of the solution was determined from the known amount of agent (ng) delivered, previous test data with the decontaminant, and test materials and divided by the amount of extraction solvent (mL). Upon analysis, the found concentration was converted to a mass recovered (ng). The mass recovered was compared to the mass delivered to determine the calculated remaining agent. This test was performed in accordance with the panel testing guidance set forth in the Source Document.[16]

Upon test completion, the samples were aliquoted into GC autosampler septa vials, diluted as needed for the calibration range and set up on the GCE for analysis. The expected concentrations of the test samples fell within the calibration range (2–2,000 ng/mL) and were analyzed with the method GCE GD_DEANS.M.

Method parameters were established on GCE in accordance with Appendix A. Prior to analysis of test samples, the instrument was calibrated using GD calibration standards prepared in high purity solvent. The GCE GD calibration curve for this test had a correlation coefficient value of 0.9990. The calibration curve was established with a weighted quadratic curve fit ($1/x^2$). The calibration curve for this test is shown in Figure 68. A summary of the method performance with calculated LOD and LOQ is shown in Table 28.

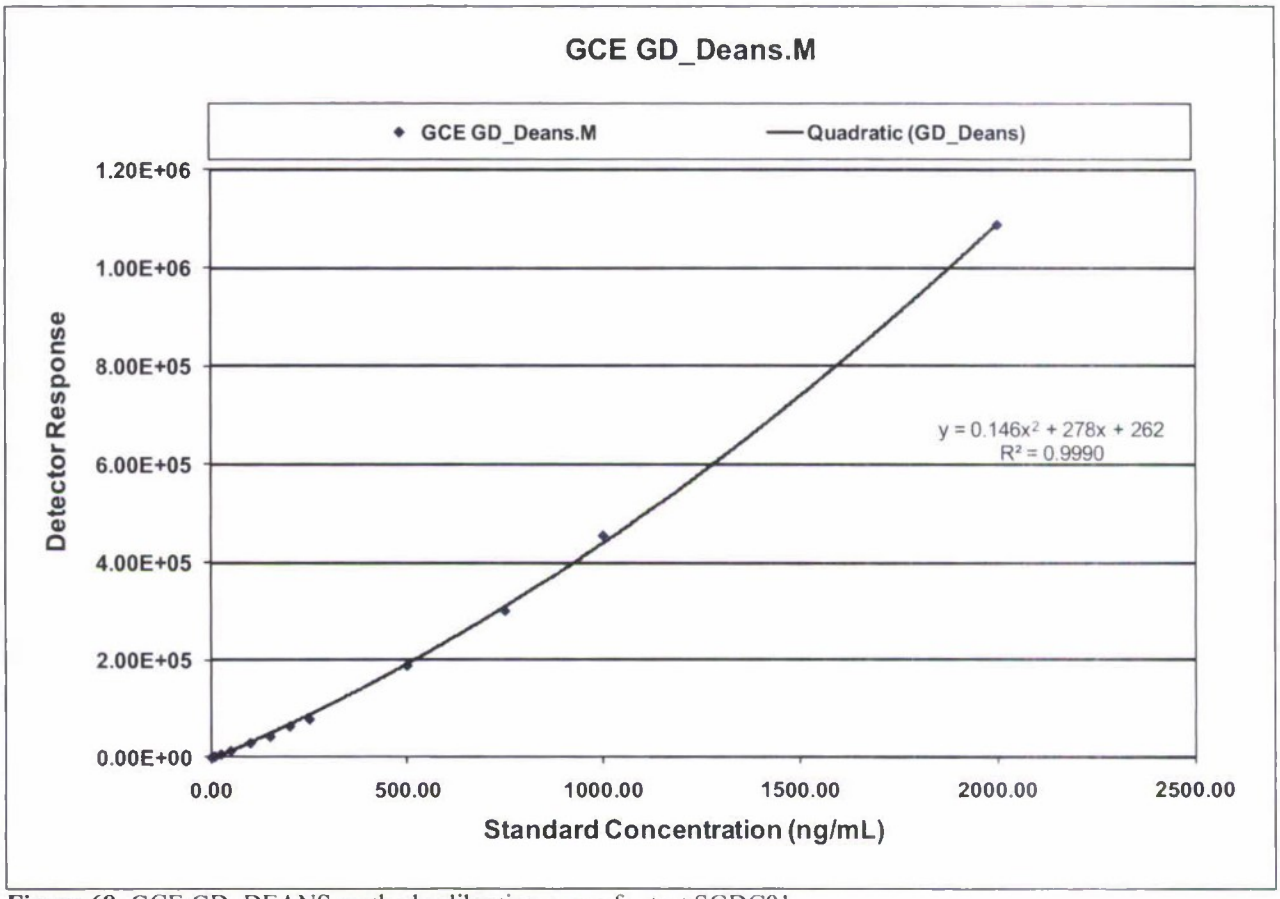


Figure 68. GCE GD_DEANS method calibration curve for test SGDC01.

Table 28. GCE GD_DEANS.M method summary for test SGDC01.

Method	GCE GD_DEANS.M
Test ID	SGDC01
Platform	GCE (GC/MS)
Sample Type	Liquid
Analyte(s)	GD
LOD	0.42 ng/mL
LOQ	1.67 ng/mL
Data Acquisition	EI SIM
Quantitation Ion	126 m/z

CCV samples were performed every ninth sample, and were found to be within 30% of their expected concentrations. Solvent blanks were performed throughout the test for continuing baseline acceptance. Analysis of the blank samples showed little carryover and no interferences. The sample queue, with analytical data, for the performance testing samples analyzed with the GCE GD_DEANS method is shown in Figure 69. All samples were successfully analyzed using this GCE GD_DEANS method.

Sample Name	Q Value	Response	Reported Conc (ng/mL)
SGDC01_blankA	57	233	BD
SGDC01_blankB	1	91	BD
SGDC01_2std01	87	795	2.1
SGDC01_Sstd01	91	144S	4.S
SGDC01_10std01	92	2709	9.0
SGDC01_2Sstd01	97	7027	24.2
SGDC01_S0std01	99	14170	49.0
SGDC01_100std01	99	303S9	102.9
SGDC01_1S0std01	97	4347S	144.6
SGDC01_200std01	98	64420	208.1
SGDC01_2S0std01	99	79136	250.7
SGDC01_S00std01	100	189118	S30.9
SGDC01_7S0std01	100	301733	771.2
SGDC01_1000std01	100	4SS278	1,0S2.8
SGDC01_2000std01	99	1086399	1,934.9
SGDC01_blankC	8S	817	2.2
SGDC01_blank01	1	311	BD
SGDC01_10ccv01	98	2843	9.S
SGDC01_1S0ccv01	99	46718	154.7
SGDC01_7S0ccv01	100	297472	762.7
SGDC01_1tool-1	100	475014	1,086.2
SGDC01_1tool-2	100	480662	1,09S.7
SGDC01_1tool-3	32	482924	1,099.4
SGDC01_1tool-4	100	47S647	1,087.3
SGDC01_1tool-5	100	497103	1,122.9
SGDC01_01SS-T2	70	S20	BQ
SGDC01_02SS-T2	61	217	BD
SGDC01_03S5-T2	49	1S5	BD
SGDC01_04SS-T2	73	154	BD
SGDC01_blank02	3	153	BD
SGDC01_10ccv02	94	2984	10.0
SGDC01_1S0ccv02	99	4498S	149.3
SGDC01_7S0ccv02	100	300700	769.1
SGDC01_0SSS-T2	48	238	BD
SGDC01_07AC-T2	1	1S7	BD
SGDC01_08AC-T2	28	207	BD
SGDC01_09AC-T2	1	102	BD
SGDC01_10AC-T2	1	130	BD
SGDC01_11AC-T2	1	80	BD
SGDC01_12AF-T2	1	102	BD
SGDC01_13AF-T2	0	0	ND
SGDC01_14AF-T2	1S	127	BD
SGDC01_blank03	0	0	ND
SGDC01_10ccv03	93	2654	8.8
SGDC01_1S0ccv03	98	426S1	142.0
SGDC01_75Occv03	32	28S438	738.S

Sample Name	Q Value	Response	Reported Conc (ng/mL)
SGDC01_1SAF-T2	68	157	BD
SGDC01_16AF-T2	0	0	ND
SGDC01_17GL-T2	1	79	BD
SGDC01_18GL-T2	0	0	ND
SGDC01_19GL-T2	0	0	ND
SGDC01_20GL-T2	0	0	ND
SGDC01_21GL-T2	80	307	BD
SGDC01_01SS-T1	1	92	BD
SGDC01_02SS-T1	6	182	BD
SGDC01_blank04	0	0	ND
SGDC01_10ccv04	96	24SS	8.1
SGDC01_1S0ccv04	99	40801	136.2
SGDC01_7S0ccv04	100	270219	707.S
SGDC01_03SS-T1	78	427	BQ
SGDC01_04SS-T1	16	164	BD
SGDC01_0SSS-T1	72	361	BQ
SGDC01_07AC-T1	1	266	BD
SGDC01_08AC-T1	20	381	BQ
SGDC01_09AC-T1	1	272	BD
SGDC01_10AC-T1	1	258	BD
SGDC01_11AC-T1	28	233	BD
SGDC01_12AF-T1	1	94	BD
SGDC01_blank0S	1	102	BD
SGDC01_10ccv0S	97	262S	8.7
SGDC01_1S0ccv0S	99	39912	133.4
SGDC01_7S0ccv0S	32	269792	706.6
SGDC01_13AF-T1	74	203	BD
SGDC01_14AF-T1	72	86	BD
SGDC01_15AF-T1	1	78	BD
SGDC01_16AF-T1	1	129	BD
SGDC01_17GL-T1	31	13244	4S.8
SGDC01_18GL-T1	88	2386	7.8
SGDC01_19GL-T1	0	0	ND
SGDC01_20GL-T1	9S	S311	18.2
SGDC01_21GL-T1	96	3287	11.0
SGDC01_blank06	0	0	ND
SGDC01_10ccv06	96	2S77	8.S
SGDC01_150ccv06	99	40462	135.2
SGDC01_7S0ccv06	32	280389	728.3

BD = Below Detect (LOD)
BQ = Below Quant (LOQ)
ND = Non Detect

Figure 69. GD_DEANS.M method demonstration queue.

8. ANALYSIS OF VX VAPOR SAMPLES

8.1 Decontamination Efficacy Requirement Documents

The method detection limit target was the lowest mass of contaminant on tube that could be accurately detected and quantified from the baseline noise and still enable sample throughput to support decontamination performance evaluations. The requirement document vapor concentration levels for threshold (T) and objective (O) of primary interest at the time of this program are listed in Table 29.[4-9] Table 29 lists the requirement, toxicology, IDLH, STEL, WPL, and GPL in order of the vapor concentration numerical value. These values represent different exposure durations, requiring different sampling protocols for sample collection and data reporting. The toxicology vapor concentrations were for a 10 h exposure.[28] The IDLH, STEL, and WPL shown are OSHA-reported values.[13] The IDLH value is for a one time, short-term exposure. The STEL is for a 15 min exposure, once per day. The WPL is for the time-weighted average vapor concentration for working population exposure of 8 h per day for five days per week. The GPL is the EPA general population value time-weighted average vapor concentration for lifetime exposure.[14] The vapor test, as described in the source document,[16] calculated a vapor emission rate. While the test measured a vapor concentration in the dynamic vapor chamber, this vapor concentration did not correspond to the vapor concentration to which unprotected personnel would be exposed, and should not be directly compared to a requirement. The emission rate was used to calculate the vapor concentration that was present in a specified scenario. This use of data required that the analytical method be capable of accurately detecting agent across a broad range of masses, ideally from a few ng to as many as 1000 ng.

Table 29. Decontamination testing detection targets for VX vapor samples.

Publication	Vapor Conc. (mg/m^3)	Publication	Vapor Conc. (mg/m^3)
JSSED 03 T	0.0400000	1% Tox Response	0.0000240
IDLH	0.0030000	JSSED 03 O	0.0000100
84% Tox Response	0.0002400	JSSED 04 T	0.0000100
16% Tox Response	0.0000700	JSSED 04 O	0.0000030
STEL	0.0000500	WPL	0.0000010
JPID 05 T	0.0000360	EPA 04 GPL	0.0000006
JPID 05 O	0.0000240		

8.2 Solid Sorbent Selection for VX Vapor Analysis

The vapor analysis of VX is typically performed by pre-treating the VX molecule with silver fluoride (AgF) to form the G-analog. It is widely accepted that the solid sorbent material, Tenax TA, is an excellent all-around adsorbent material for chemical agent. Other sorbent materials are available and may have a higher affinity for agent retention during the sampling process. All initial method development work for VX, analyzed as the G-analog, was performed on Tenax TA.

As part of method development and overall vapor-sampling verification, extensive breakthrough studies were performed to demonstrate the ability of the selected sorbent to retain the agent of interest, and to determine the safe-samplingvolume for the agent/sorbent pairing. *Breakthrough* is defined as the inability of the sorbent to retain the compound of interest during sampling, which allows some/all of the compound to pass through the sorbent. During sampling, breakthrough may occur if the sorbent material cannot retain the compound of interest under sampling extremes such as high sampling flow rates, long sampling periods, or a slight elevation in temperature. It is vital to ensure that the

sampling methodology avoids collecting samples that may experience breakthrough, as this would result in underestimating the vapor concentration (and ultimately lead to underestimating the hazard). As expected, breakthrough studies demonstrated that the G-analog of VX vapor samples showed significant breakthrough when sampled on Tenax TA. A second sorbent material, Chromosorb 106, was evaluated and no breakthrough occurred during the breakthrough studies.[29]

VX vapor-sampling methods are presented for Tenax TA sorbent sampling as well as Chromosorb 106 sorbent sampling. Note that the Chromosorb 106 methodology is preferred for the collection of VX vapors from decontamination coupon and small item testing, due to the demonstrated breakthrough of VX (G-analog) through the Tenax TA adsorbent material.

8.3 Initial Method Development

8.3.1 Tenax TA Method Development

Analysis of analytical vapor samples is commonly performed using solid sorbent sampling. Air samples are collected on a tube containing a selective sorbent material, followed by thermal desorption and analysis using a GC/MS. Method development for the analysis of VX vapor samples was performed on the instrument designated as GCV.

Historically, the analysis of the VX in vapor form has been problematic given the physical and chemical properties associated with the molecule. In particular, the overall size and volatility of the VX molecule caused sampling issues. The agent VX has been known to be highly adhesive to various materials that would be utilized for testing. As a result, VX is very difficult to transport along the overall sample pathway from sample collection, to thermal desorption, through the analytical system, to ultimate detection. Altering the VX molecule made it much more similar to the G-series nerve agents by making it smaller and more volatile.[30-31] This molecule alteration was accomplished using a polyester filter pad, impregnated with silver fluoride at the distal end of the sampling apparatus, which cleaved the VX molecule between the phosphorus-sulfur bond. The silver fluoride pad (AgF pad), which is more commonly referred to as V-to-G pads, converted the VX molecule to the G-analog molecule—a molecule ~141 amu smaller and 1500 times more volatile. The G-analog (CAS number 673-97-2) has a chemical name of ethyl methylphosphonofluoridate, a molecular formula of $C_3H_8FO_2P$, and a molecular weight of 126 amu. The remainder of the VX molecule stays on the conversion pad as a silver-organic salt. Figure 70 shows a simple representation of the VX to G-analog conversion, as well as the chemical structure of the G-analog.

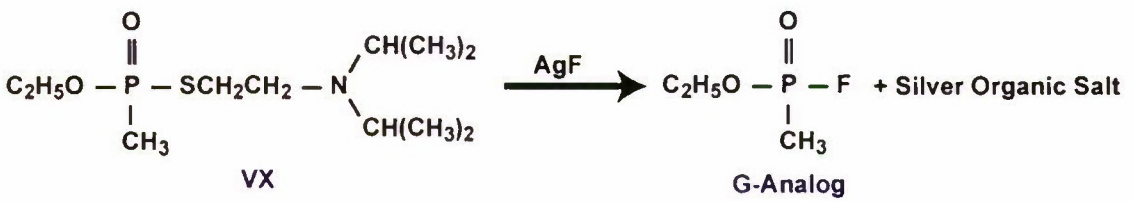


Figure 70. VX to G-analog conversion using silver fluoride (AgF).

When utilizing these pads, the primary concern was conversion efficiency of VX to the G-analog. Conversion efficiency was related to the amount of surface area available, so to ensure that the maximum amount of VX was converted to the G-analog, multiple pads were used in series. The V-to-G pads are commercially available as ¼ in. pads that can be placed into fittings for conversion of VX. In this form, two pads were used for conversion. A plain, white polyester filter pad was placed behind the V-to-G pads to protect the sorbent tubes from silver fluoride. The V-to-G pads are also commercially

available as 1/8 in. pads packed into a ¼ in. Teflon tube, which allows the V-to-G pads to be more easily placed into the sampling stream. These V-to-G tubes contained six 1/8 in. pads and were purchased through the Chemical Agent Monitoring Supply Company (CAMSCO). Method development used both styles of V-to-G pads with little difference noted in performance. The V-to-G pads are light and moisture sensitive; therefore, along with the organic salt material left on the pad post-conversion, the pads will wear and need to be replaced with fresh pads on a regular basis. All sample collection and/or method development for VX on the vapor system was performed with VX evaluated as the G-analog.

The goal of method development for VX as the G-analog was to determine the lowest mass-on-column value that gave consistent results with acceptable detection. For future testing, sample collection parameters will be adjusted to give expected masses at or above the method capabilities.

Initially, an NIST 2.0 library search was performed using the Agilent ChemStation software Enhanced Data Analysis. The search was performed for the G-analog by CAS number (673-97-2) to obtain the NIST database entry giving the mass spectrum and major ions for this analyte. The NIST library entry number is 226118 and the 10 largest peaks for G-analog, per the NIST library are detailed in Table 30.

Table 30. G-Analog mass spectra characteristics.

Compound	G- Analog
NIST No.	226118
ID No.	56237
DB	mainlib
Peak 1	99 (999)
Peak 2	81 (530)
Peak 3	111 (250)
Peak 4	82 (230)
Peak 5	27 (80)
Peak 6	47 (80)
Peak 7	67 (80)
Peak 8	83 (70)
Peak 9	29 (60)
Peak 10	45 (60)

After examining the mass spectrum for G-analog, the MSD was set up to simultaneously analyze in the SCAN and SIM modes of acquisition. Based on the ion relative abundances from the expected mass spectrum, the ions selected for the SIM mode were m/z 99 (base peak), 81, 111, and 125. Note that the molecular ion of G-analog (m/z 126) was not present in the mass spectrum. In addition, m/z 81 was sometimes detected above the expected relative abundance and was found to be a natural interferent. The presence of m/z 81 can potentially skew the overall expected relative abundances and, therefore, the reported Q-value. Although it was not one of the more abundant ions, m/z 125 was chosen as the highest m/z ion to help qualify the G-analog mass spectrum. If the peak found during analysis was at the correct G-analog retention time, and m/z 81 was not in the appropriate relative abundance, m/z 81 was excluded while quantifying and qualifying G-analog.

In an effort to correctly identify the G-analog peak, generic method parameters were selected for the GCV instrument for initial sample analysis. Initial Markes system settings were established based on manufacturer recommendations for the Tenax-TA sorbent material used. No Markes TDS split was initially needed for the VX method. Chemical agent standards were prepared in high purity IPA for instrument calibration. Standards were introduced onto the sorbent tubes by spiking the tubes with 5 µL of known agent standard concentrations. Note that tubes were spiked with agent standards through V-to-G tubes to convert the VX to the G-analog, as previously described. Experimentation with spiking agent standards through the V-to-G tubes indicated that the optimal conversion efficiency was obtained with a flow rate of 200 mL/min, aspirated for 3 min. Solvent blanks and a higher concentration of VX standard (10 ng/µL) were spiked onto conditioned tubes for a spike mass of 50 ng to be introduced to the GCV. Solvent blanks were analyzed before and after the VX standard to ensure that there were no baseline issues that might interfere with the analyte of interest. The goals of injecting a higher concentration standard were to identify the correct peak via library matching, determine RT, and evaluate peak shape on the TIC. Evaluating peak shape (efficiency and resolution) allowed the method to be altered for optimal chromatography. Typically, peaks on the TIC were identified by the mass spectrum, which contained selected ions in certain relative abundances. As lower concentrations of the standard were evaluated to determine detection limit capabilities, selected ion relative abundances deviated from the ideal. By initially identifying the correct peak via library matching and noting the RT, the identification of the peak was possible at much lower concentrations. When the ions of interest were not in their ideal relative abundances at these lower concentrations, analyte peaks could be identified by RT. After analysis of the initial samples, the peak was successfully identified by PBM of the mass spectrum from the SCAN mode of acquisition with the G-analog entry in the NIST 2.0 library. The PBM of a 50 ng VX standard, analyzed as the G-analog, identified the analyte peak at 2.70 min as G-Analog with an 83% match quality. The next highest match, identified as the very similar n-propyl methylphosphonofluoridate, gave a 64% match quality. From these results, the analyte peak was correctly identified as G-analog. Note that the mass spectrum for the G-analog in SIM mode of acquisition also showed the ions in the expected relative abundances. The solvent blank did not show a chromatographic peak at the RT of the peak identified as G-analog. An example of the VX standard, analyzed as G-analog in the SIM and SCAN modes of acquisition on the MSD, and an example of the solvent blank are shown in Figure 71 and Figure 72, respectively.

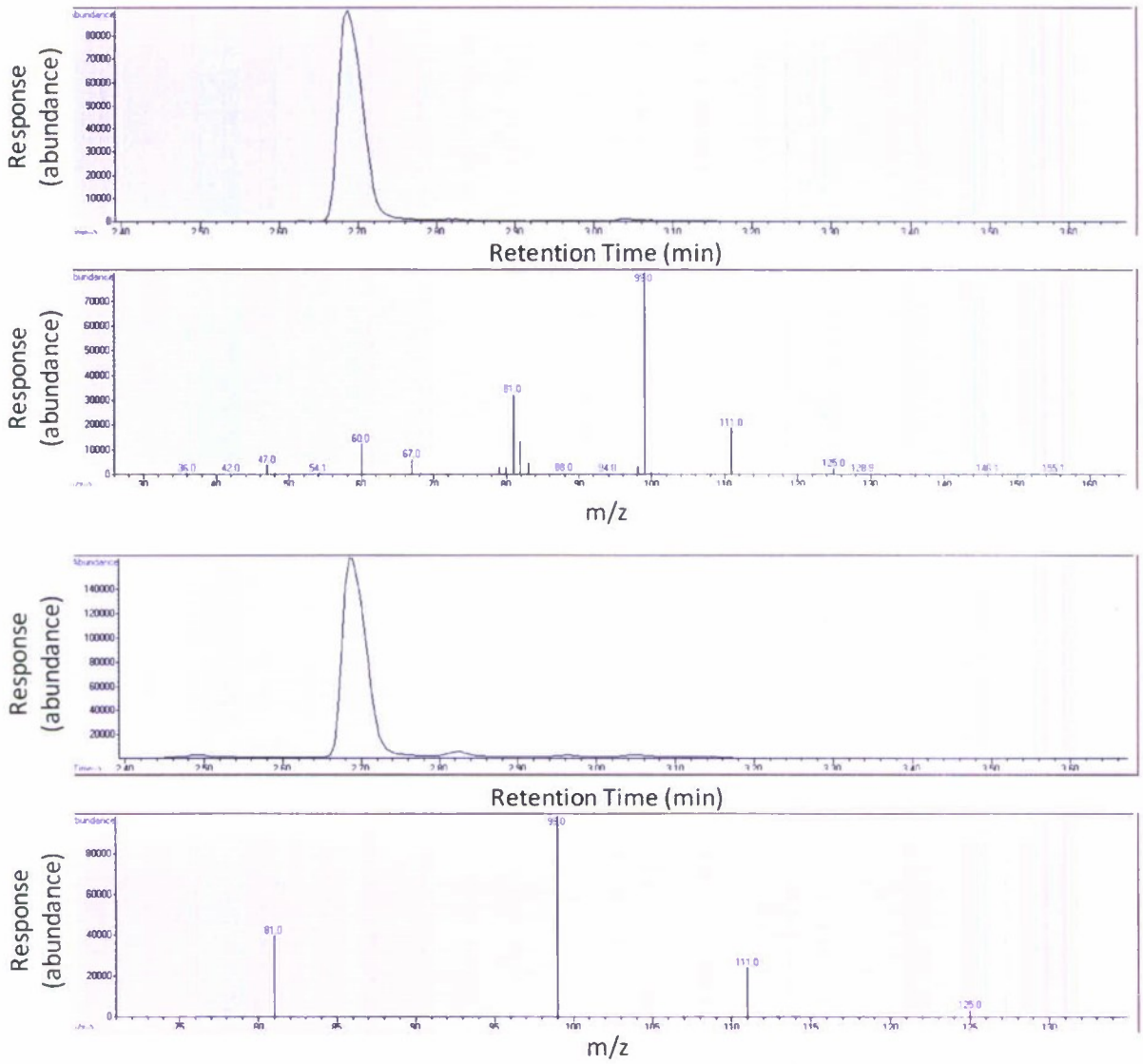


Figure 71. GC/MS SCAN and SIM analysis of 50 ng VX as the G-analog on GCV.

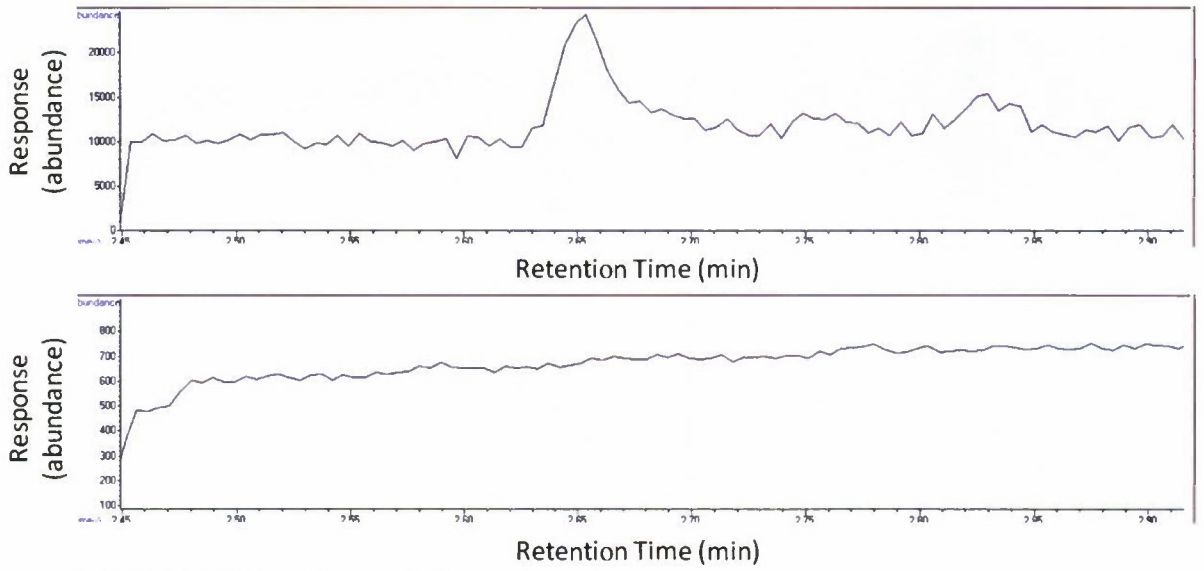


Figure 72. GC/MS SCAN and SIM analysis of an IPA solvent blank on GCV zoomed at the G-Analog RT of 2.70 min.

Additional tubes were analyzed after spiking with decreasing masses in an attempt to identify the observed method detection limits. The range of VX detection was sought to determine the lowest mass on column in nanograms (ng) that would yield the NIST 2.0 library search characterization ions in the correct ratios, and the greatest mass on column that could be analyzed without detector saturation or excessive carryover. Varying masses of analyte were spiked and analyzed. The low-end detection limit capabilities for VX as the G-analog on the GCV were observed to be 3 ng mass on column. The same procedure was performed to establish an upper-end capability, which was determined to be 500 ng. No Markes TDS split was needed for this mass range at this time.

8.3.2 Initial Method Capabilities

8.3.2.1 Method GCV VX-DEANS.M — Initial

The GCV VX-DEANS.M method was developed with the Markes TDS, Agilent 6890N GC coupled with an Agilent Deans Switch, Agilent 5975 MSD, and an AgF pad for conversion to the G-analog of VX.

The purpose of the GCV VX-DEANS.M method was to detect and quantify the chemical agent VX as ethyl methylphosphonofluoridate (G-analog) in vapor samples. The research target was established as the lowest mass on column that can be confidently measured, allowing reasonable sample throughput in support of decontaminant performance evaluations. This method detected VX as the G-analog with a mass-on-column range of 3–500 ng. No significant method interferences were identified. However, users should confirm that new test materials do not off-gas a compound that could elute in the detection region of interest. Sample throughput of this method was approximately four samples per hour.

The GCV VX-DEANS.M method was appropriate for vapor samples containing the chemical agent VX as the G-analog. This method utilized seven calibration standards at masses of ~3, 10, 25, 50, 100, 250, and 500 ng VX. The method use, detection limit, and quantitation limit were based on using the full standard set. The standards were prepared in IPA.

The *initial* method performance for VX as G-analog is as follows:

- Quantitative Method
- Calibration Range: ~3–500 ng VX on column
- Calibration Model: Linear with intercept
- Calibration Weighting: One over the mass squared ($1/x^2$)
- LOD: 0.86 ng
- LOQ: 2.62 ng
- Spiking Sample Solvent: IPA
- Quantitation Ion: 99

Calibration standards were prepared and analyzed six times for calibration consistency and detection limit determination. The calibration range of 3–500 ng/mL showed a linear response. This method used a linear with intercept calibration model with one over the mass squared weighting. This method had an LOD of 0.86 ng and an LOQ of 2.62 ng. The precision of the method was determined by analysis of the multiple CCV samples analyzed. Six replicate analyses of the 3 ng standard produced a standard deviation of 0.19 and an RSD of 6.65%, which indicated very good reproducibility at the lowest standard in the calibration curve. For the other standards, six replicates were analyzed producing standard deviations ranging from 0.61 to 34.31 and RSDs ranging from 2.00 to 14.01%. The calibration curves were reproducible—the %RSD of the slope and intercept for six calibration curves were 4.68% and 94.14%, respectively, and the correlation coefficients ranged from 0.9989 to 1.

The research target was established as the lowest mass on column that can be confidently measured, allowing reasonable sample throughput in support of decontaminant performance evaluations. The low-level VX vapor method (GCV VX-DEANS.M) detected and quantified VX as the G-analog with a mass-on-column range of 3–500 ng using a GC/MS.

8.4 Final Method Refinement

8.4.1 Chromosorb 106 Method Development

As with all aspects of a quality system that undergoes continuous improvement, method development and validation continues to ensure that the methods used are the best, most robust methods, giving the greatest possible precision and accuracy available for the technology. The VX (G-analog) vapor method was improved to offer more reliable sampling, a greater dynamic range when collecting vapor samples, and better chromatography.

After initial method development and validation with Tenax TA, stringent method testing continued showing breakthrough of G-analog. In review, vapor sampling occurs through the ability of a solid-sorbent material to retain the compound of interest present in a vapor stream. Originally, Tenax-TA was selected for VX (G-analog) testing; however, initial breakthrough studies showed that VX (G-analog) was not consistently retained on the Tenax-TA at the higher flow rates and/or sampling times needed to determine the safe-sampling volume. To compensate, a more selective sorbent material, Chromosorb 106 was chosen. Breakthrough studies demonstrated that Chromosorb 106 successfully retained G-analog.

Method development with Chromosorb 106 consisted of verification of the initial method parameters determined using the Tenax TA sorbent. It was observed that there was little difference in method performance when using Chromosorb 106 as the sorbent material. Breakthrough studies also validated, with high confidence that the Chromosorb 106 material retained the entire VX (G-analog) sample presented during the sampling process. Using the Chromosorb 106 tubes did show a slightly elevated chromatographic baseline; however, this did not interfere with the ability of the system to confidently detect VX (G-analog). Additionally, it was also observed that tube spiking required higher flow rates with long aspiration times to effectively remove the standard delivery solvent (IPA), which contributed to the slightly elevated baseline. The new spiking aspiration parameters entailed a flow rate of 750 mL/min with an aspiration time of 20 min. After the initial method capabilities assessment it was determined that the original method range of 3–500 ng, with no Markes split, presented multiple limitations for sampling and analysis.

From the sampling standpoint, the dynamic range was not large enough to feasibly predict vapor sample collection sampling schedules so that expected masses would not exceed the high end mass of the method capability. This created a situation where samples would be consistently higher than the upper detection capability of the method. This caused scenarios whereby experiments had to be repeated, adjusting the sampling times to compensate for the expected lower mass-on-tube amounts. Analytically, the splitless method was potentially overloading the column at the upper end of the mass range and beyond, causing chromatographic issues such as peak tailing, carryover, and detector saturation. Due to these and other issues, the VX method was refined into two methods with two working mass ranges using Chromosorb 106 as the sorbent material. The two new VX methods, a low-level (LL) and high-level (HL), allowed a much larger dynamic mass range for sampling purposes and were designated VXLL and VXHL, respectively. The new, overall mass range for both methods combined was 0.5–1500 ng.

- The low-level method mass range was 0.5–100 ng and remained a splitless method.
- The high-level method mass range was 50–1500 ng and incorporated a Markes split of ~20:1.

The high-level method split is a *double split,* whereby part of the sample is split at the inlet (during sample transfer from the sample collection tube to the cold trap) and the rest of the sample is split at the outlet (during sample transfer from the cold trap to the analytical column). Sample splitting is achieved via adjusting the Markes TDS parameters controlled by the Unity software. The chromatographic/detection parameters are adjusted independently by the GC/MS ChemStation software.

The most notable change on the GC part of the GCV instrument was to the Deans switch configuration and method, which was changed to more closely mimic the Deans settings of the GCE instrument. This allowed more consistent heartcut and analyte retention times and incorporated a more consistent maintenance schedule and consumable parts list among all instruments. Additionally, the chromatographic parameters were adjusted for better performance in overall peak shape and detection. For example, the oven temperature ramp was made less aggressive to facilitate better separation and resolution of the analyte from other, potentially interfering peaks that may be present in the chromatogram. Finally, to improve detection, the m/z 125 was removed and dwell times in the SIM mode of acquisition were also decreased, allowing more cycles per second (i.e., scans across the peak).

The only difference between the VXLL and VXHL methods are the split parameters established on the Markes TDS and set by the Unity software. The Unity software parameters are established as Unity method files with the file extension ".mth". The Unity methods were designated as VXLL.mth and VXHL.meth. The MSD ChemStation parameters for the VXLL-DEANS.M and VXHL-DEANS.M methods are identical; separate method names are used for consistency with the standalone

Unity software method parameters. The calibration curve fit and weighting was adjusted for optimal performance and consistency across the new method ranges.

8.4.2 Final Method Capabilities

After refinement was complete, the final method capabilities were examined. The final method capabilities were then used to determine the most appropriate methodology for decontamination test sample analysis.

8.4.2.1 Method GCV VXLL-DEANS.M with VXLL.mth — Final

Method Purpose and Performance Overview: The purpose of the GCV VXLL-DEANS.M method is to detect and quantify the chemical agent VX as ethyl methylphosphonofluoridate (G-analog) in vapor samples. The research target was established as the lowest mass on column that can be confidently measured, allowing reasonable sample throughput in support of decontaminant performance evaluations. This method detects VX (G-analog) with a mass-on-column range of 0.5–100 ng. The method's detection requirements were optimized for the analysis of a 2 in. diameter test surface area. This method can support larger item vapor testing. No significant method interferents were identified during method development or subsequent interference testing. The sample throughput of this method is approximately four samples per hour.

Method Summary and Operation Details: The GCV VXLL-DEANS.M method is appropriate for vapor samples containing the chemical agent VX as the G-analog. This method utilizes nine calibration standards at masses of ~0.5, 0.5, 0.5, 1.0, 2.5, 5.0, 10, 50, and 100 ng VX. The method use, detection limit, and quantitation limit are based on using the full standard set. The standards were prepared in IPA.

The *final* method performance for VX as G-analog is as follows:

- Quantitative Method
- Calibration Range: 0.5–100 ng VX (G-analog) on column
- CCV Levels: 0, 10, 10 ng
- Calibration Model: Weighted linear regression
- Calibration Weighting: One over the mass squared ($1/x^2$)
- LOD: 0.14 ng
- LOQ: 0.46 ng
- Spiking Sample Solvent: IPA
- Quantitation Ion: 99

LOD and Quantitation and Measurement Uncertainty: Calibration standards were prepared and analyzed multiple times for calibration consistency and detection limit determination. The final method evaluation pulled calibration and CCV performance data sets from decontamination performance testing queues. The high concentration standard of the calibration set was followed by tube blanks to determine method carryover. The carryover from the high-level calibration standard has no affect on the method or the ability of the method to detect and quantify the low end of the calibration

range. The calibration range of 0.5–100 ng yielded a linear response. This method uses a weighted linear regression calibration model with an applied weighting of $1/x^2$. The application of $1/x^2$ weighting ensures accuracy at the lower levels of the calibration curve. Accuracy at the upper end of the calibration mass range was maintained with the $1/x^2$ weighting. A demonstration of the GCV VXLL-DEANS calibration performance can be seen in Figure 73.

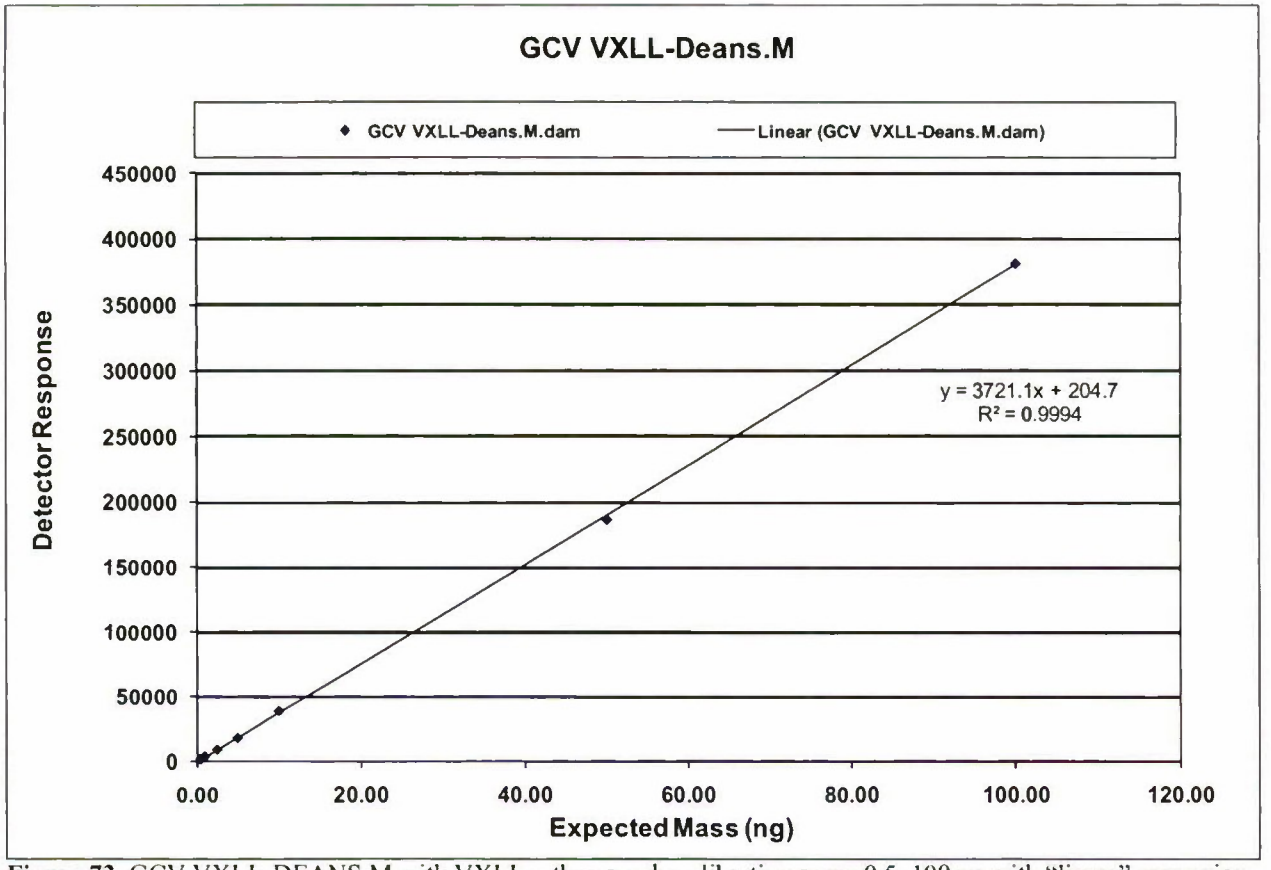


Figure 73. GCV VXLL-DEANS.M with VXLL.mth example calibration curve 0.5–100 ng with "linear" regression ("$1/x^2$" weighting).

The GCV VXLL-DEANS.M method yielded a calculated LOD of 0.14 ng and an LOQ of 0.46 ng. The precision of the method was determined by analysis of multiple CCV samples analyzed. From one representative test, there were 12 replicate analyses of the 10 ng CCV, which produced an average found mass of 10.1 ng with a standard deviation of 1.61 and an RSD of 15.94%. This indicated good reproducibility at the CCV level within the calibration curve. Furthermore, there were 13 replicate analyses of the 0.5 ng low-level standard from multiple tests, which produced a standard deviation of 0.05 and an RSD of 9.02%. This indicated very good reproducibility at the lowest standard in the calibration curve. For the other standards, replicates were analyzed, producing standard deviations ranging from 0.1 to 8.4 and RSDs ranging from 5.54 to 10.7% indicating very good, overall method performance.

The accuracy of the method is demonstrated in Table 31 as uncertainty in the measurements at each standard in the calibration curve with 95% confidence. For a standard or sample, containing 10 ng of analyte, the analytical result reported would be 10 ± 0.04 ng.

Table 31. List of standard masses and the associated confidence intervals.

Standard Mass on Tube (ng)	95% Confidence Interval (ng)	Lower Confidence Interval (ng)	Upper Confidence Interval (ng)
0.50	0.023	0.477	0.523
0.50	0.023	0.477	0.523
0.50	0.023	0.477	0.523
1.00	0.023	0.977	1.02
2.5	0.023	2.48	2.52
5.0	0.027	4.97	5.03
10.0	0.040	9.96	10.04
50.0	0.241	49.8	50.2
100.0	0.526	99.5	100.5

8.4.2.2 Method GCV VXHL-DEANS.M with VXHL.mth — Final

Method Purpose and Performance Overview: The purpose of the GCV VXHL-DEANS.M method is to detect and quantify the chemical agent VX as ethyl methylphosphonofluoridate (G-analog) in vapor samples. This method was developed as a complementary method to the low-level method (GCV VXLL-DEANS.M) for the evaluations of vapor sample sorbent tubes containing high masses of VX (G-analog). This method detects VX (G-analog) with a mass-on-tube range of 50–1500 ng. No significant method interferents were identified during method development or subsequent interference testing. The sample throughput of this method is approximately four samples per hour.

Method Summary and Operation Details: The GCV VXHL-DEANS.M method is appropriate for vapor samples containing the chemical agent VX as the G-analog. This method utilizes seven calibration standards at masses of ~50, 100, 250, 500, 750, 1000, and 1500 ng VX. The method use, detection limit, and quantitation limit were based on using the full standard set. The standards are prepared in IPA.

The *final* method performance for VX as G-analog is as follows:

- Quantitative Method
- Calibration Range: 50–1500 ng VX (G-analog) on column
- CCV Levels: 0, 500, 500 ng
- Calibration Model: Weighted linear regression
- Calibration Weighting: One over the mass squared ($1/x^2$)
- LOD: 1.34 ng
- LOQ: 4.47 ng
- Spiking Sample Solvent: IPA
- Quantitation Ion: 99

LOD and Quantitation and Measurement Uncertainty: Calibration standards were prepared and analyzed multiple times for calibration consistency and detection limit determination. The final method evaluation pulled calibration and CCV performance data sets from decontamination performance testing queues. The high mass standard of the calibration set was followed by tube blanks to determine method carryover. The carryover from the high-level calibration standard has no affect on the method or the ability of the method to detect and quantify the low end of the calibration range. The calibration range of 50–1500 ng yielded a linear response. This method uses a weighted linear regression calibration model with an applied weighting of $1/x^2$. The application of $1/x^2$ weighting ensures accuracy at the lower levels of the calibration curve. Accuracy at the upper end of the calibration mass range is maintained with the $1/x^2$ weighting. A demonstration of the GCV VXHL-DEANS calibration performance can be seen in Figure 74.

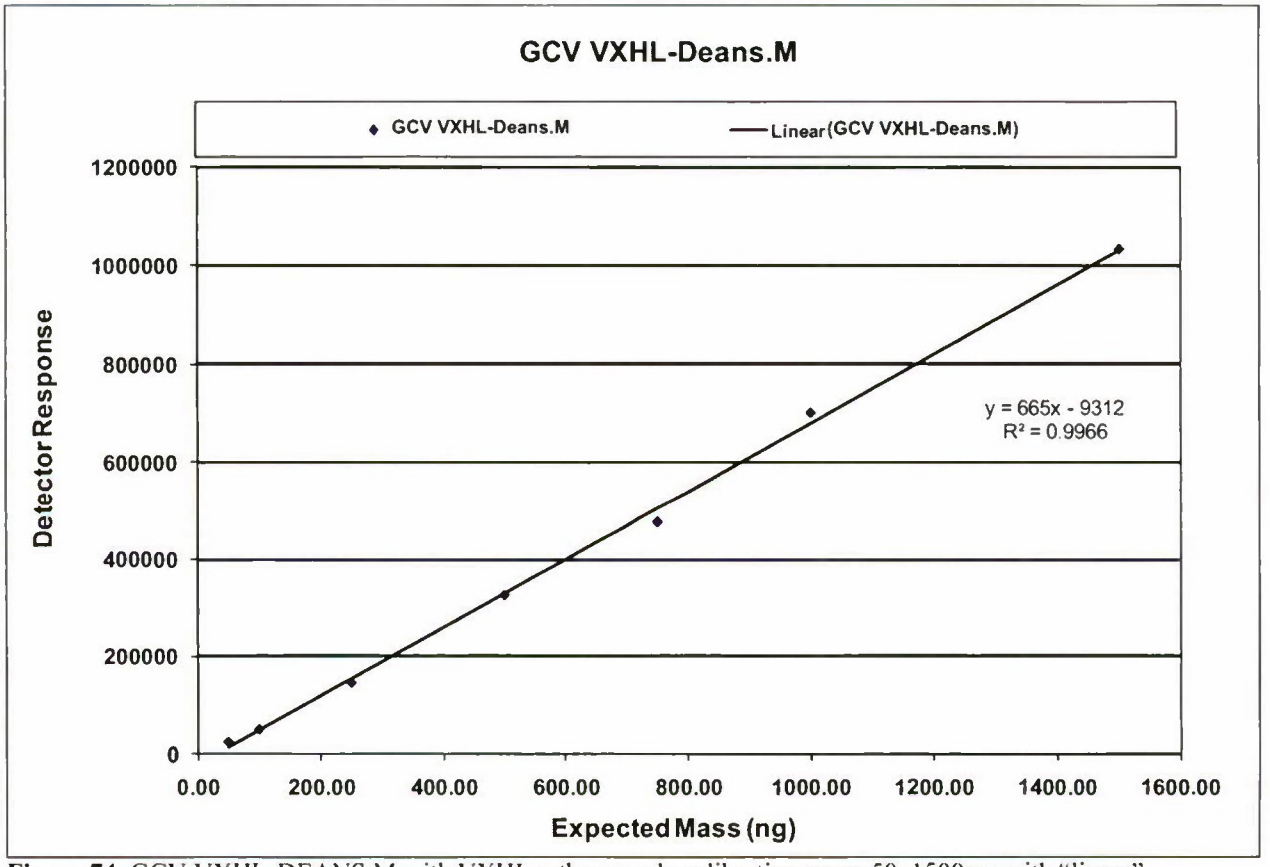


Figure 74. GCV VXHL-DEANS.M with VXHL.mth example calibration curve 50–1500 ng with "linear" regression ("$1/x^2$" weighting).

The GCV VXHL-DEANS.M method yielded a calculated LOD of 1.34 ng and an LOQ of 4.47 ng. The precision of the method was determined by analysis of multiple CCV samples analyzed. From one representative test, there were 12 replicate analyses of the 500 ng CCV, which produced an average found mass of 518 ng with a standard deviation of 22.8 and an RSD of 4.4%. This indicated very good reproducibility at the CCV level within the calibration curve. Furthermore, there were multiple replicate analyses of the 50 ng low-level standard from multiple tests, which produced a standard deviation of 0.45 and an RSD of 0.89%. This indicated very good reproducibility at the lowest standard in the calibration curve. For the other standards, replicates were analyzed, producing standard deviations

ranging from 4.3 to 134.5 and RSDs ranging from 4.28 to 8.70% indicating excellent, overall method performance.

The accuracy of the method is demonstrated in Table 32 as uncertainty in the measurements at each standard in the calibration curve with 95% confidence. For a standard or sample, containing 10 ng of analyte, the analytical result reported would be 10 ± 0.04 ng.

Table 32. List of standard masses and the associated confidence intervals.

Standard Mass on Tube (ng)	95% Confidence Interval (ng)	Lower Confidence Interval (ng)	Upper Confidence Interval (ng)
50	14.154	35.8	64.2
100	14.155	85.8	114.2
250	14.426	235.6	264.4
500	14.981	485.0	515.0
750	15.547	734.5	765.5
1000	16.114	983.9	1016
1500	17.251	1483	1517

8.5 Method Demonstration for Decontaminant Performance Testing

8.5.1 GCV VXLL-DEANS.M Method Demonstration

The VX LL vapor method (GCV VXLL-DEANS.M) was used to analyze vapor samples collected from multiple coupons of the material Navy TopCoat spiked with a known amount of the chemical agent VX and decontaminated with multiple test decontaminants. This set of vapor samples was collected as part of test number SSVV17. The objective of SSVV17 was to show the decontamination performance of the test decontaminants as applied to the emission of agent vapors collected from the treated coupons.

The materials were spiked with known amounts of VX and were then decontaminated. The coupons were moved to the microchamber vapor manifold for vapor sampling onto Markes TDS tubes packed with Chromosorb 106. The microchamber vapor-sampling manifold had previously been cleaned, verified blank, and loaded with tubes ready for analysis. Operating conditions of the instrument had been met prior to test sample collection. Samples were collected at specific time intervals and flow rates (mL/min), which were pre-determined by the test designer and previously loaded to the instrument as a set of instructions. This test was performed in accordance with the panel testing guidance set forth in the Source Document.[16]

Upon test completion, the sample tubes were removed from the sampling manifold and analyzed on a GCV instrument. The expected masses of VX (G-analog) on the vapor sample spanned the range of both VX vapor methods; therefore, the tubes were sorted into two analytical queues for analysis. The tubes with expected masses within the low-level calibration range (0.5–100 ng) were analyzed with the method GCV VXLL-DEANS.M.

Method parameters were established on GCV in accordance with Appendix A. Prior to analysis of the test samples, the instrument was calibrated using VX calibration standards prepared in high purity solvent and spiked to Markes TDS tubes. The GCV VX calibration curve for this test had a correlation coefficient (r) value of 0.9994. The calibration curve was established with a weighted linear

curve fit ($1/x^2$). The calibration curve for this test is shown in Figure 75. A summary of the method performance with calculated LOD and LOQ is shown in Table 33.

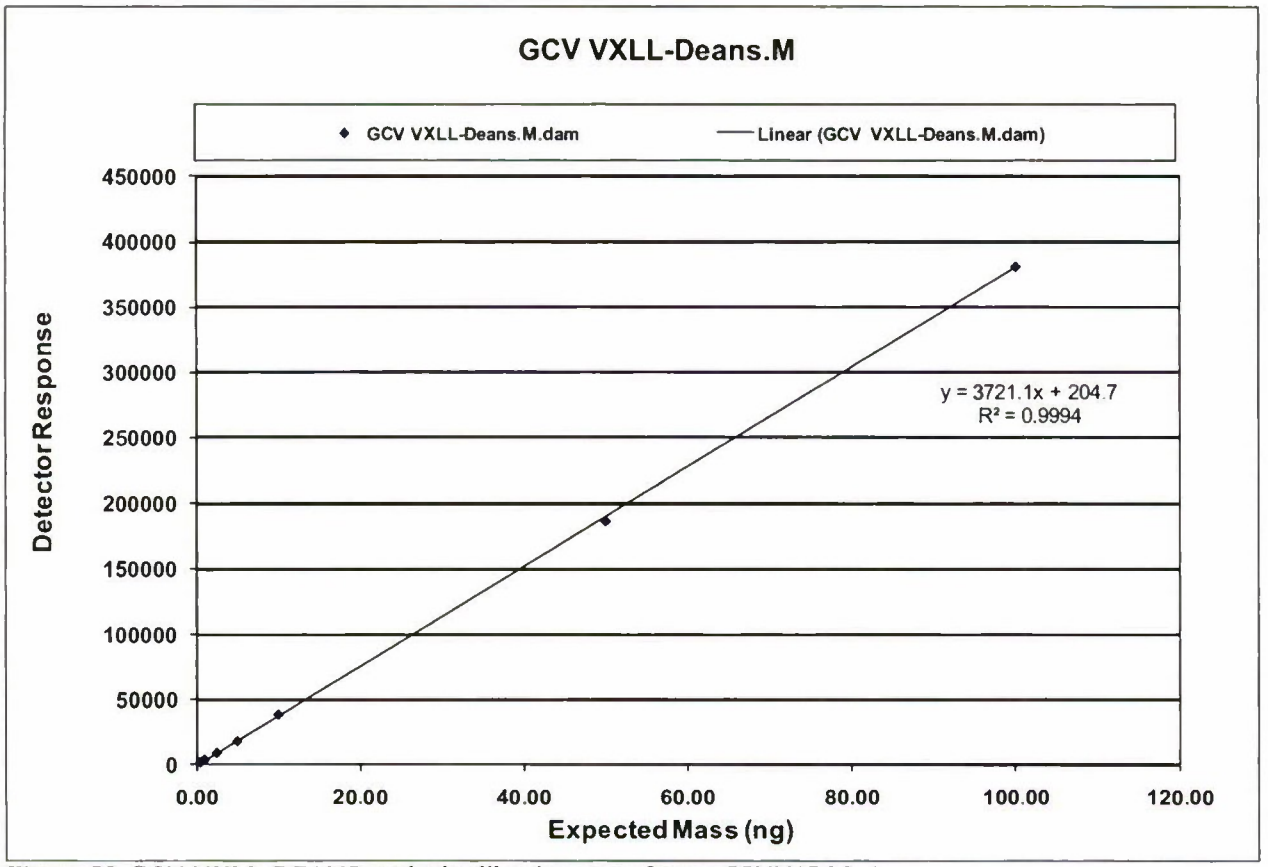


Figure 75. GCV VXLL-DEANS method calibration curve for test SSVV17 LL-1.

Table 33. GCV VXLL-DEANS method summary for test SSVV17 LL-1.

Method	GCV VXLL-DEANS.M
Test ID	SSVV17, LL-1
Platform	GCV (GC/MS)
Sample Type	Vapor
Analyte(s)	VX (G-analog)
LOD	0.055 ng
LOQ	0.314 ng
Data Acquisition	EI SIM
Quantitation Ion	99 m/z

CCV samples were performed every 12 samples, and were found to be within ±30% RPD of their expected mass in ng. Note that one CCV failed; however, the redundant CCV was within allowable tolerance at 4.6% (RPD to expected mass), validating instrument control. Tube blanks were analyzed throughout the test for continuing baseline acceptance. Analysis of the blank samples showed little carryover and no interferences. The sample queue, with analytical data, for the performance testing

samples analyzed with the GCV VXLL-DEANS method is shown in Figure 76. All samples were successfully analyzed using this GCV VXLL-DEANS method.

Sample Name	Q Value	Response	Mass on Tube (ng)
S5VV17_LL1_Pos01_Std_0d0	0	0	ND
SSVV17_LL1_Pos02_Std_0dS	S2	1976	0.48
S5VV17_LL1_Pos03_Std_0dS	54	2094	0.S1
SSVV17_LL1_Pos04_Std_0d5	62	2155	0.52
SSVV17_LL1_Pos0S_Std_1d0	80	3865	0.98
SSVV17_LL1_Pos06_Std_2dS	92	9213	2.42
SSVV17_LL1_Pos07_Std_Sd0	96	18170	4.83
SSVV17_LL1_Pos08_Std_10d0	97	38888	10.41
S5VV17_LL1_Pos09_Std_50d0	98	186665	50.18
SSVV17_LL1_Pos10_Std_100d0	97	381S38	102.62
SSVV17_LL1_Pos11_Std_0d0	98	2254	0.55
SSVV17_LL1_Pos12_0d0_CCV01	41	7S9	BQ
SSVV17_LL1_Pos13_10d0_CCV01	99	42230	11.31
5SVV17_LL1_Pos14_10d0_CCV01	99	4211S	11.28
SSVV17_01NV_C01_T00	97	17SS76	47.19
SSVV17_02NV_C02_T00	98	369S	0.94
SSVV17_03NV_C03_T00	97	25181	6.72
55VV17_04NV_C04_T00	98	7910	2.07
SSVV17_0SNV_C0S_T00	97	73867	19.82
55VV17_06NV_C06_T00	99	4734	1.22
SSVV17_07NV_C07_T00	74	1164	BQ
55VV17_08NV_C08_T00	98	6780	1.77
SSVV17_09NV_C09_T00	41	969	BQ
SSVV17_10NV_C10_T00	71	124S	BQ
SSVV17_11NV_C11_T00	80	1916	0.46
5SVV17_12NV_C12_T00	41	916	BQ
SSVV17_LL1_Pos27_0d0_CCV02	0	0	ND
SSVV17_LL1_Pos28_10d0_CCV02	98	20660	5.50
SSVV17_LL1_Pos29_10d0_CCV02	99	39075	10.46
5SVV17_13NV_C13_T00	67	1323	BQ
55VV17_14NV_C14_T00	97	3230	0.81
S5VV17_15NV_C1S_T00	71	1367	BQ
5SVV17_16NV_C16_T00	98	2767	0.69
55VV17_17NV_C17_T00	41	776	BQ
S5VV17_18NV_C18_T00	0	0	ND
SSVV17_19NV_C19_T00	41	661	BQ
55VV17_20NV_C20_T00	0	0	ND
SSVV17_LL1_Pos38_0d0_CCV03	0	0	ND
SSVV17_LL1_Pos39_10d0_CCV03	99	41062	10.99
SSVV17_LL1_Pos40_10d0_CCV03	99	41379	11.08

BD = Below Detect (LOD)

BQ = Below Quant (LOQ)

ND = Non Detect

*The shading is for ease of reading. Light green indicates calibration and CCV levels. Light blue indicates samples. Yellow indicates detector response information. Red indicates a failing CCV value.

Figure 76. VXLL sequence and results for SSVV17 LL1.

8.5.2 GCV VXHL-DEANS.M Method Demonstration

The VX HL vapor method (GCV VXHL-DEANS.M) was used to analyze vapor samples from multiple coupons of the material Navy TopCoat, spiked with a known amount of the chemical agent VX and decontaminated with multiple test decontaminants. This set of vapor samples was collected as part of test number SSVV17. The objective of SSVV17 was to show the decontamination performance of the test decontaminants as applied to the emission of agent vapors collected from the treated coupons.

The materials were spiked with known amounts of VX and were then decontaminated. The coupons were moved to the microchamber vapor manifold for vapor sampling onto Markes TDS tubes packed with Chromosorb 106. The microchamber vapor-sampling manifold had previously been cleaned, verified blank, and loaded with tubes ready for analysis. Operating conditions of the instrument had been met prior to test sample collection. Samples were collected at specific time intervals and flow rates (mL/min), which were pre-determined by the test designer and previously loaded to the instrument as a set of instructions. This test was performed in accordance with the panel testing guidance set forth in the Source Document.[16]

Upon test completion, the sample tubes were removed from the sampling manifold and analyzed on a GCV instrument. The expected masses of VX (G-analog) on the vapor sample spanned the range of both VX vapor methods; therefore, the tubes were sorted into two analytical queues for analysis. The tubes with expected masses within the high-level calibration range (50–1500 ng) were analyzed with the method GCV VXHL-DEANS.M.

Method parameters were established on GCV in accordance with Appendix A. Prior to analysis of test samples, the instrument was calibrated using VX calibration standards prepared in high purity solvent and spiked to Markes TDS tubes. The GCV VX calibration curve for this test had a correlation coefficient value of 0.9966. The calibration curve was established with a weighted linear curve fit ($1/x^2$). The calibration curve for this test is shown in Figure 77. A summary of the method performance with calculated LOD and LOQ is shown in Table 34.

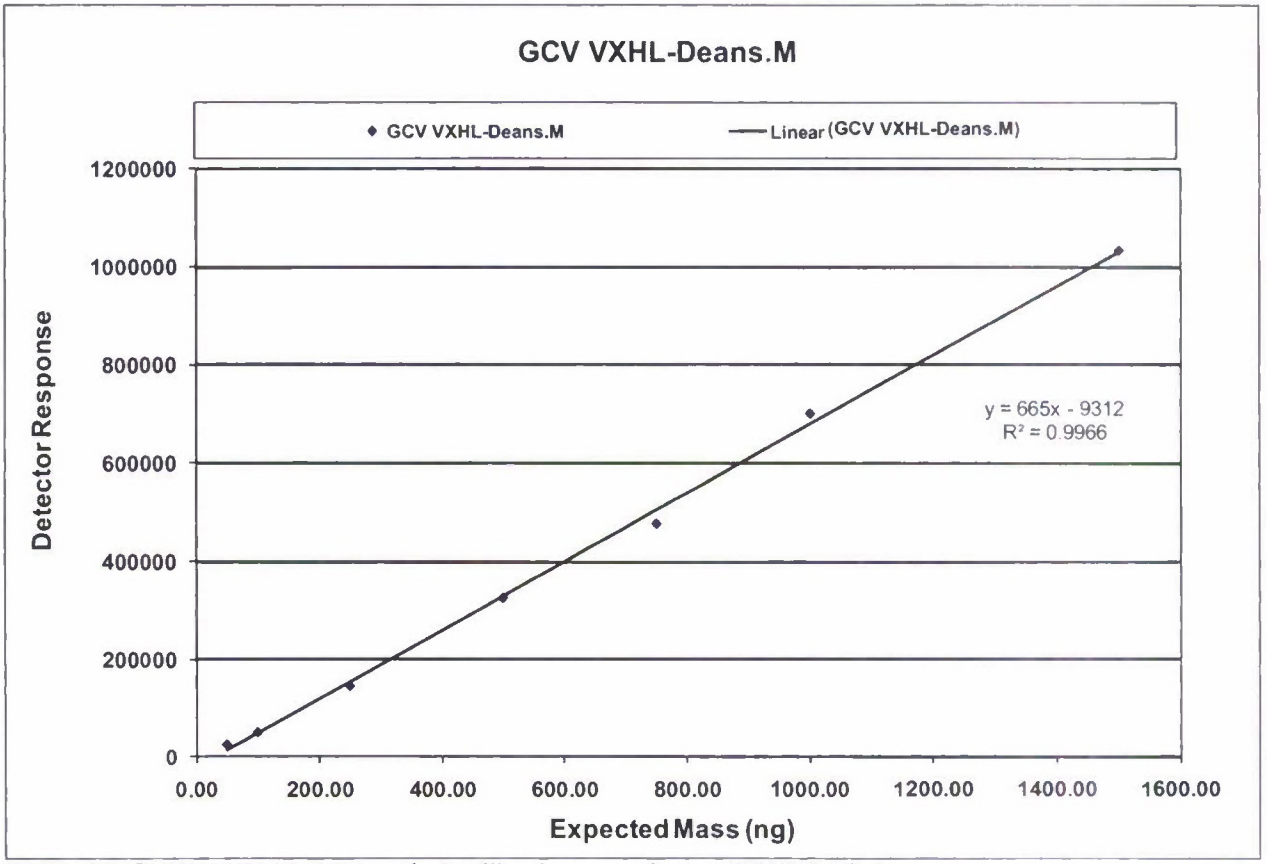


Figure 77. GCV VXHL-DEANS method calibration curve for test SSVV17 HL-1.

Table 34. GCV VXHL-DEANS method summary for test SSVV17 HL-1.

Method	GCV VXHL-DEANS.M
Test ID	SSVV17, HL-1
Platform	GCV (GC/MS)
Sample Type	Vapor
Analyte(s)	VX (G-analog)
LOD	13.6 ng
LOQ	14.1 ng
Data Acquisition	EI SIM
Quantitation Ion	99 m/z

CCV samples were performed every 12 samples, and were found to be within ±30% RPD of their expected mass. Tube blanks were analyzed throughout the test for continuing baseline acceptance. Analysis of the blank samples showed little carryover and no interferences. The sample queue, with analytical data for the performance testing samples analyzed with the GCV VXHL-DEANS method, is shown in Figure 78. All samples were successfully analyzed using this GCV VXHL-DEANS method.

Sample Name	Q Val	Response	Mass on Tube (ng)
SSVV17_HL1_Pos01_Std_0d0	0	0	ND
SSVV17_HL1_Pos02_Std_50d0	99	2S659	S2.01
SSVV17_HL1_Pos03_Std_100d0	99	S1430	90.79
SSVV17_HL1_Pos04_Std_250d0	98	146492	233.85
SSVV17_HL1_Pos0S_Std_500d0	98	326254	504.37
SSVV17_HL1_Pos06_Std_750d0	98	477158	731.47
SSVV17_HL1_Pos07_Std_1000d0	98	701200	1068.62
SSVV17_HL1_Pos08_Std_1500d0	98	1033097	1568.09
SSVV17_HL1_Pos09_Std_0d0	100	2096	16.SS
SSVV17_HL1_Pos10_0d0_CCV01	42	646	14.37
SSVV17_HL1_Pos11_500d0_CCV01	98	319112	493.62
SSVV17_HL1_Pos12_500d0_CCV01	98	338446	S22.72
SSVV17_01NV_C01_T01	98	211189	331.21
SSVV17_01NV_C01_T02	98	201682	316.90
SSVV17_01NV_C01_T03	98	216224	338.79
SSVV17_01NV_C01_T04	98	2S8169	401.91
SSVV17_01NV_C01_T0S	98	234085	36S.67
SSVV17_01NV_C01_T06	98	265017	412.22
SSVV17_02NV_C02_T01	98	289984	449.79
SSVV17_02NV_C02_T02	99	4913	20.79
SSVV17_02NV_C02_T03	98	287737	446.41
SSVV17_02NV_C02_T04	98	311463	482.11
SSVV17_02NV_C02_T0S	98	29SS8	458.18
SSVV17_02NV_C02_T06	98	314508	486.70
SSVV17_HL1_Pos2S_0d0_CCV02	42	1089	15.03
SSVV17_HL1_Pos26_500d0_CCV02	98	344061	S31.17
SSVV17_HL1_Pos27_500d0_CCV02	98	303287	469.81
SSVV17_03NV_C03_T01	98	2329SS	363.97
SSVV17_03NV_C03_T02	98	29664S	4S9.81
SSVV17_03NV_C03_T03	98	308894	478.2S
SSVV17_03NV_C03_T04	98	33261S	S13.94
SSVV17_03NV_C03_T0S	98	301798	467.S7
SSVV17_03NV_C03_T06	98	329918	509.89
SSVV17_04NV_C04_T01	98	185393	292.39
SSVV17_04NV_C04_T02	98	241507	376.84
SSVV17_04NV_C04_T03	98	26383S	410.44
SSVV17_04NV_C04_T04	98	29S76S	4S8.49
SSVV17_04NV_C04_T0S	98	2S6299	399.10
SSVV17_04NV_C04_T06	98	313624	48S.36
SSVV17_HL1_Pos40_0d0_CCV03	42	946	14.82
SSVV17_HL1_Pos41_500d0_CCV03	98	308239	477.26
SSVV17_HL1_Pos42_500d0_CCV03	98	348372	S37.66

Sample Name	Q Val	Response	Mass on Tube (ng)
SSVV17_0SNV_C0S_T01	98	2127SS	333.S7
SSVV17_0SNV_C0S_T02	98	2040S6	320.48
SSVV17_0SNV_C0S_T03	98	202598	318.28
SSVV17_0SNV_C0S_T04	98	23386S	36S.34
SSVV17_0SNV_C0S_T0S	98	225640	3S2.96
SSVV17_0SNV_C0S_T06	98	240726	37S.66
SSVV17_06NV_C06_T01	98	238338	372.07
SSVV17_06NV_C06_T02	98	271109	421.38
SSVV17_06NV_C06_T03	98	49S171	758.S7
SSVV17_06NV_C06_T04	98	41388S	636.2S
SSVV17_06NV_C06_T0S	98	439379	674.61
SSVV17_06NV_C06_T06	98	4480SS	687.67
SSVV17_HL1_PosSS_0d0_CCV04	42	1324	15.39
SSVV17_HL1_Pos56_500d0_CCV04	98	328660	507.99
SSVV17_HL1_PosS7_500d0_CCV04	98	3386SS	S23.03
SSVV17_07NV_C07_T01	98	2S0287	390.0S
SSVV17_07NV_C07_T02	98	3S7133	S50.84
SSVV17_07NV_C07_T03	98	618992	944.91
SSVV17_07NV_C07_T04	98	508961	779.33
SSVV17_07NV_C07_T0S	98	542619	829.98
SSVV17_07NV_C07_T06	98	469699	720.24
SSVV17_08NV_C08_T01	98	24845 2	387.29
SSVV17_08NV_C08_T02	98	317877	491.77
SSVV17_08NV_C08_T03	98	54S941	834.98
SSVV17_08NV_C08_T04	98	4S8629	703.S8
SSVV17_08NV_C08_T0S	98	469712	720.26
SSVV17_08NV_C08_T06	98	4S0370	691.15
SSVV17_HL1_Pos70_0d0_CCV0S	80	1308	15.36
SSVV17_HL1_Pos71_500d0_CCV0S	98	32S710	503.SS
SSVV17_HL1_Pos72_500d0_CCV0S	98	350620	541.04
SSVV17_09NV_C09_T01	98	2S7862	401.4S
SSVV17_09NV_C09_T02	98	381540	587.S7
SSVV17_09NV_C09_T03	98	670540	1022.48
SSVV17_09NV_C09_T04	98	489013	749.31
SSVV17_09NV_C09_T0S	98	507582	777.2S
SSVV17_09NV_C09_T06	98	49S2S2	7S8.70
SSVV17_10NV_C10_T01	98	2S783S	401.41
SSVV17_10NV_C10_T02	98	338718	S23.13
SSVV17_10NV_C10_T03	98	S808S3	887.S2
SSVV17_10NV_C10_T04	98	49828S	763.26
SSVV17_10NV_C10_T0S	98	S16739	791.03
SSVV17_10NV_C10_T06	98	4989SS	764.27
SSVV17_HL1_Pos85_0d0_CCV06	80	1583	15.78
SSVV17_HL1_Pos86_500d0_CCV06	98	338725	S23.14
SSVV17_HL1_Pos87_500d0_CCV06	98	313544	48S.24

BD = Below Detect (LOD)
BQ = Below Quant (LOQ)
ND = Non Detect

*The shading is for ease of reading. Light green indicates calibration and CCV levels. Light blue indicates samples. Yellow indicates detector response information.

Figure 78. VXHL sequence and results for SSVV17 HL1.

9. ANALYSIS OF HD VAPOR SAMPLES

9.1 Decontamination Efficacy Requirement Documents

The method detection limit target was the lowest mass of contaminant on a tube that could be accurately detected and quantified from the baseline noise and still enable sample throughput to support decontamination performance evaluations. The requirement document vapor concentration levels

for threshold (T) and objective (O) of primary interest, at the time of the Low-Level program, are listed in Table 35.[4-9] The requirement, toxicology, IDLH, STEL, WPL, and GPL appear in order of the vapor concentration numerical value. These values represent different exposure durations, requiring different sampling protocols for sample collection and data reporting. The toxicology vapor concentrations were for a 10 h exposure.[28] The IDLH, STEL, and WPL shown are OSHA-reported values. The IDLH value is for a one time, short-term exposure. The STEL is for a 15 min exposure, once per day. The WPL is the time-weighted average vapor concentration for a working population exposure of 8 h per day for five days per week. The GPL is the EPA general population value time-weighted average vapor concentration for lifetime exposure.[14] The vapor test, as described in the source document,[16] calculated a vapor emission rate. A vapor concentration was measured in the dynamic vapor chamber, but it did not correspond to the vapor concentration to which unprotected personnel would be exposed, and should not be directly compared to a requirement. The emission rate was used to calculate the vapor concentration that was present in a specified scenario. This use of data required that the analytical method be capable of accurately detecting agent across a broad range of masses, ideally from a few ng to as many as 1000 ng.

Table 35. Decontamination testing detection targets for HD vapor samples.

Publication	Vapor Conc. (mg/m^3)	Publication	Vapor Conc. (mg/m^3)
IDLH	0.7000000	JSSED 03 O	0.0030000
84% Tox Response	0.2000000	JSSED 04 T	0.0030000
JSSED 03 T	0.1000000	STEL	0.0030000
16% Tox Response	0.0400000	WPL	0.0004000
JPID T	0.0058000	JSSED 04 O	0.0001000
1% Tox Response	0.0040000	EPA 04 GPL	0.0000200
JPID 03 O	0.0030000		

9.2 Solid Sorbent Selection for HD Vapor Analysis

It is widely accepted that the solid sorbent material Tenax TA is an excellent all-around adsorbent material for chemical agent. Other sorbent materials are available and may have a higher affinity for agent retention during the sampling process. All initial method development work was performed on Tenax TA.

As part of method development and overall vapor sampling verification, extensive breakthrough studies were performed to demonstrate the ability of the selected sorbent to retain the agent of interest and to determine the safe-samplingvolume for the agent/sorbent pairing. *Breakthrough* is defined as the inability of the sorbent to retain the compound of interest during sampling, which allows some/all of the compound to pass through the sorbent. During sampling, breakthrough may occur if the sorbent material cannot retain the compound of interest under sampling extremes such as high sampling flow rates, long sampling periods, or a slight elevation in temperature. It is vital to ensure that the sampling methodology avoids collecting samples that may experience breakthrough, as this would result in underestimating the vapor concentration (and ultimately lead to underestimating the hazard). Breakthrough studies demonstrated that the chemical agent HD vapor samples showed no breakthrough when sampled on Tenax TA[29]

Given no observed breakthrough of HD through Tenax TA, all method development and validation acitivities were performed with this sorbent material.

9.3 Initial Method Development

Analysis of analytical vapor samples is commonly performed using solid sorbent sampling. Air samples are collected on a tube containing a selective sorbent material, followed by thermal desorption and analysis using a GC/MS. Method development for the analysis of HD vapor samples was performed on the instrument designated as GCV.

No sample pretreatment is required for collection and analyzis of HD vapor samples. The goal of method development for HD was to determine the lowest mass-on-column value that gave excellent, reproducible results. For future testing, sample collection parameters will be adjusted to give expected masses at or above the method capabilities.

Initially, a NIST 2.0 library search was performed using the Agilent ChemStation software Enhanced Data Analysis. The search was performed for HD by CAS number (505-60-2) to obtain the NIST database entry giving the mass spectrum and major ions for this analyte. The NIST library entry number is 289463 and the 10 largest peaks for HD, per the NIST library are detailed in Table 36.

Table 36. HD mass spectrum characteristics.

Compound	HD
NIST No.	289463
ID No.	65664
DB	mainlib
Peak 1	109 (999)
Peak 2	111 (383)
Peak 3	63 (347)
Peak 4	27 (252)
Peak 5	158 (238)
Peak 6	160 (162)
Peak 7	47 (158)
Peak 8	45 (132)
Peak 9	65 (112)
Peak 10	59 (95)

After examining the mass spectrum for HD, the MSD was set up to simultaneously analyze in the SCAN and SIM modes of acquisition. Based on the ion relative abundances from the expected mass spectrum, the ions selected for the SIM mode were m/z 109, 111, 158, and 160. It is important to note that the ion m/z 158 is the molecular ion of HD. The ion at m/z 109 is the base peak (most abundant) of the mass spectrum while the ion m/z 111 is the same ion fragment as m/z 109, but contains the isotope ^{37}Cl, which accounts for the additional 2 amu. Ion m/z 111 was chosen as the quantitation ion to help mitigate interferences, if present, that may fragment to m/z 109.

In an effort to correctly identify the HD peak, generic method parameters were selected for initial sample analysis using the GCV instrument. The initial Markes system settings were established based on manufacturer recommendations for the Tenax-TA sorbent material used. No Markes TDS split was initially needed for the HD method. Chemical agent standards were prepared in high purity chloroform for instrument calibration. Standards were introduced onto the sorbent tubes by spiking the

tubes with 5 µL of known agent standard concentrations. Experimentation with spiking HD agent standards onto the vapor tubes indicated that optimal spiking results were obtained using a flow rate of 200 mL/min, aspirated for 3 min. A higher concentration of HD standard (10 ng/µL) was spiked onto conditioned tubes for a spike mass of 50 ng to be introduced to the GCV. Solvent blanks were analyzed before and after the HD standard to ensure that there were no baseline issues that might interfere with the analyte of interest. Injecting a higher mass standard was done to identify the correct peak via PBM library matching, determine RT, and evaluate peak shape on the TIC. Evaluating the peak shape (efficiency and resolution) allowed the method to be altered for optimal chromatography.

Typically, peaks on the chromatogram can be identified by the mass spectrum, which contains selected ions in certain relative abundances. As lower concentrations of the standard were evaluated to determine detection limit capabilities, selected ion relative abundances began deviating from the ideal. By initially identifying the correct peak via library matching and noting the RT, the identification of the peak was possible at much lower concentrations. When the ions of interest were not in their ideal relative abundances at these lower concentrations, analyte peaks were identified by RT. After analysis of the initial samples, the peak was successfully identified by PBM of the mass spectrum from the SCAN mode of acquisition with the HD entry in the NIST 2.0 library. The PBM of a 50 ng HD standard identified the analyte peak at 4.13 min as HD with a 94% match quality. The next highest match for the analyte peak only gave a 23% match quality. From these results, the analyte peak was correctly identified as HD. Note that the mass spectrum for the HD in SIM mode of acquisition also showed the ions in the expected relative abundances. The solvent blank did not show a chromatographic peak at the RT of the peak identified as HD. For an example of the HD standard, analyzed in the SIM and SCAN modes of acquisition on the MSD, as well as an example of the solvent blank, see Figure 79 and Figure 80, respectively.

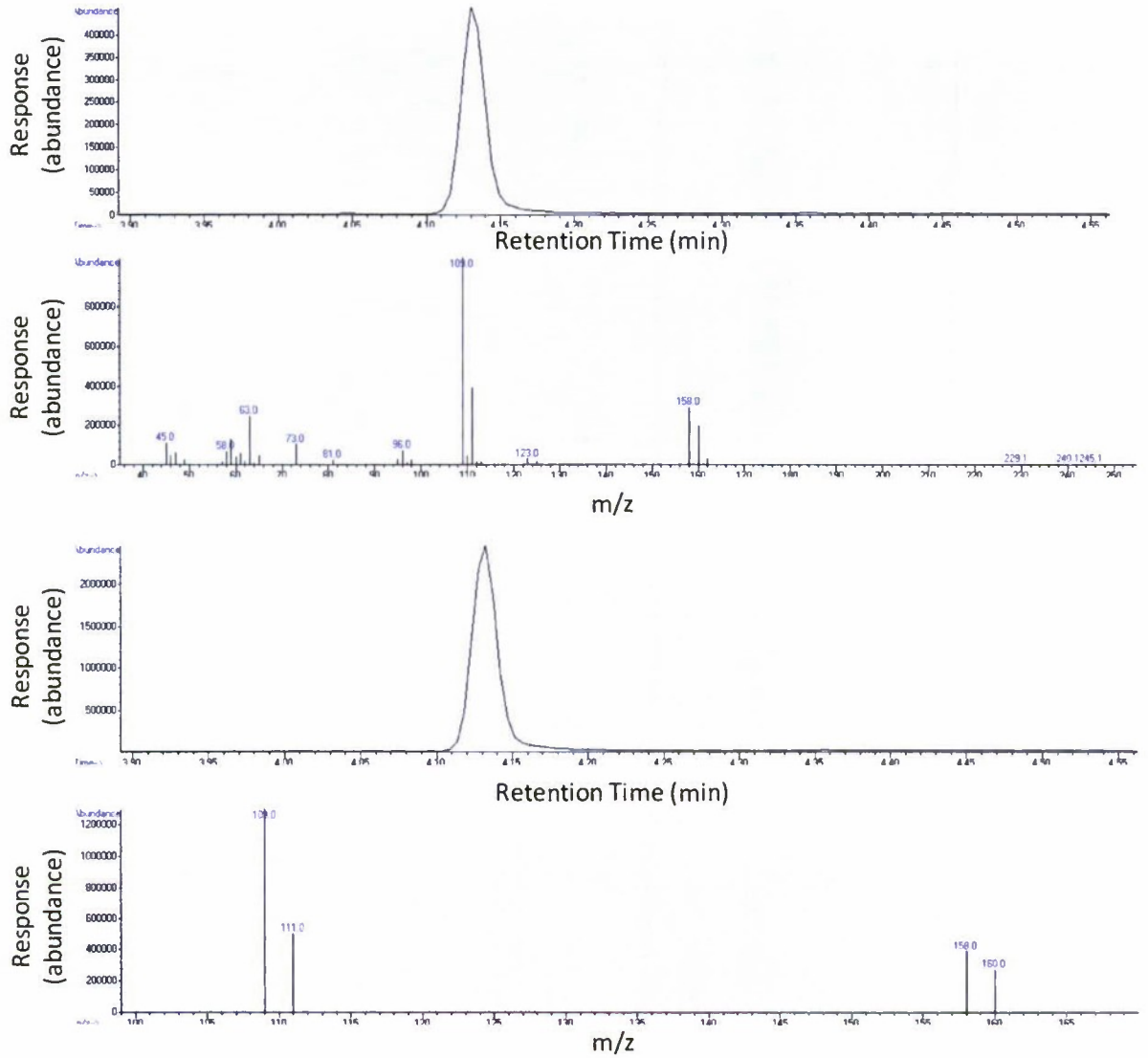


Figure 79. GC/MS SCAN and SIM analysis of 50 ng HD on GCV.

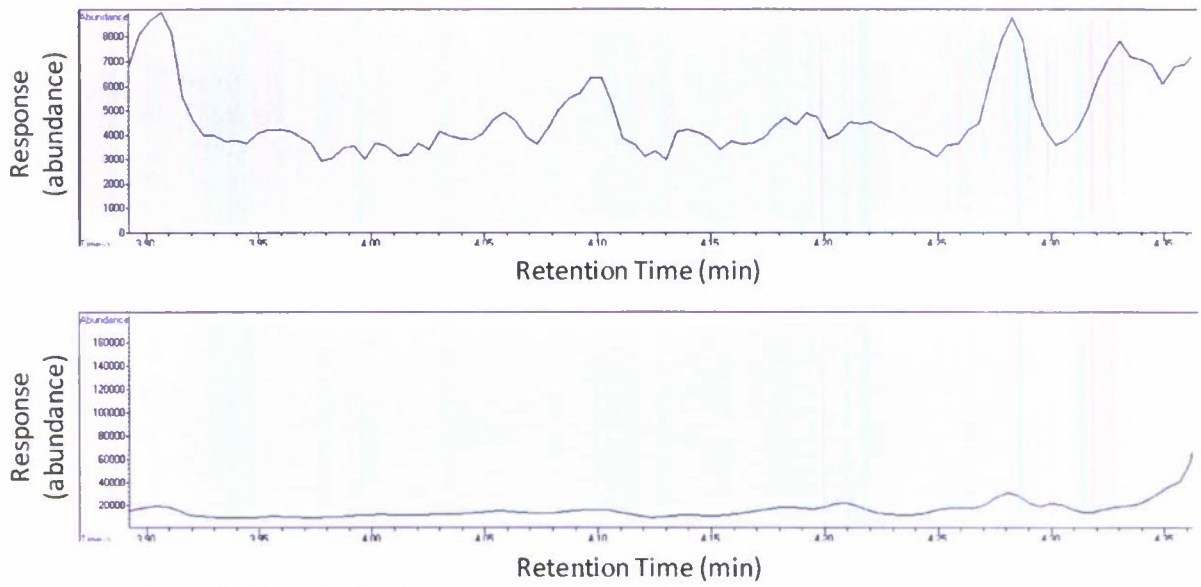


Figure 80. GC/MS SCAN and SIM analysis of a chloroform solvent blank on GCV zoomed at the HD RT of 4.13 min.

Additional tubes were analyzed after spiking them with decreasing masses in an attempt to identify the observed method detection limits. The range of HD detection was sought to determine the lowest mass on column (ng) that would yield the NIST 2.0 library search characterization ions in the correct ratios and the greatest mass on column that could be analyzed without detector saturation or excessive carryover. Varying masses of analyte were spiked and analyzed. The low-level detection limit of HD on GCV was determined to be 0.1 ng HD mass on column. After the low-end detection limit was established, increasing masses were examined. As the masses on column increased, they began saturating the detector at the more abundant ions (m/z 109, 111). Based upon these results, a Markes TDS split was required for the larger masses. To achieve the greatest mass range, analysis of HD required two methods.

- A low-level HD method (HDLL) was established that required no split on the Markes TDS. Analyzing samples determined the low-level mass range to be 0.1–500 ng HD.

- A high-level HD method (HDHL) was established that required a 95 mL/min split on the Markes TDS. Analyzing samples determined the high-level mass range to be 100–2000 ng HD.

9.3.1 Initial Method Capabilities

9.3.1.1 Method GCV HDLL-DEANS.M — Initial

The purpose of the GCV HDLL-DEANS.M method was to detect and quantify the chemical agent HD in vapor samples. The research target was established as the lowest mass on column that can be confidently measured, allowing reasonable sample throughput in support of decontaminant performance evaluations. This method detected HD with a mass-on-column range of 0.1–500 ng. No significant method interferents were identified. The sample throughput of this method was approximately four samples per hour.

The GCV HDLL-DEANS.M method was appropriate for vapor samples containing the chemical agent HD. This method utilized five calibration standards at masses of ~0.1, 1, 10, 100, and

500 ng HD. The method use, deteetion limit, and quantitation limit were based on using the full standard set. The standards were prepared in ehloroform.

The initial method performanee for HD was as follows:

- Quantitative Method
- Calibration Range: ~0.1–500 ng HD on eolumn
- Calibration Model: Linear foreed-zero ealibration model
- Calibration Weighting: None
- LOD: 0.47 ng
- LOQ: 1.42 ng
- Spiking Sample Solvent: Chloroform
- Quantitation Ion: 111

Calibration standards were prepared and analyzed two times for ealibration eonsisteney and deteetion limit determination. The ealibration range of 0.1–500 ng/mL showed a linear response. The low-level method used a linear foreed-zero ealibration model with no weighting. This method had an LOD of 0.47 ng and an LOQ of 1.42 ng. The preeision of the method was determined by analysis of the multiple CCV samples analyzed. Four replieate analyses of the 0.1 ng standard produeed a standard deviation of 0.14 and an RSD of 46.32%. For the other standards, two replieates were analyzed, produeing standard deviations ranging from 0.13 to 3.44 and RSDs ranging from 0.43 to 33.00%. The ealibration eurves were reprodueible—the %RSD of the slope for two ealibration eurves was 2.41% and the eorrelation eoeffieients ranged from 0.999 to 1.00.

9.3.1.2 Method GCV HDHL-DEANS.M — Initial

The GCV HDHL-DEANS.M method was a eomplementary method to the low-level method for the evaluation of sample sorbent tubes eontaining higher masses of HD. This method deteeted HD with a mass-on-eolumn range of 100–2000 ng. No signifieant method interferents were identified during method development. Sample throughput was approximately four samples per hour.

The GCV HDHL-DEANS.M method was appropriate for vapor samples eontaining the ehemieal agent HD. This method utilized five ealibration standards at masses of ~100, 500, 1000, 1500, and 2000 ng HD. The method use, deteetion limit, and quantitation limit were based on using the full standard set. The standards were prepared in ehloroform.

The method performanee for HD was as follows:

- Quantitative Method
- Calibration Range: ~100–2000 ng HD on eolumn
- Calibration Model: Linear with intereept
- Calibration Weighting: One over mass squared ($1/x^2$)

- LOD: 39.20
- LOQ: 118.79
- Spiking Sample Solvent: Chloroform
- Quantitation Ion: 111

LOD and Quantitation and Measurement Uncertainty: Calibration standards were prepared and analyzed six times for calibration consistency and detection limit determination. This method used a linear with intercept calibration model with $1/x^2$ weighting. This method had an LOD of 39.20 ng and an LOQ of 118.79 ng. The precision of the method was determined by analysis of the multiple CCV samples analyzed. Six replicate analyses of the 100 ng standard produced a standard deviation of 3.61 and an RSD of 3.67%, which indicated very good reproducibility at the lowest standard in the calibration curve. For the other standards, six replicates were analyzed, producing standard deviations ranging from 26.71 to 65.00 and RSDs ranging from 2.67 to 5.35%. The calibration curves were reproducible—the %RSD of the slope and intercept for six calibration curves were 4.33 and 48.52%, respectively, and the correlation coefficients ranged from 0.997 to 1.

9.4 Final Method Refinement

As with all aspects of a quality system that undergoes continuous improvement, method development and validation continues to ensure that the methods used are the best, most robust methods giving the greatest possible precision and accuracy available for the technology. The HD vapor method was improved to offer more reliable sampling, an improved dynamic range when collecting vapor samples, and better detection.

The initial methodology had an overall dynamic range of 0.1–2000 ng. The low-level HD method (HDLL) had a mass range of 0.1–500 ng on a splitless method. The high-level HD method (HDHL) had a mass range of 100–2000 ng and utilized a single Markes split at the inlet (during sample transfer from the sample collection tube to the cold trap). The actual, calculated split ratio for this method was ~2:1, or, two parts of the sample split to the exhaust with one part sent to the GC for analysis. Method validation showed calculated LOD and LOQ values above the lowest calibration level for the low-level method, and a calculated LOQ value above the lowest calibration level for the high-level method. Additionally, once put into practical use, these methods showed inconsistencies in found masses from calibration to calibration, CCV to CCV, and sample to sample. While the calibration curves were valid and passed quality control criteria, the inconsistencies often caused issues that challenged the overall confidence of these methods and the reported mass results obtained.

Further investigation determined that the likely culprits for these inconsistencies included high masses that overloaded the column causing poor peak shape, a dynamic range that challenged the upper and lower detection capabilities of the instrument, and an unreliable split on the high-level method. To address these issues, the overall dynamic range of both methods was adjusted, the Markes split parameters were addressed to be more appropriate, and the GC Deans Switch configuration was changed. The new, overall mass range for both methods was 0.5–2500 ng. The low-level method mass range was 0.5–100 ng and remained a splitless method. Further examination showed that adjusting the low-level calibration point to 0.5 ng allowed more reliable and reproduceable results.

To obtain the low-level detection, the high calibration level was scaled back to 100 ng. This adjustment prevented the column from being overloaded in a splitless method, which was evident in the peak shape of the higher mass levels. The high-level method mass range was adjusted to 50–2500 ng and incorporated a Markes split of ~30:1. The high-level method split was now a double split. Part of the sample was split at the inlet (during sample transfer from the sample collection tube to the cold trap) and

the rest of the sample was split at the outlet (during sample transfer from the cold trap to the analytical column). Sample splitting was achieved via adjusting the Markes TDS parameters controlled by the Unity software. Additionally, the double split at 30:1 gave the high-level method a wider mass range and made performance much more consistent with excellent RPD values. The wider mass range of the high-level method also provided overlap with the low-level method, which was an important capability improvement from the initial methodology. A change to the GCV Deans switch configuration and method was performed to more closely mimic the Deans settings of the GCE instrument. This allowed more consistent heartcut and analyte retention times and incorporated a more consistent maintenance schedule and consumable parts list among all instruments. Only minor changes were required to the chromatographic/detection method parameters. (The changes to the mass ranges and the Markes TDS split ratio parameters prevented the column from being overloaded with analyte, which was adversely affecting chromatography). Examples of minor changes include adjusting the heart-cut set points for the analyte RT, and adjusting the Dwell times in the SIM mode of acquisition to allow more cycles per second (i.e., scans across the peak) and improve detection.

The only difference between the HDLL and HDHL methods are the split parameters established on the Markes TDS and set by the Unity software. The MSD ChemStation parameters for the HDLL and HDHL methods are identical. The calibration curve fit and weighting has been adjusted for optimal performance and consistency across the new method ranges. Both methods now utilize a quadratic curve fith with one over mass squared ($1/x^2$) weighting.

The refinement allowed significant method improvement to ensure accuracy, precision, and confidence of reported masses.

9.4.1 Final Method Capabilities

After refinement was complete, the final method capabilities were examined. The final method capabilities were then used to determine the most appropriate methodology for decontamination test sample analysis.

9.4.1.1 Method GCV HDLL-DEANS.M with HDLL.mth — Final

Method Purpose and Performance Overview: The purpose of the GCV HDLL-DEANS.M method is to detect and quantify the chemical agent HD in vapor samples. The research target was established as the lowest mass on column that can be confidently measured, allowing reasonable sample throughput in support of decontaminant performance evaluations. This method detects HD with a mass-on-column range of 0.5–100 ng. The method's detection capabilities were optimized for the analysis of a 2 in. diameter test surface area. This method can support larger item vapor testing. No significant method interferents were identified; however, users should confirm that new test materials do not off-gas a compound that could elute at the expected RT, causing method interference. The sample throughput of each method was approximately four samples per hour.

Method Summary and Operation Details: The GCV HDLL-DEANS.M method is appropriate for vapor samples containing the chemical agent HD. This method utilizes nine calibration standards at masses of ~0.5, 0.5, 0.5, 1.0, 2.5, 5.0, 10, 50, and 100 ng HD. The method use, detection limit, and quantitation limit were based on using the full standard set. The standards were prepared in chloroform.

The *final* method performance for HD is as follows:

- Quantitative Method
- Calibration Range: 0.5–100 ng HD

- CCV Levels: 0, 10, 10 ng
- Calibration Model: Quadratic calibration model
- Calibration Weighting: One over the mass squared ($1/x^2$)
- LOD: 0.13 ng
- LOQ: 0.44 ng
- Spiking Sample Solvent: Chloroform
- Quantitation Ion: 111

LOD and Quantitation and Measurement Uncertainty: Calibration standards were prepared and analyzed multiple times for calibration consistency and detection limit determination. The final method evaluation pulled calibration and CCV performance data sets from decontamination performance testing queues. The high mass standard of the calibration set was followed by tube blanks to determine method carryover. The carryover from the high-level calibration standard has no affect on the method or the ability of the method to detect and quantify the low end of the calibration range. The calibration range of 0.5–100 ng yielded a quadratic response. This method uses a weighted quadratic calibration model with an applied weighting of $1/x^2$. The application of $1/x^2$ weighting ensures accuracy at the lower levels of the calibration curve. Accuracy at the upper end of the calibration mass range is maintained with the $1/x^2$ weighting. A demonstration of the GCV HDLL-DEANS calibration performance can be seen in Figure 81.

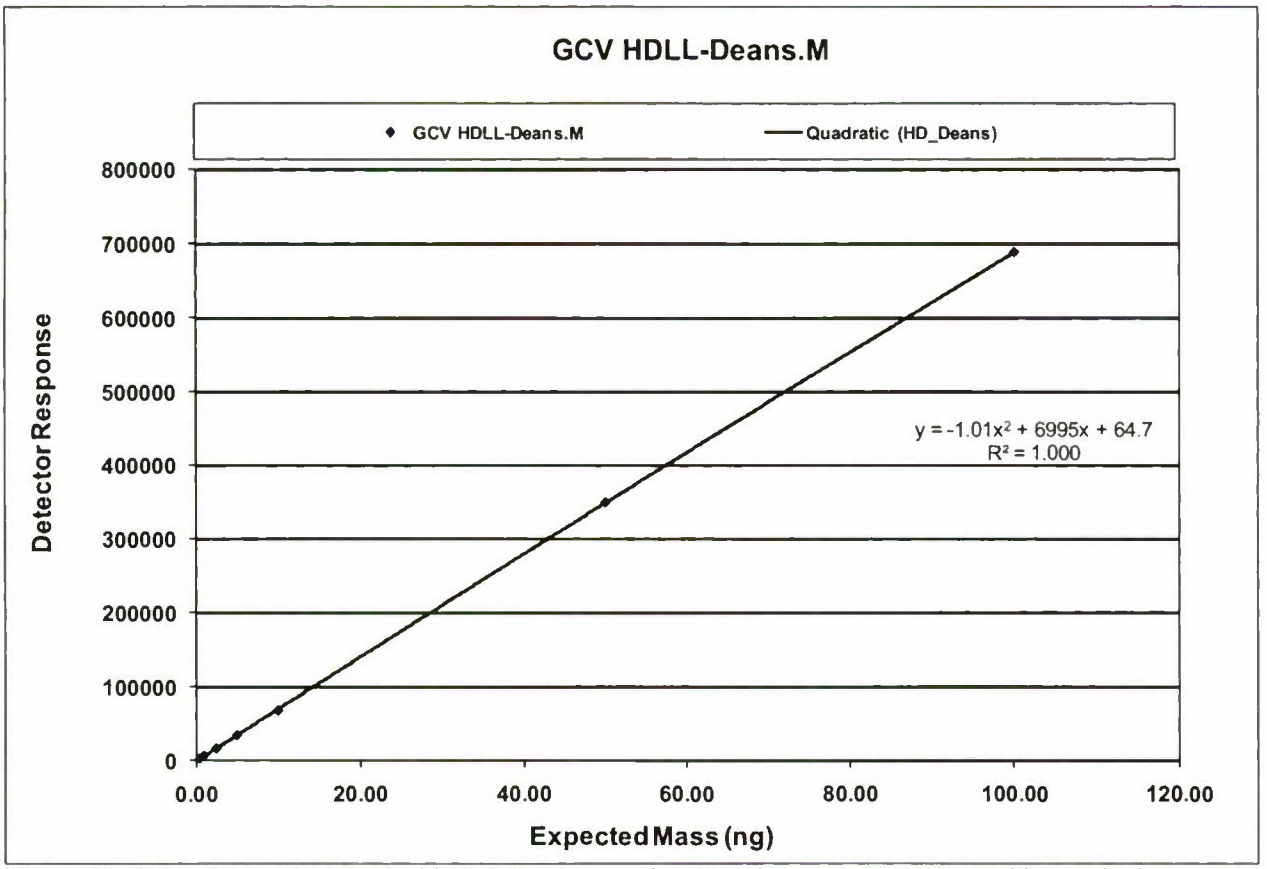


Figure 81. GCV HDLL-DEANS.M with HDLL.mth example calibration curve 0.5–100 ng with "quadratic" regression ("$1/x^2$" weighting).

The GCV HDLL-DEANS.M method yielded a calculated LOD of 0.13 ng and an LOQ of 0.44 ng. The precision of the method was determined by analysis of multiple CCV samples analyzed. From one representative test, there were 12 replicate analyses of the 10 ng CCV, which produced an average found mass of 11.58 ng with a standard deviation of 0.7 and an RSD of 6.1%. This indicated very good reproducibility at the CCV level within the calibration curve. Furthermore, there were multiple replicate analyses of the 0.5 ng low-level standard from multiple tests, which produced a standard deviation of 0.04 and an RSD of 8.58%. This indicated very good reproducibility at the lowest standard in the calibration curve. For the other standards, replicates were analyzed, producing standard deviations ranging from 0.06 to 2.30 and RSDs ranging from 1 to 9.3% indicating excellent, overall method performance.

The accuracy of the method is demonstrated in Table 37 as uncertainty in the measurements at each standard in the calibration curve with 95% confidence. For a standard or sample, containing 10 ng of analyte, the analytical result reported was 10 ± 0.4 ng.

Table 37. List of standard masses and the associated confidence intervals.

Standard Mass on Tube (ng)	95% Confidence Interval (ng)	Lower Confidence Interval (ng)	Upper Confidence Interval (ng)
0.50	0.010	0.490	0.510
0.50	0.010	0.490	0.510
0.50	0.010	0.490	0.510
1.00	0.026	0.974	1.03
2.5	0.083	2.42	2.58
5.0	0.179	4.82	5.18
10.0	0.367	9.63	10.37
50.0	1.84	48.16	51.84
100.0	4.47	95.53	104.5

9.4.1.2 Method GCV HDHL-DEANS.M with HDHL.mth – Final

Method Purpose and Performance Overview: The purpose of the GCV HDHL-DEANS.M method was to detect and quantify the chemical agent HD in vapor samples. This method was developed as a complementary method to the low-level method (GCV HDLL-DEANS.M) for the evaluations of sample sorbent tubes containing higher masses of HD. This method detected HD with a mass-on-column range of 50–2500 ng. The method's detection capabilities were optimized for the analysis of a 2 in. diameter test surface area. This method can support larger item vapor testing. No significant method interferents were identified; however, users should confirm that new test materials do not off-gas a compound that could elute at the expected RT causing method interference. The sample throughput of each method was approximately four samples per hour.

Method Summary and Operation Details: The GCV HDHL-DEANS.M method is appropriate for vapor samples containing the chemical agent HD. This method utilizes nine calibration standards at masses of ~50, 100, 250, 500, 750, 1000, 1500, 2000, and 2500 ng HD. The method use, detection limit, and quantitation limit were based on using the full standard set. The standards were prepared in chloroform.

The *final* method performance for HD is as follows:

- Quantitative Method
- Calibration Range: 50–2500 ng HD
- CCV Levels: 0, 500, 500 ng
- Calibration Model: Quadratic calibration model
- Calibration Weighting: One over the mass squared ($1/x^2$)
- LOD: 5.65 ng
- LOQ: 18.82 ng

- Spiking Sample Solvent: Chloroform
- Quantitation Ion: 111

LOD and Quantitation and Measurement Uncertainty: Calibration standards were prepared and analyzed multiple times for calibration consistency and detection limit determination. The final method evaluation pulled calibration and CCV performance data sets from decontamination performance testing queues. The high mass standard of the calibration set was followed by tube blanks to determine method carryover. The carryover from the high-level calibration standard has no affect on the method or the ability of the method to detect and quantify the low end of the calibration range. The calibration range of 50–2500 ng yielded a quadratic response. This method used a weighted quadratic calibration model with an applied weighting of $1/x^2$. The application of $1/x^2$ weighting ensures accuracy at the lower levels of the calibration curve. Accuracy at the upper end of the calibration concentration range is maintained with the $1/x^2$ weighting. A demonstration of the GCV HDHL-DEANS calibration performance can be seen in Figure 82.

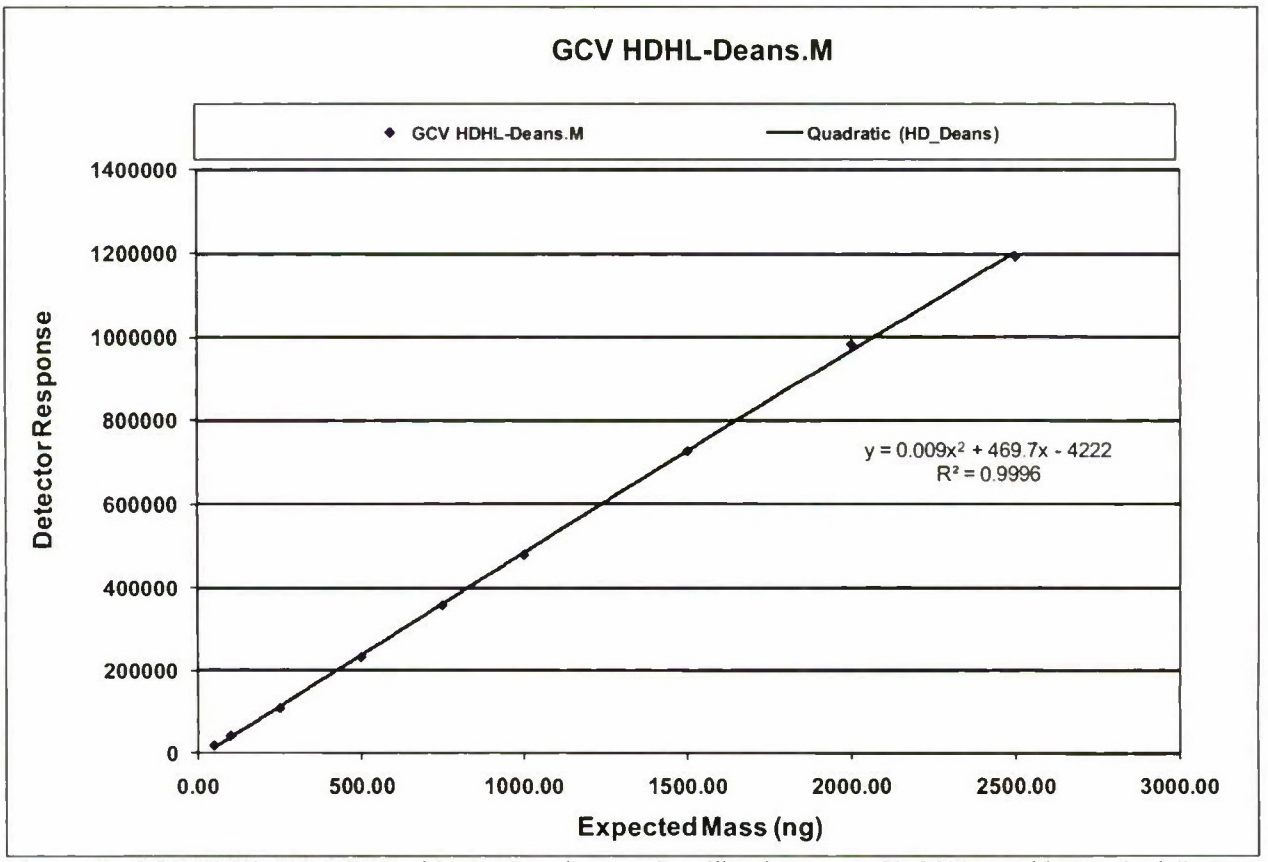


Figure 82. GCV HDHL-DEANS.M with HDHL.mth example calibration curve, 50–2500 ng with "quadratic" regression ("$1/x^2$" weighting).

The GCV HDHL-DEANS.M method yielded a calculated LOD of 5.65 ng and an LOQ of 18.82 ng. The precision of the method was determined by analysis of multiple CCV samples analyzed. From one representative test, there were 12 replicate analyses of the 500 ng CCV, which produced an average found mass of 506.7 ng with a standard deviation of 8.4 and an RSD of 1.66%. This indicated very good reproducibility at the CCV level within the calibration curve. Furthermore, there were

multiple replicate analyses of the 50 ng low calibration standard from multiple tests, which produced a standard deviation of 1.88 and an RSD of 3.71%. This indicated very good reproducibility at the lowest standard in the calibration curve. For the other standards, replicates were analyzed, producing standard deviations ranging from 4.4 to 45.5 and RSDs ranging from 1.84 to 4.43% indicating excellent, overall method performance.

The accuracy of the method is demonstrated in Table 38 as uncertainty in the measurements at each standard in the calibration curve with 95% confidence. For a standard or sample, containing 10 ng of analyte, the analytical result reported was 10 ± 0.04 ng.

Table 38. List of standard masses and the associated confidence intervals.

Standard Mass on Tube (ng)	95% Confidence Interval (ng)	Lower Confidence Interval (ng)	Upper Confidence Interval (ng)
50	10.48	39.52	60.48
100	11.42	88.58	111.42
250	15.14	234.86	265.14
500	21.21	478.79	521.21
750	26.94	723.06	776.94
1000	32.54	967.46	1032.54
1500	44.35	1455.65	1544.35
2000	58.67	1941.33	2058.67
2500	77.08	2422.92	2577.08

9.5 Method Demonstration for Decontaminant Performance Testing

9.5.1 GCV HDLL-DEANS.M Method Demonstration

The HD LL vapor method (GCV HDLL-DEANS.M) was used to analyze vapor samples from multiple coupon materials, including CARC and silicone that were spiked with a known amount of the chemical agent HD and decontaminated with multiple test decontaminants. This set of vapor samples was collected as part of test number CR118. The objective of CR118 was to show the decontamination performance of the test decontaminants as applied to the emission of agent vapors collected from the treated coupons.

The materials were spiked with known amounts of HD and were then decontaminated. The coupons were moved to the microchamber vapor manifold for vapor sampling onto Markes TDS tubes packed with Tenax TA. The microchamber vapor-sampling manifold had previously been cleaned, verified blank, and loaded with tubes ready for analysis. Operating conditions of the instrument had been met prior to test sample collection. Samples were collected at specific time intervals and flow rates (mL/min), which were pre-determined by the test designer and previously loaded to the instrument as a set of instructions. This test was performed in accordance with the panel testing guidance set forth in the Source Document.[16]

Upon test completion, the sample tubes were removed from the sampling manifold and analyzed on a GCV instrument. The expected masses of HD on the vapor sample spanned the range of both HD vapor methods; therefore, the tubes were sorted into two analytical queues for analysis. The tubes with expected masses within the low-level calibration range (0.5–100 ng) were analyzed with the method GCV HDLL-DEANS.M.

Method parameters were established on GCV in accordance with Appendix A. Prior to analysis of test samples, the instrument was calibrated using HD calibration standards prepared in high purity solvent and spiked to Markes TDS tubes. The GCV HD calibration curve for this test had a correlation coefficient value of 1.000. The calibration curve was established with a weighted quadratic curve fit ($1/x^2$). The calibration curve for this test is shown in Figure 83. A summary of the method performance with calculated LOD and LOQ is shown in Table 39.

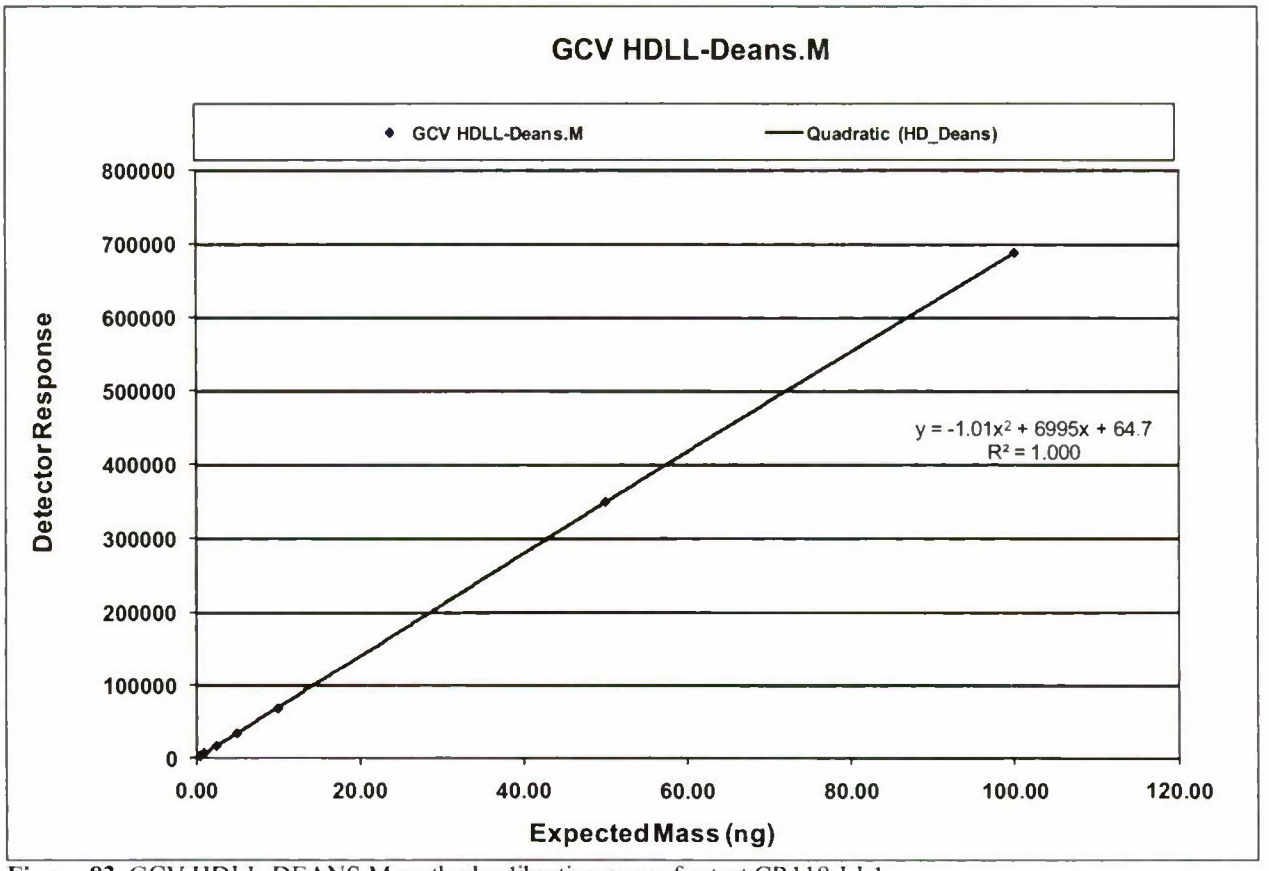


Figure 83. GCV HDLL-DEANS.M method calibration curve for test CR118-LL1.

Table 39. GCV HDLL-DEANS method summary for test CR118-LL1.

Method	GCV HDLL-DEANS.M
Test ID	CR118, LL1
Platform	GCV (GC/MS)
Sample Type	Vapor
Analyte(s)	HD
LOD	0.116 ng
LOQ	0.365 ng
Data Acquisition	EI SIM
Quantitation Ion	111 m/z

CCV samples were performed every 12 samples, and were found to be within 30% of their expected masses. Tube blanks were analyzed throughout the test for continuing baseline acceptance. Analysis of the blank samples showed little carryover and no interferences. The sample queue, with analytical data, for the performance testing samples analyzed with the GCV HDLL-DEANS method is shown in Figure 84. All samples were successfully analyzed using this GCV HDLL-DEANS method.

Sample Name	Q Value	Response	Mass on Tube (ng)
CR118_LL1_Pos01_Std_0d0	85	327	8D
CR118_LL1_Pos02_Std_0d5	93	3465	0.49
CR118_LL1_Pos03_Std_0d5	99	3663	0.51
CR118_LL1_Pos04_Std_0d5	94	3484	0.49
CR118_LL1_Pos05_Std_1d0	92	7533	1.07
CR118_LL1_Pos06_Std_2d5	99	17122	2.44
CR118_LL1_Pos07_Std_5d0	100	34946	4.98
CR118_LL1_Pos08_Std_10d0	100	68660	9.81
CR118_LL1_Pos09_Std_49d9	99	349772	50.27
CR118_LL1_Pos10_Std_99d8	99	688369	99.63
CR118_LL1_Pos11_Std_0d0	89	1574	8Q
CR118_LL1_Pos12_0d0_CCV01	95	580	8D
CR118_LL1_Pos13_10d0_CCV01	100	72726	10.39
CR118_LL1_Pos14_10d0_CCV01	100	72675	10.38
CR118_01C_C01_T00	64	2128	8Q
CR118_02C_C02_T00	72	1342	8Q
CR118_03C_C03_T00	64	1797	8Q
CR118_04C_C04_T00	45	1446	8Q
CR118_05C_C05_T00	53	1607	8Q
CR118_06S_C06_T00	68	3336	0.47
CR118_07S_C07_T00	0	0	ND
CR118_08S_C08_T00	0	0	ND

Sample Name	Q Value	Response	Mass on Tube (ng)
CR118_09S_C09_T00	34	11448	1.63
CR118_10S_C10_T00	0	0	ND
CR118_11C_C11_T00	55	16188	2.30
CR118_12C_C12_T00	0	0	ND
CR118_LL1_Pos27_0d0_CCV02	0	0	ND
CR118_LL1_Pos28_10d0_CCV02	100	72131	10.30
CR118_LL1_Pos29_10d0_CCV02	100	69499	9.93
CR118_13C_C13_T00	72	1665	0.23
CR118_14C_C14_T00	82	4483	0.63
CR118_15C_C15_T00	0	0	ND
CR118_16S_C16_T00	74	9469	1.34
CR118_17S_C17_T00	0	0	ND
CR118_18S_C18_T00	0	0	ND
CR118_19S_C19_T00	0	0	ND
CR118_20S_C20_T00	0	0	ND
CR118_21C_C21_T00	0	0	ND
CR118_22C_C22_T00	0	0	ND
CR118_23S_C23_T00	93	7200	1.02
CR118_24S_C24_T00	96	9516	1.35
CR118_LL1_Pos42_0d0_CCV03	0	0	ND
CR118_LL1_Pos43_10d0_CCV03	98	58245	8.31
CR118_LL1_Pos44_10d0_CCV03	99	70317	10.04

BD = Below Detect (LOD)
8Q = 8elow Quant (LOQ)
ND = Non Detect

*The shading is for ease of reading. Light green indicates calibration and CCV levels. Light blue indicates samples. Yellow indicates detector response information.

Figure 84. HDLL sequence and results for CR118 LL1.

9.5.2 GCV HDHL-DEANS.M Method Demonstration

The HD HL vapor method (GCV HDHL-DEANS.M) was used to analyze vapor samples from multiple coupon materials including CARC and silicone that were spiked with a known amount of the chemical agent HD and decontaminated with multiple test decontaminants. This set of vapor samples was collected as part of test number CR118. The objective of CR118 was to show the decontamination performance of the test decontaminants as applied to the emission of agent vapors collected from the treated coupons.

The materials were spiked with known amounts of HD and were then decontaminated. The coupons were moved to the microchamber vapor manifold for vapor sampling onto Markes TDS tubes packed with Tenax TA. The microchamber vapor-sampling manifold had previously been cleaned, verified blank, and loaded with tubes ready for analysis. Operating conditions of the instrument had been met prior to test sample collection. Samples were collected at specific time intervals and flow rates (mL/min), which were pre-determined by the test designer and previously loaded to the instrument as a

set of instructions. This test was performed in accordance with the panel testing guidance set forth in the Source Document.[16]

Upon test completion, the sample tubes were removed from the sampling manifold and analyzed on a GCV instrument. The expected masses of HD on the vapor sample spanned the range of both HD vapor methods; therefore, the tubes were sorted into two analytical queues for analysis. The tubes with expected masses within the low-level calibration range (50–2500 ng) were analyzed with the method GCV HDHL-DEANS.M.

Method parameters were established on GCV in accordance with Appendix A. Prior to analysis of test samples, the instrument was calibrated using HD calibration standards prepared in high purity solvent and spiked to Markes TDS tubes. The GCV HD calibration curve for this test had a correlation coefficient value of 0.9996. The calibration curve was established with a weighted quadratic curve fit ($1/x^2$). The calibration curve for this test is shown in Figure 85. A summary of the method performance with calculated LOD and LOQ is shown in Table 40.

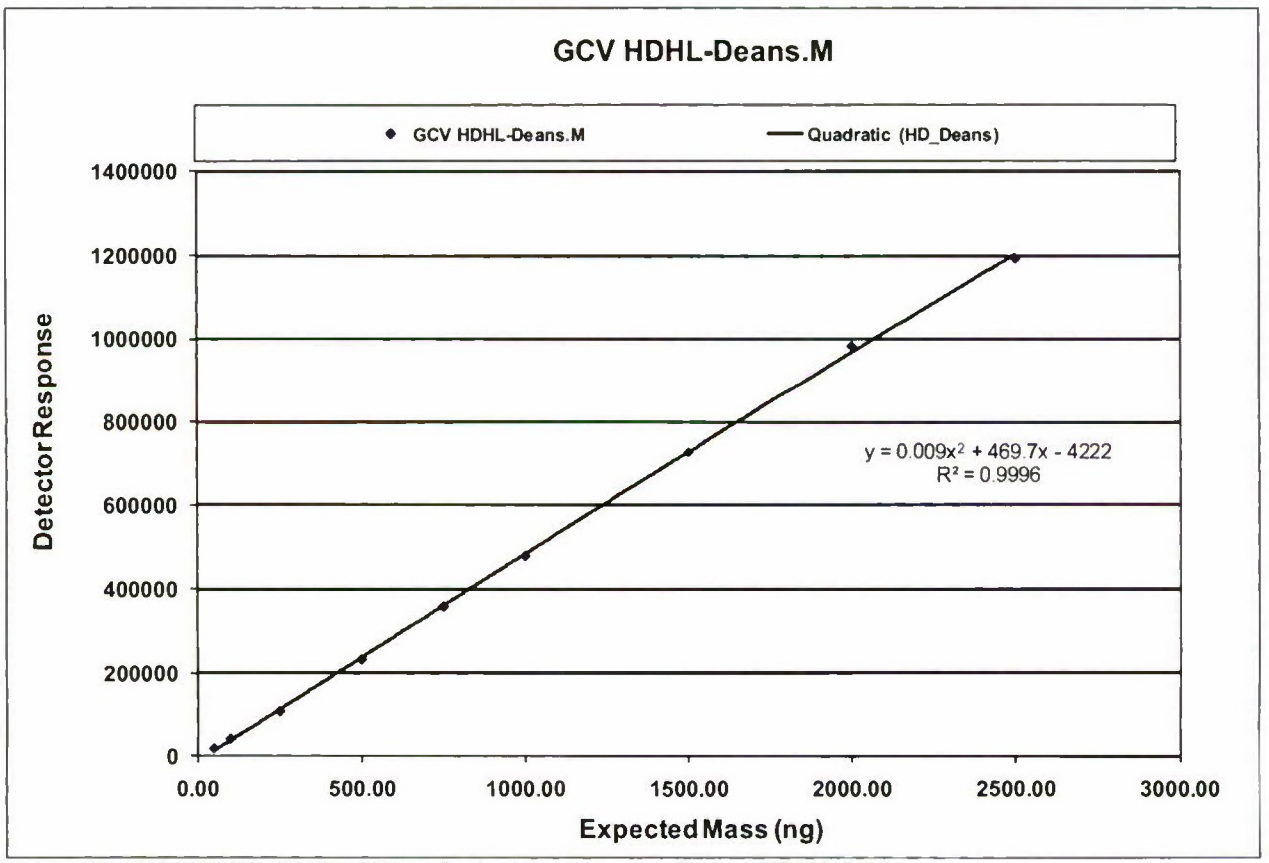


Figure 85. GCV HDHL-DEANS.M method calibration curve for test CR118-HL1.

Table 40. GCV HDHL-DEANS method summary for test CR118-HL1.

Method	GCV HDHL-DEANS.M
Test ID	CR118, HL1
Platform	GCV (GC/MS)
Sample Type	Vapor
Analyte(s)	HD
LOD	10.03 ng
LOQ	10.19 ng
Data Acquisition	EI SIM
Quantitation Ion	111 m/z

CCV samples were performed every 12 samples, and were found to be within 30% of their expected masses. Tube blanks were analyzed throughout the test for continuing baseline acceptance. Analysis of the blank samples showed little carryover and no interferences. The sample queue, with analytical data, for the performance testing samples analyzed with the GCV HDHL-DEANS method is shown in Figure 86. All samples were successfully analyzed using this GCV HDHL-DEANS method.

Sample Name	Q Value	Response	Mass on Tube (ng)
CR118_HL1_Pos01_Std_0d0	1	23	BD
CR118_HL1_Pos02_Std_49d9	99	19002	50.30
CR118_HL1_Pos03_Std_99d8	99	42050	99.14
CR118_HL1_Pos04_Std_250d0	99	109195	240.96
CR118_HL1_Pos05_Std_499d0	100	233152	500.92
CR118_HL1_Pos06_Std_749d0	99	358670	761.75
CR118_HL1_Pos07_Std_998d0	99	479609	1010.84
CR118_HL1_Pos08_Std_1497d0	98	726836	1513.50
CR118_HL1_Pos09_Std_2000d0	97	983903	2027.24
CR118_HL1_Pos10_Std_2500d0	96	1193281	2439.26
CR118_HL1_Pos11_Std_0d0	99	2113	14.45
CR118_HL1_Pos12_0d0_CCV01	93	368	10.75
CR118_HL1_Pos13_499d0_CCV01	100	259533	555.93
CR118_HL1_Pos14_499d0_CCV01	100	242576	520.58
CR118_01C_C01_T01	99	20295	53.04
CR118_01C_C01_T02	99	24167	61.25
CR118_01C_C01_T03	99	32354	78.61
CR118_01C_C01_T04	99	46676	108.94
CR118_01C_C01_T05	99	62211	141.80
CR118_01C_C01_T06	99	72816	164.21
CR118_01C_C01_T07	99	80667	180.79
CR118_01C_C01_T08	99	87826	195.91
CR118_01C_C01_T09	99	94027	208.99
CR118_02C_C02_T01	97	825	11.72
CR118_02C_C02_T02	99	742	11.54
CR118_02C_C02_T03	98	1247	12.61
CR118_HL1_Pos27_0d0_CCV02	31	27	BD
CR118_HL1_Pos28_499d0_CCV02	100	235254	505.30
CR118_HL1_Pos29_499d0_CCV02	100	234468	503.66
CR118_02C_C02_T04	98	3689	17.80
CR118_02C_C02_T05	99	8659	28.35
CR118_02C_C02_T06	99	19756	51.90
CR118_02C_C02_T07	99	30271	74.19
CR118_02C_C02_T08	99	35769	85.84
CR118_02C_C02_T09	99	43203	101.59
CR118_03C_C03_T01	96	188	10.36
CR118_03C_C03_T02	98	682	11.41
CR118_03C_C03_T03	97	115	10.21
CR118_03C_C03_T04	91	123	10.23
CR118_03C_C03_T05	94	86	8Q
CR118_03C_C03_T06	92	96	8Q
CR118_HL1_Pos42_0d0_CCV03	75	11	BD
CR118_HL1_Pos43_499d0_CCV03	100	241199	517.71
CR118_HL1_Pos44_499d0_CCV03	100	241680	518.71

Sample Name	Q Value	Response	Mass on Tube (ng)
CR118_03C_C03_T07	90	393	10.80
CR118_03C_C03_T08	39	387	10.79
CR118_03C_C03_T09	96	234	10.46
CR118_04C_C04_T01	93	141	10.26
CR118_04C_C04_T02	98	145	10.27
CR118_04C_C04_T03	82	169	10.32
CR118_04C_C04_T04	79	195	10.38
CR118_04C_C04_T05	97	179	10.34
CR118_04C_C04_T06	94	167	10.32
CR118_04C_C04_T07	90	183	10.35
CR118_04C_C04_T08	98	184	10.36
CR118_04C_C04_T09	96	206	10.40
CR118_HL1_Pos57_0d0_CCV04	1	10	BD
CR118_HL1_Pos58_499d0_CCV04	100	248826	533.62
CR118_HL1_Pos59_499d0_CCV04	100	242701	520.84
CR118_05C_C05_T01	97	357	10.72
CR118_05C_C05_T02	97	117	10.21
CR118_05C_C05_T03	94	101	BQ
CR118_05C_C05_T04	96	86	BQ
CR118_05C_C05_T05	98	73	BQ
CR118_05C_C05_T06	71	96	BQ
CR118_05C_C05_T07	91	97	BQ
CR118_05C_C05_T08	82	136	10.25
CR118_05C_C05_T09	80	227	10.45
CR118_06S_C06_T01	99	75476	169.83
CR118_06S_C06_T02	99	82447	184.55
CR118_06S_C06_T03	99	91647	203.97
CR118_HL1_Pos72_0d0_CCV05	87	123	10.23
CR118_HL1_Pos73_499d0_CCV05	100	243395	522.29
CR118_HL1_Pos74_499d0_CCV05	100	247643	531.15
CR118_06S_C06_T04	100	120535	264.84
CR118_06S_C06_T05	100	136465	298.36
CR118_06S_C06_T06	100	137304	300.12
CR118_06S_C06_T07	100	131427	287.76
CR118_06S_C06_T08	100	117006	257.41
CR118_06S_C06_T09	100	117021	257.44
CR118_07S_C07_T01	99	75948	170.83
CR118_07S_C07_T02	99	84947	189.83
CR118_07S_C07_T03	99	96286	213.75
CR118_07S_C07_T04	100	125123	274.50
CR118_07S_C07_T05	100	141984	309.96
CR118_07S_C07_T06	100	143744	313.66
CR118_HL1_Pos87_0d0_CCV06	97	372	10.75
CR118_HL1_Pos88_499d0_CCV06	100	247504	530.86
CR118_HL1_Pos89_499d0_CCV06	100	246065	527.86

BD = Below Detect (LOD)
8Q = Below Quant (LOQ)
ND = Non Detect

*The shading is for ease of reading. Light green indicates calibration and CCV levels. Light blue indicates samples. Yellow indicates detector response information.

Figure 86. HDHL sequence and results for CR118 HL1.

10. ANALYSIS OF GD VAPOR SAMPLES

10.1 Decontamination Efficacy Requirement Documents

The method detection limit target was the lowest mass of contaminant on tube that could be accurately detected and quantified from the baseline noise and still enable sample throughput to support decontamination performance evaluations. The requirement document vapor concentration levels for threshold (T) and objective (O) of primary interest at the time of this program are listed in Table 41.[4-9] The requirement, toxicology, IDLH, STEL, WPL, and GPL are listed in order of the vapor concentration numerical value. These values represent different exposure durations requiring different sampling protocols for sample collection and data reporting. The toxicology vapor concentrations are for a 10 h exposure. The IDLH, STEL, and WPL shown are OSHA-reported values. The IDLH value is for a one time, short-term exposure. The STEL is for a 15 min exposure, once per day. The WPL is the time-weighted average vapor concentration for a working population exposure of 8 h per day for five days per week. The GPL is the EPA general population value time-weighted average vapor concentration for lifetime exposure. The vapor test, as described in the source document,[16] calculated a vapor emission rate. A vapor concentration was measured in the dynamic vapor chamber, but it did not correspond to the vapor concentration to which unprotected personnel would be exposed and should not be directly compared to a requirement. The emission rate was used to calculate the vapor concentration that was present in a specified scenario. This use of data required that the analytical method be capable of accurately detecting agent across a broad range of masses, ideally from a few ng to as many as 1000 ng.

Table 41. Decontamination testing detection targets for GD vapor samples.

Publication	Vapor Conc. (mg/m^3)	Publication	Vapor Conc. (mg/m^3)
84% Tox Response	0.1800000	JSSED 03 O	0.0001000
JSSED 03 T	0.1000000	WPL	0.0000300
IDLH	0.0500000	JSSED 04 T	0.0000300
16% Tox Response	0.0010000	STEL	0.0000100
JPID 03 T	0.0008700	JSSED 04 O	0.0000030
1% Tox Response	0.0006000	EPA 04 GPL	0.0000010
JPID 03 O	0.0002000		

10.2 Solid Sorbent Selection for GD Vapor Analysis

It is widely accepted that the solid sorbent material Tenax TA is an excellent all-around adsorbent material for chemical agent. Other sorbent materials are available and may have a higher affinity for agent retention during the sampling process. All initial method development work was performed on Tenax TA.

As part of method development and overall vapor sampling verification, extensive breakthrough studies were performed to demonstrate the ability of the selected sorbent to retain the agent of interest and to determine the safe-samplingvolume for the agent/sorbent pairing. *Breakthrough* is defined as the inability of the sorbent to retain the compound of interest during sampling, which allows some/all of the compound to pass through the sorbent. During sampling, breakthrough may occur if the sorbent material cannot retain the compound of interest under sampling extremes such as high sampling flow rates, long sampling periods, or a slight elevation in temperature. It is vital to ensure that the sampling methodology avoids collecting samples that may experience breakthrough, as this would result in underestimating the vapor concentration (and ultimately lead to underestimating the hazard).

Breakthrough studies demonstrated that the chemical agent GD vapor samples showed no breakthrough when sampled on Tenax TA.[29]

Given no observed breakthrough of GD through Tenax TA, all method development and validation acitivities were performed with this sorbent material.

10.3 Initial Method Development

Analysis of analytical vapor samples is commonly performed using solid sorbent sampling. Air samples are collected on a tube containing a selective sorbent material, followed by thermal desorption and analysis using a GC/MS. Method development for the analysis of GD vapor samples was performed on the instrument designated as GCV.

No sample pretreatment is required for collection and analysis of GD vapor samples. The goal of method development for GD was to determine the lowest mass-on-column value that gave excellent, reproducible results. For future testing, sample collection parameters will be adjusted to give expected masses at or above the method capabilities.

Initially, a NIST 2.0 library search was performed using the Agilent ChemStation software Enhanced Data Analysis. The search was performed for GD by CAS number (96-64-0) to obtain the NIST database entry giving the mass spectrum and major ions for this analyte. The NIST library entry number is 226124 and the 10 largest peaks for GD, per the NIST library are detailed in Table 42.

Table 42. GD mass spectrum characteristics.

Compound	GD
NIST No.	226124
ID No.	78999
DB	mainlib
Peak 1	126 (999)
Peak 2	99 (850)
Peak 3	82 (500)
Peak 4	69 (490)
Peak 5	41 (370)
Peak 6	57 (210)
Peak 7	43 (150)
Peak 8	83 (140)
Peak 9	84 (100)
Peak 10	85 (90)

After examining the mass spectrum for GD, the MSD was set up to simultaneously analyze in the SCAN and SIM modes of acquisition. Based on the ion relative abundances from the expected mass spectrum, the ions selected for the SIM mode were m/z 126, 99, and 82. The molecular ion of GD (m/z 182) was not present on the mass spectrum. Ion m/z 69 was detected above the expected relative abundance, and was found to be a natural interferent, which can potentially skew the overall expected relative abundances and, therefore, the reported Q-value. If the found peak was at the correct

GD RT during analysis, and m/z 69 was not in the appropriate relative abundance, m/z 69 was excluded in quantifying and qualifying GD.

In an effort to correctly identify the GD peak, generic method parameters were selected for initial sample analysis using the GCV instrument. The initial Markes system settings were established based on manufacturer recommendations for the sorbent material used, Tenax-TA. It was determined that no Markes TDS split would initially be needed for the GD method. Chemical agent standards were prepared in high purity acetonitrile for instrument calibration. Standards were introduced onto the sorbent tubes by spiking the tubes with 5 µL of known agent standard concentrations. Experimentation with spiking GD agent standards onto the vapor tubes indicated that optimal spiking results were obtained using a flow rate of 200 mL/min, aspirated for 3 min. A higher concentration of GD standard (10 ng/µL) was spiked onto conditioned tubes for a spike mass of 50 ng to be introduced to the GCV. Solvent blanks were analyzed before and after the GD standard to ensure that there were no baseline issues that might interfere with the analyte of interest. Injecting a higher mass standard was done to identify the correct peak via library matching, to determine RT, and to evaluate peak shape on the TIC. Evaluating the peak shape (efficiency and resolution) allowed the method to be altered for optimal chromatography.

Typically, peaks on the TIC can be identified by the chromatographic RT and by the mass spectrum, which contains selected ions in certain relative abundances. As lower concentrations of the standard were evaluated to determine detection limit capabilities, selected ion relative abundances were observed to deviate from the ideal. By initially identifying the correct peak via library matching and noting the RT, the identification of the peak at much lower concentrations was possible. When the ions of interest were not in their ideal relative abundances at these lower concentrations, analyte peaks could be identified by RT alone. After analysis of the initial samples, the peak was successfully identified by PBM of the mass spectrum from the SCAN mode of acquisition with the GD entry in the NIST 2.0 library. The PBM of a 50 ng GD standard identified the analyte peak at 3.62 min as GD with a 90% match quality. The next highest match for the analyte peak only gave a 45% match quality. From these results, the analyte peak was correctly identified as GD. Note that the mass spectrum for the GD in SIM mode of acquisition also showed the ions in the expected relative abundances. The solvent blank did not show a chromatographic peak at the RT of the peak identified as GD. For an example of the GD standard analyzed in the SIM and SCAN modes of acquisition on the MSD, and an example of the solvent blank, see Figure 87 and Figure 88, respectively.

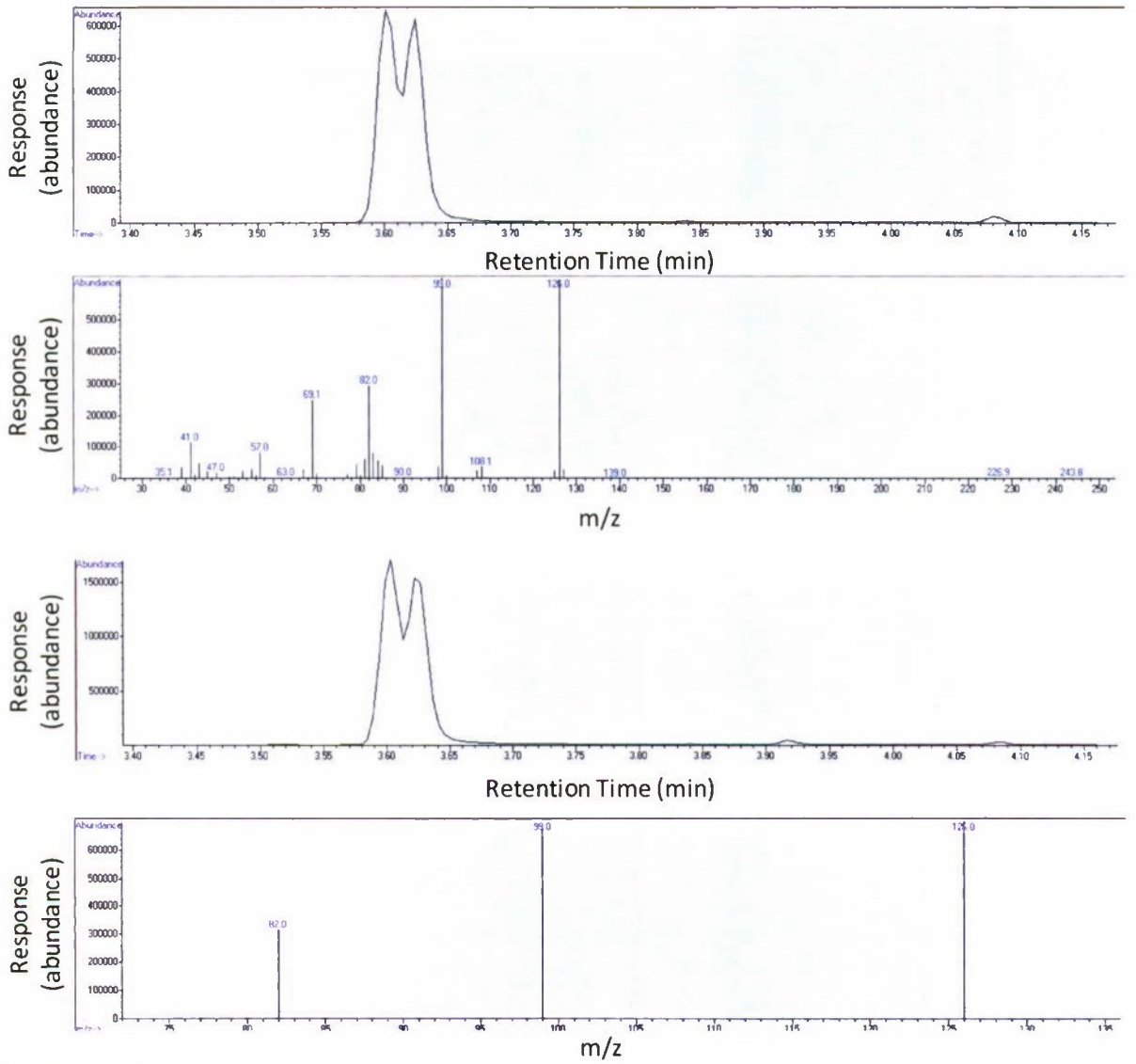


Figure 87. GC/MS SCAN and SIM analysis of 50 ng GD on GCV.

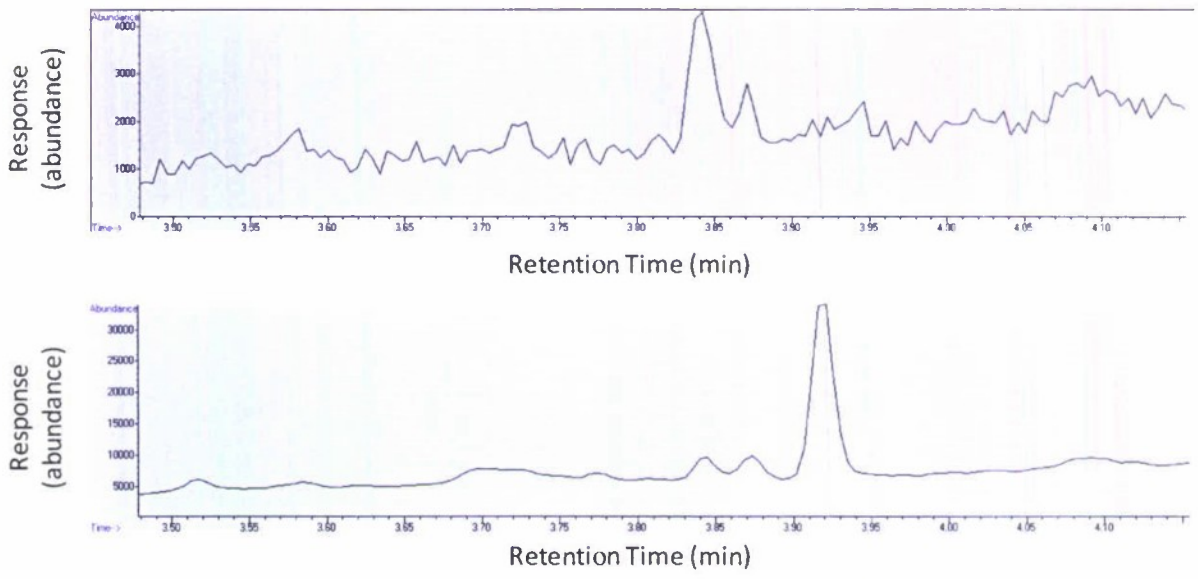


Figure 88. GC/MS SCAN and SIM analysis of an acetonitrile solvent blank on GCV zoomed at the GD RT of 3.62 min.

Additional tubes were analyzed after spiking them with decreasing masses in an attempt to identify the observed method detection limits. The range of GD detection was sought to determine the lowest mass on column in ng that would yield the NIST 2.0 library search characterization ions in the correct ratios, and the greatest mass on column that could be analyzed without detector saturation or excessive carryover. Varying masses of analyte were spiked and analyzed. The low-level detection limit of GD on GCV was observed to be 0.5 ng mass on column. After the low-end detection limit was established, increasing masses were examined. As the masses on column increased, they began saturating the detector at the more abundant ions (m/z 126, 99). Based upon these results, a Markes TDS split was required for the larger masses. To achieve the greatest mass range, analysis of GD required two methods.

- A low-level GD method (GDLL) was established that required no split on the Markes TDS. Analyzing samples determined the low-level mass range to be 0.5–10 ng GD.

- A high-level GD method (GDHL) was established that required a 95 mL/min split on the Markes TDS. Analyzing samples determined the high-level mass range to be 10–500 ng GD.

10.3.1 Initial Method Capabilities

10.3.1.1 Method GCV GDLL-DEANS.M — Initial

The purpose of the GCV GDLL-DEANS.M method was to detect and quantify the chemical agent GD in vapor samples. The research target was established as the lowest mass on column that can be confidently measured, allowing reasonable sample throughput in support of decontaminant performance evaluations. This method detected GD with a mass-on-column range of 0.5–10 ng. No significant method interferents were identified. Sample throughput of this method was approximately four samples per hour.

The GCV GDLL-DEANS.M method was appropriate for vapor samples containing the chemical agent GD. This method utilized five calibration standards at masses of ~0.5, 1, 3, 5, and 10 ng

GD. The method use, detection limit, and quantitation limit were based on using the full standard set. The standards were prepared in acetonitrile.

The initial method performance for GD is as follows:

- Quantitative Method
- Calibration Range: ~0.5–10 ng GD on column
- Calibration Model: Linear forced-zero calibration model
- Calibration Weighting: None
- LOD: 0.01 ng
- LOQ: 0.02 ng
- Spiking Sample Solvent: Acetonitrile
- Quantitation Ion: 126

Calibration standards were prepared and analyzed five times for calibration consistency and detection limit determination. The calibration range of 0.5–10 ng/mL shows a linear response. The low-level method used a linear forced-zero calibration model with no weighting. This method had an LOD of 0.01 ng and an LOQ of 0.02 ng. The precision of the method was determined by analysis of multiple CCV samples. There were six replicate analyses of the 0.5 ng standard, which produced a standard deviation of 0.18 and an RSD of 44.90%. For the other standards, six replicates were analyzed producing standard deviations ranging from 0.08 to 2.5 and RSDs ranging from 7.10–39.90%. The calibration curves were reproducible—the %RSD of the slope for six calibration curves was 5.46%, and the correlation coefficients ranged from 0.9785 to 0.9994.

10.3.1.2 Method GCV GDHL-DEANS.M — Initial

The GCV GDHL-DEANS.M method was a complementary method to the low-level method for the detection of higher masses of GD from vapor sorbent tube samples. This method detected GD with a mass-on-column range of 10–500 ng. No significant method interferents were identified. The sample throughput was approximately four samples per hour.

The GCV GDHL-DEANS.M method was appropriate for vapor samples containing the chemical agent GD. This method utilized five calibration standards at masses of ~10, 25, 50, 250, and 500 ng GD. The method use, detection limit, and quantitation limit were based on using the full standard set. The standards were prepared in acetonitrile.

The initial method performance for GD is as follows:

- Quantitative Method
- Calibration Range: ~10–500 ng GD on column
- Calibration Model: Linear forced-zero calibration model
- Calibration Weighting: None

- LOD: 2.65 ng
- LOQ: 8.02 ng
- Spiking Sample Solvent: Acetonitrile
- Quantitation Ion: 126

Calibration standards were prepared and analyzed five times for calibration consistency and detection limit determination. The calibration range of 0.5–10 ng/mL showed a linear response. The high-level method used a linear forced-zero calibration model with no weighting. This method had an LOD of 2.65 ng and an LOQ of 8.02 ng. The precision of the method was determined by analysis of multiple CCV samples. There were six replicate analyses of the 10 ng standard. These analyses produced a standard deviation of 0.8 and an RSD of 8.70%, which indicated very good reproducibility at the lowest standard in the calibration curve. For the other standards, six replicates were analyzed, producing standard deviations ranging from 1.44 to 13.97 and RSDs ranging from 1.37 to 6.79%. The calibration curves were reproducible—the %RSD of the slope and y-intercept for six calibration curves was 5.32% and 6.53%, respectively, and the correlation coefficients ranged from 0.9942 to 0.9999.

10.4 Final Method Refinement

As with all aspects of a quality system that undergoes continuous improvement, method development and validation continues to ensure that the methods used are the best, most robust methods giving the greatest possible precision and accuracy for the available technology. The GD vapor method was improved to offer more reliable sampling, an improved dynamic range when collecting vapor samples, and better detection.

The original method had an overall dynamic range of 0.5–500 ng. The low-level GD method (GDLL) had a mass range of 0.5–10 ng with a splitless method. The high-level GD method (GDHL) had a mass range of 10–500 ng and utilized a single Markes split at the inlet (during sample transfer from the sample collection tube to the cold trap). The actual split ratio for this method was ~2:1. As with VX vapor sampling, the dynamic range was not large enough to feasibly predict sampling schedules so that expected masses would not exceed the high end mass of the method capability. This created a situation where samples were consistently higher than the upper detection capability of the method, causing scenarios whereby experiments needed to be repeated, adjusting sampling times for less expected mass on tube.

The limited split ratio prevented masses from being increased without overwhelming the detector. Therefore, the split ratio was adjusted on the high-level method. The new, overall mass range for both methods was 0.5–2500 ng. The low-level method mass range was 0.5–100 ng and remained a splitless method. This low-level range was expanded to increase the detection range of the method as well as to provide overlap with the high-level method. The high-level method mass range was expanded to 50–2500 ng and incorporated a Markes split of ~30:1. The high-level method split was now a double split whereby part of the sample was split at the inlet (during sample transfer from the sample collection tube to the cold trap) and the rest of the sample was split at the outlet (during sample transfer from the cold trap to the analytical column). Sample splitting was achieved via adjusting the Markes TDS parameters controlled by the Unity software. Additionally, the double split at 30:1 gave the high-level method a wider mass range and made performance much more consistent and reproducible. A change to the GCV Deans switch configuration and method was performed to more closely mimic the Deans settings of the GCE instrument. This change allowed more consistent heartcut and analyte retention times and incorporated a more consistent maintenance schedule and consumable parts list among all instruments. Additional changes were required to the chromatographic/detection method parameters. Chromatographic parameters were adjusted for better performance in overall peak shape and detection.

For example, the oven temperature ramp was made less aggressive to facilitate better separation and resolution of the analyte from other, potentially interfering peaks that may be present in the chromatogram. Other changes included adjusting the heart-cut set points for the analyte RT and adjusting the Dwell times in the SIM mode of acquisition to allow more cycles per second (i.e., scans across the peak) and improve detection.

The only difference between the GDLL and GDHL methods are the split parameters established on the Markes TDS and set by the Unity software. The MSD ChemStation parameters for the GDLL and GDHL methods are identical. The calibration curve fit and weighting has been adjusted for optimal performance and consistency across the new method ranges. Both methods now utilize a quadratic curve fith with one over mass squared ($1/x^2$) weighting.

The methodology refinement allowed significant method improvement to ensure accuracy, precision, and confidence of reported masses.

10.4.1 Final Method Capabilities

10.4.1.1 Method GCV GDLL-DEANS.M with GDLL.mth — Final

Method Purpose and Performance Overview: The purpose of the GCV GDLL-DEANS.M method is to detect and quantify the chemical agent GD in vapor samples. The research target was established as the lowest mass on column that can be confidently measured, allowing reasonable sample throughput in support of decontaminant performance evaluations. This method detects GD with a mass-on-column range of 0.5–100 ng. The method's detection requirements were optimized for the analysis of a 2 in. diameter test surface area. This method can support larger item vapor testing. No significant method interferents were identified; however, users should confirm that new test materials do not off-gas a compound that could elute at the expected RT causing method interference. The sample throughput of this method is approximately five samples per hour.

Method Summary and Operation Details: The GCV GDLL-DEANS.M method is appropriate for vapor samples containing the chemical agent GD. This method utilizes nine calibration standards at masses of ~0.5, 0.5, 0.5, 1.0, 2.5, 5.0, 10, 50, and 100 ng GD. The method use, detection limit, and quantitation limit are based on using the full standard set. The standards were prepared in acetonitrile.

The *final* method performance for GD is as follows:

- Quantitative Method
- Calibration Range: 0.5–100 ng GD
- CCV Levels: 0, 10, 10 ng
- Calibration Model: Quadratic calibration model
- Calibration Weighting: One over the mass squared ($1/x^2$)
- LOD: 0.07 ng
- LOQ: 0.23 ng
- Spiking Sample Solvent: Acetonitrile

- Quantitation Ion: 126

LOD and Quantitation and Measurement Uncertainty: Calibration standards were prepared and analyzed multiple times for calibration consistency and detection limit determination. The final method evaluation pulled calibration and CCV performance data sets from decontamination performance testing queues. The high mass standard of the calibration set was followed by tube blanks to determine method carryover. The carryover from the high-level calibration standard has no affect on the method or the ability of the method to detect and quantify the low end of the calibration range. The calibration range of 0.5–100 ng yielded a quadratic response. This method uses a weighted quadratic calibration model with an applied weighting of $1/x^2$. The application of $1/x^2$ weighting ensures accuracy at the lower levels of the calibration curve. Accuracy at the upper end of the calibration mass range is maintained with the $1/x^2$ weighting. A demonstration of the GCV GDLL-DEANS.M calibration performance can be seen in Figure 89.

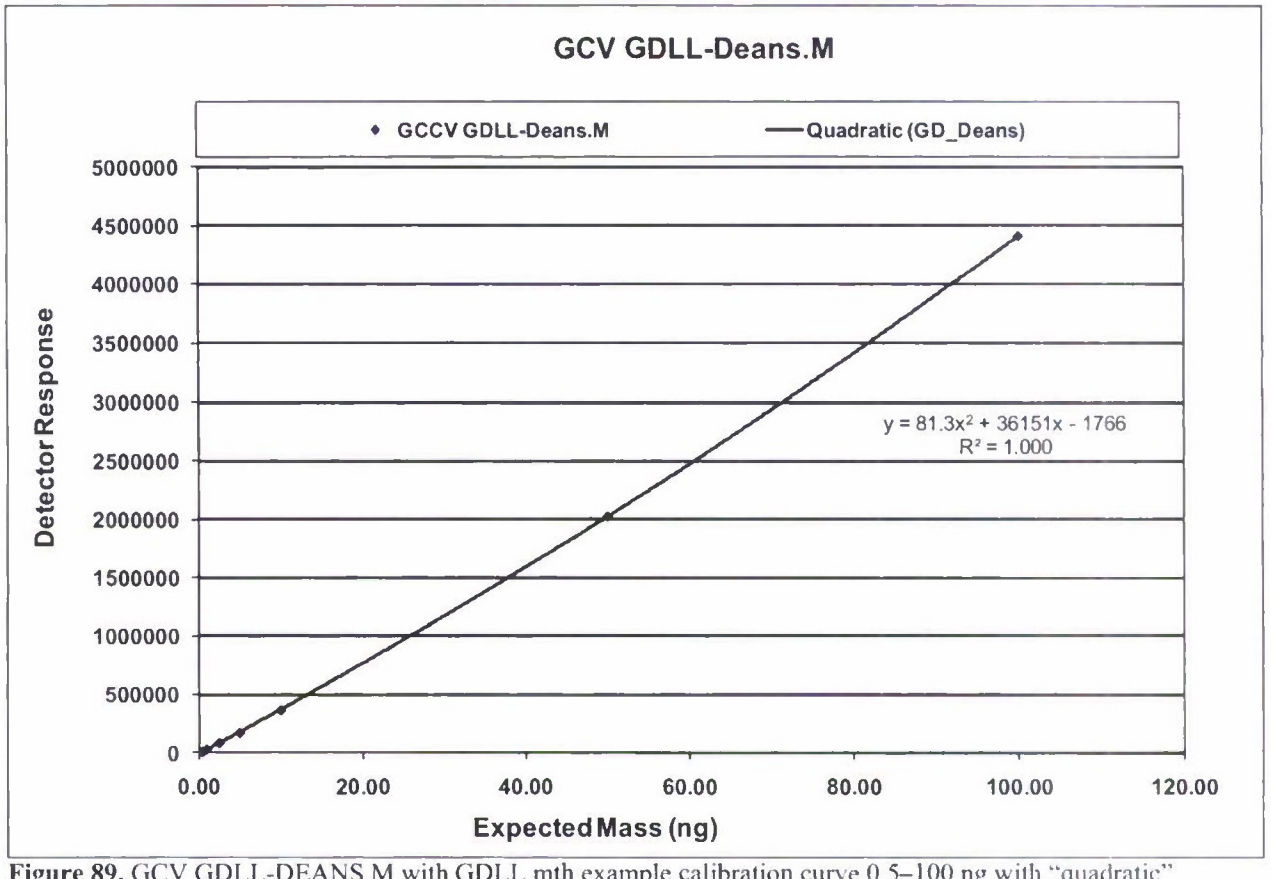


Figure 89. GCV GDLL-DEANS.M with GDLL.mth example calibration curve 0.5–100 ng with "quadratic" regression ("$1/x^2$" weighting).

The GCV GDLL-DEANS.M method had an LOD of 0.07 ng and an LOQ of 0.23 ng. The precision of the method was determined by analysis of multiple CCV samples. From one representative test, there were 12 replicate analyses of the 10 ng CCV, which produced an average found concentration of 9.5 ng with a standard deviation of 1.65 and an RSD of 17.36%. This indicated good reproducibility at the CCV level within the calibration curve. Furthermore, there were multiple replicate analyses of the 0.5 ng low-level standard from multiple tests, which produced a standard deviation of 0.023 and an RSD of 4.53%. This indicated very good reproducibility at the lowest standard in the

calibration curve. For the other standards, replicates were analyzed, producing standard deviations ranging from 0.02 to 5.66 and RSDs ranging from 3.65 to 12.86% indicating very good, overall method performance.

The accuracy of the method is demonstrated in Table 43 as uncertainty in the measurements at each standard in the calibration curve with 95% confidence. For a standard or sample, containing 10 ng of analyte, the analytical result reported was 10 ± 0.44 ng.

Table 43. List of standard masses and the associated confidence intervals.

Standard Mass on Tube (ng)	95% Confidence Interval (ng)	Lower Confidence Interval (ng)	Upper Confidence Interval (ng)
0.50	0.07	0.43	0.57
0.50	0.07	0.43	0.57
0.50	0.07	0.43	0.57
1.00	0.09	0.91	1.09
2.5	0.14	2.36	2.64
5.0	0.24	4.76	5.24
10.0	0.44	9.56	10.44
50.0	1.96	48.04	51.96
100.0	4.65	95.35	104.65

10.4.1.2 Method GCV GDHL-DEANS.M with GDHL.mth — Final

Method Purpose and Performance Overview: The purpose of the GCV GDHL-DEANS.M method is to detect and quantify the chemical agent GD in vapor samples. This method was developed as a complementary method to the low-level method (GCV GDLL-DEANS.M) for the evaluations of sample sorbent tubes containing higher masses of GD. This method detected GD with a mass-on-column range of 50–2500 ng. The method's detection requirements were optimized for the analysis of a 2 in. diameter test surface area. No significant method interferents were identified; however, users should confirm that new test materials do not off-gas a compound that could elute at the expected RT causing method interference. The sample throughput of this method is approximately four samples per hour.

Method Summary and Operation Details: The GCV GDHL-DEANS.M method is appropriate for vapor samples containing the chemical agent GD. This method utilizes nine calibration standards at masses of ~50, 100, 250, 500, 750, 1000, 1500, 2000, and 2500 ng GD. The method use, detection limit, and quantitation limit are based on using the full standard set. The standards are prepared in chloroform.

The *final* method performance for GD is as follows:

- Quantitative Method
- Calibration Range: 50–2500 ng GD
- CCV Levels: 0, 500, 500 ng.
- Calibration Model: Quadratic calibration model

- Calibration Weighting: One over the mass squared ($1/x^2$)
- LOD: 6.54 ng
- LOQ: 21.79 ng
- Spiking Sample Solvent: Acetonitrile
- Quantitation Ion: 126

LOD and Quantitation and Measurement Uncertainty: Calibration standards were prepared and analyzed multiple times for calibration consistency and detection limit determination. The final method evaluation pulled calibration and CCV performance data sets from decontamination performance testing queues. The high mass standard of the calibration set was followed by tube blanks to determine method carryover. The carryover from the high-level calibration standard has no affect on the method or the ability of the method to detect and quantify the low end of the calibration range. The calibration range of 50–2500 ng yielded a quadratic response. This method used a weighted quadratic calibration model with an applied weighting of $1/x^2$. The application of $1/x^2$ weighting ensures accuracy at the lower levels of the calibration curve. Accuracy at the upper end of the calibration mass range is maintained with the $1/x^2$ weighting. A demonstration of the GCV GDHL-DEANS.M calibration performance can be seen in Figure 90.

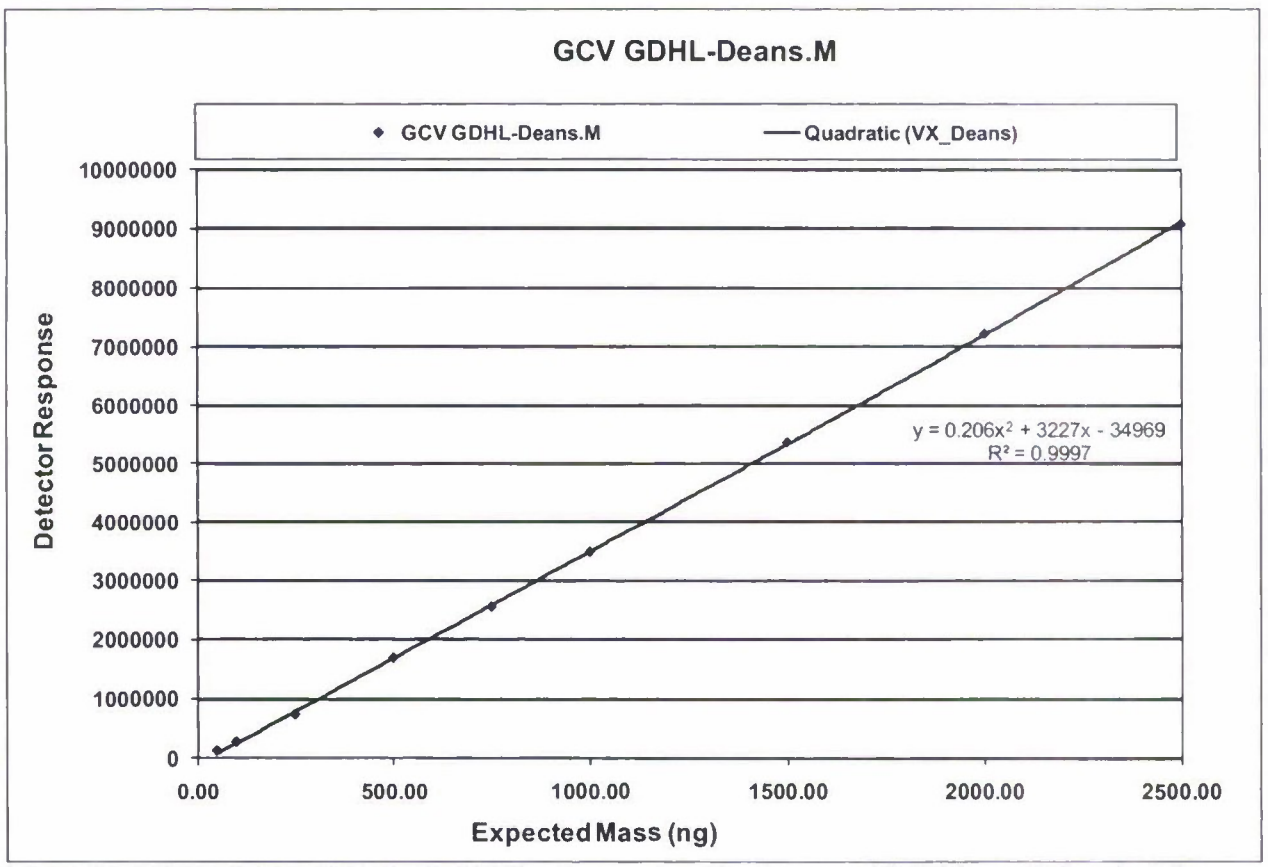


Figure 90. GCV GDHL-DEANS.M with GDHL.mth example calibration curve 50–2500 ng with "quadratic" regression ("$1/x^2$" weighting).

The GCV GDHL-DEANS.M method had an LOD of 6.54 ng and an LOQ of 21.79 ng. The precision of the method was determined by analysis of multiple CCV samples. From one representative test, there were 12 replicate analyses of the 500 ng CCV, which produced an average found mass of 499.3 ng with a standard deviation of 18.4 and an RSD of 3.68%. This indicated very good reproducibility at the CCV level within the calibration curve. Furthermore, there were multiple replicate analyses of the 50 ng low-level standard from multiple tests, which produced a standard deviation of 2.18 and an RSD of 4.35%. This indicated very good reproducibility at the lowest standard in the calibration curve. For the other standards, replicates were analyzed, producing standard deviations ranging from 3.86 to 31.6 and RSDs ranging from 0.71 to 4.03% indicating excellent, overall method performance.

The accuracy of the method is demonstrated in Table 44 as uncertainty in the measurements at each standard in the calibration curve with 95% confidence. For a standard or sample, containing 500 ng of analyte, the analytical result reported was 500 ± 32 ng.

Table 44. List of standard masses and the associated confidence intervals.

Standard Mass on Tube (ng)	95% Confidence Interval (ng)	Lower Confidence Interval (ng)	Upper Confidence Interval (ng)
50	13.40	36.60	63.40
100	15.04	84.96	115.04
250	21.46	228.54	271.46
500	31.95	468.05	531.95
750	41.84	708.16	791.84
1000	51.49	948.51	1051.49
1500	71.81	1428.19	1571.81
2000	96.39	1903.61	2096.39
2500	127.90	2372.10	2627.90

10.5 Method Demonstration for Decontaminant Performance Testing

10.5.1 GCV GDLL-DEANS.M Method Demonstration

The GD LL vapor method (GCV GDLL-DEANS.M) was used to analyze vapor samples from multiple coupon materials including stainless steel and CARC-coated stainless steel. This set of vapor samples was collected as part of test number SGDV02. The objective of SGDV02 was to show the decontamination performance of the test decontaminant as applied to the emission of agent vapors collected from the treated coupons.

The materials were spiked with known amounts of GD and were then decontaminated. The coupons were moved to the microchamber vapor manifold for vapor sampling onto Markes TDS tubes packed with Tenax TA. The microchamber vapor-sampling manifold had previously been cleaned, verified blank, and loaded with tubes ready for analysis. Operating conditions of the instrument had been met prior to test sample collection. Samples were collected at specific time intervals and flow rates (mL/min), which were pre-determined by the test designer and previously loaded to the instrument as a set of instructions. This test was performed in accordance with the panel testing guidance set forth in the Source Document.[16]

Upon test completion, the sample tubes were removed from the sampling manifold and analyzed on a GCV instrument. The expected masses of GD on the vapor sample tubes fell within the calibration range (0.5–100 ng) and were analyzed with the method GCV GDLL-DEANS.M.

Method parameters were established on GCV in accordance with Appendix A. Prior to analysis of test samples, the instrument was calibrated using GD calibration standards prepared in high purity solvent and spiked to Markes TDS tubes. The GCV GD calibration curve for this test had a correlation coefficient value of 1.000. The calibration curve was established with a weighted quadratic curve fit ($1/x^2$). The calibration curve for this test is shown in Figure 91. A summary of the method performance with calculated LOD and LOQ is shown in Table 45.

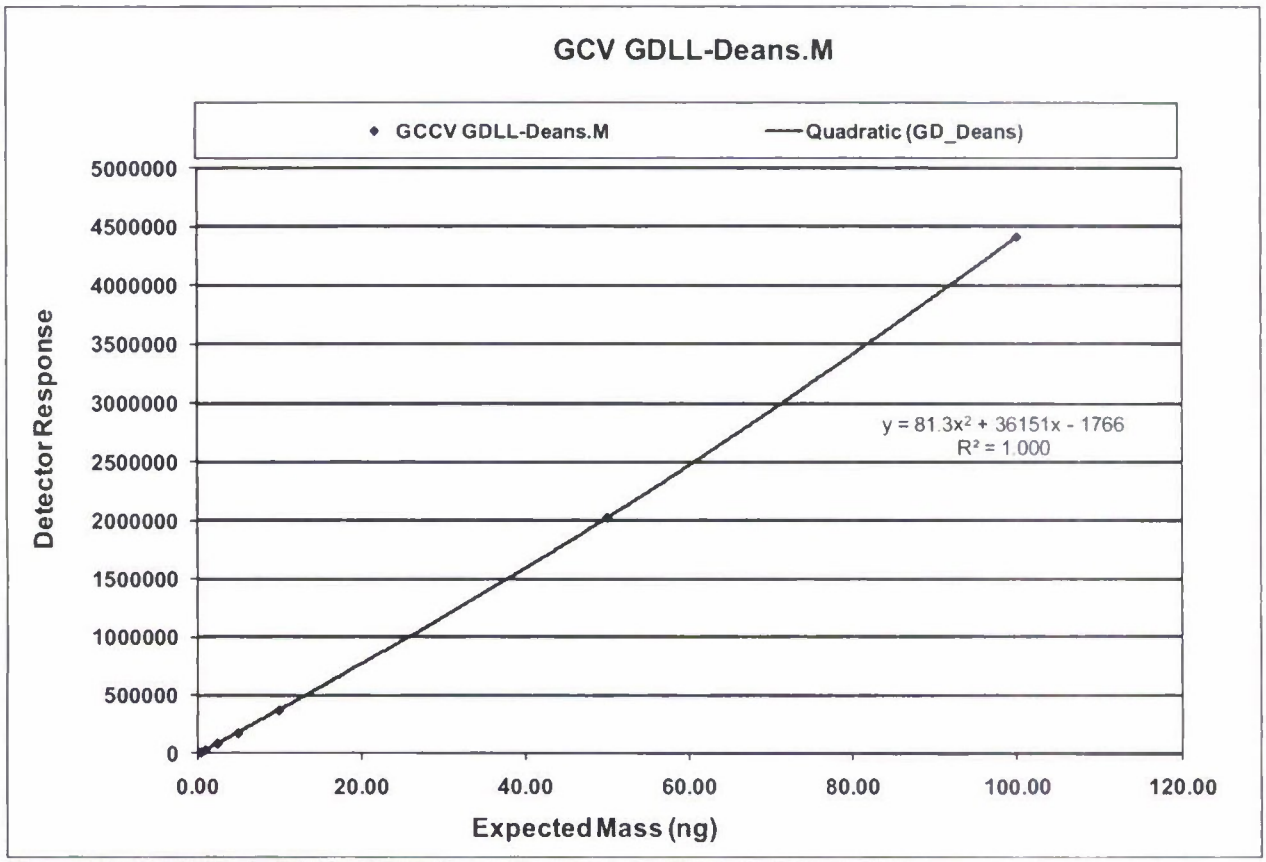


Figure 91. GCV GDLL-DEANS.M method calibration curve for test SGDV02-LL1.

Table 45. GCV GDLL-DEANS method summary for test SGDV02-LL1.

Method	GCV GDLL-DEANS.M
Test ID	SGDV02, LL1
Platform	GCV (GC/MS)
Sample Type	Vapor
Analyte(s)	GD
LOD	0.16 ng
LOQ	0.42 ng
Data Acquisition	EI SIM
Quantitation Ion	126 m/z

CCV samples were performed every 12 samples, and were found to be within 30% of their expected masses. Tube blanks were analyzed throughout the test for continuing baseline acceptance. Analysis of the blank samples showed little carryover and no interferences. The sample queue, with analytical data, for the performance testing samples analyzed with the GCV GDLL-DEANS method is shown in Figure 92. All samples were successfully analyzed using this GCV GDLL-DEANS method.

Sample Name	Q Value	Response	Mass on Tube (ng)
SGDV02_LL1_Pos01_Std_0d0	1	315	BD
SGDV02_LL1_Pos02_Std_0dS	S8	17117	0.S3
SGDV02_LL1_Pos03_Std_0dS	58	15036	0.47
SGDV02_LL1_Pos04_Std_0dS	76	16287	0.50
SGDV02_LL1_Pos05_Std_1d0	72	34014	0.99
SGDV02_LL1_Pos06_Std_2dS	96	90482	2.54
SGDV02_LL1_Pos07_Std_Sd0	97	176397	4.88
SGDV02_LL1_Pos08_Std_10d0	99	370158	10.07
SGDV02_LL1_Pos09_Std_50d0	97	2027754	50.43
SGDV02_LL1_Pos10_Std_100d0	97	440793S	99.6S
SGDV02_LL1_Pos11_Std_0d0	98	103036	2.89
SGDV02_LL1_Pos12_0d0_CCV01	92	62329	1.77
SGDV02_LL1_Pos13_10d0_CCV01	96	436625	11.82
SGDV02_LL1_Pos14_10d0_CCV01	96	434458	11.76
SGDV02_01SS_C01_T00	70	38960	1.13
SGDV02_01SS_C01_T01	8S	27308	0.81
SGDV02_01SS_C01_T02	77	32154	0.94
SGDV02_01SS_C01_T03	83	27708	0.82
SGDV02_01SS_C01_T04	80	21191	0.64
SGDV02_01SS_C01_T05	89	24754	0.74
SGDV02_01SS_C01_T06	68	13301	0.42
SGDV02_02SS_C02_T00	20	16188	0.50
SGDV02_02SS_C02_T01	81	21281	0.64
SGDV02_02SS_C02_T02	73	18098	0.5S
SGDV02_02SS_C02_T03	84	24391	0.73
SGDV02_02SS_C02_T04	77	15923	0.49
SGDV02_LL1_Pos27_0d0_CCV02	82	9242	8Q
SGDV02_LL1_Pos28_10d0_CCV02	96	387950	10.54
SGDV02_LL1_Pos29_10d0_CCV02	95	414554	11.24
SGDV02_02SS_C02_T05	29	21529	0.65
SGDV02_02SS_C02_T06	38	18840	0.S7
SGDV02_03SS_C03_T00	3S	20428	0.62
SGDV02_03SS_C03_T01	74	17326	0.53
SGDV02_03SS_C03_T02	87	25058	0.75
SGDV02_03SS_C03_T03	74	14842	0.46
SGDV02_03SS_C03_T04	72	10523	8Q
SGDV02_03SS_C03_T0S	69	837S	BQ
SGDV02_03SS_C03_T06	64	8058	BQ
SGDV02_04SS_C04_T00	3	12637	8Q
SGDV02_04SS_C04_T01	81	17743	0.54
SGDV02_04SS_C04_T02	41	10426	8Q
SGDV02_LL1_Pos42_0d0_CCV03	54	6108	BQ
SGDV02_LL1_Pos43_10d0_CCV03	96	409458	11.10
SGDV02_LL1_Pos44_10d0_CCV03	97	399466	10.84

Sample Name	Q Value	Response	Mass on Tube (ng)
SGDV02_04SS_C04_T03	85	23870	0.71
SGDV02_04SS_C04_T04	92	21826	0.66
SGDV02_04SS_C04_T05	76	12070	BQ
SGDV02_04SS_C04_T06	24	7762	BQ
SGDV02_0SSS_C05_T00	10	10015	BQ
SGDV02_0SSS_C05_T01	79	13907	0.44
SGDV02_0SSS_C0S_T02	40	11919	BQ
SGDV02_05SS_C0S_T03	67	14S26	0.46
SGDV02_0SSS_C0S_T04	26	29459	0.87
SGDV02_05SS_C0S_T05	61	37337	1.08
SGDV02_0SSS_C05_T06	S9	12080	BQ
SGDV02_06AC_C06_T00	34	142S5	0.45
SGDV02_LL1_Pos57_0d0_CCV04	69	5356	BQ
SGDV02_LL1_Pos58_10d0_CCV04	97	399209	10.83
SGDV02_LL1_PosS9_10d0_CCV04	97	434913	11.77
SGDV02_06AC_C06_T01	93	1508824	38.46
SGDV02_06AC_C06_T02	92	930068	24.44
SGDV02_06AC_C06_T03	89	549386	14.76
SGDV02_06AC_C06_T04	99	227676	6.26
SGDV02_06AC_C06_T05	89	306342	8.37
SGDV02_06AC_C06_T06	92	276793	7.58
SGDV02_07AC_C07_T00	48	34491	1.01
SGDV02_07AC_C07_T01	90	1403461	3S.97
SGDV02_07AC_C07_T02	90	1096429	28.SS
SGDV02_07AC_C07_T03	90	938927	24.66
SGDV02_07AC_C07_T04	88	63392S	16.94
SGDV02_07AC_C07_T05	88	417898	11.33
SGDV02_LL1_Pos72_0d0_CCV0S	93	42800	1.23
SGDV02_LL1_Pos73_10d0_CCV0S	96	440186	11.91
SGDV02_LL1_Pos74_10d0_CCV0S	97	436378	11.81
SGDV02_07AC_C07_T06	88	301804	8.2S
SGDV02_08AC_C08_T00	53	3509S	1.02
SGDV02_08AC_C08_T01	94	2067880	S1.33
SGDV02_08AC_C08_T02	93	1468266	37.S1
SGDV02_08AC_C08_T03	93	1206471	31.23
SGDV02_08AC_C08_T04	93	862S12	22.7S
SGDV02_08AC_C08_T05	93	650259	17.36
SGDV02_08AC_C08_T06	93	483468	13.0S
SGDV02_09AC_C09_T00	81	10S61S	2.96
SGDV02_09AC_C09_T01	94	2331465	57.19
SGDV02_09AC_C09_T02	93	1668285	42.20
SGDV02_09AC_C09_T03	93	1298449	33.46
SGDV02_LL1_Pos87_0d0_CCV06	98	124893	3.48
SGDV02_LL1_Pos88_10d0_CCV06	96	481447	12.99
SGDV02_LL1_Pos89_10d0_CCV06	96	458002	12.38

BD = 8elow Detect (LOD)
8Q = 8elow Quant (LOQ)
ND = Non Detect

*The shading is for ease of reading. Light green indicates calibration and CCV levels. Light blue indicates samples. Yellow indicates detector response information.

Figure 92. GDLL sequence and results for SGDV02 LL1.

10.5.2 GCV GDHL-DEANS.M Method Demonstration

The GD HL vapor method (GCV GDHL-DEANS.M) was used to analyze vapor samples from multiple coupon materials including stainless steel, CARC-coated stainless steel, AirForce Topcoat, and glass that were spiked with a known amount of the chemical agent GD. This set of vapor samples

was collected as part of test number SGDV02S. The objective of SGDV02S was to show the decontamination performance of the test decontaminant as applied to the emission of agent vapors collected from the treated coupons.

The materials were spiked with known amounts of GD and were then decontaminated. The coupons were moved to the microchamber vapor manifold for vapor sampling onto Markes TDS tubes packed with Tenax TA. The microchamber vapor-sampling manifold had previously been cleaned, verified blank and loaded with tubes ready for analysis. Operating conditions of the instrument had been met prior to test sample collection. Samples were collected at specific time intervals and flow rates (mL/min), which were pre-determined by the test designer and previously loaded to the instrument as a set of instructions. This test was performed in accordance with the panel testing guidance set forth in the Source Document.[16]

Upon test completion, the sample tubes were removed from the sampling manifold and analyzed on a GCV instrument. The expected masses of GD on the vapor sample tubes fell within the calibration range (50–2500 ng) and were analyzed with the method GCV GDHL-DEANS.M.

Method parameters were established on GCV in accordance with Appendix A. Prior to analysis of test samples, the instrument was calibrated using GD calibration standards prepared in high purity solvent and spiked to Markes TDS tubes. The GCV GD calibration curve for this test had a correlation coefficient value of 0.9997. The calibration curve was established with a weighted quadratic curve fit ($1/x^2$). The calibration curve for this test is shown in Figure 93. A summary of the method performance with calculated LOD and LOQ is shown in Table 46.

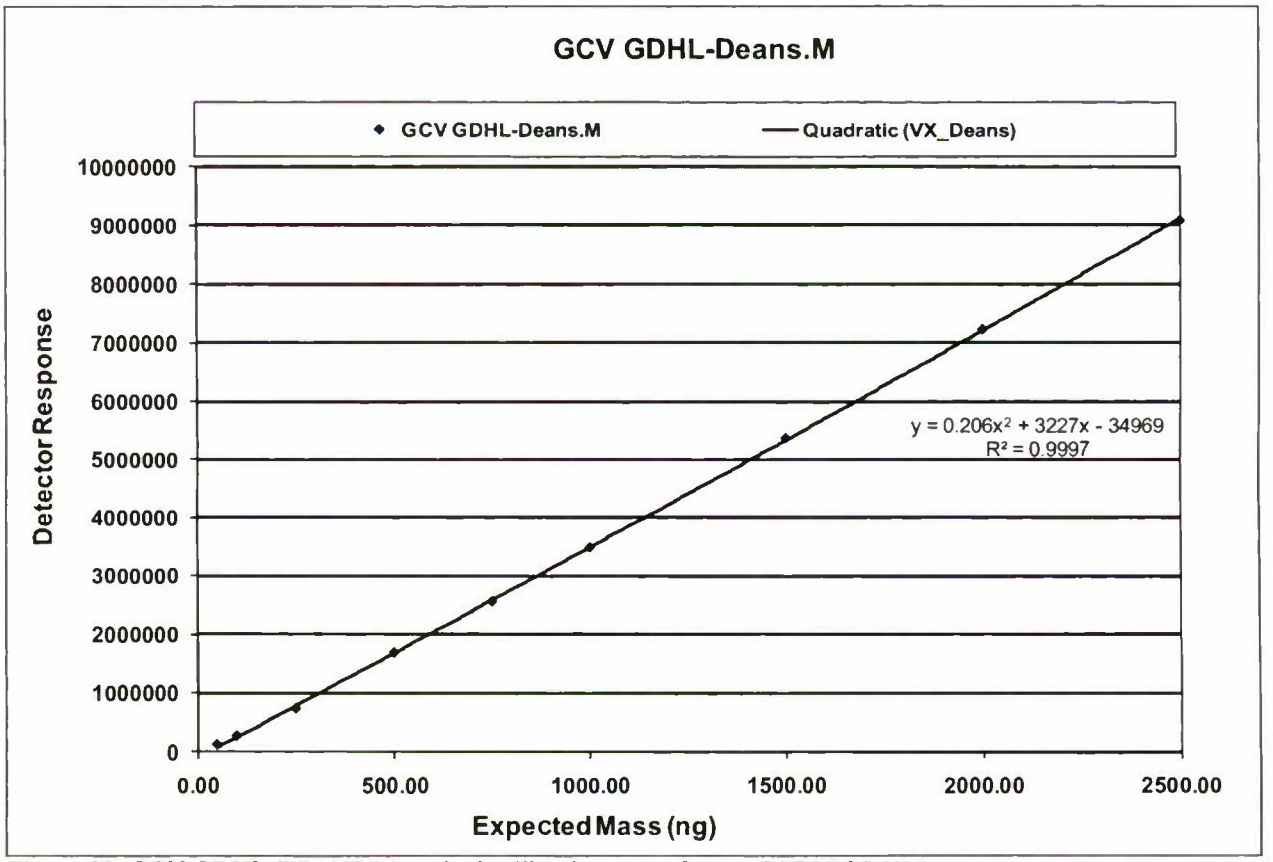


Figure 93. GCV GDHL-DEANS.M method calibration curve for test SGDV02S-HL1.

Table 46. GCV GDHL-DEANS method summary for test SGDV02S-HL1.

Method	GCV GDHL-DEANS.M
Test ID	SGDV02S, HL1
Platform	GCV (GC/MS)
Sample Type	Vapor
Analyte(s)	GD
LOD	8.38 ng
LOQ	8.67 ng
Data Acquisition	EI SIM
Quantitation Ion	126 m/z

CCV samples were performed every 12 samples, and were found to be within 30% of their expected masses. Tube blanks were analyzed throughout the test for continuing baseline acceptance. Analysis of the blank samples showed little carryover and no interferences. The sample queue, with analytical data, for the performance testing samples analyzed with the GCV GDHL-DEANS method is shown in Figure 94. All samples were successfully analyzed using this GCV GDHL-DEANS method.

Sample Name	Q Value	Response	Mass on Tube (ng)
SGDV02S_HL1_Pos01_Std_0d0	1	205	BD
SGDV02S_HL1_Pos02_Std_50d0	99	140260	50.94
SGDV02S_HL1_Pos03_Std_100d0	99	296442	98.24
SGDV02S_HL1_Pos04_Std_250d0	100	740380	231.46
SGDV02S_HL1_Pos05_Std_500d0	100	1700068	513.42
SGDV02S_HL1_Pos06_Std_750d0	100	2579745	765.11
SGDV02S_HL1_Pos07_Std_1000d0	99	3539009	1032.77
SGDV02S_HL1_Pos08_Std_1500d0	99	5386575	1530.09
SGDV02S_HL1_Pos09_Std_2000d0	98	7200942	1997.68
SGDV02S_HL1_Pos10_Std_2500d0	98	8977864	2437.96
SGDV02S_HL1_Pos11_Std_0d0	99	42467	21.20
SGDV02S_HL1_Pos12_0d0_CCV01	100	16540	13.30
SGDV02S_HL1_Pos13_500d0_CCV01	100	1648569	498.49
SGDV02S_HL1_Pos14_500d0_CCV01	100	1706604	515.31
SGDV02S_01SS_C01_T01	99	20559	14.52
SGDV02S_01SS_C01_T02	99	7351	10.50
SGDV02S_01SS_C01_T03	100	6885	10.35
SGDV02S_01SS_C01_T04	98	3901	9.44
SGDV02S_01SS_C01_T05	99	4391	9.59
SGDV02S_01SS_C01_T06	98	3396	9.29
SGDV02S_02SS_C02_T01	98	3386	9.29
SGDV02S_02SS_C02_T02	97	2272	8.95
SGDV02S_02SS_C02_T03	99	2426	8.99
SGDV02S_02SS_C02_T04	98	1751	8.79
SGDV02S_02SS_C02_T05	97	2068	8.88
SGDV02S_02SS_C02_T06	97	1861	8.82
SGDV02S_HL1_Pos27_0d0_CCV02	92	1199	BQ
SGDV02S_HL1_Pos28_500d0_CCV02	100	1575232	477.19
SGDV02S_HL1_Pos29_500d0_CCV02	100	1616648	489.22
SGDV02S_03SS_C03_T01	99	7671	10.59
SGDV02S_03SS_C03_T02	96	3309	9.26
SGDV02S_03SS_C03_T03	97	2994	9.17
SGDV02S_03SS_C03_T04	98	1715	8.78
SGDV02S_03SS_C03_T05	96	1609	8.74
SGDV02S_03SS_C03_T06	97	1328	BQ
SGDV02S_04AC_C04_T01	98	4979	9.77
SGDV02S_04AC_C04_T02	97	3892	9.44
SGDV02S_04AC_C04_T03	95	3412	9.29
SGDV02S_04AC_C04_T04	98	1835	8.81
SGDV02S_04AC_C04_T05	97	1760	8.79
SGDV02S_04AC_C04_T06	93	1136	8.60
SGDV02S_HL1_Pos42_0d0_CCV03	89	765	BQ
SGDV02S_HL1_Pos43_500d0_CCV03	100	1589937	481.46
SGDV02S_HL1_Pos44_500d0_CCV03	100	1593547	482.51

Sample Name	Q Value	Response	Mass on Tube (ng)
SGDV02S_05AC_C05_T01	98	46986	22.57
SGDV02S_05AC_C05_T02	97	15013	12.83
SGDV02S_05AC_C05_T03	95	6894	10.36
SGDV02S_05AC_C05_T04	92	2930	9.15
SGDV02S_05AC_C05_T05	92	2763	9.10
SGDV02S_05AC_C05_T06	92	3396	9.29
SGDV02S_06AC_C06_T01	99	185417	64.64
SGDV02S_06AC_C06_T02	99	114425	43.09
SGDV02S_06AC_C06_T03	99	86389	34.56
SGDV02S_06AC_C06_T04	99	48676	23.09
SGDV02S_06AC_C06_T05	99	33654	18.51
SGDV02S_06AC_C06_T06	99	21981	14.96
SGDV02S_HL1_Pos57_0d0_CCV04	91	933	BQ
SGDV02S_HL1_Pos58_500d0_CCV04	100	1531978	464.61
SGDV02S_HL1_Pos59_500d0_CCV04	100	1525139	462.62
SGDV02S_07AF_C07_T01	99	5833	10.03
SGDV02S_07AF_C07_T02	92	2499	9.02
SGDV02S_07AF_C07_T03	97	1656	8.76
SGDV02S_07AF_C07_T04	95	1186	BQ
SGDV02S_07AF_C07_T05	96	1269	BQ
SGDV02S_07AF_C07_T06	96	977	BQ
SGDV02S_08AF_C08_T01	94	1156	BQ
SGDV02S_08AF_C08_T02	95	839	BQ
SGDV02S_08AF_C08_T03	85	847	BQ
SGDV02S_08AF_C08_T04	88	568	BQ
SGDV02S_08AF_C08_T05	89	741	BQ
SGDV02S_08AF_C08_T06	82	475	BQ
SGDV02S_HL1_Pos72_0d0_CCV05	95	458	BQ
SGDV02S_HL1_Pos73_500d0_CCV05	100	1470881	446.81
SGDV02S_HL1_Pos74_500d0_CCV05	100	1514646	459.56
SGDV02S_09AF_C09_T01	98	5997	10.08
SGDV02S_09AF_C09_T02	96	2958	9.16
SGDV02S_09AF_C09_T03	92	2067	8.88
SGDV02S_09AF_C09_T04	96	1719	8.78
SGDV02S_09AF_C09_T05	84	1086	BQ
SGDV02S_09AF_C09_T06	89	846	BQ
SGDV02S_10GL_C10_T01	93	795	BQ
SGDV02S_10GL_C10_T02	98	841	BQ
SGDV02S_10GL_C10_T03	96	645	BQ
SGDV02S_10GL_C10_T04	97	448	BQ
SGDV02S_10GL_C10_T05	57	395	BD
SGDV02S_10GL_C10_T06	87	601	BQ
SGDV02S_HL1_Pos87_0d0_CCV06	95	366	BD
SGDV02S_HL1_Pos88_500d0_CCV06	100	1500726	455.50
SGDV02S_HL1_Pos89_500d0_CCV06	100	1527335	463.25

8D = Below Detect (LOD)

8Q = Below Quant (LOQ)

ND = Non Detect

*The shading is for ease of reading. Light green indicates calibration and CCV levels. Light blue indicates samples. Yellow indicates detector response information.

Figure 94. GDHL sequence and results for SGDV02S HL1.

LITERATURE CITED

1. *Field Manual 3-11.5: CBRN Decontamination: Multiservice Tactics, Techniques, and Procedures for Chemical, Biological, Radiological, and Nuclear Decontamination*; U.S. Army: April 2006.

2. *CSTE-DTC-TT-M Test Operations Procedure (TOP) 8-2-061: Chemical and Biological Decontamination Testing*; West Desert Test Center: Dugway Proving Ground, UT, 19 November 2002. UNCLASSIFIED Report (AD-A409 136).

3. JRO Joint Requirements Office for CBRN Defense. https://jro-cbrnd.cbiac.apgea.army.mil/Home.aspx (Accessed January 2008).

4. JRO *Operational Requirements Document (ORD) for Joint Service Sensitive Equipment Decontamination (JSSED)*; JROC: Washington, DC, 2003.

5. JRO *Operational Requirements Document (ORD) for Joint Platform Interior Decontamination (JPID)*; (V1.4); Joint Requirements Office for Chemical, Biological, and Nuclear Defense, 8000 Joint Staff Pentagon: Washington, DC, 1 November 2004.

6. JRO *Operational Requirements Document (ORD) for Joint Service Sensitive Equipment Decontamination (JSSED)*; JROC: Washington, DC, March 2004.

7. JRO *Operational Requirements Document (ORD) for Joint Platform Interior Decontamination (JPID) (V1.4)*; JROC: Washington, DC, Updated May 2005.

8. JRO *Capability Development Document (CDD) for Joint Platform Interior Decontamination (JPID)*; (V1.1); JROC: Washington, DC, February 2006.

9. JRO *Capability Development Document (CDD) for Joint Service Sensitive Equipment Decontamination (JSSED) (V1.1)*; JROC: Washington, DC, February 2006.

10. EPA Test Methods for Evaluating Solid Waste, Physical/Chemical Methods (SW-846). http://www.epa.gov/epaoswer/hazwaste/test/sw846.htm (Accessed January 2008).

11. U.S. Army Chemical Materials Agency. http://www.cma.army.mil/ (Accessed January 2008).

12. Congress Department of Defense Authorization Act of 1986. In 1986; Vol. 99-1145.

13. *Toxic Chemical Agent Safety Standards*; DA PAM 385-61; Department of the Army: March 2002.

14. *Implementation Guidance Policy for Revised Airborne Exposures Limits for GB, GA, GD, GF, VX, H, HD, and HT*; Department of the Army Office of the Assistant Secretary of the Army Installations and Environment: June 2004.

15. *Programmatic Laboratory and Monitoring Quality Assurance Plan*; U.S. Army Chemical Materials Agency: Aberdeen Proving Ground, MD, June 2004.

16. Lalain, T.; Mantooth, B.; Lynn, T.; Zander, Z.; Humphreys, P. *Development of the 2007 Chemical Decontaminant Source Document*; ECBC-TR-671; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, March 2009. UNCLASSIFIED Report (AD-A511 356).

17. *Environmental Quality: Guidance for Evaluating Performance-Based Chemical Data*; EM 200-1-10; U.S. Army Corps of Engineers: 2005.

18. *American National Standard Z1.4 Sampling Procedures and Tables for Inspection by Attributes*; ANSI/ASQ Z1.4; American Society for Quality: Milwaukee, WI, 2008.

19. Wisconsin *Analytical Detection Limit Guidance & Laboratory Guide for Determining Method Detection Limits*; PUBL-TS-056-96; Wisconsin Department of Natural Resources Laboratory Certification Program: April 1996.

20. *Guidance for Industry: Q2B Validation of Analytical Procedures: Methodology*; U.S. Department of Health and Human Services, Food and Drug Administration: Washington DC, November 1996. UNCLASSIFIED Report.

21. FDA Guidance for Industry: Bioanalytical Method Validation. http://www.fda.gov/cder/guidance/index.htm (Accessed December, 2007).

22. Kiser, M. M.; Dolan, J. W. Selecting the Best Curve Fit. *LC GC Europe* **2004,** 17 (3), pp 138-43.

23. *Field Manual 3-11.9: Potential Military Chemical/Biological Agents and Compounds*; FM 3-11.9; U.S. Army: 2005.

24. Small, M. J. *Compounds Formed from the Chemical Decontamination of HD, GB, and VX and Their Environmental Fate*; Technical Report 8304; U.S. Army Medical Bioengineering Research and Development Laboratory: Fort Detrick, MD, June 1984. UNCLASSIFIED Report (AD-A149 515).

25. Munro, N. B.; Talmage, S. S.; Griffin, G. D.; Waters, L. C.; Watson, A. P.; King, J. F.; Hauschild, V. The Sources, Fate, and Toxicity of Chemical Warfare Agent Degradation Products. *Environmental Health Perspectives* **1999,** 107 (12), pp 933-74.

26. D'Agostino, P. A.; Chenier, C. L.; Hancock, J. R. *Electrospray Mass Spectrometry of Chemical Warfare Agents, Degradation Products, and Related Compounds*; DRES TR 2002-028; Defense Research Establishment, Suffield (DRES): Alberta, Canada, March 2002. UNCLASSIFIED Report.

27. Yeung, D. T.; Smith, J. R.; Sweeney, R. E.; Lenz, D. E.; Cerasoli, D. M. Direct Detection of Stereospecific Soman Hydrolysis by Wild-type Human Serum Paraoxonase. *The Federation of European Biochemical Societies Journal* **2007,** 274, pp 1183-91.

28. Reutter, S. A.; Moretz, R. W.; Murray, M. M.; Sommerville, D. R. *Review of Toxicological Data Regarding Contact Hazards of Chemical Agents*; ECBC-TR-514; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, August 2006. UNCLASSIFIED Report (AD-B321 921).

29. Lalain, T.; Mantooth, B.; Lynn, J. T.; Hover, M.; Shue, M.; Myers, J. *Development of the Small-Item Vapor Test Method*; ECBC-TR-742; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, February 2010. UNCLASSIFIED Report (AD-B357 963).

30. Barrett, W. J.; Brady, D. V.; Hostettler, J. B.; Crawford, D. E. *Investigation of VG Conversion Prefilter for Point-Sampling Alarms*; Report Number 4; U.S. Army Chemical Research and Development Laboratories: Edgewood Arsenal, MD, March 1965.

31. Smith, J. E.; Boyd, W. D.; Mason, D. W. *Depot Area Air Monitoring System and VX Study*; ARCSL-CR-82052; U.S. Army Chemical Systems Laboratory: Aberdeen Proving Ground, MD, November 1982. UNCLASSIFIED Report (AD-B070 559L).

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ACRONYMS

ACh	Acetylcholine
AChE	Acetyleholinesterase Enzyme
AEL	Airborne Exposure Limit
ALS	Automatic Liquid Sampler
AMU	Atomic Mass Unit
APCI	Atmospheric Pressure Chemieal Ionization
APG	Aberdeen Proving Ground
AQL	Acceptanee Quality Level
ASTM	American Society for Testing and Materials
CARC	Chemical Agent Resistant Coating
CAS	Chemical Abstract Service
CASARM	Chemieal Agent Standard Analytieal Reference Material
CB	Chemical, Biologieal
CBRN	Chemical, Biological, Radiologieal, and Nuclear
CCV	Continuing Calibration Verification
CDF	Chemical Demilitarization Faeility
CI	Chemical Ionization (for mass spectroscopy)
CIS	Cooled Injection System
CMA	U.S. Army Chemieal Materials Agency
DAAMS	Depot Area Air Monitoring System
DoD	Department of Defense
DTRA	Defense Threat Reduction Ageney
ECBC	Edgewood Chemieal Biologieal Center
EI	Electron Impact Ionization (for mass speetroscopy)
EMPA	Ethyl Methylphosphonie Acid
EPA	U.S. Environmental Proteetion Ageney
ESI	Electro Spray Ionization
FDA	Food and Drug Administration
FID	Flame Ionization Deteetor
FPD	Flame Photometrie Deteetor
GC	Gas Chromatograph
GPL	General Population Limit
HL	High Level
ICV	Initial Calibration Verification
IDLH	Immediately Dangerous to Life and Health
ISO	International Standards Organization
JPID	Joint Platform Interior Decontamination
JRO	Joint Requirements Office
JSSED	Joint Service Sensitive Equipment Decontamination
LC	Liquid Chromatograph
LL	Low Level
LOD	Limit of Detection
LOQ	Limit of Quantitation
MOPP	Mission Oriented Proteetive Posture
MPA	Methylphosphonic Aeid
MPS	MultiPurpose Sampler
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometer
MS/MS	Mass Spectrometer/Mass Spectrometer (detector)
MSD	Mass Selective Detector
MSDS	Material Safety Data Sheet

NIST	National Institute of Standards and Technology
NMR	Nuclear Magnetic Resonance
ORD	Operations Requirement Document
OSHA	Occupational Safety and Health Administration
PBM	Probability Based Matching
PMT	Photomultiplier Tube
PPE	Personnel Protective Equipment
QA/QC	Quality Assurance/Quality Control
QQQ	Triple Quadrupole Mass Spectrometer
RDECOM	Research, Development, and Engineering Command
RMSE	Root Mean Square Error
RPD	Relative Percent Deviation
RSD	Relative Standard Deviation
RT	Retention Time
S/N	Signal to Noise
SIM	Selected Ion Monitoring
SSE	Sum of the Square of the Errors
STB	Supertropical Bleach
STEL	Short-Term Exposure Limit
SVE	Solvent Vapor Exit
TCC	Thermal Column Compartment
TDG	Thiodiglycol
TDS	Thermal Desorption System
TGD	Thickened GD
TIC	Total Ion Chromatogram
ULL	Ultra-Low-Level
WPL	Worker Population Limit
XIC	Extracted Ion Chromatogram

GLOSSARY

Terminology and/or acronyms used in this report that are either unique to this project or have specific meaning that need to be defined for clarification are provided in the following list.

- **base peak:** Typically the most abundant ion on a mass spectrum and the ion that other ions are compared to when determining relative abundances.
- **breakthrough:** The inability of the sorbent to retain the compound of interest during sampling, allowing some/all of the compound to pass through the sorbent.
- **carryover:** The presence of analyte in a subsequent analytical evaluation following a high concentration extract or large mass vapor sample creating a positive response or bias.
- **chemical agent:** A chemical agent is a toxic chemical for use in military operations. A comprehensive listing of chemical agents can be found in Army Field Manual number 3-11.9. The term agent is used interchangeably with the term contaminant.
- **contact hazard:** The amount of contaminant remaining on the surface that, based on toxicological human estimates, could pose a threat to unprotected personnel touching the contaminated surface.
- **contact mass:** A uniform mass used to apply pressure during the contact test. The masses are typically prepared from stainless steel. The masses should evenly exert 0.7–1.0 psi pressure on the coupon surface. For the 2 in. diameter disk, this is equivalent to a 2 in. diameter cylindrical mass weighing 1 kg.
- **contact sampler:** Material used in this contact test as a surrogate for human skin. The sample sorbs the available surface contamination, which is then extracted to determine the mass of agent potentially bioavailable or available for contact transfer.
- **contact transfer:** The capability for a contaminant present on a specific surface to be moved to another through touching the contaminated surface.
- **contaminant:** A chemical compound with harmful effects to humans to be neutralized or removed from surfaces of interest. Typical contaminants include chemical agents, chemical agent simulants, toxic industrial chemicals, and toxic industrial materials.
- **coupon handling:** Treatment of the coupons upon leaving inventory through disposal. Handling may include contamination, decontamination, extraction, etc.
- **decontaminant:** For these procedures, a substance with ability to remove and/or neutralize chemical agents on/in surfaces of interest. The decontaminant can be liquid-phase, solid-phase (powders, wipes), or gas-phase (fumigants, including aerosols).
- **decontamination process:** The process of making any person, object, or area low-risk by absorbing, destroying, neutralizing, making harmless, or removing a contaminant (reference FM 3-11.9). More specifically for these procedures, the specific series of coupon treatment tasks performed, which may include contaminating, aging, decontaminating, rinsing and drying.
- **detection limit:** The lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) within a stated level of confidence.
- **hazard:** A condition with the potential to cause injury, illness, or death of personnel; damage to or loss of equipment or property; or mission degradation (reference FM 3-11.9).

- **molecular ion:** An ion representing the ionized form of the analyte molecule on a mass spectrum.
- **quantitation limit:** The lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy.
- **Q-value:** A qualitative value, calculated by the Agilent MSD ChemStation, representing a match quality by comparison of the ion relative abundances in a sample versus the ion relative abundances in the calibration database.
- **relative standard deviation:** (RSD or %RSD) Refers to the absolute value of the coefficient of variation expressed as a percentage.
- **requirement levels:** The documented amount of permissible agent remaining after a decontaminant process, typically expressed as a vapor concentration (mg/m^3) or a surface concentration (mg/m^2).
- **residual agent:** The amount of contaminant present in/on the material of interest after the decontaminant process and hazard test have been conducted.
- **vapor chamber:** A dynamic vapor microchamber that fully encloses a coupon to enable vapor emission analysis. The chamber must facilitate the ability to control airflow and mixing, collect vapor samples, and measure environmental conditions such as temperature and relative humidity.
- **vapor hazard:** A value specified in requirements documents, usually specified as a concentration (mg/m^3), that should have an accompanying exposure time. The value corresponds to an exposure that presents an acceptable risk level to unprotected personnel exposed to the vapor concentration. The toxic load model will be applied to calculate a vapor hazard.

APPENDIX

2007 Low-Level Analytical Methods to Support Decontaminant Performance Evaluations

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Method Deliverable
Project CA06DEC407

Final Methods
December 2007

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LOW-LEVEL ANALYTICAL METHODOLOGY UPDATES TO SUPPORT DECONTAMINANT PERFORMANCE EVALUATIONS

METHODS INTRODUCTION

CA06DEC407 was a DTRA JSTO-funded effort designed to address the challenges associated with quantifying low-level residual agent to support decontaminant contact and vapor test evaluations. The program had three main objectives. The primary program objective was to develop improved analytical methods to enable the confident detection of chemical agents VX, HD and GD at low published requirement levels. The lowest requirements at the time of this program, used to establish the required detection limits, were the Joint Platform Interior Decontamination (JPID) program 2003 and the Joint Service Sensitive Equipment Decontamination (JSSED) program 2005 requirement documents. An *analytical method* is an electronic file containing the settings the analytical equipment uses during operation. These settings are optimized for the specific objective (i.e., low-level VX). The *analytical equipment* is the hardware that uses the analytical methods. The second program objective was to establish methods for the detection of common agent byproducts that could form during decontaminant testing. The third program objective was to make the new methods available to establish uniformity in test procedures across testing locations. This document contains the set of test methods meeting all three project objectives.

This document is formally published as an appendix to the final project technical report, which contains the method development and demonstration, and cited references. The final technical report is written by Mr. Matthew Shue, et al., and titled "Low-Level Analytical Methodology Updates to Support Decontaminant Performance Evaluations." These methods are used to support decontaminant performance testing based on the following methods:

- West Desert Test Center, Dugway Proving Grounds, Test Operations Procedure (TOP) 8-2-061, Chemical and Biological Decontaminant Testing, 19 November 2002.
- Technical report by T. Lalain, et al., titled "The 2007 Chemical Decontaminant Source Document."
- Technical report by T. Lalain, et al., titled "Development of the 2007 Chemical Decontaminant Source Document."

Each method is documented as an individual method. The methods are constructed using standardized fields with all pertinent information. The "Analyte Concentration Range" section provides an overview of the method target, the calibration range, calibration curve-fitting model and weighting, limit of detection (LOD), limit of quantitation (LOQ), solvent and quantitation ion(s). The methods are identified as quantitative or qualitative. The program objective was byproduct identification. The byproduct methods as presented here are qualitative. All qualitative methods in this document can be quantitative, if a second set of calibration standards using the byproduct are prepared and analyzed. The LOD and LOQ are calculated based on the laboratory evaluation of the final method. Each laboratory should recalculate these values based on their method performance. The LOD and LOQ are a function of instrument sensitivity, which can decay over time/use. Instrument sensitivity can often be restored by regular scheduled maintenance, thus illustrating the need for a regular maintenance schedule. It is anticipated that a laboratory can achieve a better LOD and LOQ by using new equipment or scheduling regular instrument maintenance. These values should be calculated over a period of time to determine the laboratory performance as discussed in the final technical report. The

"Apparatus" section details the analytical equipment and standard preparation tools. The "Method Parameters" section provides the complete listing of instrumentation settings for the method.

NOTE: The methods developed for this Low-Level program are provided in the output format for the specific instruments used. The methods and parameters detailed in this appendix should yield similar results when applied to instruments with similar specifications and configurations. The methods detailed in this program may be applied to other instrument configurations; however, some method modification may be required to account for software and hardware specifications. All methods should be verified to confirm performance prior to use for sample analysis.

FINAL LIQUID EXTRACTION SAMPLE ANALYSIS METHODOLOGY

The following methodology represents the final methodology and parameters for the liquid extraction sample analyses detailed in this technical report.

METHOD A: LC/MS/MS METHOD FOR ULTRA LOW-LEVEL VX EA-2192 IDENTIFICATION (LCE VX_ULL.DAM)

ANALYTICAL METHOD

TITLE

Ultra low-level detection of VX and EA2192 in liquid extraction samples for chemical agent decontamination testing using an LC/MS/MS system.

AUTHORS

Matthew Shue (ECBC Decontamination Sciences Branch)
Phillip Smith, Ph.D. (SAIC)
Teri Lalain, Ph.D. (Branch Chief, ECBC Decontamination Sciences Branch)

KEYWORDS

VX, O-Ethyl S-2-diisopropylaminoethyl methyl phosphonothiolate, CAS 50782-69-9
EA2192, S-(2-Diisopropylaminoethyl) methylphosphonothioic acid, CAS 73207-98-4

REVISION HISTORY

This document is version 1, released December 2007.

PURPOSE AND APPLICATIONS

The primary purpose of this method is to detect and quantify ultra low concentrations of the chemical agent VX in liquid extraction samples. The secondary purpose of this method is to screen liquid extraction samples for EA2192, a hydrolysis byproduct of VX. This method is optimized to support the analysis of liquid extracts following the chemical agent decontamination performance evaluation testing. Contact sampler and coupon extracts for a 2 in. diameter test surface area are extracted in no greater than 20 mL of extraction solvent. Sample throughput is approximately seven samples per hour.

ANALYTE CONCENTRATION RANGE

This method was developed to exceed the low-level JPID ORD requirements as detailed in the Low-Level Test Execution and Resource Integration Plan. This method can detect VX solution concentrations from 0.05 to 10.0 ng/mL.

This method utilizes eight calibration standards at concentrations of ~0.05, 0.1, 0.2, 0.5, 1.0, 2.5, 5, and 10 ng/mL VX. The method use, detection limit, and quantitation limit are based on using the full standard set. The calibration standards are prepared in isopropyl alcohol. The mobile phase is a 50:50 mixture of the aqueous and organic phases. The organic phase consists of 95% IPA, 5% deionized water, 0.1% formic acid, and 5 mM ammonium acetate. The

aqueous phase consists of 95% deionized water, 5% IPA, 0.1% formic acid, and 5 mM ammonium acetate. The method is conducted in an isocratic configuration.

The method performance for VX_ULL is:

- Quantitative Method
- Calibration Range: 0.05 to 10 ng/mL VX
- CCV Levels: 0, 0.5, 2.5 ng/mL
- Calibration Model: Weighted quadratic regression
- Calibration Weighting: $1/x^2$
- Limit of Detection (LOD): 0.002 ng/mL
- Limit of Quantitation (LOQ): 0.006 ng/mL
- Sample Solvent: IPA
- Quantitative Ion Pair: VX 268.1 / 128

The method performance for EA2192 is as follows:

- Qualitative Method
- Sample Solvent: IPA
- Quantitative Ion Pair: EA-2192 240.1 / 128

SAMPLE MATRICES AND INTERFERENCES

This method is appropriate for liquid samples extracted with the solvent isopropyl alcohol (C_3H_8O; CAS #67-63-0) and containing the chemical agent VX and/or the VX byproduct EA2192. No significant method interferents have been identified. Users of this method should confirm that the test material and solvent are compatible prior to testing.

RISK AND SAFETY ASSESSMENT

This document does not claim to address all of the safety concerns associated with chemical decontaminant testing as the requirements may vary based on facility, state and other regulatory requirements. It is the responsibility of the user of this method, prior to use, to establish appropriate environment, health, and safety practices for the use of this method and handling of generated wastes in compliance with applicable regulations. Users of this method should conduct testing in appropriate facilities and follow proper laboratory practices, including the use of appropriate personal protective equipment and material safety data sheets.

SCIENTIFIC BASIS

This method is based on liquid chromatography and mass spectrometry techniques.

TRAINING

Users of this method must have the basic skill level for analytical chromatography instrument operation, interpretation of mass spectrometry results, and associated wet lab techniques (e.g., sample dilution and standard preparation). Training covering instrument operation and maintenance of equipment is suggested.

APPARATUS

The liquid extraction samples are analyzed on an Applied Biosystems API5000 Triple-quadrupole Mass Spectrometer equipped with the TurboV Ion Source. Sample introduction and chromatography are performed with an Agilent 1200 series Liquid Chromatograph (LC). Sample effluent is directed from the LC directly to the TurboV ion source of the API5000 MS. The system is fitted with an Agilent Technologies Zorbax SB-C18 analytical column (4.6 mm inside diameter [ID] x 75 mm length [L], 3.5 µm particle size [part number 866953-902]). Instrumentation operation, maintenance manuals, and miscellaneous and consumable equipment lists are available from the instrument manufacturers: Applied Biosystems and Agilent Technologies. All equipment should be used in accordance with the manufacturer's instructions.

Chemicals required include dilute VX standards prepared in high purity isopropyl alcohol solvent.

Volumetric glassware used for standard preparation should be Class A and meet the specifications in the most current version of ASTM standards E288 and E69. Pipettes used for standard preparation should be compliant with the required performance specifications listed in the most current versions of ISO 8655 Parts 1 and 2 and/or ASTM E 1154 for the volume measured, if standards are made volumetrically. Balances used for gravimetric standard preparation should be sensitive enough to measure the appropriate mass delivered. All equipment should be used in accordance with the manufacturer's requirements, and should be properly maintained and calibrated.

Amber glass analytical sample vials are preferred. The septum cap should be lined with inert material, preferably PTFE/Teflon, to prevent extraction of plasticizers or other impurities into the sample.

PROCEDURE

The specific equipment and column are identified in the "Apparatus" section. Procedures for equipment operation, instrumentation problems, and corrective actions are found in the manufacturer's operating manuals. The specific instrumentation settings for this method are provided in the "Method Parameters" section. The equipment should be confirmed as operational prior to use for test support.

Standards are prepared for calibration either volumetrically or gravimetrically, as required by the method. The LC mobile phase solutions (aqueous and organic) are prepared in accordance with the method specifications.

Liquid extract samples above the method calibration range are diluted to be within the method calibration range. Extrapolation of results outside the calibrated range is not recommended.

Calibration curves are generated from the standard sample results. The curves are fit and weighted as specified in this method. For quality control, the analysis of test samples must include the use of Initial Calibration Verification (ICV), Continuing Calibration Verifications (CCV) and solvent blanks. Specific guidance for method use for decontaminant performance evaluations is provided in the "2007 Chemical Decontaminant Performance Evaluation Testing Source Document" by T. Lalain, et al. Standard preparation, sample queues, interferences, and other general analytical guidance are provided in the corresponding technical report to this document titled, "Low-Level Analytical Methodology Updates to Support Decontaminant Performance Evaluations" by M. Shue, et al.

DATA ACCEPTANCE & REPORTING

Analytical results for samples are reported as solution concentrations (ng/mL). Solution concentration can be calculated using the instrument control software (e.g., Applied Biosystems Analyst, Chemstation, etc.) or using external statistical software (e.g., Excel, Sigma plot, etc.) with the specified calibration model. The reported solution concentration should account for sample dilutions made after the original sample generation (e.g., contact, residual, and remaining agent testing) such that the analyzed sample is within the calibration range. Calibration curves are not extrapolated; samples must fall within the range or be reported as "above calibration range" or "below detection" as appropriate. The reported concentration is acceptable if all QA/QC criteria are met as specified (e.g., CCVs before and after the sample are within 30% RSD).

METHOD A PARAMETERS

"LCE VX_ULL"
ECBC Decontamination Sciences Branch
LCE Method Summary

INSTRUMENT CONTROL PARAMETERS: LCE (1200 Series LC / API5000 MS)

Acquisition Method Properties

Comment: LC/MS/MS Method (MRM) for ultra low-level VX in liquid extraction samples. This method can also screen for the VX byproduct EA2192.

Synchronization Mode:	LC Sync
Auto-Equilibration:	Off
Acquisition Duration:	7min0s
Number of Scans:	667
Periods in File:	1
Acquisition Module:	Acquisition Method
Software version:	Analyst 1.4.2

API5000 Mass Spec

MS Method Properties:
Period 1:

Scans in Period:	667
Relative Start Time:	0.00 ms
Experiments in Period:	1

Period 1 Experiment 1:

Scan Type:	MRM (MRM)
Polarity:	Positive
Scan Mode:	N/A
Ion Source:	Turbo Spray
Resolution Q1:	Unit
Resolution Q3:	High
Intensity Thres.:	0.00 cps
Settling Time:	0.0000 ms
MR Pause:	5.0070 ms
MCA:	No
Step Size:	0.00 amu

```
Q1 Mass (amu)     Q3 Mass (amu)     Dwell(ms)
268.15            128.00            100.00

Q1 Mass (amu)     Q3 Mass (amu)     Dwell(ms)
268.15            167.00            100.00

Q1 Mass (amu)     Q3 Mass (amu)     Dwell(ms)
268.15            139.00            100.00

Q1 Mass (amu)     Q3 Mass (amu)     Dwell(ms)
240.10            128.00            100.00

Q1 Mass (amu)     Q3 Mass (amu)     Dwell(ms)
240.10            162.00            100.00

Q1 Mass (amu)     Q3 Mass (amu)     Dwell(ms)
240.10            139.00            100.00

Parameter Table(Period 1  Experiment   1):
CUR:        15.00
GS1:        50.00
GS2:        55.00
IS:         5500.00
TEM:        550.00
ihe:        ON
CAD:        3.00
DP          60.00
EP          10.00
CE          25.00
CXP         12.00


END OF INSTRUMENT CONTROL PARAMETERS
-------------------------------------
```

```
================================================================================
                    AGILENT 1200 SERIES LC PARAMETERS
================================================================================

Agilent LC Pump Method Properties

Pump Model:                          Agilent 1200 Binary Pump
Minimum Pressure (psi):              0.0
Maximum Pressure (psi):              5801.0
Dead Volume (µL):                    40.0
Maximum Flow Ramp (mL/min²):         100.0
Maximum Pressure Ramp (psi/s):       290.0

Step Table:
@Step      Total Time(min)   Flow Rate(µL/min)      A (%)       B (%)
0                0.05              200               50.0        50.0
1                7.00              200               50.0        50.0

Left Compressibility:                50.0
Right Compressibility:               100.0
Left Dead Volume (µL):               40.0
Right Dead Volume (µL):              40.0
Left Stroke Volume (µL):             -1.0
Right Stroke Volume (µL):            -1.0
Left Solvent:                        A1
Right Solvent:                       B1


Agilent Autosampler Properties

Autosampler Model:                   Agilent 1200 High Performance
                                     Autosampler
Syringe Size (µL):                   100
Injection Volume (µL):               4.00
Draw Speed (µL/min):                 200.0
Eject Speed (µL/min):                200.0
Needle Level (mm):                   0.00
Temperature Control                  Not Used
Wash Location:                       Wash Vial
Wash Cycles (1 - 5):                 2
Wash Vial Number:                    1
Wash Rack Number:                    1

Automatic Delay Volume Reduction     Enabled
Sample Flush-out Factor:             10.0
Equilibration Time (s):              2
Enable Vial/Well Bottom Sensing      No
Use Custom Injector Program          Yes

Contents of Custom Injector Program
1: DRAW def. amount from sample    def. speed def. offset
2: WASH NEEDLE with default wash parameters
3: INJECT
```

4: WAIT 0.50 min
5: VALVE bypass
6: WAIT 0.20 min
7: VALVE mainpass
8: WAIT 0.20 min
9: VALVE bypass
10: WAIT 0.20 min
11: VALVE mainpass

Agilent Column Oven Properties

Left Temperature (°C):	25.00
Right Temperature (°C):	25.00
Temperature Tolerance +/- (°C):	10.00
Start Acquisition Tolerance +/- (°C):	5.00
Time Table	(Not Used)
Column Switching Valve	Installed
Position for first sample in the batch:	Left

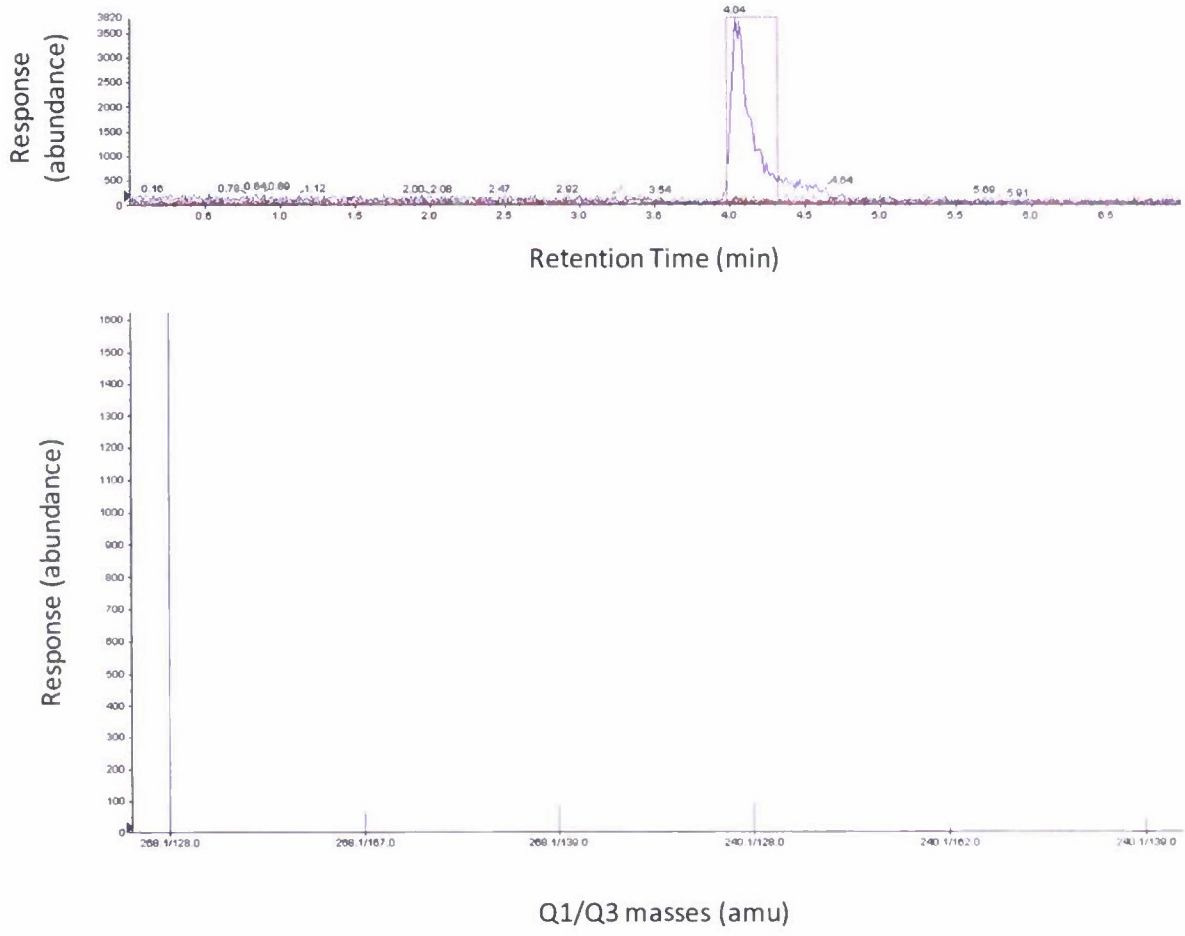
Use same position for all samples in the batch

Column Type:
Agilent ZORBAX SB-C18
PN: 866953-902
SN: USDZ010845
4.6 mm x 75 mm 3.5 µm

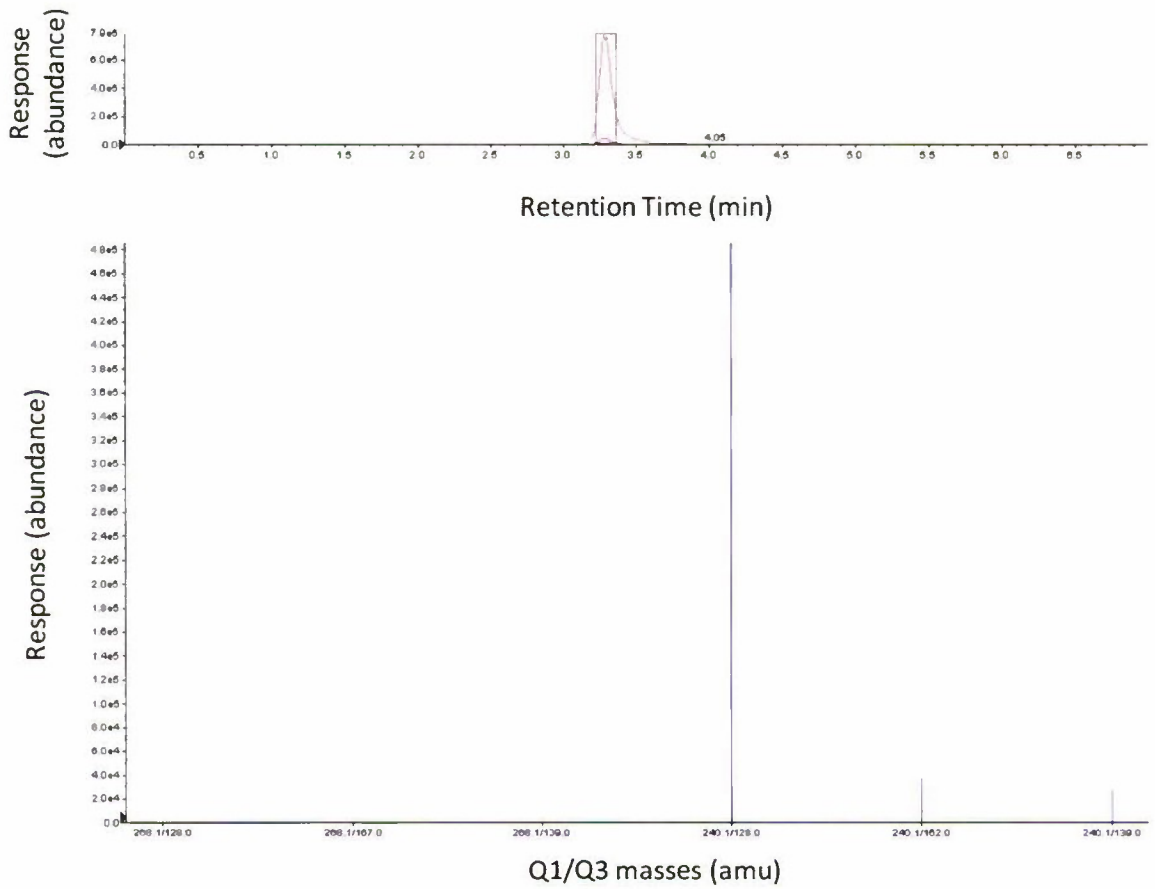
Mobile Phase
Aqueous:
95% dH20
5% IPA
0.1% Formic Acid
5mM Ammonium Acetate

Organic:
95% IPA
5% dH20
0.1% Formic Acid
5 mM Ammonium Acetate

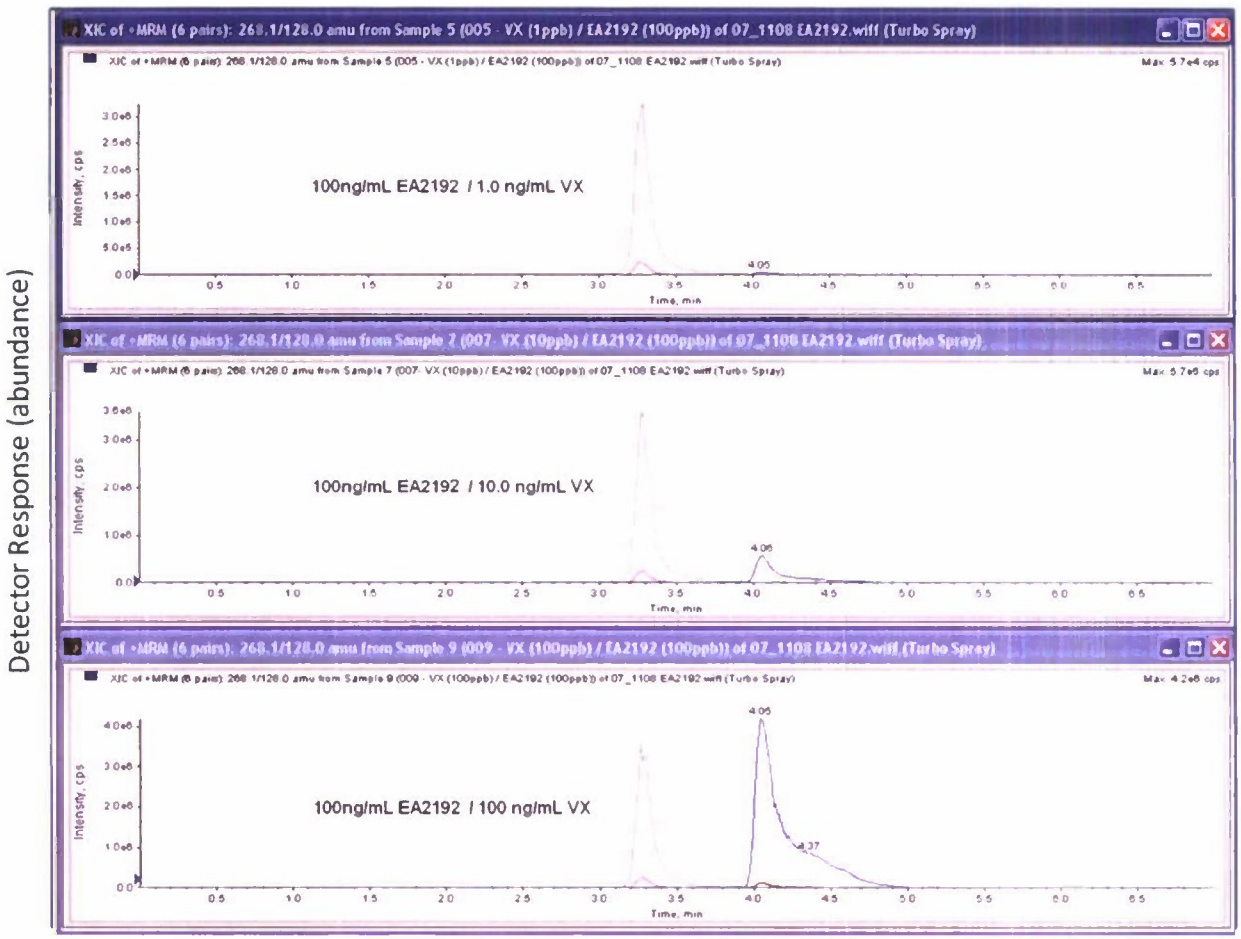
VX (RT 4.0 min) XIC and Mass Spectrum - LCE DATA ANALYSIS



EA2192 (RT 3.3 min) XIC and Mass Spectrum - LCE DATA ANALYSIS



VX and EA2192 XIC - LCE DATA ANALYSIS



METHOD B: LC/MS/MS METHOD FOR LOW-LEVEL VX AND EA-2192 IDENTIFICATION (LCE VX_LL.DAM)

ANALYTICAL METHOD

TITLE
Low-level detection of VX and EA2192 in liquid extraction samples for chemical agent decontamination testing using an LC/MS/MS system.

AUTHORS
Matthew Shue (ECBC Decontamination Sciences Branch)
Phillip Smith, Ph.D. (SAIC)
Teri Lalain, Ph.D. (Branch Chief, ECBC Decontamination Sciences Branch)

KEYWORDS
VX, O-Ethyl S-2-diisopropylaminoethyl methyl phosphonothiolate, CAS 50782-69-9
EA2192, S-(2-Diisopropylaminoethyl) methylphosphonothioic acid, CAS 73207-98-4

REVISION HISTORY
This document is version 1, released December 2007.

PURPOSE AND APPLICATIONS
The primary purpose of this method is to detect and quantify low-level concentrations of the chemical agent VX in liquid extraction samples. The secondary purpose of this method is to screen liquid extraction samples for EA2192, a hydrolysis byproduct of VX. This method is optimized to support the analysis of liquid extracts following the chemical agent decontamination performance evaluation testing. Contact sampler and coupon extracts for a 2 in. diameter test surface area are extracted in no greater than 20 mL of extraction solvent. Sample throughput is approximately nine samples per hour.

ANALYTE CONCENTRATION RANGE
This method was developed as a complementary method to the ultra low-level method (LCE VX_ULL.dam) to allow for VX decontamination testing at levels at or slightly higher concentrations than the ultra low-level method. As a complementary method, this method can detect VX solution concentrations from 1.0 to 750 ng/mL.

This method utilizes ten calibration standards at concentrations of ~1, 2.5, 5, 10, 25, 50, 100, 250, 500, and 750 ng/mL VX. The method use, detection limit, and quantitation limit are based on using the full standard set. The calibration standards are prepared in IPA. The mobile phase is a 50:50 mixture of the organic and aqueous phases. The organic phase consists of 95% IPA, 5% de-ionized water, 0.1% formic acid, and 5 mM ammonium acetate. The aqueous phase consists of 95% de-ionized water, 5% IPA, 0.1% formic acid, and 5 mM ammonium acetate. The method is conducted in an isocratic configuration.

The method performance for VX is as follows:

- Quantitative Method
- Calibration Range: 1 to 750 ng/mL VX
- CCV Levels: 0, 5, 50, 250 ng/mL
- Calibration Model: Weighted quadratic calibration model
- Calibration Weighting: $1/x^2$
- Limit of Detection (LOD): 0.17 ng/mL
- Limit of Quantitation (LOQ): 0.34 ng/mL
- Sample Solvent: IPA
- Quantitative Ion Pair: VX 268.1 / 128

The method performance for EA2192 is as follows:

- Qualitative Method
- Sample Solvent: IPA
- Quantitative Ion Pair: EA-2192 240.1 / 128

SAMPLE MATRICES AND INTERFERENCES

This method is appropriate for liquid samples extracted with the solvent isopropyl alcohol (C_3H_8O; CAS #67-63-0) and containing the chemical agent VX and/or the VX byproduct EA2192. No significant method interferents have been identified. Users of this method should confirm that the test material and solvent are compatible prior to testing.

RISK AND SAFETY ASSESSMENT

This document does not claim to address all of the safety concerns associated with chemical decontaminant testing as the requirements may vary based on facility, state and other regulatory requirements. It is the responsibility of the user of this method, prior to use, to establish appropriate environment, health, and safety practices for the use of this method and handling of generated wastes in compliance with applicable regulations. Users of this method should conduct testing in appropriate facilities and follow proper laboratory practices including the use of appropriate personal protective equipment and material safety data sheets.

SCIENTIFIC BASIS

This method is based on liquid chromatography and mass spectrometry techniques.

TRAINING

Users of this method must have the basic skill level for analytical chromatography instrument operation, interpretation of mass spectrometry results and associated wet lab techniques (e.g. sample dilution and standard preparation). Training covering instrument operation and maintenance of equipment is suggested.

APPARATUS

The liquid extraction samples are analyzed on an Applied Biosystems API5000 Triple-quadrupole Mass Spectrometer equipped with the TurboV Ion Source. Sample introduction and chromatography are performed with an Agilent 1200 series LC. Sample effluent is directed from the LC directly to the TurboV ion source of the API5000 MS. The system is fitted with an Agilent Technologies Zorbax SB-C18 analytical column (4.6 mm inside diameter [ID] x 75 mm length [L], 3.5 µm particle size [part number 866953-902]). Instrumentation operation, maintenance manuals, and miscellaneous and consumable equipment lists are available from the instrument manufacturers: Applied Biosystems and Agilent Technologies. All equipment should be used in accordance with the manufacturer's instructions.

Chemicals required include dilute VX standards prepared in high purity isopropyl alcohol solvent.

Volumetric glassware used for standard preparation should be Class A and meet the specifications in the most current version of ASTM standards E288 and E69. Pipettes used for standard preparation should be compliant with the required performance specifications listed in the most current versions of ISO 8655 Parts 1 and 2 and/or ASTM E 1154 for the volume measured if standards are made volumetrically. Balances used for gravimetric standard preparation should be sensitive enough to measure the appropriate mass delivered. All equipment should be used in accordance with the manufacturer's requirements, and should be properly maintained and calibrated.

Amber glass analytical sample vials are preferred. The septum cap should be lined with inert material, preferably PTFE/Teflon, to prevent extraction of plasticizers or other impurities into the sample.

PROCEDURE

The specific equipment and column are identified in the "Apparatus" section. Procedures for equipment operation, instrumentation problems, and corrective actions are found in the manufacturer's operating manuals. The specific instrumentation settings for this method are provided in the "Method Parameters" section. The equipment should be confirmed as operational prior to use for test support.

Standards are prepared for calibration either volumetrically or gravimetrically, as required by the method. The LC mobile phase solutions (aqueous and organic) are prepared in accordance with the method specifications.

Liquid extract samples above the method calibration range are diluted to be within the method calibration range. Extrapolation of results outside the calibrated range is not recommended.

Calibration curves are generated from the standard sample results. The curves are fit and weighted as specified in this method. For quality control, the analysis of test samples must include the use of ICV, CCV, and solvent blanks for quality control. Specific guidance for method use for decontaminant performance evaluations is provided in the "2007 Chemical Decontaminant Performance Evaluation Testing Source Document" by T. Lalain, et al. Standard preparation, sample queues, interferences and other general analytical guidance are provided in the corresponding technical report to this document titled, "Low-Level Analytical Methodology Updates to Support Decontaminant Performance Evaluations" by M. Shue, et al.

DATA ACCEPTANCE & REPORTING

Analytical results for samples are reported as solution concentrations (ng/mL). Solution concentration can be calculated using the instrument control software (e.g., Applied Biosystems Analyst, Chemstation, etc.) or using external statistical software (e.g. Excel, Sigma plot, etc.) with the specified calibration model. The reported solution concentration should account for sample dilutions made after the original sample generation (e.g., contact, residual, and remaining agent testing) such that the analyzed sample is within the calibration range. Calibration curves are not extrapolated; samples must fall within the range or be reported as "above calibration range" or "below detection" as appropriate. The reported concentration is acceptable if all QA/QC criteria are met as specified (e.g., CCVs before and after the sample are within 30% RSD).

METHOD B PARAMETERS

"LCE VX_LL"
ECBC Decontamination Sciences Branch
LCE Method Summary

INSTRUMENT CONTROL PARAMETERS: LCE (1200 Series LC / API5000 MS)

Acquisition Method Properties

Comment: LC/MS/MS Method (MRM) for low-level VX in liquid extraction samples. This method can also screen for the VX byproduct EA2192.

Synchronization Mode:	LC Sync
Auto-Equilibration:	Off
Acquisition Duration:	6min0s
Number of Scans:	490
Periods in File:	1
Acquisition Module:	Acquisition Method
Software version:	Analyst 1.4.2

API5000 Mass Spec

MS Method Properties:
Period 1:

Scans in Period:	490
Relative Start Time:	0.00 ms
Experiments in Period:	1

Period 1 Experiment 1:

Scan Type:	MRM (MRM)
Polarity:	Positive
Scan Mode:	N/A
Ion Source:	Turbo Spray
Resolution Q1:	Unit
Resolution Q3:	High
Intensity Thres.:	0.00 cps
Settling Time:	0.0000 ms
MR Pause:	5.0070 ms
MCA:	No
Step Size:	0.00 amu

@Q1 Mass (amu)	Q3 Mass (amu)	Dwell(ms)
268.15	128.00	100.00

```
@Q1 Mass (amu)    Q3 Mass (amu)     Dwell(ms)
268.15            167.00            100.00

@Q1 Mass (amu)    Q3 Mass (amu)     Dwell(ms)
268.15            139.00            100.00

@Q1 Mass (amu)    Q3 Mass (amu)     Dwell(ms)
240.10            128.00            100.00

@Q1 Mass (amu)    Q3 Mass (amu)     Dwell(ms)
240.10            162.00            100.00

@Q1 Mass (amu)    Q3 Mass (amu)     Dwell(ms)
240.10            139.00            100.00

Parameter Table(Period 1  Experiment   1):
CUR:        15.00
GS1:        40.00
GS2:        50.00
IS:         5500.00
TEM:        100.00
ihe:        ON
CAD:        6.00
DP          60.00
EP          10.00
CE          25.00
CXP         12.00


END OF INSTRUMENT CONTROL PARAMETERS
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================================================================================
                    AGILENT 1200 SERIES LC PARAMETERS
================================================================================

Agilent LC Pump Method Properties

Pump Model:                          Agilent 1200 Binary Pump
Minimum Pressure (psi):              0.0
Maximum Pressure (psi):              5801.0
Dead Volume (µL):                    40.0
Maximum Flow Ramp (mL/min²):         100.0
Maximum Pressure Ramp (psi/s):       290.0

Step Table:
@Step       Total Time(min)   Flow Rate(µL/min)      A (%)       B (%)
0                 0.00              200               50.0        50.0
1                 6.00              200               50.0        50.0

Left Compressibility:                50.0
Right Compressibility:               100.0
Left Dead Volume (µL):               40.0
Right Dead Volume (µL):              40.0
Left Stroke Volume (µL):             -1.0
Right Stroke Volume (µL):            -1.0
Left Solvent:                        A1
Right Solvent:                       B1


Agilent Autosampler Properties

Autosampler Model:                   Agilent 1200 High Performance
                                     Autosampler
Syringe Size (µL):                   100
Injection Volume (µL):               2.00
Draw Speed (µL/min):                 200.0
Eject Speed (µL/min):                200.0
Needle Level (mm):                   0.00
Temperature Control                  Not Used
Wash Location:                       Flush Port
Wash Time (1 - 999 s):               5

Automatic Delay Volume Reduction     Enabled
Sample Flush-out Factor:             10.0
Equilibration Time (s):              2
Enable Vial/Well Bottom Sensing      No
Use Custom Injector Program          Yes

Contents of Custom Injector Program
1: DRAW def. amount from sample    def. speed def. offset
2: WASH NEEDLE with default wash parameters
3: INJECT
4: WAIT 0.50 min
5: VALVE bypass
```

6: WAIT 0.20 min
7: VALVE mainpass
8: WAIT 0.20 min
9: VALVE bypass
10: WAIT 0.20 min
11: VALVE mainpass

Agilent Column Oven Properties

Left Temperature (°C):	25.00
Right Temperature (°C):	25.00
Temperature Tolerance +/- (°C):	10.00
Start Acquisition Tolerance +/- (°C):	5.00
Time Table	(Not Used)
Column Switching Valve	Installed
Position for first sample in the batch:	Left

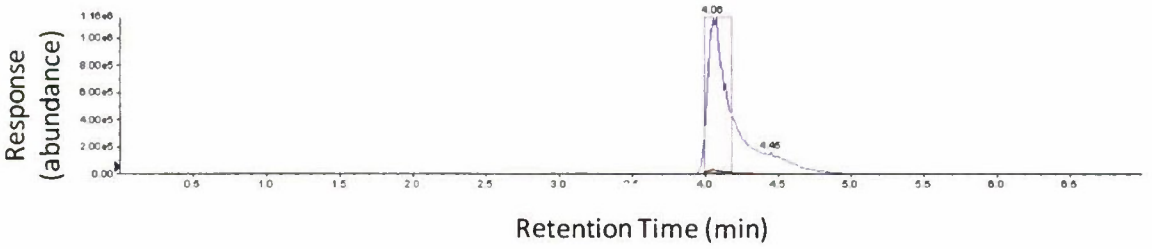
Use same position for all samples in the batch

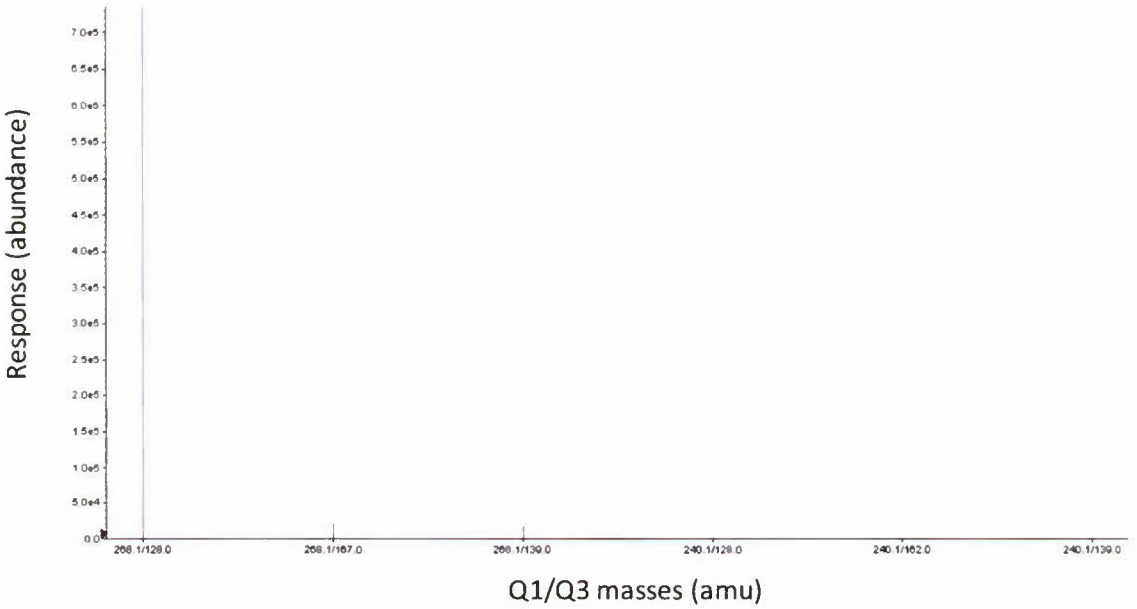
Column Type:
Agilent ZORBAX SB-C18
PN: 866953-902
SN: USDZ010845
4.6 mm x 75 mm 3.5 µm

Mobile Phase
Aqueous:
95% dH20
5% IPA
0.1% Formic Acid
5mM Ammonium Acetate

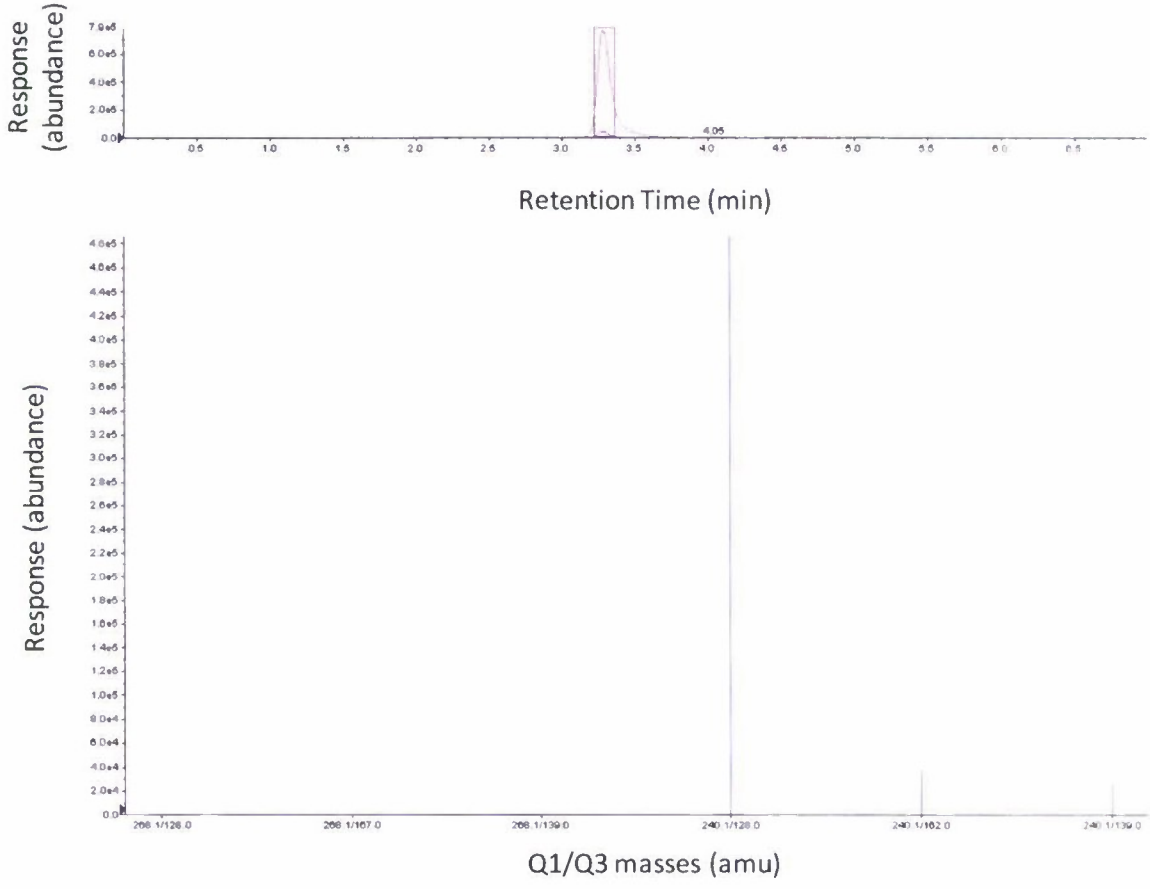
Organic:
95% IPA
5% dH20
0.1% Formic Acid
5 mM Ammonium Acetate

VX (RT 4.0 min) XIC and Mass Spectrum - LCE DATA ANALYSIS

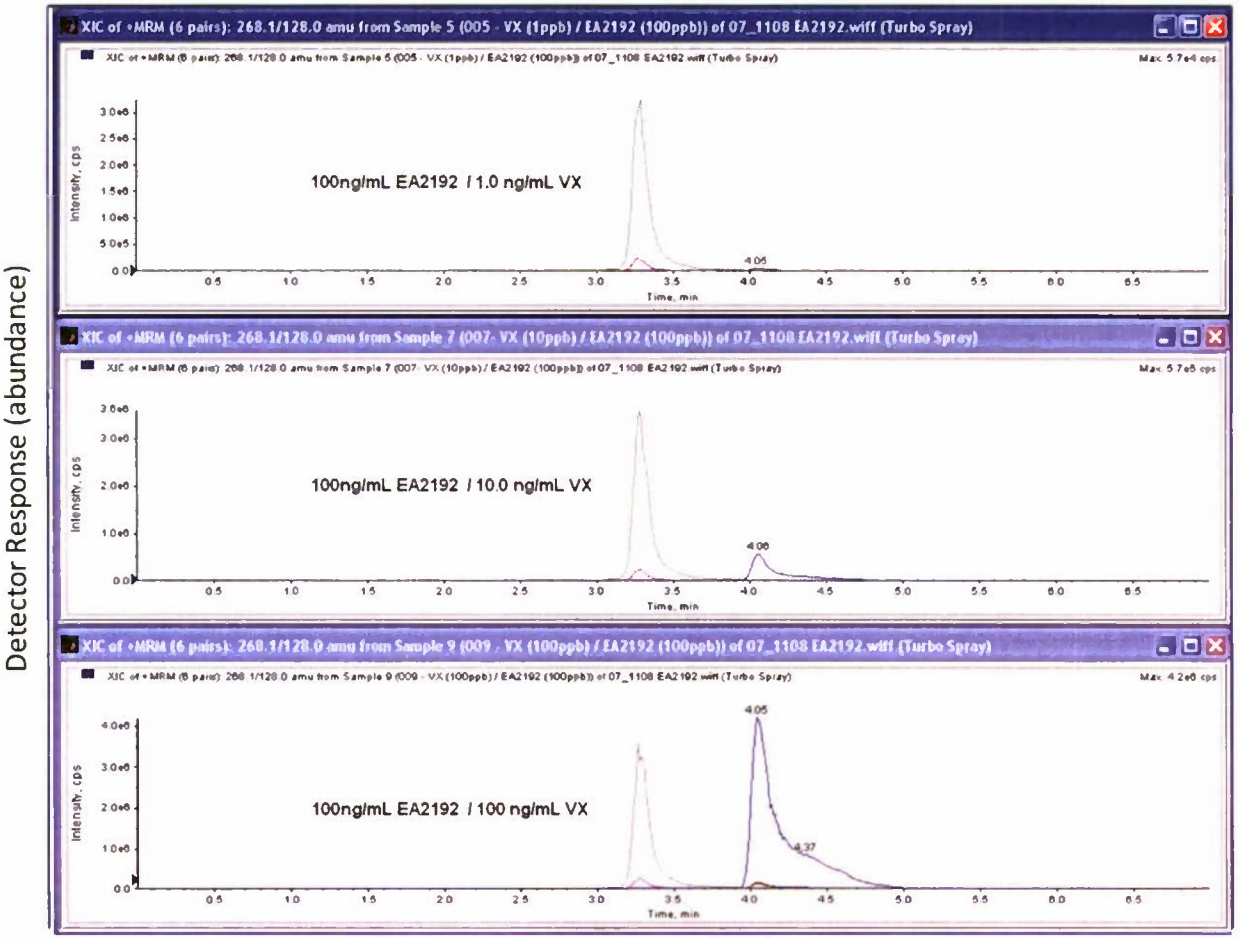




EA2192 (RT 3.3 min) XIC and Mass Spectrum - LCE DATA ANALYSIS



VX and EA2192 - LCE DATA ANALYSIS



METHOD C: LC/MS/MS METHOD FOR EMPA IDENTIFICATION (LCE EMPA.DAM)

ANALYTICAL METHOD

TITLE
Detection of EMPA, a hydrolysis product of VX, in liquid extraction samples from chemical agent decontamination testing using an LC/MS/MS system.

AUTHORS
Matthew Shue (ECBC Decontamination Sciences Branch)
Phillip Smith, Ph.D. (SAIC)
Teri Lalain, Ph.D. (Branch Chief, ECBC Decontamination Sciences Branch)

KEYWORDS
EMPA, Ethyl methylphosphonate, CAS 1832-53-7

REVISION HISTORY
This document is version 1, released December 2007.

PURPOSE AND APPLICATIONS
The purpose of this method is to detect ethyl methyphosphonate (EMPA). EMPA is a hydrolysis byproduct of the chemical agent VX. This method is optimized to support the analysis of liquid extracts following the chemical agent decontamination performance evaluation testing. Contact sampler and coupon extracts for a 2 in. diameter test surface area are extracted in no greater than 20 mL of extraction solvent. Sample throughput is approximately seven samples per hour.

ANALYTE CONCENTRATION RANGE
This method was developed to screen for a VX byproduct, EMPA. The approximate concentration range for this method is from 5.0 to 500 ng/mL.

The method performance for EMPA is as follows:

- Qualitative Method
- Sample Solvent: IPA
- Quant Ion: 123 / 95

SAMPLE MATRICES AND INTERFERENCES
This method is appropriate for liquid samples extracted with the solvent isopropyl alcohol (C_3H_8O; CAS #67-63-0) and suspected to contain the VX byproduct EMPA. No significant method interferents have been identified. Users of this method should confirm that the test material and solvent are compatible prior to testing.

RISK AND SAFETY ASSESSMENT

This document does not claim to address all of the safety concerns associated with chemical decontaminant testing as the requirements may vary based on facility, state and other regulatory requirements. It is the responsibility of the user of this method, prior to use, to establish appropriate environment, health, and safety practices for the use of this method and handling of generated wastes in compliance with applicable regulations. Users of this method should conduct testing in appropriate facilities and follow proper laboratory practices, including the use of appropriate personal protective equipment and material safety data sheets.

SCIENTIFIC BASIS

This method is based on liquid chromatography and mass spectrometry techniques.

TRAINING

Users of this method must have the basic skill level for analytical chromatography instrument operation, interpretation of mass spectrometry results, and associated wet lab techniques (e.g., sample dilution and standard preparation). Training covering instrument covering operation and maintenance of equipment is suggested.

APPARATUS

The liquid extraction samples are analyzed on an Applied Biosystems API5000 Triple-quadrupole Mass Spectrometer equipped with the TurboV Ion Source. Sample introduction and chromatography are performed with an Agilent 1200 series LC. Sample effluent is directed from the LC directly to the TurboV ion source of the API5000 MS. The system is fitted with an Agilent ZORBAX SB-C18 4.6 mm x 75 mm 3.5 µm column. Instrumentation operation, maintenance manuals, and miscellaneous and consumable equipment lists are available from the instrument manufacturers: Applied Biosystems and Agilent Technologies. All equipment should be used in accordance with the manufacturer's instructions.

Chemicals required include a dilute standard of EMPA prepared in high purity isopropyl alcohol solvent between the working concentration ranges of this method.

Volumetric glassware used for standard preparation should be Class A and meet the specifications in the most current version of ASTM standards E288 and E69. Pipettes used for standard preparation should be compliant with the required performance specifications listed in the most current versions of ISO 8655 Parts 1 and 2 and/or ASTM E 1154 for the volume measured, if standards are made volumetrically. Balances used for gravimetric standard preparation should be sensitive enough to measure the appropriate mass delivered. All equipment should be used in accordance with the manufacturer's requirements, and should be properly maintained and calibrated.

Amber glass analytical sample vials are preferred. The septum cap should be lined with inert material, preferably PTFE/Teflon, to prevent extraction of plasticizers or other impurities into the sample.

PROCEDURE

The specific equipment and column are identified in the "Apparatus" section. Procedures for equipment operation, instrumentation problems, and corrective actions are found in the manufacturer's operating manuals. The specific instrumentation settings for this method are provided in the "Method Parameters" section. The equipment should be confirmed as operational prior to use for test support.

Standards are prepared for calibration either volumetrically or gravimetrically, as required by the method. The LC mobile phase solutions (aqueous and organic) are prepared in accordance with the method specifications.

Liquid extract samples above the method calibration range are diluted to be within the method calibration range. Extrapolation of results outside the calibrated range is not recommended.

Calibration curves are generated from the standard sample results. The curves are fit and weighted as specified in this method. For quality control, the analysis of test samples must include the use of ICV, CCV, and solvent blanks for quality control. Specific guidance for method use for decontaminant performance evaluations is provided in the "2007 Chemical Decontaminant Performance Evaluation Testing Source Document" by T. Lalain, et al. Standard preparation, sample queues, interferences, and other general analytical guidance are provided in the corresponding technical report to this document titled, "Low-Level Analytical Methodology Updates to Support Decontaminant Performance Evaluations" by M. Shue, et al.

DATA ACCEPTANCE & REPORTING

Analytical results for samples are reported as solution concentrations (ng/mL). Solution concentration can be calculated using the instrument control software (e.g., Applied Biosystems Analyst, Chemstation, etc.) or using external statistical software (e.g., Excel, Sigma plot, etc.) with the specified calibration model. The reported solution concentration should account for sample dilutions made after the original sample generation (e.g., contact, residual, and remaining agent testing) such that the analyzed sample is within the calibration range. Calibration curves are not extrapolated; samples must fall within the range or be reported as "above calibration range" or "below detection" as appropriate. The reported concentration is acceptable if all QA/QC criteria are met as specified (e.g., CCVs before and after the sample are within 30% RSD).

METHOD C PARAMETERS

"LCE EMPA"
ECBC Decontamination Sciences Branch
LCE Method Summary

INSTRUMENT CONTROL PARAMETERS: LCE (1200 Series LC / API5000 MS)

Acquisition Method Properties

Comment: LC/MS/MS Method (MRM)for EMPA.

Synchronization Mode:	LC Sync
Auto-Equilibration:	Off
Acquisition Duration:	7min0s
Number of Scans:	683
Periods in File:	1
Acquisition Module:	Acquisition Method
Software version:	Analyst 1.4.2

API5000 Mass Spec

MS Method Properties:
Period 1:

Scans in Period:	683
Relative Start Time:	0.00 ms
Experiments in Period:	1

Period 1 Experiment 1:

Scan Type:	MRM (MRM)
Polarity:	Negative
Scan Mode:	N/A
Ion Source:	Turbo Spray
Resolution Q1:	Unit
Resolution Q3:	High
Intensity Thres.:	0.00 cps
Settling Time:	0.0000 ms
MR Pause:	5.0070 ms
MCA:	No
Step Size:	0.00 amu

@Q1 Mass (amu)	Q3 Mass (amu)	Dwell(ms)

```
123.00              95.00               200.00

@Q1 Mass (amu)     Q3 Mass (amu)      Dwell(ms)
123.00              79.00               200.00

@Q1 Mass (amu)     Q3 Mass (amu)      Dwell(ms)
123.00              77.00               200.00

Parameter Table(Period 1  Experiment   1):
CUR:         10.00
GS1:         50.00
GS2:         55.00
TEM:         500.00
ihe:         ON
CAD:         6.00
IS:          -4200.00
DP           -100.00
EP           -10.00
CE           -25.00
CXP          -15.00


END OF INSTRUMENT CONTROL PARAMETERS
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================================================================================
                    AGILENT 1200 SERIES LC PARAMETERS
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Agilent LC Pump Method Properties

Pump Model:                          Agilent 1200 Binary Pump
Minimum Pressure (psi):              0.0
Maximum Pressure (psi):              5801.0
Dead Volume (µL):                    40.0
Maximum Flow Ramp (mL/min²):         100.0
Maximum Pressure Ramp (psi/s):       290.0

Step Table:
@Step      Total Time(min)   Flow Rate(µL/min)      A (%)       B (%)
0                0.50              200               50.0        50.0
1                7.00              200               50.0        50.0

Left Compressibility:                50.0
Right Compressibility:               100.0
Left Dead Volume (µL):               40.0
Right Dead Volume (µL):              40.0
Left Stroke Volume (µL):             -1.0
Right Stroke Volume (µL):            -1.0
Left Solvent:                        A1
Right Solvent:                       B1


Agilent Autosampler Properties

Autosampler Model:                   Agilent 1200 High Performance
                                     Autosampler
Syringe Size (µL):                   100
Injection Volume (µL):               4.00
Draw Speed (µL/min):                 200.0
Eject Speed (µL/min):                200.0
Needle Level (mm):                   0.00
Temperature Control                  Not Used
Wash Location:                       Wash Vial
Wash Cycles (1 - 5):                 2
Wash Vial Number:                    1
Wash Rack Number:                    1

Automatic Delay Volume Reduction     Not Used
Equilibration Time (s):              2
Enable Vial/Well Bottom Sensing      No
Use Custom Injector Program          No

Agilent Column Oven Properties

Left Temperature (°C):               25.00
Right Temperature (°C):              25.00
Temperature Tolerance +/- (°C):      10.00
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Start Acquisition
Tolerance +/- (°C): 5.00
Time Table (Not Used)
Column Switching Valve Installed
Position for first
sample in the batch: Left

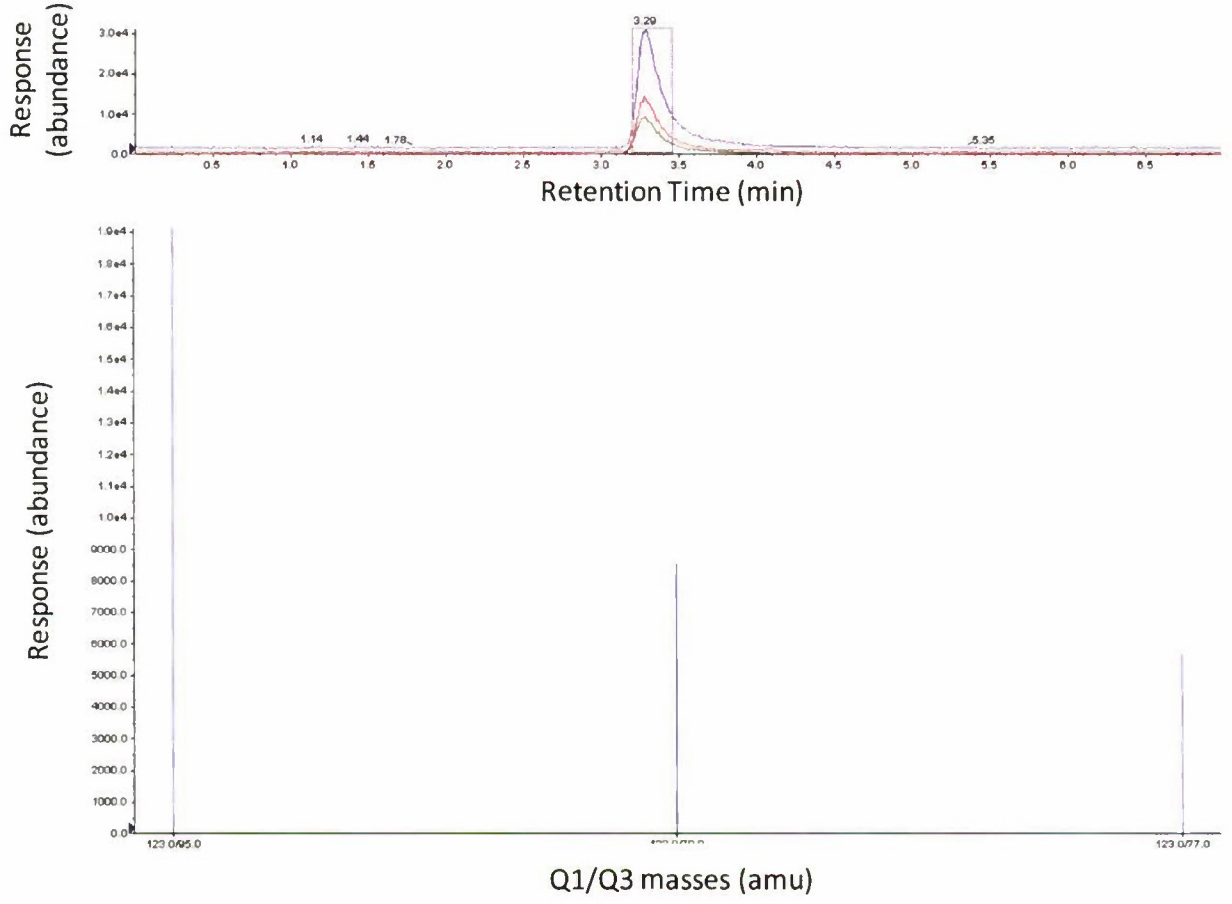
Use same position for all samples in the batch

Column Type:
Agilent ZORBAX
SB-C18
PN: 866953-902
SN: USDZ010845
4.6mm x 75mm 3.5µm

Mobile Phase
Aqueous:
95% dH20
5% IPA
0.1% Formic Acid
5mM Ammonium Acetate

Organic:
95% IPA
5% dH20
0.1% Formic Acid
5mM Ammonium Acetate

EMPA (RT 3.3 min) XIC and Mass Spectrum - LCE DATA ANALYSIS



METHOD D: GC/MSD METHOD FOR VX (GCE VX_DEANS.M)

ANALYTICAL METHOD

TITLE
Detection of VX in liquid extraction samples for chemical agent decontamination testing using a GC/MSD with an autosampler.

AUTHORS
Matthew Shue (ECBC Decontamination Sciences Branch)
Michelle Sheahy (SAIC)
Teri Lalain, Ph.D. (Branch Chief, ECBC Decontamination Sciences Branch)

KEYWORDS
VX, O-Ethyl S-2-diisopropylaminoethyl methyl phosphonothiolate, CAS 50782-69-9

REVISION HISTORY
This document is version 1, released December 2007.

PURPOSE AND APPLICATIONS
The purpose of this method is to detect and quantify the chemical agent VX in liquid extraction samples. This method is optimized to support the analysis of liquid extracts following the chemical agent decontamination performance evaluation testing. Contact sampler and coupon extracts for a 2 in. diameter test surface area are extracted in no greater than 20 mL of extraction solvent. This method was outside the low-level scope; however, some testing may need use of higher range. Sample throughput is approximately six samples per hour.

ANALYTE CONCENTRATION RANGE
This method is intended for samples containing higher quantities of VX. This method can detect VX solution concentrations from 250 to 2000 ng/mL..

This method utilizes five calibration standards at concentrations of ~250, 500, 750, 1000, and 2000 ng/mL VX. The method use, detection limit, and quantitation limit are based on using the full standard set. The standards are prepared in isopropyl alcohol.

The method performance for VX is as follows:

- Quantitative Method
- Calibration Range: 250 to 2000 ng/mL VX
- CCV Levels: 0, 500, 500 ng/mL
- Calibration Model: Weighted quadratic calibration model
- Calibration Weighting: $1/x^2$
- Limit of Detection (LOD): 38.8 ng/mL
- Limit of Quantitation (LOQ): 39.6 ng/mL

- Sample Solvent: IPA
- Quantitative Ion: 114

SAMPLE MATRICES AND INTERFERENCES

This method is appropriate for liquid samples extracted with the solvent Isopropyl alcohol (C_3H_8O; CAS #67-63-0) and containing the chemical agent VX. No significant method interferents have been identified. Users of this method should confirm that the test material and solvent are compatible prior to testing.

RISK AND SAFETY ASSESSMENT

This document does not claim to address all of the safety concerns associated with chemical decontaminant testing as the requirements may vary based on facility, state and other regulatory requirements. It is the responsibility of the user of this method, prior to use, to establish appropriate environment, health, and safety practices for the use of this method and handling of generated wastes in compliance with applicable regulations. Users of this method should conduct testing in appropriate facilities and follow proper laboratory practices, including the use of appropriate personal protective equipment and material safety data sheets.

SCIENTIFIC BASIS

This method is based on gas chromatography and mass spectrometry techniques.

TRAINING

Users of this method must have the basic skill level for analytical chromatography instrument operation, interpretation of mass spectrometry results, and associated wet lab techniques (e.g., sample dilution and standard preparation). Training covering instrument operation and maintenance of equipment is suggested.

APPARATUS

The liquid extraction samples are analyzed on an Agilent 6890 Gas Chromatograph (GC) equipped with a 5975 Mass Selective Detector (MSD). The GC is outfitted with an Agilent Deans Switch allowing for flow switching during analysis so that only a small portion of the sample effluent, including the analyte of interest, is directed to the MSD. All other flow is directed to a Flame Photometric Detector (FPD). Sample introduction is performed using a GERSTEL Multi Purpose Sampler (MPS2) and a GERSTEL Cooled Injection System inlet (CIS4). The system is fitted with a HP-5MS 5% Phenyl Methyl Siloxane column (30 m x 0.25 mm ID x 0.25 µm film thickness). Instrumentation operation, maintenance manuals, and miscellaneous and consumable equipment lists are available from the instrument manufacturers: Agilent and GERSTEL. All equipment should be used in accordance with the manufacturer's instructions.

Chemicals required include dilute VX standards prepared in high purity isopropyl alcohol solvent.

Volumetric glassware used for standard preparation should be Class A and meet the specifications in the most current version of ASTM standards E288 and E69. Pipettes used for

standard preparation should be compliant with the required performance specifications listed in the most current versions of ISO 8655 Parts 1 and 2 and/or ASTM E 1154 for the volume measured, if standards are made volumetrically. Balances used for gravimetric standard preparation should be sensitive enough to measure the appropriate mass delivered. All equipment should be used in accordance with the manufacturer's requirements, and should be properly maintained and calibrated.

Amber glass analytical sample vials are preferred. The septum cap should be lined with inert material, preferably PTFE/Teflon, to prevent extraction of plasticizers or other impurities into the sample.

PROCEDURE

The specific equipment and column are identified in the "Apparatus" section. Procedures for equipment operation, instrumentation problems, and corrective actions are found in the manufacturer's operating manuals. The specific instrumentation settings for this method are provided in the "Method Parameters" section. The equipment should be confirmed as operational prior to use for test support.

Standards are prepared for calibration either volumetrically or gravimetrically, as required by the method.

Liquid extract samples above the method calibration range are diluted to be within the method calibration range. Extrapolation of results outside the calibrated range is not recommended.

Calibration curves are generated from the standard sample results. The curves are fit and weighted as specified in this method. For quality control, the analysis of test samples must include the use of ICV, CCV, and solvent blanks. Specific guidance for method use for decontaminant performance evaluations is provided in the "2007 Chemical Decontaminant Performance Evaluation Testing Source Document" by T. Lalain, et al. Standard preparation, sample queues, interferences, and other general analytical guidance are provided in the corresponding technical report to this document titled, "Low-Level Analytical Methodology Updates to Support Decontaminant Performance Evaluations" by M. Shue, et al.

DATA ACCEPTANCE & REPORTING

Analytical results for samples are reported as solution concentrations (ng/mL). Solution concentration can be calculated using the instrument control software (e.g., Applied Biosystems Analyst, Chemstation, etc.) or using external statistical software (e.g. Excel, Sigma plot, etc.) with the specified calibration model. The reported solution concentration should account for sample dilutions made after the original sample generation (e.g., contact, residual, and remaining agent testing) such that the analyzed sample is within the calibration range. Calibration curves are not extrapolated; samples must fall within the range or be reported as "above calibration range" or "below detection" as appropriate. The reported concentration is acceptable if all QA/QC criteria are met as specified (e.g., CCVs before and after the sample are within 30% RSD).

METHOD D PARAMETERS

"VX_DEANS.M"
ECBC Decontamination Sciences Branch
GCE Method Summary

INSTRUMENT CONTROL PARAMETERS: GCE (6890GC - 5975MSD)

Control Information
Sample Inlet : GC
Injection Source : External Device
Injection Location : Rear
Mass Spectrometer : Enabled

AGILENT DEANS SWITCH PARAMETERS

METHOD: GCE_DEANS.M

Comment: Deans Switch Parameters for GC/MSD methods on GCE.

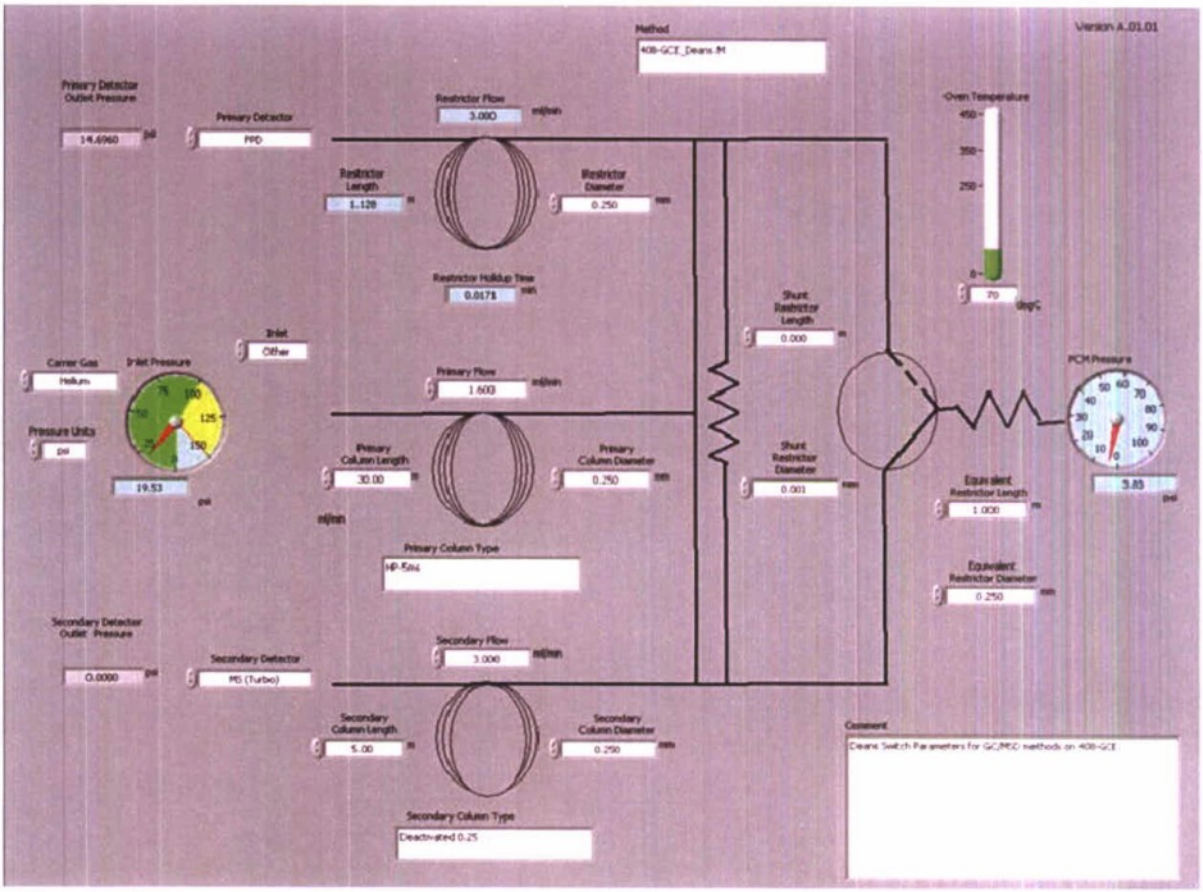
CALCULATION RESULTS:

	Primary Column	Secondary Column
Inlet Pressure:	19.53	3.83
Avg. Linear Velocity (cm/s):	34.72	154.76
Hold up Time (min):	1.44	0.054
Restrictor Length (m):		1.128
Restrictor Hold Up Time (min):		0.017

CALCULATION INPUTS

	Primary Column	Secondary Column
Length (m):	30.00	5.00
i.d. (mm):	0.25	0.25
Flow (mL/min):	1.60	3.00
Detector:	FPD	MS (Turbo)
Detector Pressure (abs):	14.70	0.00
Carrier Gas:		Helium
Pressure Units:		psi
Oven Temperature:		70.00
Restrictor Diameter:		0.25
Equivalent Restrictor Diameter (mm):		0.25
Equivalent Restrictor Length (m):		1.000

DEANS SWITCH CALCULATOR


Method
408-GCE_Deans.M
Version A.01.01
Primary Detector Outlet Pressure
14.6960
psi
Primary Detector
FPD
Restrictor Flow
3.080
ml/min
Restrictor Length
1.120
m
Restrictor Diameter
0.250
mm
Restrictor Holdup Time
0.0171
min
Oven Temperature
450
350
250
0
70
deg C
Inlet
Other
Carrier Gas
Helium
Inlet Pressure
Pressure Units
psi
19.53
psi
Primary Flow
1.600
ml/min
Primary Column Length
30.00
m
Primary Column Diameter
0.250
mm
Primary Column Type
HP-5ms
Shunt Restrictor Length
0.000
m
Shunt Restrictor Diameter
0.001
mm
PCM Pressure
5.85
psi
Equivalent Restrictor Length
1.000
m
Equivalent Restrictor Diameter
0.250
mm
Secondary Detector Outlet Pressure
0.0000
psi
Secondary Detector
MS (Turbo)
Secondary Flow
3.000
ml/min
Secondary Column Length
5.00
m
Secondary Column Diameter
0.250
mm
Secondary Column Type
Deactivated 0.25
Comment
Deans Switch Parameters for GC/MSD methods on 408-GCE

```
================================================================================
                                    GERSTEL CIS
================================================================================

TEMPERATURE PROGRAM
Initial Temperature      : 12 °C
Equilibration Time       : 0.05 min
Initial Time             : 0.20 min
Ramp 1
Rate 1                : 12.0 °C/s
End Temp 1            : 270 °C
Hold Time 1           : 4.00 min
Ramp 2
Rate 2                : 0.0 °C/s

CRYO COOLING
Cryo Cooling             : used

GERSTEL MPS Liquid Injection

Syringe                  : 10 µL

SAMPLE PARAMETERS
Inj. Volume              : 2.0 µL
Inj. Speed               : 25.00 µL/s
Fill Volume              : 10.0 µL
Fill Strokes             : 3

Fill Speed               : 5.00 µL/s
Eject Speed              : 100.00 µL/s
Viscosity Delay          : 1.0 s

Air Volume               : 0.0 µL
Pre Inj. Delay           : 0.00 s
Post Inj. Delay          : 0.00 s
Inj. Penetration         : 40.00 mm
Vial Penetration         : 31.00 mm

CLEANING PARAMETERS
Preclean Sample          : 0

Preclean Solv.1          : 1
Postclean Solv.1         : 3
Fill Speed Solv.1        : 5.00 µL/s
Viscosity Delay Solv.1   : 1.0 s
Eject Speed Solv.1       : 100.00 µL/s

Preclean Solv.2          : 1
Postclean Solv.2         : 3
Fill Speed Solv.2        : 5.00 µL/s
Viscosity Delay Solv.2   : 1.0 s
Eject Speed Solv.2       : 100.00 µL/s
```

```
================================================================================
                                6890 GC METHOD
================================================================================

OVEN
Initial temp:  70 °C (On)                    Maximum temp:  325 °C
Initial time:  1.00 min                      Equilibration time:  0.25 min
Ramps:
#  Rate  Final temp  Final time
1 30.00      280        3.00
2   0.0(Off)
Post temp:  50 °C
Post time:  0.00 min
Run time:  11.0 min

FRONT INLET (SPLIT/SPLITLESS)                BACK INLET (CIS3)
Mode:  Split                                 Mode:  Solvent Vent
Initial temp:  200 °C (Off)                  Initial temp:  250 °C (Off)
Pressure:  0.00 psi (Off)                    Pressure:  19.53 psi (On)
Total flow:  3.7 mL/min                      Vent time:  0.20 min
Gas saver:  Off                              Vent flow:  20.0 mL/min
Gas type:  Helium                            Vent Pressure:  19.5 psi
                                             Purge flow:  50.0 mL/min
                                             Purge time:  1.00 min
                                             Total flow:  54.4 mL/min
                                             Gas saver:  Off
                                             Gas type:  Helium

COLUMN 1                                     COLUMN 2
Capillary Column                             Capillary Column
Model Number:  Agilent 19091S-433            Model Number:  Agilent 19091S-433
HP-5MS  5% Phenyl Methyl Siloxane            HP-5MS  5% Phenyl Methyl Siloxane
Max temperature:  325 °C                     Max temperature:  325 °C
Nominal length:  30.0 m                      Nominal length:  30.0 m
Nominal diameter:  250.00 µm                 Nominal diameter:  250.00 µm
Nominal film thickness:  0.25 µm             Nominal film thickness:  0.25 µm
Mode:  constant pressure                     Inlet:  (unspecified)
Pressure:  19.53 psi                         Outlet:  Other
Nominal initial flow:  1.7 mL/min
Average velocity:  39 cm/s
Inlet:  Back Inlet
Outlet:  Other
Outlet pressure:  ambient

FRONT DETECTOR (NO DET)                      BACK DETECTOR (FPD)
                                               Temperature:  250 °C (On)
                                               Hydrogen flow:  75.0 mL/min (On)
                                               Oxidizer flow:  100.0 mL/min (On)
                                               Oxidizer Gas Type: Air
                                               Mode:  Constant makeup flow
                                               Makeup flow:  25.0 mL/min (On)
                                               Makeup Gas Type: Helium
                                               Flame:  On
                                               Lit offset:  2.00
                                               Photo multiplier:  On
```

```
SIGNAL 1
Data rate:  20 Hz
Type:  back detector
Save Data:  On
Zero:  0.0 (Off)
Range:  0
Fast Peaks:  Off
Attenuation:  0

SIGNAL 2
Data rate:  20 Hz
Type:  test plot
Save Data:  Off
Zero:  0.0 (Off)
Range:  0
Fast Peaks:  Off
Attenuation:  0

COLUMN COMP 1
(No Detectors Installed)

COLUMN COMP 2
(No Detectors Installed)

THERMAL AUX 1
Use:  MSD Transfer Line Heater
Description:  MSD Transferline
Initial temp:  280 °C (On)
Initial time:  0.00 min
#  Rate  Final temp  Final time
1   0.0(Off)

THERMAL AUX 2
Unknown Thermal Aux Type

AUX PRESSURE 3
Description:  Deans Switch
Gas Type:  Helium
Initial pressure:  3.83 psi (On)
      Initial time:  0.00 min
      #  Rate  Final pres  Final time
      1   0.0(Off)

AUX PRESSURE 4
Description:  No Vent
Gas Type:  Helium
Initial pressure:  0.00 psi (Off)

AUX PRESSURE 5
Description:
Gas Type:  Helium
Initial pressure:  0.00 psi (Off)

VALVES
Valve 1  Switching Off
Description:

POST RUN
Post Time: 0.00 min

TIME TABLE
Time       Specifier                Parameter & Setpoint
7.00       Valve 1:                 On
8.00       Valve 1:                 Off


GC Injector


Front Injector:
No parameters specified

Back Injector:
No parameters specified

Column 1 Inventory Number : AB001
Column 2 Inventory Number : AB002
```

```
================================================================================
                        5975 MS ACQUISTION PARAMETERS
================================================================================

General Information
------- -----------

Tune File                 : atune.u
Acquistion Mode           : SIM


MS Information
-- -----------

Solvent Delay             : 6.00 min

EMV Mode                  : Gain Factor
Gain Factor               : 1.0
Resulting EM Voltage      : 1165.0*

[Sim Parameters]

GROUP 1
Group ID                  : VX
Resolution                : High
Plot 1 Ion                : 114.00

Ions/Dwell In Group:
(Mass, Dwell)
(72.00, 50)
(114.00, 50)
(127.00, 50)
(167.00, 50)


[MSZones]

MS Quad                   : 150 C   maximum 200 C
MS Source                 : 230 C   maximum 250 C

END OF MS ACQUISITION PARAMETERS

                              TUNE PARAMETERS*
                              ---------------
*MSD Specific Values automatically determined and set when performing an
Autotune.
END OF TUNE PARAMETERS
------------------------------------

END OF INSTRUMENT CONTROL PARAMETERS
------------------------------------
```

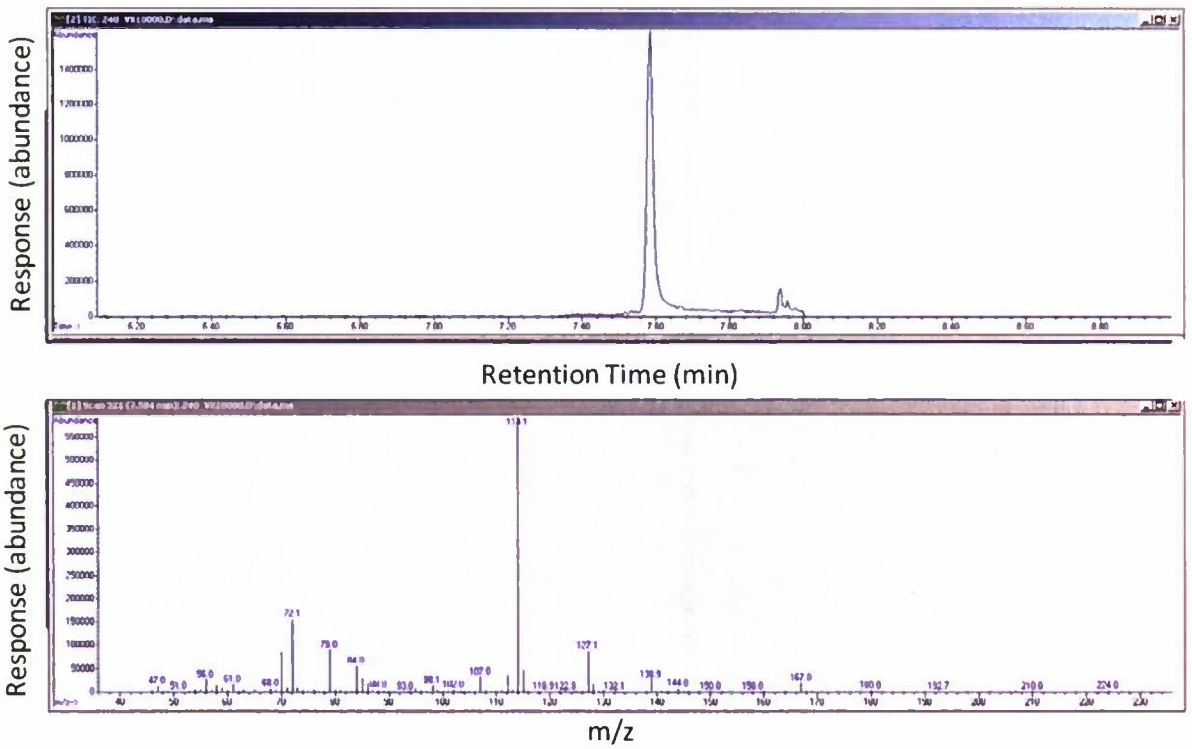
VX SPECTRUM (SCAN) - NIST LIBRARY SEARCH 2.0

The NIST library contains a sample spectrum, which can be found as identified below:

Name: VX Formula: $C_{11}H_{26}NO_2PS$ MW: 267
CAS#: 50782-69-9 NIST#: 226161 ID#: 68951 DB: mainlib

A sample spectrum from this program is provided.

VX (RT 7.6 min) TIC and SPECTRUM - GCE DATA ANALYSIS



METHOD E: GC/MS METHOD FOR LOW-LEVEL HD (GCE HD_DEANS.M)

ANALYTICAL METHOD

TITLE
Detection of HD in liquid extraction samples for chemical agent decontamination testing using a GC/MS with Autosampler.

AUTHORS
Matthew Shue (ECBC Decontamination Sciences Branch)
Zachary Zander (SAIC)
Teri Lalain, Ph.D. (Branch Chief, Decontamination Sciences Branch)

KEYWORDS
Mustard, HD, Bis(2-chloroethyl)sulfide CAS 505-60-2

REVISION HISTORY
This document is version 1, released December 2007.

PURPOSE AND APPLICATIONS
The purpose of this method is to detect and quantify the chemical agent HD in liquid extraction samples. This method is optimized to support the analysis of liquid extracts following the chemical agent decontamination performance evaluation testing. Contact sampler and coupon extracts for a 2 in. diameter test surface area are extracted in no greater than 20 mL of extraction solvent. Sample throughput is approximately four samples per hour.

ANALYTE CONCENTRATION RANGE
This method can detect HD solution concentrations from 2.0 to 2000 ng/mL. This method utilizes 13 calibration standards at concentrations of ~2, 5, 10, 25, 50, 100, 150, 200, 250, 500, 750, 1000, and 2000 ng/mL HD. The method use, detection limit, and quantitation limit are based on using the full standard set. The standards are prepared in chloroform.

The final method performance for GCE HD_DEANS.M is as follows:
- Quantitative Method
- Calibration Range: 2–2000 ng/mL HD
- CCV Levels: 0, 10, 150, 750 ng/mL
- Calibration Model: Weighted quadratic calibration model
- Calibration Weighting: 1/x2
- LOD: 0.37 ng/mL
- LOQ: 1.23 ng/mL
- Sample Solvent: Chloroform
- Quantitation Ion: 111

SAMPLE MATRICES AND INTERFERENCES

This method is appropriate for liquid samples extracted with the solvent chloroform ($CHCl_3$; CAS #67-66-3) and containing the chemical agent HD. No significant method interferents have been identified. Users of this method should confirm that the test material and solvent are compatible prior to testing.

RISK AND SAFETY ASSESSMENT

This document does not claim to address all of the safety concerns associated with chemical decontaminant testing as the requirements may vary based on facility, state and other regulatory requirements. It is the responsibility of the user of this method, prior to use, to establish appropriate environment, health, and safety practices for the use of this method and handling of generated wastes in compliance with applicable regulations. Users of this method should conduct testing in appropriate facilities and follow proper laboratory practices, including the use of appropriate personal protective equipment and material safety data sheets.

SCIENTIFIC BASIS

This method is based on gas chromatography and mass spectrometry techniques.

TRAINING

Users of this method must have the basic skill level for analytical chromatography instrument operation, interpretation of mass spectrometry results and associated wet lab techniques (e.g. sample dilution and standard preparation). Training covering instrument operation and maintenance of equipment is suggested.

APPARATUS

The liquid extraction samples are analyzed on an Agilent 6890 GC equipped with a 5975 MSD. The GC is outfitted with an Agilent Deans Switch allowing flow switching during analysis so that only a small portion of the sample effluent, including the analyte of interest, is directed to the MSD. All other flow is directed to a FPD. Sample introduction is performed using a GERSTEL MPS2 and a GERSTEL CIS4. The system is fitted with a HP-5MS 5% phenyl methyl siloxane column. Instrumentation operation, maintenance manuals, and miscellaneous and consumable equipment lists are available from the instrument manufacturers: Agilent and GERSTEL. All equipment should be used in accordance with manufacturer's instructions.

Chemicals required include dilute HD standards prepared in high purity chloroform solvent.

Volumetric glassware used for standard preparation should be Class A and meet the specifications in the most current version of ASTM standards E288 and E69. Pipettes used for standard preparation should be compliant with the required performance specifications listed in the most current versions of ISO 8655 Parts 1 and 2 and/or ASTM E 1154 for the volume measured if standards are made volumetrically. Balances used for gravimetric standard preparation should be sensitive enough to measure the appropriate mass delivered. All equipment should be used in accordance with the manufacturer's requirements, and should be properly maintained and calibrated.

Amber glass analytical sample vials are preferred. The septum cap should be lined with inert material, preferably PTFE/Teflon, to prevent extraction of plasticizers or other impurities into the sample.

PROCEDURE

The specific equipment and column are identified in the "Apparatus" section. Procedures for equipment operation, instrumentation problems, and corrective actions are found in the manufacturer's operating manuals. The specific instrumentation settings for this method are provided in the "Method Parameters" section. The equipment should be confirmed operational prior to use for test support.

Standards are prepared for calibration either volumetrically or gravimetrically, as required by the method.

Liquid extract samples above the method calibration range are diluted to be within the method calibration range. Extrapolation of results outside the calibrated range is not recommended.

Calibration curves are generated from the standard sample results. The curves are fit and weighted as specified in this method. For quality control, the analysis of test samples must include the use of ICV, CCV, and solvent blanks for quality control. Specific guidance method use for decontaminant performance evaluations is provided in the "2007 Chemical Decontaminant Performance Evaluation Testing Source Document" by T. Lalain, et al. Standard preparation, sample queues, interferences and other general analytical guidance are provided in the corresponding technical report to this document titled, "Low-Level Analytical Methodology Updates to Support Decontaminant Performance Evaluations" by M. Shue, et al.

DATA ACCEPTANCE & REPORTING

Analytical results for samples are reported as solution concentrations (ng/mL). Solution concentration can be calculated using the instrument control software (e.g., Applied Biosystems Analyst, Chemstation, etc.) or using external statistical software (e.g., Excel, Sigma plot, etc.) with the specified calibration model. The reported solution concentration should account for sample dilutions made post original sample generation (e.g., contact, residual, and remaining agent testing) such that the analyzed sample is within the calibration range. Calibration curves are not extrapolated; samples must fall within the range or be reported as "above calibration range" or "below detection" as appropriate. The reported concentration is acceptable if all QA/QC criteria are met as specified (e.g., CCVs before and after the sample are within 30% RSD).

METHOD E PARAMETERS

"HD_DEANS.M"
ECBC Decontamination Sciences Branch
GCE Method Summary

INSTRUMENT CONTROL PARAMETERS: GCE (6890GC - 5975MSD)

Control Information
Sample Inlet : GC
Injection Source : External Device
Injection Location : Rear
Mass Spectrometer : Enabled

AGILENT DEANS SWITCH PARAMETERS

METHOD: GCE_DEANS.M

Comment: Deans Switch Parameters for GC/MS methods on GCE.

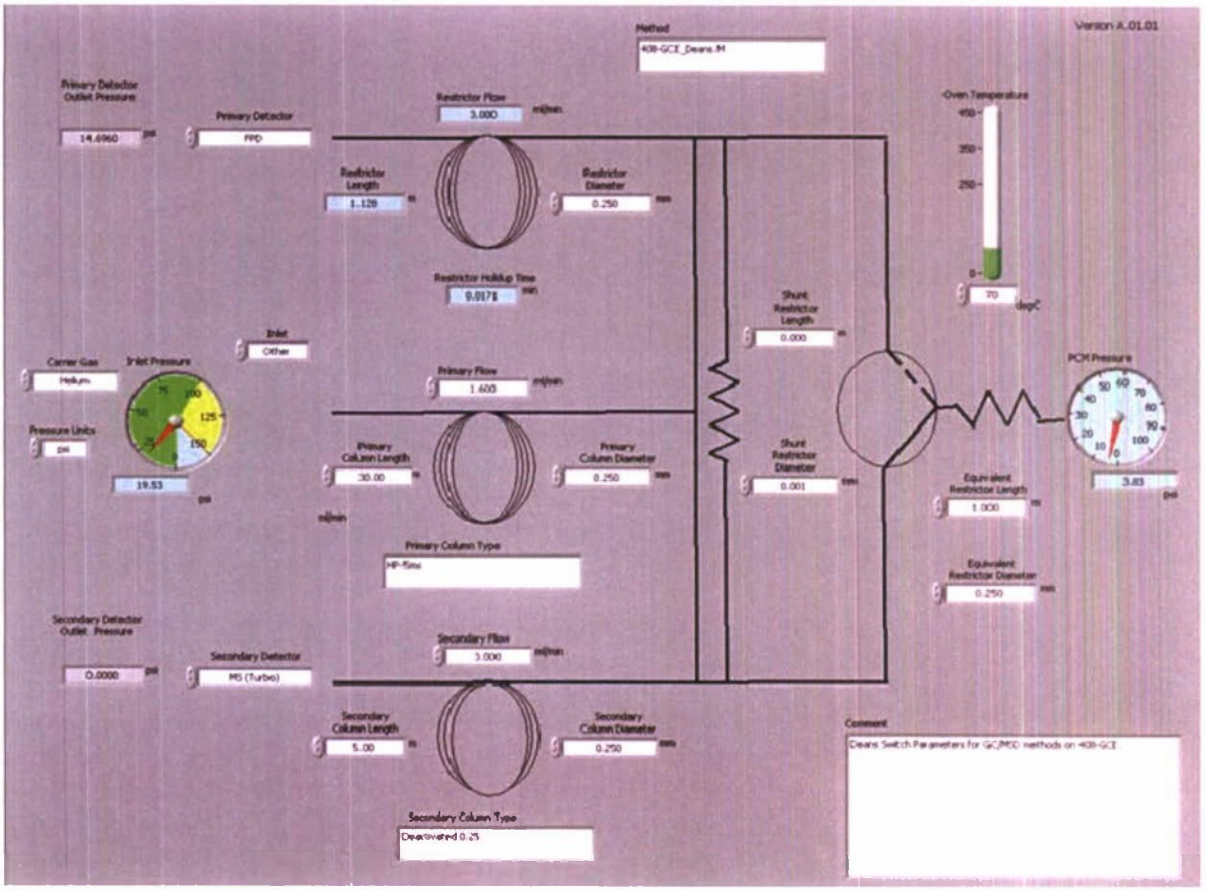
CALCULATION RESULTS:

	Primary Column	Secondary Column
Inlet Pressure:	19.53	3.83
Avg. Linear Velocity (cm/s):	34.72	154.76
Hold up Time (min):	1.44	0.054
Restrictor Length (m):		1.128
Restrictor Hold Up Time (min):		0.017

CALCULATION INPUTS

	Primary Column	Secondary Column
Length (m):	30.00	5.00
i.d. (mm):	0.25	0.25
Flow (mL/min):	1.60	3.00
Detector:	FPD	MS (Turbo)
Detector Pressure (abs):	14.70	0.00
Carrier Gas:		Helium
Pressure Units:		psi
Oven Temperature:		70.00
Restrictor Diameter:		0.25
Equivalent Restrictor Diameter (mm):		0.25
Equivalent Restrictor Length (m):		1.000

DEANS SWITCH CALCULATOR



```
================================================================================
                                  GERSTEL CIS
================================================================================

TEMPERATURE PROGRAM
Initial Temperature      : 12 °C
Equilibration Time       : 0.05 min
Initial Time             : 0.40 min
Ramp 1
Rate 1               : 12.0 °C/s
End Temp 1           : 260 °C
Hold Time 1          : 7.00 min
Ramp 2
Rate 2               : 0.0 °C/s

CRYO COOLING
Cryo Cooling             : used

GERSTEL MPS Liquid Injection

Syringe                  : 10 µL

SAMPLE PARAMETERS
Inj. Volume              : 2.0 µL
Inj. Speed               : 25.00 µL/s
Fill Volume              : 7.0 µL
Fill Strokes             : 3

Fill Speed               : 1.00 µL/s
Eject Speed              : 100.00 µL/s
Viscosity Delay          : 0.0 s

Air Volume               : 1.0 µL
Pre Inj. Delay           : 0.00 s
Post Inj. Delay          : 0.00 s
Inj. Penetration         : 40.00 mm
Vial Penetration         : 31.00 mm

CLEANING PARAMETERS
Preclean Sample          : 0

Preclean Solv.1          : 1
Postclean Solv.1         : 3
Fill Speed Solv.1        : 5.00 µL/s
Viscosity Delay Solv.1   : 1.0 s
Eject Speed Solv.1       : 100.00 µL/s

Preclean Solv.2          : 1
Postclean Solv.2         : 3
Fill Speed Solv.2        : 5.00 µL/s
Viscosity Delay Solv.2   : 1.0 s
Eject Speed Solv.2       : 100.00 µL/s
```

6890 GC METHOD

OVEN
Initial temp: 70 °C (On)
Initial time: 0.75 min
Ramps:

#	Rate	Final temp	Final time
1	35.00	120	0.00
2	10.00	150	0.00
3	40.00	280	0.00
4	0.0(Off)		

Post temp: 70 °C
Post time: 0.00 min
Run time: 8.4286 min
Maximum temp: 325 °C
Equilibration time: 0.25 min

FRONT INLET (SPLIT/SPLITLESS)
Mode: Split
Initial temp: 200 °C (Off)
Pressure: 0.00 psi (Off)
Total flow: 3.7 mL/min
Gas saver: Off
Gas type: Helium

BACK INLET (CIS3)
Mode: Solvent Vent
Initial temp: 250 °C (Off)
Pressure: 19.53 psi (On)
Vent time: 0.30 min
Vent flow: 20.0 mL/min
Vent Pressure: 19.5 psi
Purge flow: 50.0 mL/min
Purge time: 1.00 min
Total flow: 54.4 mL/min
Gas saver: Off
Gas type: Helium

COLUMN 1
Capillary Column
Model Number: Agilent 19091S-433
HP-5MS 5% Phenyl Methyl Siloxane
Max temperature: 325 °C
Nominal length: 30.0 m
Nominal diameter: 250.00 µm
Nominal film thickness: 0.25 µm
Mode: constant pressure
Pressure: 19.53 psi
Nominal initial flow: 1.7 mL/min
Average velocity: 39 cm/s
Inlet: Back Inlet
Outlet: Other
Outlet pressure: ambient

COLUMN 2
Capillary Column
Model Number: Agilent 19091S-433
HP-5MS 5% Phenyl Methyl Siloxane
Max temperature: 325 °C
Nominal length: 30.0 m
Nominal diameter: 250.00 µm
Nominal film thickness: 0.25 µm
Inlet: (unspecified)
Outlet: Other

FRONT DETECTOR (NO DET)

BACK DETECTOR (FPD)
Temperature: 250 °C (On)
Hydrogen flow: 75.0 mL/min (On)
Oxidizer flow: 100.0 mL/min (On)
Oxidizer Gas Type: Air
Mode: Constant makeup flow
Makeup flow: 25.0 mL/min (On)
Makeup Gas Type: Helium
Flame: On
Lit offset: 2.00

Photo multiplier: On

SIGNAL 1
Data rate: 20 Hz
Type: back detector
Save Data: On
Zero: 0.0 (Off)
Range: 0
Fast Peaks: Off
Attenuation: 0

SIGNAL 2
Data rate: 20 Hz
Type: test plot
Save Data: Off
Zero: 0.0 (Off)
Range: 0
Fast Peaks: Off
Attenuation: 0

COLUMN COMP 1
(No Detectors Installed)

COLUMN COMP 2
(No Detectors Installed)

THERMAL AUX 1
Use: MSD Transfer Line Heater
Description: MSD Transferline
Initial temp: 280 °C (On)
Initial time: 0.00 min
Rate Final temp Final time
1 0.0(Off)

THERMAL AUX 2
Unknown Thermal Aux Type

AUX PRESSURE 3
Description: Deans Switch
Gas Type: Helium
Initial pressure: 3.83 psi (On)
Initial time: 0.00 min
Rate Final pres Final time
1 0.0(Off)

AUX PRESSURE 4
Description: No Vent
Gas Type: Helium
Initial pressure: 0.00 psi (Off)

AUX PRESSURE 5
Description:
Gas Type: Helium
Initial pressure: 0.00 psi (Off)

VALVES
Valve 1 Switching Off
Description:

POST RUN
Post Time: 0.00 min

TIME TABLE

Time	Specifier	Parameter & Setpoint
5.10	Valve 1:	On
6.10	Valve 1:	Off

GC Injector

Front Injector:
No parameters specified

Back Injector:
No parameters specified

Column 1 Inventory Number : AB001
Column 2 Inventory Number : AB002

```
================================================================================
                        5975 MS ACQUISITION PARAMETERS
================================================================================
General Information
------- -----------

Tune File                 : atune.u
Acquistion Mode           : SIM

MS Information
-- -----------

Solvent Delay             : 5.00 min

EMV Mode                  : Gain Factor
Gain Factor               : 5
Resulting EM Voltage      : 1271.0*

[Sim Parameters]

GROUP 1
Group ID                  : HD
Resolution                : High
Plot 1 Ion                : 111.00
Ions/Dwell In Group:

(Mass, Dwell)
(109.00, 30)
(111.00, 30)
(158.00, 30)
(160.00, 30)

[MSZones]

MS Quad                   : 150 C   maximum 200 C
MS Source                 : 230 C   maximum 250 C
Timed Events
----- ------

[Timed MS Detector Entries]

END OF MS ACQUISITION PARAMETERS

                              TUNE PARAMETERS*
                               --------------
*MSD Specific Values automatically determined and set when performing an
Autotune.
END OF TUNE PARAMETERS
-------------------------------------

END OF INSTRUMENT CONTROL PARAMETERS
-----------------------------------
```

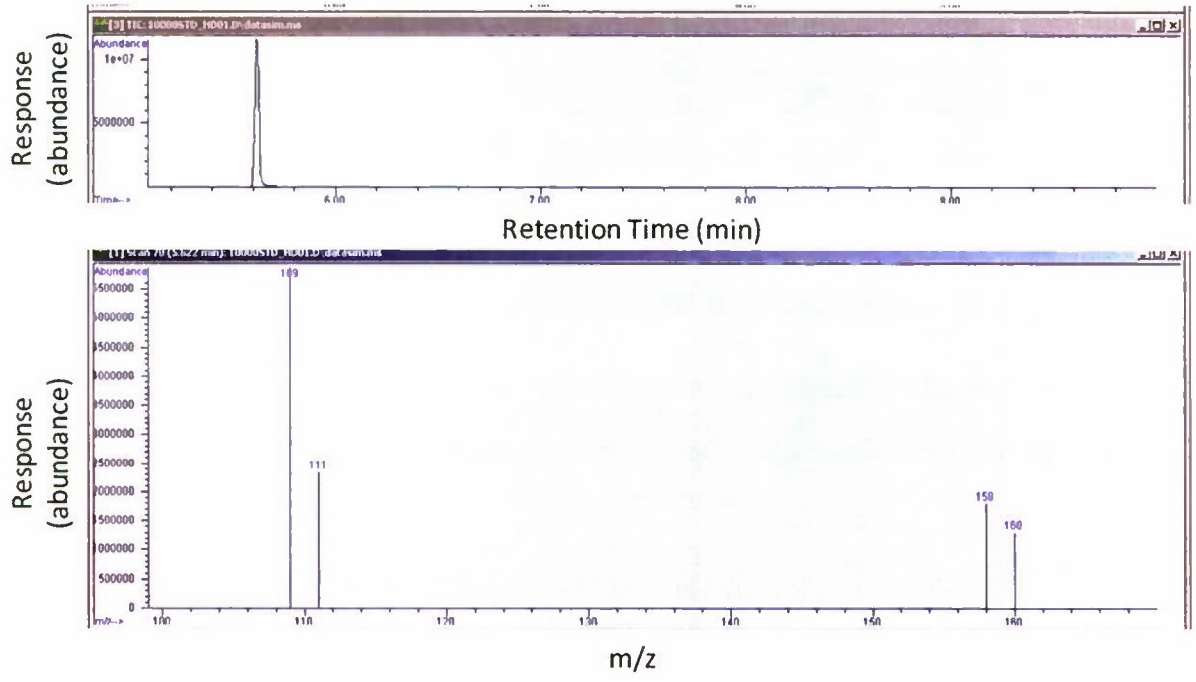
HD SPECTRUM (SCAN) - NIST LIBRARY SEARCH 2.0

The NIST library contains a sample spectrum, which can be found as identified below:

Name: Mustard Gas Formula: $C_4H_8Cl_2S$ MW: 158
CAS#: 505-60-2 NIST#: 289463 ID#: 65664 DB: mainlib

A sample spectrum from this program is provided.

HD (RT 6.5 min) TIC and SPECTRUM (SIM) - GCE DATA ANALYSIS



METHOD F: GC/MS METHOD FOR HD SULFONE IDENTIFICATION (GCE H-SULFONE_DEANS.M)

ANALYTICAL METHOD

TITLE
Detection of Mustard agent (HD) and the oxidation by-product of HD, Mustard Sulfone (H-Sulfone) in liquid extraction samples for chemical agent decontamination testing using a GC/MS with Autosampler.

AUTHORS
Matthew Shue (ECBC Decontamination Sciences Branch)
Teri Lalain, Ph.D. (Branch Chief, Decontamination Sciences Branch)

KEYWORDS
Mustard, HD, Bis(2-chloroethyl)sulfide CAS 505-60-2
Mustard Sulfone, H-Sulfone, Bis(2-chloroethyl)sulfone, CAS 471-03-4

REVISION HISTORY
This document is version 1, released December 2007.

PURPOSE AND APPLICATIONS
The purpose of this method is to detect and identify the chemical agent HD and H-sulfone, the oxidation by-product of HD, in liquid extraction samples. This method is to support the analysis of liquid extracts following the chemical agent decontamination performance evaluation testing. Contact sampler and coupon extracts for a 2 in. diameter test surface area are extracted in no greater than 20 mL of extraction solvent. Sample throughput is approximately four samples per hour.

ANALYTE CONCENTRATION RANGE
This method was developed to identify the chemical agent HD and/or the HD by-product H-sulfone when present independently or within the same sample. This method can detect and identify HD solution concentrations from ~2.0 to 2000 ng/mL. This method can identify H-sulfone solution concentrations from ~10 to 2000 ng/mL.

The method performance for H-sulfone is as follows:

- Qualitative Method
- Sample Solvent: chloroform.
- Quantitative Ion: 63.

SAMPLE MATRICES AND INTERFERENCES

This method is appropriate for liquid samples extracted with the solvent chloroform ($CHCl_3$; CAS #67-66-3) and containing the chemical agent HD and/or the HD by-product H-sulfone. No significant method interferents have been identified. Users of this method should confirm that the test material and solvent are compatible prior to testing.

RISK AND SAFETY ASSESSMENT

This document does not claim to address all of the safety concerns associated with chemical decontaminant testing as the requirements may vary based on facility, state, and other regulatory requirements. It is the responsibility of the user of this method, prior to use, to establish appropriate environment, health, and safety practices for the use of this method and handling of generated wastes in compliance with applicable regulations. Users of this method should conduct testing in appropriate facilities and follow proper laboratory practices, including the use of appropriate personal protective equipment and material safety data sheets.

SCIENTIFIC BASIS

This method is based on gas chromatography and mass spectrometry techniques.

TRAINING

Users of this method must have the basic skill level for analytical chromatography instrument operation, interpretation of mass spectrometry results, and associated wet lab techniques (e.g., sample dilution and standard preparation). Training covering instrument operation and maintenance of equipment is suggested.

APPARATUS

The liquid extraction samples are analyzed on an Agilent 6890 GC equipped with a 5975 MSD. The GC is outfitted with an Agilent Deans Switch allowing flow switching during analysis so that only a small portion of the sample effluent, including the analyte(s) of interest, is directed to the MSD. All other flow is directed to a FPD. Sample introduction is performed using a GERSTEL MPS2 and a GERSTEL CIS4. The system is fitted with a HP-5MS 5% phenyl methyl siloxane column. Instrumentation operation, maintenance manuals, and miscellaneous and consumable equipment lists are available from the instrument manufacturers: Agilent and GERSTEL. All equipment should be used in accordance with the manufacturer's instructions.

Chemicals required include dilute HD and H-sulfone standards prepared in high purity chloroform solvent.

Volumetric glassware used for standard preparation should be Class A and meet the specifications in the most current version of ASTM standards E288 and E69. Pipettes used for standard preparation should be compliant with the required performance specifications listed in the most current versions of ISO 8655 Parts 1 and 2 and/or ASTM E 1154 for the volume measured if standards are made volumetrically. Balances used for gravimetric standard preparation should be sensitive enough to measure the appropriate mass delivered. All equipment should be used in accordance with the manufacturer's requirements, and should be properly maintained and calibrated.

Amber glass analytical sample vials are preferred. The septum cap should be lined with inert material, preferably PTFE/Teflon, to prevent extraction of plasticizers or other impurities into the sample.

PROCEDURE

The specific equipment and column are identified in the "Apparatus" section. Procedures for equipment operation, instrumentation problems, and corrective actions are found in the manufacturer's operating manuals. The specific instrumentation settings for this method are provided in the "Method Parameters" section. The equipment should be confirmed as operational prior to use for test support.

Standards are prepared for calibration either volumetrically or gravimetrically, as required by the method.

Liquid extract samples above the method calibration range are diluted to be within the method calibration range. Extrapolation of results outside the calibrated range is not recommended.

Calibration curves are generated from the standard sample results. The curves are fit and weighted as specified in this method. For quality control, the analysis of test samples must include the use of ICV, CCV, and solvent blanks. Specific guidance for method use for decontaminant performance evaluations is provided in the "2007 Chemical Decontaminant Performance Evaluation Testing Source Document" by T. Lalain, et al. Standard preparation, sample queues, interferences and other general analytical guidance are provided in the corresponding technical report to this document titled, "Low-Level Analytical Methodology Updates to Support Decontaminant Performance Evaluations" by M. Shue, et al.

DATA ACCEPTANCE & REPORTING

Analytical results for samples are reported as solution concentrations (ng/mL). Solution concentration can be calculated using the instrument control software (e.g., Applied Biosystems Analyst, Chemstation, etc.) or using external statistical software (e.g., Excel, Sigma plot, etc.) with the specified calibration model. The reported solution concentration should account for sample dilutions made after the original sample generation (e.g., contact, residual, and remaining agent testing) such that the analyzed sample is within the calibration range. Calibration curves are not extrapolated; samples must fall within the range or be reported as "above calibration range" or "below detection" as appropriate. The reported concentration is acceptable if all QA/QC criteria are met as specified (e.g., CCVs before and after the sample are within 30% RSD).

METHOD F PARAMETERS

"H-Sulfone_DEANS.M"
ECBC Decontamination Sciences Branch
GCE Method Summary

INSTRUMENT CONTROL PARAMETERS: GCE (6890GC - 5975MSD)

Control Information
Sample Inlet : GC
Injection Source : External Device
Injection Location : Rear
Mass Spectrometer : Enabled

AGILENT DEANS SWITCH PARAMETERS

METHOD: GCE_DEANS.M

Comment: Deans Switch Parameters for GC/MS methods on GCE.

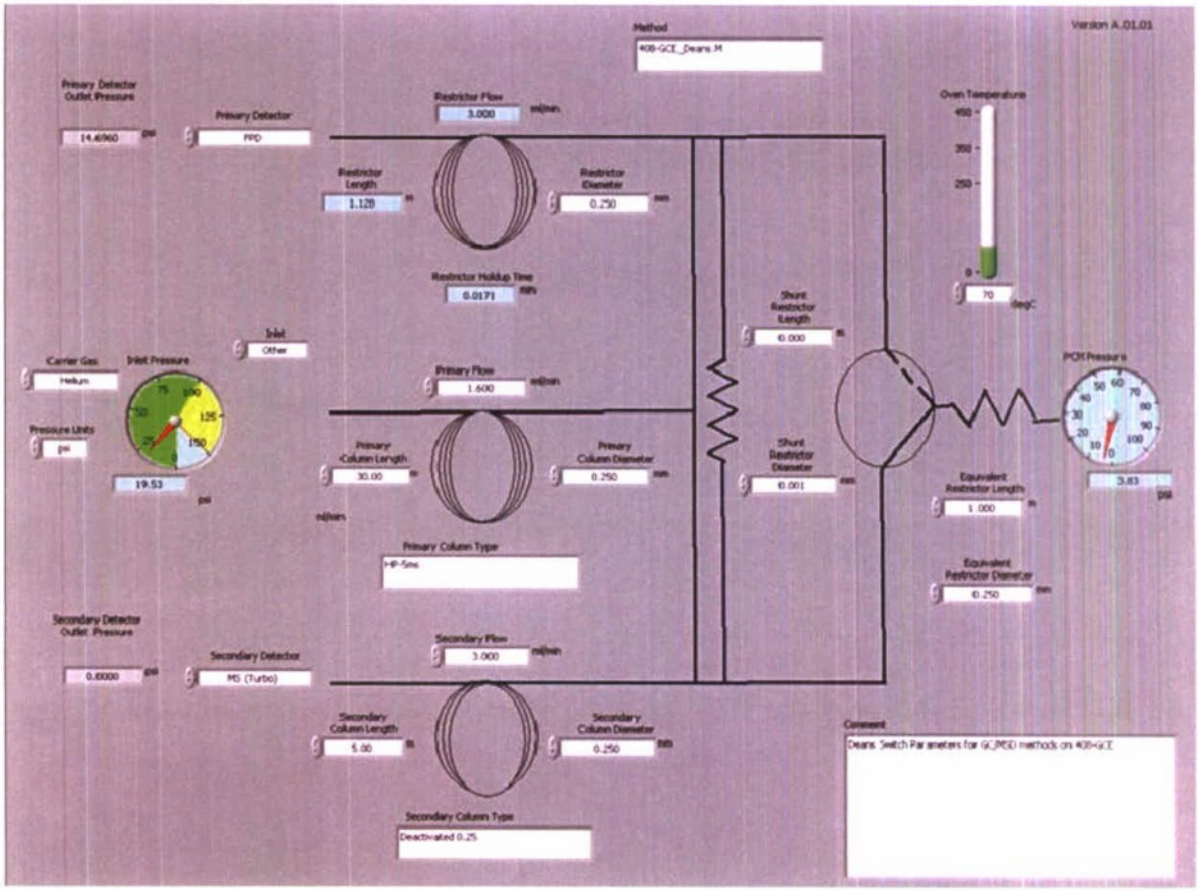
CALCULATION RESULTS:

	Primary Column	Secondary Column
Inlet Pressure:	19.53	3.83
Avg. Linear Velocity (cm/s):	34.72	154.76
Hold up Time (min):	1.44	0.054
Restrictor Length (m):		1.128
Restrictor Hold Up Time (min):		0.017

CALCULATION INPUTS

	Primary Column	Secondary Column
Length (m):	30.00	5.00
i.d. (mm):	0.25	0.25
Flow (mL/min):	1.60	3.00
Detector:	FPD	MS (Turbo)
Detector Pressure (abs):	14.70	0.00
Carrier Gas:		Helium
Pressure Units:		psi
Oven Temperature:		70.00
Restrictor Diameter:		0.25
Equivalent Restrictor Diameter (mm):		0.25
Equivalent Restrictor Length (m):		1.000

DEANS SWITCH CALCULATOR



```
================================================================================
                                  GERSTEL CIS
================================================================================
TEMPERATURE PROGRAM
Initial Temperature      : 12 °C
Equilibration Time       : 0.05 min
Initial Time             : 0.40 min
Ramp 1
Rate 1               : 12.0 °C/s
End Temp 1           : 260 °C
Hold Time 1          : 4.00 min
Ramp 2
Rate 2               : 0.0 °C/s

CRYO COOLING
Cryo Cooling             : used

GERSTEL MPS Liquid Injection

Syringe                  : 10 µL

SAMPLE PARAMETERS
Inj. Volume              : 2.0 µL
Inj. Speed               : 25.00 µL/s
Fill Volume              : 7.0 µL
Fill Strokes             : 3

Fill Speed               : 1.00 µL/s
Eject Speed              : 100.00 µL/s
Viscosity Delay          : 0.0 s

Air Volume               : 1.0 µL
Pre Inj. Delay           : 0.00 s
Post Inj. Delay          : 0.00 s
Inj. Penetration         : 40.00 mm
Vial Penetration         : 31.00 mm

CLEANING PARAMETERS
Preclean Sample          : 0

Preclean Solv.1          : 1
Postclean Solv.1         : 3
Fill Speed Solv.1        : 5.00 µL/s
Viscosity Delay Solv.1   : 1.0 s
Eject Speed Solv.1       : 100.00 µL/s

Preclean Solv.2          : 1
Postclean Solv.2         : 3
Fill Speed Solv.2        : 5.00 µL/s
Viscosity Delay Solv.2   : 1.0 s
Eject Speed Solv.2       : 100.00 µL/s
```

```
================================================================================
                                6890 GC METHOD
================================================================================
OVEN
Initial temp:  70 °C (On)                  Maximum temp:  325 °C
Initial time:  0.75 min                    Equilibration time:  0.25 min
Ramps:
#  Rate  Final temp  Final time
1 35.00      120        0.00
2 10.00      150        0.00
3 40.00      280        0.00
4   0.0(Off)
Post temp:  70 °C
Post time:  0.00 min
Run time:  8.4286 min

FRONT INLET (SPLIT/SPLITLESS)              BACK INLET (CIS3)
Mode:  Split                               Mode:  Solvent Vent
Initial temp:  200 °C (Off)                Initial temp:  250 °C (Off)
Pressure:  0.00 psi (Off)                  Pressure:  19.53 psi (On)
Total flow:  3.7 mL/min                    Vent time:  0.30 min
Gas saver:  Off                            Vent flow:  20.0 mL/min
Gas type:  Helium                          Vent Pressure:  19.5 psi
                                           Purge flow:  50.0 mL/min
                                           Purge time:  1.00 min
                                           Total flow:  54.4 mL/min
                                           Gas saver:  Off
                                           Gas type:  Helium

COLUMN 1                                   COLUMN 2
Capillary Column                           Capillary Column
Model Number:  Agilent 19091S-433          Model Number:  Agilent 19091S-433
HP-5MS  5% Phenyl Methyl Siloxane          HP-5MS  5% Phenyl Methyl Siloxane
Max temperature:  325 °C                   Max temperature:  325 °C
Nominal length:  30.0 m                    Nominal length:  30.0 m
Nominal diameter:  250.00 µm               Nominal diameter:  250.00 µm
Nominal film thickness:  0.25 µm           Nominal film thickness:  0.25 µm
Mode:  constant pressure                   Inlet:  (unspecified)
Pressure:  19.53 psi                       Outlet:  Other
Nominal initial flow:  1.7 mL/min
Average velocity:  39 cm/s
Inlet:  Back Inlet
Outlet:  Other
Outlet pressure:  ambient

FRONT DETECTOR (NO DET)                    BACK DETECTOR (FPD)
                                             Temperature:  250 °C (On)
                                             Hydrogen flow:  75.0 mL/min (On)
                                             Oxidizer flow:  100.0 mL/min (On)
                                             Oxidizer Gas Type:  Air
                                             Mode:  Constant makeup flow
                                             Makeup flow:  25.0 mL/min (On)
                                             Makeup Gas Type:  Helium
                                             Flame:  On
                                             Lit offset:  2.00
```

```
                                        Photo multiplier:  On

SIGNAL 1                                SIGNAL 2
Data rate:  20 Hz                       Data rate:  20 Hz
Type:  back detector                    Type:  test plot
Save Data:  On                          Save Data:  Off
Zero:  0.0 (Off)                        Zero:  0.0 (Off)
Range:  0                               Range:  0
Fast Peaks:  Off                        Fast Peaks:  Off
Attenuation:  0                         Attenuation:  0

COLUMN COMP 1                           COLUMN COMP 2
(No Detectors Installed)                (No Detectors Installed)

THERMAL AUX 1                           THERMAL AUX 2
Use:  MSD Transfer Line Heater          Unknown Thermal Aux Type
Description:  MSD Transferline
Initial temp:  280 °C (On)
Initial time:  0.00 min
#  Rate  Final temp  Final time
1   0.0(Off)

AUX PRESSURE 3                          AUX PRESSURE 4
Description:  Deans Switch              Description:  No Vent
Gas Type:  Helium                       Gas Type:  Helium
Initial pressure:  3.83 psi (On)        Initial pressure:  0.00 psi (Off)
      Initial time:  0.00 min
      #  Rate  Final pres  Final time
      1   0.0(Off)

AUX PRESSURE 5
Description:
Gas Type:  Helium
Initial pressure:  0.00 psi (Off)

VALVES                                  POST RUN
Valve 1  Switching Off                  Post Time: 0.00 min
Description:

TIME TABLE
Time       Specifier                     Parameter & Setpoint
5.00       Valve 1:                      On
6.00       Valve 1:                      Off
7.00       Valve 1:                      On
8.00       Valve 1:                      Off

GC Injector

Front Injector:
No parameters specified

Back Injector:
No parameters specified

Column 1 Inventory Number : AB001
Column 2 Inventory Number : AB002
```

```
================================================================================
                        5975 MS ACQUISITION PARAMETERS
================================================================================


General Information
------- -----------

Tune File                 : atune.u
Acquistion Mode           : SIM


MS Information
-- -----------

Solvent Delay             : 5.00 min

EMV Mode                  : Gain Factor
Gain Factor               : 5
Resulting EM Voltage      : 1271.0*

[Sim Parameters]

GROUP 1
Group ID                  : HD
Resolution                : High
Plot 1 Ion                : 111.00
Ions/Dwell In Group:

(Mass, Dwell)
(109.00, 30)
(111.00, 30)
(158.00, 30)
(160.00, 30)

GROUP 2
Group ID                  : H-Sulfone
Resolution                : High
Group Start Time          : 6.50
Plot 1 Ion                : 27.00
Ions/Dwell In Group:

(Mass, Dwell)
(27.00, 30)
(28.00, 30)
(63.00, 30)
(65.00, 30)


[MSZones]

MS Quad                   : 150 C   maximum 200 C
MS Source                 : 230 C   maximum 250 C
```

Timed Events
----- ------

[Timed MS Detector Entries]

END OF MS ACQUISITION PARAMETERS

TUNE PARAMETERS*

**MSD Specific Values automatically determined and set when performing an Autotune.*

END OF TUNE PARAMETERS

END OF INSTRUMENT CONTROL PARAMETERS

HD SPECTRUM (SCAN) - NIST LIBRARY SEARCH 2.0

The NIST library contains a sample spectrum, which can be found as identified below:

Name: Mustard Gas Formula: $C_4H_8Cl_2S$ MW: 158
CAS#: 505-60-2 NIST#: 289463 ID#: 65664 DB: mainlib

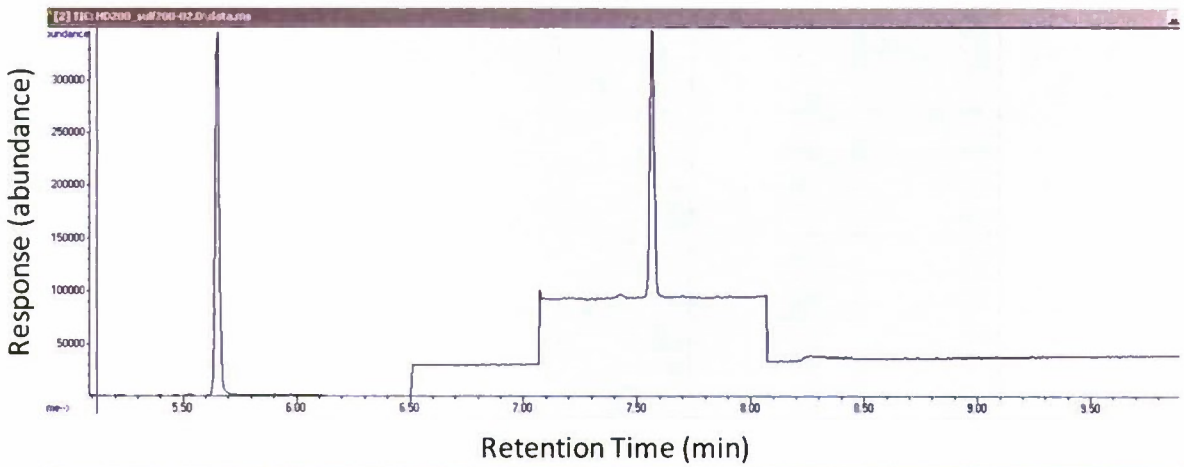
H-Sulfone SPECTRUM (SCAN) - NIST LIBRARY SEARCH 2.0

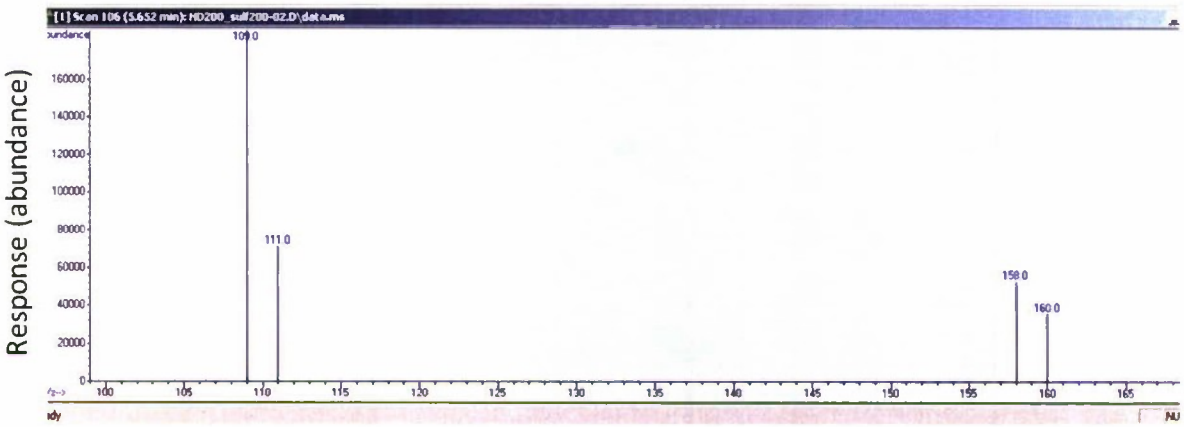
The NIST library contains a sample spectrum, which can be found as identified below:

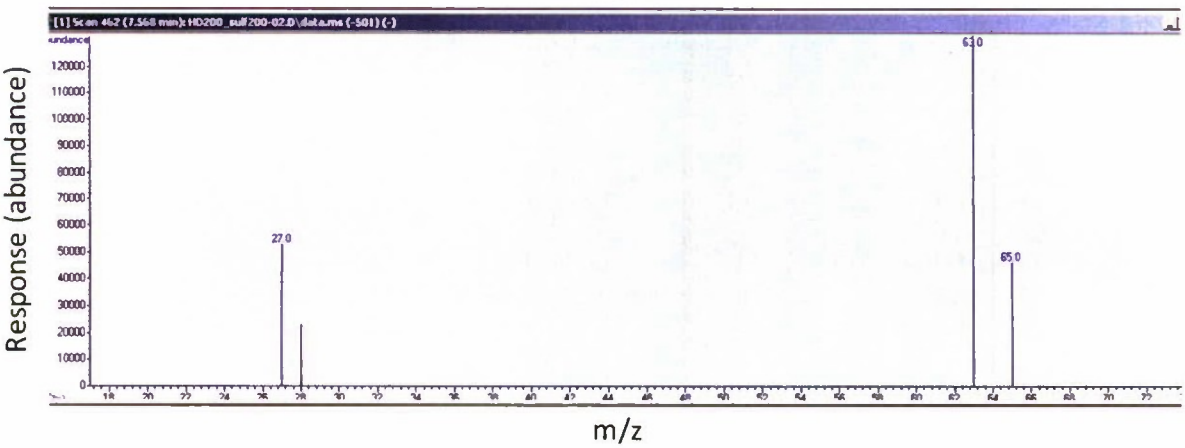
Name: Bis(á-chloroethyl) sulfone Formula: $C_4H_8Cl_2O_2S$ MW: 190
CAS#: 471-03-4 NIST#: 289499 ID#: 75 DB: mainlib

A sample spectrum from this program is provided.

HD (RT 5.6 min)and H-Sulfone (RT 7.6 min) TIC and Spectra (SIM) - GCE DATA ANALYSIS







METHOD G: GC/MS METHOD FOR TDG IDENTIFICATION (GCE TDG_DEANS.M)

ANALYTICAL METHOD

TITLE
Detection of the hydrolysis by-product of HD, Thiodiglycol (TDG) in liquid extraction samples for chemical agent decontamination testing using a GC/MS with Autosampler.

AUTHORS
Matthew Shue (ECBC Decontamination Sciences Branch)
Teri Lalain, Ph.D. (Branch Chief, Decontamination Sciences Branch)

KEYWORDS
Thiodiglycol, 2,2'-Thiodiethanol CAS 111-48-8

REVISION HISTORY
This document is version 1, released December 2007.

PURPOSE AND APPLICATIONS
The purpose of this method is to detect and identify the hydrolysis by-product of HD, thiodiglycol (TDG), in liquid extraction samples. This method is to support the analysis of liquid extracts following the chemical agent decontamination performance evaluation testing. Contact sampler and coupon extracts for a 2 in. diameter test surface area are extracted in no greater than 20 mL of extraction solvent. Sample throughput is approximately five samples per hour.

ANALYTE CONCENTRATION RANGE
This method was developed to identify the HD by-product TDG. This method can detect and identify TDG solution concentrations from ~25,000 to 100,000 ng/mL. NOTE: Due to the large concentrations required, this method is not appropriate for analyzing HD and TDG simultaneously.

The method performance for TDG is as follows:

- Qualitative Method
- Sample Solvent: chloroform.
- Quantitative Ion: 61.

SAMPLE MATRICES AND INTERFERENCES
This method is appropriate for liquid samples extracted with the solvent chloroform ($CHCl_3$; CAS #67-66-3) and containing the HD by-product Thiodiglycol. No significant method interferents have been identified. Users of this method should confirm that the test material and solvent are compatible prior to testing.

RISK AND SAFETY ASSESSMENT

This document does not claim to address all of the safety concerns associated with chemical decontaminant testing as the requirements may vary based on facility, state, and other regulatory requirements. It is the responsibility of the user of this method, prior to use, to establish appropriate environment, health, and safety practices for the use of this method and handling of generated wastes in compliance with applicable regulations. Users of this method should conduct testing in appropriate facilities and follow proper laboratory practices, including the use of appropriate personal protective equipment and material safety data sheets.

SCIENTIFIC BASIS

This method is based on gas chromatography and mass spectrometry techniques.

TRAINING

Users of this method must have the basic skill level for analytical chromatography instrument operation, interpretation of mass spectrometry results, and associated wet lab techniques (e.g., sample dilution and standard preparation). Training covering instrument operation and maintenance of equipment is suggested.

APPARATUS

The liquid extraction samples are analyzed on an Agilent 6890 GC equipped with a 5975 MSD. The GC is outfitted with an Agilent Deans Switch allowing flow switching during analysis so that only a small portion of the sample effluent, including the analyte(s) of interest, is directed to the MSD. All other flow is directed to a FPD. Sample introduction is performed using a GERSTEL MPS2 and a GERSTEL CIS4. The system is fitted with a HP-5MS 5% phenyl methyl siloxane column. Instrumentation operation, maintenance manuals, and miscellaneous and consumable equipment lists are available from the instrument manufacturers: Agilent and GERSTEL. All equipment should be used in accordance with the manufacturer's instructions.

Chemicals required include dilute thiodiglycol standards prepared in high purity chloroform solvent.

Volumetric glassware used for standard preparation should be Class A and meet the specifications in the most current version of ASTM standards E288 and E69. Pipettes used for standard preparation should be compliant with the required performance specifications listed in the most current versions of ISO 8655 Parts 1 and 2 and/or ASTM E 1154 for the volume measured if standards are made volumetrically. Balances used for gravimetric standard preparation should be sensitive enough to measure appropriate mass delivered. All equipment should be used in accordance with manufacturer's requirements, properly maintained, and calibrated.

Amber glass analytical sample vials are preferred. The septum cap should be lined with inert material, preferably PTFE/Teflon, to prevent extraction of plasticizers or other impurities into the sample.

PROCEDURE

The specific equipment and column are identified in the "Apparatus" section. Procedures for equipment operation, instrumentation problems, and corrective actions are found in the manufacturer's operating manuals. The specific instrumentation settings for this method are provided in the "Method Parameters" section. The equipment should be confirmed as operational prior to use for test support.

Standards are prepared for calibration either volumetrically or gravimetrically, as required by the method.

Liquid extract samples above the method calibration range are diluted to be within the method calibration range. Extrapolation of results outside the calibrated range is not recommended.

Calibration curves are generated from the standard sample results. The curves are fit and weighted as specified in this method. For quality control, the analysis of test samples must include the use of ICV, CCV, and solvent blanks. Specific guidance for method use for decontaminant performance evaluations is provided in the "2007 Chemical Decontaminant Performance Evaluation Testing Source Document" by T. Lalain, et al. Standard preparation, sample queues, interferences, and other general analytical guidance are provided in the corresponding technical report to this document titled, "Low-Level Analytical Methodology Updates to Support Decontaminant Performance Evaluations" by M. Shue, et al.

DATA ACCEPTANCE & REPORTING

Analytical results for samples are reported as solution concentrations (ng/mL). Solution concentration can be calculated using the instrument control software (e.g., Applied Biosystems Analyst, Chemstation, etc.) or using external statistical software (e.g., Excel, Sigma plot, etc.) with the specified calibration model. The reported solution concentration should account for sample dilutions made after the original sample generation (e.g., contact, residual, and remaining agent testing) such that the analyzed sample is within the calibration range. Calibration curves are not extrapolated; samples must fall within the range or be reported as "above calibration range" or "below detection" as appropriate. The reported concentration is acceptable if all QA/QC criteria are met as specified (e.g., CCVs before and after the sample are within 30% RSD).

METHOD G PARAMETERS

"TDG_DEANS.M"
ECBC Decontamination Sciences Branch
GCE Method Summary

INSTRUMENT CONTROL PARAMETERS: GCE (6890GC - 5975MSD)

Control Information
Sample Inlet : GC
Injection Source : External Device
Injection Location : Rear
Mass Spectrometer : Enabled

AGILENT DEANS SWITCH PARAMETERS

METHOD: GCE_DEANS.M

Comment: Deans Switch Parameters for GC/MS methods on GCE.

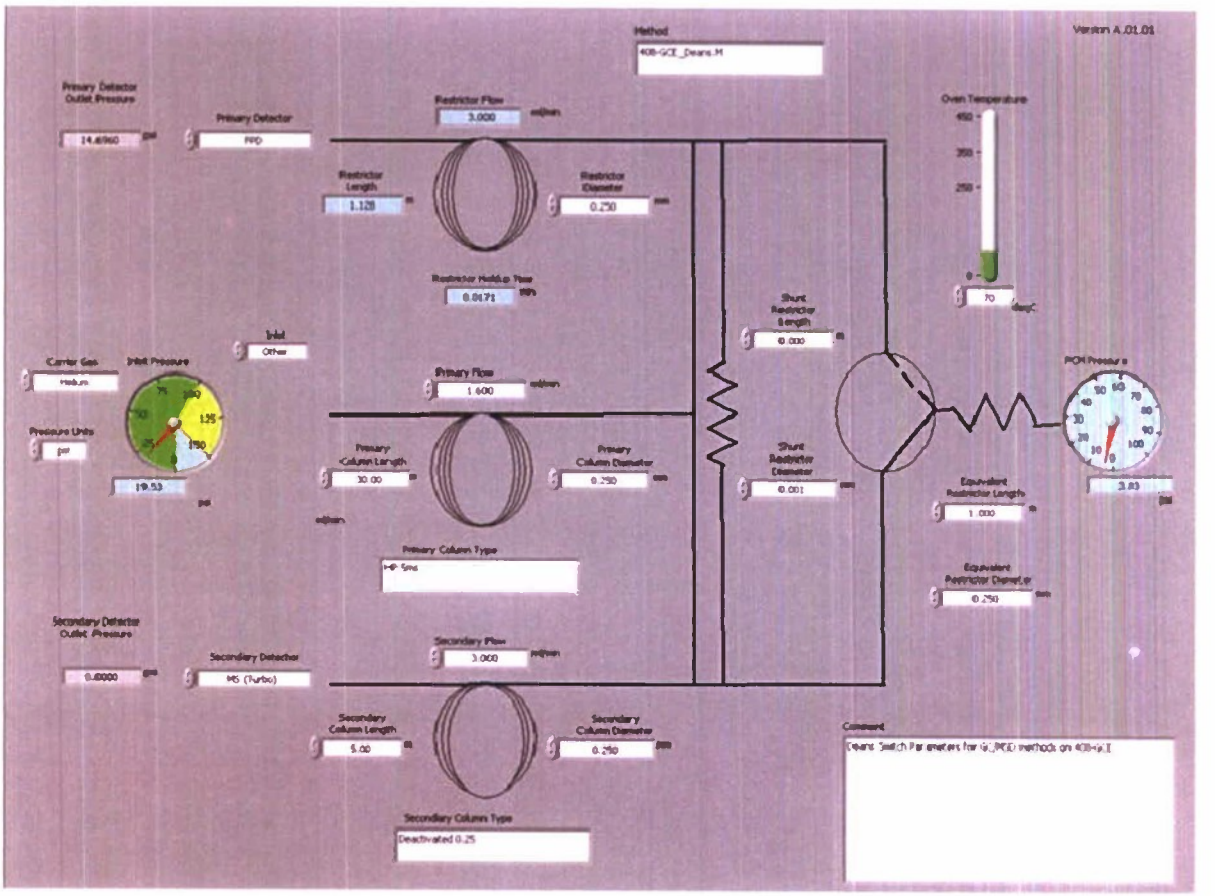
CALCULATION RESULTS:

	Primary Column	Secondary Column
Inlet Pressure:	19.53	3.83
Avg. Linear Velocity (cm/s):	34.72	154.76
Hold up Time (min):	1.44	0.054
Restrictor Length (m):		1.128
Restrictor Hold Up Time (min):		0.017

CALCULATION INPUTS

	Primary Column	Secondary Column
Length (m):	30.00	5.00
i.d. (mm):	0.25	0.25
Flow (mL/min):	1.60	3.00
Detector:	FPD	MS (Turbo)
Detector Pressure (abs):	14.70	0.00
Carrier Gas:		Helium
Pressure Units:		psi
Oven Temperature:		70.00
Restrictor Diameter:		0.25
Equivalent Restrictor Diameter (mm):		0.25
Equivalent Restrictor Length (m):		1.000

DEANS SWITCH CALCULATOR



```
================================================================================
                                  GERSTEL CIS
================================================================================

TEMPERATURE PROGRAM
Initial Temperature      : 12 °C
Equilibration Time       : 0.05 min
Initial Time             : 0.20 min
Ramp 1
Rate 1               : 12.0 °C/s
End Temp 1           : 270 °C
Hold Time 1          : 3.00 min
Ramp 2
Rate 2               : 0.0 °C/s

CRYO COOLING
Cryo Cooling             : used

GERSTEL MPS Liquid Injection

Syringe                  : 10 µL

SAMPLE PARAMETERS
Inj. Volume              : 2.0 µL
Inj. Speed               : 25.00 µL/s
Fill Volume              : 10.0 µL
Fill Strokes             : 3

Fill Speed               : 5.00 µL/s
Eject Speed              : 100.00 µL/s
Viscosity Delay        : 1.0 s

Air Volume               : 0.0 µL
Pre Inj. Delay           : 0.00 s
Post Inj. Delay          : 0.00 s
Inj. Penetration         : 40.00 mm
Vial Penetration         : 31.00 mm

CLEANING PARAMETERS
Preclean Sample          : 0

Preclean Solv.1          : 1
Postclean Solv.1         : 3
Fill Speed Solv.1        : 5.00 µL/s
Viscosity Delay Solv.1   : 1.0 s
Eject Speed Solv.1       : 100.00 µL/s

Preclean Solv.2          : 1
Postclean Solv.2         : 3
Fill Speed Solv.2        : 5.00 µL/s
Viscosity Delay Solv.2   : 1.0 s
Eject Speed Solv.2       : 100.00 µL/s
```

```
================================================================================
                                6890 GC METHOD
================================================================================
OVEN
Initial temp:  70 °C (On)                  Maximum temp:  325 °C
Initial time:  0.00 min                    Equilibration time:  0.25 min
Ramps:
#  Rate  Final temp  Final time
1 35.00      280        1.00
2 0.00       000        0.00
3 0.00       000        0.00
4   0.0(Off)
Post temp:  70 °C
Post time:  0.00 min
Run time:   7.00 min

FRONT INLET (SPLIT/SPLITLESS)              BACK INLET (CIS3)
Mode:  Split                               Mode:  Solvent Vent
Initial temp:  200 °C (Off)                Initial temp:  250 °C (Off)
Pressure:  0.00 psi (Off)                  Pressure:  19.53 psi (On)
Total flow:  3.7 mL/min                    Vent time:  0.20 min
Gas saver:  Off                            Vent flow:  20.0 mL/min
Gas type:  Helium                          Vent Pressure:  19.5 psi
                                           Purge flow:  50.0 mL/min
                                           Purge time:  1.5 min
                                           Total flow:  54.4 mL/min
                                           Gas saver:  Off
                                           Gas type:  Helium

COLUMN 1                                   COLUMN 2
Capillary Column                           Capillary Column
Model Number:  Agilent 19091S-433          Model Number:  Agilent 19091S-433
HP-5MS  5% Phenyl Methyl Siloxane          HP-5MS  5% Phenyl Methyl Siloxane
Max temperature:  325 °C                   Max temperature:  325 °C
Nominal length:  30.0 m                    Nominal length:  30.0 m
Nominal diameter:  250.00 µm               Nominal diameter:  250.00 µm
Nominal film thickness:  0.25 µm           Nominal film thickness:  0.25 µm
Mode:  constant pressure                   Inlet:  (unspecified)
Pressure:  19.53 psi                       Outlet:  Other
Nominal initial flow:  1.7 mL/min
Average velocity:  39 cm/s
Inlet:  Back Inlet
Outlet:  Other
Outlet pressure:  ambient

FRONT DETECTOR (NO DET)                    BACK DETECTOR (FPD)
                                             Temperature:  250 °C (On)
                                             Hydrogen flow:  75.0 mL/min (On)
                                             Oxidizer flow:  100.0 mL/min (On)
                                             Oxidizer Gas Type: Air
                                             Mode:  Constant makeup flow
                                             Makeup flow:  25.0 mL/min (On)
                                             Makeup Gas Type: Helium
                                             Flame:  On
                                             Lit offset:  2.00
```

Photo multiplier: On

SIGNAL 1
Data rate: 20 Hz
Type: back detector
Save Data: On
Zero: 0.0 (Off)
Range: 0
Fast Peaks: Off
Attenuation: 0

SIGNAL 2
Data rate: 20 Hz
Type: test plot
Save Data: Off
Zero: 0.0 (Off)
Range: 0
Fast Peaks: Off
Attenuation: 0

COLUMN COMP 1
(No Detectors Installed)

COLUMN COMP 2
(No Detectors Installed)

THERMAL AUX 1
Use: MSD Transfer Line Heater
Description: MSD Transferline
Initial temp: 280 °C (On)
Initial time: 0.00 min

#	Rate	Final temp	Final time
1	0.0	(Off)	

THERMAL AUX 2
Unknown Thermal Aux Type

AUX PRESSURE 3
Description: No vent
Gas Type: Helium
Initial pressure: 0.00 psi (Off)

AUX PRESSURE 4
Description: Deans Switch
Gas Type: Helium
Initial pressure: 3.83 psi (On)
Initial time: 0.00 min

#	Rate	Final pres	Final time
1	0.0	(Off)	

AUX PRESSURE 5
Description:
Gas Type: Helium
Initial pressure: 0.00 psi (Off)

VALVES
Valve 1 Switching Off
Description:

POST RUN
Post Time: 0.00 min

TIME TABLE

Time	Specifier	Parameter & Setpoint
3.90	Valve 1:	On
4.90	Valve 1:	Off

GC Injector

Front Injector:
No parameters specified

Back Injector:
No parameters specified

Column 1 Inventory Number : AB001
Column 2 Inventory Number : AB002

```
================================================================================
                        5975 MS ACQUISITION PARAMETERS
================================================================================
General Information
------- -----------

Tune File                 : atune.u
Acquistion Mode           : SIM

MS Information
-- -----------
Solvent Delay             : 3.50 min

EM Absolute               : False
EM Offset                 : 400
Resulting EM Voltage      : 2400.0*

[Sim Parameters]

GROUP 1
Group ID                  : TDG
Resolution                : High
Plot 1 Ion                : 61.00
Ions/Dwell In Group:

(Mass, Dwell)
(45.00, 30)
(61.00, 30)
(91.00, 30)
(104.00, 30)
(122.00, 30)

[MSZones]

MS Quad                   : 150 C   maximum 200 C
MS Source                 : 230 C   maximum 250 C
Timed Events
----- ------
[Timed MS Detector Entries]

END OF MS ACQUISITION PARAMETERS

                              TUNE PARAMETERS*
                               ---------------
 *MSD Specific Values automatically determined and set when performing an
Autotune.

END OF TUNE PARAMETERS
------------------------------------
END OF INSTRUMENT CONTROL PARAMETERS
------------------------------------
```

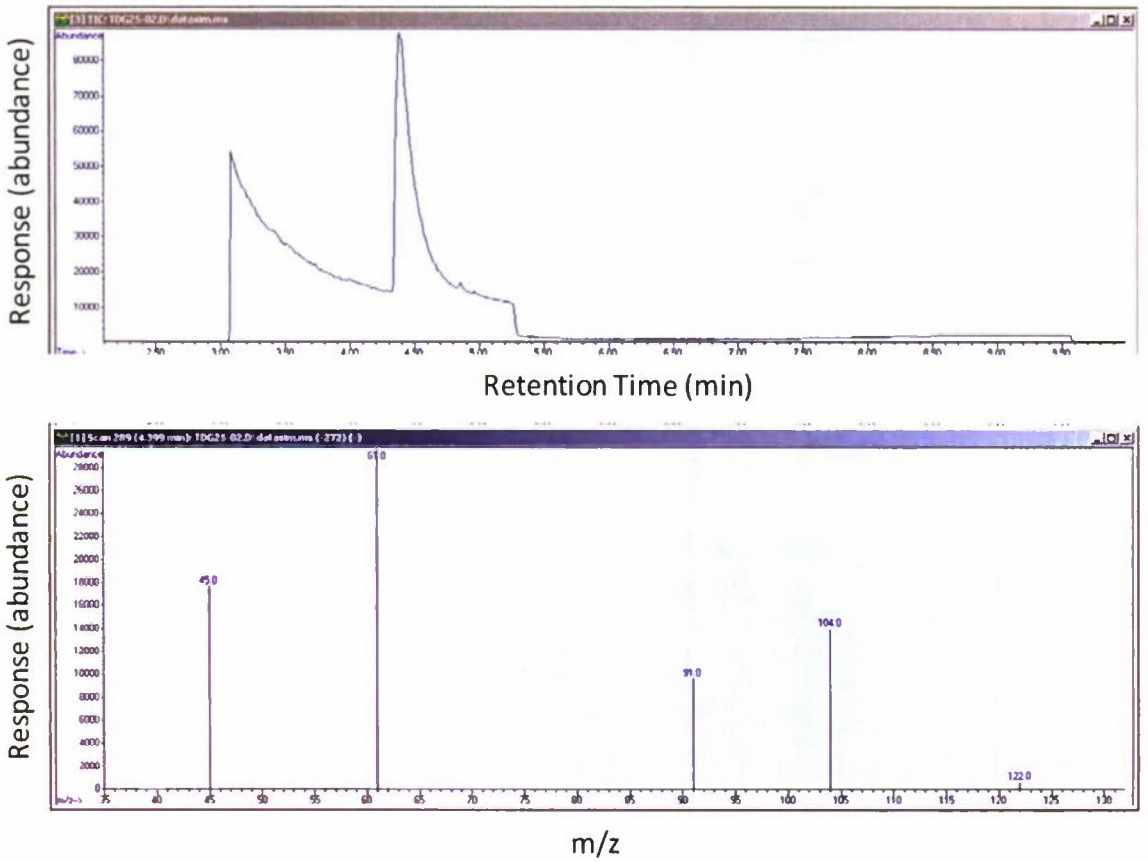
Thiodiglycol (SCAN) - NIST LIBRARY SEARCH 2.0

The NIST library contains a sample spectrum, which can be found as identified below:

Name: Thiodiglycol Formula: $C_4H_{10}O_2S$ MW: 122
CAS#: 111-48-8 NIST#: 229583 ID#: 25714 DB: mainlib

A sample spectrum from this program is provided.

TDG (RT 4.5 min) TIC and SPECTRUM (SIM) - GCE DATA ANALYSIS



METHOD H: LC-MS METHOD FOR HD SULFOXIDE IDENTIFICATION (LCE H-SULFOXIDE.DAM)

ANALYTICAL METHOD

TITLE
Detection of Mustard Sulfoxide (H-Sulfoxide), an oxidation product of HD, in liquid extraction samples from chemical agent decontamination testing using an LC/MS/MS system.

AUTHORS
Matthew Shue (ECBC Decontamination Sciences Branch)
Phillip Smith, Ph.D. (SAIC)
Teri Lalain, Ph.D. (Branch Chief, Decontamination Sciences Branch)

KEYWORDS
Mustard Sulfoxide, H-Sulfoxide, Bis(2-chloroethyl) sulfoxide,
CAS 5819-08-9

REVISION HISTORY
This document is version 1, released December 2007.

PURPOSE AND APPLICATIONS
The purpose of this method is to detect mustard sulfoxide (H-sulfoxide). H-sulfoxide is an oxidation by-product of the chemical agent HD. This method is to support the analysis of liquid extracts following the chemical agent decontamination performance evaluation testing for contact sampler and coupon extracts for a 2 in. diameter test surface area extracted in no greater than 20 mL of extraction solvent. Sample throughput is approximately seven samples per hour.

ANALYTE CONCENTRATION RANGE
This method was developed to screen for a HD byproduct, H-sulfoxide. The approximate concentration range for this method is from 10.0 to 500 ng/mL.

The method performance for H-sulfoxide is as follows:

- Qualitative Method
- Sample Solvent: chloroform.
- Quantitative Ion Pair: 175/63.

SAMPLE MATRICES AND INTERFERENCES
This method is appropriate for liquid samples extracted with the solvent chloroform ($CHCl_3$; CAS #67-66-3) and suspected to contain the HD byproduct H-sulfoxide. No significant method

interferents have been identified. Users of this method should confirm that the test material and solvent are compatible prior to testing.

RISK AND SAFETY ASSESSMENT

This document does not claim to address all of the safety concerns associated with chemical decontaminant testing as the requirements may vary based on facility, state and other regulatory requirements. It is the responsibility of the user of this method, prior to use, to establish appropriate environment, health, and safety practices for the use of this method and handling of generated wastes in compliance with applicable regulations. Users of this method should conduct testing in appropriate facilities and follow proper laboratory practices, including the use of appropriate personal protective equipment and material safety data sheets.

SCIENTIFIC BASIS

This method is based on liquid chromatography and mass spectrometry techniques.

TRAINING

Users of this method must have the basic skill level for analytical chromatography instrument operation, interpretation of mass spectrometry results, and associated wet lab techniques (e.g., sample dilution and standard preparation). Training covering instrument operation and maintenance of equipment is suggested.

APPARATUS

The liquid extraction samples are analyzed on an Applied Biosystems API5000 Triple-quadrupole Mass Spectrometer equipped with the TurboV Ion Source. Sample introduction and chromatography are performed with an Agilent 1200 series LC. Sample effluent is directed from the LC directly to the TurboV ion source of the API5000 MS. The system is fitted with a HP-5MS 5% phenyl methyl siloxane column. Instrumentation operation, maintenance manuals, and miscellaneous and consumable equipment lists are available from the instrument manufacturers: Applied Biosystems and Agilent Technologies. All equipment should be used in accordance with the manufacturer's instructions.

Chemicals required include dilute standard of H-sulfoxide prepared in high purity chloroform solvent within the working concentration range of this method.

Volumetric glassware used for standard preparation should be Class A and meet the specifications in the most current version of ASTM standards E288 and E69. Pipettes used for standard preparation should be compliant with the required performance specifications listed in the most current versions of ISO 8655 Parts 1 and 2 and/or ASTM E 1154 for the volume measured, if standards are made volumetrically. Balances used for gravimetric standard preparation should be sensitive enough to measure the appropriate mass delivered. All equipment should be used in accordance with the manufacturer's requirements, and should be properly maintained and calibrated.

Amber glass analytical sample vials are preferred. The septum cap should be lined with inert material, preferably PTFE/Teflon, to prevent extraction of plasticizers or other impurities into the sample.

PROCEDURE

The specific equipment and column are identified in the "Apparatus" section. Procedures for equipment operation, instrumentation problems, and corrective actions are found in the manufacturer's operating manuals. The specific instrumentation settings for this method are provided in the "Method Parameters" section. The equipment should be confirmed as operational prior to use for test support.

Standards are prepared for calibration either volumetrically or gravimetrically, as required by the method.

Liquid extract samples above the method calibration range are diluted to be within the method calibration range. Extrapolation of results outside the calibrated range is not recommended.

Calibration curves are generated from the standard sample results. The curves are fit and weighted as specified in this method. For quality control, the analysis of test samples must include the use of ICV, CCV, and solvent blanks. Specific guidance for method use for decontaminant performance evaluations is provided in the "2007 Chemical Decontaminant Performance Evaluation Testing Source Document" by T. Lalain, et al. Standard preparation, sample queues, interferences and other general analytical guidance are provided in the corresponding technical report to this document titled, "Low-Level Analytical Methodology Updates to Support Decontaminant Performance Evaluations" by M. Shue, et al.

DATA ACCEPTANCE & REPORTING

Analytical results for samples are reported as solution concentrations (ng/mL). Solution concentration can be calculated using the instrument control software (e.g., Applied Biosystems Analyst, Chemstation, etc.) or using external statistical software (e.g., Excel, Sigma plot, etc.) with the specified calibration model. The reported solution concentration should account for sample dilutions made after the original sample generation (e.g., contact, residual, and remaining agent testing) such that the analyzed sample is within the calibration range. Calibration curves are not extrapolated; samples must fall within the range or be reported as "above calibration range" or "below detection" as appropriate. The reported concentration is acceptable if all QA/QC criteria are met as specified (e.g., CCVs before and after the sample are within 30% RSD).

METHOD H PARAMETERS

"LCE H-Sulfoxide"
ECBC Decontamination Sciences Branch
LCE Method Summary

INSTRUMENT CONTROL PARAMETERS: LCE (1200 Series LC / API5000 MS)

Acquisition Method Properties
------- ---------------------

Comment: LC/MS/MS Method (MRM)for H-Sulfoxide.

Synchronization Mode:	LC Sync
Auto-Equilibration:	Off
Acquisition Duration:	7min0s
Number of Scans:	683
Periods in File:	1
Acquisition Module:	Acquisition Method
Software version:	Analyst 1.4.2

==========

API5000 Mass Spec

==========

MS Method Properties:
Period 1:

Scans in Period:	683
Relative Start Time:	0.00 ms
Experiments in Period:	1

Period 1 Experiment 1:

Scan Type:	MRM (MRM)
Polarity:	Positive
Scan Mode:	N/A
Ion Source:	Turbo Spray
Resolution Q1:	Unit
Resolution Q3:	High
Intensity Thres.:	0.00 cps
Settling Time:	0.0000 ms
MR Pause:	5.0070 ms
MCA:	No
Step Size:	0.00 amu

@Q1 Mass (amu)	Q3 Mass (amu)	Dwell(ms)
175.00	63.00	200.00

@Q1 Mass (amu)	Q3 Mass (amu)	Dwell(ms)

```
175.00              95.00               200.00

@Q1 Mass (amu)     Q3 Mass (amu)      Dwell(ms)
177.00              63.00               200.00

Parameter Table(Period 1  Experiment   1):
CUR:          15.00
GS1:          50.00
GS2:          55.00
TEM:          500.00
ihe:          ON
CAD:          3.00
IS:           5500.00
DP            60.00
EP            10.00
CE            25.00
CXP           12.00


END OF INSTRUMENT CONTROL PARAMETERS
-------------------------------------
```

==========

AGILENT 1200 SERIES LC PARAMETERS

==========

Agilent LC Pump Method Properties

Pump Model:	Agilent 1200 Binary Pump
Minimum Pressure (psi):	0.0
Maximum Pressure (psi):	5801.0
Dead Volume (µL):	40.0
Maximum Flow Ramp (mL/min²):	100.0
Maximum Pressure Ramp (psi/s):	290.0

Step Table:

@Step	Total Time(min)	Flow Rate(µL/min)	A (%)	B (%)
0	1.00	200	50.0	50.0
1	7.00	200	50.0	50.0

Left Compressibility:	50.0
Right Compressibility:	115.0
Left Dead Volume (µL):	40.0
Right Dead Volume (µL):	40.0
Left Stroke Volume (µL):	-1.0
Right Stroke Volume (µL):	-1.0
Left Solvent:	A1
Right Solvent:	B1

Agilent Autosampler Properties

Autosampler Model:	Agilent 1200 High Performance Autosampler
Syringe Size (µL):	100
Injection Volume (µL):	4.00
Draw Speed (µL/min):	200.0
Eject Speed (µL/min):	200.0
Needle Level (mm):	0.00
Temperature Control	Not Used
Wash Location:	Wash Vial
Wash Cycles (1 - 5):	2
Wash Vial Number:	1
Wash Rack Number:	1
Automatic Delay Volume Reduction	Not Used
Equilibration Time (s):	2
Enable Vial/Well Bottom Sensing	No
Use Custom Injector Program	No

Agilent Column Oven Properties

Left Temperature (°C):	25.00
Right Temperature (°C):	25.00
Temperature Tolerance +/- (°C):	10.00

Start Acquisition
Tolerance +/- (°C): 5.00
Time Table (Not Used)
Column Switching Valve Installed
Position for first
sample in the batch: Left

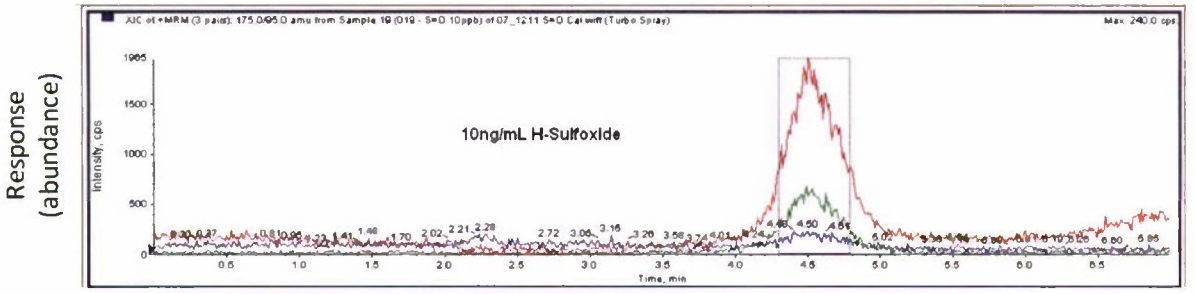
Use same position for all samples in the batch

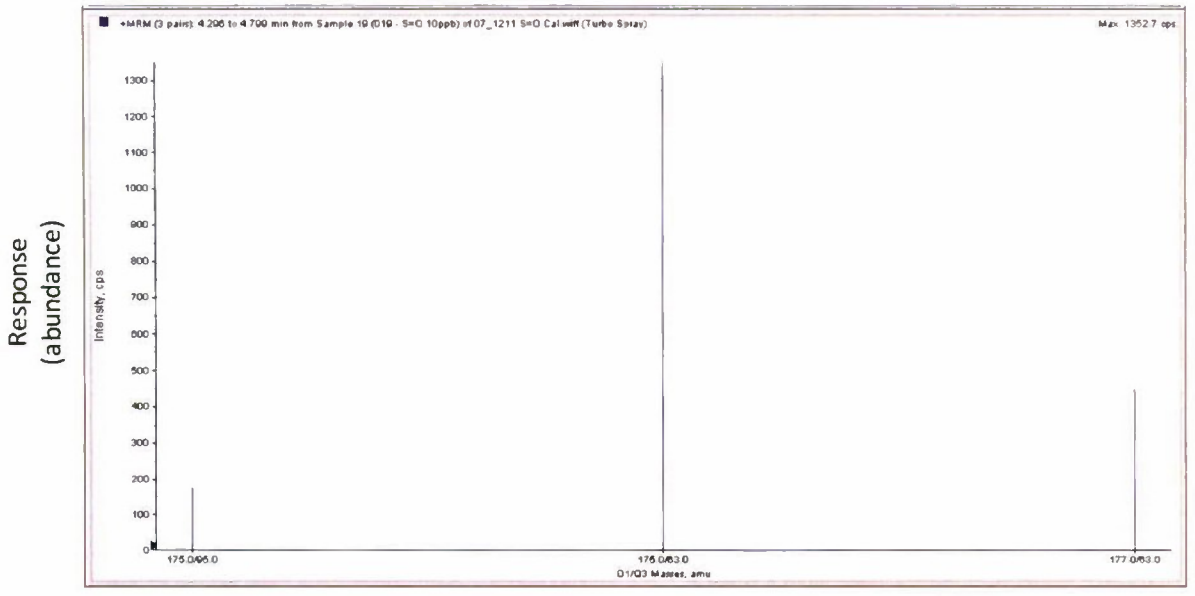
Column Type:
Agilent ZORBAX
SB-C18
PN: 866953-902
SN: USDZ010845
4.6mm x 75mm 3.5µm

Mobile Phase
Aqueous:
95% dH20
5% IPA
0.1% Formic Acid
5mM Ammonium Acetate

Organic:
95% IPA
5% dH20
0.1% Formic Acid
5mM Ammonium Acetate

H-Sulfoxide (RT 4.5 min) XIC and Mass Spectrum - LCE DATA ANALYSIS





METHOD I: GC/MS METHOD FOR LOW-LEVEL GD (GCE GD_DEANS.M)

ANALYTICAL METHOD

TITLE
Detection of GD in liquid extraction samples for chemical agent decontamination testing using a GC/MS with Autosampler.

AUTHORS
Matthew Shue (ECBC Decontamination Sciences Branch)
Michelle Sheahy (SAIC)
Teri Lalain, Ph.D. (Branch Chief, Decontamination Sciences Branch)

KEYWORDS
Soman, GD, O-Pinacolyl methylphosphonofluoridate CAS 69-64-0

REVISION HISTORY
This document is version 1, released December 2007.

PURPOSE AND APPLICATIONS
The purpose of this method is to detect and quantify the chemical agent GD in liquid extraction samples. This method is optimized to support the analysis of liquid extracts following the chemical agent decontamination performance evaluation testing. Contact sampler and coupon extracts for a 2 in. diameter test surface area are extracted in no greater than 20 mL of extraction solvent. Sample throughput is approximately eight samples per hour.

ANALYTE CONCENTRATION RANGE
This method can detect GD solution concentrations from 2.0 to 2000 ng/mL. This method utilizes 13 calibration standards at concentrations of ~2.0, 5, 10, 25, 50, 100, 250, 500, 750, 1000, and 2000 ng/mL GD. The method use, detection limit, and quantitation limit are based on using the full standard set. The standards are prepared in acetonitrile.

The method performance for GD_DEANS.M is as follows:

- Quantitative Method
- Calibration Range: 2.0 to 2000 ng/mL GD.
- CCV Levels: 0, 10, 150, 750 ng/mL
- Calibration Model: Weighted quadratic calibration model
- Calibration Weighting: $1/x^2$
- Limit of Detection (LOD): 0.11 ng/mL
- Limit of Quantitation (LOQ): 0.38 ng/mL
- Sample Solvent: acetonitrile
- Quantitative Ion: 126

SAMPLE MATRICES AND INTERFERENCES

This method is appropriate for liquid samples extracted with the solvent acetonitrile (CH_3CN; CAS #75-05-8) and containing the chemical agent GD. No significant method interferents have been identified. Users of this method should confirm that the test material and solvent are compatible prior to testing.

RISK AND SAFETY ASSESSMENT

This document does not claim to address all of the safety concerns associated with chemical decontaminant testing as the requirements may vary based on facility, state, and other regulatory requirements. It is the responsibility of the user of this method, prior to use, to establish appropriate environment, health, and safety practices for the use of this method and handling of generated wastes in compliance with applicable regulations. Users of this method should conduct testing in appropriate facilities and follow proper laboratory practices, including the use of appropriate personal protective equipment and material safety data sheets.

SCIENTIFIC BASIS

This method is based on gas chromatography and mass spectrometry techniques.

TRAINING

Users of this method must have the basic skill level for analytical chromatography instrument operation, interpretation of mass-spectrometry results and associated wet lab techniques (e.g., sample dilution and standard preparation). Training covering instrument operation and maintenance of equipment is suggested.

APPARATUS

The liquid extraction samples are analyzed on an Agilent 6890 GC equipped with a 5975 MSD. The GC is outfitted with an Agilent Deans Switch, allowing flow switching during analysis so that only a small portion of the sample effluent, including the analyte of interest, is directed to the MSD. All other flow is directed to a FPD. Sample introduction is performed using a GERSTEL MPS2 and a GERSTEL CIS4. The system is fitted with a HP-5MS 5% phenyl methyl siloxane column. Instrumentation operation, maintenance manuals, and miscellaneous and consumable equipment lists are available from the instrument manufacturers: Applied Agilent and GERSTEL. All equipment should be used in accordance with the manufacturer's instructions.

Chemicals required include dilute GD standards prepared in high purity acetonitrile solvent.

Volumetric glassware used for standard preparation should be Class A and meet the specifications in the most current version of ASTM standards E288 and E69. Pipettes used for standard preparation should be compliant with the required performance specifications listed in the most current versions of ISO 8655 Parts 1 and 2 and/or ASTM E 1154 for the volume measured if standards are made volumetrically. Balances used for gravimetric standard preparation should be sensitive enough to measure the appropriate mass delivered. All equipment should be used in accordance with the manufacturer's requirements, and should be properly maintained and calibrated.

Amber glass analytical sample vials are preferred. The septum cap should be lined with inert material, preferably PTFE/Teflon, to prevent extraction of plasticizers or other impurities into the sample.

PROCEDURE

The specific equipment and column are identified in the "Apparatus" section. Procedures for equipment operation, instrumentation problems, and corrective actions are found in the manufacturer's operating manuals. The specific instrumentation settings for this method are provided in the "Method Parameters" section. The equipment should be confirmed as operational prior to use for test support.

Standards are prepared for calibration either volumetrically or gravimetrically, as required by the method.

Liquid extract samples above the method calibration range are diluted to be within the method calibration range. Extrapolation of results outside the calibrated range is not recommended.

Calibration curves are generated from the standard sample results. The curves are fit and weighted as specified in this method. For quality control, the analysis of test samples must include the use of ICV, CCV, and solvent blanks for quality control. Specific guidance for method use for decontaminant performance evaluations is provided in the "2007 Chemical Decontaminant Performance Evaluation Testing Source Document" by T. Lalain, et al. Standard preparation, sample queues, interferences, and other general analytical guidance are provided in the corresponding technical report to this document titled, "Low-Level Analytical Methodology Updates to Support Decontaminant Performance Evaluations" by M. Shue, et al.

DATA ACCEPTANCE & REPORTING

Analytical results for samples are reported as solution concentrations (ng/mL). Solution concentration can be calculated using the instrument control software (e.g., Applied Biosystems Analyst, Chemstation, etc.) or using external statistical software (e.g., Excel, Sigma plot, etc.) with the specified calibration model. The reported solution concentration should account for sample dilutions made after the original sample generation (e.g., contact, residual, and remaining agent testing) such that the analyzed sample is within the calibration range. Calibration curves are not extrapolated; samples must fall within the range or be reported as "above calibration range" or "below detection" as appropriate. The reported concentration is acceptable if all QA/QC criteria are met as specified (e.g., CCVs before and after the sample are within 30% RSD).

METHOD I PARAMETERS

"GD_DEANS.M"
ECBC Decontamination Sciences Branch
GCE Method Summary

INSTRUMENT CONTROL PARAMETERS: GCE (6890GC - 5975MSD)

Control Information

Sample Inlet : GC
Injection Source : External Device
Injection Location : Rear
Mass Spectrometer : Enabled

AGILENT DEANS SWITCH PARAMETERS

METHOD: GCE_DEANS.M

Comment: Deans Switch Parameters for GC/MS methods on GCE.

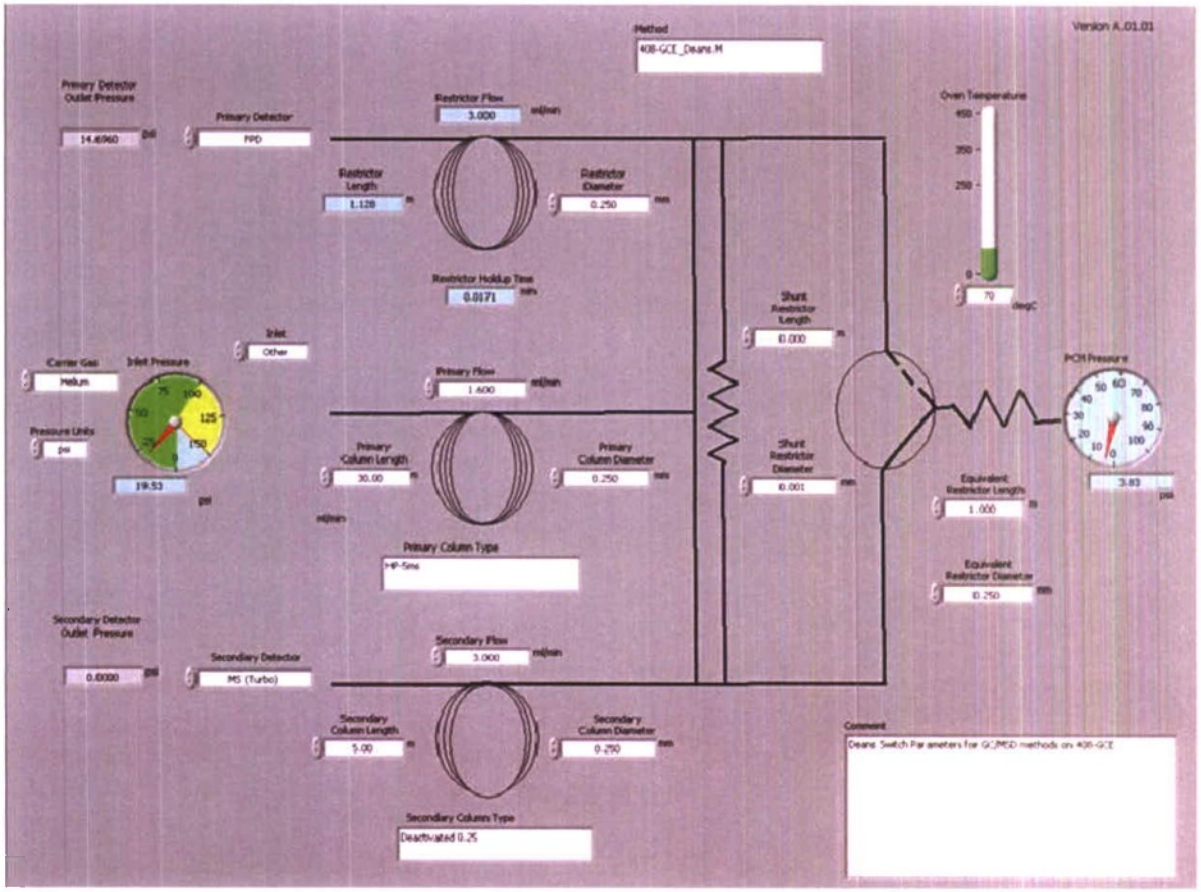
CALCULATION RESULTS:

	Primary Column	Secondary Column
Inlet Pressure:	19.53	3.83
Avg. Linear Velocity (cm/s):	34.72	154.76
Hold up Time (min):	1.44	0.054
Restrictor Length (m):		1.128
Restrictor Hold Up Time (min):		0.017

CALCULATION INPUTS

	Primary Column	Secondary Column
Length (m):	30.00	5.00
i.d. (mm):	0.25	0.25
Flow (mL/min):	1.60	3.00
Detector:	FPD	MS (Turbo)
Detector Pressure (abs):	14.70	0.00
Carrier Gas:		Helium
Pressure Units:		psi
Oven Temperature:		70.00
Restrictor Diameter:		0.25
Equivalent Restrictor Diameter (mm):		0.25
Equivalent Restrictor Length (m):		1.000

DEANS SWITCH CALCULATOR



```
================================================================================
                                  GERSTEL CIS
================================================================================
TEMPERATURE PROGRAM
Initial Temperature      : 12 °C
Equilibration Time       : 0.05 min
Initial Time             : 0.20 min
Ramp 1
Rate 1               : 12.0 °C/s
End Temp 1           : 240 °C
Hold Time 1          : 3.00 min
Ramp 2
Rate 2               : 0.0 °C/s

CRYO COOLING
Cryo Cooling             : used


GERSTEL MPS Liquid Injection

Syringe                  : 10 µL

SAMPLE PARAMETERS
Inj. Volume              : 1.0 µL
Inj. Speed               : 25.00 µL/s
Fill Volume              : 10.0 µL
Fill Strokes             : 3

Fill Speed               : 5.00 µL/s
Eject Speed              : 100.00 µL/s
Viscosity Delay        : 1.0 s

Air Volume               : 0.0 µL
Pre Inj. Delay           : 0.00 s
Post Inj. Delay          : 0.00 s
Inj. Penetration         : 40.00 mm
Vial Penetration         : 31.00 mm

CLEANING PARAMETERS
Preclean Sample          : 0

Preclean Solv.1          : 1
Postclean Solv.1         : 2
Fill Speed Solv.1        : 5.00 µL/s
Viscosity Delay Solv.1   : 1.0 s
Eject Speed Solv.1       : 100.00 µL/s

Preclean Solv.2          : 1
Postclean Solv.2         : 2
Fill Speed Solv.2        : 5.00 µL/s
Viscosity Delay Solv.2   : 1.0 s
Eject Speed Solv.2       : 100.00 µL/s
```

```
================================================================================
                                6890 GC METHOD
================================================================================

OVEN
   Initial temp:  80 °C (On)                  Maximum temp:  325 °C
   Initial time:  0.75 min                    Equilibration time:  0.25 min
   Ramps:
      #  Rate  Final temp  Final time
      1 35.00      260        0.00
      2   0.0(Off)
   Post temp:  80 °C
   Post time:  0.00 min
   Run time:  5.8929 min

FRONT INLET (SPLIT/SPLITLESS)              BACK INLET (CIS3)
   Mode:  Split                               Mode:  Solvent Vent
   Initial temp:  200 °C (Off)                Initial temp:  250 °C (Off)
   Pressure:  0.00 psi (Off)                  Pressure:  19.53 psi (On)
   Total flow:  3.7 mL/min                    Vent time:  0.20 min
   Gas saver:  Off                            Vent flow:  20.0 mL/min
   Gas type:  Helium                          Vent Pressure:  19.5 psi
                                              Purge flow:  50.0 mL/min
                                              Purge time:  0.7 min
                                              Total flow:  54.4 mL/min
                                              Gas saver:  Off
                                              Gas type:  Helium

COLUMN 1                                   COLUMN 2
   Capillary Column                           Capillary Column
   Model Number:  Agilent 19091S-433          Model Number:  Agilent 19091S-433
   HP-5MS  5% Phenyl Methyl Siloxane          HP-5MS  5% Phenyl Methyl Siloxane
   Max temperature:  325 °C                   Max temperature:  325 °C
   Nominal length:  30.0 m                    Nominal length:  30.0 m
   Nominal diameter:  250.00 µm               Nominal diameter:  250.00 µm
   Nominal film thickness:  0.25 µm           Nominal film thickness:  0.25 µm
   Mode:  constant pressure                   Inlet:  (unspecified)
   Pressure:  19.53 psi                       Outlet:  Other
   Nominal initial flow:  1.7 mL/min
   Average velocity:  39 cm/s
   Inlet:  Back Inlet
   Outlet:  Other
   Outlet pressure:  ambient

FRONT DETECTOR (NO DET)                    BACK DETECTOR (FPD)
                                              Temperature:  250 °C (On)
                                              Hydrogen flow:  75.0 mL/min (On)
                                              Oxidizer flow:  100.0 mL/min (On)
                                              Oxidizer Gas Type:  Air
                                              Mode:  Constant makeup flow
                                              Makeup flow:  25.0 mL/min (On)
                                              Makeup Gas Type:  Helium
                                              Flame:  On
                                              Lit offset:  2.00
                                              Photo multiplier:  On
```

```
SIGNAL 1
   Data rate:  20 Hz
   Type:  back detector
   Save Data:  On
   Zero:  0.0 (Off)
   Range:  0
   Fast Peaks:  Off
   Attenuation:  0

COLUMN COMP 1
   (No Detectors Installed)

THERMAL AUX 1
   Use:  MSD Transfer Line Heater
   Description:  MSD Transferline
   Initial temp:  280 °C (On)
   Initial time:  0.00 min
      #  Rate  Final temp  Final time
      1   0.0(Off)

AUX PRESSURE 3
   Description:  Deans Switch
   Gas Type:  Helium
   Initial pressure:  3.83 psi (On)
      Initial time:  0.00 min
      #  Rate  Final pres  Final time
      1   0.0(Off)

AUX PRESSURE 5
   Description:
   Gas Type:  Helium
   Initial pressure:  0.00 psi (Off)

VALVES
   Valve 1  Switching On
      Description:

TIME TABLE
   Time        Specifier                 Parameter & Setpoint
      3.30        Valve 1:                  On
      3.90        Valve 1:                  Off
```

```
SIGNAL 2
   Data rate:  20 Hz
   Type:  test plot
   Save Data:  Off
   Zero:  0.0 (Off)
   Range:  0
   Fast Peaks:  Off
   Attenuation:  0

COLUMN COMP 2
   (No Detectors Installed)

THERMAL AUX 2
   Unknown Thermal Aux Type

AUX PRESSURE 4
   Description:  No Vent
   Gas Type:  Helium
   Initial pressure:  0.00 psi (Off)

POST RUN
   Post Time: 0.00 min
```

```
================================================================================
                        5975 MS ACQUISITION PARAMETERS
================================================================================
General Information
------- -----------

Tune File                 : atune.u
Acquistion Mode           : SIM

MS Information
-- -----------

Solvent Delay             : 3.00 min
EMV Mode                  : Gain Factor
Gain Factor               : 15
Resulting EM Voltage      : 1353.0*

[Sim Parameters]

GROUP 1
Group ID                  : GD
Resolution                : High
Plot 1 Ion                : 126.00

Ions/Dwell In Group

(Mass, Dwell)

(82.00, 40)
(99.00, 40)
(126.00, 40)


[MSZones]
MS Quad                   : 150 C   maximum 200 C
MS Source                 : 230 C   maximum 250 C

END OF MS ACQUISITION PARAMETERS


TUNE PARAMETERS*
----------------

 *MSD Specific Values automatically determined and set when performing an
Autotune.

END OF TUNE PARAMETERS
----------------------

                    END OF INSTRUMENT CONTROL PARAMETERS
```

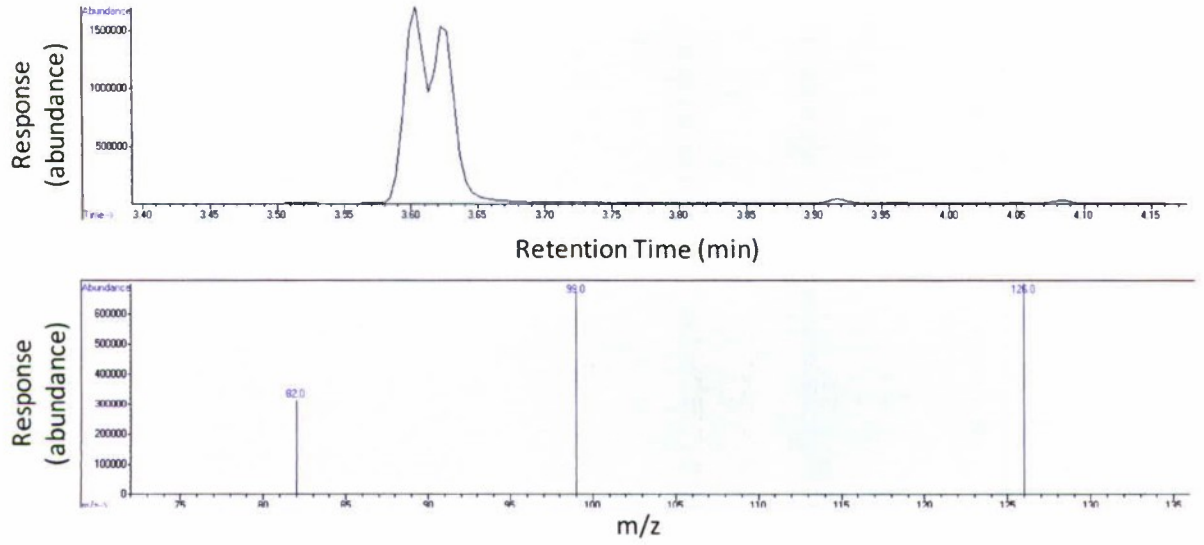
GD SPECTRUM (SCAN) - NIST LIBRARY SEARCH 2.0

The NIST library contains a sample spectrum, which can be found as identified below:

Name: Soman Formula: $C_7H_{16}FO_2P$ MW: 182
CAS#: 96-64-0 NIST#: 226124 ID#: 78999 DB: mainlib

A sample spectrum from this program is provided.

GD (RT 3.6 min) TIC and SPECTRUM (SIM) - GCE DATA ANALYSIS



METHOD J: GC/MS METHOD FOR GD-ACID IDENTIFICATION (GCE GD-ACID_DEANS.M)

ANALYTICAL METHOD

TITLE
Detection of the hydrolysis by-product of GD, Pinacolyl Methylphosphonic Acid (GD-Acid) in liquid extraction samples for chemical agent decontamination testing using a GC/MS with Autosampler.

AUTHORS
Matthew Shue (ECBC Decontamination Sciences Branch)
Teri Lalain, Ph.D. (Branch Chief, Decontamination Sciences Branch)

KEYWORDS
Pinacolyl Methylphosphonic Acid, GD-Acid, Pinacolyl Methylphosphonate CAS 616-52-4

REVISION HISTORY
This document is version 1, released December 2007.

PURPOSE AND APPLICATIONS
The purpose of this method is to detect and identify the hydrolysis by-product of GD, pinacolyl methylphosphonic acid (GD-acid), in liquid extraction samples. This method is optimized to support the analysis of liquid extracts following the chemical agent decontamination performance evaluation testing. Contact sampler and coupon extracts for a 2 in. diameter test surface area are extracted in no greater than 20 mL of extraction solvent. Sample throughput is approximately six samples per hour.

ANALYTE CONCENTRATION RANGE
This method was developed to identify the GD by-product GD-acid. This method can detect and identify GD-acid solution concentrations from ~5,000 to 500,000 ng/mL. NOTE: Due to the large concentrations required, this method is not appropriate for analyzing GD and GD-acid simultaneously.

The method performance for GD-acid is as follows:

- Qualitative Method
- Sample Solvent: acetonitrile.
- Quantitative Ion Pair: 97.

SAMPLE MATRICES AND INTERFERENCES

This method is appropriate for liquid samples extracted with the solvent acetonitrile (CH_3CN; CAS #75-05-8) and containing the GD by-product GD-acid. No significant method interferents have been identified. Users of this method should confirm that the test material and solvent are compatible prior to testing.

RISK AND SAFETY ASSESSMENT

This document does not claim to address all of the safety concerns associated with chemical decontaminant testing as the requirements may vary based on facility, state, and other regulatory requirements. It is the responsibility of the user of this method, prior to use, to establish appropriate environment, health, and safety practices for the use of this method and handling of generated wastes in compliance with applicable regulations. Users of this method should conduct testing in appropriate facilities and follow proper laboratory practices, including the use of appropriate personal protective equipment and material safety data sheets.

SCIENTIFIC BASIS

This method is based on gas chromatography and mass spectrometry techniques.

TRAINING

Users of this method must have the basic skill level for analytical chromatography instrument operation, interpretation of mass spectrometry results, and associated wet lab techniques (e.g., sample dilution and standard preparation). Training covering instrument operation and maintenance of equipment is suggested.

APPARATUS

The liquid extraction samples are analyzed on an Agilent 6890 GC equipped with a 5975 MSD. The GC is outfitted with an Agilent Deans Switch allowing flow switching during analysis so that only a small portion of the sample effluent, including the analyte(s) of interest, is directed to the MSD. All other flow is directed to a FPD. Sample introduction is performed using a GERSTEL MPS2 and a GERSTEL CIS4. The system is fitted with a HP-5MS 5% Phenyl Methyl Siloxane column. Instrumentation operation, maintenance manuals, and miscellaneous and consumable equipment lists are available from the instrument manufacturers: Agilent and GERSTEL. All equipment should be used in accordance with the manufacturer's instructions.

Chemicals required include dilute GD-acid standards prepared in high purity acetonitrile solvent.

Volumetric glassware used for standard preparation should be Class A and meet the specifications in the most current version of ASTM standards E288 and E69. Pipettes used for standard preparation should be compliant with the required performance specifications listed in the most current versions of ISO 8655 Parts 1 and 2 and/or ASTM E 1154 for the volume measured, if standards are made volumetrically. Balances used for gravimetric standard preparation should be sensitive enough to measure the appropriate mass delivered. All equipment should be used in accordance with the manufacturer's requirements, and should be properly maintained and calibrated.

Amber glass analytical sample vials are preferred. The septum cap should be lined with inert material, preferably PTFE/Teflon, to prevent extraction of plasticizers or other impurities into the sample.

PROCEDURE

The specific equipment and column are identified in the "Apparatus" section. Procedures for equipment operation, instrumentation problems, and corrective actions are found in the manufacturer's operating manuals. The specific instrumentation settings for this method are provided in the "Method Parameters" section. The equipment should be confirmed as operational prior to use for test support.

Standards are prepared for calibration either volumetrically or gravimetrically, as required by the method.

Liquid extract samples above the method calibration range are diluted to be within the method calibration range. Extrapolation of results outside the calibrated range is not recommended.

Calibration curves are generated from the standard sample results. The curves are fit and weighted as specified in this method. For quality control, the analysis of test samples must include the use of ICV, CCV, and solvent blanks. Specific guidance for method use for decontaminant performance evaluations is provided in the "2007 Chemical Decontaminant Performance Evaluation Testing Source Document" by T. Lalain, et al. Standard preparation, sample queues, interferences, and other general analytical guidance are provided in the corresponding technical report to this document titled, "Low-Level Analytical Methodology Updates to Support Decontaminant Performance Evaluations" by M. Shue, et al.

DATA ACCEPTANCE & REPORTING

Analytical results for samples are reported as solution concentrations (ng/mL). Solution concentration can be calculated using the instrument control software (e.g., Applied Biosystems Analyst, Chemstation, etc.) or using external statistical software (e.g., Excel, Sigma plot, etc.) with the specified calibration model. The reported solution concentration should account for sample dilutions made post original sample generation (e.g., contact, residual, and remaining agent testing) such that the analyzed sample is within the calibration range. Calibration curves are not extrapolated; samples must fall within the range or be reported as "above calibration range" or "below detection" as appropriate. The reported concentration is acceptable if all QA/QC criteria are met as specified (e.g., CCVs before and after the sample are within 30% RSD).

METHOD J PARAMETERS

"GD-Acid_DEANS.M"
ECBC Decontamination Sciences Branch
GCE Method Summary

INSTRUMENT CONTROL PARAMETERS: GCE (6890GC - 5975MSD)

Control Information
Sample Inlet : GC
Injection Source : External Device
Injection Location : Rear
Mass Spectrometer : Enabled

AGILENT DEANS SWITCH PARAMETERS

METHOD: GCE_DEANS.M

Comment: DEANS Switch Parameters for GC/MS methods on GCE.

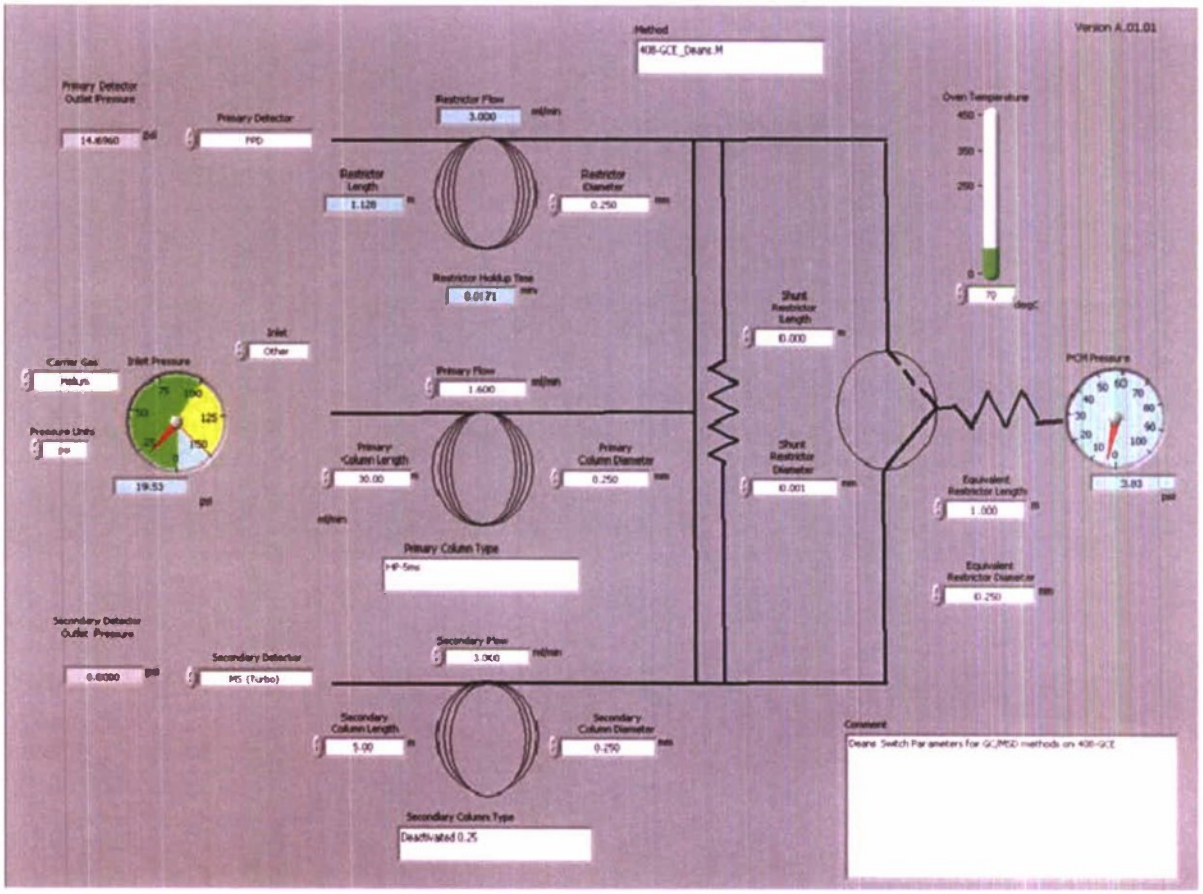
CALCULATION RESULTS:

	Primary Column	Secondary Column
Inlet Pressure:	19.53	3.83
Avg. Linear Velocity (cm/s):	34.72	154.76
Hold up Time (min):	1.44	0.054
Restrictor Length (m):		1.128
Restrictor Hold Up Time (min):		0.017

CALCULATION INPUTS

	Primary Column	Secondary Column
Length (m):	30.00	5.00
i.d. (mm):	0.25	0.25
Flow (mL/min):	1.60	3.00
Detector:	FPD	MS (Turbo)
Detector Pressure (abs):	14.70	0.00
Carrier Gas:		Helium
Pressure Units:		psi
Oven Temperature:		70.00
Restrictor Diameter:		0.25
Equivalent Restrictor Diameter (mm):		0.25
Equivalent Restrictor Length (m):		1.000

DEANS SWITCH CALCULATOR



```
================================================================================
                                GERSTEL CIS
================================================================================
TEMPERATURE PROGRAM
Initial Temperature      : 50 °C
Equilibration Time       : 0.05 min
Initial Time             : 0.20 min
Ramp 1
Rate 1               : 12.0 °C/s
End Temp 1           : 280 °C
Hold Time 1          : 3.00 min
Ramp 2
Rate 2               : 0.0 °C/s

CRYO COOLING
Cryo Cooling             : used


GERSTEL MPS Liquid Injection

Syringe                  : 10 µL

SAMPLE PARAMETERS
Inj. Volume              : 1.0 µL
Inj. Speed               : 25.00 µL/s
Fill Volume              : 10.0 µL
Fill Strokes             : 3

Fill Speed               : 5.00 µL/s
Eject Speed              : 100.00 µL/s
Viscosity Delay         : 1.0 s

Air Volume               : 0.0 µL
Pre Inj. Delay           : 0.00 s
Post Inj. Delay          : 0.00 s
Inj. Penetration         : 40.00 mm
Vial Penetration         : 31.00 mm

CLEANING PARAMETERS
Preclean Sample          : 0

Preclean Solv.1          : 1
Postclean Solv.1         : 2
Fill Speed Solv.1        : 5.00 µL/s
Viscosity Delay Solv.1   : 1.0 s
Eject Speed Solv.1       : 100.00 µL/s

Preclean Solv.2          : 1
Postclean Solv.2         : 2
Fill Speed Solv.2        : 5.00 µL/s
Viscosity Delay Solv.2   : 1.0 s
Eject Speed Solv.2       : 100.00 µL/s
```

6890 GC METHOD

OVEN
Initial temp: 70 °C (On)
Initial time: 0.75 min
Ramps:

#	Rate	Final temp	Final time
1	45.00	280	2.6
2	0.0(Off)		

Post temp: 50 °C
Post time: 0.00 min
Run time: 8.02 min
Maximum temp: 325 °C
Equilibration time: 0.25 min

FRONT INLET (SPLIT/SPLITLESS)
Mode: Split
Initial temp: 200 °C (Off)
Pressure: 0.00 psi (Off)
Total flow: 3.7 mL/min
Gas saver: Off
Gas type: Helium

BACK INLET (CIS3)
Mode: Solvent Vent
Initial temp: 250 °C (Off)
Pressure: 19.53 psi (On)
Vent time: 0.20 min
Vent flow: 20.0 mL/min
Vent Pressure: 19.5 psi
Purge flow: 50.0 mL/min
Purge time: 3.00 min
Total flow: 54.4 mL/min
Gas saver: Off
Gas type: Helium

COLUMN 1
Capillary Column
Model Number: Agilent 19091S-433
HP-5MS 5% Phenyl Methyl Siloxane
Max temperature: 325 °C
Nominal length: 30.0 m
Nominal diameter: 250.00 µm
Nominal film thickness: 0.25 µm
Mode: constant pressure
Pressure: 19.53 psi
Nominal initial flow: 1.7 mL/min
Average velocity: 39 cm/s
Inlet: Back Inlet
Outlet: Other
Outlet pressure: ambient

COLUMN 2
Capillary Column
Model Number: Agilent 19091S-433
HP-5MS 5% Phenyl Methyl Siloxane
Max temperature: 325 °C
Nominal length: 30.0 m
Nominal diameter: 250.00 µm
Nominal film thickness: 0.25 µm
Inlet: (unspecified)
Outlet: Other

FRONT DETECTOR (NO DET)

BACK DETECTOR (FPD)
Temperature: 250 °C (On)
Hydrogen flow: 75.0 mL/min (On)
Oxidizer flow: 100.0 mL/min (On)
Oxidizer Gas Type: Air
Mode: Constant makeup flow
Makeup flow: 25.0 mL/min (On)
Makeup Gas Type: Helium
Flame: On
Lit offset: 2.00
Photo multiplier: On

```
SIGNAL 1
   Data rate:  20 Hz
   Type:  back detector
   Save Data:  On
   Zero:  0.0 (Off)
   Range:  0
   Fast Peaks:  Off
   Attenuation:  0

COLUMN COMP 1
   (No Detectors Installed)

THERMAL AUX 1
   Use:  MSD Transfer Line Heater
   Description:  MSD Transferline
   Initial temp:  280 °C (On)
   Initial time:  0.00 min
      #  Rate  Final temp  Final time
      1   0.0(Off)

AUX PRESSURE 3
   Description:  No vent
   Gas Type:  Helium
   Initial pressure:  0.00 psi (Off)

AUX PRESSURE 5
   Description:
   Gas Type:  Helium
   Initial pressure:  0.00 psi (Off)

VALVES
   Valve 1  Switching On
      Description:

TIME TABLE
   Time       Specifier                Parameter & Setpoint
      5.30       Valve 1:                 On
      6.30       Valve 1:                 Off
```

```
SIGNAL 2
   Data rate:  20 Hz
   Type:  test plot
   Save Data:  Off
   Zero:  0.0 (Off)
   Range:  0
   Fast Peaks:  Off
   Attenuation:  0

COLUMN COMP 2
   (No Detectors Installed)

THERMAL AUX 2
   Unknown Thermal Aux Type

AUX PRESSURE 4
   Description:  Deans Switch
   Gas Type:  Helium
   Initial pressure:  3.80 psi (On)
   Initial time:  0.00 min
      #  Rate  Final pres  Final time
      1   0.0(Off)

POST RUN
   Post Time: 0.00 min
```

```
================================================================================
                        5975 MS ACQUISITION PARAMETERS
================================================================================
General Information
------- -----------

Tune File                 : atune.u
Acquistion Mode           : SIM

MS Information
-- -----------

Solvent Delay             : 5.00 min

EM Absolute               : False
EM Offset                 : 400
Resulting EM Voltage      : 2400.0*

[Sim Parameters]
GROUP 1
Group ID                  : GD-Acid
Resolution                : High
Plot 1 Ion                : 97.00
Ions/Dwell In Group
(Mass, Dwell)
(41.00, 75)
(80.00, 75)
(97.00, 75)
(124.00, 75)

[MSZones]
MS Quad                   : 150 C   maximum 200 C
MS Source                 : 230 C   maximum 250 C
                     END OF MS ACQUISITION PARAMETERS

                           TUNE PARAMETERS*
                           ----------------


*MSD Specific Values automatically determined and set when performing an
Autotune.

                          END OF TUNE PARAMETERS
                          ----------------------


END OF INSTRUMENT CONTROL PARAMETERS
```

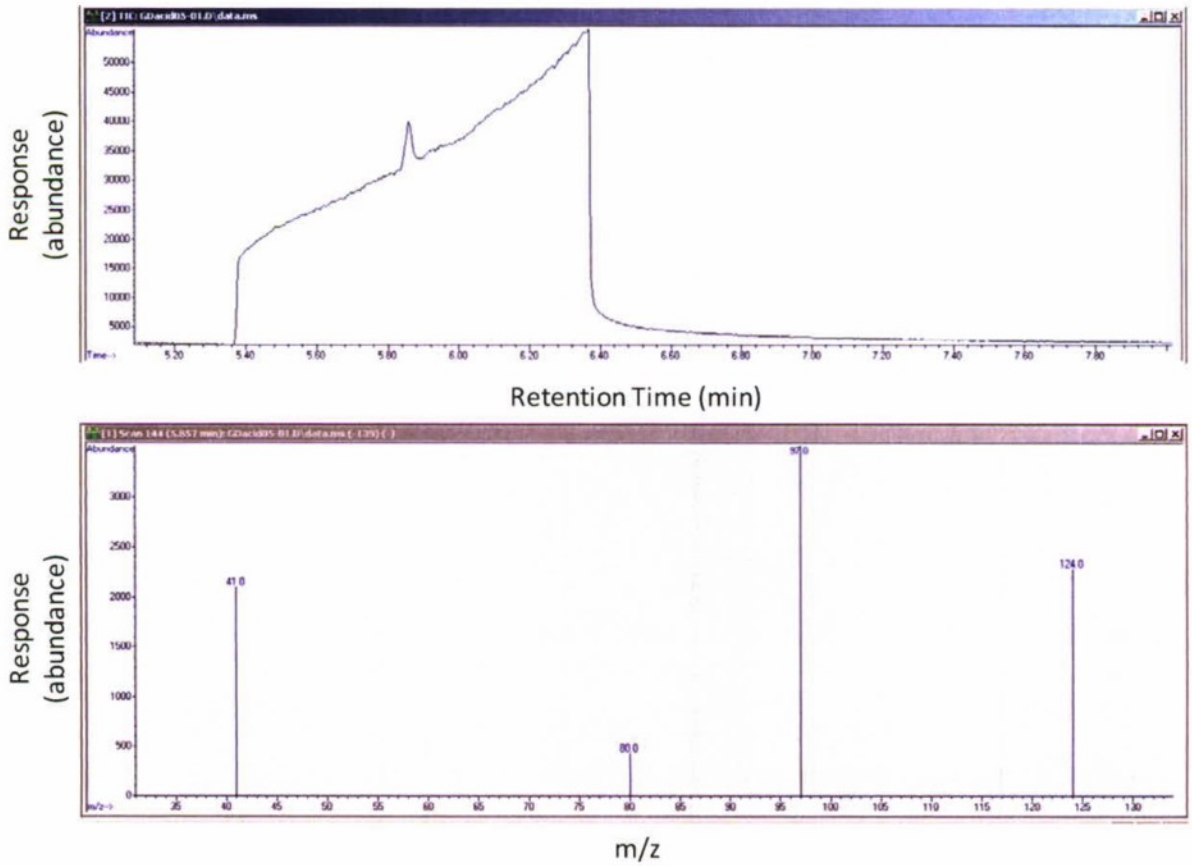
Pinacolyl Methylphosphonic Acid** (SCAN) - NIST LIBRARY SEARCH 2.0

The NIST library contains a sample spectrum, which can be found as identified below:

Name: Methylphosphonic acid, 1,2,2,-trimethylpropyl ester Formula: $C_7H_{17}O_3P$
MW: 180 CAS#: 616-52-4 NIST#: 273479 ID#: 54739 DB: mainlib
**Identified in NIST Library by CAS Number.

A sample spectrum from this program is provided.

GD-Acid (RT 5.9 min) TIC and SPECTRUM (SIM)-GCE DATA ANALYSIS



FINAL VAPOR SAMPLE ANALYSIS METHODOLOGY

The following methodology represents the final methodology and parameters for the vapor sample analyses detailed in this technical report.

METHOD K: GC/MS METHOD FOR LOW-LEVEL VX VAPOR SAMPLE ANALYSIS (GCV VXLL-DEANS.M)

ANALYTICAL METHOD

TITLE

Detection of VX as the G-analog in vapor samples for chemical agent decontamination testing using a GC/MSD with a thermal desorption unit.

AUTHORS

Matthew Shue (ECBC Decontamination Sciences Branch)
Morgan Hall (ECBC)
Teri Lalain, Ph.D. (Branch Chief, ECBC Decontamination Sciences Branch)

KEYWORDS

VX, O-Ethyl S-2-diisopropylaminoethyl methyl phosphonothiolate, CAS 50782-69-9.

G-analog, Ethyl methylphosphonofluoridate, CAS 673-97-2

REVISION HISTORY

This document is version 1, released December 2007.

PURPOSE AND APPLICATIONS

The purpose of this method is to detect and quantify the chemical agent VX as ethyl methylphosphonofluoridate (G-analog) in vapor samples. This method is based on the collection of vapor samples on tubes containing solid sorbent material following chemical agent decontamination performance evaluation testing. The method is optimized for the detection requirements for the analysis of a 2 in. diameter test surface area. It is anticipated that this method can support larger item vapor testing. Sample throughput is approximately four samples per hour.

ANALYTE CONCENTRATION RANGE

This method was developed to detect and quantify the chemical agent VX as ethyl methylphosphonofluoridate (G-analog) in vapor samples. This method can detect VX as the G-analog with a mass-on-column range of 0.5 to 100 ng. Sample collection parameters are adjusted to collect vapor samples in this mass range. V-to-G conversion pads are required to convert VX to the G-analog. G-analog is collected/spiked onto tubes containing the sorbent Chromosorb-106.

This method utilizes nine calibration levels at masses of ~0.5, 0.5, 0.5, 1, 2.5, 5, 10, 50, and 100 ng VX. The method use, detection limit, and quantitation limit are based on using the full standard set. The standards are prepared in isopropyl alcohol.

The method performance for VX (G-analog). LL is as follows:

- Quantitative Method
- Calibration Range: 0.5 to 100 ng VX (G-analog) on column
- CCV Levels: 0, 10, 10 ng
- Calibration Model: Weighted linear regression
- Calibration Weighting: 1/x2
- Limit of Detection (LOD): 0.14 ng
- Limit of Quantitation (LOQ): 0.46 ng
- Spiking sample Solvent: isopropyl alcohol
- Quantitative Ion: 99

SAMPLE MATRICES AND INTERFERENCES

This method is appropriate for vapor samples containing the chemical agent VX as the G-analog. No significant method interferents have been identified. Users of this method should confirm that the test material does not off gas an interferent in the detection region of interest.

RISK AND SAFETY ASSESSMENT

This document does not claim to address all of the safety concerns associated with chemical decontaminant testing as the requirements may vary based on facility, state and other regulatory requirements. It is the responsibility of the user of this method, prior to use, to establish appropriate environment, health, and safety practices for the use of this method and handling of generated wastes in compliance with applicable regulations. Users of this method should conduct testing in appropriate facilities and follow proper laboratory practices, including the use of appropriate personal protective equipment and material safety data sheets.

SCIENTIFIC BASIS

This method is based on gas chromatography and mass spectrometry techniques.

TRAINING

Users of this method must have the basic skill level for analytical chromatography instrument operation, interpretation of mass spectrometry results and associated wet lab techniques (e.g., sample dilution and standard preparation). Training covering instrument operation and maintenance of equipment is suggested.

APPARATUS

The vapor samples are analyzed on an Agilent 6890 GC equipped with a 5975 MSD. The GC is outfitted with an Agilent Deans Switch allowing for flow switching during analysis so that only a small portion of the sample effluent, including the analyte of interest, is directed to the MSD. All other flow is directed to a FPD. Sample introduction of a vapor sample is performed using a MARKES Thermal Desorption System (TDS). The system is fitted with a HP-5MS 5% Phenyl Methyl Siloxane column. Instrumentation operation, maintenance manuals, and miscellaneous and consumable equipment lists are available from the instrument manufacturers: Agilent and

MARKES. All equipment should be used in accordance with the manufacturer's instructions.

Chemicals required include dilute VX standards prepared in high purity isopropyl alcohol solvent.

Volumetric glassware used for standard preparation should be Class A and meet the specifications in the most current version of ASTM standards E288 and E69. Pipettes used for standard preparation should be compliant with the required performance specifications listed in the most current versions of ISO 8655 Parts 1 and 2 and/or ASTM E 1154 for the volume measured, if standards are made volumetrically. Balances used for gravimetric standard preparation should be sensitive enough to measure the appropriate mass delivered. All equipment should be used in accordance with the manufacturer's requirements, and should be properly maintained and calibrated.

Amber glass analytical sample vials are preferred. The septum cap should be lined with inert material, preferably PTFE/Teflon, to prevent extraction of plasticizers or other impurities into the sample.

PROCEDURE

The specific equipment and column are identified in the "Apparatus" section. Procedures for equipment operation, instrumentation problems, and corrective actions are found in the manufacturer's operating manuals. The specific instrumentation settings for this method are provided in the "Method Parameters" section. The equipment should be confirmed as operational prior to use for test support.

Standards are prepared for calibration either volumetrically or gravimetrically as required by the method.

The mass-of-agent on tube should be estimated for vapor samples to minimize saturation of the detector.

Calibration curves are generated from the standard sample results. The curves are fit and weighted as specified in this method. For quality control, the analysis of test samples must include the use of ICV, CCV, and solvent blanks for quality control. Specific guidance for method use for decontaminant performance evaluations is provided in the "2007 Chemical Decontaminant Performance Evaluation Testing Source Document" by T. Lalain, et al. Standard preparation, tube spiking, sample queues, interferences, and other general analytical guidance are provided in the corresponding technical report to this document titled, "Low-Level Analytical Methodology Updates to Support Decontaminant Performance Evaluations" by M. Shue, et al.

DATA ACCEPTANCE & REPORTING

Analytical results for samples are reported as mass on tube (ng). Mass on tube can be calculated using the instrument control software (e.g., Chemstation, etc.) or using external statistical software (e.g. Excel, Sigma plot, etc.) with the specified calibration model. Calibration curves are not extrapolated; samples must fall within the range or be reported as "above calibration range" or "below detection" as appropriate. The reported mass is acceptable if all QA/QC criteria are met as specified (e.g., CCVs before and after the sample are within 30% RSD).

METHOD K PARAMETERS

"VXLL-DEANS.M"
ECBC Decontamination Sciences Branch
GCV Method Summary

NOTE: VX is analyzed as the G-analog.

INSTRUMENT CONTROL PARAMETERS: GCV (6890GC - 5975MSD)

Control Information

Sample Inlet : GC
Injection Source : External Device
Mass Spectrometer : Enabled

AGILENT DEANS SWITCH PARAMETERS

METHOD: GCV_DEANS.M

Comment: Deans Switch Parameters for GCV w/ Markes Thermal Desorption System.

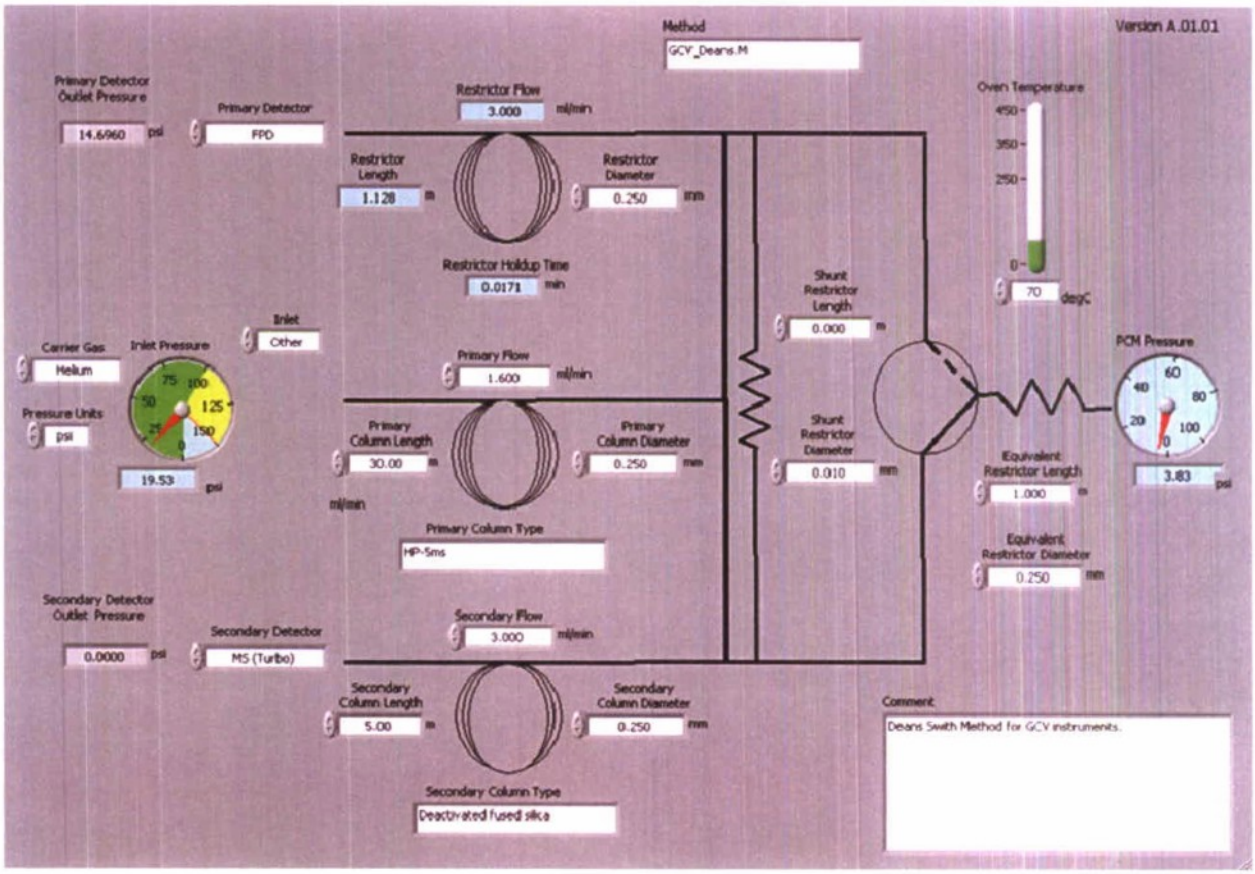
CALCULATION RESULTS:

	Primary Column	Secondary Column
Inlet Pressure:	19.53	3.83
Avg. Linear Velocity (cm/s):	34.72	154.76
Hold up Time (min):	1.44	0.054
Restrictor Length (m):		1.128
Restrictor Hold Up Time (min):		0.017

CALCULATION INPUTS

	Primary Column	Secondary Column
Length (m):	30.00	5.00
i.d. (mm):	0.25	0.25
Flow (mL/min):	1.60	3.00
Detector:	FPD	MS (Turbo)
Detector Pressure (abs):	14.70	0.00
Carrier Gas:		Helium
Pressure Units:		psi
Oven Temperature:		70.00
Restrictor Diameter:		0.25
Equivalent Restrictor Diameter (mm):		0.25
Equivalent Restrictor Length (m):		1.000

DEANS SWITCH CALCULATOR



MARKES TDS UNITY METHOD

UNITY Method Name = VXLL.mth

Method Type:	Standard 2(3) stage desorption
Standby Split:	ON
Standby Flow Rate (mL/min):	20.0
PrePurge Time (min):	0.5
PrePurge Trap In Line:	Not in line
PrePurge Split:	ON
PrePurge Flow Rate (mL/min):	40.0
Tube Desorb Time 1 (min):	4.5
Tube Desorb Temp 1 (°C):	45
Tube Desorb Split 1 On (mL/min):	40.0
Tube Desorb Time 2 (min):	5.0
Tube Desorb Temp 2 (°C):	200
Tube Desorb Split 2 On (mL/min):	No split
Trap Desorb Low Temp (°C):	30
Trap Desorb High Temp (°C):	220
Trap Desorb Hold Time (min):	3.0
Trap Desorb Split On:	No split
Trap Heating Rate (°C/s):	MAX
Flow Path Temp ((°C):	180
GC Cycle Time (min):	7.5
Minimum Carrier Pressure (psi):	5.0
Split Ratios:	Splitless
Desorb Flow Rate (mL/min):	40.0

Cold Trap Info: Chemical Weapon Agent Trap (Markes P/N U-T10CW)

```
================================================================================
                                6890 GC METHOD
================================================================================

OVEN
   Initial temp:  70 °C (On)                  Maximum temp:  300 °C
   Initial time:  1.50 min                    Equilibration time:  0.25 min
   Ramps:
      # Rate Final temp Final time
      1 15.00     90          0.00
      2 30.00     220         0.00
   Post temp:  70 °C
   Post time:  0.00 min
   Run time:  7.17 min

FRONT INLET (SPLIT/SPLITLESS)              BACK INLET (UNKNOWN)
   Mode:  Splitless
   Initial temp:  250 °C (On)
   Pressure:  19.53 psi (On)
   Purge flow:  50.0 mL/min
   Purge time:  999.99 min
   Total flow:  56.1 mL/min
   Gas saver:  Off
   Gas type:  Helium

*Markes modified inlet may be front or back depending on inlet availability at
install.


COLUMN 1                                   COLUMN 2
   Capillary Column                           (not installed)
   Model Number:  Agilent 19091S-433
   HP-5MS  5% Phenyl Methyl Siloxane
   Max temperature:  325 °C
   Nominal length:  30.0 m
   Nominal diameter:  250.00 µm
   Nominal film thickness:  0.25 µm
   Mode:  constant pressure
   Pressure:  19.53 psi
   Nominal initial flow:  1.7 mL/min
   Average velocity:  39 cm/s
   Inlet:  Front Inlet
   Outlet:  Other
   Outlet pressure:  ambient

FRONT DETECTOR (NO DET)                    BACK DETECTOR (FPD)
                                              Temperature:  220 °C (On)
                                              Hydrogen flow:  75.0 mL/min (On)
                                              Oxidizer flow:  100.0 mL/min (On)
                                              Oxidizer Gas Type: Air
                                              Mode:  Constant makeup flow
                                              Makeup flow:  25.0 mL/min (On)
                                              Makeup Gas Type: Helium
                                              Flame:  On
                                              Lit offset:  2.00
                                              Photo multiplier:  On
```

```
SIGNAL 1
   Data rate:  20 Hz
   Type:  back detector
   Save Data:  On
   Zero:  0.0 (Off)
   Range:  0
   Fast Peaks:  Off
   Attenuation:  0

SIGNAL 2
   Data rate:  20 Hz
   Type:  test plot
   Save Data:  Off
   Zero:  0.0 (Off)
   Range:  0
   Fast Peaks:  Off
   Attenuation:  0

COLUMN COMP 1
   (No Detectors Installed)

COLUMN COMP 2
   (No Detectors Installed)

THERMAL AUX 1
   Use:  MSD Transfer Line Heater
   Description:
   Initial temp:  280 °C (On)
   Initial time:  0.00 min
      #  Rate  Final temp  Final time
      1   0.0(Off)

THERMAL AUX 2
   Unknown Thermal Aux Type

AUX PRESSURE 3
   Description: Deans Switch
   Gas Type:  Helium
   Initial pressure:  3.83 psi (On)
   Initial time:  0.00 min
      #  Rate  Final pres  Final time
      1   0.0(Off)

AUX PRESSURE 4
   Description:
   Gas Type:  Helium
   Initial pressure:  0.0 psi (Off)

AUX PRESSURE 5
   Description:
   Gas Type:  Helium
   Initial pressure:  0.00 psi (Off)

VALVES
   Valve 1  Switching Off
      Description:

POST RUN
   Post Time: 0.00 min

TIME TABLE
   Time       Specifier                  Parameter & Setpoint
      2.00       Valve 1:                   On
      3.00       Valve 1:                   Off

                              GC Injector
     Front Injector:
No parameters specified

     Back Injector:
No parameters specified

 Column 1 Inventory Number : AB002
 Column 2 Inventory Number :
```

MS ACQUISITION PARAMETERS

General Information
------- -----------
Tune File : atune.u
Acquistion Mode : SIM

MS Information
-- -----------
Solvent Delay : 1.80 min

EMV Mode : Gain Factor
Gain Factor : 1.0
Resulting EM Voltage : 2047*

[Sim Parameters]

GROUP 1
Group ID : VX
Resolution : High
Plot 1 Ion : 99.00
Ions/Dwell In Group

(Mass, Dwell)
(81.00, 50)
(99.00, 50)
(111.00, 50)

[MSZones]

MS Quad : 150 C maximum 200 C
MS Source : 230 C maximum 250 C
Timed Events
----- ------

[Timed MS Detector Entries]

Time (min) State (MS on/off)
3.00 Off

TUNE PARAMETERS*
---

*MSD Specific Values automatically determined and set when performing an Autotune.

END OF TUNE PARAMETERS

END OF INSTRUMENT CONTROL PARAMETERS

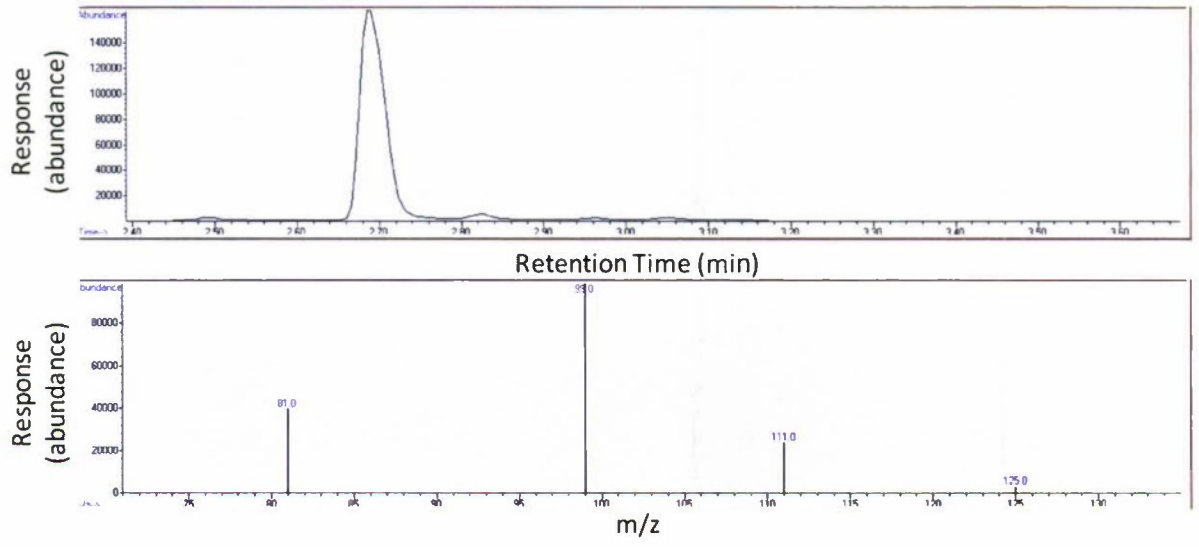
G-ANALOG SPECTRUM (SCAN) - NIST LIBRARY SEARCH 2.0

The NIST library contains a sample spectrum, which can be found as identified below:

Name: Ethyl methylphosphonofluoridate (G-analog) Formula: $C_3H_8FO_2P$
MW: 126 CAS#: 673-97-2 NIST#: 226118 ID#: 56237 DB: mainlib

A sample spectrum from this program is provided.

G-ANALOG (RT 2.7 min) TIC and SPECTRUM (SIM) - GCV DATA ANALYSIS



METHOD L: GC/MS METHOD FOR HIGH-LEVEL VX VAPOR SAMPLE ANALYSIS (GCV VXHL-DEANS.M)

ANALYTICAL METHOD

TITLE
Detection of VX as the G-analog in vapor samples for chemical agent decontamination testing using a GC/MS with a thermal desorption unit.

AUTHORS
Matthew Shue (ECBC Decontamination Sciences Branch)
Morgan Hall (ECBC)
Teri Lalain, Ph.D. (Branch Chief, ECBC Decontamination Sciences Branch)

KEYWORDS
VX, O-Ethyl S-2-diisopropylaminoethyl methyl phosphonothiolate, CAS 50782-69-9.

G-analog, Ethyl methylphosphonofluoridate, CAS 673-97-2

REVISION HISTORY
This document is version 1, released December 2007.

PURPOSE AND APPLICATIONS
The purpose of this method is to detect and quantify the chemical agent VX as ethyl methylphosphonofluoridate (G-analog) in vapor samples. This method is based on the collection of vapor samples on tubes containing solid sorbent material following chemical agent decontamination performance evaluation testing. The method is optimized for the detection requirements for the analysis of a 2 in. diameter test surface area. It is anticipated that this method can support larger item vapor testing. Sample throughput is approximately four samples per hour.

ANALYTE CONCENTRATION RANGE
This method is a complementary method to the low-level method for the detection of higher masses of VX from vapor sorbent tube samples. This method can detect VX as the G-analog with a mass on column range of 100 to 1500 ng. Sample collection parameters are adjusted to collect vapor samples in this mass range. V-to-G conversion pads are required to convert VX to the G-analog. G-analog is collected/spiked onto tubes containing the sorbent Chromosorb-106.

This method utilizes seven calibration levels at masses of ~50, 100, 250, 500, 750, 1000, and 1500 ng VX. The method use, detection limit, and quantitation limit are based on using the full standard set. The standards are prepared in isopropyl alcohol.

The method performance for VX (G-analog) HL is as follows:

- Quantitative Method
- Calibration Range: 50 to 1500 ng VX (G-analog) on column

- CCV Levels: 0, 500, 500 ng
- Calibration Model: Weighted linear regression
- Calibration Weighting: $1/x^2$
- Limit of Detection (LOD): 1.34 ng
- Limit of Quantitation (LOQ): 4.47 ng
- Spiking Sample Solvent: isopropyl alcohol
- Quantitative Ion: 99

SAMPLE MATRICES AND INTERFERENCES

This method is appropriate for vapor samples containing the chemical agent VX as the G-analog. No significant method interferents have been identified. Users of this method should confirm that the test material does not off gas an interferent in the detection region of interest.

RISK AND SAFETY ASSESSMENT

This document does not claim to address all of the safety concerns associated with chemical decontaminant testing as the requirements may vary based on facility, state, and other regulatory requirements. It is the responsibility of the user of this method, prior to use, to establish appropriate environment, health and safety practices for the use of this method and handling of generated wastes in compliance with applicable regulations. Users of this method should conduct testing in appropriate facilities and follow proper laboratory practices, including the use of appropriate personal protective equipment and material safety data sheets.

SCIENTIFIC BASIS

This method is based on gas chromatography and mass spectrometry techniques.

TRAINING

Users of this method must have the basic skill level for analytical chromatography instrument operation, interpretation of mass spectrometry results, and associated wet lab techniques (e.g., sample dilution and standard preparation). Training covering instrument operation and maintenance of equipment is suggested.

APPARATUS

The vapor samples are analyzed on an Agilent 6890 GC equipped with a 5975 MSD. The GC is outfitted with an Agilent Deans Switch allowing for flow switching during analysis so that only a small portion of the sample effluent, including the analyte of interest, is directed to the MSD. All other flow is directed to a FPD. Introduction of a vapor sample is performed using a MARKES TDS. The system is fitted with a HP-5MS 5% phenyl methyl siloxane column. Instrumentation operation, maintenance manuals, and miscellaneous and consumable equipment lists are available from the instrument manufacturers: Agilent and MARKES. All equipment should be used in accordance with the manufacturer's instructions.

Chemicals required include dilute VX standards, prepared in high purity isopropyl alcohol solvent.

Volumetric glassware used for standard preparation should be Class A and meet the

specifications in the most current version of ASTM standards E288 and E69. Pipettes used for standard preparation should be compliant with the required performance specifications listed in the most current versions of ISO 8655 Parts 1 and 2 and/or ASTM E 1154 for the volume measured, if standards are made volumetrically. Balances used for gravimetric standard preparation should be sensitive enough to measure the appropriate mass delivered. All equipment should be used in accordance with the manufacturer's requirements, and should be properly maintained and calibrated.

Amber glass analytical sample vials are preferred. The septum cap should be lined with inert material, preferably PTFE/Teflon, to prevent extraction of plasticizers or other impurities into the sample.

PROCEDURE

The specific equipment and column are identified in the "Apparatus" section. Procedures for equipment operation, instrumentation problems, and corrective actions are found in the manufacturer's operating manuals. The specific instrumentation settings for this method are provided in the "Method Parameters" section. The equipment should be confirmed as operational prior to use for test support.

Standards are prepared for calibration either volumetrically or gravimetrically, as calibration required by the method.

The mass-of-agent on tube should be estimated for vapor samples to minimize saturation of the detector.

Calibration curves are generated from the standard sample results. The curves are fit and weighted as specified in this method. For quality control, the analysis of test samples must include the use of ICV, CCV, and solvent blanks for quality control. Specific guidance for method use for decontaminant performance evaluations is provided in the "2007 Chemical Decontaminant Performance Evaluation Testing Source Document" by T. Lalain, et al. Standard preparation, tube spiking, sample queues, interferences, and other general analytical guidance are provided in the corresponding technical report to this document titled, "Low-Level Analytical Methodology Updates to Support Decontaminant Performance Evaluations" by M. Shue, et al.

DATA ACCEPTANCE & REPORTING

Analytical results for samples are reported as mass on tube (ng). Mass on tube can be calculated using the instrument control software (e.g., Chemstation, etc.) or using external statistical software (e.g., Excel, Sigma plot, etc.) with the specified calibration model. Calibration curves are not extrapolated; samples must fall within the range or be reported as "above calibration range" or "below detection" as appropriate. The reported mass is acceptable if all QA/QC criteria are met as specified (e.g., CCVs before and after the sample are within 30% RSD).

METHOD L PARAMETERS

"VXHL-DEANS.M"
ECBC Decontamination Sciences Branch
GCV Method Summary

NOTE: VX is analyzed as the G-analog.

INSTRUMENT CONTROL PARAMETERS: GCV (6890GC - 5975MSD)

Control Information

Sample Inlet : GC
Injection Source : External Device
Mass Spectrometer : Enabled

AGILENT DEANS SWITCH PARAMETERS

METHOD: GCV_DEANS.M

Comment: Deans Switch Parameters for GCV w/ Markes Thermal Desorption System.

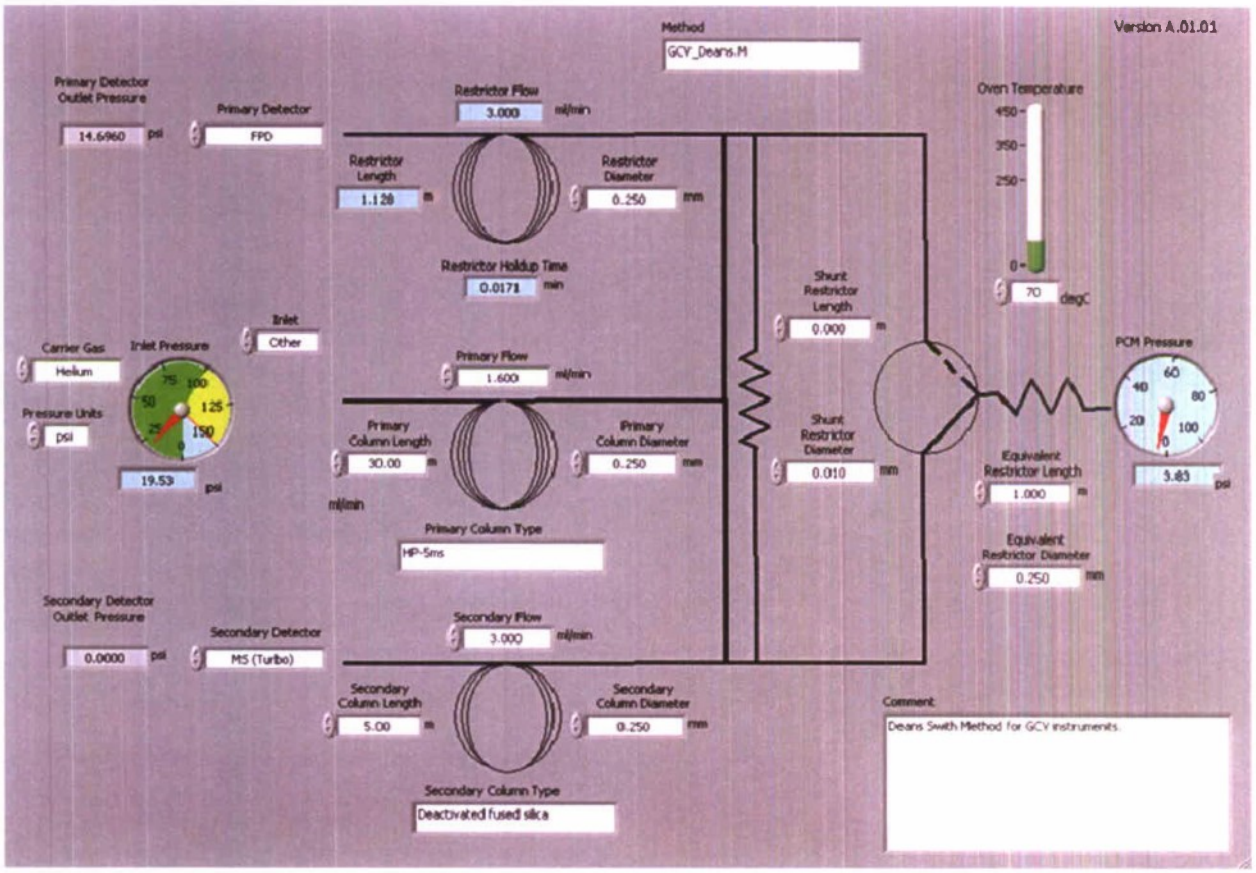
CALCULATION RESULTS:

	Primary Column	Secondary Column
Inlet Pressure:	19.53	3.83
Avg. Linear Velocity (cm/s):	34.72	154.76
Hold up Time (min):	1.44	0.054
Restrictor Length (m):		1.128
Restrictor Hold Up Time (min):		0.017

CALCULATION INPUTS

	Primary Column	Secondary Column
Length (m):	30.00	5.00
i.d. (mm):	0.25	0.25
Flow (mL/min):	1.60	3.00
Detector:	FPD	MS (Turbo)
Detector Pressure (abs):	14.70	0.00
Carrier Gas:		Helium
Pressure Units:		psi
Oven Temperature:		70.00
Restrictor Diameter:		0.25
Equivalent Restrictor Diameter (mm):		0.25
Equivalent Restrictor Length (m):		1.000

DEANS SWITCH CALCulATOR



MARKES TDS UNITY METHOD

UNITY Method Name = VXHL.mth

Method Type:	Standard 2(3) stage desorption
Standby Split:	ON
Standby Flow Rate (mL/min):	20.0
PrePurge Time (min):	0.5
PrePurge Trap In Line:	Not in line
PrePurge Split:	ON
PrePurge Flow Rate (mL/min):	40.0
Tube Desorb Time 1 (min):	1.0
Tube Desorb Temp 1 (°C):	45
Tube Desorb Split 1 On (mL/min):	40.0
Tube Desorb Time 2 (min):	3.5
Tube Desorb Temp 2 (°C):	200
Tube Desorb Split 2 On (mL/min):	40.0
Trap Desorb Low Temp (°C):	30
Trap Desorb High Temp (°C):	220
Trap Desorb Hold Time (min):	3.0
Trap Desorb Split On (mL/min):	11.0
Trap Heating Rate (°C/s):	MAX
Flow Path Temp ((°C):	180
GC Cycle Time (min):	7.5
Minimum Carrier Pressure (psi):	5.0
Split Ratios*:	14.9:1
Desorb Flow Rate (mL/min):	40.0

Cold Trap Info: Chemical Weapon Agent Trap (Markes P/N U-T10CW)

* Split ratio is calculated based on the column flow of 1.7 mL/min, the desorb flow of 40 mL/min and the inlet/outlet split flow rates of 40.0 and 11.0 mL/min, respectively.

```
================================================================================
                                  6890 GC METHOD
================================================================================

OVEN
   Initial temp:  70 °C (On)                    Maximum temp:  300 °C
   Initial time:  1.50 min                      Equilibration time:  0.25 min
   Ramps:
      # Rate Final temp Final time
      1 15.00     90          0.00
      2 30.00     220         0.00
   Post temp:  70 °C
   Post time:  0.00 min
   Run time:  7.17 min

FRONT INLET (SPLIT/SPLITLESS)                BACK INLET (UNKNOWN)
   Mode:  Splitless
   Initial temp:  250 °C (On)
   Pressure:  19.53 psi (On)
   Purge flow:  50.0 mL/min
   Purge time:  999.99 min
   Total flow:  56.1 mL/min
   Gas saver:  Off
   Gas type:  Helium

*Markes modified inlet may be front or back depending on inlet availability at
install.


COLUMN 1                                     COLUMN 2
   Capillary Column                             (not installed)
   Model Number:  Agilent 19091S-433
   HP-5MS  5% Phenyl Methyl Siloxane
   Max temperature:  325 °C
   Nominal length:  30.0 m
   Nominal diameter:  250.00 µm
   Nominal film thickness:  0.25 µm
   Mode:  constant pressure
   Pressure:  19.53 psi
   Nominal initial flow:  1.7 mL/min
   Average velocity:  39 cm/s
   Inlet:  Front Inlet
   Outlet:  Other
   Outlet pressure:  ambient

FRONT DETECTOR (NO DET)                      BACK DETECTOR (FPD)
                                                Temperature:  220 °C (On)
                                                Hydrogen flow:  75.0 mL/min (On)
                                                Oxidizer flow:  100.0 mL/min (On)
                                                Oxidizer Gas Type: Air
                                                Mode:  Constant makeup flow
                                                Makeup flow:  25.0 mL/min (On)
                                                Makeup Gas Type: Helium
                                                Flame:  On
                                                Lit offset:  2.00
                                                Photo multiplier:  On
```

```
SIGNAL 1
   Data rate:  20 Hz
   Type:  back detector
   Save Data:  On
   Zero:  0.0 (Off)
   Range:  0
   Fast Peaks:  Off
   Attenuation:  0

SIGNAL 2
   Data rate:  20 Hz
   Type:  test plot
   Save Data:  Off
   Zero:  0.0 (Off)
   Range:  0
   Fast Peaks:  Off
   Attenuation:  0

COLUMN COMP 1
   (No Detectors Installed)

COLUMN COMP 2
   (No Detectors Installed)

THERMAL AUX 1
   Use:  MSD Transfer Line Heater
   Description:
   Initial temp:  280 °C (On)
   Initial time:  0.00 min
      #  Rate  Final temp  Final time
      1   0.0(Off)

THERMAL AUX 2
   Unknown Thermal Aux Type

AUX PRESSURE 3
   Description: Deans Switch
   Gas Type:  Helium
   Initial pressure:  3.83 psi (On)
   Initial time:  0.00 min
      #  Rate  Final pres  Final time
      1   0.0(Off)

AUX PRESSURE 4
   Description:
   Gas Type:  Helium
   Initial pressure:  0.0 psi (Off)

AUX PRESSURE 5
   Description:
   Gas Type:  Helium
   Initial pressure:  0.00 psi (Off)

VALVES
   Valve 1  Switching Off
      Description:

POST RUN
   Post Time: 0.00 min

TIME TABLE
   Time       Specifier                  Parameter & Setpoint
      2.00       Valve 1:                   On
      3.00       Valve 1:                   Off

                              GC Injector
     Front Injector:
No parameters specified

     Back Injector:
No parameters specified

 Column 1 Inventory Number : AB002
 Column 2 Inventory Number :
```

MS ACQUISITION PARAMETERS

General Information
------- -----------
Tune File : atune.u
Acquistion Mode : SIM

MS Information
-- -----------
Solvent Delay : 1.80 min

EMV Mode : Gain Factor
Gain Factor : 1.0
Resulting EM Voltage : 2047*

[Sim Parameters]

GROUP 1
Group ID : VX
Resolution : High
Plot 1 Ion : 99.00
Ions/Dwell In Group

(Mass, Dwell)
(81.00, 50)
(99.00, 50)
(111.00, 50)

[MSZones]

MS Quad : 150 C maximum 200 C
MS Source : 230 C maximum 250 C
Timed Events
----- ------

[Timed MS Detector Entries]

Time (min) State (MS on/off)
3.00 Off

TUNE PARAMETERS*
----

*MSD Specific Values automatically determined and set when performing an Autotune.

END OF TUNE PARAMETERS

END OF INSTRUMENT CONTROL PARAMETERS

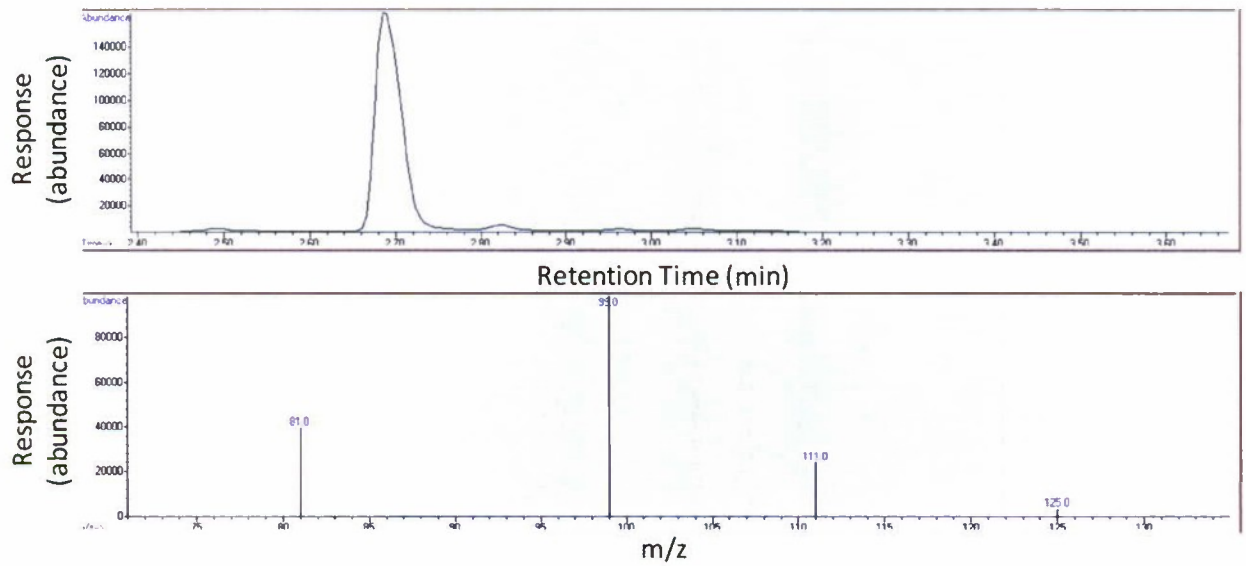
G-ANALOG SPECTRUM (SCAN) - NIST LIBRARY SEARCH 2.0

The NIST library contains a sample spectrum, which can be found as identified below:

Name: Ethyl methylphosphonofluoridate (G-analog) Formula: $C_3H_8FO_2P$
MW: 126 CAS#: 673-97-2 NIST#: 226118 ID#: 56237 DB: mainlib

A sample spectrum from this program is provided.

G-ANALOG (RT 2.7 min) TIC and SPECTRUM (SIM) - GCV DATA ANALYSIS



METHOD M: GC/MS METHOD FOR LOW-LEVEL HD VAPOR SAMPLE ANALYSIS (GCV HDLL-DEANS.M)

ANALYTICAL METHOD

TITLE
Detection of HD in vapor samples for chemical agent decontamination testing using a GC/MS with a thermal desorption unit.

AUTHORS
Matthew Shue (ECBC Decontamination Sciences Branch)
Morgan Hall (ECBC)
Teri Lalain, Ph.D. (Branch Chief, ECBC Decontamination Sciences Branch)

KEYWORDS
Mustard, HD, Bis(2-chloroethyl)sulfide CAS 505-60-2

REVISION HISTORY
This document is version 1, released December 2007.

PURPOSE AND APPLICATIONS
The purpose of this method is to detect and quantify the chemical agent HD in vapor samples. This method is based on the collection of vapor samples on tubes containing solid sorbent material following chemical agent decontamination performance evaluation testing. The method is optimized for the detection requirements for the analysis of a 2 in. diameter test surface area. It is anticipated that this method can support larger item vapor testing. Sample throughput is approximately four samples per hour.

ANALYTE CONCENTRATION RANGE
This method is for the analysis of vapor sorbent tube samples for low-level HD. This HD low-level method can detect HD with a mass on column range of 0.5 to 100 ng. Sample collection parameters are adjusted to collect vapor samples in this mass range. No sample split on the Markes unit is needed. HD is collected/spiked onto tubes containing the sorbent Tenax-TA.

This method utilizes nine calibration levels at masses of 0.5, 0.5, 0.5, 1, 2.5, 5, 10, 50, and 100 ng HD. The method use, detection limit, and quantitation limit are based on using the full standard set. The standards are prepared in chloroform.

The method performance for HD LL is as follows:

- Quantitative Method
- Calibration Range: 0.5 to 100 ng HD on column.
- CCV Levels: 0, 10, 10 ng

- Calibration Model: Weighted quadratic calibration model
- Calibration Weighting: $1/x^2$
- Limit of Detection (LOD): 0.13 ng
- Limit of Quantitation (LOQ): 0.44 ng
- Spiking Sample Solvent: chloroform.
- Quantitative Ion: 111

SAMPLE MATRICES AND INTERFERENCES

This method is appropriate for vapor samples containing the chemical agent HD. No significant method interferents have been identified. Users of this method should confirm that the test material does not off gas an interferent in the detection region of interest.

RISK AND SAFETY ASSESSMENT

This document does not claim to address all of the safety concerns associated with chemical decontaminant testing as the requirements may vary based on facility, state, and other regulatory requirements. It is the responsibility of the user of this method, prior to use, to establish appropriate environment, health, and safety practices for the use of this method and handling of generated wastes in compliance with applicable regulations. Users of this method should conduct testing in appropriate facilities and follow proper laboratory practices, including the use of appropriate personal protective equipment and material safety data sheets.

SCIENTIFIC BASIS

This method is based on gas chromatography and mass spectrometry techniques.

TRAINING

Users of this method must have the basic skill level for analytical chromatography instrument operation, interpretation of mass-spectrometry results and associated wet lab techniques (e.g., sample dilution and standard preparation). Training covering instrument operation and maintenance of equipment is suggested.

APPARATUS

The vapor samples are analyzed on an Agilent 6890 GC equipped with a 5975 MSD. The GC is outfitted with an Agilent Deans Switch allowing for flow switching during analysis so that only a small portion of the sample effluent, including the analyte of interest, is directed to the MSD. All other flow is directed to a FPD. Sample introduction of a vapor sample is performed using a MARKES TDS. The system is fitted with a HP-5MS 5% phenyl methyl siloxane column. Instrumentation operation, maintenance manuals, and miscellaneous and consumable equipment lists are available from the instrument manufacturers: Agilent and MARKES. All equipment should be used in accordance with the manufacturer's instructions.

Chemicals required include dilute HD standards prepared in high purity hexane or chloroform solvent.

Volumetric glassware used for standard preparation should be Class A and meet the specifications in the most current version of ASTM standards E288 and E69. Pipettes used for

standard preparation should be compliant with the required performance specifications listed in the most current versions of ISO 8655 Parts 1 and 2 and/or ASTM E 1154 for the volume measured if standards are made volumetrically. Balances used for gravimetric standard preparation should be sensitive enough to measure the appropriate mass delivered. All equipment should be used in accordance with the manufacturer's requirements, and should be properly maintained and calibrated.

Amber glass analytical sample vials are preferred. The septum cap should be lined with inert material, preferably PTFE/Teflon, to prevent extraction of plasticizers or other impurities into the sample.

PROCEDURE

The specific equipment and column are identified in the "Apparatus" section. Procedures for equipment operation, instrumentation problems, and corrective action are found in the manufacturer's operating manuals. The specific instrumentation settings for this method are provided in the "Method Parameters" section. The equipment should be confirmed as operational prior to use for test support.

Standards are prepared for calibration either volumetrically or gravimetrically, as required by the method.

The mass-of-agent on tube should be estimated for vapor samples to minimize saturation of the detector.

Calibration curves are generated from the standard sample results. The curves are fit and weighted as specified in this method. For quality control, the analysis of test samples must include the use of ICV, CCV, and solvent blanks. Specific guidance for method use for decontaminant performance evaluations is provided in the "2007 Chemical Decontaminant Performance Evaluation Testing Source Document" by T. Lalain, et al. Standard preparation, tube spiking, sample queues, interferences, and other general analytical guidance are provided in the corresponding technical report to this document titled, "Low-Level Analytical Methodology Updates to Support Decontaminant Performance Evaluations" by M. Shue, et al.

DATA ACCEPTANCE & REPORTING

Analytical results for samples are reported as mass on tube (ng). Mass on tube can be calculated using the instrument control software (e.g., Chemstation, etc.) or using external statistical software (e.g., Excel, Sigma plot, etc.) with the specified calibration model. Calibration curves are not extrapolated; samples must fall within the range or be reported as "above calibration range" or "below detection" as appropriate. The reported mass is acceptable if all QA/QC criteria are met as specified (e.g., CCVs before and after the sample are within 30% RSD).

METHOD M PARAMETERS

"HDLL-DEANS.M"
ECBC Decontamination Sciences Branch
GCV Method Summary

INSTRUMENT CONTROL PARAMETERS: GCV (6890GC - 5975MSD)

Control Information

Sample Inlet : GC
Injection Source : External Device
Mass Spectrometer : Enabled

AGILENT DEANS SWITCH PARAMETERS

METHOD: GCV_DEANS.M

Comment: Deans Switch Parameters for GCV w/ Markes Thermal Desorption System.

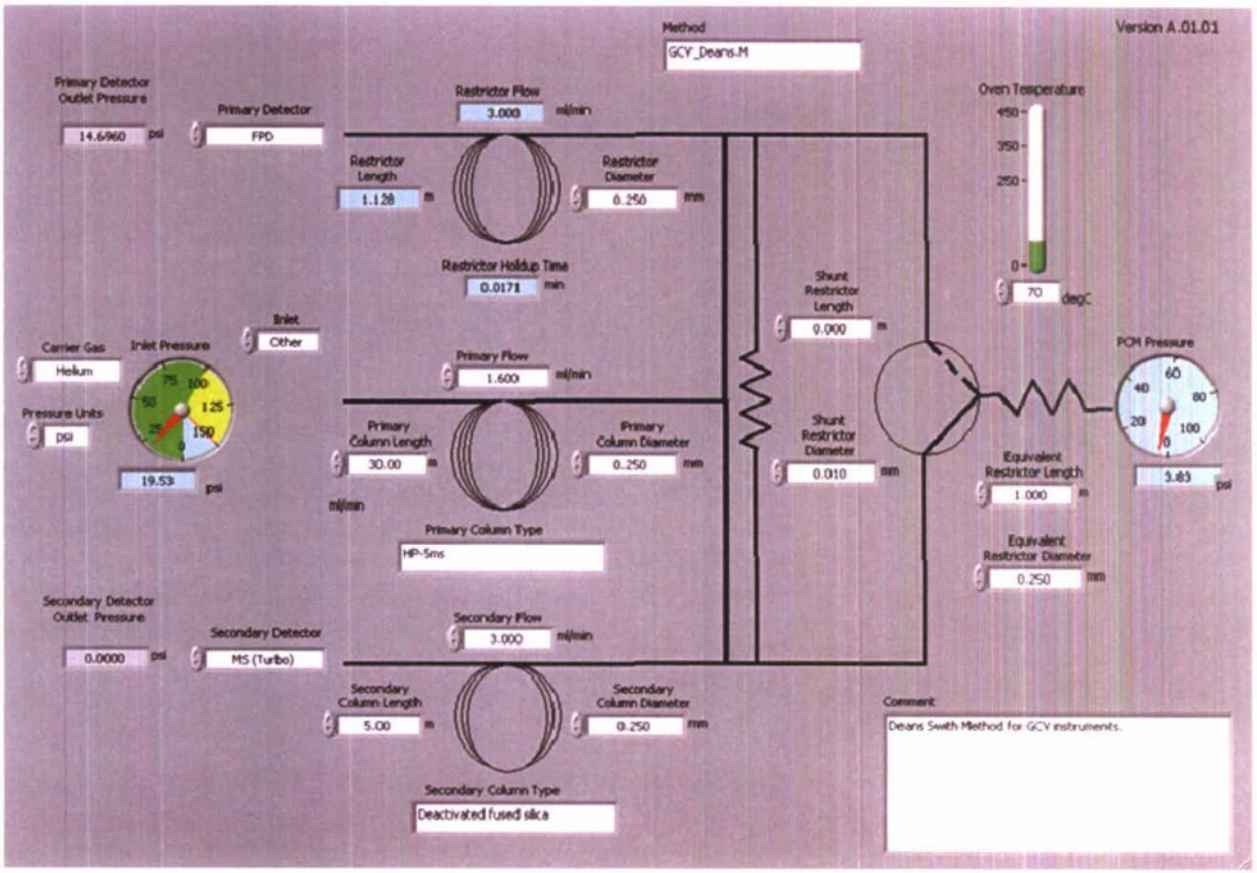
CALCULATION RESULTS:

	Primary Column	Secondary Column
Inlet Pressure:	19.53	3.83
Avg. Linear Velocity (cm/s):	34.72	154.76
Hold up Time (min):	1.44	0.054
Restrictor Length (m):		1.128
Restrictor Hold Up Time (min):		0.017

CALCULATION INPUTS

	Primary Column	Secondary Column
Length (m):	30.00	5.00
i.d. (mm):	0.25	0.25
Flow (mL/min):	1.60	3.00
Detector:	FPD	MS (Turbo)
Detector Pressure (abs):	14.70	0.00
Carrier Gas:		Helium
Pressure Units:		psi
Oven Temperature:		70.00
Restrictor Diameter:		0.25
Equivalent Restrictor Diameter (mm):		0.25
Equivalent Restrictor Length (m):		1.000

DEANS SWITCH CALCULATOR


Method
GCY_Deans.M
Version A.01.01
Primary Detector Outlet Pressure
14.6960 psi
Primary Detector
FPD
Restrictor Flow
3.000 ml/min
Restrictor Length
1.128 m
Restrictor Diameter
0.250 mm
Restrictor Holdup Time
0.0171 min
Oven Temperature
70 degC
Shunt Restrictor Length
0.000 m
Inlet
Other
Carrier Gas
Helium
Inlet Pressure
19.53 psi
Primary Flow
1.600 ml/min
Pressure Units
psi
Primary Column Length
30.00 m
Primary Column Diameter
0.250 mm
ml/min
Shunt Restrictor Diameter
0.010 mm
PCM Pressure
3.83 psi
Equivalent Restrictor Length
1.000 m
Primary Column Type
HP-5ms
Equivalent Restrictor Diameter
0.250 mm
Secondary Detector Outlet Pressure
0.0000 psi
Secondary Detector
MS (Turbo)
Secondary Flow
3.000 ml/min
Secondary Column Length
5.00 m
Secondary Column Diameter
0.250 mm
Comment
Deans Swith Method for GCV instruments.
Secondary Column Type
Deactivated fused silica

==========

MARKES TDS UNITY METHOD

==========

UNITY Method Name = HDLL.mth

Method Type:	Standard 2(3) stage desorption
Standby Split:	ON
Standby Flow Rate (mL/min):	20.0
PrePurge Time (min):	0.5
PrePurge Trap In Line:	Not in line
PrePurge Split:	ON
PrePurge Flow Rate (mL/min):	40.0
Tube Desorb Time 1 (min):	4.0
Tube Desorb Temp 1 (°C):	280
Tube Desorb Time 2 (min):	0.0
Tube Desorb Split On:	No split
Trap Desorb Low Temp (°C):	0
Trap Desorb Low Temp (°C):	280
Trap Desorb Hold Time (min):	3.0
Trap Desorb Split On:	No split
Trap Heating Rate (°C/s):	MAX
Flow Path Temp ((°C):	180
GC Cycle Time (min):	7.5
Minimum Carrier Pressure (psi):	5.0
Split Ratios:	Splitless
Desorb Flow Rate (mL/min):	40.0

Cold Trap Info: Chemical Weapon Agent Trap (Markes P/N U-T10CW)

```
================================================================================
                                  6890 GC METHOD
================================================================================

OVEN
   Initial temp:  70 °C (On)                    Maximum temp:  300 °C
   Initial time:  0.00 min                      Equilibration time:  0.00 min
   Ramps:
      # Rate Final temp Final time
      1 30.00      220        0.50
      2 0.0(Off)
   Post temp:  70 °C
   Post time:  0.00 min
   Run time:  5.50 min

FRONT INLET (SPLIT/SPLITLESS)*          BACK INLET (UNKNOWN)
   Mode:  Splitless
   Initial temp:  250 °C (On)
   Pressure:  19.53 psi (On)
   Purge flow:  50.0 mL/min
   Purge time:  999.99 min
   Total flow:  56.1 mL/min
   Gas saver:  Off
   Gas type:  Helium

*Markes modified inlet may be front or back depending on inlet availability at
install.

COLUMN 1                                COLUMN 2
   Capillary Column                        (not installed)
   Model Number:  Agilent 19091S-433
   HP-5MS  5% Phenyl Methyl Siloxane
   Max temperature:  325 °C
   Nominal length:  30.0 m
   Nominal diameter:  250.00 µm
   Nominal film thickness:  0.25 µm
   Mode:  constant pressure
   Pressure:  19.53 psi
   Nominal initial flow:  1.7 mL/min
   Average velocity:  39 cm/s
   Inlet:  Front Inlet
   Outlet:  Other
   Outlet pressure:  ambient

FRONT DETECTOR (NO DET)                 BACK DETECTOR (FPD)
                                           Temperature:  220 °C (On)
                                           Hydrogen flow:  75.0 mL/min (On)
                                            Oxidizer flow:  100.0 mL/min (On)
                                            Oxidizer Gas Type: Air
                                            Mode:  Constant makeup flow
                                            Makeup flow:  25.0 mL/min (On)
                                            Makeup Gas Type: Helium
                                            Flame:  On
                                            Lit offset:  2.00
                                            Photo multiplier:  On
```

SIGNAL 1
Data rate: 20 Hz
Type: back detector
Save Data: On
Zero: 0.0 (Off)
Range: 0
Fast Peaks: Off
Attenuation: 0

SIGNAL 2
Data rate: 20 Hz
Type: test plot
Save Data: Off
Zero: 0.0 (Off)
Range: 0
Fast Peaks: Off
Attenuation: 0

COLUMN COMP 1
(No Detectors Installed)

COLUMN COMP 2
(No Detectors Installed)

THERMAL AUX 1
Use: MSD Transfer Line Heater
Description:
Initial temp: 280 °C (On)
Initial time: 0.00 min
Rate Final temp Final time
1 0.0(Off)

THERMAL AUX 2
Unknown Thermal Aux Type

AUX PRESSURE 3
Description: Deans Switch
Gas Type: Helium
Initial pressure: 3.83 psi (On)
Initial time: 0.00 min
Rate Final temp Final time
1 0.0(Off)

AUX PRESSURE 4
Description:
Gas Type: Helium
Initial pressure: 0.00 psi (Off)

AUX PRESSURE 5
Description:
Gas Type: Helium
Initial pressure: 0.00 psi (Off)

VALVES
Valve 1 Switching Off
Description:

POST RUN
Post Time: 0.00 min

TIME TABLE

Time	Specifier	Parameter & Setpoint
3.70	Valve 1:	On
4.60	Valve 1:	Off

GC Injector

Front Injector:
No parameters specified

Back Injector:
No parameters specified

Column 1 Inventory Number : AB002
Column 2 Inventory Number :

MS ACQUISITION PARAMETERS

General Information

Tune File : atune.u
Acquistion Mode : SIM

MS Information

Solvent Delay : 3.00 min

EMV Mode : Gain Factor
Gain Factor : 1.0
Resulting EM Voltage : 2047*

[Sim Parameters]

GROUP 1
Group ID : HD
Resolution : High
Plot 1 Ion : 111.00
Ions/Dwell In Group
(Mass, Dwell)
(109.00, 30)
(111.00, 30)
(158.00, 30)
(160.00, 30)

[MSZones]

MS Quad : 150 C maximum 200 C
MS Source : 230 C maximum 250 C

Timed Events

[Timed MS Detector Entries]

Time (min)	State (MS on/off)
5.00	Off

END OF MS ACQUISITION PARAMETERS

TUNE PARAMETERS*

*MSD Specific Values automatically determined and set when performing an Autotune.

END OF TUNE PARAMETERS

END OF INSTRUMENT CONTROL PARAMETERS

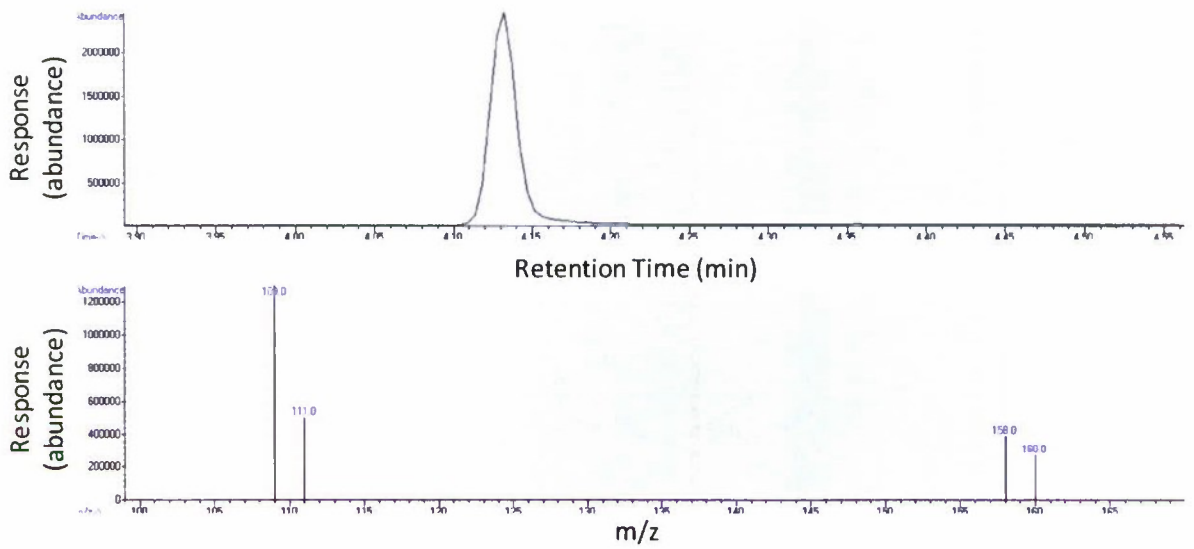
HD SPECTRUM (SCAN) - NIST LIBRARY SEARCH 2.0

The NIST library contains a sample spectrum, which can be found as identified below:

Name: Mustard Gas Formula: $C_4H_8Cl_2S$ MW: 158
CAS#: 505-60-2 NIST#: 289463 ID#: 65664 DB: mainlib

A sample spectrum from this program is provided.

HD (RT 4.1 min) TIC and SPECTRUM (SIM) - GCV DATA ANALYSIS



METHOD N: GC/MS METHOD FOR HIGH-LEVEL HD VAPOR SAMPLE ANALYSIS (GCV HDHL-DEANS.M)

ANALYTICAL METHOD

TITLE
Detection of HD in vapor samples for chemical agent decontamination testing using a GC/MS with a thermal desorption unit.

AUTHORS
Matthew Shue (ECBC Decontamination Sciences Branch)
Morgan Hall (ECBC)
Teri Lalain, Ph.D. (Branch Chief, ECBC Decontamination Sciences Branch)

KEYWORDS
Mustard, HD, Bis(2-chloroethyl)sulfide CAS 505-60-2

REVISION HISTORY
This document is version 1, released December 2007.

PURPOSE AND APPLICATIONS
The purpose of this method is to detect and quantify the chemical agent HD in vapor samples. This method is based on the collection of vapor samples, on tubes containing solid sorbent material, following chemical agent decontamination performance evaluation testing. The method is optimized for the detection requirements needed to analyze a 2 in. diameter test surface area. It is anticipated that this method can support larger item vapor testing. Sample throughput is approximately four samples per hour.

ANALYTE CONCENTRATION RANGE
This is a complementary method to the low-level method for the analysis of higher masses of HD from vapor sorbent tube samples. This method can detect HD with a mass-on-column range of 50 to 2500 ng. Sample collection parameters are adjusted to collect vapor samples in this mass range. The masses for this high-level method require sample splitting prior to analysis. The desired split is achieved via the Markes system; no parameter changes are needed with the Agilent GC/MS system. HD is collected/spiked onto tubes containing the sorbent Tenax-TA.

This method utilizes nine calibration levels at masses of ~50, 100, 250, 500, 750, 1000, 1500, 2000 and 2500 ng HD. The method use, detection limit, and quantitation limit are based on using the full standard set. The standards are prepared in chloroform.

The method performance for HD HL is as follows:

- Quantitative Method
- Calibration Range: 50 to 2500 ng HD on column

- CCV Levels: 0, 500, 500 ng
- Calibration Model: quadratic calibration model
- Calibration Weighting: $1/x^2$
- Limit of Detection (LOD): 5.65 ng
- Limit of Quantitation (LOQ): 18.82 ng
- Spiking Sample Solvent: chloroform
- Quantitative Ion: 111

SAMPLE MATRICES AND INTERFERENCES

This method is appropriate for vapor samples containing the chemical agent HD. No significant method interferents have been identified. Users of this method should confirm that the test material does not off gas an interferent in the detection region of interest.

RISK AND SAFETY ASSESSMENT

This document does not claim to address all of the safety concerns associated with chemical decontaminant testing as the requirements may vary based on facility, state, and other regulatory requirements. It is the responsibility of the user of this method, prior to use, to establish appropriate environment, health, and safety practices for the use of this method and handling of generated wastes in compliance with applicable regulations. Users of this method should conduct testing in appropriate facilities and follow proper laboratory practices, including the use of appropriate personal protective equipment and material safety data sheets.

SCIENTIFIC BASIS

This method is based on gas chromatography and mass spectrometry techniques.

TRAINING

Users of this method must have the basic skill level for analytical chromatography instrument operation, interpretation of mass spectrometry results, and associated wet lab techniques (e.g., sample dilution and standard preparation). Training covering instrument operation and maintenance of equipment is suggested.

APPARATUS

The vapor samples are analyzed on an Agilent 6890 GC equipped with a 5975 MSD. The GC is outfitted with an Agilent Deans Switch allowing for flow switching during analysis so that only a small portion of the sample effluent, including the analyte of interest, is directed to the MSD. All other flow is directed to a FPD. Sample introduction of a vapor sample is performed using a MARKES TDS. The system is fitted with a HP-5MS 5% phenyl methyl siloxane column. Instrumentation operation, maintenance manuals, and miscellaneous and consumable equipment lists are available from the instrument manufacturers: Agilent and MARKES. All equipment should be used in accordance with manufacturer's instructions.

Chemicals required include dilute HD standards prepared in high purity hexane or chloroform solvent.

Volumetric glassware used for standard preparation should be Class A and meet the

specifications in the most current version of ASTM standards E288 and E69. Pipettes used for standard preparation should be compliant with the required performance specifications listed in the most current versions of ISO 8655 Parts 1 and 2 and/or ASTM E 1154 for the volume measured if standards are made volumetrically. Balances used for gravimetric standard preparation should be sensitive enough to measure the appropriate mass delivered. All equipment should be used in accordance with the manufacturer's requirements, and should be properly maintained and calibrated.

Amber glass analytical sample vials are preferred. The septum cap should be lined with inert material, preferably PTFE/Teflon, to prevent extraction of plasticizers or other impurities into the sample.

PROCEDURE

The specific equipment and column are identified in the "Apparatus" section. Procedures for equipment operation, instrumentation problems, and corrective actions are found in the manufacturer's operating manuals. The specific instrumentation settings for this method are provided in the "Method Parameters" section. The equipment should be confirmed as operational prior to use for test support.

Standards are prepared for calibration either volumetrically or gravimetrically, as required by the method.

The mass-of-agent on tube should be estimated for vapor samples to minimize saturation of the detector.

Calibration curves are generated from the standard sample results. The curves are fit and weighted as specified in this method. For quality control, the analysis of test samples must include the use of ICV, CCV, and solvent blanks. Specific guidance for method use for decontaminant performance evaluations is provided in the "2007 Chemical Decontaminant Performance Evaluation Testing Source Document" by T. Lalain, et al. Standard preparation, tube spiking, sample queues, interferences, and other general analytical guidance are provided in the corresponding technical report to this document titled, "Low-Level Analytical Methodology Updates to Support Decontaminant Performance Evaluations" by M. Shue, et al.

DATA ACCEPTANCE & REPORTING

Analytical results for samples are reported as mass on tube (ng). Mass on tube can be calculated using the instrument control software (e.g., Chemstation, etc.) or using external statistical software (e.g., Excel, Sigma plot, etc.) with the specified calibration model. Calibration curves are not extrapolated; samples must fall within the range or be reported as "above calibration range" or "below detection" as appropriate. The reported mass is acceptable if all QA/QC criteria are met as specified (e.g., CCVs before and after the sample are within 30% RSD).

METHOD N PARAMETERS

"HDHL-DEANS.M"
ECBC Decontamination Sciences Branch
GCV Method Summary

INSTRUMENT CONTROL PARAMETERS: GCV (6890GC - 5975MSD)

Control Information

Sample Inlet : GC
Injection Source : External Device
Mass Spectrometer : Enabled

AGILENT DEANS SWITCH PARAMETERS

METHOD: GCV_DEANS.M

Comment: Deans Switch Parameters for GCV w/ Markes Thermal Desorption System.

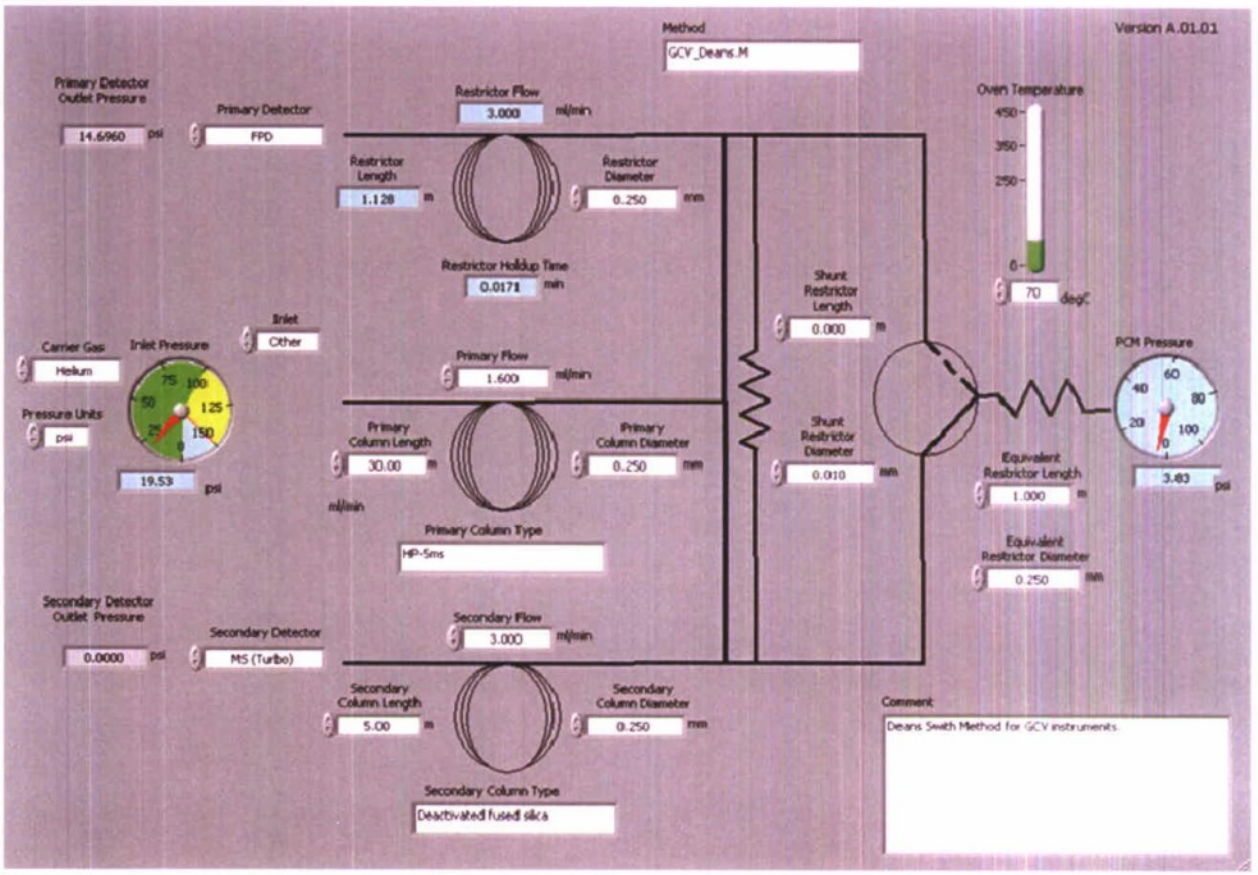
CALCULATION RESULTS:

	Primary Column	Secondary Column
Inlet Pressure:	19.53	3.83
Avg. Linear Velocity (cm/s):	34.72	154.76
Hold up Time (min):	1.44	0.054
Restrictor Length (m):		1.128
Restrictor Hold Up Time (min):		0.017

CALCULATION INPUTS

	Primary Column	Secondary Column
Length (m):	30.00	5.00
i.d. (mm):	0.25	0.25
Flow (mL/min):	1.60	3.00
Detector:	FPD	MS (Turbo)
Detector Pressure (abs):	14.70	0.00
Carrier Gas:		Helium
Pressure Units:		psi
Oven Temperature:		70.00
Restrictor Diameter:		0.25
Equivalent Restrictor Diameter (mm):		0.25
Equivalent Restrictor Length (m):		1.000

DEANS SWITCH CALCULATOR


Method
GCV_Deans.M
Version A.01.01
Primary Detector Outlet Pressure
14.6960 psi
Primary Detector
FPD
Restrictor Flow
3.000 ml/min
Restrictor Length
1.128 m
Restrictor Diameter
0.250 mm
Restrictor Holdup Time
0.0171 min
Oven Temperature
450
350
250
0
70 degC
Shunt Restrictor Length
0.000 m
Shunt Restrictor Diameter
0.010 mm
Inlet
Other
Carrier Gas
Helium
Inlet Pressure
75
100
50
125
25
150
0
19.53 psi
Pressure Units
psi
Primary Flow
1.600 ml/min
Primary Column Length
30.00 m
Primary Column Diameter
0.250 mm
ml/min
Primary Column Type
HP-5ms
PCM Pressure
60
40
80
20
100
0
3.83 psi
Equivalent Restrictor Length
1.000 m
Equivalent Restrictor Diameter
0.250 mm
Secondary Detector Outlet Pressure
0.0000 psi
Secondary Detector
MS (Turbo)
Secondary Flow
3.000 ml/min
Secondary Column Length
5.00 m
Secondary Column Diameter
0.250 mm
Secondary Column Type
Deactivated fused silica
Comment
Deans Swith Method for GCV instruments

MARKES TDS UNITY METHOD

UNITY Method Name = HDHL.mth

Method Type:	Standard 2(3) stage desorption
Standby Split:	ON
Standby Flow Rate (mL/min):	20.0
PrePurge Time (min):	0.5
PrePurge Trap In Line:	Not in line
PrePurge Split:	ON
PrePurge Flow Rate (mL/min):	40.0
Tube Desorb Time 1 (min):	4.0
Tube Desorb Temp 1 (°C):	280
Tube Desorb Time 2 (min):	0.0
Tube Desorb Split On:	40.0
Trap Desorb Low Temp (°C):	0
Trap Desorb Low Temp (°C):	280
Trap Desorb Hold Time (min):	3.0
Trap Desorb Split On:	25.0
Trap Heating Rate (°C/s):	MAX
Flow Path Temp ((°C):	180
GC Cycle Time (min):	7.5
Minimum Carrier Pressure (psi):	5.0
Split Ratios*:	31.4:1
Desorb Flow Rate (mL/min):	40.0

Cold Trap Info: Chemical Weapon Agent Trap (Markes P/N U-T10CW)

* Split ratio is calculated based on the column flow of 1.7 mL/min, the desorb flow of 40 mL/min and the inlet/outlet split flow rates of 40.0 and 25.0 mL/min, respectively.

```
================================================================================
                                  6890 GC METHOD
================================================================================

OVEN
   Initial temp:  70 °C (On)                    Maximum temp:  300 °C
   Initial time:  0.00 min                      Equilibration time:  0.00 min
   Ramps:
      # Rate Final temp Final time
      1 30.00      220        0.50
      2 0.0(Off)
   Post temp:  70 °C
   Post time:  0.00 min
   Run time:  5.50 min

FRONT INLET (SPLIT/SPLITLESS)*          BACK INLET (UNKNOWN)
   Mode:  Splitless
   Initial temp:  250 °C (On)
   Pressure:  19.53 psi (On)
   Purge flow:  50.0 mL/min
   Purge time:  999.99 min
   Total flow:  56.1 mL/min
   Gas saver:  Off
   Gas type:  Helium

*Markes modified inlet may be front or back depending on inlet availability at
install.

COLUMN 1                                COLUMN 2
   Capillary Column                        (not installed)
   Model Number:  Agilent 19091S-433
   HP-5MS  5% Phenyl Methyl Siloxane
   Max temperature:  325 °C
   Nominal length:  30.0 m
   Nominal diameter:  250.00 µm
   Nominal film thickness:  0.25 µm
   Mode:  constant pressure
   Pressure:  19.53 psi
   Nominal initial flow:  1.7 mL/min
   Average velocity:  39 cm/s
   Inlet:  Front Inlet
   Outlet:  Other
   Outlet pressure:  ambient

FRONT DETECTOR (NO DET)                 BACK DETECTOR (FPD)
                                           Temperature:  220 °C (On)
                                           Hydrogen flow:  75.0 mL/min (On)
                                            Oxidizer flow:  100.0 mL/min (On)
                                            Oxidizer Gas Type: Air
                                            Mode:  Constant makeup flow
                                            Makeup flow:  25.0 mL/min (On)
                                            Makeup Gas Type: Helium
                                            Flame:  On
                                            Lit offset:  2.00
                                            Photo multiplier:  On
```

```
SIGNAL 1
   Data rate:  20 Hz
   Type:  back detector
   Save Data:  On
   Zero:  0.0 (Off)
   Range:  0
   Fast Peaks:  Off
   Attenuation:  0

COLUMN COMP 1
   (No Detectors Installed)

THERMAL AUX 1
   Use:  MSD Transfer Line Heater
   Description:
   Initial temp:  280 °C (On)
   Initial time:  0.00 min
      # Rate Final temp Final time
      1   0.0(Off)

AUX PRESSURE 3
   Description:  Deans Switch
   Gas Type:  Helium
   Initial pressure:  3.83 psi (On)
   Initial time:  0.00 min
      # Rate Final temp Final time
      1   0.0(Off)

AUX PRESSURE 5
   Description:
   Gas Type:  Helium
   Initial pressure:  0.00 psi (Off)

VALVES
   Valve 1  Switching Off
      Description:

TIME TABLE
   Time       Specifier                 Parameter & Setpoint
      3.70       Valve 1:                  On
      4.60       Valve 1:                  Off
```

```
SIGNAL 2
   Data rate:  20 Hz
   Type:  test plot
   Save Data:  Off
   Zero:  0.0 (Off)
   Range:  0
   Fast Peaks:  Off
   Attenuation:  0

COLUMN COMP 2
   (No Detectors Installed)

THERMAL AUX 2
   Unknown Thermal Aux Type

AUX PRESSURE 4
   Description:
   Gas Type:  Helium
   Initial pressure:  0.00 psi (Off)

POST RUN
   Post Time: 0.00 min
```

GC Injector

```
     Front Injector:
No parameters specified

     Back Injector:
No parameters specified

 Column 1 Inventory Number : AB002
 Column 2 Inventory Number :
```

MS ACQUISITION PARAMETERS

General Information

Tune File	: atune.u
Acquistion Mode	: SIM

MS Information

Solvent Delay	: 3.00 min
EMV Mode	: Gain Factor
Gain Factor	: 1.0
Resulting EM Voltage	: 2047*

[Sim Parameters]

GROUP 1	
Group ID	: HD
Resolution	: High
Plot 1 Ion	: 111.00

Ions/Dwell In Group

(Mass,	Dwell)
(109.00,	30)
(111.00,	30)
(158.00,	30)
(160.00,	30)

[MSZones]

MS Quad	: 150 C	maximum 200 C
MS Source	: 230 C	maximum 250 C

Timed Events

[Timed MS Detector Entries]

Time (min)	State (MS on/off)
5.00	Off

END OF MS ACQUISITION PARAMETERS

TUNE PARAMETERS*

*MSD Specific Values automatically determined and set when performing an Autotune.

END OF TUNE PARAMETERS

END OF INSTRUMENT CONTROL PARAMETERS

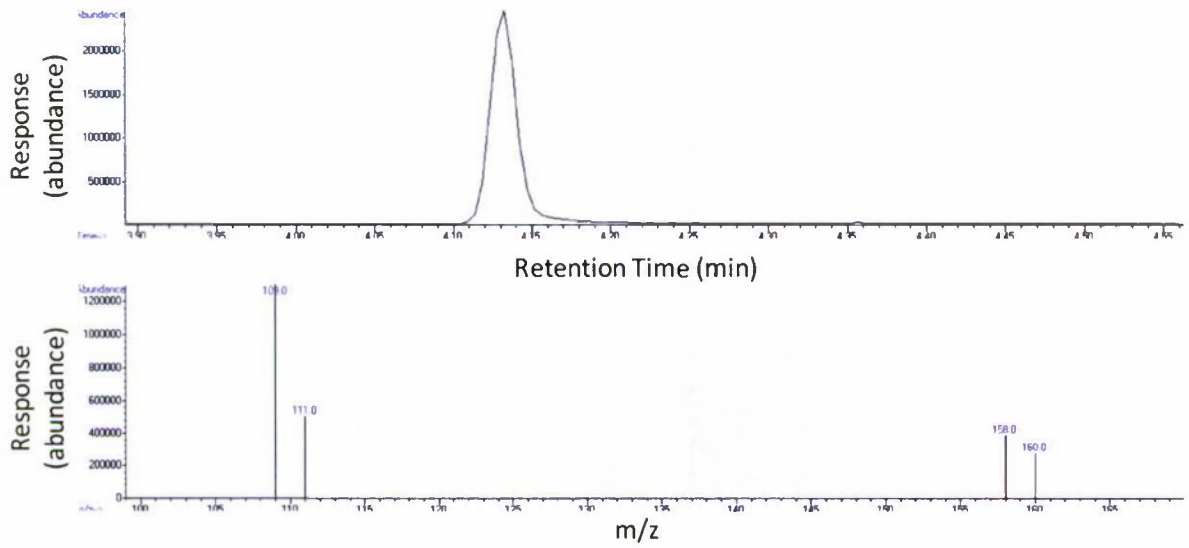
HD SPECTRUM (SCAN) - NIST LIBRARY SEARCH 2.0

The NIST library contains a sample spectrum, which can be found as identified below:

Name: Mustard Gas Formula: $C_4H_8Cl_2S$ MW: 158
CAS#: 505-60-2 NIST#: 289463 ID#: 65664 DB: mainlib

A sample spectrum from this program is provided.

HD (RT 4.1 min) TIC SPECTRUM (SIM) - GCV DATA ANALYSIS



METHOD O: GC/MS METHOD FOR LOW-LEVEL GD VAPOR SAMPLE ANALYSIS (GCV GDLL-DEANS.M)

ANALYTICAL METHOD

TITLE
Detection of GD in vapor samples for chemical agent decontamination testing using a GC/MS with a thermal desorption unit.

AUTHORS
Matthew Shue (ECBC Decontamination Sciences Branch)
Morgan Hall (ECBC)
Teri Lalain, Ph.D. (Branch Chief, ECBC Decontamination Sciences Branch)

KEYWORDS
Soman, GD, O-Pinacolyl methylphosphonofluoridate; CAS 96-64-0

REVISION HISTORY
This document is version 1, released December 2007.

PURPOSE AND APPLICATIONS
The purpose of this method is to detect and quantify the chemical agent GD in vapor samples. This method is based on the collection of vapor samples on tubes containing solid sorbent material, following chemical agent decontamination performance evaluation testing. The method is optimized for the detection requirements for the analysis of a 2 in. diameter test surface area. It is anticipated that this method can support larger item vapor testing. Sample throughput is approximately four samples per hour.

ANALYTE CONCENTRATION RANGE
This method analyzes vapor sorbent tube samples for low-level GD. This GD low-level method can detect GD with a mass-on-column range of 0.5 to 100 ng. Collection parameters are adjusted to collect vapor samples in this mass range. No sample split on the Markes unit is needed. GD is collected/spiked onto tubes containing the sorbent Tenax-TA.

This method utilizes nine calibration levels at masses of ~0.5, 0.5, 0.5, 1, 2.5, 5, 10, 50, and 100 ng GD. The method use, detection limit, and quantitation limit are based on using the full standard set. The standards are prepared in acetonitrile.

The method performance for GD LL is as follows:

- Quantitative Method
- Calibration Range: 0.5 to 100 ng GD on column
- CCV Levels: 0, 10, 10 ng
- Calibration Model: Weighted quadratic calibration model
- Calibration Weighting: $1/x^2$

- Limit of Detection (LOD): 0.07 ng
- Limit of Quantitation (LOQ): 0.23 ng
- Spiking Sample Solvent: acetonitrile
- Quantitative Ion: 126

SAMPLE MATRICES AND INTERFERENCES

This method is appropriate for vapor samples containing the chemical agent GD. No significant method interferents have been identified. Users of this method should confirm that the test material does not off gas an interferent in the detection region of interest.

RISK AND SAFETY ASSESSMENT

This document does not claim to address all of the safety concerns associated with chemical decontaminant testing as the requirements may vary based on facility, state, and other regulatory requirements. It is the responsibility of the user of this method, prior to use, to establish appropriate environment, health, and safety practices for the use of this method and handling of generated wastes in compliance with applicable regulations. Users of this method should conduct testing in appropriate facilities and follow proper laboratory practices, including the use of appropriate personal protective equipment and material safety data sheets.

SCIENTIFIC BASIS

This method is based on gas chromatography and mass spectrometry techniques.

TRAINING

Users of this method must have the basic skill level for analytical chromatography instrument operation, interpretation of mass spectrometry results and associated wet lab techniques (e.g., sample dilution and standard preparation). Training covering instrument operation and maintenance of equipment is suggested.

APPARATUS

The vapor samples are analyzed on an Agilent 6890 GC equipped with a 5975 MSD. The GC is outfitted with an Agilent Deans Switch allowing flow switching during analysis so that only a small portion of the sample effluent, including the analyte of interest, is directed to the MSD. All other flow is directed to a FPD. Introduction of a vapor sample is performed using a MARKES TDS. The system is fitted with a HP-5MS 5% phenyl methyl siloxane column. Instrumentation operation, maintenance manuals, and miscellaneous and consumable equipment lists are available from the instrument manufacturers: Agilent and MARKES Technologies. All equipment should be used in accordance with manufacturer's instructions.

Chemicals required include dilute GD standards prepared in high purity hexane or chloroform solvent.

Volumetric glassware used for standard preparation should be Class A and meet the specifications in the most current version of ASTM standards E288 and E69. Pipettes used for standard preparation should be compliant with the required performance specifications listed in the most current versions of ISO 8655 Parts 1 and 2 and/or ASTM E 1154 for the volume

measured if standards are made volumetrically. Balances used for gravimetric standard preparation should be sensitive enough to measure the appropriate mass delivered. All equipment should be used in accordance with the manufacturer's requirements, and should be properly maintained and calibrated.

Amber glass analytical sample vials are preferred. The septum cap should be lined with inert material, preferably PTFE/Teflon, to prevent extraction of plasticizers or other impurities into the sample.

PROCEDURE

The specific equipment and column are identified in the "Apparatus" section. Procedures for equipment operation, instrumentation problems, and corrective actions are found in the manufacturer's operating manuals. The specific instrumentation settings for this method are provided in the "Method Parameters" section. The equipment should be confirmed as operational prior to use for test support.

Standards are prepared for calibration either volumetrically or gravimetrically, as required by the method.

The mass-of-agent on tube should be estimated for vapor samples to minimize saturation of the detector.

Calibration curves are generated from the standard sample results. The curves are fit and weighted as specified in this method. For quality control, the analysis of test samples must include the use of ICV, CCV, and solvent blanks for quality control. Specific guidance for method use for decontaminant performance evaluations is provided in the "2007 Chemical Decontaminant Performance Evaluation Testing Source Document" by T. Lalain, et al. Standard preparation, tube spiking, sample queues, interferences and other general analytical guidance are provided in the corresponding technical report to this document titled, "Low-Level Analytical Methodology Updates to Support Decontaminant Performance Evaluations" by M. Shue, et al.

DATA ACCEPTANCE & REPORTING

Analytical results for samples are reported as mass on tube (ng). Mass on tube can be calculated using the instrument control software (e.g., Chemstation, etc.) or using external statistical software (e.g., Excel, Sigma plot, etc.) with the specified calibration model. Calibration curves are not extrapolated; samples must fall within the range or be reported as "above calibration range" or "below detection" as appropriate. The reported mass is acceptable if all QA/QC criteria are met as specified (e.g., CCVs before and after the sample are within 30% RSD).

METHOD O PARAMETERS

"GDLL-DEANS.M"
ECBC Decontamination Sciences Branch
GCV Method Summary

INSTRUMENT CONTROL PARAMETERS: GCV (6890GC - 5975MSD)

Control Information

Sample Inlet : GC
Injection Source : External Device
Mass Spectrometer : Enabled

AGILENT DEANS SWITCH PARAMETERS

METHOD: GCV_DEANS.M

Comment: Deans Switch Parameters for GCV w/ Markes Thermal Desorption System.

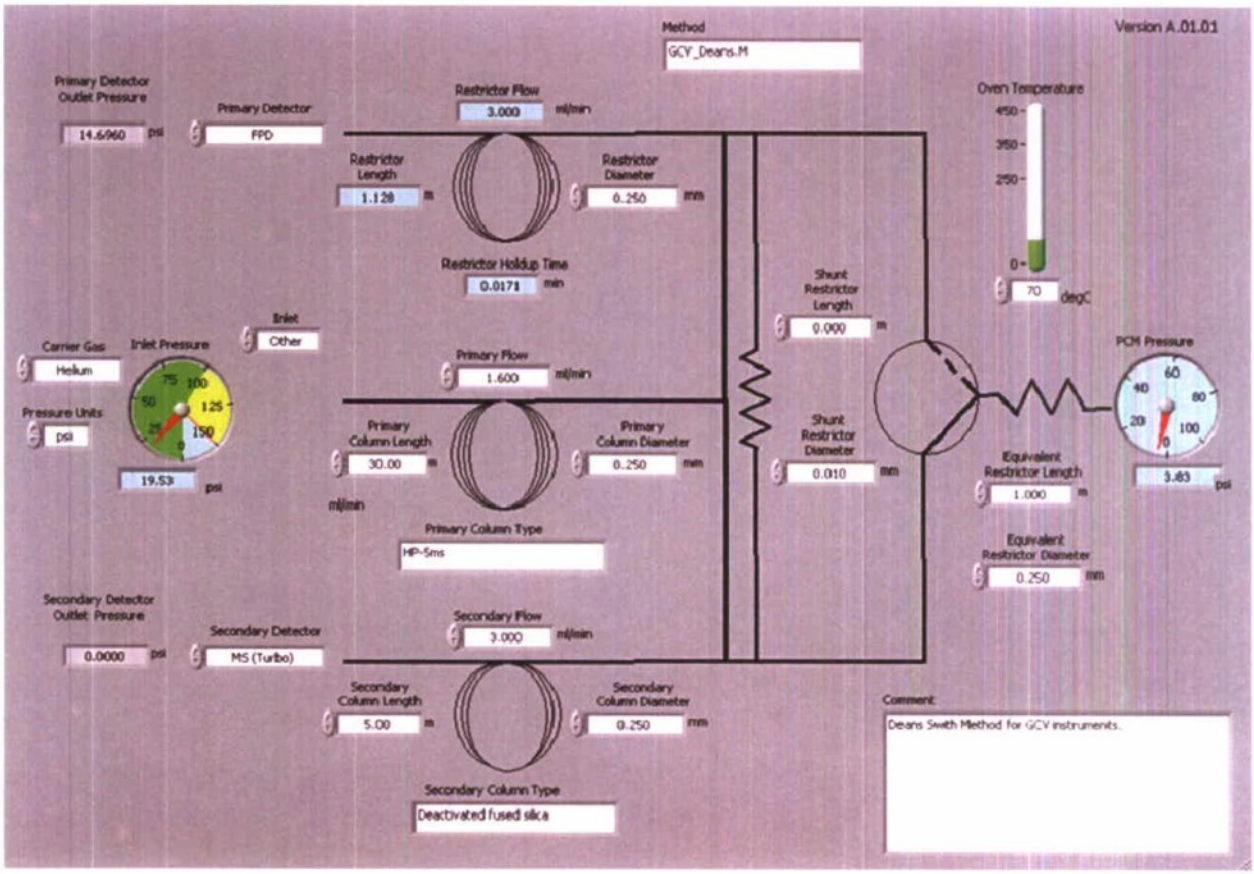
CALCULATION RESULTS:

	Primary Column	Secondary Column
Inlet Pressure:	19.53	3.83
Avg. Linear Velocity (cm/s):	34.72	154.76
Hold up Time (min):	1.44	0.054
Restrictor Length (m):		1.128
Restrictor Hold Up Time (min):		0.017

CALCULATION INPUTS

	Primary Column	Secondary Column
Length (m):	30.00	5.00
i.d. (mm):	0.25	0.25
Flow (mL/min):	1.60	3.00
Detector:	FPD	MS (Turbo)
Detector Pressure (abs):	14.70	0.00
Carrier Gas:		Helium
Pressure Units:		psi
Oven Temperature:		70.00
Restrictor Diameter:		0.25
Equivalent Restrictor Diameter (mm):		0.25
Equivalent Restrictor Length (m):		1.000

DEANS SWITCH CALCULATOR


Method
GCV_Deans.M
Version A.01.01
Primary Detector Outlet Pressure
14.6960 psi
Primary Detector
FPD
Restrictor Flow
3.000 ml/min
Oven Temperature
450
350
250
0
70 degC
Restrictor Length
1.128 m
Restrictor Diameter
0.250 mm
Restrictor Holdup Time
0.0171 min
Shunt Restrictor Length
0.000 m
Inlet
Other
Carrier Gas
Helium
Inlet Pressure
75 100 50 125 25 150 0
19.53 psi
Primary Flow
1.600 ml/min
PCM Pressure
60 40 80 20 100 0
3.83 psi
Pressure Units
psi
Primary Column Length
30.00 m
Primary Column Diameter
0.250 mm
Shunt Restrictor Diameter
0.010 mm
Equivalent Restrictor Length
1.000 m
ml/min
Primary Column Type
HP-5ms
Equivalent Restrictor Diameter
0.250 mm
Secondary Detector Outlet Pressure
0.0000 psi
Secondary Detector
MS (Turbo)
Secondary Flow
3.000 ml/min
Secondary Column Length
5.00 m
Secondary Column Diameter
0.250 mm
Comment
Deans Swith Method for GCV instruments.
Secondary Column Type
Deactivated fused silica

==========

MARKES TDS UNITY METHOD

==========

UNITY Method Name = GDLL.mth

Method Type:	Standard 2(3) stage desorption
Standby Split:	ON
Standby Flow Rate (mL/min):	20.0
PrePurge Time (min):	0.5
PrePurge Trap In Line:	Not in line
PrePurge Split:	ON
PrePurge Flow Rate (mL/min):	40.0
Tube Desorb Time 1 (min):	4.0
Tube Desorb Temp 1 (°C):	200
Tube Desorb Time 2 (min):	0.0
Tube Desorb Split On:	No split
Trap Desorb Low Temp (°C):	0
Trap Desorb Low Temp (°C):	240
Trap Desorb Hold Time (min):	3.0
Trap Desorb Split On:	No split
Trap Heating Rate (°C/s):	MAX
Flow Path Temp ((°C):	180
GC Cycle Time (min):	7.5
Minimum Carrier Pressure (psi):	5.0
Split Ratios:	No Split
Desorb Flow Rate (mL/min):	40.0

Cold Trap Info: Chemical Weapon Agent Trap (Markes P/N U-T10CW)

6890 GC METHOD

OVEN

Initial temp: 70 °C (On)
Initial time: 0.50 min
Ramps:

#	Rate	Final temp	Final time
1	35.00	240	0.00
2	0.0(Off)		

Post temp: 70 °C
Post time: 0.00 min
Run time: 5.36 min

Maximum temp: 300 °C
Equilibration time: 0.25 min

FRONT INLET (SPLIT/SPLITLESS)

Mode: Splitless
Initial temp: 250 °C (On)
Pressure: 19.53 psi (On)
Purge flow: 50.0 mL/min
Purge time: 999.99 min
Total flow: 56.1 mL/min
Gas saver: Off
Gas type: Helium

BACK INLET (UNKNOWN)

*Markes modified inlet may be front or back depending on inlet availability at install.

COLUMN 1

Capillary Column
Model Number: Agilent 19091S-433
HP-5MS 5% Phenyl Methyl Siloxane
Max temperature: 325 °C
Nominal length: 30.0 m
Nominal diameter: 250.00 µm
Nominal film thickness: 0.25 µm
Mode: constant pressure
Pressure: 19.53 psi
Nominal initial flow: 1.7 mL/min
Average velocity: 39 cm/sec
Inlet: Front Inlet
Outlet: Other
Outlet pressure: ambient

COLUMN 2

(not installed)

FRONT DETECTOR (NO DET)

BACK DETECTOR (FPD)

Temperature: 220 °C (On)
Hydrogen flow: 75.0 mL/min (On)
Oxidizer flow: 100.0 mL/min (On)
Oxidizer Gas Type: Air
Mode: Constant makeup flow
Makeup flow: 25.0 mL/min (On)
Makeup Gas Type: Helium
Flame: On
Lit offset: 2.00
Photo multiplier: On

```
SIGNAL 1
   Data rate:  20 Hz
   Type:  back detector
   Save Data:  On
   Zero:  0.0 (Off)
   Range:  0
   Fast Peaks:  Off
   Attenuation:  0

SIGNAL 2
   Data rate:  20 Hz
   Type:  test plot
   Save Data:  Off
   Zero:  0.0 (Off)
   Range:  0
   Fast Peaks:  Off
   Attenuation:  0

COLUMN COMP 1
   (No Detectors Installed)

COLUMN COMP 2
   (No Detectors Installed)

THERMAL AUX 1
   Use:  MSD Transfer Line Heater
   Description:
   Initial temp:  280 °C (On)
   Initial time:  0.00 min
      #  Rate  Final temp  Final time
      1   0.0(Off)

THERMAL AUX 2
   Unknown Thermal Aux Type

AUX PRESSURE 3
   Description: Deans Switch
   Gas Type:  Helium
   Initial pressure:  3.83 psi (On)
   Initial time:  0.00 min
      #  Rate  Final pres  Final time
      1   0.0(Off)

AUX PRESSURE 4
   Description:
   Gas Type:  Helium
   Initial pressure:  0.0 psi (Off)

AUX PRESSURE 5
   Description:
   Gas Type:  Helium
   Initial pressure:  0.00 psi (Off)

VALVES
   Valve 1  Switching Off
      Description:

POST RUN
   Post Time: 0.00 min

TIME TABLE
   Time       Specifier                  Parameter & Setpoint
      3.20       Valve 1:                   On
      3.90       Valve 1:                   Off

                          GC Injector


     Front Injector:
No parameters specified

     Back Injector:
No parameters specified

 Column 1 Inventory Number : AB002
 Column 2 Inventory Number :
```

MS ACQUISITION PARAMETERS

General Information
------- -----------
Tune File : atune.u
Acquistion Mode : SIM

MS Information
-- -----------
Solvent Delay : 3.0 min

EMV Mode : Gain Factor
Gain Factor : 1.0
Resulting EM Voltage : 2047*

[Sim Parameters]

GROUP 1
Group ID : GD
Resolution : High
Plot 1 Ion : 126.00
Ions/Dwell In Group
(Mass, Dwell)
(82.00, 40)
(99.00, 40)
(126.00, 40)

[MSZones]

MS Quad : 150 C maximum 200 C
MS Source : 230 C maximum 250 C
Timed Events
----- ------

[Timed MS Detector Entries]

Time (min) State (MS on/off)
4.00 Off

END OF MS ACQUISITION PARAMETERS

TUNE PARAMETERS*
---

*MSD Specific Values automatically determined and set when performing an Autotune.

END OF TUNE PARAMETERS

END OF INSTRUMENT CONTROL PARAMETER

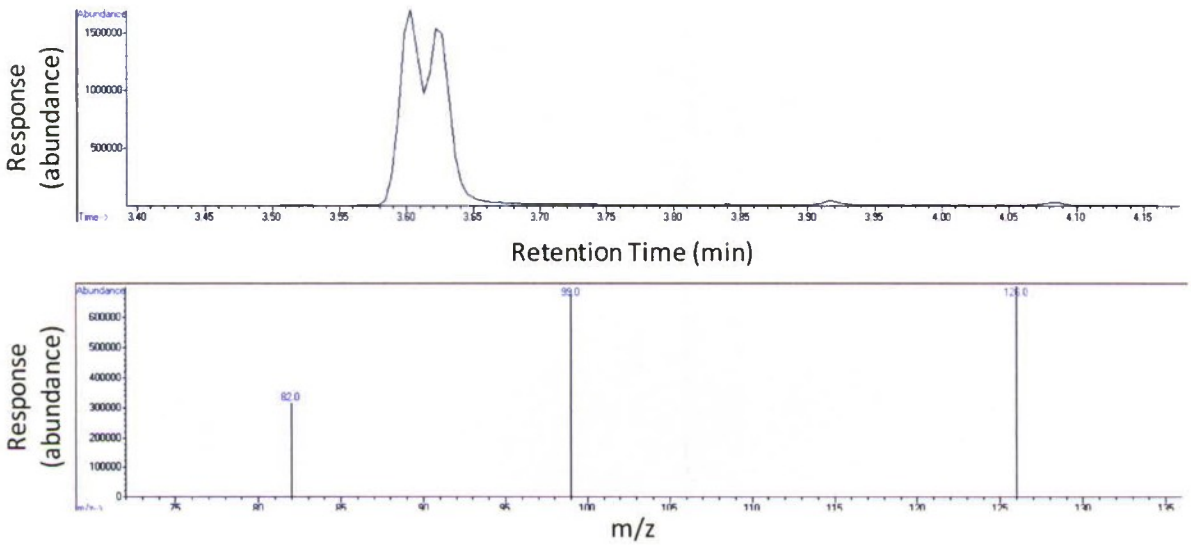
GD SPECTRUM (SCAN) - NIST LIBRARY SEARCH 2.0

The NIST library contains a sample spectrum, which can be found as identified below:

Name: Soman Formula: $C_7H_{16}FO_2P$ MW: 182
CAS#: 96-64-0 NIST#: 226124 ID#: 78999 DB: mainlib

A sample spectrum from this program is provided.

GD (RT 3.6 min) TIC and SPECTRUM (SIM) - GCV DATA ANALYSIS



METHOD P: GC/MS METHOD FOR HIGH-LEVEL GD VAPOR SAMPLE ANALYSIS (GCV GDHL-DEANS.M)

ANALYTICAL METHOD

TITLE
Detection of GD in vapor samples for chemical agent decontamination testing using a GC/MS with a thermal desorption unit.

AUTHORS
Matthew Shue (ECBC Decontamination Sciences Branch)
Morgan Hall (ECBC)
Teri Lalain, Ph.D. (Branch Chief, ECBC Decontamination Sciences Branch)

KEYWORDS
Soman, GD, O-Pinacolyl methylphosphonofluoridate; CAS 96-64-0

REVISION HISTORY
This document is version 1, released December 2007.

PURPOSE AND APPLICATIONS
The purpose of this method is to detect and quantify the chemical agent GD in vapor samples. This method is based on the collection of vapor samples on tubes containing solid sorbent material, following chemical agent decontamination performance evaluation testing. The method is optimized for the detection requirements for the analysis of a 2 in. diameter test surface area. It is anticipated that this method can support larger item vapor testing. Sample throughput is approximately four samples per hour.

ANALYTE CONCENTRATION RANGE
This method is a complementary method to the low-level method for the detection of higher masses of GD from vapor sorbent tube samples. This GD high-level method can detect GD with a mass-on-column range of 50.0 ng to 2500 ng. Sample collection parameters are adjusted to collect vapor samples in this mass range. The masses for this high-level method require sample splitting prior to analysis. The desired split is achieved via the Markes system; no parameter changes are needed with the Agilent GC/MS system. GD is collected/spiked onto tubes containing the sorbent Tenax-TA.

This method utilizes nine calibration levels at masses of ~50, 100, 250, 500, 750, 1000, 1500, 2000, and 2500 ng GD. The method use, detection limit, and quantitation limit are based on using the full standard set. The standards are prepared in acetonitrile.

The method performance for GD HL is as follows:

- Quantitative Method
- Calibration Range: 50 to 2500 ng GD on column

- CCV Levels: 0, 500, 500 ng
- Calibration Model: Weighted quadratic calibration model
- Calibration Weighting: $1/x^2$
- Limit of Detection (LOD): 6.54 ng
- Limit of Quantitation (LOQ): 21.79 ng
- Spiking Sample Solvent: acetonitrile
- Quantitative Ion: 126

SAMPLE MATRICES AND INTERFERENCES

This method is appropriate for vapor samples containing the chemical agent GD. No significant method interferents have been identified. Users of this method should confirm that the test material does not off gas an interferent in the detection region of interest.

RISK AND SAFETY ASSESSMENT

This document does not claim to address all of the safety concerns associated with chemical decontaminant testing as the requirements may vary based on facility, state and other regulatory requirements. It is the responsibility of the user of this method, prior to use, to establish appropriate environment, health, and safety practices for the use of this method and handling of generated wastes in compliance with applicable regulations. Users of this method should conduct testing in appropriate facilities and follow proper laboratory practices, including the use of appropriate personal protective equipment and material safety data sheets.

SCIENTIFIC BASIS

This method is based on gas chromatography and mass spectrometry techniques.

TRAINING

Users of this method must have the basic skill level for analytical chromatography instrument operation, interpretation of mass spectrometry results and associated wet lab techniques (e.g., sample dilution and standard preparation). Instrument training covering operation and maintenance of equipment is suggested.

APPARATUS

The vapor samples are analyzed on an Agilent 6890 GC equipped with a 5975 MSD. The GC is outfitted with an Agilent Deans Switch allowing flow switching during analysis so that only a small portion of the sample effluent, including the analyte of interest, is directed to the MSD. All other flow is directed to a FPD. Introduction of a vapor sample is performed using a MARKES TDS. The system is fitted with a HP-5MS 5% phenyl methyl siloxane column. Instrumentation operation, maintenance manuals, and miscellaneous and consumable equipment lists are available from the instrument manufacturers: Agilent and MARKES. All equipment should be used in accordance with manufacturer's instructions.

Chemicals required include dilute GD standards prepared in high purity hexane or chloroform solvent.

Volumetric glassware used for standard preparation should be Class A and meet the

specifications in the most current version of ASTM standards E288 and E69. Pipettes used for standard preparation should be compliant with the required performance specifications listed in the most current versions of ISO 8655 Parts 1 and 2 and/or ASTM E 1154 for the volume measured if standards are made volumetrically. Balances used for gravimetric standard preparation should be sensitive enough to measure the appropriate mass delivered. All equipment should be used in accordance with the manufacturer's requirements, and should be properly maintained and calibrated.

Amber glass analytical sample vials are preferred. The septum cap should be lined with inert material, preferably PTFE/Teflon, to prevent extraction of plasticizers or other impurities into the sample.

PROCEDURE

The specific equipment and column are identified in the "Apparatus" section. Procedures for equipment operation, instrumentation problems, and corrective actions are found in the manufacturer's operating manuals. The specific instrumentation settings for this method are provided in the "Method Parameters" section. The equipment should be confirmed as operational prior to use for test support.

Standards are prepared for calibration either volumetrically or gravimetrically, as required by the method.

The mass-of-agent on tube should be estimated for vapor samples to minimize saturation of the detector.

Calibration curves are generated from the standard sample results. The curves are fit and weighted as specified in this method. For quality control, the analysis of test samples must include the use of ICV, CCV, and solvent blanks. Specific guidance for method use for decontaminant performance evaluations is provided in the "2007 Chemical Decontaminant Performance Evaluation Testing Source Document" by T. Lalain, et al. Standard preparation, tube spiking, sample queues, interferences and other general analytical guidance are provided in the corresponding technical report to this document titled, "Low-Level Analytical Methodology Updates to Support Decontaminant Performance Evaluations" by M. Shue, et al.

DATA ACCEPTANCE & REPORTING

Analytical results for samples are reported as mass on tube (ng). Mass on tube can be calculated using the instrument control software (e.g., Chemstation, etc.) or using external statistical software (e.g., Excel, Sigma plot, etc.) with the specified calibration model. Calibration curves are not extrapolated; samples must fall within the range or be reported as "above calibration range" or "below detection" as appropriate. The reported mass is acceptable if all QA/QC criteria are met as specified (e.g., CCVs before and after the sample are within 30% RSD).

METHOD P PARAMETERS

"GDHL-DEANS.M"
ECBC Decontamination Sciences Branch
GCV Method Summary

INSTRUMENT CONTROL PARAMETERS: GCV (6890GC - 5975MSD)

Control Information

Sample Inlet : GC
Injection Source : External Device
Mass Spectrometer : Enabled

AGILENT DEANS SWITCH PARAMETERS

METHOD: GCV_DEANS.M

Comment: Deans Switch Parameters for GCV w/ Markes Thermal Desorption System.

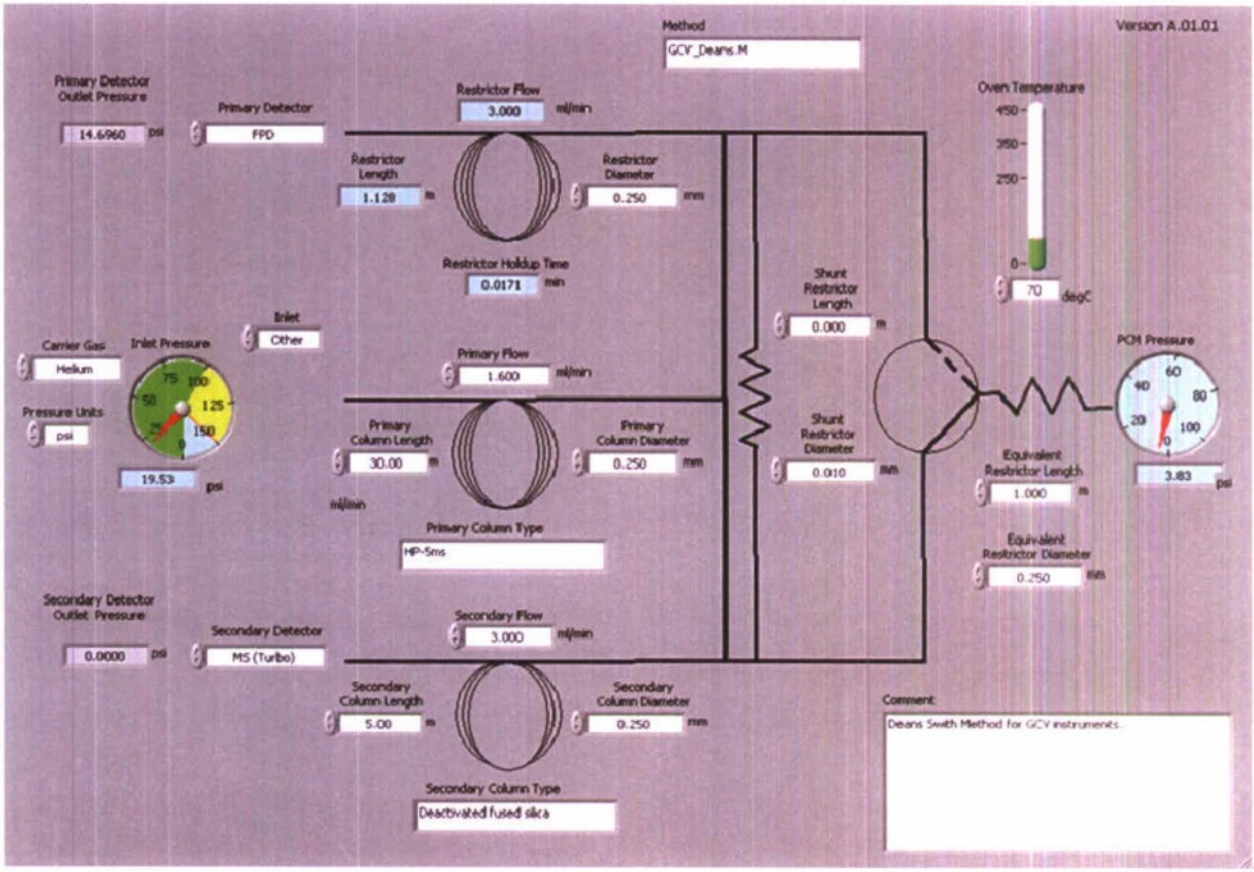
CALCULATION RESULTS:

	Primary Column	Secondary Column
Inlet Pressure:	19.53	3.83
Avg. Linear Velocity (cm/s):	34.72	154.76
Hold up Time (min):	1.44	0.054
Restrictor Length (m):		1.128
Restrictor Hold Up Time (min):		0.017

CALCULATION INPUTS

	Primary Column	Secondary Column
Length (m):	30.00	5.00
i.d. (mm):	0.25	0.25
Flow (mL/min):	1.60	3.00
Detector:	FPD	MS (Turbo)
Detector Pressure (abs):	14.70	0.00
Carrier Gas:		Helium
Pressure Units:		psi
Oven Temperature:		70.00
Restrictor Diameter:		0.25
Equivalent Restrictor Diameter (mm):		0.25
Equivalent Restrictor Length (m):		1.000

DEANS SWITCH CALCULATOR


Method
GCV_Deans.M
Version A.01.01
Primary Detector Outlet Pressure
14.6960
psi
Primary Detector
FPD
Restrictor Flow
3.000
ml/min
Restrictor Length
1.120
m
Restrictor Diameter
0.250
mm
Restrictor Holdup Time
0.0171
min
Oven Temperature
450
350
250
0
70
degC
Shunt Restrictor Length
0.000
m
Carrier Gas
Helium
Inlet Pressure
Inlet
Other
Pressure Units
psi
19.53
psi
Primary Flow
1.600
ml/min
PCM Pressure
3.83
psi
Primary Column Length
30.00
m
Primary Column Diameter
0.250
mm
ml/min
Shunt Restrictor Diameter
0.010
mm
Equivalent Restrictor Length
1.000
m
Equivalent Restrictor Diameter
0.250
mm
Primary Column Type
HP-5ms
Secondary Detector Outlet Pressure
0.0000
psi
Secondary Detector
MS (Turbo)
Secondary Flow
3.000
ml/min
Secondary Column Length
5.00
m
Secondary Column Diameter
0.250
mm
Comment
Deans Swith Method for GCV instruments.
Secondary Column Type
Deactivated fused silica

==========

MARKES TDS UNITY METHOD

==========

UNITY Method Name = GDHL.mth

Method Type:	Standard 2(3) stage desorption
Standby Split:	ON
Standby Flow Rate (mL/min):	20.0
PrePurge Time (min):	0.5
PrePurge Trap In Line:	Not in line
PrePurge Split:	ON
PrePurge Flow Rate (mL/min):	40.0
Tube Desorb Time 1 (min):	4.0
Tube Desorb Temp 1 (°C):	200
Tube Desorb Time 2 (min):	0.0
Tube Desorb Split On:	40.0
Trap Desorb Low Temp (°C):	0
Trap Desorb Low Temp (°C):	240
Trap Desorb Hold Time (min):	3.0
Trap Desorb Split On:	25.0
Trap Heating Rate (°C/s):	MAX
Flow Path Temp ((°C):	180
GC Cycle Time (min):	7.5
Minimum Carrier Pressure (psi):	5.0
Split Ratios8:	31.4:1
Desorb Flow Rate (mL/min):	40.0

Cold Trap Info: Chemical Weapon Agent Trap (Markes P/N U-T10CW)

* Split ratio is calculated based on the column flow of 1.7 mL/min, the desorb flow of 40 mL/min and the inlet/outlet split flow rates of 40.0 and 25.0 mL/min, respectively.

```
================================================================================
                                6890 GC METHOD
================================================================================


OVEN
   Initial temp:  70 °C (On)                    Maximum temp:  300 °C
   Initial time:  0.50 min                      Equilibration time:  0.25 min
   Ramps:
      # Rate Final temp Final time
      1 35.00      240        0.00
      2   0.0(Off)
   Post temp:  70 °C
   Post time:  0.00 min
   Run time:  5.36 min

FRONT INLET (SPLIT/SPLITLESS)                BACK INLET (UNKNOWN)
   Mode:  Splitless
   Initial temp:  250 °C (On)
   Pressure:  19.53 psi (On)
   Purge flow:  50.0 mL/min
   Purge time:  999.99 min
   Total flow:  56.1 mL/min
   Gas saver:  Off
   Gas type:  Helium

*Markes modified inlet may be front or back depending on inlet availability at
install.


COLUMN 1                                     COLUMN 2
   Capillary Column                             (not installed)
   Model Number:  Agilent 19091S-433
   HP-5MS  5% Phenyl Methyl Siloxane
   Max temperature:  325 °C
   Nominal length:  30.0 m
   Nominal diameter:  250.00 µm
   Nominal film thickness:  0.25 µm
   Mode:  constant pressure
   Pressure:  19.53 psi
   Nominal initial flow:  1.7 mL/min
   Average velocity:  39 cm/sec
   Inlet:  Front Inlet
   Outlet:  Other
   Outlet pressure:  ambient

FRONT DETECTOR (NO DET)                      BACK DETECTOR (FPD)
                                                Temperature:  220 °C (On)
                                                Hydrogen flow:  75.0 mL/min (On)
                                                Oxidizer flow:  100.0 mL/min (On)
                                                Oxidizer Gas Type: Air
                                                Mode:  Constant makeup flow
                                                Makeup flow:  25.0 mL/min (On)
                                                Makeup Gas Type: Helium
                                                Flame:  On
                                                Lit offset:  2.00
                                                Photo multiplier:  On
```

SIGNAL 1
Data rate: 20 Hz
Type: back detector
Save Data: On
Zero: 0.0 (Off)
Range: 0
Fast Peaks: Off
Attenuation: 0

SIGNAL 2
Data rate: 20 Hz
Type: test plot
Save Data: Off
Zero: 0.0 (Off)
Range: 0
Fast Peaks: Off
Attenuation: 0

COLUMN COMP 1
(No Detectors Installed)

COLUMN COMP 2
(No Detectors Installed)

THERMAL AUX 1
Use: MSD Transfer Line Heater
Description:
Initial temp: 280 °C (On)
Initial time: 0.00 min

#	Rate	Final temp	Final time
1	0.0	(Off)	

THERMAL AUX 2
Unknown Thermal Aux Type

AUX PRESSURE 3
Description: Deans Switch
Gas Type: Helium
Initial pressure: 3.83 psi (On)
Initial time: 0.00 min

#	Rate	Final pres	Final time
1	0.0	(Off)	

AUX PRESSURE 4
Description:
Gas Type: Helium
Initial pressure: 0.0 psi (Off)

AUX PRESSURE 5
Description:
Gas Type: Helium
Initial pressure: 0.00 psi (Off)

VALVES
Valve 1 Switching Off
Description:

POST RUN
Post Time: 0.00 min

TIME TABLE

Time	Specifier	Parameter & Setpoint
3.20	Valve 1:	On
3.90	Valve 1:	Off

GC Injector

Front Injector:
No parameters specified

Back Injector:
No parameters specified

Column 1 Inventory Number : AB002
Column 2 Inventory Number :

```
                        MS ACQUISITION PARAMETERS

General Information
------- -----------
Tune File                : atune.u
Acquistion Mode          : SIM


MS Information
-- -----------
Solvent Delay            : 3.0 min

EMV Mode                 : Gain Factor
Gain Factor              : 1.0
Resulting EM Voltage     : 2047*

[Sim Parameters]

GROUP 1
Group ID                 : GD
Resolution               : High
Plot 1 Ion               : 126.00
Ions/Dwell In Group
(Mass,  Dwell)
(82.00, 40)
(99.00, 40)
(126.00, 40)

[MSZones]

MS Quad                  : 150 C   maximum 200 C
MS Source                : 230 C   maximum 250 C
Timed Events
----- ------

[Timed MS Detector Entries]

Time (min)          State (MS on/off)
4.00                     Off


                    END OF MS ACQUISITION PARAMETERS


                          TUNE PARAMETERS*
                          ----------------
*MSD Specific Values automatically determined and set when performing an
Autotune.

END OF TUNE PARAMETERS
----------------------
END OF INSTRUMENT CONTROL PARAMETER
```

GD SPECTRUM (SCAN) - NIST LIBRARY SEARCH 2.0

The NIST library contains a sample spectrum, which can be found as identified below:

Name: Soman Formula: $C_7H_{16}FO_2P$ MW: 182
CAS#: 96-64-0 NIST#: 226124 ID#: 78999 DB: mainlib

A sample spectrum from this program is provided.

GD (RT 3.6 min) TIC and SPECTRUM (SIM) - GCV DATA ANALYSIS

