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Packed capillary liquid chromatography–electrospray ionization (tandem) mass spectrometry of mustard hydrolysis products in soil

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Abstract

A packed capillary liquid chromatography–electrospray ionization tandem mass spectrometry (LC–ESI-MS/MS) method was developed and applied to the identification of mustard hydrolysis products in aqueous extracts of soil. In the first application the LC–ESI-MS/MS method was used to identify thiodiglycol and nine longer chain diols in soil samples taken at different locations and depths from a former mustard storage site as part of an ongoing environmental assessment. Aqueous extracts of the soil samples were analysed by LC–ESI-MS/MS using a quadrupole/time-of-flight tandem mass spectrometer operating with a resolution of 9000. High resolution product mass spectra were acquired for thiodiglycol, the hydrolysis product of mustard and nine other sulfur containing diols, including five longer chain diols that could not be identified during prior LC–ESI-MS analyses. The high resolution LC–ESI-MS/MS method was also incorporated into an analytical approach designed to provide rapid chemical warfare agent identification in cases where the chemical and/or biological warfare agent content of a sample is unknown. A sample handling method involving aqueous extraction of the soil sample in biocontainment level 3 (BL-3), followed by autoclave sterilization of the aqueous extract was developed. Once sterilized, the container and aqueous extract can then be safely manipulated outside of BL-3 in the analytical laboratories and may be analysed for the presence or absence of chemical warfare agents, their hydrolysis products or related compounds by LC–ESI-MS/MS.

Keywords: Mustard; Hydrolysis; Chemical warfare agents

1. Introduction

More than 150 States Parties have ratified the Chemical Weapons Convention (CWC) and agreed not to develop, produce, stockpile, transfer or use chemical weapons and to destroy their own chemical weapons and production facilities. The CWC has reduced the likelihood of chemical weapons use by States Parties, but there remains a serious concern that other parties may make use of these weapons against civilian or military targets. Concerns over possible terrorist use, continued interest within the defence community and the requirements of a verifiable CWC, have all driven the development and application of analytical methods for the detection, characterization and confirmation of chemical warfare agents. Analytical techniques play an important role in this process as sampling and analysis will be conducted to ensure treaty compliance, to investigate allegations of use and to verify the use of these weapons for forensic purposes.

Chemical warfare agents have been grouped into several categories with the nerve agents and vesicants being the two most important classes. Chromatographic methods for the separation and analysis of these compounds have been reviewed [1–3], with capillary column gas chromatography–mass spectrometry (GC–MS) being the most commonly employed analytical technique for the identification of chemical warfare agents and their hydrolysis products in military, environmental, biological, and decontamination samples. Bis(2-chloroethyl)sulfide (mustard or H), a vesicant known for its blistering properties, may be purposely degraded by hydrolysis or may undergo hydrolysis in the environment over time, resulting in the formation of

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thiodiglycol [4], a non-toxic compound that may be easily handled.

Munitions grade mustard formulations typically contain additional sulfur vesicants, including bis(2-chloroethylthio)ethane (sesquimustard or Q), bis[(2-chloroethylthio)ethyl] ether (T) and longer chain sulfur vesicants [5,6]. In some cases the munitions were purposely developed to contain multiple vesicants, with HT and HQ being two munitions containing relatively crude mixtures of H and T and, H and Q, respectively [5]. These munitions grade mustard formulations often contain only 50 to 80% mustard with most of the remaining content being other sulfur vesicants which would decompose to other products. GC–MS under both electron impact (EI) and chemical ionization (CI) conditions has been used for the identification of thiodiglycol in a Kurdish soil sample [7] and several hydrolysis products of longer chain sulfur vesicants have been characterized in hydrolysed samples of munitions grade mustard [8]. The GC chromatographic peak shape of diols generally improves with derivatization and butyldimethylsilyl or trimethylsilyl derivatives of thiodiglycol and the longer chain diols were generated in both studies [7,8].

Thiodiglycol [9–14] and several related longer chain diols [11,13] have been analysed without the need for derivatization by liquid chromatography–mass spectrometry using either electrospray or atmospheric pressure chemical ionization. These studies have focused on the development of screening methods for thiodiglycol and application of low resolution LC–ESI-MS methods for the determination of thiodiglycol in spiked environmental samples. High resolution LC–ESI-MS and LC–ESI-MS/MS offers the analyst the opportunity to accurately determine elemental composition, a considerable advantage when confirming compound presence or when dealing with the identification of previously uncharacterized compounds in environmental samples.

A high resolution LC–ESI-MS/MS method was developed for the determination of thiodiglycol and longer chain diols in aqueous soil extracts. The developed method was used for two different applications involving the identification of mustard hydrolysis products in contaminated soil. In the first application the LC–ESI-MS/MS method was used to identify thiodiglycol and nine longer chain diols in soil samples taken at different locations and depths from a former mustard storage site as part of an ongoing environmental assessment. Preliminary LC–ESI-MS data indicated the presence of thiodiglycol, three longer chain diols and a number of other compounds, the results of which were incorporated into a published conference proceeding that focused on the comparative advantages of GC–MS and LC–ESI-MS for chemical warfare agent analyses [15]. In-depth analysis of these same soil samples by high resolution LC–ESI-MS/MS in the current investigation resulted in the characterization of five additional longer chain diols. The second application incorporated the developed LC–ESI-MS/MS method as part of a new analytical approach developed for the rapid determination of chemical warfare agent contamination in spiked soil samples suspected to contain chemical and/or biological warfare agents.

2. Experimental

2.1. Former mustard storage site soil samples

A portion of each of six soil samples was weighed (1.5–2.0 g) and ultrasonically extracted with 2 mL water in a 15 mm × 125 mm screw-capped, Teflon-lined, glass culture tube for 10 min. The contents were centrifuged for 10 min at 2000 rpm and an aliquot of the aqueous layer (1 mL) was removed and stored in a 1.8 mL screw-capped, Teflon-lined, glass sample vial prior to LC–ESI-MS analysis. (Note: Higher speed centrifugation, required for the spiked Ottawa sand described below, was not required to remove the fines in the aqueous layer above these soil samples).

2.2. Spiked Ottawa sand samples

Ottawa sand samples (3.0 g) were each weighed into 15 mm × 125 mm screw-capped, Teflon-lined, glass culture tubes. The sand samples were then spiked with either triethyl phosphate, isopropyl methylphosphonic acid or thiodiglycol (25 μL aliquot of a 10 mg/mL solution in water) and allowed to stand at room temperature for 1 h to simulate collected contaminated soil samples.

The spiked samples were ultrasonically extracted with water (10 mL) in the glass culture tubes for 10 min and then centrifuged in the same tubes at 2000 rpm for 10 min to settle out most of the sand. An aliquot of the aqueous layer (6–7 mL) was removed and transferred into a screw-capped, Teflon-lined, 20 mL glass scintillation vial. The lid was left finger-tight and the vial was autoclaved for 2 h at 121 °C at 15 psi (liquid cycle). The sterilized aqueous extract was allowed to cool and an aliquot (1 mL) was removed, transferred into a 1.5 mL plastic microcentrifuge tube, and centrifuged for 10 min at 10,000 rpm. A portion of the resulting extract was removed and stored in a 1.8 mL screw-capped, Teflon-lined, glass sample vial prior to LC–ESI-MS analysis.

An aliquot (1 mL) from the initial aqueous soil extracts was taken prior to autoclaving for comparative purposes. It was transferred into a 1.5 mL plastic microcentrifuge tube, and centrifuged for 10 min at 10,000 rpm. A portion of the resulting extract was removed and stored in a 1.8 mL screw-capped Teflon-lined glass sample vial prior to LC–ESI-MS analysis.

2.3. LC–ESI-MS and LC–ESI-MS/MS analyses

Chromatographic separations were performed with a Waters CapLC using a 5–75%B gradient over 30 min and a flow rate of 10 μL/min. The following solvent compositions were prepared for the mobile phase: solvent A (0.1% trifluoroacetic

acid (TFA) in water) and solvent B (0.1% TFA in acetonitrile/water, 95:5). All LC separations were performed with a MicroTech 150 mm × 0.32 mm i.d. fused-silica capillary column packed with Zorbax C_{18} SB (5 μm particle size). The CapLC autosampler was used to introduce 1–3 μL samples of the aqueous soil extracts.

LC–ESI-MS and LC–ESI-MS/MS data were acquired using a Waters/Micromass QTOF Ultima tandem mass spectrometer equipped with a Z-spray electrospray interface. The electrospray capillary was operated at 1 kV with a sampling cone voltage of 35 V. The collision energy was maintained at 5 V for LC–ESI-MS operation and was varied from 2 to 12 V (depending on the precusor ion selected) for LC–ESI-MS/MS operation. Argon was continually flowing into the collision cell at 11 psi during both LC–ESI-MS and LC–ESI-MS/MS operation. Nitrogen desolvation gas was introduced into the interface (80 °C) at a flow rate of 500 L/h and nitrogen cone gas was introduced at a flow rate of 50 L/h. ESI-MS data were acquired from 40 to 700 Da (1 s with a 0.1 s interscan delay) and ESI-MS/MS (product mass spectra) data were acquired from 40 to 350 Da (1 s with a 0.1 s interscan delay). All data were acquired in the continuum mode with a resolution of 9000 (V-mode, 50% valley definition) using a lock mass.

3. Results and discussion

3.1. Analysis of soil from a former mustard storage site

During World War II over 700 tonnes of the chemical warfare agent mustard were shipped to DRDC Suffield and stored in lead-lined concrete vaults. In the early 1970s it was decided that this stockpile of mustard would be destroyed by hydrolysis. Batch hydrolysis using 1000 gallons of mustard, 5000 pounds of lime ($Ca(OH)_2$) and 2500 gallons of water was carried out according to a method developed at DRDC Suffield. Following batch hydrolysis the mustard hydrolysate was transferred from the reaction vessel into one of five empty storage vaults. Although the majority of the mustard hydrolysate was removed from the vaults, a residual amount remained. The remaining hydrolysate was contained and buried with the remains of the vault after mustard destruction. Monitoring wells were established at the site to enable future water sampling. Sampling was performed in 1984, 1986, 1987, and 1997 at which times thiodiglycol was found at 3.9, 0.6, 2.2, and 0.2 mg/mL levels, respectively [11,16], in the water at one of the locations near the buried vaults. The 1997 aqueous sample containing thiodiglycol was analysed by LC–ESI-MS and found to also contain a small amount of the hydrolysis product of bis[(2-chloroethylthio)ethyl] ether (T), 6-oxa-3,9-dithia-1,11-undecanediol [11]. Hexane extracts of aqueous samples were analysed by capillary column gas chromatography–mass spectrometry under electron impact (EI) and isobutane chemical ionization (CI) conditions. Mustard was not detected, but the extracts did contain a number of sulfur containing compounds [16].

In 2002, a consultant was contracted to take numerous soil samples at different locations and depths at the former mustard storage site. Six of the collected soil sample were selected by the consultant for GC–MS and LC–ESI-MS analysis. The headspace above the collected soil samples was sampled for safety reasons using a Chemical Agent Monitor (handheld military chemical warfare agent detection device). Mustard was not detected with the Chemical Agent Monitor and was not detected during GC–MS of the dichloromethane extracts of the soil samples [15]. Preliminary LC–ESI-MS data indicated the presence of thiodiglycol and three longer chain diols in aqueous extracts of these soil samples [15]. However, this approach was not sensitive or selective enough to enable characterization of the remaining components in these soil extracts.

A recently developed sample handling and LC–ESI-MS analysis method for soils contaminated with sarin, soman and their hydrolysis products was used with slight modification for thiodiglycol determination. Aqueous extraction and LC–ESI-MS analysis offered significantly improved recoveries over dichloromethane extraction, derivatization and GC–MS analysis for the determination of organophosphorus chemical warfare agent hydrolysis products in soil [17]. A similar advantage was expected for the hydrolysis product of mustard, thiodiglycol. A sandy clay loam, a loamy sand, and two control soil samples taken from near the former mustard storage site, were spiked in triplicate at the 20 μg/g level with thiodiglycol to estimate recovery efficiency. Thiodiglycol recovery, while variable due to differences in soil composition, was sufficient to confirm the presence of this chemical warfare agent hydrolysis product in contaminated soils. It was recovered from all four soils, with 52 ± 4% and 51 ± 3% efficiency for the two control soils, 85 ± 7% efficiency for the sandy clay loam and 54 ± 6% efficiency for the loamy sand [15].

The aqueous extracts of the soil samples were screened for the presence of thiodiglycol by LC–ESI-MS. Fig. 1 illustrates the LC–ESI-MS total-ion-current chromatogram obtained for one of two soil sample extracts found to contain thiodiglycol and related mustard hydrolysis products (compounds identified in Table 1). This sample was selected for all subsequent LC–ESI-MS and LC–ESI-MS/MS investigations as it contained the same sample components as the other contaminated soil sample but in slightly higher concentration. Thiodiglycol was detected in water extracts of this soil sample at approximately 300 μg/g (200 μg/g in the second soil sample containing thiodiglycol). A major sample component(s) with a retention time in the 2–3 min range was also detected in the water extract of all the soil samples, including those that did not contain thiodiglycol, but it did not appear to contain any compounds associated with mustard hydrolysis and the component(s) was not identified.

High resolution LC–ESI-MS/MS data were acquired with collision energies between 2 and 12 V in order to determine an appropriate setting for each diol that resulted in the acquisition of product mass spectra containing both precursor and

Table 1
Mass spectrometric identification of organosulfur compounds in a soil sample taken from a former mustard storage site

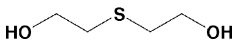
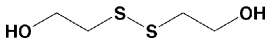
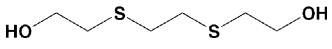
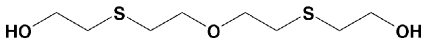
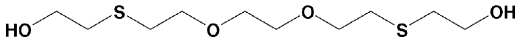
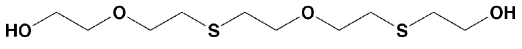
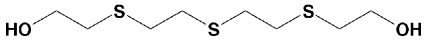
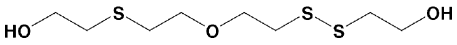
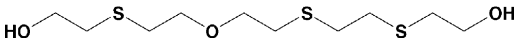
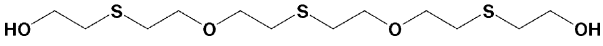
Peak #[a]	Compound name	Ion identity	Observed mass (Da)[b]	Theoretical mass (Da)	Error (Da)
1	Thiodiglycol 	$[M+H^+]$	123.0480	123.0480	0.0000
		$[M+H-H_2O]^+$	105.0377	105.0374	0.0003
2	Bis(hydroxyethyl)disulfide 	$[M+H]^+$	155.0197	155.0200	0.0003
		$[M+H-H_2O]^+$	137.0098	137.0095	0.0003
3	3,6-Dithia-1,8-octanediol 	$[M+H]^+$	183.0518	183.0513	0.0005
		$[M+H-H_2O]^+$	165.0412	165.0408	0.0004
		$[M+H-HOC_2H_5]^+$	137.0101	137.0095	0.0006
		$[M+H-HOC_2H_4SH)^+$	105.0373	105.0374	0.0001
4	6-Oxa-3,9-dithia-1,11-undecanediol 	$[M+H]^+$	227.0777	227.0775	0.0002
		$[M+H-H_2O]^+$	209.0668	209.0670	0.0002
		$[M+H-HOC_2H_5]^+$	181.0360	181.0357	0.0003
		$[M+H-HOC_2H_4SC_2H_4OH]^+$	105.0373	105.0374	0.0001
5	6,9-Dioxa-3,12-dithia-1,14-tetradecanediol 	$[M+H]^+$	271.1044	271.1038	0.0006
		$[M+H-H_2O]^+$	253.0934	253.0932	0.0002
		$[M+H-HOC_2H_5]^+$	225.0631	225.0619	0.0012
		$[M+H-HOC_2H_4SC_2H_4OH]^+$	149.0617	149.0636	0.0019
		$[MH-HOC_2H_4SC_2H_4OC_2H_4OH]^+$	105.0382	105.0374	0.0008
6	6,12-Dioxa-3,9-dithia-1,14-tetradecanediol 	$[M+H]^+$	271.1040	271.1038	0.0002
		$[M+H-H_2O]^+$	253.0937	253.0932	0.0005
		$[M+H-HOC_2H_5]^+$	225.0628	225.0619	0.0009
		$[M+H-HOC_2H_4OH]^+$	209.0688	209.0670	0.0018
		$[M+H-HOC_2H_4OC_2H_5]^+$	181.0382	181.0357	0.0025
		$[M+H-HOC_2H_4OC_2H_4SH]^+$	149.0648	149.0636	0.0012
		$[M+H-HOC_2H_4OC_2H_4SC_2H_4OH]^+$	105.0384	105.0374	0.0010
7	3,6,9-Trithia-1,11-undecanediol 	$[M+H]^+$	243.0560	243.0547	0.0013
		$[M+H-H_2O]^+$	225.0465	225.0441	0.0024
		$[M+H-HOC_2H_5]^+$	197.0163	197.0128	0.0035
		$[M+H-HOC_2H_4SH]^+$	165.0432	165.0408	0.0024
		$[M+H-HOC_2H_4SC_2H_5]^+$	137.0109	137.0095	0.0014
		$[M+H-HOC_2H_4SC_2H_4SH]^+$	105.0377	105.0374	0.0003

Table 1 (*Continued*)

Peak #[a]	Compound name	Ion identity	Observed mass (Da)[b]	Theoretical mass (Da)	Error (Da)
	6-Oxa-3,9,10-trithia-1,12-dodecanediol				
8		$[M+H]^+$	259.0489	259.0496	0.0007
		$[M+H–H_2O]^+$	241.0397	241.0390	0.0007
		$[M+H–HOC_2H_5]^+$	213.0109	213.0077	0.0032
		$[M+H–SC_2H_4]^+$	199.0478	199.0462	0.0016
		$[M+H–HOC_2H_4SH]^+$	181.0370	181.0357	0.0013
		$[M+H–HOC_2H_4SC_2H_4OH]^+$	137.0104	137.0095	0.0009
		$[C_4H_9S_1O_1]^+$	105.0373	105.0374	0.0001
	6-Oxa-3,9,12-trithia-1,14-tetradecanediol				
9		$[M+H]^+$	287.0813	287.0809	0.0004
		$[M+H–H_2O]^+$	269.0699	269.0703	0.0004
		$[M+H–HOC_2H_4SC_2H_4OH]^+$	165.0416	165.0408	0.0008
		$[M+H–HOC_2H_4SC_2H_4OC_2H_5]^+$	137.0084	137.0095	0.0011
		$[M+H–HOC_2H_4SC_2H_4OC_2H_4SH]^+$	105.0389	105.0374	0.0015
	6,12-Dioxa-3,9,15-trithia-1,17-heptadecanediol				
10		$[M+H]^+$	331.1072	331.1071	0.0001
		$[M+H–H_2O]^+$	313.0967	313.0966	0.0001
		$[M+H–HOC_2H_4SC_2H_4OH]^+$	209.0680	209.0670	0.0010
		$[M+H–HOC_2H_4SC_2H_4OC_2H_5]^+$	181.0376	181.0357	0.0019
		$[M+H–HOC_2H_4SC_2H_4OC_2H_4SH]^+$	149.0632	149.0636	0.0004
		$[M+H–HOC_2H_4SC_2H_4OC_2H_4S\ C_2H_4OH]^+$	105.0378	105.0374	0.0004

$[M+H]^+$ data were obtained during LC–ESI-MS analysis. $[M+H]^+$ product ion data for each compound were obtained during LC–ESI-MS/MS analysis.

[a] Refer to Fig. 1.

[b] Average of (typically) four to seven full scans (40–350 Da) across the chromatographic peak.

structurally significant product ion data. Figs. 2–4 illustrate representative ESI-MS/MS data acquired for each of the diols at the selected collision energy setting and Table 1 summarizes the acquired high resolution data that was used for identification purposes. $[M+H]^+$ data were acquired during high resolution LC–ESI-MS analyses and $[M+H]^+$ product ion data were acquired during high resolution LC–ESI-MS/MS. The average error and standard deviation associated with the 10 $[M+H]^+$ measurements was 0.0004 ± 0.0004 (equivalent to 2 ppm at *m/z* 200). Slightly larger average errors, due in part to weaker signals for some of the product ions, were observed for the 38 product ions measured. An average error and standard deviation of 0.0010 ± 0.0009 (equivalent to 5 ppm at *m/z* 200) was associated with these mass measurements.

Fig. 2 illustrates typical ESI-MS/MS data for thiodiglycol, the hydrolysis product of mustard, and the hydrolysis products of three longer chain sulfur vesicants, bis(2-chloroethyl) disulfide, bis(2-chloroethylthio)ethane (sesquimustard or Q), and bis[(2-chloroethylthio)ethyl] ether (T) initially identified by LC–ESI-MS in the soil extracts [15]. A significant product ion due to the loss of water for the precusor ion ($[M+H]^+$) was observed for these and the other diols, along with structurally significant product ions that could be used to establish chain length. The ESI-MS/MS data obtained for the hydrolysis product of sesquimustard, 3,6-dithia-1,8-octanediol, was typical of a longer chain diol. Product ions due to $[M+H-HOC_2H_5]^+$ and $[M+H-HOC_2H_4SH]^+$ were observed at *m/z* 137 and 105, respectively. The product ion at *m/z* 105, due to protonated 1,4-thioxane, proved to be a common product ion for all the diols analysed and could be considered for extract screening purposes.

Fig. 3 illustrates the different ESI-MS/MS data obtained for two isomers with identical elemental composition that have not been previously characterized. 6,9-Dioxa-3,12-dithia-1,14-tetradecanediol (Fig. 3a) and 6,12-dioxa-3,9-dithia-1,14-tetradecanediol (Fig. 3b), two compounds which differ in their relative O and S positioning, were identified based on the acquired ESI-MS/MS data. Both compounds exhibited common ions due to $[M+H–H_2O]^+$ and $[M+H–HOC_2H_5]^+$ at *m/z* 253 and *m/z* 225 and a

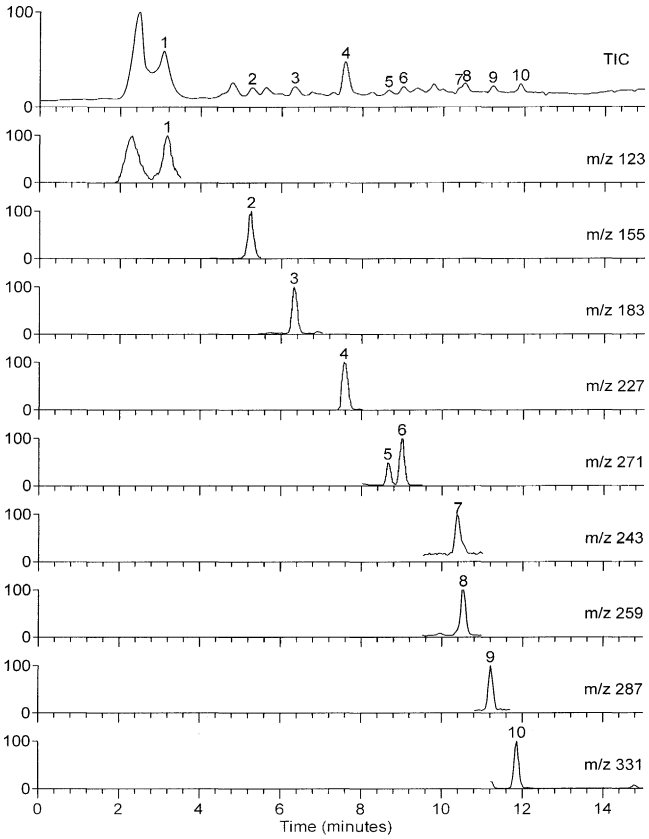


Fig. 1. Packed capillary LC–ESI-MS and LC–ESI-MS/MS chromatograms obtained for an aqueous extract of a soil sample taken from a former mustard storage site. The top chromatogram illustrates the total-ion-current (40–700 Da) obtained during LC–ESI-MS analysis. The lower nine chromatograms illustrate the LC–ESI-MS/MS chromatograms that were obtained during a single analysis, with the mass of the selected precursor ion $[M+H^+]$ changing with time. Numbered sample components are identified in Table 1.

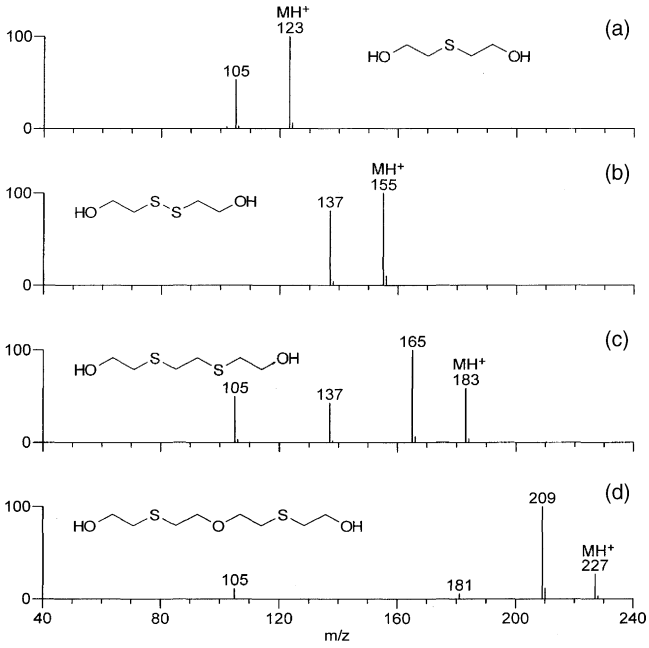


Fig. 2. ESI-MS/MS product mass spectra obtained for the $[M+H]^+$ ion of (a) thiodiglycol (collision energy: 2 V), (b) bis(hydroxyethyl)disulfide (collision energy: 2 V), (c) 3,6-dithia-1,8-octanediol (collision energy: 4 V), and (d) 6-oxa-3,9-dithia-1,11-undecanediol (collision energy: 5 V) during LC–ESI-MS/MS analysis of an aqueous extract of a soil sample taken from a former mustard storage site.

protonated 1,4-thioxane ion at *m/z* 105. 6,12-Dioxa-3,9-dithia-1,14-tetradecanediol was differentiated from 6,9-dioxa-3,12-dithia-1,14-tetradecanediol by the presence of ions at *m/z* 209 and *m/z* 181 due to $[M+H–HOC_2H_4OH]^+$ and $[M+H–HOC_2H_4OC_2H_5]^+$. The remaining ions at *m/z* 149 and 105 due to $[M+H–HOC_2H_4OC_2H_4SH]^+$ and $[M+H–HOC_2H_4OC_2H_4SC_2H_4OH]^+$ suggested a structure consistent with alternating O and S positioning within the diol. The ion at *m/z* 149 for 6,9-dioxa-3,12-dithia-1,14-tetradecanediol results from the loss of 1,4-thioxane (and water) from one end of the protonated molecule. The only possible relative positioning of the S and O atoms, other than the alternating pattern proposed for the other isomer, would be a structure with both oxygens occupying the central positions in the diol.

Fig. 4 illustrates the ESI-MS/MS data for the remaining longer chain diols that could not be identified in the prior LC–ESI-MS study [15]. Table 1 indicates the identity of the product ions that were observed, leading to identification of 3,6,9-trithia-1,11-undecanediol (Fig. 4a), 6-oxa-3,9,10-trithia-1,12-dodecanediol (Fig. 4b), 6-oxa-3,9,12-trithia-1,14-tetradecanediol (Fig. 4c), and 6,12-dioxa-3,9,15-trithia-1,17-heptadecanediol (Fig. 4d) in an aqueous extract of the soil sample.

The relative positioning of the S and O atoms was augmented by the acquisition of complementary ESI-MS/MS data for the $[M+H–H_2O]^+$ precursor ions for each of the diols as well. In all cases the best product mass spectra were obtained with slightly higher collision energies (7–12 V) than were used to acquire $[M+H]^+$ product mass spectra. Fig. 5 illustrates this ESI-MS/MS data for two of the diols, 3,6,9-trithia-1,11-undecanediol (Fig. 5a) and 6-oxa-3,9,12-trithia-

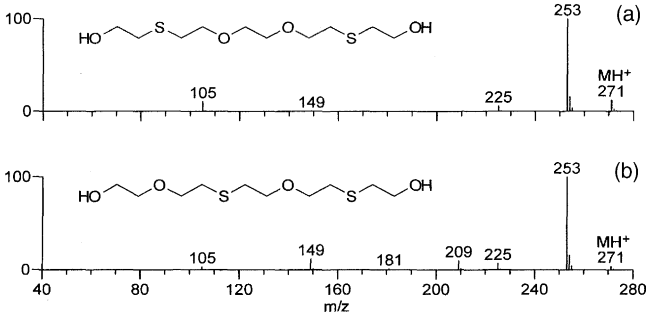
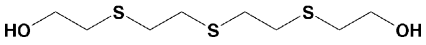
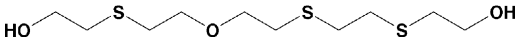


Fig. 3. ESI-MS/MS product mass spectra obtained for the $[M+H]^+$ ion of (a) 6,9-dioxa-3,12-dithia-1,14-tetradecanediol (collision energy: 7 V) and (b) 6,12-dioxa-3,9-dithia-1,14-tetradecanediol (collision energy: 7 V) during LC–ESI-MS/MS analysis of an aqueous extract of a soil sample taken from a former mustard storage site.

Table 2
Product ion data obtained for $[M+H–H_2O]^+$ precusor ions of 3,6,9-trithia-1,11-undecanediol and 6-oxa-3,9,12-trithia-1,14-tetradecanediol (found in soil sample taken from a former mustard storage site)

Peak #[a]	Compound name	Ion identity	Observed mass (Da)[b]	Theoretical mass (Da)	Error (Da)
	3,6,9-Trithia-1,11-undecanediol				
7		$[M+H–H_2O-C_2H_4]^+$	197.0125	197.0128	0.0003
		$[M+H–H_2O-C_2H_4S]^+$	165.0399	165.0408	0.0009
		$[M+H–H_2O-C_2H_4SC_2H_4]^+$	137.0103	137.0095	0.0008
		$[M+H–H_2O-C_2H_4SC_2H_4S]^+$	105.0373	105.0374	0.0001
	6-Oxa-3,9,12-trithia-1,14-tetradecanediol				
9		$[M+H–H_2O-C_2H_4]^+$	241.0386	241.0390	0.0004
		$[M+H–H_2O-C_2H_4S]^+$	209.0667	209.0670	0.0003
		$[M+H–H_2O-C_2H_4SC_2H_4)^+$	181.0383	181.0357	0.0026
		$[M+H–H_2O-C_2H_4SC_2H_4S)^+$	149.0630	149.0636	0.0006
		$[M+H–H_2O-C_2H_4SC_2H_4SC_2H_4O]^+$	105.0381	105.0374	0.0007

[a] Refer to Fig. 1.
[b] Average of (typically) 4 to 7 full scans (40 to 350 Da) across the chromatographic peak.

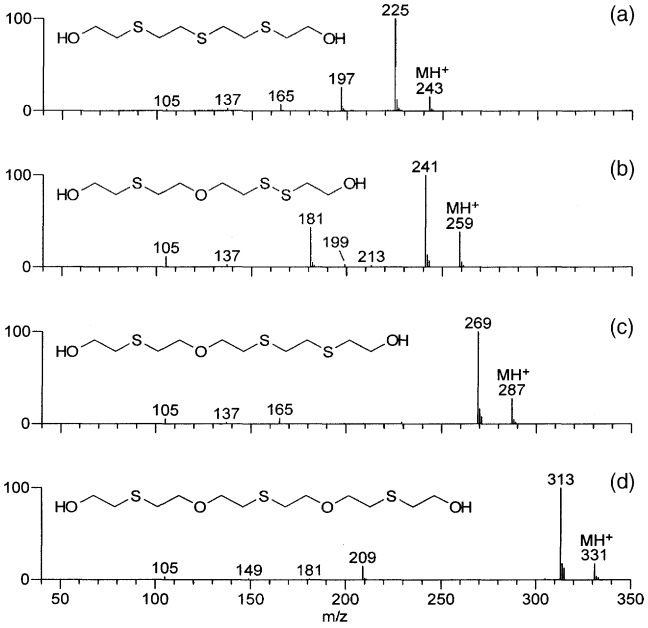


Fig. 4. ESI-MS/MS product mass spectra obtained for the $[M+H]^+$ ion of (a) 3,6,9-trithia-1,11-undecanediol (collision energy: 5 V), (b) 6-oxa-3,9,10-trithia-1,12-dodecanediol (collision energy: 5 V), (c) 6-oxa-3,9,12-trithia-1,14-tetradecanediol (collision energy: 5 V) and (d) 6,12-dioxa-3,9,15-trithia-1,17-heptadecanediol (collision energy: 7 V) during LC–ESI-MS/MS analysis of an aqueous extract of a soil sample taken from a former mustard storage site.

1,14-tetradecanediol (Fig. 5b) and Table 2 summarizes product ion identities and associated mass measurements. In both cases it was possible to assign the relative O and S positioning based on the observed product ions. The mass differences in the product ions of 3,6,9-trithia-1,11-undecanediol differed by 28 Da (C_2H_4) and 32 Da (S), consistent with the assigned structure where sulfur atoms were present at positions 3, 6, and 9. The same initial alternating pattern for the product ions, differing in mass by 28 Da (*m*/*z* 241), followed by 32 Da (*m*/*z* 209), 28 Da (*m*/*z* 181), and 32 Da (*m*/*z* 149) was also observed for 6-oxa-3,9,12-trithia-1,14-tetradecanediol, indicating the presence of a sulfur atom at positions 9 and 12. In addition, there was a mass difference of 44 Da (OC_2H_4) between the product ions at *m*/*z* 149 and *m*/*z* 105 (protonated 1,4-thioxane), indicating an oxygen atom at the 6 position. The remaining sulfur was assigned to the 3 position.

Additional product ions, all of which could be assigned based on their accurate mass data, were also observed in

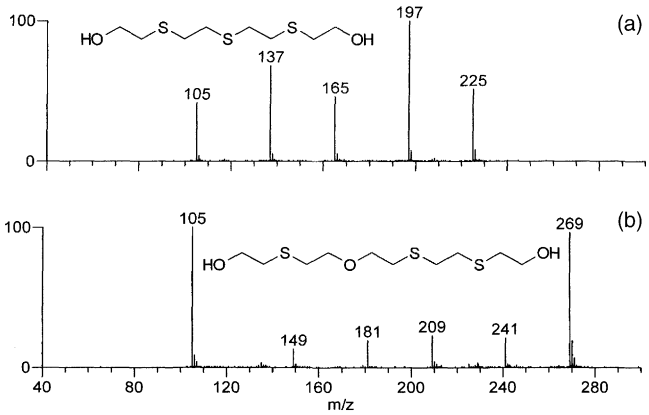


Fig. 5. ESI-MS/MS product mass spectra obtained for the $[M+H–H_2O]^+$ ion of (a) 3,6,9-trithia-1,11-undecanediol (collision energy: 10 V) and (b) 6-oxa-3,9,12-trithia-1,14-tetradecanediol (collision energy: 10 V) during LC–ESI-MS/MS analysis of an aqueous extract of a soil sample taken from a former mustard storage site.

the $[M+H–H_2O]^+$ product mass spectra of several other diols. A single product ion at *m*/*z* 87 (relative intensity ~3%) due to loss of H_2O was observed for thiodiglycol. Three mass deficient, product ions at *m*/*z* 119 (relative intensity ~6%), 109 (relative intensity ~60%), and 91 (relative intensity ~4%), due to loss of H_2O, C_2H_4, and HOC_2H_5, respectively, from the $[M+H–H_2O]^+$ precursor ion were observed for bis(hydroxyethyl)disulfide. Both 6,9-dioxa-3,12-dithia-1,14-tetradecanediol and 6,12-dioxa-3,9-dithia-1,14-tetradecanediol exhibited a weak product ion at *m*/*z* 89 (relative intensity ~1–2%) due to protonated 1,4-dioxane, $[C_4H_9O_2]^+$, consistent with the fact that these were the only two diols containing $HO–C_2H_4O–C_2H_4–$ or $–O–C_2H_4–OC_2H_4–$ substructures that could give rise to this ion. It should be noted that an alternative ion structure with the same nominal mass, $[C_4H_9S]^+$, was not considered as its theoretical mass was at least an order of magnitude higher than the errors typically associated with the observed mass measurements.

3.2. Rapid identification of chemical warfare agent contamination

Samples that may be contaminated with a combination of chemical and/or biological warfare agents pose a unique problem to chemical and biological specialists tasked with determining the presence of chemical or biological warfare agents. Such a sample would initially be received into biocontainment level 3 (BL-3) at DRDC Suffield where biological identification may be safely carried out. Under normal circumstances, a sample extract requiring removal from BL-3 for use in a BL-2 or chemical laboratory would be sterilized by 0.22 μm filtration. A sterility check of the filtered extract would then be conducted in BL-3, a process that may take up to two weeks. During this time chemical detection within BL-3 would be limited to devices such as the Chemical Agent Monitor. A more rapid sample handling and analysis method that could determine the presence (or absence) of chemical warfare agent contamination would be valuable to the defence community or during crime scene investigations. Rapid determination of the absence of chemical warfare agent would also benefit those working in BL-3 as the level of precautions required could be reduced.

The most rapid and effective means of sterilizing a sample contaminated with biological warfare agents that allows removal of the sample from BL-3, without a sterility check, involves autoclaving the sample for 2 h. Any sample undergoing this process is necessarily exposed to water vapour at a high temperature, making the likelihood of chemical warfare agent hydrolysis high. An analytical method for chemical warfare agent identification must therefore be able to identify the principal hydrolysis products of the common chemical warfare agents. LC–ESI-MS may be used for this purpose and has the added benefit of being able to also detect and identify intact organophosphorus chemical warfare agents and many related compounds in aqueous sample extracts [15,17].

The developed sample handling method involves aqueous extraction of the soil (or other media) sample in BL-3, followed by sterilization of the aqueous extract in the autoclave between BL-3 and the chemistry laboratories. The sterilized container and aqueous contents can then be safely manipulated in the chemical analysis laboratory and analysed for the presence or absence of chemical warfare agents, their hydrolysis products or related compounds.

Three compounds were selected for evaluation of the proposed sample handling method, based in part on prior experiences with aqueous extraction and analysis of contaminated soil samples [17]. Triethyl phosphate, an organophosphorus compound that has been used as a nerve agent simulant, was selected to investigate the extent of hydrolysis during autoclaving, as this compound is much more resistant to hydrolysis than the common organophosphorus chemical warfare agents (nerve agents). Isopropyl methylphosphonic acid, the hydrolysis product of the nerve agent sarin, was selected as a typical nerve agent hydrolysis product and to investigate the likelihood of further hydrolysis to methylphosphonic acid during the process. Finally, thiodiglycol was selected since this product would be expected following mustard hydrolysis. It should be noted that mustard cannot be readily detected by LC–ESI-MS, but hydrolyses readily in the presence of water to thiodiglycol, a compound that may be detected by LC–ESI-MS.

All three spiked compounds were readily extracted from the Ottawa sand at the 80 μg/g level (recovery efficiencies not estimated). This spiking level was below typical battlefield contamination levels, estimated to be in the 100–1000 μg/g range, based on a contamination density of 1–10 g/m^2 (soil density about 1 g/cm^3 and a 1 cm sampling depth) and considered typical of soil contamination levels that might be expected hours to days after an attack. Soil samples were extracted with water using ultrasonic vibration and a portion of the aqueous extract was removed and autoclaved to eliminate biological activity. This aqueous extract was centrifuged and analysed by LC–ESI-MS to confirm compound identity.

Fig. 6 illustrates typical chromatograms obtained during LC–ESI-MS and LC–ESI-MS/MS analysis of the soil sample spiked with thiodiglycol. The product mass spectrum acquired for thiodiglycol during LC–ESI-MS/MS analysis (Fig. 6b) was identical to that obtained in Fig. 2a during analysis of the mustard storage site soil samples, confirming the presence of thiodiglycol in the autoclaved extract. Triethyl phosphate underwent some hydrolysis (about 10–20%) to diethyl hydrogen phosphate, suggesting that hydrolysis of nerve agents to their initial acids would be significant following autoclaving. Hydrolysis of isopropyl methylphosphonic acid to methylphosphonic acid, the common hydrolysis product for many nerve agents, was not observed.

The chromatograms obtained for portions of the spiked aqueous extracts that did not undergo autoclaving were similar with two exceptions. The concentration of the spiked

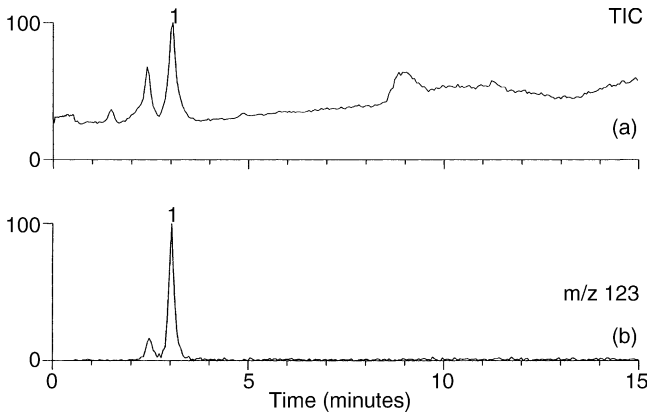


Fig. 6. (a) Packed capillary LC–ESI-MS total-ion-current (40–700 Da) and (b) LC–ESI-MS/MS chromatogram obtained for the $[M+H]^+$ precursor ion (*m/z* 123 for thiodiglycol) during a single analysis of the autoclaved aqueous extract of an Ottawa sand sample spiked with thiodiglycol. The lower chromatogram contains the ion current for *m/z* 123 and the formed product ions.

analyte was lower in these samples since the aqueous extracts underwent a 20% volume reduction during the autoclaving procedure. Finally, hydrolysis product(s) of triethyl phosphate were not found in the spiked aqueous extracts that did not undergo autoclaving, a finding that was consistent with prior experiences.

4. Conclusions

A packed capillary LC–ESI-MS/MS method was developed and applied to the identification of mustard hydrolysis products in aqueous extracts of soil. In the first application the LC–ESI-MS/MS method was used to identify mustard hydrolysis products in soil samples taken at different locations and depths from a former mustard storage site as part of an ongoing environmental assessment. Preliminary LC–ESI-MS data indicated the presence of thiodiglycol, three longer chain diols and a number of other compounds. In-depth analysis of these same soil samples by high resolution LC–ESI-MS/MS using a quadrupole/time-of-flight tandem mass spectrometer resulted in the characterization of five additional longer chain diols. In all cases the hydrolysis products exhibited protonated molecular ions and structurally significant product ions that were used for identification of previously uncharacterized compounds.

The high resolution LC–ESI-MS/MS method was also incorporated into an analytical approach designed to provide rapid chemical warfare agent identification in cases where the chemical and/or biological warfare agent content of a sample is unknown. A sample handling method involving aqueous extraction of the soil sample in biocontainment level 3 (BL-3), followed by autoclave sterilization of the aqueous extract was developed. Once sterilized, the container and aqueous extract can then be safely manipulated outside of BL-3 in the analytical laboratories and may be analysed for the presence or absence of chemical warfare agents, their hydrolysis products or related compounds by LC–ESI-MS/MS. The method was successfully validated with soil samples spiked with thiodiglycol, triethyl phosphate, and isopropyl methylphosphonic acid at the 80 μg/g level. Extension of the developed chemical warfare agent screening method to aqueous extracts of other sample media is anticipated.

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