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Experiments with the LECO Pegasus® Gas Chromatograph/ Time-of-Flight Mass Spectrometer Phase 1: Fast GC Separations and Comparison of the GC/TOF-MS with Conventional Quadrupole GC/MS and Fast Quadrupole GC/MS

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Disclaimer

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Auspices Statement

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Abbreviations/Acronyms

1-D – One dimensional, specifically in reference to gas chromatography

2-D – Two dimensional, specifically in reference to gas chromatography

AMDIS – Automated Mass Spectral Deconvolution and Identification System

CCV – Continuing Calibration Verification

CWA – Chemical Warfare Agent, in the context of this report, the CWAs of interest are HD, GB, GD, GF, and VX

CWA-SAP – *Standard Analytical Protocol for Extractable Semivolatile Organic Compounds* (1)

DC – Direct current

DFTPP – Decafluorotriphenylphosphine

ECBC – Edgewood Chemical Biological Center (ECBC), Aberdeen Proving Grounds, MD

EPA – United States Environmental Protection Agency

FBP – 2-Fluorobiphenyl

RF – Radio-frequency

GB – Sarin

GC – Gas Chromatography

GC/MS – Gas Chromatography/Mass Spectrometry

GC/TOF-MS – Gas Chromatography coupled with Time-of-Flight Mass Spectrometry

GD – Soman

GF – Cyclosarin

HD – Distilled sulfur mustard

IAG – Interagency Agreement

i.d. – Internal diameter

IDL – Instrument Detection Limit

LLNL – Lawrence Livermore National Laboratory

MDL – Method Detection Limit

MS – Mass spectrometry

m/z – Mass to charge ratio, with reference to an ion

NB-d_5 – Deuterated (d_5) nitrobenzene

ND – Not Detected

NHSRC – EPA's National Homeland Security Research Center, Cincinnati, OH

NIST – National Institute of Standards and Technology

NMR – Nuclear Magnetic Resonance Spectroscopy

PCP-d_5 – Deuterated (d_5) phencyclidine

PFTBA – Perfluorotributylamine

ppb – Part(s) per billion

ppm – Part(s) per million

QAPP – Quality Assurance Project Plan

SIM – Selected Ion Monitoring (operating mode of a mass spectrometer)

S:N – Signal-to-Noise ratio

TEA – Triethylamine

TIC – Total Ion Chromatogram (produced by GC/MS analysis)

TIC – Toxic Industrial Compounds

Ter-d_{14} – Deuterated (d_{14}) terphenyl

TOC – Total organic carbon

TOF – Time of flight

TPP – Triphenyl phosphate

UD-CWA – Ultra-dilute (10 ppm) Chemical Warfare Agent standards

VX – *O*-ethyl-*S*-[2-(diisopropylamino)ethyl] methylphosphonothioate

Executive Summary

Conventional analysis by gas chromatography/quadrupole mass spectrometry (GC/MS) can be time-consuming (30 – 60 minutes) and prone to interferences. The use of fast gas chromatography coupled with time-of-flight mass spectrometry (GC/TOF-MS) offers the advantages of faster (13 min) analysis times, improved GC resolution afforded by the use of narrower (0.1– 0.18 mm i.d.) columns, and improved mass resolution and data acquisition speed provided by the TOF-MS. In addition, GC/TOF-MS offers the promise of better detection limits than quadrupole GC/MS, while still providing the full mass spectral data which offers an additional level of confidence in analyte identification.

In this study, the LECO Pegasus® 4D GC/TOF-MS was used to detect chemical warfare agents (CWAs) and to compare this instrument's performance to the speed of analysis and detection limits of conventional quadrupole-based GC/MS. Analytes studied were sulfur mustard (HD), sarin (GB), soman (GD), cyclosarin (GF), and *O*-ethyl-*S*-[2-(diisopropylamino)ethyl] methylphosphonothioate (VX). Measured concentrations of analytes were determined by GC/MS and GC/TOF-MS and compared for reagent water, surface water, sand, three types of soils, and wipes. Limited comparisons between analyte concentrations measured with GC/TOF-MS and fast GC/MS were also performed.

Instrument detection limits (IDLs) for GC/TOF-MS were lower than those observed for GC/MS; IDLs were analyte-dependent and ranged from 0.0025 to 0.025 ng. The reproducibilities of retention times for replicate (n=7) injections of 0.5 ng each CWA were within 0.4% and reproducibilities of peak areas were less than 3%. In general, matches between TOF-MS spectra and those contained in the NIST database were good (i.e., greater than 700 out of 1000), even at the lowest levels detected in standards.

Analyte concentrations determined by GC/TOF-MS were reasonably comparable to those measured by quadrupole GC/MS for standards, water, soils, and wipe extracts. On average, analyte concentrations determined for control and water samples by GC/TOF-MS and conventional GC/MS were comparable (agreement within <20%). For sand and soils, concentrations measured by GC/MS were often higher than those measured by GC/TOF-MS and were not always in good agreement. For most (but not all) analytes in wipes, concentrations measured by GC/TOF-MS and GC/MS agreed within 30%. The reasons for the differences are unclear, but the fact that some recoveries of analytes from wipes were >150% when measured by GC/MS suggests that the GC/MS might be prone to matrix interferences that are not being satisfactorily separated by the conditions used for GC/MS.

GC/TOF-MS appears to be a good technique to measure concentrations of CWAs in environmental matrices. Such data can be produced with faster analysis times (by a factor of three) than with conventional GC/MS. And GC-TOF-MS provides low detection limits while retaining full mass spectral data. The collection of a complete mass spectrum provides greater confidence in correct analyte identification. Currently, the only disadvantage of GC/TOF-MS is that many analysts do not have sufficient experience with the technique; however, such expertise can be developed by knowledgeable GC/MS operators. In addition, standard mass spectrometric tune criteria (i.e., based on decafluorotriphenylphosphine(DFTPP) ions of specified relative abundances) used to determine that quadrupole GC/MS systems are operating correctly must be adapted to allow the use of GC/TOF-MS. Once tune criteria are met, the GC/TOF-MS has been observed to operate well for several months, with no need to "retune" the system. Data suggest that GC/TOF-MS can be used routinely for the analysis of sample extracts containing CWAs.

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1.0 Introduction and Background

Gas chromatography/mass spectrometry (GC/MS) is the method of choice for the analysis of volatile and semivolatile organic compounds in environmental samples. Most laboratories performing this technique use quadrupole mass spectrometers. The quadrupole GC/MS performs mass filtering of ions based on changing DC and RF fields and has low detection limits. It is capable of detecting low- to sub-nanogram quantities of chemicals when operated in the full scan mode and low picogram amounts of materials when operated in the selected ion monitoring (SIM) mode. However, in order to achieve the low detection limits of SIM, the collection of full mass spectral data is sacrificed. Quadrupole GC/MS is commonly used in laboratories because advances in software made by instrument manufacturers have simplified the use of such instruments — it is easy to collect data with quadrupole mass spectrometers and the systems are rugged, reliable, and relatively inexpensive (~$85,000, for a basic Agilent GC/MS). In addition, mass spectral libraries have been developed for use with quadrupole GC/MS to assist in the identification of unknown compounds and software packages assist in the processing and presentation of quantitative data. The main disadvantages of quadrupole GC/MS are that a typical analysis takes 30–60 minutes and that some older systems do not possess the electronic components necessary to provide the fast scan speeds required for operation with fast GC separations.

Gas chromatography coupled with time-of-flight mass spectrometry (GC/TOF-MS) offers the promise of improved analytical speed. Because its principle of operation differs from that of a quadrupole GC/MS (i.e., in GC/TOF-MS, ions of different masses travel through the flight tube at different speeds, thus reaching the detector at different times), TOF-MS does not have to "scan" a mass spectrum (by changing DC and RF fields) as does the quadrupole MS. Due to this operational difference, the cycle of ion production, acceleration, and detection is faster (on the order of 100 µsec) for TOF-MS than for quadrupole MS. The speed of the cycle makes the TOF-MS an ideal instrument to couple with fast GC separations (i.e., separations that provide improved GC resolution afforded by the use of narrow-bore [0.1– 0.18 mm i.d.] capillary columns). Complete analyses using a fast GC method can be performed in less than half the time required for separations using conventional 30 m x 0.25 mm i.d. columns. Thus, the GC/TOF-MS is expected to be a valuable tool in situations where a large number of sample analyses are required in a short amount of time. In addition, GC/TOF-MS provides low picogram detection limits, retains complete mass spectral data for each compound it detects, and is comparable in price to the quadrupole GC/MS ($101,000 for a basic LECO TruTOF® HT, LECO Corporation, St. Joseph, MI). Retention of complete mass spectral data offers an additional level of confidence in analyte identification (i.e., the more ions upon which to base analyte identification, the greater the confidence in that identification)

In this study, the LECO Pegasus® 4D GC/TOF-MS was used to detect chemical warfare agents (CWAs) and to compare this instrument's performance, with regards to speed of analysis and detection limits, to conventional quadrupole GC/MS. Analytes studied were sulfur mustard (HD), sarin (GB), soman (GD), cyclosarin (GF), and *O*-ethyl-*S*-[2-(diisopropylamino)ethyl]methylphosphonothioate (VX). We also compared the use of the GC/TOF-MS and quadrupole GC/MS for analysis of CWAs in various matrices, including

waters, sand, soils, and wipes, that were spiked with CWAs and prepared by standard procedures (1). We also collected limited data with fast GC separations coupled with quadrupole GC/MS.

2.0 Study Objectives

The focus of this work was to determine how best to utilize the GC/TOF-MS for the analysis of CWAs. Specifically, our goals were:

1) To establish appropriate separation conditions for the analysis of HD, GB, GD, GF, and VX by GC/TOF-MS, while minimizing the analysis time.

2) To determine instrument detection limits (IDLs) by GC/TOF-MS (electron ionization mode) for HD, GB, GD, GF, and VX.

3) To establish calibration curves and response factors using EPA Method 8270 internal standards.

4) To compare analytical concentrations determined for analytes in sample extracts measured by GC/MS (quadrupole system) and GC/TOF-MS. Sample extracts were derived from various spiked matrices, including water, sand, soils, and wipes.

3.0 Experimental Conditions

The experimental strategy used in our studies was to first optimize separation and analysis conditions for HD, GB, GD, GF, and VX and then to analyze the same standard solutions and sample extracts by GC/TOF-MS and by quadrupole GC/MS.

3.1 Standards

CWA standards used for this study were synthesized by LLNL and were characterized for purity by NMR and GC/MS analyses. Dilute standards were prepared gravimetrically from neat materials. As determined by proton NMR, the purities for GB, GD, GF, HD, and VX were 97.2%, 92.9%, 94.4%, 94.0%, and 94.0%, respectively.

Surrogate and internal standards used were those of EPA Method 8270D (3) and those suggested by a previous Battelle study (4). The surrogate standard mix included nitrobenzene-d_5 (NB-d_5), 2-fluorobiphenyl (FBP), phencyclidine-d_5 (PCP-d_5), terphenyl-d_{14} (Ter-d_{14}), and triphenyl phosphate (TPP). Specific solutions purchased for this work included: Base/Neutrals Surrogate Standard, 1000 µg/mL, in dichloromethane (Catalog number ERB-076, Cerilliant, Round Rock, TX), Triphenylphosphate, 5000 µg/mL, in methyl *tert*-butyl ether (Catalog number ERT-108S, Cerilliant), and PCP-d_5 (phencyclidine-d_5), 1000 µg/mL, in methanol (Catalog number P-006, Cerilliant).

Internal standards used included 1,4-dichlorobenzene-d_4, naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, and perylene-d_{12}. These standards were

purchased as a Semivolatile Internal Standard Mix, 2000 µg/mL, in dichloromethane (Catalog number 861238, Supelco, Bellefonte, PA). Internal standards were spiked into all sample extracts such that their concentrations were 1 ng/µL for all analyses.

Decafluorotriphenylphosphine (DFTPP) was used to verify that the GC/MS systems were functioning optimally. DFTPP was purchased as a solution with a concentration of 1000 µg/mL in acetone (Catalog number, 47941, Supelco, Bellefonte, PA).

All standards and samples were stored at 4–8 º C.

3.2 Sample preparation

Our sample preparation procedures, described below, were consistent with the CWA-SAP (1) now under development at EPA. The extraction materials and protocols, described below, have been previously employed by our laboratory (5).

Soil samples

Briefly, 10-g aliquots of sand and soils were spiked with 500 ng of surrogates (see Section 3.1 above) and extracted for one-hour by waterbath sonication with 25.00 mL of 25/50/25 (v/v/v) acetone/dichloromethane/ethyl acetate. The resulting extract was separated from the sand or soil by centrifugation and the supernatant removed. The sand and soils were then extracted for a second time, as described above, with 5% triethylamine (TEA) in ethyl acetate. Extracts from the two extraction procedures were kept separate, reduced in volume to 1.00 mL, and spiked with internal standards (also per Section 3.1) prior to analysis.

Soil samples used included Nebraska Aglands Ap soil (5.1% sand, 57.5% silt, 31.7% clay, and 1.9% TOC), Georgia Bt2 soil (46% sand, 22% silt, 32% clay, and 0.2% TOC), and Virginia soil (64.5% sand, 28% silt, 7.5% clay, and 2.6% TOC). All soils were obtained from National Exposure Research Laboratory, US EPA, Las Vegas, NV.

Water samples

Water samples (35-mL) were spiked with 2 µg each surrogate (see Section 3.1 above) and extracted by vortexing for 2 minutes with 2.00 mL dichloromethane. Measured amounts of the resulting extract were spiked with internal standards (also per Section 3.1) and analyzed. The pH of the water samples was measured; pH of the reagent water (HPLC-grade, Aldrich, P/N 27,073-3) used was 9.85; pH of Milli-Q™ water was 8.14; and surface water was pH 7.80. The surface water used in this study was collected at the Zone 7 Water Agency Water Quality Laboratory, Livermore, California, and came from the South Bay Aqueduct, which collects water from the Sacramento River Delta and includes snowmelt water from the northern Sierra Nevada Mountains.

Wipe samples

Wipes (3” x 3”, Kendall-Curity, 12-ply, P/N 1903, available from Tyco Healthcare Group LP, Mansfield, MA) were spiked with 500 ng surrogates (see Section 3.1 above) extracted by waterbath sonication for 30 minutes (twice) with 15.00 mL 25/50/25 (v/v/v) acetone/dichloromethane/ethyl acetate. The resulting extracts were combined, evaporated to 1.00

mL, spiked with internal standards (also per Section 3.1), and analyzed.

All sample extracts were stored at 4–8 º C until the time of analysis and each batch of sample extracts was analyzed with corresponding method blanks.

3.3 GC/TOF-MS conditions

GC/TOF-MS experiments were performed with an Agilent 6890 gas chromatograph (Agilent Technologies, Inc., Santa Clara, CA) coupled with a LECO Pegasus® 4D mass spectrometer (LECO Corp., St. Joseph, MI). Prior to use, the GC/TOF-MS was tuned with the vendor's standard protocols and perfluorotributylamine (PFTBA) as a calibrant. An injection of 15 ng decafluorotriphenylphosphine (DFTPP) was used to check the performance of the instrument prior to analyzing samples. The amount of DFTPP used for instrument checks was lower than the 50 ng amount recommended by the CWA-SAP (1) to check the performance of a quadrupole GC/MS system. The reduction in DFTPP was necessary so as not to overload the GC column and TOF-MS detector. Experimental data were collected using the same instrument conditions, including electron multiplier voltages, as those used to produce the DFTPP check samples. During analysis sequences, a continuing calibration verification (CCV) standard near the midpoint of the calibration range was analyzed every 10 samples. The CWA concentrations calculated for the CCV, using the most recent calibration curve, were required to be within 20% of the expected value in order for the data collected between CCV checks to be considered valid.

Standard operating parameters for the GC/TOF-MS were as described below:

Injection size:	1 µL
Inlet type:	split/splitless
Injection mode:	pulsed-splitless
Pulse pressure:	40 psi for 0.5 min
Purge time:	35 sec at 30 mL/min
Carrier gas:	He with constant flow of 1.2 mL/min
GC injection port:	250ºC
GC columns:	15 m x 0.18 mm i.d. x 0.18 µm film thickness, HP5-MS UI (Agilent Technologies, Inc., Santa Clara, CA) 1 m x 0.1 mm i.d. x 0.1 µm film thickness, Rxi-17 (Restek, Bellefonte, PA)
GC oven (primary):	55 ºC held for 0.5 min, 20 ºC/min to 100 ºC, 40 ºC/min to 280 ºC, held for 2.75 min
GC oven (secondary):	70 ºC held for 0.5 min, 20 ºC/min to 115 ºC, 40 ºC/min to 295 ºC, held for 1.64 min
GC transfer line:	295 ºC

The following MS conditions were used for detection.

MS filament delay:	1.5 min
MS scan range:	35–500, at a data acquisition rate of 15 spectra/sec
MS source:	250 ºC
Electron energy:	70 eV

Although the GC/TOF-MS used for this study was capable of performing two-dimensional (2-D) GC separations, it was operated to perform only one-dimensional (1-D) separations, using the HP5-MS UI column. In 2-D chromatography, a second GC column with a different chemical phase than that of the first GC column is used to provide additional separation of analytes as they elute from the first column. Because the chemistry of the second GC column is different from the first, compounds that co-elute from the first column may be easily resolved after a separation on the second GC column (i.e., the peak capacity of the system is increased and the specificity of analyte detection is improved). In order to maintain a configuration that allowed for easy transition between 1-D and 2-D modes of operation (i.e., did not require the venting and down-time of the TOF-MS associated with column changes and installations), the Rxi-17 column was left in place when 1-D experiments were performed. The modulator and secondary oven conditions were optimized so that the second GC column acted as a transfer line into the TOF-MS. No separations occurred on the secondary GC column. By keeping the secondary GC oven at a temperature 15 ºC higher than the primary oven (to ensure that analytes did not condense in the secondary column) and by ensuring that the modulator was not used to cryofocus effluent from the primary GC column, the Rxi-17 column segment performed the function of a transfer line into the mass spectrometer.

To collect GC/TOF-MS data, separate autosampler, GC, MS, and data processing methods were created and linked. The method parameters routinely used to collect and process data are recorded in Appendices A, B, D, and E of this report. Because the values of the parameters that were entered into the LECO software were of great importance, screen capture images of the method setup pages have been provided so that the conditions of our analyses can be replicated. Appendix A contains the autosampler method used for all experiments and Appendix B contains the GC conditions used by LLNL. Because the GC/TOF-MS used in this study was capable of operating with 2-D GC separations, there were additional parameters that would not be used with a GC/TOF-MS that is capable of only 1-D GC separations. For this reason, also included in Appendix C are parameters that would be used to replicate our methods on a GC/TOF-MS that is capable of performing a chromatographic separation using a single GC column. Appendix D contains the relevant MS method and Appendix E contains the data analysis method used in this study.

3.4 GC/MS conditions (conventional quadrupole)

GC/MS was performed with an Agilent 6890 GC coupled with an Agilent 5973 MS (Agilent Technologies, Inc., Santa Clara, CA). Prior to use, the GC/MS was tuned with the vendor's standard protocols and PFTBA as a calibrant. An injection of 50 ng DFTPP was used to check the performance of the instrument prior to sample analysis. CCVs were also performed every 10 samples, as prescribed by EPA protocols, during the course of run sequences. Acceptance criteria used for the CCVs required that their measured concentrations were 80–120% of the expected values.

The standard GC parameters were:

Carrier gas:	Helium, at a constant flow of 32 cm/s
Injection mode:	Splitless for 0.75 min
Injector temperature:	250 ºC
Sample injection volume:	1 µL
GC Column:	Agilent HP-5MS, (5%-phenyl)-methylpolysiloxane
Column dimensions:	30 m x 0.25 mm x 0.25 µm (length x i.d. x film thickness)
GC temperature program:	40 ºC held for 3 min, 10 ºC/min to 150 ºC, 25 ºC/min to 280 ºC, held for 10.8 min

The standard MS conditions for full scan analyses performed in electron ionization mode were:

MS transfer line temperature:	280 ºC
MS source temperature:	230 ºC
MS quadrupole temperature:	150 ºC
Solvent delay time:	3 min
Scan range:	35-500 m/z
Electron energy:	70 eV
Scan time:	3.15 scans/sec
Ionization polarity:	Positive

The standard MS conditions for selected ion monitoring analyses performed in electron ionization mode were:

MS transfer line temperature:	280 ºC
MS source temperature:	230 ºC
MS quadrupole temperature:	150 ºC
Electron energy:	70 eV
Ion dwell time:	100 msec per ion (each analyte was assigned its own SIM group; depending on the number of ions monitored, cycle times ranged from 1.44 – 2.86 cycles/sec)
Ionization polarity:	Positive

3.5 GC/MS conditions (fast separations, quadrupole MS)

GC/MS analyses were performed with an Agilent 5973 system, which was tuned with DFTPP and checked with 50 ng DFTPP. During analyses, CCVs (continuing calibration verification) were analyzed at a frequency of every 10 samples. Acceptance criteria used for the CCVs required that their measured concentrations were 80–120% of the expected values.

The GC conditions were modified to allow faster chromatographic separations. Parameters that were changed from the conditions of Section 3.4 are shown below:

Carrier gas:	Helium, at a constant pressure of 17.8 PSI

Injection mode:	Splitless for 0.75 min
Injector temperature:	250 ºC
Sample injection volume:	1 µL
GC Column:	Agilent HP-5MS-UI, (5%-phenyl)-methylpolysiloxane
Column dimensions:	20 m x 0.18 mm x 0.18 µm (length x i.d. x film thickness)
GC temperature program:	40 ºC for 1.25 min, 24 ºC/min to 150 ºC, 45 ºC/min to 280 ºC, held for 7.3 min

The standard MS conditions for full scan analyses performed in electron ionization mode were:

MS transfer line temperature:	280 ºC
MS source temperature:	230 ºC
MS quadrupole temperature:	150 ºC
Solvent delay time:	3 min
Scan range:	35-500 m/z
Electron energy:	70 eV
Scan time:	5.92 scans/sec
Ionization polarity:	Positive

The standard MS conditions for selected ion monitoring analyses performed in electron ionization mode were:

MS transfer line temperature:	280 ºC
MS source temperature:	230 ºC
MS quadrupole temperature:	150 ºC
Electron energy:	70 eV
Ion dwell time:	40 – 300 msec per ion (each analyte was assigned its own SIM group; depending on the number of ions monitored, cycle times ranged from 1.05 – 5.88 cycles/sec)
Ionization polarity:	Positive

4.0 Results

4.1 Chromatographic separation of CWAs

Using the GC/TOF-MS, with conditions described above, chromatographic analysis of CWAs was performed in less than 13 minutes; see Figure 1. This speed of chromatographic analysis represents a two- to three-fold reduction in time from the 30-minute analysis that was required using GC/MS with a 30 m x 0.25 mm i.d. GC column. This analysis time was longer than the six-minute separation reported by ECBC, using a 10 m x 0.25 mm i.d. GC column (6). While we originally tried to implement the ECBC method, we found it not practical with our LECO Pegasus® IV for several reasons. First, because our LECO Pegasus® IV was configured for 2-D separations, the instrument could not physically accommodate the oven insert that was needed to provide consistent GC oven heating with higher temperature ramp rates. The secondary oven and modulator occupied space in the GC oven which prohibited the installation

of the oven insert. Because the instrument used by ECBC was configured for 1-D separations, ECBC was able to use an oven insert to reduce GC oven volume, thereby obtaining consistent temperature ramp rates near the vendor's recommended maximum values for an Agilent 240V fast, 6890 GC. In addition, because the insert effectively reduced the volume of the GC oven, the insert allowed ECBC to reduce GC cycle times and, therefore, increase sample throughput.

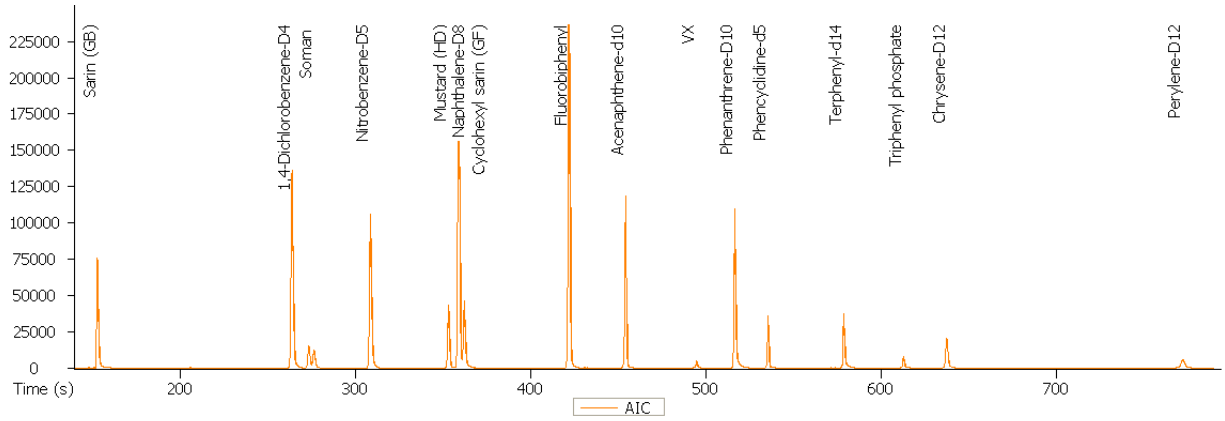

Figure 1. GC/TOF-MS TIC of 0.5 ng each CWA and 1 ng each surrogate standard.

Second, analyte separations using a 10 m GC column (HP-5MS UI 10 m x 0.18mm i.d. x 0.18 µm film thickness) and fast temperature ramps were not reproducible between equivalent GC columns. While the separation conditions used by ECBC (6) provided complete resolution of all analytes in the calibration solution on one GC column (see additional data provided in Appendix F), complete separation of HD, naphthalene-d8, and GF was not achieved when two other GC columns of the same stationary phase and column dimensions were used. Subsequent discussion with two other CWA labs suggested that they, too, observed similar separation problems. Even when complete separation of analytes was observed, because the separation between HD and naphthalene-d8 was only approximately 1 second, there were concerns that the compression of the chromatography would not be sufficient to allow separation of the analytes from the many components expected to be present in environmental matrices. Thus, we opted to use the less aggressive GC temperature ramp rates reported in Section 3.3, which resulted in a 13-minute separation (in contrast to the 6-minute separation shown in Appendix F).

Third, we were concerned that a GC temperature ramp rate that was too fast would not provide adequate chromatographic separation of analytes from the many interfering compounds expected to be present in complex sample matrices. While LECO's software includes algorithms that aid in the deconvolution of spectral data, this software deconvolution is no substitute for good chromatographic separation. In addition to good chromatographic separation, a secondary concern is to make sure that there are a sufficient number of data points to adequately define

each chromatographic peak present. When developing any GC-based method for analyte detection and quantification, care must be taken to collect an adequate number of data points across a GC peak to accurately define its shape. One common rule of thumb is that at least 10 data points are needed to define a chromatographic peak. LECO (GC/TOF-MS vendor) recommends that 15–20 mass spectra be collected across each chromatographic peak. Without an adequate number of data points to accurately define a chromatographic peak, peak shape, reproducibility (which affects quantitation), the ability to use LECO's deconvolution software, and the potential to later perform 2-D separations (which can provide important information when confirmatory analyses are needed) are compromised. Thus, the strategy used to develop a GC/TOF-MS method was to first optimize the chromatography and then to make sure that the data acquisition rate of the TOF-MS was set so that 15–20 mass spectra would be collected across the narrowest GC peak observed. The collection of 15–20 mass spectra allows LECO's deconvolution software to assign the proper spectra and signal intensities to any coeluting peaks. Using the chromatographic conditions previously described, peak widths on the order of 0.8–1 second were typically observed and data acquisition rates on the order of 20–25 mass spectra per minute were found to be adequate. While the GC/TOF-MS is capable of acquisition speeds of 500 spectra/sec, there are trade-offs between the number of spectra collected and the ease of data processing and the number of spectra collected and the size of the data file that is collected during the course of an analysis.

Using the separation conditions previously discussed (i.e., Section 3.3), reproducibility of retention times and analyte responses for seven replicate injections of a standard containing 0.5 µg/mL of CWAs were documented and are shown in Table 1. As was evident from the data, for seven replicate injections, retention times were stable and varied by less than 0.4% (relative standard deviation) for all compounds. This stability is reasonably consistent with reports that a sample of 32 Agilent 6890 Plus GCs consistently demonstrated relative standard deviation of less than 0.1 percent, with some lower than 0.01 percent (7). All analyte responses, measured as peak areas, were also reproducible and varied by less than 3%.

Table 1. Average Retention Times (± Standard Deviations) and Average Analyte Responses (± Standard Deviations) for Seven Replicate Injections of 0.5 ng of each CWA into the GC/TOF-MS

Analyte	Average Retention Time (sec)	Average Analyte Response (peak area in arbitrary units)
GB	152.49 ± 0.67	1267684 ± 23832
GD 1	272.68 ± 0.83	318556 ± 6419
GD 2	275.54 ± 0.83	284388 ± 4875
GF	361.51 ± 0.65	874301 ± 22988
HD	352.45 ± 0.68	803696 ± 18309
VX	494.59 ± 0.22	66214 ± 1580

Before data could be collected using established separation conditions, acceptable performance of the GC/MS-TOF needed to be documented. As for other GC/MS-based methods used by EPA, DFTPP was used to check acceptability of the system tune. DFTPP mass spectral attributes required to document acceptable performance of the GC/TOF-MS were evaluated. Several sets of DFTPP criteria are in use for various EPA methods; see Table 2. DFTPP criteria include those which were derived for contract laboratory program work (required by the original version of the CWA-SAP), those which were used by EPA Methods 8270D (3) and 527 (8), and those suggested by LECO (9, 10). While performing instrument detection limit and calibration studies, the DFTPP tune check was observed to predictably fail against the CWA-SAP criteria (abundance of *m/z* 442 was outside the allowed range and the abundances of *m/z* 365 and *m/z* 441 were almost outside their allowed ranges). The DFTPP check also failed the requirements of Method 8270D (abundances of *m/z* 441 and *m/z* 442 failed and *m/z* 365 was near failure). The DFTPP check suggested by LECO always met acceptance criteria if the instrument was operating properly. LECO also found this to be the case and, in 2005, petitioned to have their DFTPP criteria accepted by the US EPA (11). Subsequently, the DFTPP criteria of EPA Method 527 were modified so that both quadrupole GC/MS and GC/TOF-MS would be able to meet them. We recommend that the same criteria, those of EPA Method 527, be adopted in the CWA-SAP so that users of the method have the flexibility to perform analyses with either a quadrupole GC/MS or GC/TOF-MS. Figure 2 shows an example of a tune report used to determine if the GC/TOF-MS was working properly. Samples were analyzed only if these DFTPP tune criteria were met.

Table 2. DFTPP Key Ions and Ion Abundance Criteria Used by Different Methods to Verify GC/MS Tune

		Ion Abundance Criteria			
Mass	**Purpose**	**EPA Method 8270D**	**CWA-SAP 9/2008**	**LECO**	**EPA Method 527**
51	Low-mass sensitivity	10–80% of base peak	10–80% of *m/z* 198	10–85% of *m/z* 198	10–85% of base peak
68	Low-mass resolution	< 2% *m/z* 69	< 2% *m/z* 69	< 2% *m/z* 69	< 2% *m/z* 69
69		Not used	Present	Not used	Not used
70	Low-mass resolution	< 2% *m/z* 69	< 2% *m/z* 69	< 2% *m/z* 69	< 2% *m/z* 69
127	Low-mid-mass resolution	10–80% of base peak	10–80% of *m/z* 198	10–80% of *m/z* 198	10–80% of base peak
197	Mid-mass resolution	< 2% *m/z* 198	< 2% *m/z* 198	< 2% *m/z* 198	< 2% *m/z* 198
198	Mid-mass resolution & sensitivity	Base peak or > 50% *m/z* 442	Base peak	Base peak	Base peak or > 50% *m/z* 442
199	Mid-mass resolution & isotope ratio	5–9% *m/z* 198	5–9% *m/z* 198	5–9% *m/z* 198	5–9% *m/z* 198
275	Mid-high-mass sensitivity	10–60% of base peak	10–60% of *m/z* 198	10–60% of *m/z* 198	10–60% of base peak
365	Baseline threshold	> 1% *m/z* 198	> 1% *m/z* 198	> 0.5% *m/z* 198	> 0.5% *m/z* 198
441	High-mass resolution	Present, < 24% of *m/z* 442	Present, < *m/z* 443	< 150% *m/z* 443	< 150% *m/z* 443
442	High-mass resolution & sensitivity	Base peak or > 50% *m/z* 198	> 50 – 100% *m/z* 198	> 30% of *m/z* 198	Base peak or > 30% *m/z* 198
443	High-mass resolution & isotope ratio	15–24% *m/z* 442	15–24% *m/z* 442	15–24% *m/z* 442	15–24% *m/z* 442

DFTPP Tune Check

Lawrence Livermore National Laboratory
Sample Name: 15 ng/uL DFTPP (EPA-STDS4-42-1)
Date: 6/8/2009

Operator: Heather Mulcany
Data File Name: DFTPP:99
Time: 7:57:02 AM

Leco Pegasus IV GC-TOF/MS

Model: 614-200-700 SN: 3271

Result: Passed

Mass	Criteria	Reference Mass	Min Rel. Abundance	Max Rel. Abundance	Relative Abundance	Pass/Fail
51	>10.00% and <85.00 % of Base Ion	Base	10.00	85.00	48.00	Passed
68	<2.00% of mass 69	69		2.00	1.18	Passed
70	<2.00% of mass 69	69		2.00	0.32	Passed
127	>10.00% and <80.00 % of Base Ion	Base	10.00	80.00	38.63	Passed
197	<2.00% of mass 198	198		2.00	0.21	Passed
198	Base Ion	442	50.00		100.00	Passed
199	>5.00% and <9.00% of mass 198	198	5.00	9.00	6.59	Passed
275	>10.00% and <60.00 % of Base Ion	Base	10.00	60.00	13.63	Passed
365	>0.50% of mass 198	198	0.50		0.76	Passed
441	<150.00% of mass 443	443		150.00	102.98	Passed
442	>30.00% of mass 198	198	30.00		45.94	Passed
443	>15.00% and <24.00 % of mass 442	442	15.00	24.00	20.30	Passed

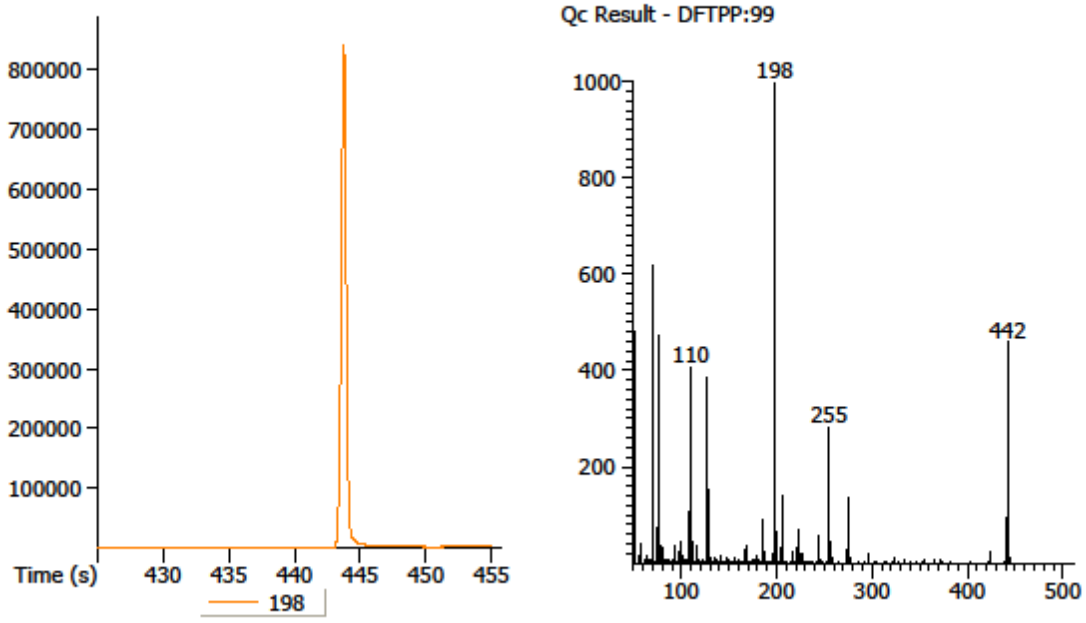

Figure 2. Example of successful DFTPP tune check (printed by LECO's software).

In summary, when choosing GC/TOF-MS separation conditions, several factors were considered including:

1) The method must have the ability to be performed on a basic GC/TOF-MS, without additional investment in equipment such as a GC oven insert.

2) The selected temperature ramp rates must be well within the limits of the GC oven.

3) Selected conditions should provide reasonable chromatographic separation of analytes for sample extracts that contain a large number of matrix interferences.

4) Analyte retention times and peak areas must be reproducible.

5) A minimum of 15–20 mass spectra must be collected across each chromatographic peak.

The final method that was proposed met all of these criteria and provided separation of analytes in just less than 13 minutes (see Section 3.3). Using this LLNL-developed GC/TOF-MS method (1-D separation, while two GC columns are installed in GC/TOF-MS), we estimate that a throughput of 82 analyses per 24 hours could be achieved using a single instrument. During this same time period, only 35 samples could be analyzed using conventional GC/MS (quadrupole), which required an analysis time of approximately 30 minutes. However, should a higher sample throughput be desired, there are several options that could be explored, including:

1) Use a GC oven insert to reduce the oven volume so that the GC column heats more quickly, yielding faster analyte separations. The reduced oven volume also allows faster cooling of the GC, thus reducing the overall cycle time and increasing throughput. This option is currently available only for GC/TOF-MS systems that perform exclusively 1-D separations.

2) Eliminate the late-eluting acenaphthene-$d_{10,}$ chrysene-d_{12} and perylene-d_{12} as internal standards that are currently proposed by the CWA-SAP (1) so that analytical run time would be reduced.

3) Use cryo-cooling to reduce GC cycle time. Experiments in our laboratory (data not shown) have suggested that the use of cryo-cooling can reduce GC cycle times by more than 50%. Note that this strategy is only feasible when a single GC column is being used.

4.2 Instrument detection limits

Instrument detection limits (IDLs) were determined by making successive injections of individual standards of decreasing analyte concentrations until a signal-to-noise ratio (S:N) of approximately 3:1–5:1, as determined manually, was obtained for the analyte peak of the second confirmation ion present in the chromatogram (detection of an analyte required the presence of

the quantitation ion and two qualifying ions). The analyte mass at which a S:N of 3:1–5:1 was obtained for the second qualifying ion of three successive injections was reported as the IDL. Blank samples (i.e., clean solvent) were analyzed before the determination of the final IDLs to ensure that carryover of higher concentrations of analytes did not influence IDL determinations.

As shown in Table 3, GC/TOF-MS IDLs ranged from 0.0025–0.025 ng. These IDLs are reasonably comparable to GC/TOF-MS IDLs observed by Virginia Division of Consolidated Laboratory Services (Richmond, VA) (data unpublished); see Table 4. Some differences in IDLs were noted and might be attributed to differences in the methods used to determine S:N.

Table 3. IDLs, Quantification (Quant) and Qualifying (Qual) Ions, and S:N Determined by GC/TOF-MS

Analyte	LLNL IDL TOF (ng)	Quant. Ion	1st Qual. Ion	2nd Qual. Ion	Avg. S/N (n=3) 2nd Qual. Ion
GB	0.025	99	125	81	4.3
GD1+GD2	0.005	99	126	82	7.7
GF	0.0025	99	67	81	5.3
HD	0.0025	109	111	63	9.8
VX	0.0025	114	72	127	5.4

Note: S:N values for the second qualifying ion were determined by manual integration

Table 4. Comparative Data for IDLs in Nanograms

Analyte	Laboratory			
	VA	LLNL	LLNL	LLNL
	Equipment Tested			
	GC/TOF-MS	GC/TOF-MS	Quadupole GC/MS, FS*	Quadupole GC/MS, SIM*
GB	0.005	0.025	0.2	0.01
GD1+GD2	0.0025	0.005	0.05	0.01
GF	0.005	0.0025	0.2	0.02
HD	0.002	0.0025	0.05	0.01
VX	0.0025	0.0025	0.2	0.05

*Data from Ref 4

Acronyms: **VA**, Virginia Division of Consolidated Laboratory Services; **LLNL**, Lawrence Livermore National Laboratory; **SIM**, selected ion monitoring mode; **FS**, full scan mode

Note: GC/TOF-MS experiments performed with 15 m, 0.18 mm i.d., GC columns and GC/MS experiments performed with 30 m, 0.25 mm i.d., GC columns.

During the course of this work, obvious differences in manual and automated S:N determinations were observed. It is important to understand the differences in the methods used to calculate S:N to ensure that IDLs are determined based on comparable numbers. Manual S:N determinations were calculated for an ion of a preselected mass and were determined based on peak height and baseline noise in the region of the ion chromatogram that immediately preceded the peak of interest. Manual S:N determinations were usually (but not always) lower than those determined using LECO's software. LECO's software provided two automated methods of S:N determinations; both of these methods calculated noise based on the background signal of the *entire* chromatogram. LECO's "Quant S:N" mode calculated S:N for a selected peak based on baseline, peak height, and the standard deviation of the noise of the baseline at a specified mass-to-charge ratio (*m/z*). LECO's "S:N" mode calculated S:N based on the signal for an unique mass and baseline selected by the software's deconvolution software and was used for peak find and peak purity calculations. "S:N" baseline was not necessarily the same baseline as that used for "Quant S:N" calculations, so the two methods of S:N determinations did not always provide equivalent results. To calculate "S:N", LECO's software allowed the selection of an unique ion. In contrast, while using "Quant S:N", the software determined an ion that was both of strong abundance and was not subject to interferences from neighboring peaks.

To provide an illustration of the results produced by different methods of S:N determination, consider the S:N values calculated when 0.025 ng of VX were introduced into the GC/TOF-MS. Manual S:N calculation for *m/z* 127 (2^{nd} qualifying ion) yielded S:N = 136. S:N values of 346 and 353 were determined using the instrument's software when the "S:N" and "Quant S:N" modes, respectively, were used. In this situation, using the software's S:N determination algorithms, and basing IDL on S:N, would produce an IDL that was approximately a factor of two lower than IDL based on the manual S:N determination. For this reason, manual S:N calculations were used to determine IDLs that are reported in Table 3 (i.e., at

IDL, a manually determined S:N of approximately 3:1–5:1 was obtained for the peak of the analyte's second confirmation ion). Manual integration was also chosen so that the method of IDL determination was comparable to that used for quadrupole GC/MS studies (5).

As shown in Table 4, GC/TOF-MS IDLs were lower than those obtained by GC/MS (quadrupole) operated both in full-scan and selected ion monitoring modes. The lower detection limits were attributed to both the different MS detector and the fact that GC/TOF-MS experiments used 0.18 mm i.d. GC columns. When equivalent amounts of analyte were introduced onto the GC column, the 0.18 mm i.d. column of the GC/TOF-MS produced narrower and taller chromatographic peaks than did the 0.25 mm i.d. column of the quadrupole GC/MS. The GC/TOF-MS produced lower IDLs than quadrupole GC/MS and, unlike GC/MS operated in the selected ion monitoring mode (which provides optimum detection limits for GC/MS by detecting only pre-selected ions), provided full mass spectral data, which, as noted in the introduction, offers an additional level of confidence in analyte identification (i.e., the more ions upon which to base analyte identification, the greater the confidence in that identification).

4.3 Calibration

Once IDLs were determined, calibration curves were established in a manner consistent with instructions of the CWA-SAP (1). At the low and high points of the calibration curve (calibration ranges were chosen to reflect expected concentrations in environmental samples), the mass spectra of the analytes of interest were determined and compared with those of the NIST library. Match factors, describing how well generated mass spectra fit to those reported in the NIST library, were reported; see Table 5. At higher concentrations, match factors were good—typically better than 840 out of a possible 1000. At the lowest detectable concentrations, the library matches for GB and GF, respectively, at 905 and 913, were good; however, the goodness-of-fit in spectral data for the other CWAs ranged from 590–650. Thus, at the lower concentrations, confidence in correct analyte assignment based on spectral data alone was less certain. For the CWAs, at 2–3 times the IDL, the match factors were better than 700 and S:N values were greater than or equal to 10 (by manual determination). Although these data represent a best case scenario (i.e., match factors for similar concentrations of CWAs in complex matrices are not expected to be as good), they are useful because they provide some perspective for data evaluation.

Table 5. Average (N=3) Match Factors (Forward Fit), Relative to the NIST Database, Determined for Various Analytes at the High and Low Points of the Calibration Curve

Analyte	Retention Time (sec.)	Curve Point	Forward Fit
GB	107.7	Low – 0.01 ng	905
		High – 0.5 ng	931
GD 1	194.3	Low – 0.0025 ng	663
		High – 0.125 ng	921
GD 2	196.0	Low – 0.0025 ng	655
		High – 0.125 ng	916
GF	248.4	Low – 0.01 ng	913
		High – 0.5 ng	929
HD	242.5	Low – 0.005 ng	650
		High – 0.25 ng	922
VX	353.5	Low – 0.01 ng	592
		High – 0.5 ng	842

Note: calibration ranges were chosen to reflect expected concentrations in environmental samples

Using the calibration data, average relative response factors, percent relative standard deviations, and R^2 values (linear regression) for five calibration levels were determined; see Table 6. Calibration ranges were chosen to reflect the largest possible range available using the ultradilute chemical agent standards. Using practices that were consistent with EPA Method 8000C (12) and the CWA-SAP (1), a procedure for quantifying GB, GD, GF, HD, and VX was implemented using the internal standards of Method 8270D, which are 1,4-dichlorobenzene-d_4, naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, and perylene-d_{12}. Because the percent relative standard deviations for the relative response factor values were not always less than or equal to 20% (based on the guidance required by U.S. EPA's 8000-series methods), all quantitation for the CWAs was based on linear regression. At 2–3 times the IDL, the differences between the expected and measured concentrations for standards (based on calibration curve values) were ≤ 15%.

Table 6. Calibration Data for CWAs: Average Relative Response Factors (RRFs), Percent Relative Standard Deviations (RSDs) for RRFs, and R^2 Values (Linear Regression); Five Calibration Levels

Analyte	Internal Standard	Calibration Range (ng/µL)	Mean RRF	% RSD of RRF	R^2
GB	1,4-Dichlorobenzene, d_4	0.025 - 10	0.976	20.6	0.9994
GD	1,4-Dichlorobenzene, d_4	0.025 - 5	0.428	18.3	0.9999
GF	Napthalene, d_8	0.025 - 10	0.312	24.6	0.9999
HD	Napthalene, d_8	0.025 - 5	0.176	30.4	0.9994
VX	Phenanthrene, d_{10}	0.05 - 10	0.316	34.6	0.9996

Note: Calibration ranges were chosen to reflect the largest possible range available using the ultradilute chemical agent standards.

4.4 Analyses of sample extracts

Selected matrices were spiked with CWAs and surrogates, extracted using procedures described in the CWA-SAP, and analyzed using the GC/TOF-MS. The same sample extracts were also analyzed by quadrupole GC/MS (30 m x 0.25 mm i.d. x 0.25 µm film thickness GC column). In order to provide somewhat similar S:N ratios for selected peaks analyzed by both GC/MS and GC/TOF-MS, all GC/MS analyses of sample extracts were performed using the SIM mode. Extracts of laboratory reagent water, surface water, clean sand, three soil types, and wipes were extracted and analyzed by both GC/TOF-MS and GC/MS to produce comparative data.

To provide a simple comparison of analyte concentrations measured by GC/TOF-MS and GC/MS, a plot of analyte concentrations (CWAs and surrogates) measured by GC/TOF-MS versus analyte concentrations measured by GC/MS (SIM mode) was generated for the data collected from three control samples analyzed during the course of our study. Each of these control samples contained five CWAs and five surrogate compounds. In plotting these concentration data, the population variances of measurements by GC/TOF-MS and by GC/MS were assumed to be equal. We also assumed, to a first approximation, that all analytes behaved similarly in their ability to be detected by GC/MS and GC/TOF-MS. Thus, all analytes were plotted on the same graph. Control samples consisted of dichloromethane that was spiked directly with CWAs and surrogates, and represented the cleanest possible samples (i.e., samples with no interfering compounds introduced from the sample matrix). The specific CWAs and surrogates in the samples have previously been described in Sections 2.0 and 3.1 of this report. The resulting plot is shown Figure 3.

Figure 3 shows a comparison of CWA and surrogate concentrations in the three control samples measured by GC/MS-TOF and GC/MS (30 m x 0.25 mm i.d. x 0.25 µm GC column). Each point represents a measured concentration of CWA or surrogate in the sample extract. In this sample set, CWAs were spiked at 0.5 ng/µL (GD isomers were measured individually at approximately 0.25 ng/µL) and surrogates were spiked at 1 ng/µL. All GC/MS data were collected in SIM mode.

A regression line was calculated for the data displayed in Figure 3; its R^2 value was 0.82,

its slope was 0.82 (with a standard error of 0.068), and its intercept was 0.068 (with a standard error of 0.048). The data show a statistically significant correlation of concentration measured by GC/MS and GC/TOF-MS (p-value << 0.001). On average, concentrations measured by GC/MS and GC/TOF-MS agreed within 16%. However, there were two apparent outliers. Two of the concentrations measured for PCP-d_5 by GC/MS were noticeably lower than the expected 1 ng/µL. The reason for observation of these lower concentrations is unclear.

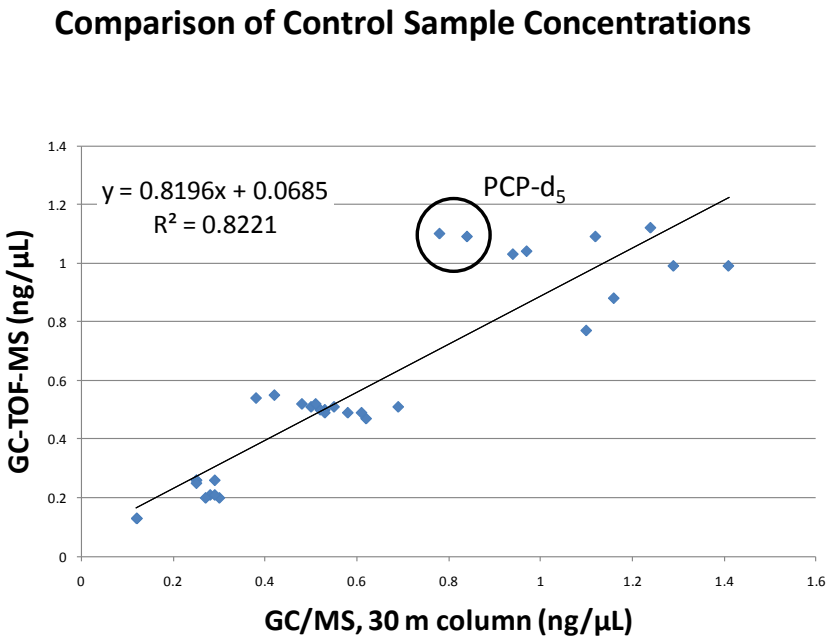

Figure 3. Comparison of CWA and surrogate concentrations in three control samples measured by GC/MS-TOF and GC/MS.

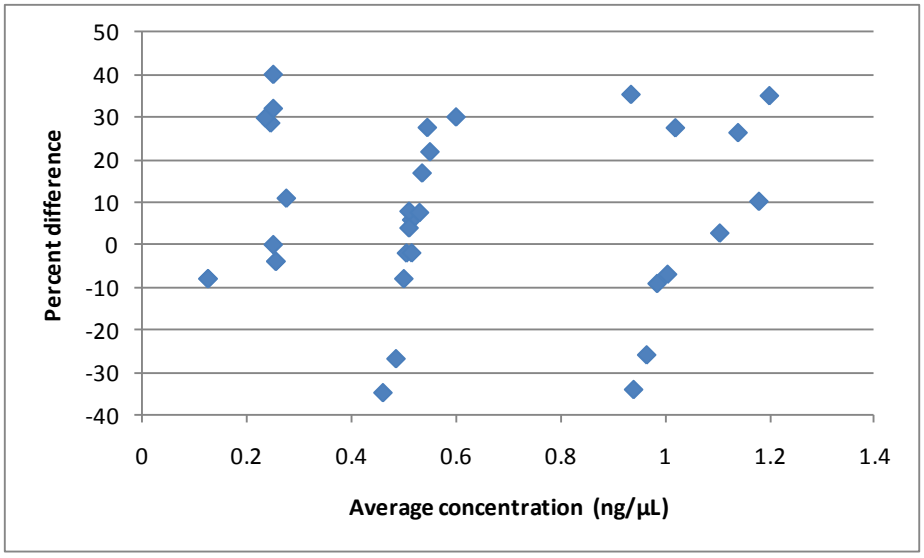

Figure 4. Percent differences between the GC/TOF-MS and GC/MS concentrations in control samples as a function of average concentration.

Because the slope of the regression line of Figure 3 suggested that slightly lower analyte concentrations were measured by GC/TOF-MS (a slope of exactly 1 would be observed if concentrations measured by the different instruments were equivalent), we plotted the percent differences in the GC/TOF-MS and GC/MS concentrations as a function of average concentration to study the bias; see Figure 4. Figure 4 shows the percent differences between average concentrations measured by GC/TOF-MS and GC/MS (percent difference = 100*(GC/MS conc – GC TOF-MS conc) / [(GC/MS conc + GC-TOF-MS conc)/2]). Visual inspection of the plot shown in Figure 4 suggests that no clear bias in the measured concentrations; however, there is considerable scatter in the data.

4.4.1 Analyses of water sample extracts

Reagent water was spiked with CWA and surrogates, extracted, and analyzed by GC/TOF-MS and GC/MS (SIM mode). Figure 5 provides a plot of analyte concentrations in sample extracts measured by GC/MS (30 m x 0.25 mm i.d. x 0.25 µm film thickness GC column) versus extract concentrations measured by GC/TOF-MS, generated from the data collected from seven replicate samples that were spiked, extracted, and analyzed. Each point represents a measured concentration of CWA or surrogate in the sample extract. In this sample set, CWAs, assuming 100% recovery, would be present at approximately 0.25 ng/µL (GD isomers were measured individually at approximately 0.14 ng/µL and VX concentrations were high at ~0.5 ng/µL) and surrogates would be present at approximately 1 ng/µL. All GC/MS data were collected in SIM mode.

Using the data in Figure 5, a regression line was calculated and its R^2 value was 0.92, its slope was 0.86 (with a standard error of 0.030), and its intercept was 0.059 (with a standard error of 0.027). The data show a statistically significant correlation of concentration measured by GC/MS and GC/TOF-MS (p-value << 0.001). On average, concentrations measured by GC/MS and GC/TOF-MS agreed within 16%.

As in the analysis of the previous data, the percent differences in the GC/TOF-MS and GC/MS concentrations were plotted as a function of average concentration to study the bias (percent difference = 100*(GC/MS conc – GC/TOF-MS conc) / [(GC/MS conc + GC/TOF-MS conc)/2]). Visual inspection of the plot shown in Figure 6 suggests that there was bias in the measured concentrations. Concentrations measured by GC/MS were slightly higher than those measured by GC/TOF-MS, as was evident from the greater number of data points that reside above the zero line of the y-axis. When plotting these data, several outliers became apparent. Two measured GB concentrations were 50% lower when measured by GC/TOF-MS and two concentration measurements of TPP were higher when measured by GC/MS.

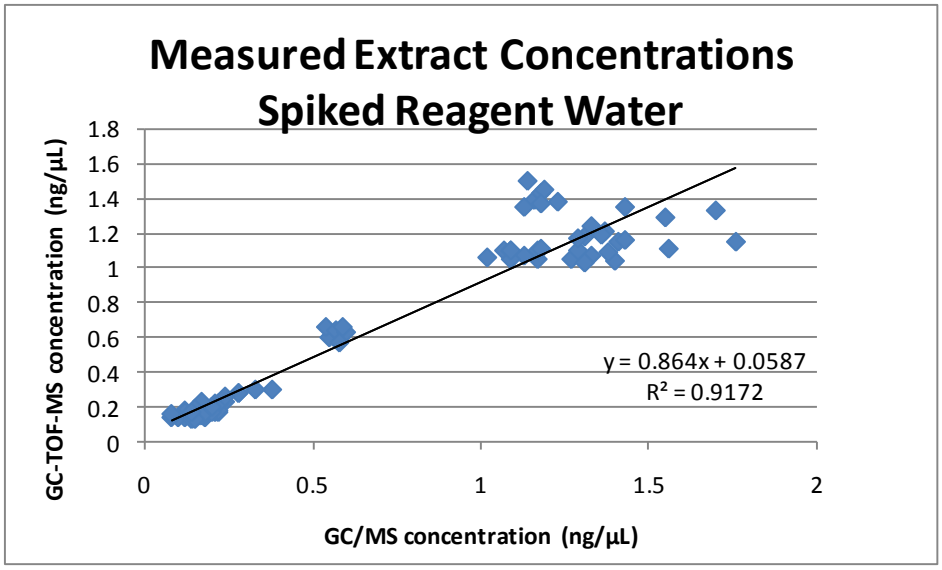

Figure 5. CWA and surrogate concentrations, in seven reagent water extracts, measured by GC/MS-TOF and GC/MS.

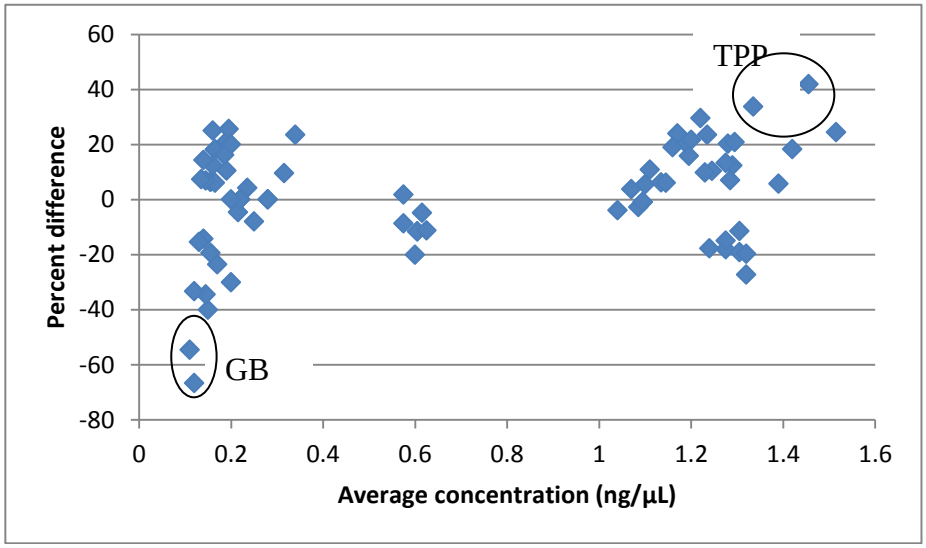

Figure 6. Percent differences between the GC/TOF-MS and GC/MS concentrations measured in reagent water extracts as a function of average concentration.

Table 7 shows the analyte concentrations in reagent water determined with the GC/TOF-MS and also comparative data generated by analyzing the same sample extracts by quadrupole GC/MS (SIM mode), operated with both a 30 m x 0.25 mm i.d. x 0.25 µm film thickness GC column (GC/MS, conventional) and a 15 m x 0.18 mm i.d. x 0.18 µm film

thickness GC column (GC/MS, fast). Average (n=7) concentrations of CWAs and surrogates (i.e., NB-d5, FBP, PCP-d5, Ter-dl4, and TPP) were measured in spiked (14 µg/L of each CWA and 56 µg/L each of surrogates) reagent water. Final sample extracts (total volume 2 mL) were spiked with 0.5 µg/mL of each internal standard. Examining these data another way, average percent differences in analyte concentrations were calculated; see Table 8. (Percent difference calculated as follows: 100*(GC/MS conc - GC/TOF-MS conc) / [(GC/MS conc) / [(GC/MS conc + GC/TOF-MS conc)/2]. Paired t-tests were performed to determine ($p<0.01$) if differences between concentrations were statistically significant.) On average, matrix-based concentrations measured by GC/TOF-MS differed by 21% from conventional GC/MS and by 9% from fast GC/MS. Paired t-tests were performed to test if significant differences were noted between the average concentrations measured by each detection system. We performed paired t-tests on the log-transformed, matrix-based concentrations. Statistical analyses were performed on the logarithms of the measured concentrations because variability of concentration units increases with concentration, whereas variability in terms of percent change tends to be more stable as a function of concentration. As shown in Table 8, some of the differences were statistically significant, even when the average measured concentrations were less than ±20%. It is generally accepted in the environmental community that, for low measured concentrations, numbers within ±20% are considered to be in reasonably good agreement.

Table 7. Average (n=7) Concentrations (Ave Conc) with Standard Deviations (Std Dev) and Percent Recoveries (Rec) of CWAs (14 µg/L) and Surrogates (56 µg/L) Using GC/MS Configurations

	GC/TOF-MS			GC/MS, conventional			GC/MS, fast		
Analyte	**Ave Conc (ng/µL)**	**Std Dev Conc (ng/µL)**	**Rec (%)**	**Ave Conc (ng/µL)**	**Std Dev Conc (ng/µL)**	**Rec (%)**	**Ave Conc (ng/µL)**	**Std Dev Conc (ng/µL)**	**Rec (%)**
GB	9.0	0.6	64	6.3	2.1	45	6.2	0.4	44
GD1	9.8	0.9	140	5.8	1.7	83	10	1.5	147
GD2	8.4	0.5	120	4.8	1.0	68	8.6	1.2	122
HD	15	3.5	106	15	3.7	109	14	4.2	101
GF	12	1.1	86	12	5.3	82	11	1.4	78
VX	37	5.2	262	33	1.7	238	35	5.6	247
NB-d5	63	2.4	113	66	2.3	118	68	4.0	122
FBP	63	1.2	113	54	3.0	97	60	2.4	107
PCP-d5	82	4.4	147	58	2.7	104	75	6.8	134
Ter-d14	73	5.6	130	72	11	128	69	4.8	124
TPP	65	5.4	116	72	16	127	72	7.2	129

Redone 2/15/2011

Table 8. Average Percent Differences in Analyte Concentrations in Reagent Water Using GC/MS Configurations

	Percent Difference Between Matrix-Based Concentrations Determined by Conventional GC/MS and GC/TOF-MS	Differences Between Conventional GC/MS and GC/TOF-MS statistically significant ($p<0.01$)?	Percent Difference* Between Matrix-Based Concentrations Determined by Fast GC/MS and GC/TOF-MS	Differences Between Fast GC/MS and GC/TOF-MS statistically significant ($p<0.01$)?
GB	-35	Yes	-36	Yes
GD1	-51	Yes	2	Yes
GD2	-54	Yes	2	No
HD	3	No	-6	No
GF	-5	No	-10	No
VX	-11	Yes	-6	No
NB-d_5	5	Yes	8	Yes
FBP	-15	Yes	-5	No
PCP-d_5	34	Yes	-9	No
Ter-d_{14}	-1.3	Yes	-5	No
TPP	10	Yes	10	Yes
Average Absolute Value of Difference	**21**		**9**	

* Percent difference calculated as follows: 100*(GC/MS conc - GC/TOF-MS conc) / [(GC/MS conc) / [(GC/MS conc + GC/TOF-MS conc)/2]. Paired t-tests were performed to determine ($p<0.01$) if differences between concentrations were statistically significant.

We also extracted and compared concentrations of CWAs and surrogates (28 µg/L of each CWA and 56 µg/L of each surrogate) measured in surface water. Figure 7 provides a plot of analyte concentrations in sample extracts measured by GC/TOF-MS versus concentrations measured by GC/MS (30 m x 0.25 mm i.d. x 0.25 µm film thickness GC column), generated from the data collected from three replicate samples that were spiked, extracted, and analyzed. Each point represents a measured concentration of CWA or surrogate in the sample extract. In this sample set, CWAs were spiked so that concentrations, at 100% recovery, would be 0.5 ng/µL for CWAs (except for each GD isomer, which was expected to be 0.25 ng/µL) and 1 ng/µL for surrogates. All GC/MS data were collected in SIM mode. A regression line was calculated and its R^2 value was 0.94, its slope was 0.94 (with a standard error of 0.042), and its intercept was 0.031 (with a standard error of 0.036). The data show a statistically significant correlation of concentration measured by GC/MS and GC/TOF-MS (p-value << 0.001). On average, concentrations measured by GC/MS and GC/TOF-MS agreed within 9%.

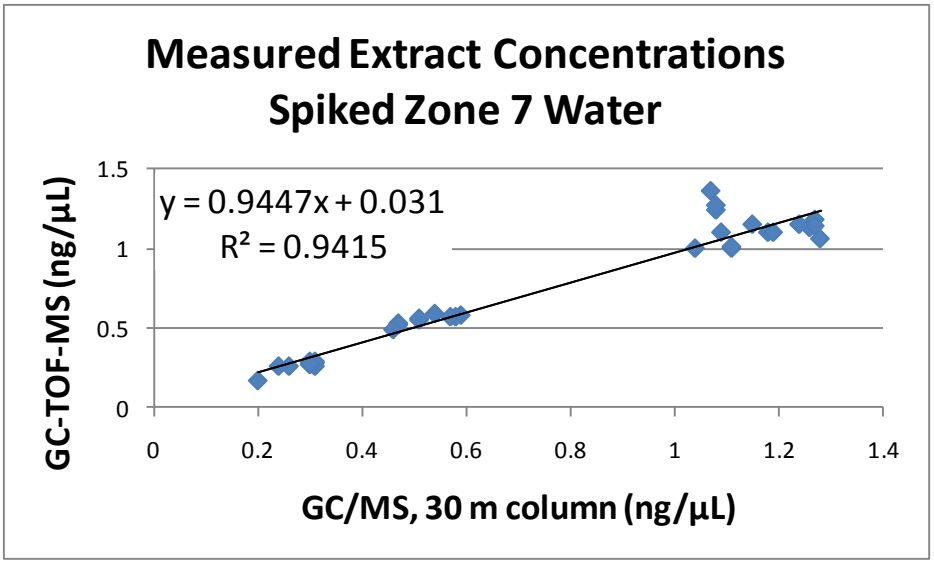

Figure 7. Comparison of CWA and surrogate concentrations, in three surface water extracts, measured by GC/MS-TOF and GC/MS.

We also plotted the percent differences (as previously defined) in the GC/TOF-MS and conventional GC/MS concentrations as a function of average concentration to study the bias. Visual inspection of the plot shown in Figure 8 suggests that there may be some bias in the data, as concentrations measured by GC/MS appear, by visual inspection, slightly higher than those measured by GC/TOF-MS. This behavior is consistent with the behavior observed for reagent water (see Figure 6).

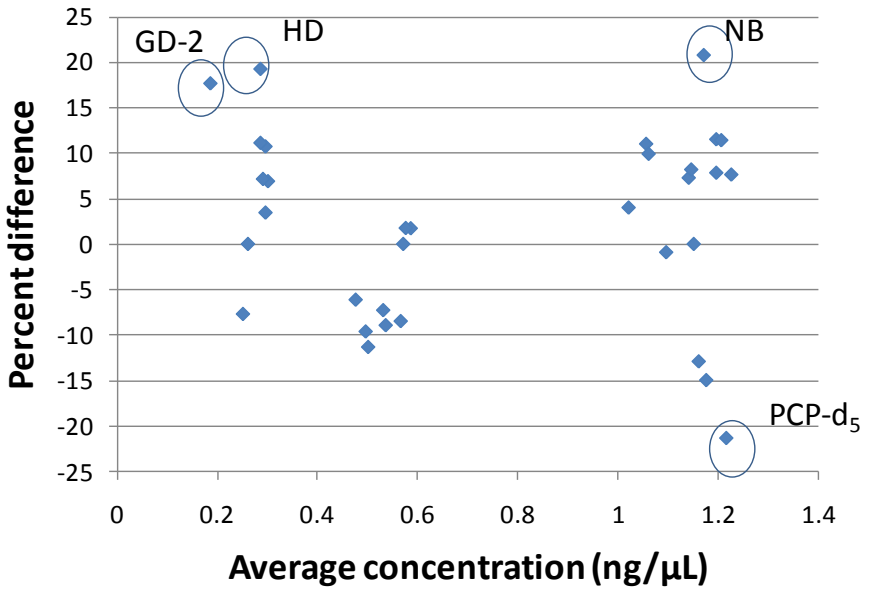

Figure 8. Percent differences between the GC/TOF-MS and conventional GC/MS (SIM mode) concentrations measured in reagent water extracts as a function of average concentration.

Detailed comparative data for these water samples are shown in Table 9. The CWA were measured in Zone 7 surface water spiked with with 28 µg/L of each CWA and 56 µg/L each of surrogate. The final sample extracts (total volume 2 mL) were spiked with 0.5 ng/µL of each internal standard. Data represent the average concentration (Conc), standard deviation in concentration (Std Dev Conc), and percent recovery (Rec) for three replicate samples that were spiked, extracted, and analyzed by GC/TOF-MS or GC/MS. Percent differences (Diff) between GC/TOF-MS and conventional GC/MS, SIM mode, and GC/TOF-MS and fast GC/MS, SIM mode, are provided.Concentrations of CWAs and surrogates were in good agreement. On average, concentrations in sample extracts measured by GC/MS and GC/TOF-MS agreed within 4%. Paired t-tests on the log-transformed, matrix-based concentrations were performed as previously described. For all analytes, with the exceptions of VX and TPP, concentrations determined by GC/TOF-MS and conventional GC/MS were statistically equivalent. Concentrations determined by GC/TOF-MS and fast GC/MS for all analytes were statistically equivalent.

Table 9. Average (n=3) Matrix-based Concentrations of CWAs and Surrogates from Spiked Surface Water Using GC/MS Configurations

	GC-TOF-MS					GC/MS, Conventional			GC/MS, Fast		
	Conc (µg/L)	Std Dev Conc (µg/L)	Rec (%)	% Diff from GC/MS, conventional [a]	% Diff from GC/MS, fast [b]	Conc (µg/L)	Std Dev Conc (µg/L)	Rec (%)	Conc (µg/L)	Std Dev Conc (µg/L)	Rec (%)
GB	29	1.2	105	-4	-4	27	0.3	95	27	0.3	97
GD1	16	0.3	117	6	3	18	0.3	125	17	0.6	118
GD2	15	0.6	110	6	0	17	0.3	124	15	0.6	110
HD	13	3.0	47	0	4	13	1.7	48	14	1.7	49
GF	33	0.3	117	0	3	33	0.6	118	35	0.3	125
VX	32	1.2	116	-3[a]	3	30	3.1	106	34	0.9	121
NB-d_5	64	2.8	112	6	2	72	1.2	126	66	1.5	115
FBP	62	4.4	108	0	-1	62	3.1	109	61	3.0	107
PCP-d_5	74	3.6	129	5	3	62	0.3	108	76	3.8	134
Ter-d_{14}	63	1.0	111	4	8	69	2.5	121	67	1.0	118
TPP	61	5.8	116	-4[a]	-4	66	5.3	116	71	2.9	124
Average Absolute Value of Difference				**4**	**3**						

Acronyms: **Conc**, average concentration; **Diff**, Percent differences **Rec**, percent recovery; **Std Dev Conc**, standard deviation in concentration

Notes: [a] Paired t-tests indicated that only concentrations for VX and TPP determined by the two techniques were statistically different ($P<0.01$).

[b] Paired t-tests indicated that the differences in concentrations, for all analytes, determined by the two techniques were not statistically significant ($p<0.01$).

4.4.2 Analyses of soil extracts

Sand and soils were spiked with CWA and surrogates, extracted, and analyzed by GC/TOF-MS and GC/MS. Table 10 provides data for analyte concentrations measured in sand by fast GC/MS (15 m x 0.18 mm i.d. x 0.18 µm film thickness GC column) and GC/TOF-MS. No conventional GC/MS data are presented in Table 10 because subsequent review of the data showed that the CCVs (continuing calibration verifications) were outside the acceptable range and, for this reason, all previously-collected data were rejected. Because fast GC/MS data were acceptable, these data were compared to those generated by GC/TOF-MS. Table 10 shows that some concentration values measured for sand with GC/TOF-MS agreed well with those measured by fast GC/MS and some did not. Paired t-tests on the log-transformed matrix-based concentrations were performed, as previously described. For all analytes, with the exceptions of

FBP and PCP-d_5, concentrations determined by GC/TOF-MS and conventional GC/MS were statistically different ($p<<0.01$). The measurement of VX was especially problematic. Data collected by analyses of different soil types, presented in Table 11, also showed this trend. Table 11 presents comparative data generated for 250 ng of each agent and 500 ng of each surrogate spiked on and extracted from 10 g of various soils (1 µg/mL internal standard in sample extract). Soil samples used included Virginia (VA) soil, Nebraska Aglands Ap (NeAp) soil, and Georgia Bt2 (GaBt) soil. All GC/MS analyses were performed in SIM mode. Our experience suggests that VX quantification is complicated by both co-eluting interferences and matrix enhancement effects; we plan to study this problem in greater detail in a subsequent study.

Table 10. Average (n=7) Water Concentrations (Ave Conc) with Standard Deviations (Std Dev) and Percent Recoveries (Rec) of CWAs (500 ng) and Surrogates (1000 ng) From Spiked Sand Using GC/MS Configurations in SIM Mode

	GC/TOF-MS			GC/MS, fast			% Diff [b]
Analyte	Conc. on sand (µg/kg)	Std Dev Conc. (µg/kg)	Rec (%)	Conc. on sand (µg/kg)	Std Dev Conc. (µg/kg)	Rec (%)	
GB	35	5	69	0	0	0	N/A
GD1	9	1	36	19	2	78	71
GD2	9	1	36	20	3	78	75
HD	33	3	65	42	5	84	24
GF	36	4	71	41	5	83	13
VX[a]	14	1	29	31	4	62	76
NB-d_5	35	4	35	71	9	71	70
FBP	81	10	81	79	9	79	-3
PCP-d_5 [a]	28	5	28	34	3	34	19
Ter-d_{14}	78	7	78	88	11	88	12
TPP	77	7	77	94	11	94	-20

[a] Note that concentrations for VX and PCP-d_5 represent the sum of these analytes in the first and second solvent extracts of sand, per the CWA-SAP.

[b] Percent difference is calculated as follows:
100*(GC/MS conc – GC/TOF-MS conc) / [(GC/TOF-MS conc + GC/MS conc)/2].
For all analytes, with the exceptions of FBP and PCP-d_5, concentrations determined by GC-TOF-MS and fast GC/MS were statistically different ($P<<0.01$).

Table 11. **Average (n=3) Soil Concentration (Conc) With Standard Deviation (Std Dev) and percent recoveries (rec) for CWA (250 ng) and Surrogate (500 ng) From Spiked Soils Using GC/MS Configurations in SIM mode.**

	VA soil, GC/TOF-MS			VA soil, conventional GC/MS			
	Soil Conc (µg/kg)	Soil Conc Std Dev (µg/kg)	Rec (%)	Soil Conc (µg/kg)	Soil Conc Std Dev (µg/kg)	Rec (%)	% Diff from GC/MS[b]
GB	3	0.6	11	18	0.58	73	143[c]
GD1	8	0.0	67	9	1	75	12
GD2	9	1.2	78	13	1.15	111	36
HD	19	0.6	78	13	0.58	53	-38 [c]
GF	28	1.2	115	27	1	108	-4
VX[a]	25	2.6	99	41	5	172	48[c]
NB-d_5	23	1.0	48	39	3.06	77	52 [c]
FBP	28	1.2	58	30	1.53	59	7
PCP-d_5[a]	108	4.4	217	56	4.2	111	-63[c]
Ter-d_{14}	91	9.3	190	39	1.53	79	-80[c]
TPP	164	9.1	342	51	1.53	103	-105[c]
	NeAp, GC-TOF-MS			**NeAp, GC/MS**			
	Soil Conc (µg/kg)	Soil Conc Std Dev (µg/kg)	Rec (%)	Soil Conc (µg/kg)	Soil Conc Std Dev (µg/kg)	Rec (%)	% Diff from GC/MS[b]
GB	3	1.5	14	15	0.58	61	133
GD1	8	0.0	67	10	0.58	81	22
GD2	8	0.6	69	10	0.58	81	22
HD	17	1.0	71	11	0.58	43	-43[c]
GF	26	1.0	108	23	0.58	91	-12
VX[a]	29	5.0	119	0	0	0[d]	N/A
NB-d_5	25	0.6	53	40	2.65	80	46[c]
FBP	31	1.2	65	33	1	66	6
PCP-d_5[a]	12	4.2	26	12	3.6	24	0
Ter-d_{14}	99	4.0	206	43	1.53	85	-79[c]
TPP	159	5.2	331	57	4.93	115	-94[c]

Table 11, continued.

	GaBt, GC-TOF-MS			GaBt, conventional GC/MS			
	Soil Conc (µg/kg)	**Soil Conc Std Dev (µg/kg)**	**Rec (%)**	**Soil Conc (µg/kg)**	**Soil Conc Std Dev (µg/kg)**	**Rec (%)**	**% Diff from GC/MS[b]**
GB	2	0.0	8	0	0	0	N/A
GD1	2	0.0	17	0	0	0	N/A
GD2	2	0.0	17	0	0	0	N/A
HD	13	5.8	56	8	0.05	31	-48
GF	5	0.6	19	0	0	0	N/A
VX[a]	7	0.6	31	26	2	103	115 [c]
NB-d_5	19	1.0	40	25	0.01	50	27
FBP	24	1.7	50	25	0.02	50	4 [c]
PCP-d_5[a]	49	0.6	101	46	4	93	-6 [c]
Ter-d_{14}	77	4.6	160	36	0.01	72	-73[c]
TPP	106	5.0	221	43	0.03	87	-85

[a] Note that concentrations for VX and PCP-d_5 represent the sum of these analytes in the first and second solvent extracts of sand, per the CWA-SAP.

[b] Percent difference is calculated as follows:
100*(GC/MS conc – GC/TOF-MS conc) / [(GC/MS conc + GC/TOF-MS conc)/2].

[c] Paired t-tests on the log-transformed, matrix-based concentrations were performed as previously described, and concentrations determined by GC/TOF-MS and conventional GC/MS were determined to be statistically different ($p<0.01$).

[d] GC/MS peaks for VX yielded S:N values less than 3:1, and therefore VX recovery was reported as "0".

4.4.3 Analyses of wipes

Wipes were also spiked directly, extracted, and analyzed by GC/TOF-MS and by GC/MS with a conventional GC column; see Table 12. In many cases, agreement was within 30%. However, some anomalies were noted. By GC/MS, interferences or matrix effects appeared to be more pronounced for GF and many of the surrogates. TPP, with a recovery of 647% by conventional GC/MS, was most problematic. Such high recoveries were not observed with the GC/TOF-MS.

Table 12. Average (n=7) Mass per Wipe, With Standard Deviation (Std Dev), and Percent Recoveries (Rec) For CWAs (250 ng) And Surrogates (500 ng) From Spiked Wipes Using GC/TOF-MS and GC/MS (SIM Mode)

	GC/TOF-MS			GC/MS, Conventional			
	Mass per Wipe (ng)	**Std Dev Mass (ng)**	**Rec (%)**	**Mass per Wipe (ng)**	**Std Dev Mass (ng)**	**Rec (%)**	**% Diff in Mass from GC/MS[a]**
GB	310	10	122	250	7	100	-21[b]
GD1	160	20	125	184	13	147	14[b]
GD2	180	10	145	188	7	151	4
GF	290	30	114	523	36	209	57[b]
HD	160	10	63	178	7	71	11[b]
VX	220	10	89	161	11	64	-31[b]
NB-d_5	410	10	83	463	18	93	12[b]
FBP	350	20	69	361	16	72	3[b]
PCP-d_5	350	10	71	103	12	20	-109[b]
Ter-d_{14}	780	90	157	707	24	141	-10
TPP	720	100	145	1617	64	323	77[b]

[a] Percent difference is calculated as follows:
100*(GC/MS conc – GC/TOF-MS conc) / [(GC/MS conc + GC/TOF-MS conc)/2].

[b] Paired t-tests on the log-transformed, matrix-based concentrations were performed as previously described, and concentrations determined by GC-TOF-MS and conventional GC/MS were determined to be statistically different ($p<0.01$).

5.0 Conclusions and Recommendations

The GC/TOF-MS appears to be a good alternative to quadrupole GC/MS. Both instruments have comparable costs. The GC/TOF-MS (LECO's Pegasus® 4) provides lower instrument limits of detection than GC/MS (Agilent 5973), while still retaining full mass spectral data. The retention of full mass spectral data is expected to be advantageous in assisting in the process of confirming an analyte's identification. In order for the GC/MS to obtain the relatively low detection levels used in this study, the GC/MS was operated in the selected ion monitoring (SIM) mode. Several EPA analysts have expressed concern during teleconferences that they are not confident using SIM analyses to provide accurate identifications and quantitations and that they favor the collection of full-scan data. Thus, the GC/TOF-MS would be a desirable detector for this group. The reproducibility of GC/TOF-MS appears comparable to GC/MS. Fast separations with the GC/TOF-MS offer increased analytical speeds and the promise of higher sample throughput; we observed that GC/TOF-MS was two- to three-times faster than conventional GC/MS.

Both GC/TOF-MS and GC/MS analyses must be performed by educated operators. Because GC/MS is most widely used in environmental analyses, many analysts already have the required knowledge to successfully implement GC/MS methods for the detection of organic compounds. Currently, there are not as many analysts that are familiar with the proper use of GC/TOF-MS systems. However, given sufficient training, analysts with the skills needed to successfully perform GC/MS analyses will be able to perform GC/TOF-MS analyses. One of the areas most critical to the correct implementation of GC/TOF-MS methods is data analysis. Because data analysis packages are not as well-developed for the GC/TOF-MS as for the GC/MS systems, care must be taken with data interpretation. While not a topic of discussion in this report, we have found that the deconvolution algorithms available for use with the GC/TOF-MS are useful to assign the proper spectra and correct signal intensity to coeluting peaks and provide quick, reliable quantification of the analyte in the presence of coeluting species. In addition, attention needs to be paid to method setup. For example, a GC/TOF-MS operator must ensure that 20 data points are generated across each chromatographic peak in order to adequately define the peak and to obtain reproducible data. While the need to have an adequate number of data points to define a peak is also critical to proper GC/MS analysis, given the maturity of GC/MS software, such requirements are often forgotten as analysts blindly use default instrument method values. Once an analyst becomes familiar with GC/TOF-MS operation and data analysis, we expect that GC/TOF-MS will be a useful tool which can be used to perform analyses in support of EPA missions.

Specific recommendations that we have for using GC/TOF-MS for future work include:

1) Because of the lower detection limits of GC/TOF-MS and the lower capacity of the narrow-bore GC columns used for fast GC analyses, we recommend reducing the amount of DFTPP required for instrument performance checks from 50 ng to 15 ng (or lower).

2) We recommend that the MS performance criteria for DFTPP listed in the

CWA-SAP be changed to those of EPA Method 527 to provide the users of the CWA-SAP the flexibility to use either a quadrupole GC/MS or GC/TOF-MS for analyses.

3) Criteria for CCVs requiring that measured concentrations fall within ± 25% of their expected values can easily be met using GC/TOF-MS.

4) EPA would benefit from further exploration of GC/TOF-MS for routine analyses.

5) EPA would benefit from additional experiments with fast GC/MS.

6.0 References

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4. Schumacher, B., Characterization and Monitoring Branch, ESD, NERL, US EPA, Las Vegas, NV, personal communication, December 18, 2006.

5. *Year One Report for Lawrence Livermore National Laboratory's Verification of Standard Analytical Protocol for Extractable Semivolatile Organic Compounds For EPA under IAG #DW89922616-01-0*, Heather Mulcahy, Roald Leif, and Carolyn Koester, LLNL-TR-412124, Revision 2, July 13, 2009.

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Environmental Protection Agency. EPA 815-R-05-005

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12. *Method 8000C: Determinative Chromatographic Separations, Rev. 3*, March 2003, U.S. Environmental Protection Agency.

13. *Proposed ERLN Approach for Determining Laboratory Detection and Quantitation Limits for Draft Standardized Analytical Protocols*, January 15, 2009.

Appendix A: Autosampler Method for LECO GC/TOF-MS

Select auto sampler type:

- (•) Agilent©
- ( ) Rail System (CTC, Gerstel, LEAP)
- ( ) Shimadzu©

- [x] 7890, 6890N or 6890 with nanoliter adapter enabled
- [ ] Enable overlapped operation when connected to an Agilent 7890 GC

Syringe Size (µL):

10

Sample Volume (µL):

1

Number of Sample Pumps (0-15):

2

Viscosity Delay (0-7 sec):

0

Sample Pre-Wash (0-15):

1

Solvent A Pre-Wash (0-15):

2

Solvent B Pre-Wash (0-15):

2

Pre-Injection Delay (0.00-1.00 min):

0

Post-Injection Delay (0.00-1.00 min):

0

Solvent A Post-Wash (0-15):

3

Solvent B Post-Wash (0-15):

3

Slow plunger enable:

( ) Yes (•) No

Sample skim enable:

( ) Yes (•) No

Appendix B: GC Method for LECO GC/TOF-MS, setup for 2-D instrument

This was the method that was used by LLNL for all 1-D experimental work.

Hardware control:

○ Agilent© 7890	Agilent© 7890 Gas Chromatograph
◉ Agilent© 6890	Agilent© 6890 Gas Chromatograph
○ Shimadzu© GC-2010	Shimadzu© GC-2010
○ Generic	Generic Gas Chromatograph
○ Direct Inlet	Direct Inlet to Calibration Compound

Option:

☐ MACH/LTM Oven

☑ LECO© GCxGC

Capillary Configuration:

No problems detected with column configuration.

Flow Path 1:

#	Type	Location	Length(m)	Int. Diameter(µ	Max Temp	Film Thickness	Phase	Bleed Masses
1*	Inlet	Back						
2	Capillary	GC Oven	15.000	250.00	350.0	0.25	HP5MS	73 149 207 281
3	Capillary	Modulator	0.100	100.00	325.0	0.10	Rxi-17	73 149 207 281
4	Capillary	Secondary	1.000	100.00	325.0	0.10	Rxi-17	73 149 207 281
5	Capillary	Detector o	0.210	100.00	325.0	0.10	Rxi-17	73 149 207 281
6	Detector	TOF						

Add | Delete | Promote | Demote | Copy | Paste

☐ Enable Flow Path 2

Mass Selection for Auto Mass Defect Tracking

◉ Excluded Masses in Auto Mass Defect Mode

(Set Auto Mass Defect Mode in MS method. Select masses between 130 to 384 inclusive.)

(For general unknown analyses. Select column bleed, matrix, interfering, and non-target masses.)

○ Included Masses in Auto Mass Defect Mode

(Generally for target analyses. Select significant masses of target analytes, minimum of 2 masses required.)

Carrier Gas:

Appendix B: GC Method for LECO GC/TOF-MS, 2-D instrument, continued.

Carrier Gas:

Helium

Back Inlet Type:

Split / Splitless

Back Inlet Mode:

Pulsed Splitless

Active Inlet Location:

○ Front ◉ Back

The active inlet must be present in the capillary configuration.

No problems detected with pressure / flow.

☑ Corrected constant flow via pressure ramps

Use this mode when in GCxGC mode or using short (< 5 m) single column or two columns.

Column 2 / Back Inlet flow(s):

#	Rate (mL/min²)	Target Flow (mL/min)	Duration (min)
1*	Initial	1.20	Entire Run

Column 2 / Back Inlet Purge Time (sec):

35

The time, after the beginning of the run, when the purge valve will open.

Column 2 / Back Inlet Purge Flow (mL/min):

30

The flow from the purge vent. This value cannot be used if your column is not defined

Column 2 / Back Inlet Total Flow (mL/min):

31.2

This is the actual flow to the inlet during a Pre-Run and during a run before purge time.

Column 2 / Back Inlet Pulse Pressure (psi):

40

Column 2 / Back Inlet Pulse Duration (minutes):

0.5

Appendix B: GC Method for LECO GC/TOF-MS, 2-D instrument, continued.

Column 2 / Back Inlet Gas Saver

◉ Yes ○ No

Column 2 / Back Inlet Gas Saver Flow (mL/min)

20

Column 2 / Back Inlet Gas Saver Time (minutes):

1

Back Inlet temperature(s):

#	Rate (°C/min)	Target Temp (°C)	Duration (min)
1*	Initial	250.00	Entire Run

No problems detected with oven temperatures.

Oven Equilibration Time (minutes):

0.5

Note: All oven temperature ramps (except the secondary oven) will have the same duration. This is accomplished by extending the final hold time.

Enter oven temperature ramp below:

#	Rate (°C/min)	Target Temp (°C)	Duration (min)
1	Initial	55.00	0.50
2*	20.00	100.00	0.00
3	40.00	280.00	2.75

Add

Remove

Coolant to Column Oven ○ On ◉ Off Coolant timeout (min) 12

☑ Enable Secondary Oven

#	Rate (°C/min)	Target Temp (°C)	Duration (min)
1	Initial	70.00	0.50
2	20.00	115.00	0.00
3*	40.00	295.00	2.75

Add

Remove

Appendix C: GC Method for LECO GC/TOF-MS, setup for 1-D instrument

LLNL's GC/TOF-MS system is capable of performing 2-D GC separations, in which two different GC columns are used to achieve separation of analytes. Thus, in configuring methods (such as the GC method shown in Appendix B), we were required to consider the installation of two GC columns. However, several of the EPA laboratories do not have GC/TOF-MS systems that offer the ability to perform 2-D separations. In order to assist these laboratories in their implementation of GC/TOF-MS protocols for the separation and detection of CWAs, we also performed several analyses with a single GC column so that we could recommend GC operating conditions.

A summary of the recommended, and LLNL-tested, operating procedures for GC/TOF-MS used in 1-D mode (i.e., when only one column is installed) are listed below. Detailed screen shots of the set-up of the GC/TOF-MS software are presented at the end of this appendix (note: this information was shared with EPA's GC/TOF-MS working group by Heather Mulcahy on February 8, 2010).

Injection size:	1 µL
Injection type:	pulsed-splitless
Pulse pressure:	40 psi for 0.5 min
Purge time:	35 sec at 30 mL/min
Carrier gas:	He with constant flow of 1.2 mL/min
GC injection port:	250 ºC
GC column:	20 m x 0.18 mm id x 0.18 µm film thickness HP5-MS UI (Agilent Technologies, Inc, Santa Clara, CA)
GC oven (primary):	50 ºC held for 0.5 min, 20 ºC/min to 110 ºC, 40 ºC/min to 170 ºC, 45 ºC/min to 300 ºC, held for 2.11 min
GC transfer line:	290 ºC

The following MS conditions were used for detection.

MS filament delay:	110 sec
MS scan range:	35–500, at an acquisition rate of 15 spectra/sec
MS source:	250 ºC
Electron energy:	70 eV

Figure C-1 shows a representative chromatogram for a 1 µL injection when one column is installed and the GC/TOF-MS is operated in 1-D mode. Using the previously described conditions, the analyses were shown to be reproducible, with respect to both retention time stability (relative standard deviations (RSDs) for all analytes were less than or equal to 2%) and peak areas (RSDs for all analytes were less than or equal to 5%, with the exception of VX, which had an RSD of 8%); see Table C-1. Note that the analysis time is decreased when using a single GC column. When two columns are used, as previously described in Appendix B, analysis time is increased due to the modulator and secondary oven, which are limited to a maximum

temperature ramp rate of 40 ºC/min and a maximum of only three temperature ramps. Despite this limitation, we chose to perform 1-D experiments with two columns installed because this configuration offered us the flexibility of performing 2-D experiments without the instrument down-time associated with venting the instrument. Thus, both 1-D and 2-D experiments could be performed using a single autosampler sequence.

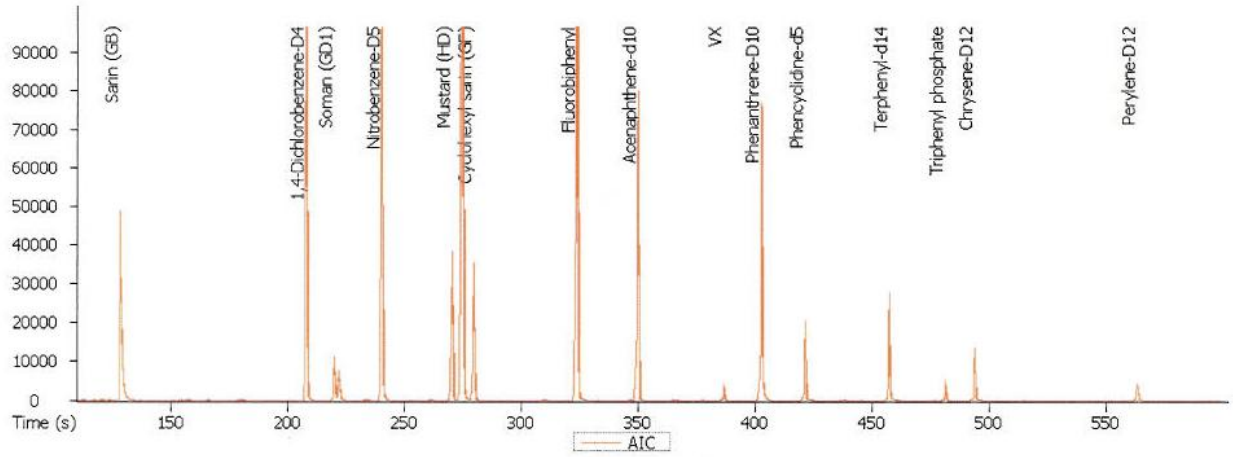


Figure C-1. Total ion chromatogram obtained when separating 0.5 ng of each CWA and 1 ng of each surrogate standard by GC/TOF-MS with a single GC column installed.

Table C-1. Average Retention Times (± Standard Deviations) and Average Analyte Responses (± Standard Deviations) for Seven Replicate Injections of 0.5 ng of Chemical Warfare Agents Into the GC/TOF-MS. Data Were Collected With Only a Single GC Column Installed (1D Operation)

Analyte	Average Retention Time (sec)	Average Analyte Response (peak area in arbitrary units)
GB	127.97 ± 0.25	674428 ± 27895
GD 1	219.75 ± 0.22	142379 ± 6703
GD 2	221.70 ± 0.23	115211 ± 5734
GF	279.56 ± 0.22	426259 ± 21011
HD	270.40 ± 0.17	527628 ± 27016
VX	386.75 ± 0.06	42527 ± 3268

Appendix C, cont'd: GC Method for LECO GC/TOF-MS, setup for 1-D instrument

Hardware control:

○ Agilent© 7890	Agilent© 7890 Gas Chromatograph
◉ Agilent© 6890	Agilent© 6890 Gas Chromatograph
○ Shimadzu© GC-2010	Shimadzu© GC-2010
○ Generic	Generic Gas Chromatograph
○ Direct Inlet	Direct Inlet to Calibration Compound

Option:

☐ MACH/LTM Oven

☐ LECO© GCxGC

Capillary Configuration:

No problems detected with column configuration.

Flow Path 1:

#	Type	Location	Length(m)	Int. Diameter(µ	Max Temp	Film Thickness	Phase	Bleed Masses
1*	Inlet	Back						
2	Capillary	GC Oven	19.800	180.00	350.0	0.18	HP5MS	73 149 207 281
3	Capillary	Detector o	0.200	180.00	325.0	0.18	HP5MS	73 149 207 281
4	Detector	TOF						

Add | Delete | Promote | Demote | Copy | Paste

☐ Enable Flow Path 2

Mass Selection for Auto Mass Defect Tracking

◉ Excluded Masses in Auto Mass Defect Mode

(Set Auto Mass Defect Mode in MS method. Select masses between 130 to 384 inclusive.)

(For general unknown analyses. Select column bleed, matrix, interfering, and non-target masses.)

○ Included Masses in Auto Mass Defect Mode

(Generally for target analyses. Select significant masses of target analytes, minimum of 2 masses required.)

Carrier Gas:

Appendix C: GC Method for LECO GC/TOF-MS, 1-D instrument, continued.

Carrier Gas:

Helium

Back Inlet Type:

Split / Splitless

Back Inlet Mode:

Pulsed Splitless

Active Inlet Location:

○ Front ◉ Back

The active inlet must be present in the capillary configuration.

No problems detected with pressure / flow.

☐ Corrected constant flow via pressure ramps

Use this mode when in GCxGC mode or using short (< 5 m) single column or two columns.

Select the column mode:

Constant Flow

Maintains a constant mass flow rate of carrier gas in the column throughout the run. If the column resistance changes due to a temperature program, the column head pressure is adjusted to keep the flow rate constant.

Column 2 / Back Inlet flow(s):

#	Rate (mL/min²)	Target Flow (mL/min)	Duration (min)
1*	Initial	1.20	Entire Run

Column 2 / Back Inlet Purge Time (sec):

35

The time, after the beginning of the run, when the purge valve will open.

Column 2 / Back Inlet Purge Flow (mL/min):

30

The flow from the purge vent. This value cannot be used if your column is not defined

Column 2 / Back Inlet Total Flow (mL/min):

31.2

This is the actual flow to the inlet during a Pre-Run and during a run before purge time.

Column 2 / Back Inlet Pulse Pressure (psi):

Appendix C: GC Method for LECO GC/TOF-MS, 1-D instrument, continued.

Column 2 / Back Inlet Pulse Pressure (psi):

40

Column 2 / Back Inlet Pulse Duration (minutes):

0.5

Column 2 / Back Inlet Gas Saver

◉ Yes ○ No

Column 2 / Back Inlet Gas Saver Flow (mL/min)

20

Column 2 / Back Inlet Gas Saver Time (minutes):

1

Back Inlet temperature(s):

#	Rate (°C/min)	Target Temp (°C)	Duration (min)
1*	Initial	250.00	Entire Run

No problems detected with oven temperatures.

Oven Equilibration Time (minutes):

0.5

Enter oven temperature ramp below:

#	Rate (°C/min)	Target Temp (°C)	Duration (min)
1*	Initial	50.00	0.50
2	20.00	110.00	0.00
3	40.00	170.00	0.00
4	45.00	300.00	2.11

Add

Remove

Coolant to Column Oven ○ On ◉ Off Coolant timeout (min) 12

Transfer Line Temperature Equilibration Time (sec):

0

Transfer Line Temperature (°C):

290

☐ Specify Additional Detectors & Auxiliary Pneumatics

Appendix D: MS Method for LECO GC/TOF-MS

Use GC method total time for MS method total time:

◉ Yes ○ No

Acquisition delay

90 ◉ Sec. ○ Min.

The length of time from injection until the data system will start storing data from the mass spectrometer.

Enter time(s) when the filament should be turned off (min of 3 sec) in the grid below

#	Start	End	Filament
1*	Start of Run	90 s	Off
2	90 s	End of Run	On

Add

Remove

Required Disk Space

NA

Enter the mass spectrometer settings:

Start Mass (u)

35

End Mass (u)

500

Acquisition Rate (spectra / second)

15

Detector Voltage

1650

Electron Energy (Volts)

-70

Mass defect mode

○ Auto (Select masses for automatic tracking in column information section of GC method.)

◉ Manual

☑ Verify offset before collecting data

Mass Defect (mu / 100 u)

0

Appendix D: MS Method for LECO GC/TOF-MS, continued.

Set the temperature for the Ion Source.

Ion Source (°C)

250

☑ Wait for ion source temperatures to reach set point before starting acquisition

Source Temperature Equilibration Time (Seconds)

0

Enter the masses to display during acquisition

t

Examples	
t	TIC
69,131	Masses 69 and 131
69+131	Sum of masses 69 and 131

Appendix E: Data Analysis Method for LECO GC/TOF-MS

Select the task or tasks you wish to perform from the list below.

- [x] Baseline - computes baseline
- [x] Peak Find - finds peaks above the baseline
- [x] Library Search - identifies all peaks found
- [x] Calculate Area / Height - computes the area and height of peaks without a calibration
- [ ] Retention Index Method
- [ ] Classifications
- [x] Apply Calibration(s) - computes the absolute concentration of peaks based upon a calibration
- [ ] Apply Reference(s) - computes the relative concentration of peaks with respect to a reference
- [ ] Semi Quantification - computes concentration based on another analytes calibration curve
- [ ] Tune Check
- [ ] Tailing Factor Check - checks to see if the analytes have an acceptable peak shape
- [ ] Calibration Check
- [ ] Blank Check - checks to make sure none of the analytes exceed their blank concentration
- [ ] EPA Method - selects the EPA Method
- [ ] Report - prints selected reports for each sample
- [ ] Export peak information in ASCII CSV format
- [ ] Export data in Andi MS format (.cdf)
- [ ] Export data file

Enter baseline tracking info below:

#	Start	End	Mode
1*	Start of Run	End of Run	Default

Add

Remove

Enter the baseline offset below (0.5-3.0):

0.8

Examples:
0.5 Through the middle of the noise
1.0 Just above the noise

Enter the number of data points that should be averaged for smoothing below:

Auto

Enter the expected peak width in seconds below: (as measured from baseline to baseline)

- [ ] Peak widths broaden throughout the chromatographic run

Peak Width	Retention Time
1.5	

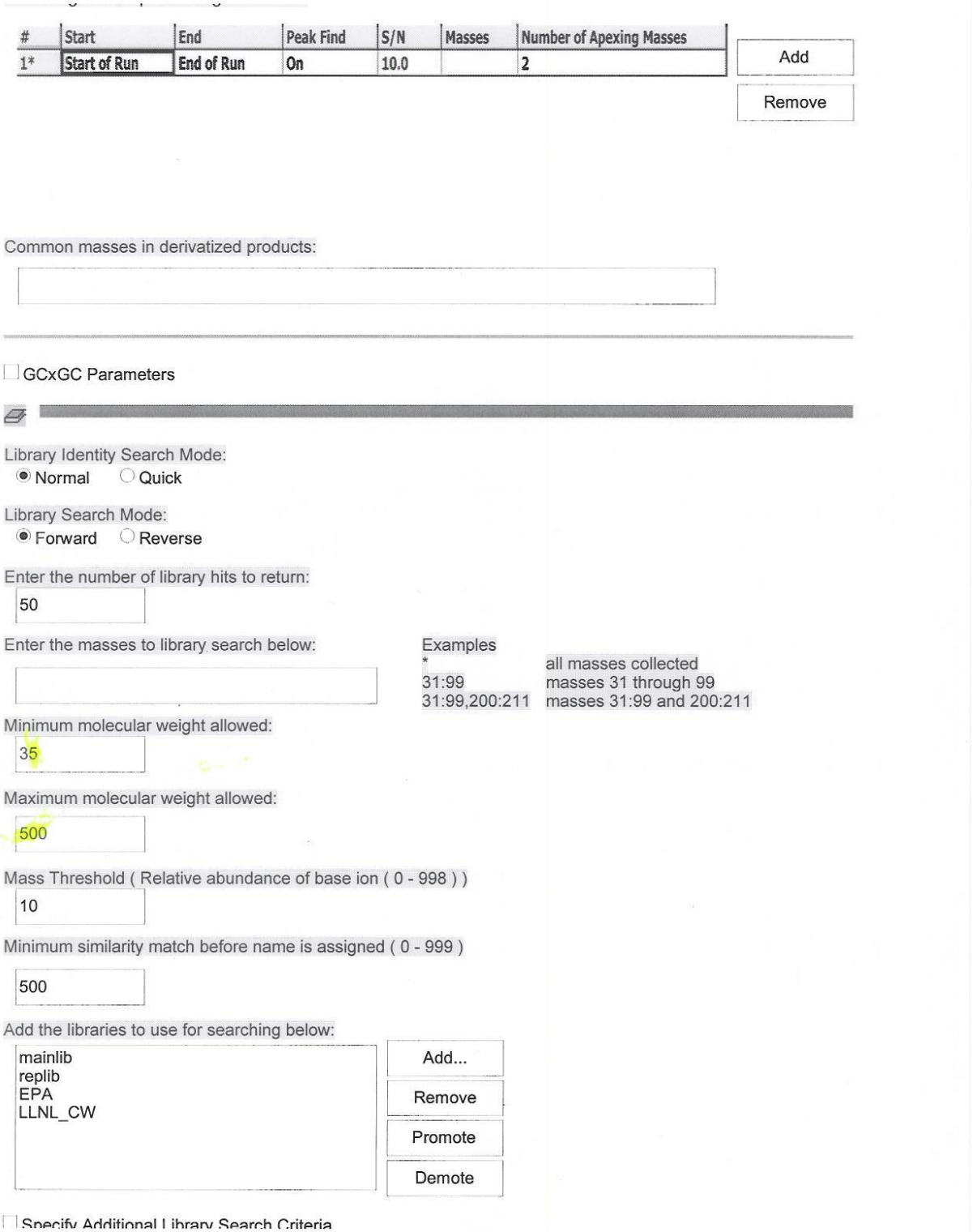
For broadening, two peak widths may be specified at two different retention times. All peak widths will be extrapolated from these two points.

Enter the maximum number of unknown peaks to find: 100

- [ ] Keep False Peaks

Enter segmented processing info below:

Appendix E: Data Analysis Method for LECO GC-TOF-MS, continued.



#	Start	End	Peak Find	S/N	Masses	Number of Apexing Masses
1*	Start of Run	End of Run	On	10.0		2

Add
Remove

Common masses in derivatized products:

GCxGC Parameters

Library Identity Search Mode:
Normal Quick

Library Search Mode:
Forward Reverse

Enter the number of library hits to return:
50

Enter the masses to library search below:

Examples	
*	all masses collected
31:99	masses 31 through 99
31:99,200:211	masses 31:99 and 200:211

Minimum molecular weight allowed:
35

Maximum molecular weight allowed:
500

Mass Threshold (Relative abundance of base ion (0 - 998))
10

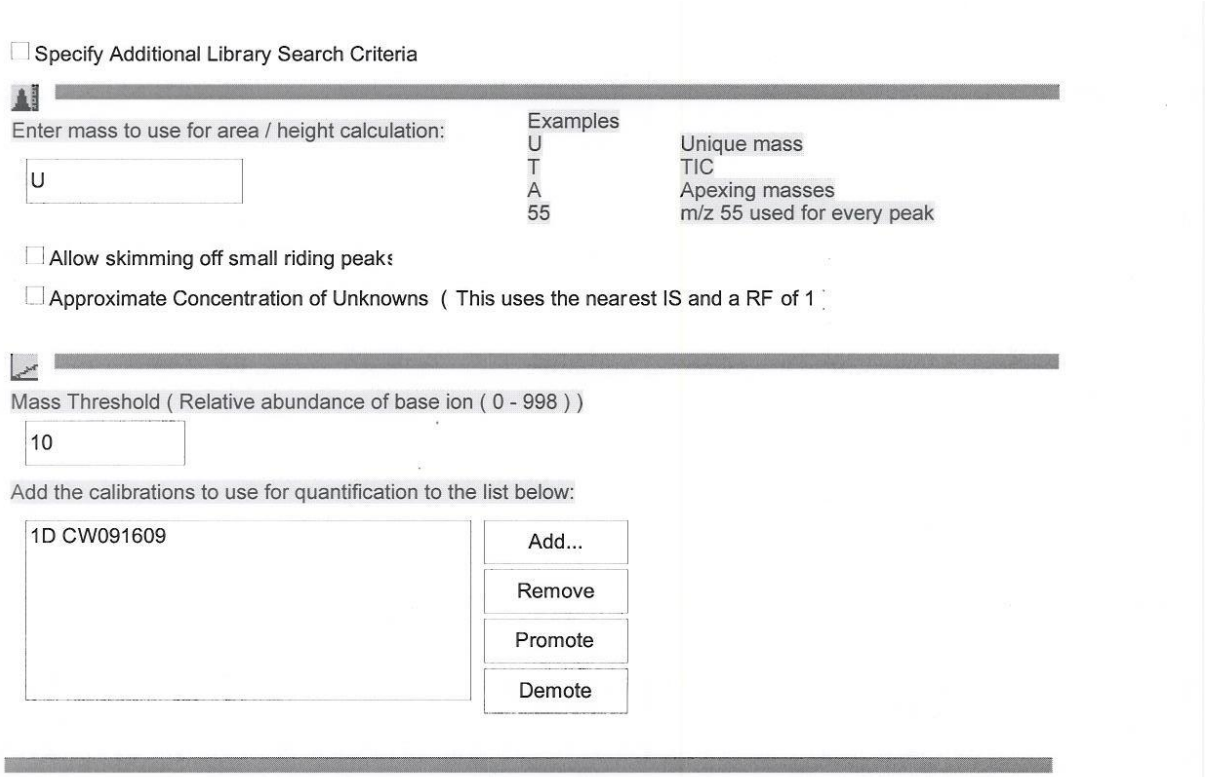
Minimum similarity match before name is assigned (0 - 999)
500

Add the libraries to use for searching below:
mainlib
replib
EPA
LLNL_CW

Add...
Remove
Promote
Demote

Specify Additional Library Search Criteria

Appendix E: Data Analysis Method for LECO GC/TOF-MS, continued.



Appendix F: Analysis Method for LECO GC/TOF-MS, Modified ECBC Conditions.

Initial experiments were performed using the faster temperature ramps of an ECBC method (6) and a 10 m x 0.18 mm i.d. x 0.18 µm film thickness, HP5-MS UI, GC column. Separation was accomplished in 6 minutes; see Figure F-1. The parameters used to achieve this separation are shown below. Screen shots of the GC/TOF-MS setup are shown at the end of this appendix.

Injection size:	1 µL
Injection type:	pulsed-splitless
Pulse pressure:	40 psi for 0.5 min
Purge time:	35 sec at 30 mL/min
Carrier gas:	He with constant flow of 1.2 mL/min
GC injection port:	250 ºC
GC column:	10 m x 0.18 mm i.d. x 0.18 µm film thickness, HP5-MS UI (Agilent Technologies, Inc, Santa Clara, CA)
GC oven (primary):	30 ºC held for 1.25 min, 85 ºC/min to 120 ºC, 65 ºC/min to 180 ºC, 45 ºC/min to 290 ºC, held for 2.35 min
GC transfer line:	290 ºC

The following MS conditions were used for detection.

MS filament delay:	90 sec
MS scan range:	50–500, at an acquisition rate of 35 spectra/sec
MS source:	250 ºC
Electron energy:	70 eV

Reproducibility of retention times and analyte responses for seven replicate injections of a standard containing 0.5 µg/mL of CWAs were documented and are shown in Table F-1. As was evident from the data, for seven replicate injections, retention times were stable and varied by less than 0.1% for all compounds. This stability is consistent with reports that a sample of 32 Agilent 6890 Plus GCs consistently demonstrated relative standard deviation of less than 0.1 percent, with some lower than 0.01 percent (7). All analyte responses, measured as peak areas, were also reproducible and varied by less than 10%.

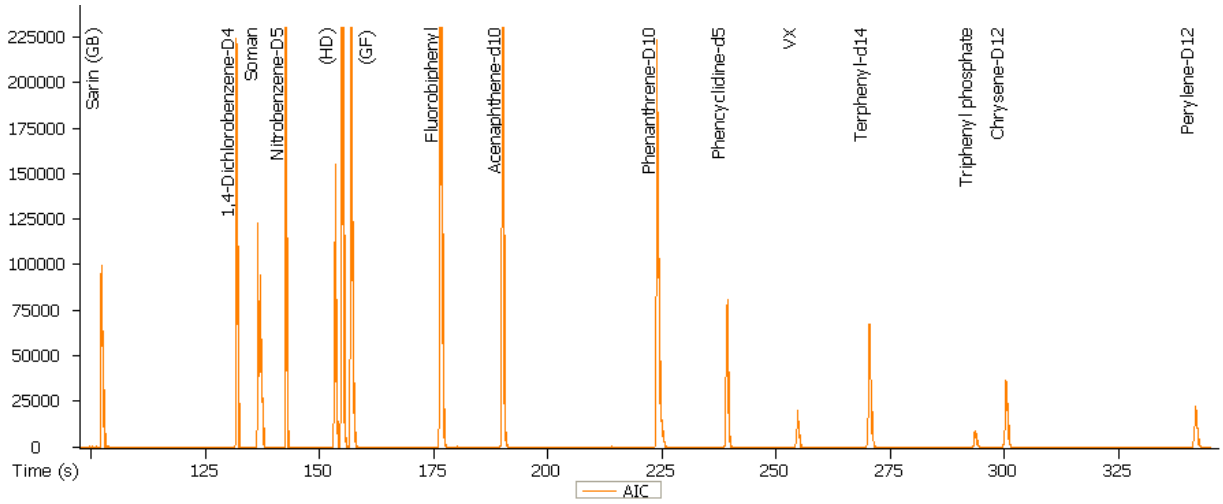


Figure F-1. Total ion chromatogram (filename = TOF:287) obtained when separating 0.5 ng of each agent and 1 ng of each surrogate standard by GC/TOF-MS. Note: Naphthalene-d_8 is the peak between HD and GF.

Table F-1. Average retention times (± standard deviations) and average analyte responses (± standard deviations) for seven replicate injections of 0.5 ng of CWAs into the GC/TOF-MS.

Analyte	Average Retention Time (sec)	Average Analyte Response (peak area in arbitrary units)
GB	102.34 ± 0.14	1167925 ± 71630
GD 1	136.51 ± 0.03	1101851 ± 109658
GD 2	137.08 ± 0.02	1208078 ± 104845
GF	156.89 ± 0.09	5799223 ± 564933
HD	153.36 ± 0.09	1711803 ± 160493
VX	213.89 ± 0.04	402713 ± 31773

Appendix F: Analysis Method for LECO GC/TOF-MS using Modified ECBC Conditions, continued.

Hardware control:

○ Agilent© 7890	Agilent© 7890 Gas Chromatograph
◉ Agilent© 6890	Agilent© 6890 Gas Chromatograph
○ Shimadzu© GC-2010	Shimadzu© GC-2010
○ Generic	Generic Gas Chromatograph
○ Direct Inlet	Direct Inlet to Calibration Compound

Option:

☐ MACH/LTM Oven

☐ LECO© GCxGC

Capillary Configuration:

No problems detected with column configuration.

Flow Path 1:

#	Type	Location	Length(m)	Int. Diameter(µ	Max Temp	Film Thickness	Phase	Bleed Masses
1*	Inlet	Back						
2	Capillary	GC Oven	10.000	180.00	300.0	0.18	HP5MS	73 149 207 281
3	Detector	TOF						

Add | Delete | Promote | Demote | Copy | Paste

☐ Enable Flow Path 2

Mass Selection for Auto Mass Defect Tracking

◉ Excluded Masses in Auto Mass Defect Mode — (Set Auto Mass Defect Mode in MS method. Select masses between 130 to 384 inclusive.)

(For general unknown analyses. Select column bleed, matrix, interfering, and non-target masses.)

○ Included Masses in Auto Mass Defect Mode — (Generally for target analytes, minimum of 2 masses required.)

Carrier Gas:

Appendix F: Analysis Method for LECO GC/TOF-MS using Modified ECBC Conditions, continued.

Carrier Gas:

Helium

Back Inlet Type:

Split / Splitless

Back Inlet Mode:

Pulsed Splitless

Active Inlet Location:

○ Front ◉ Back

The active inlet must be present in the capillary configuration.

No problems detected with pressure / flow.

☐ Corrected constant flow via pressure ramps

Use this mode when in GCxGC mode or using short (< 5 m) single column or two columns.

Select the column mode:

Constant Flow

Maintains a constant mass flow rate of carrier gas in the column throughout the run. If the column resistance changes due to a temperature program, the column head pressure is adjusted to keep the flow rate constant.

Column 2 / Back Inlet flow(s):

#	Rate (mL/min²)	Target Flow (mL/min)	Duration (min)
1*	Initial	1.20	Entire Run

Column 2 / Back Inlet Purge Time (sec):

35

The time, after the beginning of the run, when the purge valve will open.

Column 2 / Back Inlet Purge Flow (mL/min):

30

The flow from the purge vent. This value cannot be used if your column is not defined

Column 2 / Back Inlet Total Flow (mL/min):

30.9

This is the actual flow to the inlet during a Pre-Run and during a run before purge time.

Column 2 / Back Inlet Pulse Pressure (psi):

Appendix F: Analysis Method for LECO GC/TOF-MS using Modified ECBC Conditions, continued.

Column 2 / Back Inlet Pulse Pressure (psi):

15

Column 2 / Back Inlet Pulse Duration (minutes):

0.5

Column 2 / Back Inlet Gas Saver

◉ Yes ○ No

Column 2 / Back Inlet Gas Saver Flow (mL/min)

20

Column 2 / Back Inlet Gas Saver Time (minutes):

1

Back Inlet temperature(s):

#	Rate (°C/min)	Target Temp (°C)	Duration (min)
1*	Initial	250.00	Entire Run

No problems detected with oven temperatures.

Oven Equilibration Time (minutes):

1

Note: All oven temperature ramps (except the secondary oven) will have the same duration. This is accomplished by extending the final hold time.

Enter oven temperature ramp below:

#	Rate (°C/min)	Target Temp (°C)	Duration (min)
1*	Initial	30.00	1.25
2	85.00	120.00	0.00
3	65.00	180.00	0.00
4	45.00	290.00	2.35

Add

Remove

Coolant to Column Oven ○ On ◉ Off Coolant timeout (min) 10

Transfer Line Temperature Equilibration Time (sec):

0

Appendix F: Analysis Method for LECO GC/TOF-MS using Modified ECBC Conditions, continued.

Transfer Line Temperature (°C):

250

☐ Specify Additional Detectors & Auxiliary Pneumatics

Appendix F: Analysis Method for LECO GC/TOF-MS using Modified ECBC Conditions, continued.

Use GC method total time for MS method total time:

(●) Yes ( ) No

Acquisition delay

90 (●) Sec. ( ) Min.

The length of time from injection until the data system will start storing data from the mass spectrometer.

Enter time(s) when the filament should be turned off (min of 3 sec) in the grid below

#	Start	End	Filament
1*	Start of Run	90 s	Off
2	90 s	End of Run	On

Add

Remove

Required Disk Space

NA

Enter the mass spectrometer settings:

Start Mass (u)

50

End Mass (u)

500

Acquisition Rate (spectra / second)

35

Detector Voltage

1650

Electron Energy (Volts)

-70

Mass defect mode

( ) Auto (Select masses for automatic tracking in column information section of GC method.)

(●) Manual

☑ Verify offset before collecting data

Mass Defect (mu / 100 u)

0

Appendix F: Analysis Method for LECO GC/TOF-MS using Modified ECBC Conditions, continued.

Set the temperature for the Ion Source.

Ion Source (°C)

250

☑ Wait for ion source temperatures to reach set point before starting acquisition

Source Temperature Equilibration Time (Seconds)

0

Enter the masses to display during acquisition

t

Examples	
t	TIC
69,131	Masses 69 and 131
69+131	Sum of masses 69 and 131