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Analytical Protocol for Cyclohexyl Sarin, Sarin, Soman and Sulfur Mustard Using Gas Chromatography/Mass Spectrometry

Office of Research and Development
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United States Environmental Protection Agency
Office of Research and Development
Homeland Security Research Program
Cincinnati, Ohio 45268

Acknowledgments

This method is based on procedures developed by Lawrence Livermore National Laboratory (LLNL) under Interagency Agreement (IAG) DW89922616-01-0 with the U.S. Environmental Protection Agency (EPA). EPA's Homeland Security Research Program (HSRP) and Office of Land and Emergency Management managed and funded laboratory testing of the procedures for analysis of water, soil, and wipe samples in a multi-laboratory study. Laboratories participating in the study and providing technical support include EPA Regions 1, 3, 6, 9, and 10; EPA's Portable High Throughput Integrated Laboratory Identification System (PHILIS) Unit mobile laboratory in Castle Rock, Colorado; the Virginia Division of Consolidated Laboratories; the Florida Department of Environmental Protection; and LLNL. Technical support, study coordination and data evaluation were provided by CSGov (formerly CSC).

Disclaimer

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1.0 SCOPE AND APPLICATION

1.1 The U.S. Environmental Protection Agency (EPA) Homeland Security Research Program (HSRP) and Office of Land and Emergency Management (OLEM), in collaboration with experts from across EPA and other federal agencies, have identified analytical methods to be used for the analysis of extractable semi-volatile chemical agents in response to a homeland security incident. This protocol is to be applied by the national network of laboratories that has been recruited to the EPA-established Environmental Response Laboratory Network (ERLN) so that their analytical results are consistent and comparable. Summaries of these methods are provided in EPA's *Selected Analytical Methods for Environmental Remediation and Recovery (SAM)* (Reference 16.1). HSRP is using the SAM methods (based on the methods listed in Section 1.2) to develop analytical protocols for laboratory identification and measurement of target agents during site remediation.

1.2 This document is for the determination and measurement of the chemical warfare agents (CWAs) listed in the table below and in Table 1, Section 17. The protocol is based on EPA Methods 8270D (Reference 16.2), 3511 (Reference 16.3), 3570 (Reference 16.4), and EPA Method 1613 (Reference 16.5) for preparation and analysis of solid, wipe, and water samples. The procedures have been multi-laboratory tested for analysis of the analytes listed below in soil, wipe, and water samples.

Contaminant	
Cyclohexyl methylphosphonofluoridate − Cyclohexyl sarin (GF)	329-99-7
Bis(2-chloroethyl) sulfide − Sulfur mustard (HD)	505-60-2
(*RS*)-Propan-2-yl methylphosphonofluoridate − Sarin (GB)	107-44-8
3-Dimethylbutan-2-yl methylphosphonofluoridate − Soman (GD)	96-64-0

* Chemical Abstracts Service (CAS) Registry Number

1.3 Procedures in this protocol have been tested for the target analytes listed in Section 1.2 in reference matrices (i.e., reagent water, Ottawa sand, and wipes), drinking water from two sources, and two dried and homogenized soils. Results of laboratory testing are provided in Sections 13.0 and 17.0.

2.0 SUMMARY OF PROTOCOL

This analytical protocol involves microscale extraction, followed by gas chromatography/mass spectrometry (GC/MS) analysis to identify and measure target semi-volatile CWAs. Soil and wipe extracts also might require a concentration step using nitrogen evaporation to achieve appropriate levels of quantitation.

3.0 ACRONYMS, ABBREVIATIONS and DEFINITIONS

3.1 Acronyms and Abbreviations

%Recovery	Percent recovery
%RSD	Percent relative standard deviation
amu	Atomic mass unit

ASTM	ASTM International (formerly American Society for Testing and Materials)
CAS RN	Chemical Abstracts Service Registry Number
CCV	Continuing calibration verification
CWA	Chemical warfare agent
DCM	Methylene chloride (dichloromethane)
DF	Dilution factor
DFTPP	Decafluorotriphenylphosphine
EI	Electron ionization
EICP	Extracted ion current profile
EPA	U.S. Environmental Protection Agency
FC-43	Perfluoro-tri-*n*-butylamine
GB	Sarin
GD	Soman
GF	Cyclohexyl sarin
GC/MS	Gas chromatograph/mass spectrometer (gas chromatography/mass spectrometry)
HD	Sulfur mustard
HSRP	Homeland Security Research Program
IDC	Initial demonstration of capability
IDL	Instrument detection limit
IS	Internal Standard
IPR	Initial precision and recovery
LCS	Laboratory control sample
MDL	Method detection limit
MS/MSD	Matrix spike/matrix spike duplicate
OLEM	Office of Land and Emergency Management
OSHA	U.S. Occupational Safety and Health Administration
PD	Percent drift
PE	Performance evaluation
PFK	Perfluorokerosene
PPE	Personal protective equipment
PTFE	Polytetrafluoroethylene (Teflon®)
QC	Quality control
QL	Quantitation limit
RPD	Relative percent difference
rpm	Revolution(s) per minute
RRF	Relative response factor
RRT	Relative retention time
RSD	Relative standard deviation
RT	Retention time
SAM	*Selected Analytical Methods for Environmental Remediation and Recovery*. http://www.epa.gov/homeland-security-research/sam/ (accessed 05/31/2016)
SDS	Safety Data Sheet
S:N	Signal-to-noise ratio
SVOC	Semi-volatile organic compound
TOF	Time-of-flight
VOA	Volatile organic analysis

3.2 Definitions

Aliquot – A measured portion of a field sample, standard, or solution taken for sample preparation and/or analysis.

Analytical Batch – A set of samples that is analyzed on the same instrument during a 24-hour period of operation or the analysis of 20 samples (whichever comes first). The analytical batch begins and ends with the analysis of the appropriate Continuing Calibration Verification (CCV) standards.

Calibration Standard – A solution prepared from the stock standard solution(s) and the internal standards and surrogate analytes. The calibration standards are used to calibrate instrument response with respect to analyte concentration.

Continuing Calibration Verification (CCV) – A calibration standard containing the target analytes, which is analyzed periodically to verify the accuracy of the existing calibration for those analytes.

Extracted Ion Current Profile (EICP) – A plot of ion abundance versus time (or scan number) for ion(s) of specified mass(es).

Holding Time – The time elapsed from sample collection until sample extraction or analysis.

Initial Calibration – Analysis of calibration standards for a series of different specified concentrations; used to define the quantitative response, linearity, and dynamic range of the instrument for target analytes.

Initial Demonstration of Capability (IDC) – The IDC is performed prior to use of the analytical procedures and is used to evaluate the capability of the laboratory in terms of analytical precision, bias and sensitivity pertaining to the target analytes.

Initial Precision and Recovery (IPR) – A set of four aliquots of a clean reference matrix (i.e., reagent water, Ottawa sand, clean wipe) to which known quantities of the target analytes are added. The IPR aliquots are processed and analyzed exactly like a sample and analyzed prior to the analysis of field samples as part of the IDC. Their purpose is to determine whether the laboratory is capable of making accurate and precise measurements.

Instrument Detection Limit (IDL) – The minimum concentration of an analyte that, when injected into the gas chromatograph/mass spectrometer (GC/MS), produces an average signal-to-noise ratio (S:N) between 3:1 and 5:1 for at least three replicate injections.

Instrument Performance Check Solution – A solution of one or more instrument tuning compounds used to evaluate the performance of the instrument system with respect to a defined set of method criteria.

Internal Standard (IS) – Analyte added to an extract or standard solution in a known amount and used to measure the relative responses of target analytes and surrogates. The internal standard must be an analyte that is not a sample component.

Laboratory Control Sample (LCS) – An aliquot of a clean reference matrix (i.e., reagent water, Ottawa sand, clean wipe) to which known quantities of the target analytes

are added. The LCS is processed and analyzed exactly like a sample. Its purpose is to determine whether the analytical process is in control.

Matrix – The predominant material of which the sample to be analyzed is composed. For the purpose of this protocol, a sample matrix is either aqueous/water, soil/sediment/sand, or wipe. Matrix is not synonymous with phase (e.g., liquid or solid).

Matrix Spike (MS) – An aliquot of a field sample to which known quantities of target analytes are added. The MS is processed and analyzed exactly like the corresponding sample. Its purpose is to determine whether the sample matrix contributes bias to the analytical results. Background concentrations of the analytes must be determined in a separate aliquot.

Matrix Spike Duplicate (MSD) – A second aliquot of the field sample used to prepare the MS, which is fortified, extracted and analyzed exactly like the MS. The MSD is used to assess matrix effects on analytical precision and bias.

Method Blank – An aliquot of a clean reference matrix (i.e., reagent water, Ottawa sand, clean wipe) that is treated exactly as a sample, including exposure to all glassware, equipment, solvents, reagents, sample preservatives, internal standards, and surrogates that are used in the extraction batch. The method blank is used to determine whether target analytes or interferences are present in the laboratory environment, reagents or equipment.

Method Detection Limit (MDL) – The minimum concentration of an analyte that can be identified, measured and reported with 99 percent confidence that the analyte concentration is greater than zero. The MDL is a statistical determination (Section 9.7), and accurate quantitation at this level is not expected.

Percent Difference – The difference between two values divided by one of the values. Used in this protocol to compare two relative response factor (RRF) values from calibration.

Percent Drift (PD) – The difference between the calculated and theoretical value divided by the theoretical value. Used in this protocol to compare calculated and theoretical values for calibration by regression techniques.

Quantitation Limit (QL) – The minimum level of quantitation. This concentration must meet the criteria defined in Section 9.8.

Reagent Water – Water in which an interferent is not observed at or above the low-level calibration standard for each analyte of interest. The purity of this water must be equivalent to ASTM International (ASTM) Type II reagent water of Specification D1193-06, "Standard Specification for Reagent Water" (Reference 16.6).

Relative Percent Difference (RPD) – The difference between two values divided by the mean of the values. RPD is reported as an absolute value (i.e., always expressed as a positive number or zero).

Relative Response Factor (RRF) – A measure of the relative mass spectral response of an analyte compared to its internal standard. RRFs are determined by analysis of standards and are used in calculating the concentrations of analytes in samples.

Retention Time (RT) – The time an analyte is retained on a GC column before elution. The RT is dependent on the analyte, nature of the column's stationary phase, the column's diameter, temperature, carrier gas flow rate, and other column parameters.

Relative Retention Time (RRT) – The ratio of the RT of a compound to the RT of a corresponding internal standard.

Safety Data Sheet (SDS) – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, flammability, and reactivity data including storage, spill, and handling precautions.

Stock Standard Solution – A concentrated solution containing one or more target analytes prepared in the laboratory using assayed reference materials or materials purchased from a reputable commercial source.

Surrogate – Analyte that is unlikely to be found in any sample to be analyzed. Surrogates are added to a sample aliquot in a known amount before extraction or other processing. Surrogates are measured with the same procedures used to measure other sample components. The purpose of the surrogate is to monitor method performance with each sample.

Working Standard Solution – A solution containing target analytes prepared from stock standard solutions. Working standard solutions are diluted as needed to prepare calibration and spiking solutions.

4.0 INTERFERENCES

4.1 Contaminants in solvents, reagents, glassware, and other sample processing hardware can cause method interferences such as discrete artifacts and/or elevated baselines in the extracted ion current profiles (EICPs). All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running method blanks. Matrix interferences can be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source and is evaluated using the results from the analysis of matrix spike and matrix spike duplicate samples (MS/MSDs).

4.2 This protocol includes conditions for collecting mass spectral data using both quadrupole mass spectrometers in full-scan mode and time-of-flight (TOF) mass spectrometers.

5.0 SAFETY

WARNING: The toxicity of CWAs presents hazards unfamiliar to most experienced laboratory personnel. Special techniques and precautions must be used even for the simplest procedures involving these agents. Because CWAs are target analytes for this protocol, laboratory personnel must be thoroughly trained in appropriate safety procedures prior to using this method.

5.1 Operations with CWAs have specific safety requirements. The laboratory must have these requirements included in a Chemical Hygiene Plan prior to conducting the analytical procedures described in this protocol.

5.2 At a minimum, personal protective equipment (PPE) requirements include safety glasses, lab coats, and protective gloves. The availability of emergency response equipment and support personnel should be as indicated in a laboratory Chemical Hygiene Plan.

5.3 Exposure to chemical agent material is possible from contact, and risk is primarily associated with compromise of PPE. Respiratory exposure can result from spills or improper use of ventilation controls and PPE.

5.4 At concentrations of CWAs that are within the calibration range of this method, likelihood of an exposure causing adverse health effects is extremely low (Reference 16.7).

Note: This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current file of Occupational Safety and Health Administration (OSHA) regulations regarding the safe handling of the chemicals (including all solvents, reagents, and standards). A reference file of safety data sheets (SDSs) must be available to all personnel involved in these analyses, chemical handling, and contaminated area cleaning, or who might potentially come in contact with the materials in their workplace.

6.0 EQUIPMENT AND SUPPLIES

Brand names, suppliers, catalog, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance can be achieved using equipment and supplies other than those specified; however, laboratories must document use of alternative equipment or supplies and provide a demonstration of equivalent performance meeting the requirements of this protocol.

6.1 General Equipment

6.1.1 Vials – Clear or amber glass, with polytetrafluoroethylene (PTFE)-lined screw or crimp top (2.0 mL capacity for GC auto sampler) (Sigma Aldrich Catalog No.SU860033, Sigma-Aldrich. St. Louis, MO) or equivalent. Glass inserts can be used to minimize sample volumes.

6.1.2 Syringes – Contaminant-free, 1.0 mL, 2.0 mL, 10 µL, 25 µL, 500 µL

6.1.3 Pasteur glass pipettes – 1.0 mL, disposable (Fisher Scientific Catalog No. NC0541803, Thermo Fisher Scientific, Westminster, MD) or equivalent.

6.1.4 Balances – Analytical, capable of accurately weighing ±0.0001 gram, and one capable of weighing 100 grams (±0.01 grams)

6.1.5 Spatula – Stainless steel or PTFE

6.1.6 Nitrogen evaporation device – Equipped with temperature control that can be maintained at 35 – 40 °C, a RapidVap® (Labconco Corporation, Kansas City, MO) or equivalent. To prevent the release of solvent fumes into the laboratory, this device must be used with suitable engineering controls.

6.1.7 Water bath (for nitrogen evaporation devices that do not have a heating apparatus) – Heated, with concentric ring cover capable of heating to 80 °C and maintaining temperature control (±5 °C). The bath should be used with appropriate engineering controls.

6.1.8 Glass funnel (Fisher Scientific Catalog No. CG172305, Thermo Fisher Scientific, Westminster, MD) or equivalent - Used in filtering soil samples that fail to settle out with centrifugation.

6.1.9 Borosilicate glass wool - Oven-cleaned (muffled) or solvent-rinsed (using extraction solvent); used in filtering soil samples that fail to settle out with centrifugation.

6.1.10 pH paper - Including narrow range capable of measuring a pH of 2.0.

6.1.11 pH meter - With a combination glass electrode. Calibrate prior to each use according to manufacturer's instructions.

6.1.12 Ottawa sand - Held at 450 °C for four hours in a 500-mL wide-mouthed amber bottle.

6.1.13 Vortexer – VWR (VWR Corporation, Radnor, PA) or equivalent, capable of accommodating 40 – 60-mL vials and 50-mL centrifuge tubes.

6.1.14 Shaker table

6.1.14.1 Glas-Col Large Capacity Mixer (Part # 099A LC1012, Glas-Col Inc., Terre Haute, IN), Glas-Col Digital Pulse Mixer (Part # 099A DPM12) or equivalent

6.1.14.2 Foam pad for 40-mL volatile organic analysis (VOA) vials (Part #099A VC4014, Glas-Col Inc., Terre Haute, IN) or equivalent

6.1.14.3 Foam pad for 60-mL VOA vials (Part #099A VC6014, Glas-Col Inc., Terre Haute, IN) or equivalent

6.2 Microscale Extraction Apparatus

6.2.1 Solid samples

6.2.1.1 VOA vials - 40-mL capacity, disposable, pre-cleaned with PTFE-lined caps (Fisher Scientific Catalog No. 05-719-400, Thermo Fisher Scientific, Westminster, MD) or equivalent. If pre-cleaned vials are not available, appropriate cleaning procedures are provided in EPA Methods 525.3 (Reference 16.8) and 1668C (Reference 16.9) and in SW-846 Chapter 4 (Reference 16.10).

6.2.1.2 Sonicator – Branson 3510 (Branson Ultrasonics Corp., Danbury, CT) or equivalent

6.2.1.3 Apparatus for determining percent dry weight

6.2.1.3.1 Drying oven – Capable of maintaining 103 – 105 °C

6.2.1.3.2 Desiccator

6.2.1.3.3 Crucibles – Disposable aluminum

6.2.1.4 Glass beads – Solvent-rinsed, baked in 400 °C oven for approximately 1 hour (Fisher Scientific Catalog No. S80024 or equivalent)

6.2.1.5 Centrifuge – Capable of at least 500 G-force units and accommodating 40- or 50-mL vials, Accuspin™ Model 400 (Thermo Fisher Scientific, Westminster, MD) or equivalent. CAUTION: Different centrifuge makes and models have different maximum centrifuge speeds that are recommended for safe operation. The maximum safe handling speed of each centrifuge will depend, in part, on the vials used and should be determined prior to centrifuging samples.

6.2.1.6 Pasteur pipettes – 1.0-mL glass, disposable or re-pipettes/autopipettes with disposal tips

6.2.2 Water samples

6.2.2.1 Conical bottom, glass screw-top tube, 50-mL or 60-mL vials, pre-cleaned with PTFE-lined caps. If pre-cleaned vials are not available, appropriate cleaning procedures are provided in EPA Methods 525.3 (Reference 16.8) and 1668C (Reference 16.9), and in SW-846 Chapter 4 (Reference 16.10).

6.2.2.2 Beakers – 400 mL

6.2.2.3 Class A graduated cylinder – 100 mL

6.2.2.4 Class A volumetric flasks – 10 mL

6.2.3 Wipe samples - Kendall Curity® Gauze Sponges (, USP Type VII Gauze, cotton, 12 ply 3 in x 3 in, Tyco Healthcare Group, Covidien, Mansfield, MA) or equivalent; pre-cleaned by extracting with methylene chloride (DCM) using an appropriate extraction method (e.g., pressurized fluid extraction, Soxhlet extraction, soaking in DCM).
Note: Wipes used for field or laboratory quality control (QC) samples should be pre-wetted with DCM prior to use.

6.3 Gas Chromatograph/Mass Spectrometer (GC/MS) System

6.3.1 Gas chromatograph – The GC system must be capable of temperature programming and have a flow controller that maintains a constant column carrier gas flow rate throughout the temperature program operations. The system must be suitable for splitless injection and have all required accessories including syringes, analytical columns, and gases. All GC carrier gas lines must

be constructed from stainless steel or copper tubing. Non-PTFE thread sealants or flow controllers with rubber components are not to be used.

Note: Due to the potential hazards associated with the analysis of CWAs, it is recommended that a split vent trap be used for the GC system (Agilent RD-1020 Universal/External Split Vent Trap, Agilent Technologies Inc., Santa Clara, CA, or equivalent). For additional safety measures, the split and purge vent lines can be vented to the hood ventilation system.

6.3.2 GC column – Recommended length 30 m X 0.25 mm inner diameter (ID) (or 0.32 mm) bonded phase silicon coated fused silica capillary: DB-5 (J&W Scientific, Agilent Technologies, Santa Clara, CA); DB-5MS; RTX®-5 (Restek Corp., Bellefonte, PA), RTX®-5MS (Restek Corp., Bellefonte, PA), RTX-5Sil MS (Restek Corp., Bellefonte, PA); Zebron® ZB-5(Phenomenex, Phenomenex Inc., Torrance, CA); SPB®-5 (Supelco, Sigma-Aldrich. St. Louis, MO); AT-5 (Alltech, Grace, Columbia, MD); HP-5 (Agilent, Agilent Technologies, Santa Clara, CA); HP-5MS or HP-5MS UI (Agilent Technologies, Santa Clara, CA,), CP-Sil 8 CB (Chrompack Raritan, NJ); 007-2 (Quadrex, Quadrex Corp., Bethany, CT); BP-5 (SGE, Trajan Scientific Americas, Inc., Austin, TX); Zebron® ZB-5MS (Phenomenex, Phenomenex Inc., Torrance, CA) or equivalent. Columns used to generate the example data provided in Section 17.0 include: Agilent HP-5 MS, RTX® – 5sil, RTX®-5MS, RTX®-5Sil MS, Zebron® ZB-5 MS, DB-5MS.

Although a film thickness of 1.0 micron might be desirable because of its larger capacity, film thicknesses of 0.25 micron and 0.05 micron were used by laboratories generating the example data presented in Section 17.0. A description of the GC column used for analysis shall be provided in the data narrative. A capillary column is considered equivalent if:

- The column does not introduce contaminants that interfere with the identification and quantification of the compounds listed in Table 1, Section 17.
- The analytical results generated using the column meet the initial calibration and CCV technical acceptance criteria listed in the protocol and the quantitation levels determined as described in Section 9.8.
- The column provides equal or better resolution of the compounds listed in Table 1, Section 17, when compared to columns listed above.

6.3.3 MS – Must be capable of recording a spectrum from 35 – 500 atomic mass units (amu) every second or less, using 70 electron volts (nominal) in the electron ionization (EI) mode, and producing a mass spectrum that meets the tuning acceptance criteria when 50 ng or less of decafluorotriphenylphosphine (DFTPP) is injected through the GC inlet. The instrument must be vented to prevent the release of contaminants into the instrument room.

7.0 REAGENTS AND STANDARDS

7.1 Reagents

7.1.1 Organic-free reagent water – Water in which an interferent is not observed at or above the quantitation limit (QL) for each analyte of interest. ASTM Type II reagent water of Specification D1193-06, "Standard Specification for Reagent Water," (Reference 16.6) or equivalent.

7.1.2 Acetone, DCM, and toluene – pesticide residue analysis grade or equivalent. DCM must contain a non-methanol olefinic stabilizer (i.e., amylene or 2-pentene). Solvents must be anhydrous or pre-dried by adding sodium sulfate until no clumping occurs and the solution becomes clear (not cloudy).

7.1.3 Sodium sulfate – Powdered or granular anhydrous reagent grade, heated at 400 °C for four hours in a shallow tray, cooled in a desiccator, and stored in a glass bottle

7.1.4 Sodium chloride – Anhydrous reagent grade, >99 %

7.1.5 Sodium thiosulfate – Reagent grade, 10 % sodium thiosulfate solution in reagent water. Prepare fresh with each use.

7.2 Standards

The laboratory must be able to verify that stock standard solutions are certified. Manufacturers' certificates of analysis must be retained by the laboratory and presented upon request. Stock standard solutions provided in sealed glass ampules may be retained and used within six months of the preparation date. Solutions used for calibration verification ideally are prepared from a separate source other than the source used to prepare calibration standards. Due to the nature of the analytes addressed in this protocol, identification of a secondary source might be difficult.

7.2.1 Stock standard solutions

Stock standard solutions used to produce working standards may contain individual target compounds or mixtures of target compounds.

7.2.2 Working standards

7.2.2.1 Surrogate standard spiking solution – Prepare a surrogate standard spiking solution in DCM or other suitable solvent that contains appropriate surrogates for the target compounds. A concentration of 25 µg/mL is recommended for each surrogate. Surrogate standards are added to all samples and calibration solutions.

Note: Table 4, Section 17 provides a list of surrogates used during laboratory studies testing this protocol, based on surrogates typically used for semi-volatile organic compounds (SVOC) analyses. Alternative surrogates might be more representative of the analytes targeted in this method and may be used instead of or

in addition to those listed in Table 4, provided the surrogates meet the criteria in Table 7, Section 17.

7.2.2.2 Matrix spiking solution – This solution is prepared in DCM and should contain all target analytes.

7.2.2.3 Instrument performance check solution – Prepare a solution of DFTPP in DCM such that a 1-µL injection will contain 50 ng or less of DFTPP. The DFTPP may also be included in the calibration standards at this level.

7.2.2.4 Initial and continuing calibration solutions

7.2.2.4.1 Prepare calibration standards in DCM at a minimum of five concentration levels. Each calibration standard should contain each target compound of interest, associated surrogate, and internal standard.

Note 1: All samples analyzed must be injected at the same volume (e.g., 1.0 or 2.0 µL) as the calibration standard.

Note 2: The concentrations listed in Table 8, Section 17, provide an example calibration range used during laboratory evaluation of this protocol. For most analytes, the low calibration standard is set at the expected QL (determined in Section 9.8). The remaining calibration standards should be prepared at concentrations that meet the specifications in Section 10.3.4.

7.2.2.4.2 The CCV standard is prepared in DCM at or near the midpoint of the calibration curve.

7.2.2.5 Internal standard solution – An internal standard solution can be prepared by dissolving 100 mg of each of the following compounds in 100 mL of DCM: 1,4-dichlorobenzene-d_4 and naphthalene-d_8, resulting in a concentration of 1.0 mg/mL of each internal standard solution. A sufficient portion of this solution will be added to each sample extract just prior to analysis by GC/MS to result in a concentration of 0.5 ng/µL (for both full-scan quadrupole and TOF mass spectrometers). Alternatively, internal standard solutions can be purchased from commercial sources (e.g., Supelco part number 861238 or equivalent).

7.2.3 Storage of standard solutions

7.2.3.1 Store unopened ampules of stock standard solutions at ≤6 °C. If no manufacturer's expiration date is provided, unopened ampuled standard solutions may be retained and used for up to six months after the preparation date (Reference 16.11). Store opened stock standard solutions at ≤6 °C in PTFE-lined screw-cap amber bottles.

Fresh standards should be provided every twelve months (for solutions containing a single target compound) or six months (for solutions containing a mixture of target compounds), or sooner if the expiration date has elapsed.

7.2.3.2 Store the working standards at ≤ 6 °C in containers with PTFE-lined caps. Certain analytes may degrade in as little as two weeks; therefore, the working standard solution should be checked against CCV standards at least weekly for stability. If stored as single-analyte solutions, standards containing soman (GD) or cyclohexyl sarin (GF) should be stable to up to 12 months; standards containing sulfur mustard (HD) should be stable for up to six months; and standards containing sarin (GB) should be stable for up to five months. Multi-component working standards should be replaced after five months. Working standard solutions must be replaced if the stock standard solutions have expired or if comparison with CCV samples indicates a problem.

7.2.3.3 Protect all standards from light. Samples, sample extracts, and standards must be stored separately.

7.2.3.4 The laboratory is responsible for maintaining the integrity of standard solutions and verifying the solution prior to use. The standards must be brought to room temperature prior to use, checked for losses, and checked to ensure that all components have remained in solution. Guidance on standard verification procedures can be found in EPA's Superfund Analytical Services / Contract Laboratory Program, Multi-Media, Multi-Concentration Organics Analysis, SOM02.3, Exhibit E, Section 4 (Reference 16.12).

8.0 SAMPLE PRESERVATION, STORAGE, AND TECHNICAL HOLDING TIMES

8.1 Sample Preservation

Samples must be stored on ice or refrigerated at 4 °C (± 2 °C) immediately after collection until receipt in the laboratory. The presence of chlorine may increase the degradation rate of G-agents in water. If chlorine is suspected to be present in water samples (e.g., treated drinking water or wastewater) that are to be measured for G-agents, add approximately four drops (~0.2 mL) of a 10 % solution of sodium thiosulfate per 35-mL sample. If clouding results, add less sodium thiosulfate to a fresh sample aliquot. If sodium thiosulfate is not added during sample collection, it should be added immediately upon sample receipt in the laboratory, prior to sample analysis or extraction.

8.2 Sample Storage

Samples must be protected from light and refrigerated at 4 °C (± 2 °C) from the time of receipt until extraction.

8.3 Sample Extract Storage

8.3.1 Sample extracts must be protected from light and stored at ≤6 °C.

8.3.2 Samples, sample extracts, and standards must be stored separately.

8.4 Technical Holding Times

8.4.1 Soil and wipe samples must be extracted within seven days of receipt at the laboratory. Aqueous samples should be analyzed as soon as possible upon receipt in the laboratory. At a minimum, DCM must be added to aqueous samples within 48 hours of receipt (DCM should be added immediately to aqueous samples that will be analyzed for HD), and samples must be completely extracted within seven days of receipt.

Note: Holding times for soils and wipes are based on holding times listed in guidance documents and similar analytical methods for SVOCs (e.g., EPA Methods 525.2 [Reference 16.13] and 525.3 [Reference 16.8], CLP Method SOM2.1 [Reference 16.14], SW-846 Chapter 4 [Reference 16.10]). Holding times for water samples were evaluated in a single laboratory using this protocol. Although the addition of DCM has not been evaluated, these CWA agents are likely to partition into the organic phase where they would be less likely to hydrolyze. Laboratories may wish to verify this holding time for the specific samples being analyzed.

8.4.2 Extracts must be analyzed within 14 days following extraction.

9.0 QUALITY CONTROL (QC)

QC requirements for this protocol include the following:

Quality Control (QC) Analyses

Requirement	Section	Frequency
Instrument Detection Limit (IDL) Determination	Section 9.6	Optional. Performed prior to Method Detection Limit (MDL) Study
Method Detection Limit (MDL) Determination	Section 9.7	Performed once, prior to first performing the protocol procedures and with each significant change as part of the Initial Demonstration of Capability (IDC)
Initial Precision and Recovery (IPR) Determination	Section 9.2	
Quantitation Limit (QL) Determination	Section 9.8	
Method Blanks	Section 9.3	At least one per batch of ≤20 samples of the same matrix
Instrument Blank	Section 10.5	Following an analysis with suspected carry-over or following analysis of samples containing high concentrations
Matrix Spike and Matrix Spike Duplicate (MS/MSD)	Section 9.4	One per each batch of ≤20 samples of the same matrix
Laboratory Control Sample (LCS)	Section 9.5	At least one per batch of ≤20 samples of the same matrix
Continuing Calibration Verification (CCV)	Section 10.4	Prior to the analysis of samples, and after instrument performance check. Analyzed at the beginning and at the end of each analytical batch of ≤20 injections

Precision and bias criteria for data generated using this method are currently set at 50 – 150 % recovery and ≤ 30 % precision (as relative standard deviation [RSD] or relative percent difference [RPD]). These criteria may change as more laboratory data become available. In cases where analyses of difficult sample matrices generate results outside these criteria, data should be flagged, and laboratories should collect additional data to support development of laboratory- and matrix-specific criteria. Example precision and bias results obtained from multiple laboratories analyzing spiked reference matrix samples (reagent water, Ottawa sand, and wipes) and field samples (water and soil) are provided in Section 17.

9.1 Initial Demonstration of Capability (IDC)

An IDC shall be performed prior to the analysis of any samples and with each significant change in instrument type, different detection technique, personnel or method. An IDC consists of the following:

- An IPR determination (Section 9.2),
- An MDL determination (Section 9.7), and
- A QL determination (Section 9.8) on a clean matrix (reagent water, Ottawa sand, pre-cleaned wipe).

The IPR consists of four replicate samples of a clean matrix spiked with CWAs around the midpoint of the calibration curve and carried through the entire analytical process. Prior to performing the IDC, a valid initial calibration (Section 10.3) must be established.

9.2 Initial Precision and Recovery (IPR) Determination

9.2.1 Preparation and analysis of IPR samples

9.2.1.1 Water samples

Prepare four replicate samples, each consisting of 35 mL of reagent water. Add a sufficient amount of surrogate standard spiking solution and matrix spiking solution to result in the addition of 1.0 µg of each surrogate (add 40 µL if prepared as in Section 7.2.2.1) and a concentration at the mid-point of the calibration range of each matrix spike compound. Extract and analyze according to the procedures for water samples (Sections 11.2 and 11.6). The total volume of DCM added will be slightly greater than the 2 mL needed for extraction, and includes the volumes added for spiking target compounds, and surrogates.

9.2.1.2 Ottawa sand

Prepare four replicate samples consisting of 10 grams of Ottawa sand and 2.5 grams of sodium sulfate. Add a sufficient amount of the surrogate standard spiking solution and the matrix spiking solution to result in the addition of 0.5 µg of each surrogate (add 20 µL if prepared as in Section 7.2.2.1) and a concentration at the mid-point calibration range of each matrix spike compound, and follow the appropriate extraction procedure in Section 11.3. Extract, concentrate and analyze according to procedures for solid samples.

9.2.1.3 Pre-cleaned wipes

Prepare four replicate samples consisting of pre-cleaned wipes. Pre-wet the wipes with DCM prior to use. Add a sufficient amount of the surrogate standard spiking solution and the matrix spiking solution to result in the addition of 0.5 µg of each surrogate (add 20 µL if prepared as in Section 7.2.2.1) and a concentration at the mid-point calibration range of each matrix spike compound, and follow the appropriate extraction procedure in Section 11.4. Extract, concentrate and analyze according to procedures for wipe samples.

9.2.2 Calculations for IPR

9.2.2.1 Calculate the percent recovery (%Recovery) of each compound in each IPR sample using Eq. 11 (Section 12.2.9.2). Calculate an average %Recovery for each compound.

9.2.2.2 Calculate a percent relative standard deviation (%RSD) for each compound in the IPR samples.

9.2.3 Technical acceptance criteria for IPR

9.2.3.1 The average recovery of each compound in the IPR should be within 50 – 150 %.

9.2.3.2 The % RSD of each compound in the IPR should be less than or equal to 30.

9.2.4 Corrective Action for IPR

If the technical acceptance criteria in Section 9.2.3 are not met, inspect the system for problems and take corrective actions to achieve the acceptance criteria.

9.3 Method Blanks

A method blank is a volume of a clean reference matrix (e.g., reagent water for water samples, clean inert sand along with purified sodium sulfate for solid samples, or clean pre-wetted wipes for wipe samples) spiked with a sufficient amount of surrogate standard spiking solution (Section 7.2.2.1) such that the same amount of surrogate is added as for the associated samples and carried through the entire analytical procedure. Internal standard solution is added just prior to analysis by GC/MS to give a concentration of 0.5 ng/µL for each internal standard for both quadrupole and TOF modes. The volume or weight of the method blank must be approximately equal to the volume or weight of the samples associated with the blank.

9.3.1 Frequency of method blanks

A method blank must be extracted each time samples are extracted. The number of samples extracted with each method blank should not exceed 20 field samples (excluding MS/MSDs and Performance Evaluation [PE] samples). In addition, a method blank is:

- Extracted by the same procedure used to extract samples
- Analyzed on each GC/MS system used to analyze associated samples and conditions (i.e., GC/MS settings)

9.3.2 Method blank preparation

9.3.2.1 A method blank for water samples consists of a 35-mL volume of reagent water spiked with a sufficient amount of the surrogate standard spiking solution to result in the addition of 1.0 µg of each surrogate (add 40 µL if prepared as in Section 7.2.2.1). The final volume of the extracts will be approximately 2.0 mL; therefore, the concentration of the surrogates in the extract is expected to be approximately 0.50 µg/mL. For solid samples, a method blank consists of 10 grams of clean inert sand and 2.5 grams of sodium sulfate spiked with a sufficient amount of the surrogate spiking solution to result in the addition of 0.5 µg of each surrogate (add 20 µL if prepared as in Section 7.2.2.1). The final volume of the extracts will be 1.0 mL; therefore, the concentration of the surrogates in the extract is expected to be 0.50 µg/mL. A method blank for wipe samples consists of a clean unused wipe spiked with a sufficient amount of the surrogate standard spiking solution to result in the addition of 0.5 µg of each surrogate (add 20 µL if prepared as in Section 7.2.2.1). The final volume of the extracts will be 1.0 mL; therefore, the concentration of the surrogates in the extract is expected to be 0.50 µg/mL. Extract, concentrate, and analyze the blank according to procedures.

9.3.2.2 Under no circumstances should method blanks be analyzed at a dilution.

9.3.3 Technical acceptance criteria for method blank analysis

9.3.3.1 All blanks should be extracted and analyzed at the frequency described in Section 9.3.1 on a GC/MS system meeting the DFTPP tuning criteria in Section 10.2.4 and Table 3, Section 17, initial calibration in Section 10.3, and CCV technical acceptance criteria in Section 10.4.5.

9.3.3.2 The % Recovery of each of the surrogates in the blank must be within the acceptance limits listed in Table 7, Section 17.

9.3.3.3 The blank must meet the sample analysis acceptance criteria listed in Section 12.3.

9.3.3.4 A method blank for solid, water, and wipe samples must contain a concentration less than the MDL for all target compounds. In cases where a blank has detects above the MDL, but associated samples have detects greater than 10 times the blank, consult the agency to determine if re-extraction is required. In cases where a method blank fails to meet technical acceptance criteria but all samples had non-detects for all target analytes, no re-extraction or qualification of data is necessary.

9.3.4 Corrective action for method blanks

9.3.4.1 If a method blank does not meet the technical acceptance criteria for method blank analysis, the analytical system is considered to be out of control.

9.3.4.2 If contamination is the problem, then the source of the contamination should be investigated and appropriate corrective measures taken before further sample analysis proceeds. It is the laboratory's responsibility to ensure that interferences caused by contaminants in solvents, reagents, glassware, and sample storage and processing hardware that lead to discrete artifacts and/or elevated baselines in the GC/MS have been eliminated. If possible, an aliquot of any sample associated with the contaminated blank should be re-extracted and reanalyzed.

9.3.4.3 If surrogate recoveries in the method blank do not meet the acceptance criteria listed in Table 7, Section 17, first reanalyze the method blank. If the surrogate recoveries do not meet the acceptance criteria after reanalysis, the method blank and an aliquot of any sample associated with that method blank should be re-extracted, if possible, and reanalyzed. If a surrogate recovery is high and all corresponding samples had non-detects for the associated target compounds, sample re-extraction and reanalysis are not required.

9.3.4.4 If the method blank does not meet the internal standard response requirements in Section 12.3.5, follow the corrective action procedure in Section 12.4.4.1. Resolve and document problem resolution before proceeding with sample analysis.

9.3.4.5 If the method blank does not meet the retention time (RT) requirements for internal standards (Section 12.3.6), check the instrument for malfunction and recalibrate. Reanalyze the method blank.

9.3.4.6 Samples that are analyzed with corresponding method blanks that do not meet any of the criteria listed in Sections 9.3.4.2 – 9.3.4.5 should be reanalyzed. If the method blank does not meet the criteria, then all corresponding sample data should be flagged.

9.4 Matrix Spike and Matrix Spike Duplicate (MS/MSD)

To evaluate the potential effects of the sample matrix on analyses, a mixture of target compounds must be spiked into two additional aliquots of a water or solid sample and analyzed in accordance with the appropriate method. Mixtures should be spiked at a concentration near the midpoint of the calibration range.

9.4.1 Frequency of MS/MSD analyses

9.4.1.1 An MS/MSD pair is analyzed with each batch of ≤ 20 samples of each water or solid matrix type. MS/MSDs are not performed on wipe samples.

9.4.1.2 For quality assurance purposes, water rinsate samples and/or field blanks (field QC) or PE samples may accompany solid, water, and/or wipe samples that are delivered to the laboratory for analysis. These field QC or PE samples are not used for MS/MSD analyses.

9.4.1.3 If the agency requesting the analysis designates a sample to be used as an MS/MSD, then that sample must be used. If there is insufficient sample remaining to perform an MS/MSD, then the laboratory shall choose another sample on which to perform an MS/MSD analysis. At the time the selection is made, the laboratory should notify the agency that insufficient sample was received and identify the agency sample selected for the MS/MSD analysis.

9.4.1.4 If there is insufficient sample remaining in any of the samples in a batch to perform the required MS/MSD, the laboratory will report this in the data narrative.

9.4.2 Procedure for Preparing MS/MSD

9.4.2.1 Water samples

Prepare two additional aliquots of the sample chosen for spiking. The volume should be equal to that of the associated samples. Add a sufficient amount of surrogate standard spiking solution and matrix spiking solution to each aliquot to result in the addition of 1.0 µg of each surrogate (add 40 µL if prepared as in Section 7.2.2.1) and a concentration at the mid-point of the calibration range of each matrix spike compound. Extract, clean up, and analyze the MS/MSD according to the procedures for water samples (Sections 11.2 and 11.6). The total volume of DCM added will be slightly greater than the 2 mL needed for extraction, and includes the volumes added for spiking target compounds, and surrogates.

9.4.2.2 Solid samples

Prepare two additional aliquots of the sample chosen for spiking in two 40-mL VOA vials with PTFE-lined caps. The amount chosen should be equal to that of the associated sample. If the sample contains moisture, add 2.5 grams of sodium sulfate for every 10 grams of sample.

Mix well. Add a sufficient amount of the surrogate standard spiking solution and the matrix spiking solution to each aliquot to result in the addition of 0.5 µg of each surrogate (add 20 µL if prepared as in Section 7.2.2.1) and a concentration at the mid-point of each matrix spike compound, and follow the appropriate extraction procedure in Section 11.3. Extract, concentrate, clean up, and analyze the MS/MSD according to procedures for solid samples.

9.4.3 Dilution of MS/MSD

Before any MS/MSD analysis, analyze the original sample, then analyze the MS/MSD at the same concentration as the most concentrated extract for which the original sample results will be reported.

9.4.4 Calculations for MS/MSD

9.4.4.1 Calculate the % Recovery of each matrix spike compound in the MS/MSD sample (see Eq. 11 in Section 12.2.9).

9.4.4.2 Calculate the RPD of the concentrations of each compound in the MS/MSD using Eq. 1. Concentrations of each compound in the MS/MSD are calculated using the same equations as used for target compounds (Eq. 6 for water samples and Eq. 7 for solid samples in Section 12.2.6).

Eq. 1 Relative Percent Difference Calculation

$$RPD = \frac{|C_1 - C_2|}{\left(\frac{C_1 + C_2}{2}\right)} \times 100$$

where:
C_1 = Measured concentration of the first sample aliquot
C_2 = Measured concentration of the second sample aliquot

9.4.5 Technical acceptance criteria for MS/MSD

9.4.5.1 All MS/MSDs must be analyzed on a GC/MS system meeting DFTPP and initial calibration and CCV technical acceptance criteria, as well as the method blank technical acceptance criteria.

9.4.5.2 The MS/MSD must be extracted and analyzed within the technical holding time (Section 8.4).

9.4.5.3 The RT shift for each of the internal standards must be within ± 0.50 minutes (30 seconds) between the MS/MSD sample and the most recent CCV standard analysis.

9.4.5.4 The limits for matrix spike compound recovery and RPD are 50 – 150 % and ≤ 30 %, respectively. For difficult matrices, laboratories are encouraged to collect sufficient data to support development of laboratory-specific criteria.

9.4.6 Corrective action for MS/MSD

If recovery or RPD limits are not met and the LCS, CCV, and method blank are within acceptable limits, this might be an indication of matrix interferences. If sufficient sample is available, an MS/MSD should be reanalyzed, along with all appropriate QC samples. If, after reanalysis, MS/MSD recovery limits are not met, flag the results of the associated sample.

9.5 Laboratory Control Sample (LCS)

An LCS consists of an aliquot of clean reference matrix of the same weight or volume as the corresponding field samples and spiked with the same compounds at the same concentrations used to spike the MS/MSD. The nominal volume of DCM added to water samples is 2.0 mL. Because target compound and surrogate spiking solutions also contain DCM, the total volume of DCM added may be slightly greater than 2.0 mL. The actual total volume of DCM must be used in the calculations in Section 12.2. When the results of the MS/MSD analysis indicate matrix interference might be present, the LCS results are used to verify that the interferences are due to the sample matrix and not from artifacts introduced in the laboratory.

9.5.1 Preparation of LCS

Extract and analyze the LCS according to the procedures in Section 11.2 (for water samples), Section 11.3 (for solid samples), or Section 11.4 (for wipe samples).

9.5.2 Frequency of LCS analyses

One LCS should be prepared, extracted, analyzed, and reported for every 20 or fewer field samples extracted in a batch of a similar matrix. The LCS must be extracted and analyzed concurrently with the samples, using the same extraction procedure, cleanup procedure (if required), and instrumentation.

9.5.3 Calculations for LCS

Calculate the recovery of each compound in the LCS using Eq. 11 (Section 12.2.9.2.

9.5.4 Technical acceptance criteria for LCS analysis

9.5.4.1 All LCSs must be extracted and analyzed at the frequency described in Section 9.5.2 on a GC/MS system meeting the tuning, initial calibration and CCV, and method blank technical acceptance criteria.

9.5.4.2 The limits for LCS compound recovery are 50 – 150 %.

9.5.5 Corrective action for LCS

9.5.5.1 If LCS recovery limits are not met, inspect the system for problems and take corrective actions to achieve the acceptance criteria.

9.5.5.2 If LCS recovery limits cannot be met, flag all associated sample and blank data accordingly.

9.6 Instrument Detection Limit (IDL) Determination

Laboratories may determine an IDL for each target compound on each instrument used for analysis. While determining IDLs is not required, IDL results can be helpful in

determining an appropriate spike level for use in determining the MDL (Section 9.7), as well as instrument sensitivity to the target analytes. It is recommended that IDLs be verified annually thereafter, or after major instrument maintenance. Major instrument maintenance includes, but is not limited to: cleaning or replacement of the mass spectrometer source, mass filters, or electron multiplier; or installing a different GC column type. An IDL is instrument-specific and independent of sample matrices.

9.6.1 An IDL is determined for each compound as the concentration that produces an average signal-to-noise ratio (S:N) of between 3:1 and 5:1 for at least three replicate injections.

9.6.2 All documentation for the IDL determination shall be maintained at the laboratory and provided to the agency or the data user upon request.

9.7 Method Detection Limit (MDL) Determination

Before any field samples are analyzed, laboratory MDLs should be determined for each target analyte in appropriate reference matrices (i.e., reagent water, Ottawa sand, or clean wipes), using the sample preparation and analytical procedures described in this protocol for each specific matrix, and following the instructions and requirements described in 40 CFR Part 136, Appendix B.

9.7.1 The laboratory must use full method procedures to prepare and analyze at least seven replicates.

9.7.2 Spike each replicate sample at concentrations of 1–5 times the IDL concentration for each analyte and analyze the samples following protocol procedures. The total volume of DCM added to water samples will be slightly greater than the 2 mL needed for extraction, and includes the volumes added for spiking target compounds and surrogates.

9.7.3 To determine analyte MDLs, the following equation is applied to the analytical results (Student's t-factor depends on the number of replicates used; a factor of 3.14 assumes seven replicates):

Eq. 2 MDL Determination

MDL = 3.14 x sd

where:
sd = the standard deviation for the analytical results, and
3.14 = the Student's t-value for seven replicate samples

9.7.4 The MDL results calculated using the equation in Section 9.7.3 must meet the following requirements as well as all other requirements specified in 40 CFR Part 136, Appendix B:

- MDL result must not be greater than the spiking level used for the MDL determination.
- MDL result must not be less than 0.10 times the spiking level used for the MDL determination.

If either requirement is not met, the laboratory must adjust their spiking level appropriately and repeat the MDL determination.

9.8 Quantitation Limit (QL) Determination

A QL determination is recommended for each laboratory/technician performing the method for the first time, or in cases where new or repaired instrumentation is being used. Laboratory QLs are determined by first assessing at least four samples containing concentrations of target analytes at the levels of the lowest calibration standard, against the criteria listed below. If any of these criteria are not met, samples are assessed at concentrations of the next (second lowest) calibration standard. These criteria are provided as guidance. If the criteria cannot be met, the laboratory should consult the analytical data requestor to determine if the QL is sufficient to address project needs:

- Results from spikes at the QL should be above the MDL.
- The QL should be at or above the lowest calibration level.
- The QL should be at least two times the MDL.
- The RSD of results from spikes at the QL should be less than 30 %.
- The mean recovery of spikes at the QL should be within 50 – 150 %.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Instrument Operating Conditions

10.1.1 GC

The following GC analytical conditions were used during laboratory studies and are provided for guidance. Other conditions may be used, provided that all technical acceptance criteria are met. Optimize GC conditions for analyte separation and sensitivity. Once optimized, the same GC conditions must be used for the analysis of all standards, samples, blanks, and MS/MSDs.

10.1.1.1 GC – Full-scan quadrupole

Initial column temperature:	40 °C for 3 minutes
Column temperature program:	40 - 150 °C at 10 °C/minute 150 - 280 °C at 25 °C/minute
Final column temperature hold:	280 °C; 10.8 minutes after the last compound (triphenyl phosphate) has eluted
Injector temperature:	250 °C
Injection mode:	Grob-type, splitless for 0.75 minutes
Sample injection volume:	1.0 µL
GC column:	30 m X 0.25 mm ID (or 0.32 mm) bonded phase silicon coated fused silica capillary (see Section 6.4.2)
Column dimensions:	30 m X 0.25 mm X 0.25 µm
Carrier gas:	Helium at 32 cm/second

10.1.1.2 GC – TOF

Initial oven temperature:	55 °C for 0.5 minutes
Column temperature program:	20 °C/minute to 100 °C (0 minute), 40 °C/minute to 280 °C
Final column temperature hold:	280 °C (2.75 minutes)
Injector temperature:	250 °C
Injection mode:	Grob-type, splitless
Sample injection volume:	1.0 µL
GC column:	HP-5MS UI or equivalent
Column dimensions:	15m X 0.18mm X 0.18µm
Carrier gas:	Helium at 1.2 mL/minute

10.1.2 MS

The following MS analytical conditions were used during laboratory studies and are provided for guidance. Other conditions may be used, provided that all technical acceptance criteria are met. Optimize MS conditions for analyte separation and sensitivity. Once optimized, the same MS conditions must be used for the analysis of all standards, samples, blanks, and MS/MSDs.

10.1.2.1 MS – Full-scan quadrupole

MS transfer line temperature:	280 °C
Source temperature:	230 °C or according to manufacturer's specifications
MS quadrupole temperature:	150 °C
Electron energy:	70 eV (nominal)
Scan range:	35 to 500 *m/z*
Ionization mode:	Electron Ionization (EI), positive
Scan time:	3.15 scan/sec (minimum of 3 scans/second)
Library searching:	NIST 05 Mass Spectral Data Base

10.1.2.2 MS – TOF

MS transfer line temperature:	295 °C
Source temperature:	250 °C or according to manufacturer's specifications
Electron energy:	70 eV (nominal)
Scan range:	35 to 500 *m/z*
Ionization mode:	Electron Ionization (EI), positive
Scan time:	15 scans/second

10.2 GC/MS Mass Calibration (Tuning) and Ion Abundance

10.2.1 Summary of GC/MS instrument performance check

The GC/MS system must be tuned to meet the manufacturer's specifications, using a suitable calibration compound such as perfluoro-tri-*n*-butylamine (FC-43) or perfluorokerosene (PFK). The mass calibration and resolution of the GC/MS system are verified by the analysis of the instrument performance check solution (Section 7.2.2.3). Prior to the analysis of any samples, including MS/MSDs, blanks, or calibration standards, the laboratory must establish that the GC/MS system meets the mass spectral ion abundance criteria for the instrument performance check solution (Table 3, Section 17) containing DFTPP.

10.2.2 Frequency of GC/MS instrument performance check – The instrument performance check solution must be injected once at the beginning of each 24-hour period, during which samples, blanks, or standards are to be analyzed. The 24-hour period begins at the moment of injection of the DFTPP solution. The time period ends after 24 hours have elapsed according to the system clock.

10.2.3 GC/MS instrument performance check

The analysis of the instrument performance check solution may be performed as an injection of 50 ng or less of DFTPP into the GC/MS or by adding a sufficient amount of DFTPP to the calibration standards to result in an on-column amount of 50 ng or less of DFTPP (Section 7.2.2.3) and analyzing the calibration standard.

10.2.4 Technical acceptance criteria for GC/MS instrument performance check

10.2.4.1 The instrument performance check solution must be analyzed at the frequency described in Section 10.2.2.

10.2.4.2 Abundance criteria are listed in Table 3, Section 17 for guidance. The mass spectrum of DFTPP must be acquired in the following manner: three scans (the peak apex scan and the scans immediately preceding and following the apex) are acquired and averaged. Background subtraction is required, and must be accomplished using a single scan acquired no more than 20 scans prior to the elution of DFTPP. The background subtraction should be designed only to eliminate column bleed or instrument background ions. Do not subtract part of the DFTPP peak.

Note 1: All subsequent standards, samples, MS/MSDs, and blanks associated with a DFTPP analysis must use the identical GC/MS instrument run conditions.

Note 2: The above tuning criteria are suggested when using DFTPP. If alternative tuning methods are used, consult the method or manufacturer notes for guidance on criteria.

10.2.5 Corrective action for GC/MS instrument performance check

The following corrective actions are minimum procedures. The analyst may try other corrective action procedures to meet criteria.

10.2.5.1 If the GC/MS instrument performance check technical acceptance criteria are not met, re-tune the GC/MS system. It may be necessary to perform maintenance to achieve the technical acceptance criteria.

10.2.5.2 The instrument performance check technical acceptance criteria in Section 10.2.4 must be met before any standards, samples, including MS/MSDs, or required blanks are analyzed.

10.3 Initial Calibration

Prior to sample analysis and after instrument performance check technical acceptance criteria have been met, each GC/MS system must be calibrated at a minimum of five concentrations (Section 7.2.2.4.1 and Table 8, Section 17) to determine instrument sensitivity and the linearity of GC/MS response for the target and surrogate compounds. If the RSD criteria cannot be met, a linear or quadratic curve may be used. Each initial calibration standard contains all the target compounds, surrogates, and internal standards.

10.3.1 Frequency of initial calibration

10.3.1.1 Each GC/MS should be calibrated whenever the laboratory takes corrective action that might change or affect the initial calibration criteria, or if the CCV technical acceptance criteria are not met.

10.3.1.2 If time remains in the 24-hour period after meeting initial calibration acceptance criteria, samples may be analyzed. In this case, it is not necessary to analyze an opening CCV standard prior to sample analysis.

10.3.2 Procedure for initial calibration

10.3.2.1 Prepare at least five calibration standards containing the detected target compounds and associated surrogates. Example concentrations for the calibration standards are provided in Section 7.2.2.4.1 and Table 8, Section 17.

10.3.2.2 Add a sufficient amount of internal standard solution (Section 7.2.2.5) to aliquots of calibration standards to result in 0.5 ng/µL of each internal standard. Standards specified in Section 7.2.2.4 should permit most of the target compounds to have relative retention times (RRTs) of approximately 0.60 to 1.70, using the assignments of internal standards given in Table 4, Section 17.

10.3.2.3 Analyze each calibration standard by injecting 1.0 µL of standard. The same injection volume must be used for all standards, samples, and blanks.

10.3.3 Calculations for initial calibration

10.3.3.1 Calculate the RRFs for each analyte and surrogate using Eq. 3 and the primary characteristic ions found in Table 5, Section 17. Assign target compounds and surrogates to internal standards according to Table 4, Section 17. For internal standards, use the primary ion listed in Table 5, Section 17 unless interferences are present (e.g., peak overlap, co-elution). Unless otherwise stated, the area response of the primary characteristic ion is the quantitation ion.

Eq. 3 RRF Calculation

$$RRF = \frac{A_x}{A_{is}} \times \frac{C_{is}}{C_x}$$

where:

A_x = Area of the characteristic ion for the compound to be measured (Table 5, Section 17)

A_{is} = Area of the characteristic ion for specific internal standard (Table 5, Section 17)

C_{is} = Amount of the internal standard injected (ng)

C_x = Amount of the target compound or surrogate injected (ng)

10.3.3.2 The Mean RRF ($\overline{RRF}$) for the Initial Calibration RRFs and mean RRFs must be calculated for all compounds. Calculate the %RSD of the RRF values for the initial calibration.

10.3.4 Technical acceptance criteria for initial calibration

10.3.4.1 An initial calibration should be performed at the frequency described in Section 10.3.1 on a GC/MS system meeting the instrument performance check technical acceptance criteria (Section 10.2.4).

10.3.4.2 The RRF for each target compound and surrogate should be greater than or equal to 0.01.

10.3.4.3 The % RSD of the RRFs over the initial calibration range for each target compound and surrogate must be less than or equal to 20. If % RSD for a target analyte or surrogate cannot meet this acceptance criterion, curve fitting by linear or quadratic regression may be used provided the R^2 value is greater than or equal to 0.99 (linear) or 0.995 (quadratic). Refer to Section 12.2.7 if linear regression is used; refer to SW-846 Method 8000C (Reference 16.15) if quadratic curve fitting is needed. If regression curve fitting is used, percent drift (PD), as calculated using Eq. 4a, Section 10.4.4.1 for each standard, should be less than or equal to 40.

10.3.5 Corrective action for initial calibration

The following corrective actions are minimum procedures. The analyst may try other corrective action procedures to meet criteria.

10.3.5.1 If technical acceptance criteria using at least one of the three optional approaches to initial calibration (% RSD of the RRFs, linear regression, or quadratic regression) are not met, inspect the system for problems, take corrective actions, remake standards and re-calibrate. If criteria are not met with re-calibration, remake the calibration standards and repeat. If criteria still are not met, the laboratory will flag all data associated with the calibration.

Note: If technical acceptance criteria for the initial calibration are not met and the initial calibration contains more than five calibration levels, the laboratory may remove calibration point(s) from either extreme end of the calibration range and reassess the calibration. Data points from within a calibration range must not be removed.

10.3.5.2 Initial calibration technical acceptance criteria must be met before any samples, including MS/MSDs or required blanks, are analyzed and reported without data qualification.

10.4 Continuing Calibration Verification (CCV)

10.4.1 Summary of CCV

Prior to the analysis of samples and after instrument performance check and initial calibration technical acceptance criteria have been met, each GC/MS system must be routinely checked by analyzing a CCV standard to ensure that the instrument continues to meet the instrument sensitivity and linearity requirements. The CCV standard contains all the target compounds, surrogates, and internal standards.

10.4.2 Frequency of CCV – Each GC/MS used for analysis must be checked at the beginning and at the end of each analytical batch of ≤ 20 injections, excluding instrument blanks. When subsequent analytical batches are run within a single 24-hour period, the closing CCV may be used as the opening CCV for a new analytical batch, provided the closing CCV meets all technical acceptance criteria for an opening CCV (see Section 10.4.5).

10.4.3 Procedure for CCV

10.4.3.1 Add a sufficient amount of internal standard solution (Section 7.2.2.5) to an aliquot of CCV standard to result in a concentration of 0.5 ng/µL for both quadrupole and TOF analyses.

10.4.3.2 Analyze the CCV standard by injecting 1.0 µL of standard.

10.4.4 Calculations for CCV

Calibration verification involves calculation of the percent drift (PD) (Eq. 4a) or the percent difference of the RRFs between the initial calibration and each subsequent CCV (Eq. 4b). The CCV approach will depend on the how the initial calibration was performed. If a regression technique (linear or quadratic)

was used, then Eq.4a is used to determine a PD. If the RRF approach is used, then Eq. 4b is used to determine the percent difference of the RRFs.

10.4.4.1 If regression techniques are used for initial calibration, the CCV must be evaluated in terms of PD, which is calculated using concentrations (see Eq. 4a).

Eq. 4a Percent Drift (PD) Calculation for CCV

$$PD = \frac{\text{Calculated Concentration} - \text{Theoretical Concentration}}{\text{Theoretical Concentration}} \text{x} 100\%$$

10.4.4.2 Calculate an RRF for each target compound and surrogate using Eq. 3 in Section 10.3.3.1, and the primary quantitation ions found in Table 5, Section 17. If regression techniques are used for the initial calibration, proceed to Section 10.4.4.3.

10.4.4.3 Calculate the Percent Difference (% Difference) between the RRF of the most recent initial calibration and the CCV RRF for each target compound and surrogate using Eq. 4b.

Eq. 4b Relative Response Factor (RRF) % Difference Calculation

$$\%\text{Difference}_{RRF} = \frac{RRF_c - \overline{RRF_i}}{\overline{RRF_i}} \times 100$$

where:
RRF_c = RRF from CCV standard.
RRF_i = Mean RRF from the most recent initial calibration meeting technical acceptance criteria.

10.4.5 Technical acceptance criteria for CCV

10.4.5.1 The CCV standard should be analyzed at or near the mid-point concentration level, at the frequency described in Section 10.4.2, on a GC/MS system meeting the instrument performance check and the initial calibration technical acceptance criteria.

10.4.5.2 The RRF for each target compound and surrogate must be greater than or equal to 0.01.

10.4.5.3 For the opening CCV, the PD or percent difference of RRFs for each target compound should be within the range of ±40.

10.4.5.4 For the closing CCV, the PD or percent difference of RRFs for each target compound should be within the range of ±50.

10.4.5.5 Excluding those ions in the solvent front, no quantitation ion may saturate the detector.

10.4.6 Corrective action for CCV

The following corrective actions are minimum procedures. The analyst may try other corrective action procedures to meet criteria.

10.4.6.1 If the CCV technical acceptance criteria in Section 10.4.5 are not met, recalibrate the GC/MS instrument according to Section 10.3.

10.4.6.2 CCV technical acceptance criteria should be met before any samples MS/MSDs, or required blanks are analyzed. If CCV criteria are not met, flag associated samples and blanks accordingly.

10.5 Instrument Blank

10.5.1 Summary of instrument blank

An instrument blank is comprised of DCM spiked with internal standards at the same concentration used for associated samples. The purpose of the instrument blank is to investigate the impact of carry-over.

10.5.2 Frequency of instrument blank

An instrument blank is recommended for analysis following suspected carry-over or during analysis of samples containing suspected high concentrations.

10.5.3 Procedure for instrument blank analysis

Add sufficient amount of internal standard solution (Section 7.2.2.5) to an aliquot of the solvent used to prepare calibration standards and sample extracts to result in a concentration of 0.5 ng/µL. Analyze each instrument blank by injecting a volume of 1.0 µL.

10.5.4 Calculations for instrument blank

Calculate the concentrations of any observed target analyte using Eq. 6 (Section 12.2.6.1), setting V_t, V_o, and dilution factor (DF) all equal to 1.

10.5.5 Technical acceptance criteria for instrument blank

If an instrument blank is analyzed, the concentration of all target analytes in the instrument blank should be less than the concentration of the target analytes in the low calibration standard. The area response of the internal standards should be within 50 – 150 % of the associated CCV or mid-level concentration of the initial calibration.

10.5.6 Corrective action for instrument blank

If an instrument blank is analyzed and the instrument blank technical acceptance criteria are not met, analyze an additional instrument blank. If the problem persists, inspect the system for problems and take corrective actions to achieve the acceptance criteria. Instrument blank technical acceptance criteria should be

met before samples are analyzed. Samples that are analyzed with corresponding instrument blanks that do not meet the instrument blank criteria should be reanalyzed, or the corresponding data should be flagged.

11.0 ANALYTICAL PROCEDURE

11.1 Sample Preparation – General

11.1.1 If less than the specified sample amount is received, the laboratory should use a reduced sample size for the analysis and adjust the calculation accordingly. For the purposes of this method, it is recommended that some sample be retained (if possible) for potential future evidentiary use.

11.1.2 If multi-phase samples (e.g., a two-phase liquid sample, oily, sludge/sandy soil sample) are received by the laboratory, the laboratory shall contact the agency requesting the analyses to apprise them of the type of sample received. If some or all phases of the sample are amenable to analysis, the agency may require the laboratory to do any of the following:

- Mix the sample and analyze an aliquot from the homogenized sample.
- Separate the phases of the sample and analyze each phase separately.
- Separate the phases and analyze one or more of the phases, but not all of the phases.
- Do not analyze the sample.

11.2 Preparation of Water Samples Using Microscale Extraction

11.2.1 Approximately 35 mL of a water sample is required for this extraction. If extraction is to be performed in the sample receipt vial, remove any excess sample such that a total sample volume of 35 mL is retained and recap the vial. Weigh the capped vial. Record the weight to the nearest 0.1 gram. Alternatively, 35.0 mL of sample can be transferred by pipette into the vial and the weighing step can be eliminated.

Note: The conical bottoms of centrifuge vials allow the DCM layer to be removed more easily than the VOA vials.

11.2.2 For GC/MS full-scan and TOF analysis, spike 1.0 µg of each surrogate (add 40 µL if prepared as in Section 7.2.2.1) into each sample, blank, etc. The final volume of the extracts will be 2.0 mL; therefore, the concentration of the surrogates in the extract is expected to be 0.50 µg/mL.

11.2.3 Add ~ 8.8 grams of sodium chloride and shake vigorously, or vortex each vial for two minutes or until the sodium chloride dissolves completely.

11.2.4 Add 2.00 mL of DCM, using a Class A volumetric pipette or syringe. Cap tightly and agitate the contents vigorously for approximately two minutes, either by hand or using a vortex mixer or shaker table.

11.2.5 Briefly allow the phases to settle by gravity for ~ five minutes. Centrifugation [three minutes at 500 revolutions per minute (rpm)] is strongly recommended to facilitate separation of the phases and affords a greater recovery of the final sample extract. CAUTION: The maximum safe handling speed of each centrifuge will depend, in part, on the vials used and should be determined prior to use.

11.2.6 Using a 2.0-mL syringe or pipette, transfer approximately 1.0 mL (or as much as possible) of the DCM (lower) layer to a 2-mL or 4-mL vial with a PTFE-lined screw cap, taking precautions to exclude any water from the syringe or pipette. Add a small amount (~ 50 mg) of anhydrous sodium sulfate to the vial, cap the vial, and shake vigorously or vortex for two minutes. Make sure that the extract is sufficiently dry and that some of the sodium sulfate added is free-flowing (i.e., not clumped).

11.2.7 Using a 1.0 mL syringe or pipette, transfer 1.0 mL (or a known volume, if less than 1.0 mL of extract is collected) of the dried extract to a 2.0-mL vial (or autosampler vial insert) with a PTFE-lined screw cap. Cap the vial.

Note: If stored prior to analysis, extracts must be protected from light and stored at ≤ 6 °C (Section 8.3).

11.2.8 Discard the remaining contents of the VOA vials according to laboratory waste disposal guidelines. Shake off the last few drops with short, brisk wrist movements. If needed, rinse the vial with a water-soluble solvent to ensure that the extraction solvent is removed. If the vial was pre-weighed (i.e., exact sample volume used in Section 11.2.1 is unknown), reweigh the capped vial, and record the weight to the nearest 0.1 gram. The difference between this weight and the weight determined in Section 11.2.1 is equal to the volume of water extracted, in milliliters. As the density of water is 1.00 g/mL (at 20 °C), the volume of water extracted, in milliliters, may be assumed to be equal to the weight of water extracted.

11.2.9 Proceed to Section 11.6 for sample analysis.

11.3 Preparation of Solid Samples Using Microscale Extraction

Note: The following procedures have been evaluated in a multi-laboratory study using Ottawa sand and dried soils and have not been evaluated for field samples. Laboratory results are provided in Section 17.0.

11.3.1 Decant and discard any water layer. Mix samples thoroughly. Discard any foreign objects such as sticks, leaves, and rocks.

11.3.2 pH determination – If pH determination is requested, transfer a 1:1 (w:w) ratio of sample:water to a 100-mL beaker and stir for one hour. Determine the pH of the sample with a pH meter or wide-range pH paper, and document this value in the data narrative. Discard this portion of the sample.

11.3.3 Percent moisture determination – If percent moisture determination is requested, immediately after weighing the sample for extraction, weigh 5 – 10 grams of the

sample into a tared vial. Dry overnight at 103 – 105 °C and cool in a desiccator before weighing. Determine the percent moisture (% Moisture) using Eq. 5. CAUTION: Due to the high toxicity associated with CWAs vaporized during this procedure, percent moisture determinations should be performed only in an oven with appropriate engineering controls.

Eq. 5 Percent Moisture Calculation

$$\%\ \text{Moisture} = \frac{\text{grams of wet sample} - \text{grams of dry sample}}{\text{grams of wet sample}} \times 100$$

11.3.4 Extraction of G-agents and HD from soil samples

11.3.4.1 Weigh 10 g of sample into a tared extraction vial (i.e., 40-mL VOA vial). Wipe the lip and threads of the vial with a clean cloth (e.g., Kimwipe® [Kimberly-Clark Professional, Roswell, GA] or equivalent). Record weight to the nearest 0.01 gram.

11.3.4.2 For full-scan quadrupole and TOF MS analyses, add 0.5 µg of each surrogate standard compound (add 20 µL if prepared as in Section 7.2.2.1) in DCM to the vial. The final volume of the extract is 1.0 mL; therefore, the concentration of the surrogates in the extract is expected to be 0.5 µg/mL.

11.3.4.3 Add approximately 2.5 grams of anhydrous sodium sulfate to a pre-cleaned 40-mL VOA vial that has a PTFE-lined screw cap. Also add 5 –10 pre-cleaned glass beads. Mix the solids together until homogenized using the glass beads and/or a metal spatula. Break up any chunks with a metal spatula, working quickly but gently.

Note: Alternatively, the anhydrous sulfate and glass beads may be added to the extraction vial prior to the addition of sample. If added prior to the sample, the anhydrous sulfate and glass beads must be included in the tared weight of the vial.

11.3.4.4 Add 25 mL of DCM to the vial, and cap tightly.

11.3.4.5 Shake the vial vigorously or vortex for approximately 20 seconds or until the slurry is free-flowing. Break up any chunks with a metal spatula, working quickly but gently. Cap immediately when finished. Add more sodium sulfate and manually mix as necessary to produce a free flowing, finely divided slurry.

11.3.4.6 Extract the sample by agitating for approximately 15 minutes using a shaker table or sonicator.

Note: Sonication at high power should be avoided for soils having high silt content; the resulting fine particles can create problems with filtering and extracting solvent from the sample.

11.3.4.7 Vortex each sample for 30 seconds. Add ~ 1 gram anhydrous sodium sulfate to each sample. Cap and briefly shake or vortex to ensure thorough mixing. Allow the solids to settle or centrifuge for 1–2 minutes at 1000 rpm. If the solid is still unsettled, repeat the centrifuge step, but increase the speed to 2500 rpm. CAUTION: The maximum safe handling speed of each centrifuge will depend, in part, on the vials used and should be determined prior to use.

Repeat until the solid is completely settled. If, after repeating the centrifuging steps several times, the solid is still unsettled, proceed to Section 11.3.4.8. Once the solid has settled, decant or pipette the solvent layer into a pre-cleaned, 40-mL VOA vial with PTFE-lined screw cap and proceed to Section 11.3.4.9.

Note: For solids that have difficulty settling, pipetting is recommended.

11.3.4.8 If solids do not settle by centrifugation (Section 11.3.4.7), filter by placing a small plug of glass wool into a small glass funnel. Add anhydrous sodium sulfate to cover the glass wool plug. Wet the sodium sulfate thoroughly with DCM, and decant the sample solvent layer into the funnel. Rinse the sodium sulfate with approximately 2 - 3 mL of DCM as soon as the surface is exposed, not allowing it to dry.

11.3.4.9 Proceed to Section 11.5 for extract concentration. Once the extract is concentrated, proceed to Section 11.6 for analysis.

11.4 Preparation of Wipe Samples by Microscale Extraction

11.4.1 Place the wipe into an extraction vial (i.e., 40-mL VOA vial). For GC/MS full-scan and TOF analyses, add 0.5 µg of each surrogate standard compound (add 20 µL if prepared as in Section 7.2.2.1) in DCM directly onto the wipe. The final volume of the extract is 1.0 mL; therefore, the concentration of the surrogates in the extract is expected to be 0.5 µg/mL.

11.4.2 Add 15 mL of DCM to the vial and cap tightly.

11.4.3 Extract the sample by agitating for approximately 15 minutes, using a shaker table or sonicator.

11.4.4 Remove the vials from the sonicator or shaker table, shake briefly by hand and allow the solvent layer to settle. Transfer the solvent layer by pipette into a pre-cleaned, 40-mL, clear glass vial with PTFE-lined screw cap.

11.4.5 Proceed to Section 11.5 for extract concentration. Once the extract is concentrated, proceed to Section 11.6 for analysis.

11.5 Final Concentration of Extract – Nitrogen Evaporation Technique (RapidVap® or equivalent) for solid and wipe samples.

11.5.1 Nitrogen evaporation technique - If using a RapidVap® or TurboVap® N_2 system (Biotage, LLC, Charlotte, NC), follow the manufacturer's guidelines. For the RapidVap®, a temperature of 40 °C is recommended. If using a water bath, place the vial in a warm water bath (30 – 40 °C recommended) and evaporate the solvent volume to just below 1.0 mL by blowing a gentle stream of clean, dry nitrogen above the extract. It is recommended that the internal wall of the vial be rinsed down several times with DCM during the operation. If using a RapidVap® the solvent rinse, at a minimum, be done during final adjustment of the extract volume. During evaporation, the tube solvent level must be kept below the water level of the bath. The extract must never be allowed to become dry. Adjust the final volume to 1.0 mL with the same solvent used for extraction. Transfer the extract to a 2-mL autosampler vial, cap, and label the vial. Store at 4 °C (± 2 °C). CAUTION: Gas lines from the gas source to the evaporation apparatus should be stainless steel, copper, or PTFE tubing. With the exception of PTFE, plastic tubing must not be used between the carbon trap and the sample since it can introduce interferences.

11.5.2 Final extract volumes – The final extract volumes in Sections 11.5.2.1 – 11.5.2.4 are recommended volumes.

11.5.2.1 Water/Liquid – As concentration of the sample extract is not needed for these sample matrices, no adjustment of the final extract volume is required. The nominal volume of DCM added to water samples is 2.0 mL. Target compound and surrogate spiking solutions also contain DCM; therefore, the total volume of DCM added may be slightly greater than 2.0 mL. The actual total volume of DCM added should be used in the calculations in Section 12.2.

11.5.2.2 Solid – Adjust the extract to a final volume of 1.0 mL with DCM.

11.5.2.3 Wipe – Adjust the extract to a final volume of 1.0 mL with DCM.

11.5.2.4 If extracts are stored prior to analysis, transfer the extract to a PTFE-lined screw-cap vial (approximately 2.0 mL), label the vial, and store at ≤ 6 °C.

11.6 Extract Analysis by GC/MS

11.6.1 Analyze extracts only after the GC/MS system has met the instrument performance check (Section 10.2.3), initial calibration (Section 10.3.4 and 10.3.5), and CCV technical acceptance requirements (Section 10.4.5). The same instrument conditions used for calibration must be used for the analysis of samples.

11.6.2 Add internal standard solution (Section 7.2.2.5) to an accurately measured aliquot of each extract, cap the vial, and invert several times to mix. For full-scan quadrupole or TOF MS analyses, add a sufficient amount of internal standard solution to result in 0.5 ng/µL concentration of each internal standard.

11.6.3 If extracts are to be diluted, add internal standards after dilution. Internal standards must be added to maintain the required 0.5 ng/µL (for both full-scan quadrupole and TOF) of each internal standard in the extract volume.

11.6.4 Inject 1.0 µL of the extract into the GC/MS.

Note: The same injection volume used for calibration standards must be used for extracts.

11.6.5 Sample extract dilution

11.6.5.1 If the response of any target compound in any sample extract exceeds the response of the same target compound in the high standard of the initial calibration, that extract must be diluted. Add additional internal standard solution such that the concentration in the diluted extract is 0.5 ng/µL for each internal standard, and analyze the diluted extract.

11.6.5.2 Use the results of the original analysis to determine the approximate dilution factor (DF) required for the largest analyte peak to fall within the initial calibration range. The DF chosen must keep the response of the largest peak for a target compound in the upper half of the calibration range of the instrument.

12.0 CALCULATIONS AND DATA ANALYSIS

12.1 Qualitative Identification of Target Compounds

12.1.1 The target compounds should be identified by an analyst competent in the interpretation of mass spectra by comparison of the sample mass spectrum to the mass spectrum of the standard of the suspected compound. Two criteria must be satisfied to verify the identifications:

1) Elution of the sample analyte within the GC RRT unit window established from the 24-hour calibration standard; and
2) Correspondence of the sample analyte and calibration standard component mass spectra.

12.1.2 For establishing correspondence of the GC RRT, the sample component must compare within ± 0.06 RRT units of the standard component. For samples analyzed during the same 24-hour time period as the initial calibration standards, compare the analyte RTs to those from the midpoint initial calibration standard. Otherwise, use the corresponding CCV standard. If coelution of interfering components prohibits accurate assignment of the sample component RRT from the total ion chromatogram, the RRT should be assigned by using EICPs for ions unique to the component of interest (see Table 5, Section 17 for appropriate characteristic ions, surrogates and internal standards).

12.1.3 For comparison of standard and sample component mass spectra, mass spectra obtained from a calibration standard at a concentration of the target analyte closest to the concentration of the analyte in the sample are required. Once obtained, these standard spectra may be used for identification purposes only if the GC/MS meets the DFTPP instrument performance requirements (see Section 10.2 for instrument performance check requirements).

12.1.4 For TOF MS and full-scan quadrupole MS analyses, all ions present in the standard mass spectrum at a relative intensity greater than 10 % (the most abundant ion in the spectrum equaling 100 %) must be present in the sample spectrum. The relative intensities of ions specified in Table 5, Section 17 must agree within ± 20 % between the standard and sample spectra (e.g., for an ion with an abundance of 50 % in the standard spectra, the corresponding sample ion abundance must be between 30 – 70 %). Ions greater than 10 % in the sample spectrum, but not present in the standard spectrum, must be considered and accounted for by the analyst making the comparison. The verification process should favor false positives. All compounds meeting the identification criteria must be reported with their spectra. When target compounds are below QLs but the spectrum meets the identification criteria, report the concentration with a "J". For example, if the QL is 5.0 µg/L and concentration of 3.0 µg/L is calculated, report as "3.0 J".

For TOF analysis, signals for the quantitation ions in Table 5, Section 17 must be present and must maximize within a period of two seconds. The S:N for the GC peak at each ion must be greater than or equal to 2.5 for each target compound and surrogate detected in a sample extract, and greater than or equal to 10 for all target compounds and surrogates in the CCV standard.

12.1.5 If a compound cannot be verified by all of the spectral identification criteria in Sections 12.1.1 – 12.1.4 but in the technical judgment of the mass spectral interpretation specialist the identification is correct, the laboratory should report the identification and proceed with quantitation.

12.2 Data Analysis and Calculations of Target Compounds

12.2.1 Target compounds identified are quantitated by the internal standard method. The internal standard used shall be the one assigned to that analyte for quantitation (Table 4, Section 17).

12.2.2 Situations are expected to arise when the automated quantitation procedures in the GC/MS software provide inappropriate quantitations. These situations normally occur when there is compound coelution, baseline noise, or matrix interferences. In these circumstances, the laboratory must perform a manual quantitation. Manual integrations are performed by integrating the area of the quantitation ion of the compound. This integration should include only the area attributable to the specific target compound. The area integrated should not include baseline background noise. The area integrated must not extend past the point where the sides of the peak intersect with the baseline noise. Manual integration is not to be used solely to meet QC criteria, nor is it to be used as a substitute for corrective action on the chromatographic system.

12.2.3 In some instances, the data system report may have been edited or manual integration or quantitation may have been performed. In all such instances, the GC/MS operator should identify such edits or manual procedures by initialing and dating the changes made to the report, and include the integration scan range. The GC/MS operator should also mark each integrated area on the quantitation report.

12.2.4 The requirements listed in Sections 12.2.1 – 12.2.3 apply to all standards, samples, and blanks.

12.2.5 The $\overline{RRF}$ from the initial calibration is used to calculate the concentration in the sample. Secondary ion quantitation is allowed only when there are sample interferences with the primary ion. If linear regression is used, a regression curve should be used to calculate the concentration in samples. Refer to Section 12.2.7 for calculating sample concentration using linear regression.

12.2.6 Calculate the concentration in the sample using the $\overline{RRF}$ and Eqs. 6–8.

12.2.6.1 Water

Eq. 6 Concentration of Water Sample

$$\text{Concentration } \mu g/L = \frac{(A_X)(I_S)(V_t)(DF)}{(A_{IS})(V_i)(V_o)\left(\overline{RRF}\right)}$$

where:

A_x = Area of the characteristic ion for the target compound

A_{IS} = Area of the characteristic ion for the internal standard

I_s = Amount of internal standard injected in ng

V_o = Volume of water extracted in mL

V_i = Volume of extract injected in µL

V_t = Volume of the extract in µL
(Extraction of water samples does not include concentration; V_t is equal to the sum of the volumes of solvent added for extraction and the addition of surrogates, and any spiked target compounds.)

$\overline{RRF}$ = Mean RRF determined from the initial calibration standard

DF = Dilution Factor. If no dilution is performed, DF = 1.0.
The DF for analysis of water samples is defined as:

$$DF = \frac{\mu L \text{ most conc. extract used to make dilution} + \mu L \text{ clean solvent}}{\mu L \text{ most conc. extract used to make dilution}}$$

12.2.6.2 Solid

Eq. 7 Concentration of Solid Sample

Eq. 7 includes a %moisture factor (D) for those cases when data are to be reported on the basis of dry sample weight. In cases where results are reported in terms of sample weight, this factor is deleted from the equation.

$$\text{Concentration } \mu g/Kg \text{ (Dry weight basis)} = \frac{(A_x)(I_s)(V_t)(DF)}{(A_{is})(V_i)(\overline{RRF})(W_s)(D)}$$

where:

A_x, I_s, A_{IS}, V_i and $\overline{RRF}$ are as given for water, above.

V_t = Volume of concentrated extract in µL

$$D = \frac{100 - \%Moisture}{100}$$

%Moisture is as given in EQ. 5

W_s = Weight of sample extracted in grams

$\overline{RRF}$ = Mean RRF determined from the initial calibration standard

DF = Dilution Factor

12.2.6.3 Wipes

Eq. 8 Concentration of Wipe Sample

$$\text{Concentration } \mu g/cm^2 = \frac{(A_X)(I_S)(V_t)(DF)}{(A_{IS})(V_i)(\text{Area})\left(\overline{RRF}\right)}$$

where:

A_x = area response for the compound to be measured, counts A_{IS} = area response for the internal standard, counts

I_s = amount of internal standard, µg

$\overline{RRF}$ = the mean RRF from the most recent initial calibration, dimensionless

Area = area of surface wiped, cm^2. If concentration is reported as µg/wipe, area = 1 wipe.

V_t = volume of concentrated extract, µL

V_i = volume of extract injected, µL

DF = dilution factor for the extract. If there was no dilution, DF equals 1. If the sample was diluted, DF is greater than 1.

12.2.7 Calculate the concentration in the sample using linear regression.

The following procedure is used to calculate analyte concentrations using a linear regression calibration curve. Refer to SW-846 Method 8000C (Reference 16.15) if calibration curves were determined using quadratic equations.

12.2.7.1 Set y = (Peak Area of Target/Peak Area of Internal Standard) and x = (Theoretical Concentration of Target/Theoretical Concentration of Internal Standard).

12.2.7.2 Plot (Peak Area of Target/Peak Area of Internal Standard [Y-axis]) vs. (Theoretical Concentration of Target/Theoretical Concentration of Internal Standard).

12.2.7.3 Determine the slope of the line (m) and the y-intercept (b).

12.2.7.4 Rearrange the line equation to solve for x; x = (y-b)/m.

12.2.7.5 Multiply x by the concentration of the internal standard to get concentration of target anayte in extract.

12.2.7.6 Multiply the concentration of target analyte in the extract by the extract volume, and divide by the sample volume to get concentration of target analyte in sample.

12.2.8 Adjusted QL calculations

Adjusted QLs are used in situations when the laboratory may not have a sample size that is sufficient for the method as written, or if the prescribed extract volume was not used or recovered.

12.2.8.1 Water samples

Eq. 9 Aqueous Adjusted QL

$$\text{Adjusted QL} = \text{Method QL} \times \frac{(V_x)(V_t)(DF)}{(V_o)(V_c)}$$

where:
V_t, DF, and V_o are as given in Eq. 6.
V_x = Recommended method sample volume (35 mL)
V_c = Recommended method concentrated extract volume (2000 µL)

12.2.8.2 Solid samples

Eq. 10 Solid Adjusted QL

$$\text{Adjusted QL} = \text{Method QL} \times \frac{(W_x)(V_t)(DF)}{(W_s)(V_c)(D)}$$

where:
V_t and DF are as given in Eq. 6.
W_s and D are as given in Eq. 7.
W_x = Recommended method sample weight (10 grams)
V_c = Recommended method concentrated extract volume (1000 µL)

12.2.9 Surrogate recoveries

12.2.9.1 Calculate surrogate recoveries for all samples, blanks, and MS/MSDs. Determine if recovery is within limits (Table 7, Section 17).

12.2.9.2 Calculate the concentrations of the surrogates using the same equations as used for the target compounds. Calculate the recovery of each surrogate using Eq. 11.

Eq. 11 Percent Recovery

$$\%\text{Recovery} = \%\text{R} = \frac{C_s}{C_n} \text{ x } 100$$

where:
C_s = Measured concentration of the spiked sample aliquot.
C_n = Nominal (theoretical) concentration increase that results from spiking the sample, or the nominal concentration of the spiked aliquot (for LCS).

12.3 Technical Acceptance Criteria for Sample Analysis

12.3.1 The samples must be analyzed on a GC/MS system meeting the instrument performance check, initial calibration, CCV, and blank technical acceptance criteria.

12.3.2 The sample must be extracted and analyzed within the technical holding times.

12.3.3 The sample must have an associated method blank meeting the blank technical acceptance criteria.

12.3.4 The percent recoveries of the surrogates in a sample should be within the recovery limits listed in Table 7, Section 17 (see Table 4, Section 17 for analyte specific surrogates). The surrogate recovery requirements do not apply to samples that have been diluted.

12.3.5 The instrumental response (EICP area) for each of the internal standards in the sample must be within the range of 50.0 – 200 % of the response of the internal standard in the most recent CCV standard analysis.

12.3.6 The RT shift for each internal standard must be within ± 0.50 minute (30 seconds) between the sample and the most recent CCV standard analysis.

12.3.7 Excluding those ions in the solvent front, no ion may saturate the detector. If a target compound concentration exceeds the upper limit of the initial calibration range, a more dilute aliquot of the sample extract must also be analyzed.

12.4 Corrective Action for Sample Analysis

12.4.1 The sample technical acceptance criteria must be met before data are reported. If the corrective actions described in this section did not solve the problem, all associated sample and blank data must be flagged accordingly.

12.4.2 Corrective action for failure to meet instrument performance checks and initial calibration and CCV must be completed before the analysis of samples. If the corrective actions described in Sections 10.2.5 (for instrument performance check), 10.3.5 (for initial calibration), or 10.4.6 (for CCV) did not solve the problem, all associated sample and blank data must be flagged accordingly.

12.4.3 Corrective Action for Surrogate Recoveries that Fail to Meet Their Acceptance Criteria (Section 12.3.4 and Table 7, Section 17).

12.4.3.1 If the surrogate recoveries in a sample fail to meet the acceptance criteria specified in Section 12.3.4, check calculations, sample preparation logs, surrogate standard spiking solutions, and the instrument operation.

12.4.3.2 If the above actions do not correct the problem, then the problem might be due to a sample matrix effect. To determine if there was a matrix effect, take the following corrective actions:

12.4.3.2.1 Reextract (if possible) and reanalyze the sample.

Note: Samples with corresponding MS and MSDs should be re-extracted and reanalyzed only if surrogate recoveries in the sample were considered unacceptable, and the surrogate recoveries met the acceptance criteria in both the corresponding MS and MSD.

12.4.3.2.2 If surrogate recoveries meet acceptance criteria in the reextracted/reanalyzed sample, the problem was within the laboratory's control.

12.4.3.2.3 If surrogate recoveries are outside the acceptance criteria in the reanalysis, flag the sample data for the associated target compounds and submit data from both analyses. Distinguish between the initial analysis and the reanalysis on all data.

12.4.4 Corrective action for internal standard compound responses and/or RTs that fail to meet the acceptance criteria (Sections 12.3.5 and 12.3.6)

12.4.4.1 If the internal standards in a sample fail to meet their acceptance criteria, check calculations, internal standard solutions, and instrument operation.

12.4.4.2 If the above actions do not correct the problem, then the problem might be due to a sample matrix effect. To determine if there was matrix effect, take the following corrective action steps:

12.4.4.2.1 Reanalyze the sample extract.

Note: Samples with corresponding MS and MSDs should be re-extracted and reanalyzed only if internal standard recoveries in the sample were considered unacceptable, and the internal standard recoveries met the acceptance criteria in both the corresponding MS and MSD.

12.4.4.2.2 If the internal standard responses and RTs meet acceptance criteria in the reanalyzed sample extract, then the problem was within the laboratory's control.

12.4.4.2.3 If the internal standard responses and RTs do not meet acceptance criteria in the reanalyzed sample extract, flag the results of the associated sample.

12.4.4.2.4 Submit data from both analyses. Distinguish between the initial analysis and the reanalysis on all data.

13.0 ANALYTICAL PROCEDURE PERFORMANCE

Performance of this protocol was evaluated in multiple laboratories for measurement of the target analytes in water, soil, and wipes. Resulting IDLs and MDLs from the multi-laboratory evaluation are listed in Table 1, Section 17. Multi-laboratory results of reference samples spiked at levels corresponding to laboratory low-calibration standards are provided in Tables 2a – 2c (Section 17). Precision (as RPD and RSD) and bias (as % Recovery) results based on multi-laboratory data are provided in Table 6 (Section 17). Additional laboratory data for real world samples are provided in Section 17 Tables 9a – 9b (multi-laboratory data for groundwater and drinking water), and 10a – 10b (multi-laboratory data for Virginia-a and ASTM soils). Characterization data for the samples used during the multi-laboratory study are provided in Tables 11 and 12, Section 17. Figures 1 and 2 provide example chromatograms generated during a multi-laboratory study using full-scan quadrupole MS and TOF MS, respectively.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that might be applicable to laboratories and research institutions consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th St., N.W. Washington, D.C. 20036, (202) 872-4477.

15.0 WASTE MANAGEMENT

EPA requires that laboratory waste management practices be conducted in a manner consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel*, available from the American Chemical Society at the address listed in Section 14.2.

Note: It is strongly recommended that all glassware and waste be decontaminated with bleach containing active chlorine at a concentration of at least 5 %, for at least six hours to provide effective decontamination of CWAs. Chemical agent decontamination procedures and inventory records should be consistent with the laboratory's Chemical Hygiene Plan for CWAs.

16.0 REFERENCES

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16.10 U.S. Environmental Protection Agency. "Organic Analytes." Chapter 4 in *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods.* EPA publication SW-846. Washington DC: U.S. Environmental Protection Agency, Office of Land and Emergency Management..

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16.13 U.S. Environmental Protection Agency. *Determination of Organic Compounds in Drinking Water by Liquid-Solid Extraction and Capillary Column Gas Chromatography/Mass Spectrometry*, Method 525.2, Revision 2.0. 1995. Cincinnati, OH: U.S. Environmental Protection Agency, Office of Research and Development.

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17.0 TABLES and FIGURES

Table 1.
Instrument Detection Limits (IDLs) and Method Detection Limits (MDLs)
Based on Multi-Laboratory Evaluation

Note: IDLs and MDLs were determined in 3–6 laboratories during a multi-laboratory study. IDLs were determined as the concentration necessary to achieve a S:N ratio of at least 3:1. MDLs were determined following Section 9.7 of this protocol, using samples spiked at levels corresponding to the lowest calibration standards.

Analyte	Full-Scan Quadrupole MS					Full-Scan TOF MS		
Reagent Water	**# Labs**	**IDL Range (ng/µL)**	**S:N[1] Range**	**MDL Range (µg/L)**	**Pooled MDL[2] (µg/L)**	**# Labs**	**MDL Range (µg/L)**	**Pooled MDL[2] (µg/L)**
Cyclohexyl sarin	6	0.04 – 0.2	3.2 – 7.4	0.510 – 1.71	0.840	6	0.0590 – 0.241	0.110
Sarin	6	0.03 – 0.2	3.0 – 5.1	0.534 – 2.11	1.03	4	0.0788 – 0.21	0.113
Soman	6	0.04 – 0.1	3.1 – 5.6	0.428 – 1.35	0.690	5	0.0511 – 0.47	0.0813
Sulfur mustard	6	0.02 – 0.1	4.4 – 6.5	0.343 – 2.29	0.831	6	0.0140 – 0.164	0.0805
Sand	**# Labs**	**IDL (ng/µL)**	**S:N[1] Range**	**MDL Range (µg/kg)**	**Pooled MDL[2] (µg/kg)**	**# Labs**	**MDL Range (µg/kg)**	**Pooled MDL[2] (µg/kg)**
Cyclohexyl Sarin	4	0.04 – 0.2	3.2 – 7.4	2.25 – 4.99	3.05	5	0.150 – 0.200	0.171
Sarin	4	0.03 – 0.2	3.0 – 5.1	1.89 – 3.38	2.10	4	0.104 – 0.261	0.164
Soman	4	0.04 – 0.1	3.1 – 5.6	0.650 – 2.46	1.30	3	0.0860 – 0.244	0.116
Sulfur mustard	4	0.02 – 0.1	4.4 – 6.5	0.810 – 1.32	1.50	5	0.0617 – 0.147	0.0923
Wipes	**# Labs**	**IDL (ng/µL)**	**S:N[1] Range**	**MDL Range (µg/wipe)**	**Pooled MDL[2] (µg/wipe)**	**# Labs**	**MDL Range (µg/wipe)**	**Pooled MDL[2] (µg/wipe)**
Cyclohexyl Sarin	5	0.04 – 0.2	3.2–7.4	0.0196 – 0.0560	0.0321	4	0.00154 – 0.00762	0.00374
Sarin	5	0.03 – 0.2	3.0–5.1	0.00699 – 0.0400	0.0253	4	0.00162 – 0.00395	0.00261
Soman	5	0.04 – 0.1	3.1–5.6	0.00801 – 0.0285	0.0150	3	0.000590 – 0.00235	0.00142
Sulfur mustard	4	0.02 – 0.1	4.4–6.5	0.0150 – 0.0495	0.0232	5	0.000830 – 0.00177	0.00123

[1] S:N value from the weaker of two secondary quantitation ions (see Table 5). S:N values were determined by measuring peak-to-peak noise using Agilent Chemstation software.

[2] Pooled MDLs are calculated by taking the square root of the sum of the squares of each lab's MDL divided by the total number of MDL values and multiplied by a weighting factor based on the degrees of freedom (e.g., for three MDL values, the weighting factor is 0.81).

Tables 2a–2c: Multi-Laboratory Results of Reference Matrix Samples Spiked at Levels Corresponding to Laboratory Low Calibration Standards

Note: Tables 2a through 2c provide summary results of reference matrix samples spiked at levels corresponding to the lowest calibration standards used by laboratories participating in a multi-laboratory study. The last column of these tables provides spike levels adjusted based on the lowest recovery result, for comparison to pooled MDLs generated using study data. With the exceptions noted in table footnotes, adjusted spike levels are above the pooled MDLs in all cases.

Table 2a.
Example Multi-Laboratory Results for Reagent Water Samples Spiked at Levels Corresponding to Laboratory Low-Calibration Standards

# of labs	Analyte	n	Spike Level (µg/L)	% Recovery Range	Pooled RSD	Pooled MDL (µg/L)	Spike Level adjusted for recovery (µg/L)
Full-Scan Quadrupole MS							
6	Cyclohexyl Sarin	42	5.71	42.6 – 117	11.0	0.840	2.44
6	Sarin	42	5.71	34.5 – 112	9.61	1.03	1.97
6	Soman	42	2.86	62.4 – 152	10.2	0.690	1.78
6	Sulfur mustard	42	2.86	35.4 – 167	9.51	0.831	1.01
Full-Scan TOF MS							
6	Cyclohexyl Sarin	49	0.571	67.2 – 155	7.72	0.110	0.384
6	Sarin	48	0.571	43.7 – 124	8.46	0.113	0.250
6	Soman	49	0.286	54.2 – 128	12.8	0.0813	0.155
5	Sulfur mustard	42	0.286	55.2 – 126	14.1	0.0805	0.158

n = number of replicates; MDL = method detection limit; RSD = relative standard deviation

Table 2b.
Example Multi-Laboratory Results for Ottawa Sand Samples Spiked at Levels Corresponding to Laboratory Low-Calibration Standards

# of labs	Analyte	n	Spike Level (µg/kg)	% Recovery Range	Pooled RSD	Pooled MDL (µg/kg)	Spike Level adjusted for recovery (µg/kg)
Full-Scan Quadrupole MS							
4	Cyclohexyl Sarin	27	10.0	46.7 – 105	14.3	3.05	4.67
4	Sarin	28	10.0	48.0 – 87.5	12.9	2.10	4.80
4	Soman	28	5.00	64.2 – 130	11.3	1.30	3.21
4	Sulfur mustard	28	5.00	43.2 – 190	10.5	1.50	2.16
Full-Scan TOF MS							
5	Cyclohexyl Sarin	35	1.00	41.3 – 177	7.71	0.171	0.413
5	Sarin	35	1.00	40.8 – 129	11.5	0.164	0.408
3	Soman	21	0.500	81.4 – 284	9.41	0.16	0.407
5	Sulfur mustard	35	0.500	29.6 – 83.4	11.9	0.0923	0.148

n = number of replicates; MDL = method detection limit; RSD = relative standard deviation

Table 2c.
Example Multi-Laboratory Results for Wipes
Spiked at Levels Corresponding to Laboratory Low-Calibration Standards

# of labs	Analyte	n	Spike Level (µg/wipe)	% Recovery Range	Pooled RSD	Pooled MDL (µg/wipe)	Spike Level adjusted for recovery (µg/wipe)
Full-Scan Quadrupole MS							
5	Cyclohexyl Sarin	35	0.100	54.6 – 110	15.2	0.0321	0.0546
5	Sarin	35	0.100	45.9 – 88.4	15.4	0.0253	0.0459
5	Soman	35	0.0500	59.0 – 122	12.7	0.0150	0.030
4	Sulfur mustard	27	0.0500	52.1 – 128	14.3	0.0232	0.0260
Full-Scan TOF MS							
5	Cyclohexyl Sarin	35	0.0100	62.0 – 187	11.0	0.00374	0.0062
5	Sarin	35	0.0100	38.9 – 105	14.7	0.00261	0.0039
3	Soman	21	0.00500	53.2 – 111	14.2	0.00142	0.0027
5	Sulfur mustard	34	0.00500	40.2 – 86.8	14.1	0.00123	0.00201

n, number of replicates; MDL, method detection limit; RSD, relative standard deviation

Table 3.
Decafluorotriphenylphosphine (DFTPP) Key Ions and Ion Abundance

Note: All ion abundances MUST be normalized to *m/z* 198.

Mass	Ion Abundance Criteria	
	Quadrupole	**Time of Flight (TOF)**
51	10.0 – 80.0 % of mass 198	10.0 – 85.0 % of mass 198
68	Less than 2.0 % of mass 69	Less than 2.0 % of mass 69
69	Present	Not used
70	Less than 2.0 % of mass 69	Less than 2.0 % of mass 69
127	10.0 – 80.0 % of mass 198	10.0 – 80.0 % of mass 198
197	Less than 2.0 % of mass 198	Less than 2.0 % of mass 198
198	Base peak 100 % relative abundance	Base peak 100 % relative abundance
199	5.0 – 9.0 % of mass 198	5.0 – 9.0 % of mass 198
275	10.0 – 60.0 % of mass 198	10.0 – 60.0 % of mass 198
365	Greater than 1.0 % of mass 198	Greater than 0.5 % of mass 198
441	Present but less than mass 443	Less than 150 % of mass 443
442	Greater than 50.0 % of mass 198	Greater than 30.0 %
443	15.0 – 24.0 % of mass 442	15.0 – 24.0 % of mass 442

Table 4.
Internal Standards and Surrogates

Note: Table 4 provides a list of surrogates used during laboratory studies testing this protocol, based on surrogates typically used for SVOC analyses. Alternative surrogates might be more representative of the analytes targeted in this method and may be used or added at the laboratory's discretion provided the surrogates meet the criteria in Table 7.

Analyte	Surrogate Compounds	Internal Standards
Cyclohexyl Sarin	Triphenyl phosphate or *p*-Terphenyl-d14	Naphthalene-d_8
Sarin	Nitrobenzene-d_5	1,4-Dichlorobenzene-d_4
Soman − GD1	Nitrobenzene-d_5	1,4-Dichlorobenzene-d_4
Soman − GD2	Nitrobenzene-d_5	1,4-Dichlorobenzene-d_4
Sulfur mustard	Triphenyl phosphate or *p*-Terphenyl-d_{14}	Naphthalene-d_8

Table 5.
Example Retention Times (RTs), Relative Retention Times (RRTs) and Quantitation Ions for Target Compounds, Surrogate Compounds, and Internal Standards

Note: Bold quantitation ions indicate the secondary quantitation ions used during single-laboratory testing.

Analyte	Retention Time (minutes)	Relative Retention Time	Primary Quantitation Ion	Secondary Quantitation Ions
Sarin	6.14 – 6.17	0.63	99	**125, 81**
1,4-Dichlorobenzene-d_4 (IS)	9.70	–	152	**150, 115,** 78
Soman − GD1	10.15 – 10.21	1.05	99	**126**, **82,** 69
Soman − GD2	10.22 – 10.29	1.06	99	**126**, **82,** 69
Nitrobenzene-d_5 (S)	10.98 – 11.01	1.13	82	**128, 54,** 98
Sulfur Mustard	12.36 – 12.39	0.98	109	158, 160, **63**, **111**
Naphthalene-d_8 (IS)	12.55 – 12.58	–	136	**68, 108**
Cyclohexyl sarin	12.79 – 12.84	1.02	99	**67**, **81, 137**, **82**
p-Terphenyl-d14 (S)	19.75 – 19.77	1.57	244	**122**
Triphenyl phosphate (S)	20.68	1.64	326	**325, 215**

(S) = Surrogate
(IS) = Internal Standard

Table 6.
Example Multi-Laboratory Precision and Bias in Reference Matrices at Mid-Calibration Levels

Full-Scan Quadrupole MS			Initial Precision and Recovery (IPR)		Laboratory Control Sample (LCS)
Analyte	***# Labs***	***n***	***% Recovery***	**Pooled % RSD**	**% Recovery**
Reagent Water[1]					
Cyclohexyl sarin	5	20	72.0 – 132	6.79	72.0 – 132
Sarin	5	20	68.9 – 110	9.19	68.9 – 110
Soman	5	20	82.9 – 125	6.00	82.9 – 125
Sulfur mustard	5	20	83.3 – 136	5.84	83.3 – 136
Ottawa Sand[2]					
Cyclohexyl sarin	4	16	56.0 – 101	5.40	56.0 – 101
Sarin	4	16	52.0 – 98.8	5.60	52.0 – 98.8
Soman	4	16	56.0 – 115	6.90	56.0 – 115
Sulfur mustard	4	16	60.0 – 123	5.15	60.0 – 123
Wipes[3]					
Cyclohexyl sarin	4	16	54.1 – 111	12.0	54.1 – 111
Sarin	4	16	52.3 – 80.0	12.1	52.3 – 80.0
Soman	4	16	53.8 – 94.0	12.5	53.8 – 94.0
Sulfur mustard	4	16	50.8 – 104	12.5	50.8 – 104
Full-Scan TOF MS			**Initial Precision and Recovery (IPR)**		**Laboratory Control Sample (LCS)**
Analyte	***# Labs***	***n***	***% Recovery***	**Pooled % RSD**	**% Recovery**
Reagent Water[1]					
Cyclohexyl sarin	5	20	63.3 – 119	6.4	63.3 – 119
Sarin	5	20	37.0 – 98.9	6.5	37.0 – 98.9
Soman	5	20	60.2 – 126	8.0	60.2 – 126
Sulfur mustard	5	20	11.7 – 127	10.4	11.7 – 127
Ottawa Sand[2]					
Cyclohexyl sarin	5	20	43.8 – 92.3	11.0	43.8 – 92.3
Sarin	5	20	42.3 – 82.1	13.2	42.3 – 82.1
Soman	5	20	38.3 – 108	14.4	38.3 – 108
Sulfur mustard	5	20	42.8 – 82.5	11.0	42.8 – 82.5
Wipes[3]					
Cyclohexyl sarin	4	16	26.7 – 105	27.2	26.7 – 105
Sarin	4	16	23.6 – 97.3	23.1	23.6 – 97.3
Soman	4	16	29.2 – 110	24.5	29.2 – 110
Sulfur mustard	4	16	29.3 – 82.0	21.3	29.3 – 82.0

[1] Reagent water spike levels: 45.7 µg/L (sarin and cyclohexyl sarin); 22.8 µg/L (soman and sulfur mustard)
[2] Soil spike levels: 80.0 µg/kg (sarin and cyclohexyl sarin); 40.0 µg/kg (soman and sulfur mustard)
[3] Wipe spike levels: 0.1 µg/wipe (sarin and cyclohexyl sarin); 0.05 µg/wipe (soman and sulfur mustard)

Table 7.
Surrogate Recovery

Note: Table 7 lists surrogates used during laboratory studies testing this protocol. Alternative surrogates might be more representative of the analytes targeted in this method, and may be used or added at the laboratory's discretion, provided the surrogates meet the criteria listed in this table.

Surrogate	Surrogate % Recovery	
	Minimum	***Maximum***
Full-Scan Quadrupole MS		
Water		
Nitrobenzene-d_5	50.0	150
p-Terphenyl-d_{14}	50.0	150
Triphenyl phosphate	50.0	150
Soil		
Nitrobenzene-d_5	50.0	150
p-Terphenyl-d_{14}	50.0	150
Triphenyl phosphate	50.0	150
Wipes		
Nitrobenzene-d_5	50.0	150
p-Terphenyl-d_{14}	50.0	150
Triphenyl phosphate	50.0	150
Full-Scan TOF MS		
Water		
Nitrobenzene-d_5	50.0	150
p-Terphenyl-d_{14}	50.0	150
Triphenyl phosphate	50.0	150
Soil		
Nitrobenzene-d_5	50.0	150
p-Terphenyl-d_{14}	50.0	150
Triphenyl phosphate	50.0	150
Wipes		
Nitrobenzene-d_5	50.0	150
p-Terphenyl-d_{14}	50.0	150
Triphenyl phosphate	50.0	150

Table 8.
Example Calibration Standard Concentrations (µg/mL)
Used During Multi-Laboratory Study

Full-Scan Quadrupole MS								
Analyte	**CAS RN**	**Cal 1**	**Cal 2**	**Cal 3**	**Cal 4**	**Cal 5**	**Cal 6**	
Cyclohexyl Sarin	329-99-7	0.1	0.2	0.4	0.8	1.0	2.0	
Sarin	107-44-8	0.1	0.2	0.4	0.8	1.0	2.0	
Soman-GD1 and GD2	96-64-0	0.05	0.1	0.2	0.4	0.5	1.0	
Sulfur mustard	505-60-2	0.05	0.1	0.2	0.4	0.5	1.0	
Nitrobenzene-d_5 (S)	4165-60-0	0.1	0.2	0.4	0.8	1.0	2.0	
p-Terphenyl-d_{14} (S)	1718-51-0	0.2	0.4	0.8	1.0	2.0	2.0	
Triphenyl phosphate (S)	115-86-6	0.2	0.4	0.8	1.0	2.0	2.0	
1,4-Dichlorobenzene-d_4 (IS)	3855-82-1	0.5	0.5	0.5	0.5	0.5	0.5	
Naphthalene-d_8 (IS)	1146-65-2	0.5	0.5	0.5	0.5	0.5	0.5	

Full-Scan TOF MS								
Analyte	**CAS RN**	**Cal 1**	**Cal 2**	**Cal 3**	**Cal 4**	**Cal 5**	**Cal 6**	**Cal 7**
Cyclohexyl Sarin	329-99-7	0.01	0.05	0.08	0.1	0.25	0.5	1.0
Sarin	107-44-8	0.01	0.05	0.08	0.1	0.25	0.5	1.0
Soman-GD1 and GD2	96-64-0	0.005	0.025	0.04	0.05	0.125	0.25	0.5
Sulfur mustard	505-60-2	0.005	0.025	0.04	0.05	0.125	0.25	0.5
Nitrobenzene-d_5 (S)	4165-60-0	0.01	0.05	0.08	0.1	0.25	0.5	1.0
p-Terphenyl-d_{14} (S)	1718-51-0	0.01	0.05	0.08	0.1	0.25	0.5	1.0
Triphenyl phosphate (S)	115-86-6	0.01	0.05	0.08	0.1	0.25	0.5	1.0
1,4-Dichlorobenzene-d_4 (IS)	3855-82-1	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Naphthalene-d_8 (IS)	1146-65-2	0.5	0.5	0.5	0.5	0.5	0.5	0.5

(S) = Surrogate; (IS) = Internal Standard

Table 9a.
Example Multi-laboratory Precision and Bias in Water Using GC Full-Scan Quadrupole MS

Drinking Water					
Analyte	**# of labs**	**n**	**Pooled % RSD**	**% Recovery Range**	**% RSD Range**
Low-Level Spike Results[1]					
Cyclohexyl sarin	3	28	6.04	76.5 – 108	5.58 – 6.22
Sarin	3	21	6.88	58.8 – 107	6.19 – 10.4
Soman	3	21	8.86	67.7 – 122	4.44 – 14.7
Sulfur mustard	3	21	6.69	69.5 – 114	4.02 – 14.7
Mid-Level Spike Results[2]					
Cyclohexyl sarin	3	21	2.97	59.5 – 115	1.85 – 5.73
Sarin	3	21	3.57	60.4 – 102	2.48 – 5.69
Soman	3	21	3.77	49.6 – 117	3.17 – 5.39
Sulfur mustard	3	21	3.99	66.7 – 115	2.43 – 7.07
Surrogates[3]					
Nitrobenzene-d_5	4	56	13.7	30.0 – 135	1.91 – 27.0
p-Terphenyl-d_{14}	3	56	7.41	58.0 – 140	2.14 – 19.1
Triphenyl phosphate	4	70	5.76	52.0 – 142	2.01 – 12.6
Groundwater					
Analyte	**# of labs**	**n**	**Pooled % RSD**	**% Recovery Range**	**% RSD Range**
Low-Level Spike Results[1]					
Cyclohexyl sarin	3	21	5.03	79.6 – 115	3.07 – 5.81
Sarin	3	21	8.22	61.1 – 108	6.37 – 9.66
Soman	3	21	9.92	70.2 – 123	4.52 – 14.1
Sulfur mustard	3	21	12.4	47.0 – 120	2.16 – 16.7
Mid-Level Spike Results[2]					
Cyclohexyl sarin	3	21	4.22	68.5 – 138	1.42 – 6.66
Sarin	3	21	4.63	66.9 – 117	2.05 – 6.05
Soman	3	21	4.31	56.1 – 126	3.29 – 4.91
Sulfur mustard	3	21	5.05	73.7 – 127	2.33 – 6.08
Surrogates[3]					
Nitrobenzene-d_5	3	42	7.33	34.0 – 136	1.17 – 10.5
p-Terphenyl-d_{14}	3	42	15.0	50.3 – 147	2.93 – 26.2
Triphenyl phosphate	3	42	9.45	60.0 – 156	4.17 – 18.1

n = number of replicates; MDL = method detection limit; RSD = relative standard deviation
[1] Low-level samples were spiked at 5.71 µg/L (sarin and cyclohexyl sarin), and 2.86 µg/L (soman and sulfur mustard).
[2] Mid-level samples were spiked at 45.7 µg/L (sarin and cyclohexyl sarin), and 22.8 µg/L (soman and sulfur mustard).
[3] Surrogates were spiked at 28.6 µg/L.

Table 9b.
Example Multi-laboratory Precision and Recovery in Water Using GC Full-Scan TOF MS

Drinking Water					
Analyte	**# of labs**	**n**	**Pooled % RSD**	**% Recovery Range**	**% RSD Range**
Low-Level Spike Results[1]					
Cyclohexyl sarin	4	28	3.59	65.1 – 112	3.66 – 5.02
Sarin	4	28	4.23	44.9 – 103	2.01 – 6.26
Soman	4	28	4.32	50.4 – 121	3.65 – 6.55
Sulfur mustard	4	28	10.4	8.65 – 120	4.08 – 17.1
Mid-Level Spike Results[2]					
Cyclohexyl sarin	4	28	3.84	63.6 – 120	1.39 – 6.66
Sarin	4	28	4.11	52.6 – 99.5	1.45 – 7.14
Soman	4	28	5.50	57.0 – 118	0.978 – 11.2
Sulfur mustard	4	28	5.04	56.8 – 120	2.34 – 6.94
Surrogates[3]					
Nitrobenzene-d_5	4	64	3.39	74.4 – 128	0.985 – 7.29
p-Terphenyl-d_{14}	4	62	10.2	17.9 – 129	1.87 – 32.0
Triphenyl phosphate	5	90	6.02	31.1 – 136	2.63 – 17.0
Groundwater					
Analyte	**# of labs**	**n**	**Pooled % RSD**	**% Recovery Range**	**% RSD Range**
Low-Level Spike Results[1]					
Cyclohexyl sarin	4	28	6.94	48.2 – 144	3.10 – 9.40
Sarin	4	28	7.32	32.6 – 124	4.28 – 9.97
Soman	4	28	9.88	41.7 – 147	4.34 – 15.4
Sulfur mustard	4	28	12.4	21.6 – 124	6.77 – 20.9
Mid-Level Spike Results[2]					
Cyclohexyl sarin	4	25	2.18	82.9 – 120	1.47 – 8.08
Sarin	4	25	5.08	65.3 – 97.2	1.02 – 8.39
Soman	4	25	4.95	69.7 – 116	2.48 – 8.04
Sulfur mustard	4	25	7.14	39.8 – 132	0.837 – 9.86
Surrogates[3]					
Nitrobenzene-d_5	4	64	4.61	66.7 – 129	1.68 – 7.32
p-Terphenyl-d_{14}	4	64	15.0	26.7 – 132	2.04 – 29.6
Triphenyl phosphate	4	64	7.82	36.6 – 143	2.35 – 18.6

n = number of replicates; MDL = method detection limit; RSD = relative standard deviation
[1] Low-level samples were spiked at 0.571 µg/L (sarin and cyclohexyl sarin), and 0.286 µg/L (soman and sulfur mustard).
[2] Mid-level samples were spiked at 5.71 µg/L (sarin and cyclohexyl sarin), and 2.86 µg/L (soman and sulfur mustard).
[3] All surrogates were spiked at 28.6 µg/L.

Table 10a.
Example Multi-laboratory Precision and Recovery in Soils Using GC Full-Scan Quadrupole MS

Virginia-a Soil					
Analyte	**# of labs**	**n**	**Pooled % RSD**	**% Recovery Range**	**% RSD Range**
Low-Level Spike Results[1]					
Cyclohexyl sarin	3	20	7.92	94.9 – 168	4.36 – 11.6
Sarin	3	21	9.65	56.9 – 91.9	6.25 – 13.0
Soman	3	21	10.9	102 – 165	10.5 – 11.6
Sulfur mustard	3	21	13.3	73.2 – 124	11.8 – 14.3
Mid-Level Spike Results[2]					
Cyclohexyl sarin	3	21	6.29	108 – 137	4.32 – 7.81
Sarin	3	21	8.12	57.7 – 80.5	6.61 – 9.47
Soman	3	21	6.66	86.6 – 114	4.96 – 8.22
Sulfur mustard	3	20	8.74	69.2 – 93.7	3.15 – 8.22
Surrogates[3]					
Nitrobenzene-d_5	3	42	10.7	52.7 – 98.7	7.18 – 15.0
p-Terphenyl-d_{14}	3	42	6.86	87.7 – 156	3.81 – 9.49
Triphenyl phosphate	3	42	8.25	113 – 202	3.37 – 12.5
ASTM Soil					
Analyte	**# of labs**	**n**	**Pooled % RSD**	**% Recovery Range**	**% RSD Range**
Low-Level Spike Results[1]					
Cyclohexyl sarin	3	21	28.0	10.4 – 31.2	16.5 – 39.1
Sarin	3	21	29.2	5.15 – 30.3	13.6 – 46.4
Soman	3	21	19.1	24.8 – 91.6	8.13 – 24.0
Sulfur mustard	3	21	17.5	38.9 –113	10.2 – 23.2
Mid-Level Spike Results[2]					
Cyclohexyl Sarin	3	21	19.3	15.4 – 37.4	7.27 – 30.3
Sarin	3	20	16.2	9.98 – 22.9	8.07 – 24.6
Soman	3	21	10.0	31.2 – 56.5	6.41 – 13.2
Sulfur Mustard	3	20	8.70	59.9 – 88.3	4.75 – 10.3
Surrogates[3]					
Nitrobenzene-d_5	3	42	10.6	44.0 – 101	3.97 – 17.3
p-Terphenyl-d_{14}	3	42	9.68	59.6 – 115	4.46 – 16.6
Triphenyl phosphate	3	42	9.52	59.9 – 158	5.83 – 16.6

n = number of replicates; MDL = method detection limit; RSD = relative standard deviation
[1] Low-level samples were spiked at 10.0 µg/kg (sarin and cyclohexyl sarin), and 5.00 µg/kg (soman and sulfur mustard).
[2] Mid-level samples were spiked at 80.0 µg/kg (sarin and cyclohexyl sarin), and 40.0 µg/kg (soman and sulfur mustard).
[3] All surrogates were spiked at 50.0 µg/kg.

Table 10b.
Example Multi-laboratory Precision and Recovery in Soils Using GC Full-Scan TOF MS

Virginia-a Soil					
Analyte	**# of labs**	**n**	**Pooled % RSD**	**% Recovery Range**	**% RSD Range**
Low-Level Spike Results[1]					
Cyclohexyl sarin	3	21	11.0	120 – 174	10.8 – 11.1
Sarin	3	21	14.0	56.9 – 129	8.14 – 17.8
Soman	3	19	8.40	67.2 – 167	1.71 – 11.9
Sulfur mustard	3	21	16.7	62.8 – 141	14.7 – 17.7
Mid-Level Spike Results[2]					
Cyclohexyl sarin	3	18	9.65	112 – 158	6.37 – 12.5
Sarin	3	18	7.24	66.1 – 129	5.14 – 10.2
Soman	3	18	6.96	130 – 171	4.95 – 9.58
Sulfur mustard	3	18	7.87	62.9 – 100	4.76 – 11.7
Surrogates[3]					
Nitrobenzene-d_5	3	39	9.06	52.0 – 91.8	3.04 – 16.2
p-Terphenyl-d_{14}	3	39	8.76	64.8 – 137	5.98 – 12.2
Triphenyl phosphate	3	25	10.9	97.9 – 155	3.89 – 18.3
ASTM Soil					
Analyte	**# of labs**	**n**	**Pooled % RSD**	**% Recovery Range**	**% RSD Range**
Low-Level Spike Results[1]					
Cyclohexyl sarin	3	21	18.6	26.9 – 49.3	12.0 – 24.5
Sarin	2	14	24.8	8.7 – 35.1	18.2 – 30.0
Soman	1	7	30.6	29.6 – 87.2	30.6
Sulfur mustard	3	21	22.2	20.6 – 118	9.23 – 34.9
Mid-Level Spike Results[2]					
Sarin	3	18	27.0	6.51 – 24.8	11.5 – 40.8
Cyclohexyl sarin	3	18	28.3	8.61 – 36.1	15.3 – 42.2
Soman	3	17	15.1	30.0 – 58.7	8.29 – 19.3
Sulfur mustard	3	18	17.1	28.4 – 92.4	3.41 – 29.1
Surrogates[3]					
Nitrobenzene-d_5	3	39	9.08	44.2 – 89.1	3.07 – 17 4
p-Terphenyl-d_{14}	3	39	8.72	52.6 – 108	2.24 – 17 8
Triphenyl phosphate	2	25	10.4	57.4 – 111	3.45 – 14 6

n = number of replicates; MDL = method detection limit; RSD = relative standard deviation
[1] Low-level samples were spiked at 1.00 µg/kg (sarin and cyclohexyl sarin), and 0.500 µg/kg (soman and sulfur mustard).
[2] Mid-level samples were spiked at 10.0 µg/kg (sarin and cyclohexyl sarin), and 5.0 µg/kg (soman and sulfur mustard).
[3] All surrogates were spiked at 50.0 µg/kg.

Table 11.
Multi-Laboratory Study Water Matrices Characterization Data

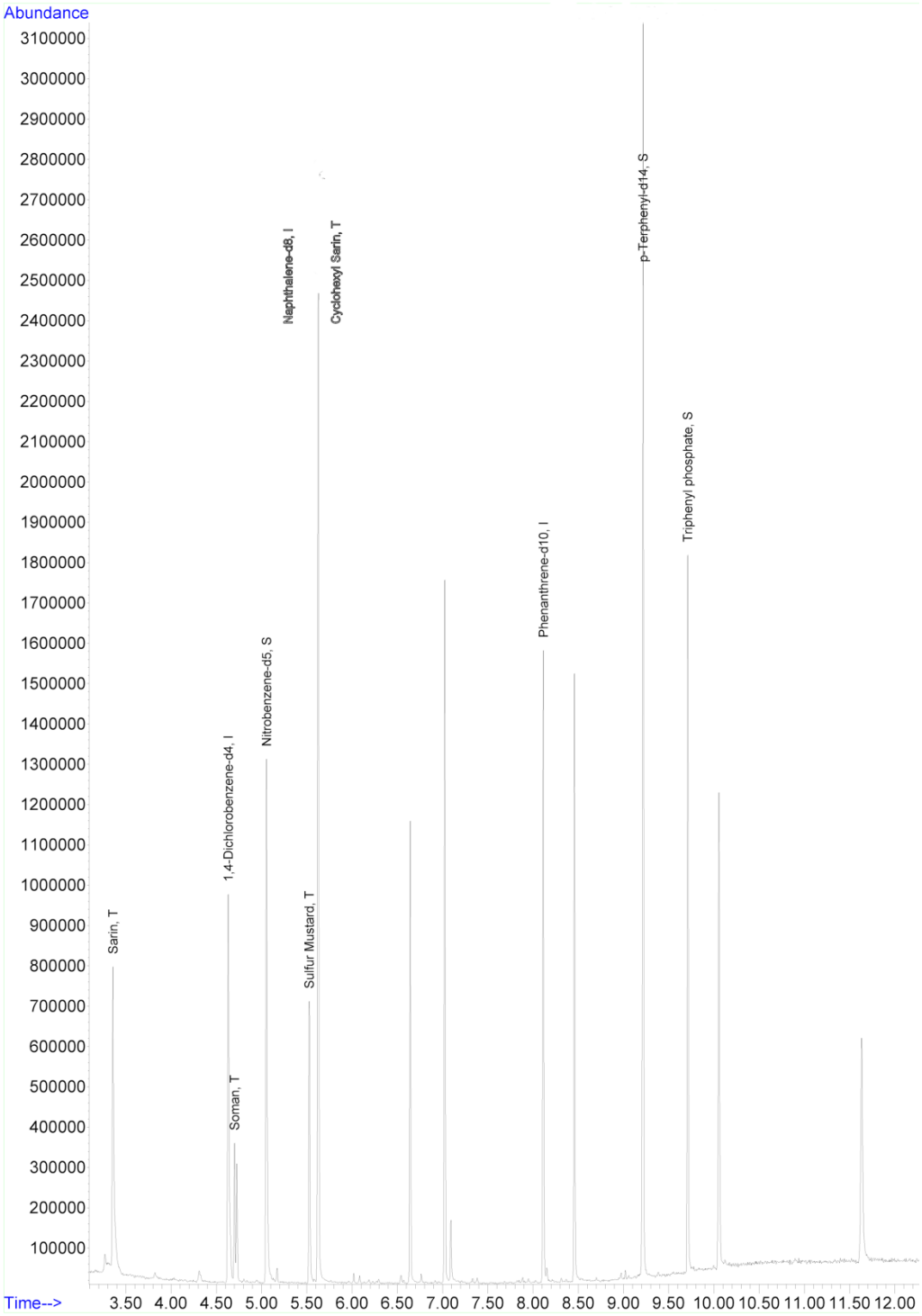
Drinking Water		**Groundwater**	
Collection Date	12/2011 – 07/2012	Collection Date	01/2012 – 02/2012
pH	8.5 – 8.7	pH	6 – 7
Chlorine (free) mg/L	1.16 – 1.35	Chlorine (free) mg/L	0.02 – 0.05
Chlorine (total) mg/L	1.20 – 1.42	Chlorine (total) mg/L	NR
Total Organic Carbon (TOC) (ppm)	0.83 – 1.14	Total Organic Carbon (TOC) (ppm)	1.08 – 1.24
Conductivity (µS)	294 – 413	Conductivity (µS)	0.82 – 0.87
Oxidation-Reduction Potential (mV)	766 – 770	Oxidation-Reduction Potential (mV)	NR
Turbidity (NTU)	0.06 – 0.07	Turbidity (NTU)	0.1 – 0.3
Total Hardness (mg/L)	114 – 131	Total Hardness (mg/L)	37 – 65
Alkalinity (mg/L)	64-80	Alkalinity (mg/L)	30 – 36

NR = Not Reported

Table 12.
Multi-Laboratory Study Soil Matrix Characterization Data

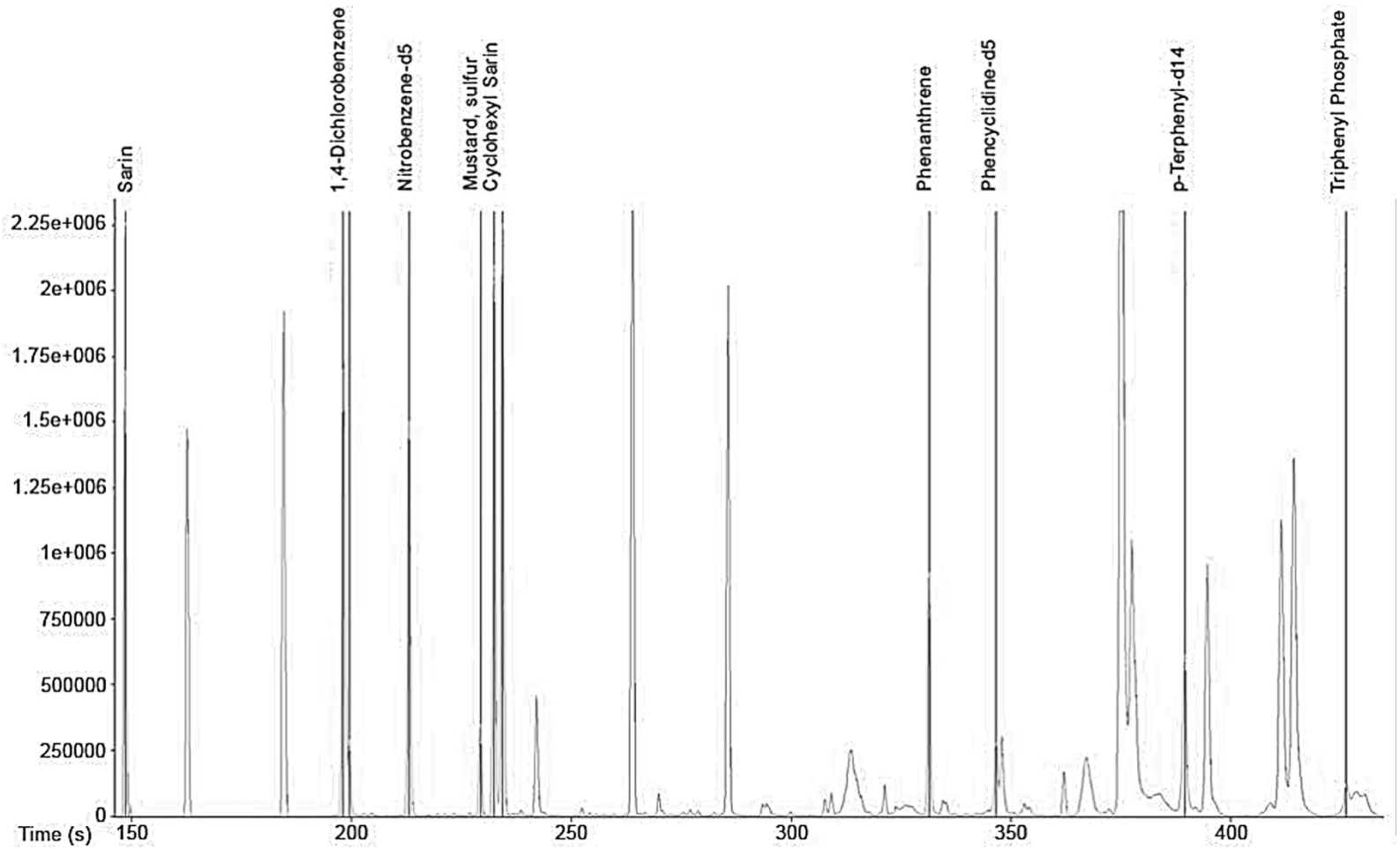
Parameter	**Matrix**	
	ASTM Soil ML-1	**Virginia-a Soil**
pH	8.68	4.41
TOC (% C)	< 0.10	2.2
% Solids	98.9	99.1

TOC = total organic carbon



Notes: (1) Concentrations of all analytes as described in Table 8, Calibration Level 6 (GC/MS– Full-Scan Quadrupole MS); (2) Unlabelled peaks represent compounds not specifically targeted by this protocol.
Time units = minutes
T = Target; S = Surrogate; I = Internal standard

Figure 1.
Example chromatogram for a calibration standard on full-scan quadrupole MS.



Notes: (1) Concentrations of all analytes as described in Table 8, Calibration Level 4 (GC/MS – TOF); (2) Unlabelled peaks represent compounds not specifically targeted by this protocol.
T = Target; S = Surrogate; I = Internal standard; (s) = seconds

Figure 2.
Example chromatogram for a midpoint calibration standard (Cal5) on time-of-flight MS.

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