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Application of headspace solid-phase microextraction and gas chromatography–mass spectrometry for detection of the chemical warfare agent bis(2-chloroethyl) sulfide in soil

Gregory L. Kimm, Gary L. Hook, Philip A. Smith*

Uniformed Services University of the Health Sciences, Department of Preventive Medicine and Biometrics, 4301 Jones Bridge Road, Bethesda, MD 20814, USA

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Abstract

A field expedient analytical method for detecting the chemical warfare agent (CWA) sulfur mustard as a soil contaminant was developed using solid-phase microextraction (SPME) and gas chromatography–mass spectrometry (GC–MS). Five commercially available SPME fibers were investigated to determine the optimal fiber, and extraction conditions. Polyacrylate and carbowax–divinylbenzene fiber coatings gave a statistically indistinguishable and best response compared to the other three types examined in a simple system studied without soil. The polyacrylate fiber coating was selected for study of a system in which sulfur mustard was spiked to an agricultural soil (Standard Reference Material 2709, San Joaquin type). With soil samples, the greatest sensitivity occurred by the addition of deionized water to spiked soil and extraction at ambient temperature for 20 min or longer. SPME sampling with GC–MS analyses afforded good reproducibility (relative standard deviation between 2 and 10%), and analyte concentrations as low as 237 ng/g were detected in soil (total ion chromatograms). As completed here, total time for sampling and analysis was just under 1 h, and use of organic solvents or special sample introduction equipment was avoided.
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Keywords: Headspace analysis; Warfare agents; Soil; Solid phase microextraction; Bis(2-chloroethyl) sulfide; Sulfur mustard; Organosulfur compounds

1. Introduction

Chemical warfare agents (CWAs) pose a serious and credible threat to civilian and military populations. CWAs were used extensively during World War I, and bis(2-chloroethyl) sulfide (sulfur mustard, or US military designation HD) caused 80% of the chemical casualties in that conflict [1]. In the more recent past, Iraq used CWAs against Iran during the Iran–Iraq war and against Kurdish refugees in the mountainous region of northwest Iraq [2].

Terrorists may use CWAs as a weapon of mass destruction as demonstrated in 1995 when a religious cult released sarin in the Tokyo subway system killing 12 and injuring more than 5000 people. Field sampling and analysis methods are needed for CWAs that are rapid, and provide adequate sensitivity and high quality data so that governmental authorities

*Corresponding author. Tel.: +1-301-295-2642; fax: +1-301-295-9298.

E-mail address: psmith@usuhs.mil (P.A. Smith).

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and public health officials may make informed decisions following CWAs exposure or use. Methods used to detect and identify CWAs in the field would also preferably allow detection and identification of toxic industrial chemicals and environmental contaminants.

Solid-phase microextraction (SPME) is a relatively new technique for passive sampling of organic analytes, with potential for rapid sampling and analysis by gas chromatography in field settings. SPME is a solvent-free process that combines sampling, extraction, concentration and instrument introduction into a single step, eliminating complicated sample preparation methods [3]. It is a non-exhaustive extraction process that concentrates organic compounds onto or into a stationary coating on a thin fused-silica fiber. The usefulness of SPME as a sample introduction method for gas chromatography (GC) in the field has been documented [4,5]. As a sampling/sample introduction technique for GC with a mass spectrometry (MS) detector, SPME has been shown to allow rapid identification of unknown compounds with analysis completed in the field [6,7]. The benefits potentially available with SPME sampling for field GC–MS analysis include simple sampling, and very little sample preparation.

In this work we explore the use of headspace SPME for sampling of sulfur mustard as a soil contaminant. Several SPME fiber types and sampling conditions were tested in a simple system without soil. Following this, SPME sampling was accomplished with sulfur mustard-contaminated soil. Analysis of SPME samples was by GC–MS.

2. Experimental

2.1. Materials

Sulfur mustard (97.5% purity) was obtained from the US Army Edgewood Chemical Biological Center (Aberdeen Proving Grounds, MD, USA). As a matter of safety, handling of neat chemical warfare agent material is to be avoided, and the high-purity sulfur mustard material used was quantitatively diluted in hexanes by the US Army Medical Research Institute for Chemical Defense to concentrations less than 10 mg/ml of solvent. Further dilutions in hexanes were prepared by the authors. Sulfur mustard solutions used were stored at −70 °C to prevent degradation. Authentic standards were purchased for 1,4-dithiane (97%), and thiodiglycol (99%) from Aldrich (Milwaukee, WI, USA).

All SPME fibers and holders used in this study are commercially available from Supelco (Bellefonte, PA, USA). The following fiber coatings were studied (film thickness as indicated): polydimethylsiloxane (PDMS, 100 μm), polyacrylate (PA, 85 μm), carbowax–divinylbenzene (CW–DVB, 65 μm), carboxen–polydimethylsiloxane (CAR–PDMS, 65 μm), and polydimethylsiloxane–divinylbenzene (PDMS–DVB, 65 μm). Prior to use, each fiber was conditioned following the manufacturer's recommendations. Blank runs were completed at least once daily before use of any fibers for sampling to ensure no carryover of analytes from previous extractions.

2.2. SPME sampling

2.2.1. Initial fiber selection

SPME fiber selection was accomplished in a simple system (no soil) by obtaining three replicate samples from 15-ml vials, each with a polytetrafluoroethane (PTFE)-lined silicone septum in an open screw top closure. A vial was spiked with sulfur mustard (2.4 mg/ml in hexanes) by piercing the septum with a syringe needle and injecting 5.0 μl of the solution. The temperature of the vial sampled was maintained at 25 °C by placing the vial in a digitally controlled hot-block heater (Barnstead/Thermolyne, Dubuque, IA, USA). Each sample was equilibrated for 10.0 min before the SPME fiber assembly outer sheath pierced the vial septum, and the SPME fiber was then lowered through the outer sheath into the vial headspace.

Sampling duration was 30 min, and immediately following sampling, the fiber was removed from the vial and GC–MS analysis commenced. The fibers giving the greatest GC–MS peak areas for the sulfur mustard peak (CW–DVB and PA) were selected for further sampling and analysis optimization.

2.2.2. Simple system: temperature selection and effect of sampling time

Another set of spiked vials was analyzed using PA and CW–DVB fiber coatings, under the same set of conditions as previously, except the temperature of extraction was 50 or 75 °C. Finally, the fibers were

exposed at the resulting optimal temperature (25 °C) over an increasing extraction time period to examine analyte loading kinetics.

2.2.3. Soil headspace SPME

Sulfur mustard was applied to Standard Reference Material (SRM) 2709, San Joaquin soil (National Institute of Standards and Technology, Gaithersburg, MD, USA), by injecting 5.0 μl of 9.5 mg/ml stock solution onto 1.0 g of soil, and shaking with a vortex mixer for 20 s. This gave a concentration of 48 μg sulfur mustard/g soil (48 ppm, m/m).

Initial experiments with spiked soil samples were completed with CW–DVB and PA type fiber coatings. The addition of water to the soil was thought to offer the potential for increased sensitivity, and two sample replicates were collected for both fiber types in soil to which 500 μl deionized water were added 10 min prior to commencement of SPME sampling. Sampling time for these samples was 30 min, at room temperature (determined to be 23±0.5 °C). Following this work, the CW–DVB fiber was not used further. Two additional sample sets were collected for comparison to the room temperature/wet soil PA fiber samples. Firstly, dry soil sample replicates were collected that were created and sampled identically to the PA fiber samples from moist soil, except no water was added. Experiments were then completed with soil prepared identically to the room temperature/wet soil PA samples, except sampling was completed at 50 and 75 °C. Only the 50 °C replicates from the last sample type mentioned were compared to the room temperature, wet soil samples as no sulfur mustard GC–MS peaks were detected in the 75 °C samples.

To produce a soil system uptake curve, spiked soil sample replicates ($n=2$, 48 μg in 1.0 g soil) were collected using a PA-type fiber at room temperature with water added as per above. Extraction times ranged from 1.0 to 60.0 min.

In order to estimate the sensitivity of the method, 30-min extractions were carried out using the PA fiber coating (wet soil) spiked at five concentrations ranging from 95 to 475,000 ng/g. The upper concentration range was set by poor chromatography, with the sulfur mustard analyte exceeding the capacity of the GC column used. Two replicates of each concentration were sampled by SPME at room temperature and at 50 °C.

2.3. GC–MS methods

The SPME samples were analyzed immediately following collection using a 6890 series gas chromatograph and 5973 quadrapole mass selective detector (Agilent Technologies, Wilmington, DE, USA). A J&W Scientific (Folsom, CA, USA) DB-5, 30 m×0.25 mm I.D. column having a film thickness of 0.25 μm was used, with helium carrier gas at 1 ml/min (constant flow mode). The oven was programmed to increase from 35 to 250 °C at 20 °C/min following a 2.00-min hold time at the initial temperature. Desorption of the SPME fiber samples was accomplished in the splitless injection mode for 2.00 min, followed by 50 ml/min injector purge. The injector temperature was maintained at 250 °C throughout an analysis, and the mass spectrometer transfer line was kept at 270 °C. Electron impact ionization (70 eV) was used and mass spectra were collected over the range of m/z 35–350.

2.4. Quantitative analysis of sulfur mustard in solvent

In order to estimate the mass of sulfur mustard loaded onto an SPME fiber, splitless injection analyses of sulfur mustard in solvent were completed by GC–MS to obtain a curve with mass of analyte on-column plotted against total ion current peak area. Three samples were analyzed at five concentrations, with the mass of sulfur mustard injected ranging from 0.7625 to 24.4 ng. The same instrument and conditions as for SPME samples were used, except a split/splitless injection port liner (Agilent) was used in place of the 0.75 mm I.D. narrow bore liner (Supelco) used for SPME samples. Sample introduction was by autosampler (7673, Agilent) using an injection volume of 1.0 μl.

3. Results and discussion

3.1. Quantitative analysis of sulfur mustard in solvent

The curve completed by liquid injection analyses of sulfur mustard in solvent showed good linearity ($r^2=0.9826$). This curve allowed estimation of the mass of sulfur mustard loaded onto an SPME fiber

by using the resulting sulfur mustard GC–MS peak area in an SPME sample to calculate the corresponding sulfur mustard mass.

3.2. Initial fiber selection

Table 1 shows the data obtained during fiber selection experiments. Fig. 1 shows a GC–MS chromatogram for SPME sampling of sulfur mustard from a simple system. The PA and CW–DVB fibers gave a statistically indistinguishable response under the conditions tested, while other fibers differed from each other with significance ($P<0.001$, analysis of variance (ANOVA) followed by Tukey's post hoc comparisons). The average GC–MS sulfur mustard peak areas for the PA and CW–DVB fibers were greater than for all other fibers tested.

3.3. Temperature selection, effect of sampling time

Table 2 shows the data obtained during temperature selection experiments using the PA and CW–DVB fiber coatings. Statistical differences existed between sulfur mustard GC–MS peak areas among the various sample conditions (Table 2 data, $P<0.001$, ANOVA followed by Tukey's post hoc comparisons). Both the PA and CW–DVB fibers gave indistinguishable peak areas at 25 °C under the conditions tested, while their peak areas differed significantly from all other combinations of fiber/temperature tested ($P<0.001$).

The SPME uptake curves completed with the simple system using PA and CW–DVB fibers are presented in Fig. 2. Equilibrium was apparently established at 30 min, with possible fugacity losses from the closed system explaining the slight decrease observed at longer extraction times.

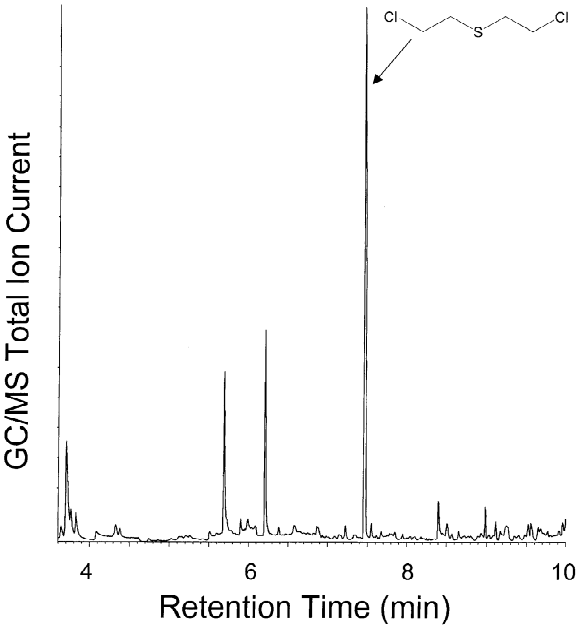

Fig. 1. Simple system total ion current chromatogram, SPME sample (12.0 μg sulfur mustard), in glass vial (no soil), 25 °C, 30-min extraction; the two peaks eluting near 6 min represent hydrocarbons present in the hexanes used to spike the sample vials (present in vials spiked only with hexanes and absent in clean vials).

3.4. Soil headspace SPME

With the initial comparisons between CW–DVB and PA fiber coatings for sampling spiked soil (wet soil, 25 °C), the average peak area given with CW–DVB sampling was only 48% that of the samples

Table 1
Simple system SPME sampling; fiber selection, GC–MS peak area counts for sulfur mustard; 30-min extraction, 25 °C

Sample no.	CW–DVB	PA	PDMS–DVB	100 PDMS	CAR–PDMS
1	1 039 823 434	1 009 583 987	841 988 999	716 206 214	182 758 826
2	987 615 869	961 340 244	821 779 662	665 113 312	195 101 473
3	1 012 615 304	965 270 820	836 856 034	668 181 715	217 265 007
Mean	1 013 351 536	978 731 684	833 541 565	683 167 080	198 375 102
SD	26 111 568	26 791 059	10 504 456	28 653 831	17 484 468
RSD (%)	2.58	2.74	1.26	4.19	8.81
Average r (%)[a]	0.35	0.34	0.29	0.24	0.06

[a] The mass of sulfur mustard recovered from fiber compared to the mass of spiked system sampled, %.

Table 2
Simple system SPME sampling; GC–MS peak area counts for sulfur mustard; temperature profile, 30-min extraction

Sample no.	CW–DVB			PA		
	25 °C	50 °C	75 °C	25 °C	50 °C	75 °C
1	1 039 823 434	868 262 540	599 511 025	1 009 583 987	790 559 359	411 214 932
2	987 615 869	797 190 447	642 802 506	961 340 244	822 950 330	338 847 589
3	1 012 615 304	784 267 005	679 874 799	965 270 820	766 860 218	379 048 981
Mean	1 013 351 536	816 573 331	640 729 443	978 731 684	793 456 636	376 370 501
SD	26 111 568	45 228 140	40 221 975	26 791 059	28 157 074	36 257 948
RSD (%)	2.58	5.54	6.28	2.74	3.55	9.63
Average r (%)[a]	0.35	0.28	0.22	0.34	0.28	0.13

[a] The mass of sulfur mustard recovered from fiber compared to the mass of the spiked system sampled, %.

collected with the PA fiber, a significant difference (two-tailed t-test, $P=0.023$). Water likely interferes with sulfur mustard adsorption in the polar CW–DVB coating, decreasing the sensitivity obtained with this coating (relative to the liquid-type PA fiber coating) compared to the simple system where water was not added. The average sulfur mustard GC–MS peak area for the dry, room temperature samples was $<1\%$ that of the samples collected at room temperature with wet soil, a significant difference (two-tailed t-test, $P=0.0024$). The average sulfur mustard GC–MS peak area for the 50 °C, wet soil SPME samples was 8.2% that of the wet soil samples collected at room temperature, a significant difference (two-tailed t-test, $P=0.003$).

Fig. 3 shows the results of analyses completed for the soil system uptake curve. Equilibrium was approached at 20 min.

Analysis of the SPME samples ranging from 95 to 475,000 ng sulfur mustard spiked per g soil (wet soil, room temperature extraction) gave results shown in Fig. 4. In examining total ion and extracted ion (m/z 109) traces for the sulfur mustard peak, it was not observed in samples spiked at 95 ng/g. At 237 ng/g, sulfur mustard peaks were observed at $>3{:}1$ signal-to-noise ratio. At 50 °C, no peaks were observed at spike concentrations <9500 ng/g. Percent recovery (ng sulfur mustard recovered on the SPME fiber compared to the total mass spiked to a soil system) for these samples is shown in Table 3.

Although high sensitivity was the desired endpoint in the work we performed, the use of room temperature extraction for as little as 10 min from a soil

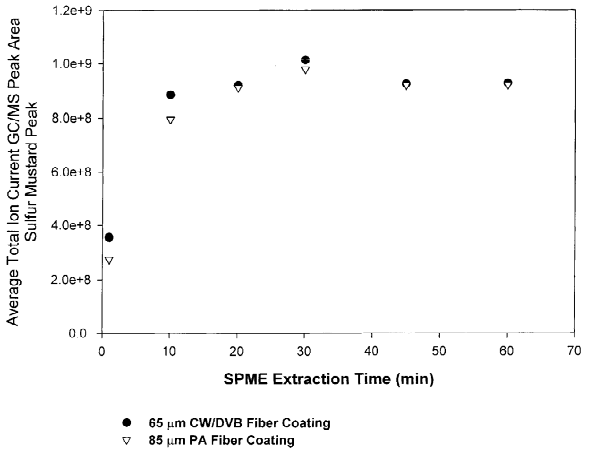

Fig. 2. Average GC–MS total ion current peak area uptake curve for sulfur mustard in a simple system (no soil), PA fiber and CW–DVB fiber types both shown, room temperature extraction.

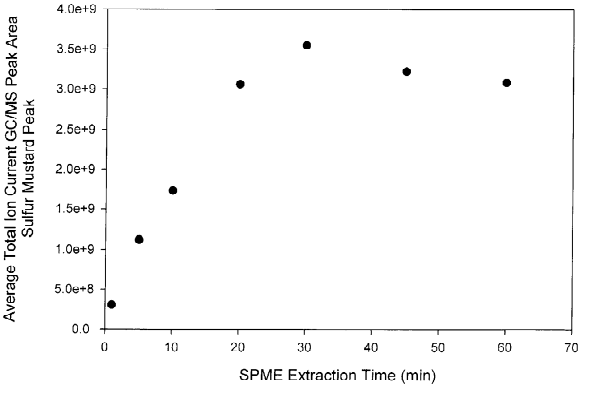

Fig. 3. Average GC–MS total ion current peak area uptake curve for sulfur mustard in a soil system, PA fiber type, room temperature extraction.

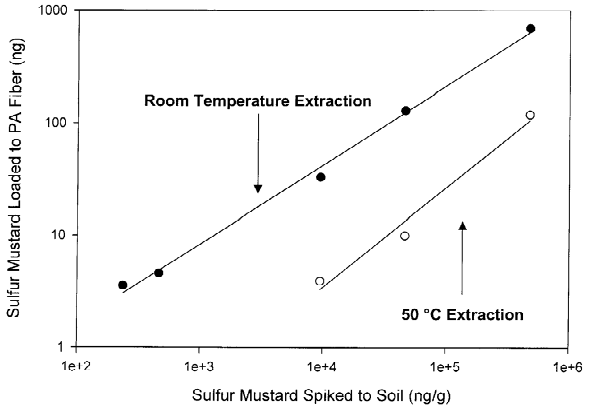

Fig. 4. Average sulfur mustard mass (ng) loaded to PA fiber by GC–MS peak area conversion to mass on column using liquid solvent injection linear regression curve; both room temperature and 50 °C headspace extractions completed from glass vials containing soil spiked with sulfur mustard (mass spiked as indicated).

system similar to the SRM soil used would give about half the sensitivity of the 20 min or longer extraction time as shown in Fig. 3. Combined with the 10-min pre-sampling equilibration time that we used following addition of water, and a GC–MS analysis time of about 15 min, a single sample could be completed in as little as 35 min. Other researchers have demonstrated the usefulness of wetting soil samples for headspace SPME sampling/analysis for analytes that are not miscible in water [8–10]. Good reproducibility (RSD between 2 and 10% for sulfur mustard GC–MS peak areas) was observed for sample replicates analyzed during this work.

Black et al. successfully detected sulfur mustard from soil samples using active headspace sampling and full scan GC–MS [11]. Their sampling and analyses were completed rapidly (about 30 min) for sulfur mustard in soil, by pumping soil headspace air through a tube loaded with Tenax for thermal desorbtion and GC–MS analysis. The thermal desorption apparatus is a relatively large piece of equipment and adds complexity to the analysis, compared to the use of SPME where no additional equipment is needed. Black et al. also used solvent extraction of soil with more traditional laboratory procedures to process the soil samples examined in their work. These methods are less suitable for field analysis than the SPME methods.

Several degradation products were identified in soil sample systems by SPME sampling and GC–MS analysis. Retention time and mass spectrum matches were obtained for authentic standards of 1,4-dithiane and thiodiglycol. The thiodiglycol was observed only at 75 °C sampling temperatures, and the resulting peaks were small and poorly shaped. Another compound was detected by GC–MS and tentatively identified as bis(2-chloroethyl) disulfide by mass spectrum library search and match only, as no chemical standard was available for this compound. 1,4-Dithiane and bis(2-chloroethyl) disulfide were identified in all soil systems to which water was added (ambient temperature, 50 and 75 °C). These analytes are known degradation products of sulfur mustard [12].

In addition to sulfur mustard, other CWA compounds are likely to be suitable for SPME sampling followed by GC–MS analysis in the field. Schneider et al. [13] showed that sarin may be rapidly sampled by SPME at low concentrations, as a vapor in air and from water. A large body of work is available to demonstrate the usefulness of SPME for sampling diverse organic analytes from air, soil, and water. Analysis by GC–MS for field detection and identification of unknown chemical compounds is easily

Table 3
Soil system SPME sampling; GC–MS peak area counts for sulfur mustard; 30-min extraction, 25 °C

Sample no.	237 ng	475 ng	9.5 μg	48 μg	475 μg
1	100 468 785	114 940 339	754 807 890	2 899 789 567	16 884 194 578
2	97 455 699	131 475 800	818 283 976	3 197 400 797	14 725 754 823
Mean	98 962 242	123 208 070	786 545 933	3 048 595 182	15 804 974 701
SD	2 130 574	11 692 337	44 884 371	210 442 919	1 526 247 388
RSD (%)	2.15	9.49	5.71	6.90	9.66
Average r (%)[a]	1.50	0.96	0.35	0.27	0.14

[a] Mass sulfur mustard recovered from fiber compared to the mass spiked system sampled, %.

combined with sample introduction by SPME. If CWA compounds must be detected and identified by GC–MS in the field, SPME sampling would simplify and facilitate the overall sampling/analysis method, and should also allow detection and identification of a range of non-CWA compounds that may be present in the matrix sampled.

4. Conclusion

Sulfur mustard was sampled by SPME in simple systems, and as a contaminant of SRM agricultural soil, with analysis by GC–MS. On examination of commercially available SPME fiber coatings and different extraction conditions using a system without soil, PA and CW–DVB fiber coatings were shown to be similar and gave larger sulfur mustard GC–MS peak areas compared to the other fibers tested. For headspace SPME sampling with contaminated soil, the addition of water to the spiked soil increased partitioning of sulfur mustard to the headspace and with sampling times of 20 min or longer at ambient temperature gave the best sensitivity. Under these conditions, the PA fiber coating was deemed a better choice compared to the CW–DVB coating. SPME sampling with GC–MS analyses afforded good reproducibility (RSD between 2 and 10%), and analyte concentrations as low as 237 ng/g were detected in soil (total ion chromatograms). As completed here, total time for sampling and analysis was just under 1 h, and use of solvents or special GC sample introduction equipment was avoided. The simplicity of SPME sampling, its apparent usefulness as a sampling method for a range of organic chemicals (including CWA compounds), and the ease with which it may be easily combined with GC–MS analysis in the field should provide impetus for further research in this area.

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