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A packed capillary liquid chromatography-electrospray ionization mass spectrometry (LC-ESI-MS) method was developed for the identification of chemical warfare agents, their degradation products and related compounds in synthetic tabun samples and in soil samples collected from a former mustard storage site. A number of organophosphorus and organosulfur compounds that had not been previously characterized were identified, based on acquired high-resolution ESI-MS data. At lower sampling cone voltages, the ESI mass spectra were dominated by protonated, sodiated and protonated acetonitrile adducts and/or their dimers that could be used to confirm the molecular mass of each compound. Structural information was obtained by inducing product ion formation in the ESI interface at higher sampling cone voltages. Representative ESI-MS mass spectra for previously uncharacterized compounds were incorporated into a database as part of an on-going effort in chemical warfare agent detection and identification. The same samples were also analyzed by capillary column gas chromatography (GC)-MS in order to compare an established method with LC-ESI-MS for chemical warfare agent identification. Analysis times and full-scanning sensitivities were similar for both methods, with differences being associated with sample matrix, ease of ionization and compound volatility. GC-MS would be preferred for organic extracts and must be used for the determination of mustard and relatively non-polar organosulfur degradation products, including 1,4-thioxane and 1,4-dithiane, as these compounds do not ionize during ESI-MS. Diols, formed following hydrolysis of mustard and longer-chain sulfur vesicants, may be analyzed using both methods with LC-ESI-MS providing improved chromatographic peak shape. Aqueous samples and extracts would, typically, be analyzed by LC-ESI-MS, since these analyses may be conducted directly without the need for additional sample handling and/or derivatization associated with GC-MS determinations. Organophosphorus compounds, including chemical warfare agents, related compounds and lower volatility hydrolysis products may all be determined during a single LC-ESI-MS analysis. Derivatization of chemical warfare agent hydrolysis products and other compounds with hydroxyl substitution would be required prior to GC-MS analysis, giving LC-ESI-MS a definite advantage over GC-MS for the analysis of samples containing chemical warfare agents and/or their hydrolysis products.

Keywords: chemical warfare agent, tabun, mustard, gas chromatography, liquid chromatography, mass spectrometry, electrospray

Introduction

More than 140 State 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 State 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.

Gas chromatography (GC) has been used extensively for the separation and identification of chemical warfare agents, with gas chromatography-mass spectrometry (GC-MS) being used frequently for the characterization of these compounds.[1,2] GC-MS, although suitable for the direct analysis of chemical warfare agents in organic extracts, is usually not preferred for the direct analysis of aqueous samples

or extracts since these samples normally require additional sample handling steps and derivatization prior to analysis.

Liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) is being used increasingly, as ESI-MS data may be used directly to identify chemical warfare agents, their degradation products and related compounds in aqueous samples or extracts. Researchers have developed atmospheric pressure ionization methods (for example, electrospray, ionspray and atmospheric pressure chemical ionization) for the characterization of polar pesticides,[3] organophosphate esters[4] and chemical warfare agents and/or their degradation products.[5–19] These ionization modes have been interfaced to liquid chromatography and capillary electrophoresis (CE), with LC-MS[9–11,13,15–19] and CE-MS[5,12] methods being reported for the identification of lower volatility chemical warfare agent hydrolysis products. Use of this analytical technique has been recently extended to include the identification of chemical warfare agents as well. In the past several years, a number of LC-ESI-MS papers have been published on the identification of chemical warfare agents and their hydrolysis products in aqueous (or snow) samples[13,14,18,19] and aqueous extracts of spiked soil samples[17] during a single analysis.

During the current study, an LC-ESI-MS method was developed for the detection and identification of chemical warfare agents in synthetic tabun samples and in soil samples collected from a former mustard storage site. A number of organophosphorus and organosulfur compounds that had not been previously characterized during LC-ESI-MS analysis were identified, based on the acquired high-resolution ESI-MS data. At lower sampling cone voltages, the ESI mass spectra were dominated by protonated, sodiated and protonated acetonitrile adducts and/or their dimers that could be used to confirm the molecular mass of each compound. Structural information was obtained by inducing product ion formation in the ESI interface at higher sampling cone voltages. LC-ESI-MS has some advantages over GC-MS for chemical warfare agent analysis;[17,18] however, a direct comparison of these techniques on the same samples has not been previously undertaken. During this investigation, GC-MS and LC-ESI-MS analyses were performed on the same samples to enable a more complete comparison of the sensitivity, speed and selectivity of these two chemical warfare agent identification methods.

Experimental

Synthetic tabun samples

A pure sample of tabun, prepared by the Canadian Single Small-Scale Facility at DRDC Suffield, was diluted in water and analyzed by LC-ESI-MS for reference purposes.

GC-MS was compared with LC-ESI-MS for the analysis of chemical defence compounds with a multi-component tabun synthetic sample that had been submitted to the DRDC Suffield Analytical Laboratory for purity analysis by mass spectrometry. The synthetic procedure failed to produce tabun. However, a number of other phosphate and pyrophosphate compounds, similar in structure to tabun, were formed in the reaction vessel. An initial portion (5 μL) of the synthetic tabun sample, used for GC-MS investigations, was diluted 6000-fold with dichloromethane (30 mL). A second portion (5 μL) was taken to dryness and diluted 6000-fold with water (30 mL) and used for LC-ESI-MS analyses.

Former mustard storage site soil samples

In 2002, a consultant was contracted to take soil samples at a number of locations and depths near a former mustard storage site on the Suffield Experimental Range as part of an environmental assessment. The soil samples (**a–f**) were extracted and analyzed by GC-MS and LC-ESI-MS for the presence of mustard and mustard degradation products.

A portion of each soil sample was weighed (1.5 – 2.0 g) and ultrasonically extracted with 4 mL dichloromethane in a 15 × 125 mm screw-capped, Teflon-lined glass culture tube for 10 min. The contents were then centrifuged and an aliquot of the dichloromethane layer (1 mL) was removed and stored in a screw-capped, Teflon-lined 1.8 mL sample vial prior to GC-MS analysis.

A second portion of each soil sample was weighed (1.5–2.0 g) and ultrasonically extracted with 2 mL water in a 15 × 125 mm screw-capped, Teflon-lined glass culture tube for 10 min. The contents were then centrifuged and an aliquot of the aqueous layer (1 mL) was removed and stored in a screw-capped, Teflon-lined 1.8 mL sample vial prior to LC-ESI-MS analysis.

GC-MS analysis

Dichloromethane samples and extracts were analyzed by GC-MS (Agilent 5973N under EI conditions: 70 eV, 0.035 mA, 230°C) using a 15 m × 0.25 mm i.d. J&W DB-35MS capillary column and the following temperature program: 40°C (2 min), 10°C min^{-1}, 280°C (5 min). All injections (1 μL) were cool on-column at 43°C. The mass spectrometer was operated in full-scanning mode and scanned from *m/z* 40 to 400 at 2.08 scans sec^{-1} (unit resolution).

LC-ESI-MS analysis

LC-ESI-MS data were acquired using a Micromass LCT time-of-flight mass spectrometer equipped with the Z-spray electrospray interface. The electrospray capillary was operated at 3.2 kV with sampling cone voltages in the 20 to 50 volts range. Nitrogen desolvation gas was introduced into the interface (80°C) at a flow rate of 480 L h^{-1}. Nitrogen nebulizer gas was introduced at a flow rate of 66 L h^{-1}. ESI-MS data were acquired from *m/z* 70 to 700 (1 sec) in the continuum mode with a resolution of 5000 (50% valley definition).

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 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). Chromatographic separations were performed with an Applied Biosystems model 140B dual syringe pump. During the analysis of the multi-component tabun synthetic sample (5 μL injection volume), a gradient of 1% to 40% B (30 min) was used to improve chromatographic separation. In order to minimize dead-volume effects and ensure reproducible mixing, the mobile phase was delivered at 200 μL min^{-1} and split prior to the injector so that the flow through the column was 16 μL min^{-1}. A 5 to 75% B (30 min) gradient was used for the analysis of the aqueous soil extracts and the tabun reference sample (1 μL injection volume). During these analyses, the mobile phase was delivered at 150 μL min^{-1} and split prior to the injector so that the flow through the column was 10 μL min^{-1}.

Results and discussion

Synthetic tabun samples

Tabun-containing samples have been analyzed on a number of occasions and several GC-MS and LC-ESI-MS papers containing acquired mass spectra have been published. An initial paper[20] containing the EI and ammonia chemical ionization (CI) mass spectra for tabun and five related compounds was followed several years later by a comprehensive paper, containing the EI and ammonia CI mass spectra of tabun and 20 related compounds found during GC-MS analysis of a munitions-grade tabun sample.[21] A similar munitions-grade tabun sample was recently analyzed by LC-ESI-MS using a time-of-flight mass spectrometer. Nineteen phosphates and pyrophosphates were characterized by ESI-MS using both higher and lower sampling cone voltages.[18]

Most syntheses are successful, with the data presented in Figure 1 being typical. Tabun was the only significant sample component, exhibiting ESI-MS data consistent with previously published data.[14] The mass spectrum obtained with a sampling cone voltage of 30 volts exhibited a protonated molecular ion at *m/z* 163 and a product ion at *m/z* 135 due loss to of C_2H_4 from the protonated molecular ion. The corresponding acetonitrile adducts were also observed at *m/z* 204 and *m/z* 176. Sample extracts often contain other species (for example, sodium, amines) and, if sufficient levels are present, adducts related to these species could also be expected.[22]

The synthesized tabun sample used in this study was initially screened by GC-MS and LC-ESI-MS for purity and later for method comparison purposes. Tabun was not detected, but in its place were 12 related organophosphorus compounds, including six compounds that had not been previously characterized during prior tabun studies.[18,20,21] The characterization of these compounds could prove valuable during future analyses since the presence of chemical warfare agent degradation products and related compounds in

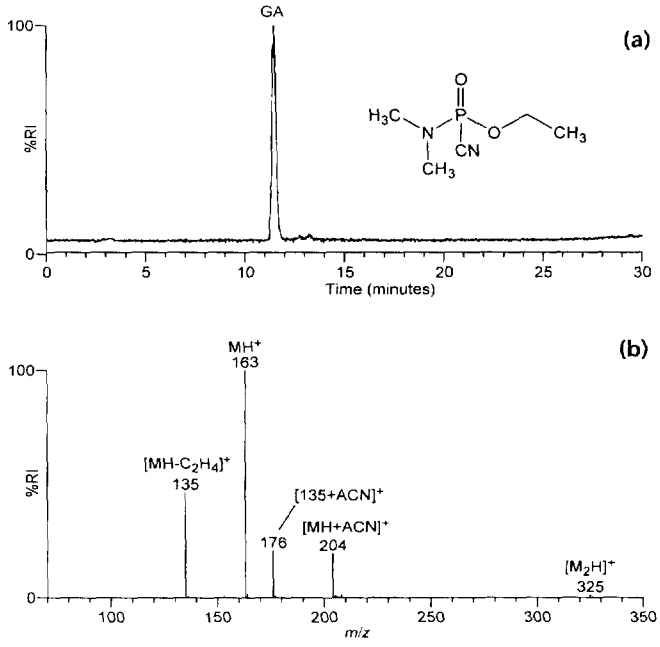


Figure 1. (a) LC-ESI-MS total-ion current (*m/z* 90 to 400) chromatogram for tabun (GA) reference standard. (b) ESI-MS data obtained for tabun with a sampling cone voltage of 30 volts.

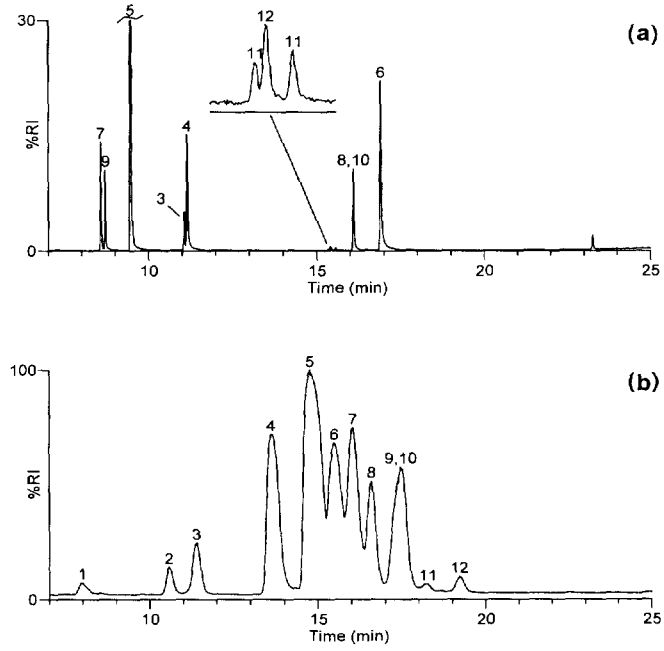


Figure 2. (a) GC-MS and (b) LC-ESI-MS total-ion current chromatograms obtained for diluted synthetic tabun samples. Compounds are identified in Table 1.

Table 1. Compounds identified in tabun synthetic sample by LC-ESI-MS.

Peak no.	Compound name	Ion	Observed mass (Da) (mean ± SD)	Theoretical mass (Da)	Average error (Da)
1	Ethyl phosphoric tetramethylphosphorodiamidic anhydride	MH^+ $[MH - C_2H_4]^+$ $[(Me_2N)_2P(OH)_2]^+$ $[(Me_2N)_2PO]^+$	261.0741 ± 0.0012 233.0464 ± 0.0019 153.0791 ± 0.0013 135.0695 ± 0.0010	261.0769 233.0456 153.0793 135.0687	0.0028 0.0008 0.0002 0.0008
2	Octamethyltetramidotriphosphoric acid (or isomer)	MH^+ $[MH - HNMe_2]^+$	367.0979 ± 0.0017 322.0473 ± 0.0019	367.1065 322.0487	0.0086 0.0014
3	Tetramethylphosphorodiamidic cyanide	MH^+	162.0979 ± 0.0017	162.0796	0.0017
4	Hexamethylphosphorotriamide	MH^+ $[(Me_2N)_2PO]^+$	180.1244 ± 0.0010 135.0681 ± 0.0004	180.1266 135.0687	0.0022 0.0006
5	Ethyl tetramethylphosphoramidate	MH^+ $[MH - C_2H_4]^+$	181.1081 ± 0.0014 153.0779 ± 0.0002	181.1106 153.0793	0.0025 0.0014
6	Bis(tetramethylphosphorodiamidic) anhydride	MH^+ $[MH - HNMe_2]^+$	287.1362 ± 0.0014 242.0815 ± 0.0037	287.1402 242.0823	0.0040 0.0008
7	Diethyl dimethylphosphoramidate	MH^+ $[MH - C_2H_4]^+$ $[MH - (C_2H_4)_2]^+$	182.0924 ± 0.0013 154.0635 ± 0.0002 126.0307 ± 0.0002	182.0946 154.0633 126.0320	0.0022 0.0002 0.0013
8	Ethyl dimethylphosphoramidic tetramethylphosphorodiamidic anhydride	MH^+ $[MH - HNMe_2]^+$ $[MH - HNMe_2 - C_2H_4]^+$	288.1212 ± 0.0012 243.0647 ± 0.0004 215.0359 ± 0.0008	288.1242 243.0664 215.0351	0.0030 0.0017 0.0008
9	Triethyl phosphate	MH^+ $[MH - C_2H_4]^+$ $[MH - (C_2H_4)_2]^+$ $[MH - (C_2H_4)_3]^+$	183.0776 ± 0.0008 155.0469 ± 0.0005 127.0155 ± 0.0001 98.9848 ± 0.0003	183.0786 155.0473 127.0160 98.9847	0.0010 0.0004 0.0005 0.0001
10	Diethyl phosphoric tetramethylphosphorodiamidic anhydride	MH^+ $[MH - C_2H_4]^+$ $[MH - HNMe_2]^+$ $[MH - (C_2H_4)_2]^+$ $[(Me_2N)_2P(OH)_2]^+$ $[(Me_2N)_2PO]^+$	289.1049 ± 0.0011 261.0790 ± 0.0006 244.0554 ± 0.0020 233.0489 ± 0.0007 153.0826 ± 0.0012 135.0705 ± 0.0009	289.1082 261.0769 244.0504 233.0456 153.0793 135.0687	0.0033 0.0021 0.0050 0.0033 0.0033 0.0018
11[a]	Bis(ethyl dimethylphosphoramidic) anhydride	MH^+ $[MH - C_2H_4]^+$ $[MH - HNMe_2]^+$ $[(Me_2N)(EtO)P(OH)_2]^+$ $[(Me_2N)P(OH)_3]^+$	289.1064 ± 0.0011 261.0753 ± 0.0015 244.0517 ± 0.0021 154.0648 ± 0.0018 126.0374 ± 0.0019	289.1082 261.0769 244.0504 154.0633 126.0320	0.0018 0.0016 0.0013 0.0015 0.0054
12	Diethyl phosphoric ethyl dimethylphosphoramidic anhydride	MH^+ $[MH - C_2H_4]^+$ $[MH - (C_2H_4)_2]^+$ $[MH - (C_2H_4)_3]^+$ $[(EtO)_2P(OH)_2]^+$ $[(EtO)P(OH)_3]^+$	290.0884 ± 0.0013 262.0613 ± 0.0013 234.0307 ± 0.0014 206.0003 ± 0.0032 155.0500 ± 0.0006 127.0184 ± 0.0016	290.0922 262.0609 234.0296 205.9983 155.0473 127.0160	0.0038 0.0004 0.0011 0.0020 0.0027 0.0024

[a]Detected as a pair of chromatographic peaks during GC-MS due to the presence of two asymmetric phosphorus atoms

collected samples may provide forensic evidence supporting the prior presence of chemical warfare agents, their source or synthetic route.

Figure 2 illustrates the GC-MS and LC-ESI-MS chromatograms obtained for the diluted synthetic tabun sample. Twelve sample components were identified during LC-ESI-MS analysis (Table 1), whereas only 10 were detected by GC-MS. The two compounds not detected by GC-MS contained hydroxyl substitution and would only be detected by GC-MS following derivatization. Both low volatility compounds, ethyl phosphoric tetramethylphosphorodiamidic anhydride and octamethyltetramidotriphosphoric acid, were detected along with the 10 more volatile phosphates and pyrophosphates during a single analysis by LC-ESI-MS. Derivatization was not required, a definite advantage for LC-ESI-MS over GC-MS for the analysis of mixtures containing organophosphorus chemical warfare agents, their hydrolysis products and other hydroxyl-substituted organophosphorus compounds.

LC-ESI-MS analyses, typically, take from 30 to 45 minutes with a 15-minute solvent equilibration between analyses. In this particular example, a 30-minute gradient program was employed. GC-MS analysis times were comparable with an analysis time of 31 minutes and up to 10 minutes to recycle the GC oven between analyses. The principal advantage of GC over LC for component separarion was in the efficiency of separation. GC chromatographic peak widths were typically an order of magnitude narrower, making it possible, potentially, to resolve a greater number of sample components.

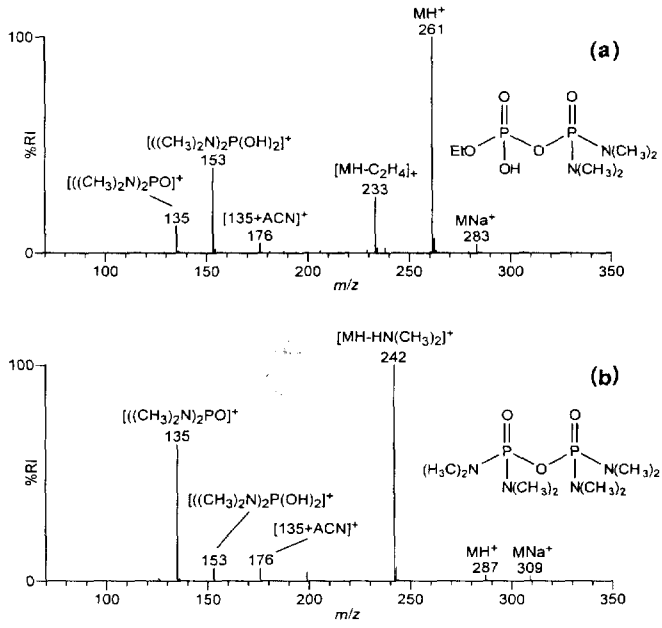


Figure 3. ESI-MS data obtained for (a) ethyl phosphoric tetramethylphosphorodiamidic anhydride (sampling cone voltage of 40 volts) and (b) bis(tetramethylphosphorodiamidic) anhydride (sampling cone voltage of 50 volts) during LC-ESI-MS analysis.

EI-MS data acquired during GC-MS analysis were consistent with EI data contained in the NIST database supplied with the Agilent 5973N data system and/or the DRDC Suffield EI Database. Several compounds had been identified during prior LC-ESI-MS analyses,[18] with the remaining compounds being candidates for inclusion in the DRDC Suffield ESI-MS Database.[22]

Table 1 lists the identities of the 12 compounds based on the interpretation of the high-resolution data obtained during LC-ESI-MS analysis with a sampling cone voltage of 24 volts (lower sampling cone voltage for protonated molecule mass determinations) and 40 volts (higher sampling cone voltage for product-ion mass determinations). Protonated molecule mass determinations (mean ± SD) were based on five measurements whereas product-ion mass determinations (mean ± SD) were based on three measurements. The acquired masses for the protonated molecule and product ions compared favorably with the theoretical values listed in Table 1, with most errors being less than 0.005 Da.

Figure 3(a) illustrates typical ESI-MS data obtained with a sampling cone voltage of 40 volts for ethyl phosphoric tetramethylphosphorodiamidic anhydride, one of the two compounds not detected during GC-MS analysis. A product ion due to loss of C_2H_4 from the ethoxyl substituent, as well as two lower-mass products at *m/z* 153 and *m/z* 135, due to loss of $(C_2H_5O)PO_2$ and $(C_2H_5O)P(O)(OH)_2$, respectively, from the protonated molecule at *m/z* 261, were observed. The principal product ion observed for octamethyltetramidotriphosphoric acid, the other compound not detected during GC-MS analysis, was due to loss of $HN(CH_3)_2$ from the protonated molecule at *m/z* 367. A minor ion at *m/z* 153, of the same elemental composition as observed for ethyl phosphoric tetramethylphosphorodiamidic anhydride, was also detected.

The ESI-MS data for the phosphates in the tabun synthetic sample have been reported previously[18] with the exception of the data for hexamethylphosphorotriamide. Hexamethylphosphorotriamide contained a protonated molecule at *m/z* 180 that was used to confirm molecular mass as well as a product ion at *m/z* 135 due to the loss of $HN(CH_3)_2$ from the protonated molecule (at a higher sampling cone voltage). The sampling cone voltage was increased to as high as 50 volts with no additional product ion formation.

A number of uncharacterized pyrophosphates were also observed in the sample. The ESI-MS data acquired for bis(tetramethylphosphorodiamidic) anhydride contained a protonated molecule at *m/z* 287 with a lower sampling cone voltage (20 volts) and a number of product ions with a sampling cone voltage of 50 volts [Figure 3(b)]. A significant product ion at *m/z* 242, due to loss of $HN(CH_3)_2$ from the protonated molecule, and two lower-mass product ions at *m/z* 153 and *m/z* 135, characteristic of an organophosphorus compound with a $[(CH_3)_2N]_2RP(O)$ substructure, were also

observed. Similar data were also observed for ethyl dimethylphosphoramidic tetramethylphosphorodiamidic anhydride, with product ions at *m/z* 243 and *m/z* 215, resulting from sequential loss of $HN(CH_3)_2$ and C_2H_4 from the protonated molecule at *m/z* 288. The characteristic lower-mass product ions at *m/z* 153 and *m/z* 135 (lower relative intensity) confirmed the presence of an organophosphorous compound with a $[(CH_3)_2N]_2RP(O)$ substructure.

Two additional pyrophosphates with identical elemental composition, diethyl phosphoric tetramethylphosphorodiamidic anhydride [Figure 4(a)] and bis(ethyl dimethylphosphoramidic) anhydride [Figure 4(b)], could not be differentiated by GC-MS and exhibited identical ESI mass spectra dominated by a protonated molecule at *m/z* 289 with a sampling cone voltage of 20 volts. Both anhydrides contained two ethoxyl and two dimethylamino substituents based on the higher-mass product ions observed at *m/z* 261, *m/z* 244 and *m/z* 233. Elemental assignments for a number of the product ions observed during accurate mass measurement have been summarized in Table 1. Differentiation of the two possible isomers was possible on the basis of the characteristic lower-mass ions observed with a sampling cone voltage of 40 volts. Bis(ethyl dimethylphosphoramidic) anhydride exhibited ions at *m/z* 126 and *m/z* 154 indicating the presence of both ethoxy and dimethylamino substitution at each phosphorus atom, whereas the product ions at *m/z* 135 and *m/z* 153 for diethyl phosphoric tetramethylphosphorodiamidic anhydride indicated that one phosphorus contained both dimethylamino substituents.

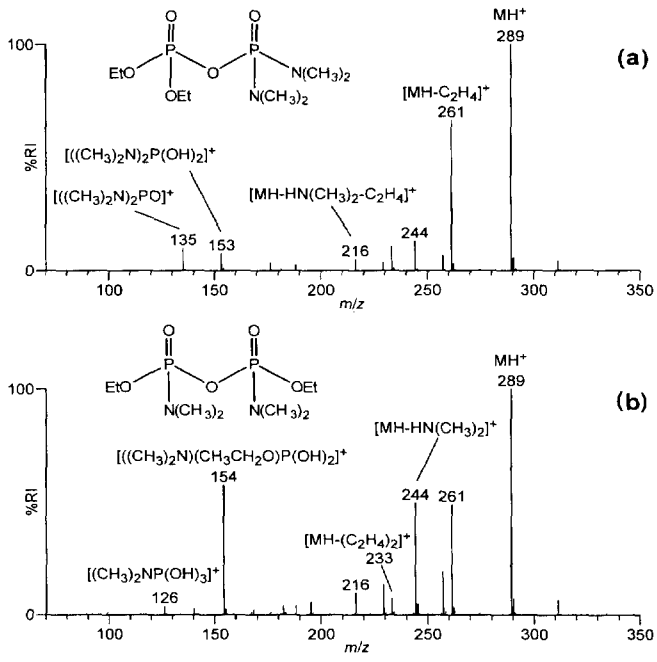


Figure 4. ESI-MS data obtained for (a) diethyl phosphoric tetramethylphosphorodiamidic anhydride and (b) bis(ethyl dimethylphosphoramidic) anhydride during LC-ESI-MS analysis (sampling cone voltage of 40 volts).

The time-of-flight mass spectrometer used in this investigation offers a significant improvement in sensitivity over the older Autospec-Q instrument for the acquisition of complete electrospray mass spectra.[11,13] The sensitivity of the LC-ESI-MS method has been estimated with triethyl phosphate, a compound that does not readily hydrolyze in water. A complete, interpretable mass spectrum (sampling cone voltage: 20 volts) was obtained for 50 pg of triethyl phosphate during LC-ESI-MS analysis. The S/N ratio for the *m/z* 183 $(M + H)^+$ reconstructed-ion current chromatogram was approximately 25 : 1.[18]

The relative sensitivity of LC-ESI-MS to GC-MS was estimated in this study since the exact contribution of each sample component in the mixture used for comparison remains unknown. Relative sensitivities were compared during the acquisition of full mass spectra for the sample components in the 6000 to 1 diluted synthetic tabun samples. The sample sensitivity of LC-ESI-MS (5 μL injection volume) for the acquisition of a full mass spectrum was comparable with GC-MS (1 μL injection volume) for compounds detected by both techniques. Interpretable full mass spectra and similar S/N ratios in the total-ion current were observed for a trace sample component (peak number 11 in Table 1 and Figure 2), estimated to be present in the low nanogram or subnanogram range (based on typical ESI-MS responses), using both methods.

Former mustard storage site soil samples

During World War II, over 700 tons 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 and 1987 and thiodiglycol was found at 3.9 mg mL^{-1}, 0.6 mg mL^{-1} and 2.2 mg mL^{-1}, respectively,[23] in the water at a location near the buried vaults. Hexane extracts of these aqueous samples were analyzed by capillary column gas chromatography-mass spectrometry (GC-MS) 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.[23] Following these analyses, munitions mustard samples were deliberately hydrolyzed to enable characterization of these compounds by EI-MS and ammonia CI-MS[24] and by ESI-MS.[11]

In February 2002, a consultant was contracted to take soil samples at a number of locations and depths at the former mustard storage site. 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.

Each of the six soil samples selected for investigation was extracted with dichloromethane and these extracts were screened for the presence of mustard by GC-MS. Mustard was not detected (sample detection limit = 0.2 μg g^{-1}, based on the acquisition of an interpretable full EI mass spectrum) in any of the soil sample extracts. However, three of the soil sample extracts were found to contain thiodiglycol and/or related mustard hydrolysis products. Figure 5 illustrates the GC-MS total-ion current chromatograms obtained for the three soil sample extracts containing thiodiglycol and/or related mustard hydrolysis products (compounds identified in Table 2).

A recently developed sample handling and LC-ESI-MS analysis method for soils contaminated with sarin, soman and their initial hydrolysis products was evaluated 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 thiodiglycol, the hydrolysis product of mustard. Two control soil samples (soil type not determined) from near the former mustard storage site, as well as a sandy clay loam and a loamy sand, were spiked in triplicate at the 20 μg g^{-1} level with thiodiglycol to estimate recovery efficiency. Thiodiglycol recovery, although 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 the 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.

Each of the aqueous extracts of the soil samples was screened for the presence of thiodiglycol by LC-ESI-MS. Figure 6 illustrates the LC-ESI-MS total-ion current chromatograms obtained for the two soil sample extracts containing thiodiglycol and related mustard hydrolysis products (compounds identified in Table 2). A major sample component(s) with a retention time in the two to three minute range was detected in the water extract of all the soil samples. It did not appear to contain any compounds associated with mustard degradation. Thiodiglycol was detected in the water extracts of soil samples **b** and **c** at 200 μg g^{-1} and 300 μg g^{-1} (semi-quantitative estimate), respectively. Thiodiglycol was not detected (sample detection limit = 1 μg g^{-1}, based on the acquisition of an interpretable full ESI mass spectrum) in the other four soil sample extracts.

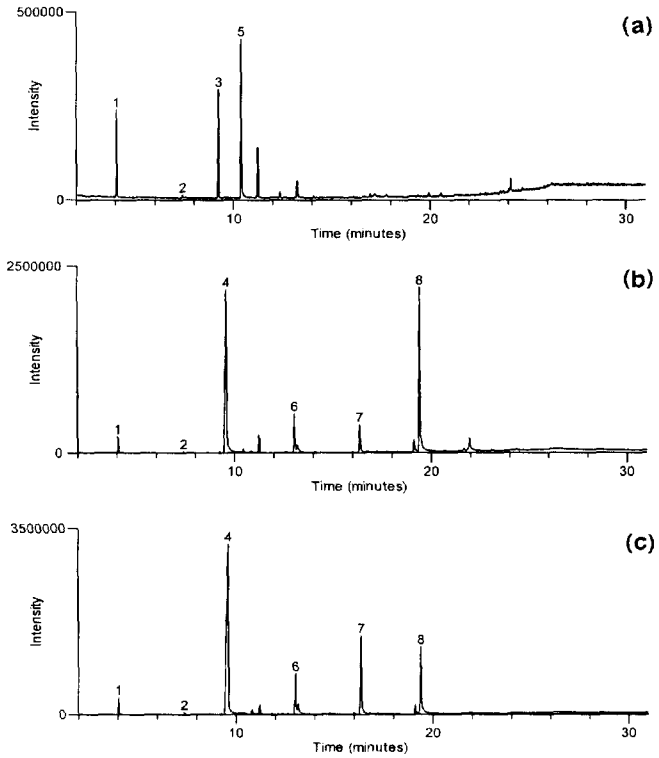


Figure 5. GC-MS total-ion current chromatograms obtained for dichloromethane extracts of (a) soil sample a, (b) soil sample b and (c) soil sample c. Compounds are identified in Table 2.

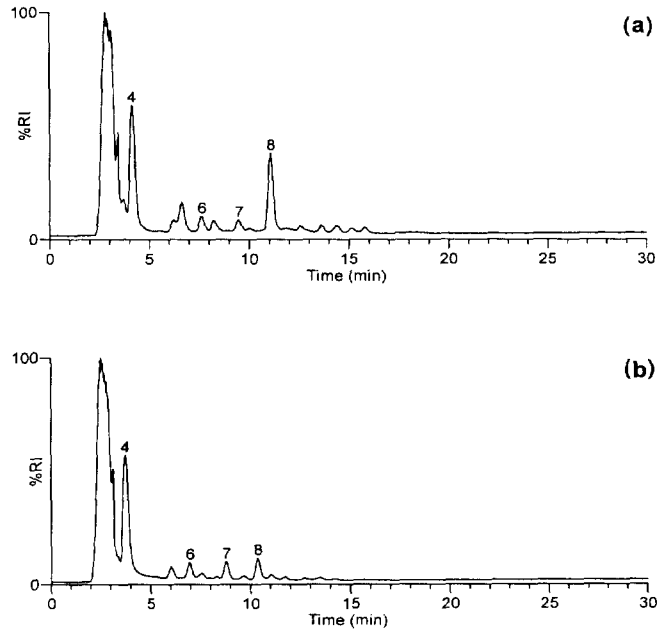
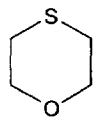
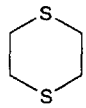
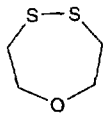
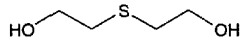
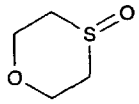
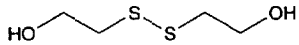
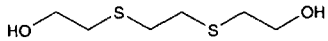
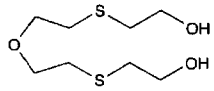


Figure 6. LC-ESI-MS total-ion current chromatograms obtained for aqueous extracts of (a) soil sample b and (b) soil sample c. Compounds are identified in Table 2.

Table 2. Compounds identified in soil sample extracts by GC-MS and LC-ESI-MS.

Peak no.	Compound name	Structure	Dichloromethane extract (GC-MS analysis)	Water extract (LC-MS analysis)
1	1,4-Oxathiane		Soil sample a Soil sample b Soil sample c	
2	1,4-Dithiane		Soil sample a Soil sample b Soil sample c	
3	1-Oxa-4,5-dithiapane		Soil sample a	
4	Thiodiglycol		Soil sample b Soil sample c	Soil sample b Soil sample c
5	1,4-Oxathiane sulfoxide		Soil sample a	
6	Bis(2-hydroxyethyl)disulfide		Soil sample a Soil sample b Soil sample c	Soil sample b Soil sample c
7	3,6-Dithia-1,8-octanediol		Soil sample b Soil sample c	Soil sample b Soil sample c
8	6-Oxa-3,9-dithia-1,11-undecanediol		Soil sample b Soil sample c	Soil sample b Soil sample c

Figures 7 and 8 illustrate typical ESI-MS data for thiodiglycol, the hydrolysis product of mustard, as well as the hydrolysis products of three longer-chain sulfur vesicants, bis(2-hydroxyethyl)disulfide, 3,6-dithia-1,8-octanediol and 6-oxa-3,9-dithia-1,11-undecanediol (sampling cone voltage: 30 volts). The diol ESI-MS data contained both molecular ion and product ion content, enabling structural identification of these hydrolysis products. The ESI-MS data obtained for 6-oxa-3,9-dithia-1,11-undecanediol [Figure 8(b)], the hydrolysis product of bis[(2-chloroethylthio)ethyl]ether (T), was typical. The mass spectrum contained a significant MH^+ ion at *m/z* 227 and an MNa^+ ion at *m/z* 249. 6-Oxa-3,9-dithia-1,11-undecanediol exhibited a product ion due to loss of H_2O at *m/z* 209 along with product ions at *m/z* 181, *m/z* 149 and *m/z* 105 due to $[MH - H_2O - C_2H_4]^+$, $[MH - H_2O - SC_2H_4]^+$ and $[MH - H_2O - SC_2H_4 - OC_2H_4]^+$, respectively, that could be used to establish relative S and O positioning.

Peak shape for the diols was better by LC-ESI-MS than by GC-MS. Some peak tailing was noted for the diols during GC-MS due to their polarity. This effect is minimized during GC analyses by the use of cool on-column injection and may be eliminated by derivatization.

Mustard cannot be detected by LC-ESI-MS. The relatively non-polar compounds related to mustard (chromatographic peak numbers 1, 2, 3 and 5 in Table 2), identified in the dichloromethane extract of the soil samples during GC-MS analysis, were not detected during LC-ESI-MS analysis of the aqueous extracts. These compounds are soluble in water and have been detected in aqueous samples taken from bore holes at the former mustard storage site in the past.[23]

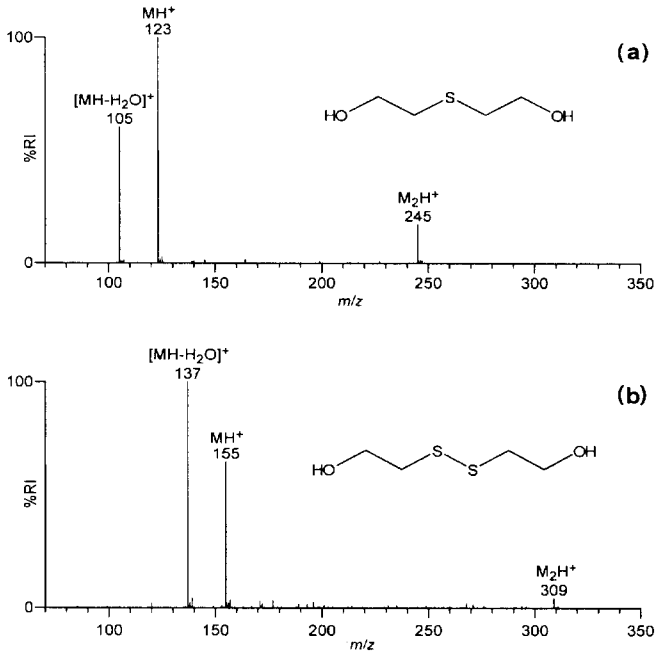


Figure 7. ESI-MS data obtained for (a) thiodiglycol (sampling cone voltage of 20 volts) and (b) bis(2-hydroxyethyl)disulfide (sampling cone voltage of 20 volts) during LC-ESI-MS analysis.

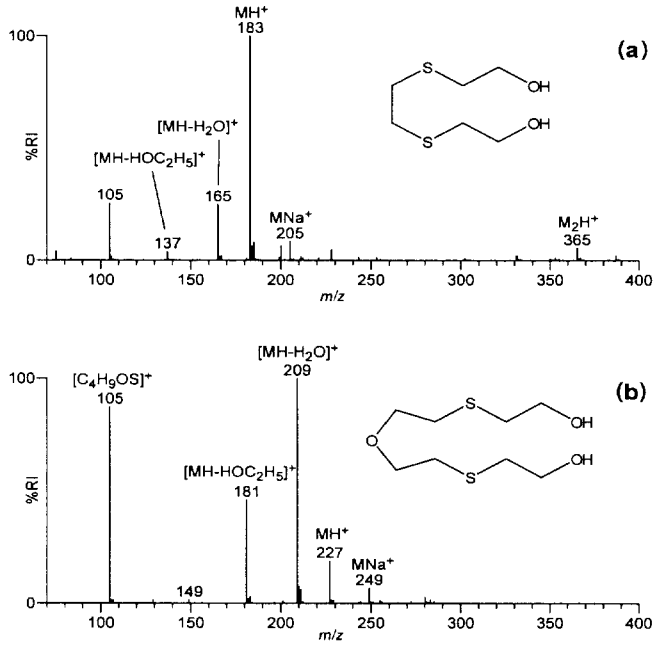


Figure 8. ESI-MS data obtained for (a) 3,6-dithia-1,8-octanediol (sampling cone voltage of 20 volts) and (b) 6-oxa-3,9-dithia-1,11-undecanediol (sampling cone voltage of 30 volts) during LC-ESI-MS analysis.

The inability to ionize these compounds by ESI-MS was confirmed by gently drying (under nitrogen) one of the dichloromethane extracts and taking it up in water for subsequent LC-ESI-MS analysis. Only the diols were detected indicating a limitation of LC-ESI-MS for mustard analyses.

In addition, several chromatographic components in both the GC-MS and LC-ESI-MS chromatograms remain unidentified. These unidentified extracted compounds may or may not be related to the mustard hydrolysate.

Conclusions

Packed capillary LC-ESI-MS and capillary column GC-MS each offer the analyst advantages for the analysis of samples containing chemical warfare agents, their degradation products and related compounds. Under well-defined circumstances, one technique may be more suitable for analysis purposes, whereas the analysis of samples with unknown contamination would best be tackled using both analytical techniques.

Tabun was identified in a pure tabun sample and 12 related sample components were identified during LC-ESI-MS analysis of a synthetic tabun sample. Only 10 sample components were detected by capillary column GC-MS. The two organophosphorus compounds not detected by GC-MS contained hydroxyl substitution and would only be detected by GC-MS following derivatization and a second analysis. Derivatization was not required during LC-ESI-MS analysis, a definite advantage for this technique over GC-MS for the analysis of mixtures containing organophosphorus chemical warfare agents, related compounds and their lower-volatility degradation products.

Both GC-MS and LC-ESI-MS were required to characterize the extracts of the soil samples from the former mustard storage site. GC-MS must be used for the analysis of mustard and relatively non-polar degradation compounds, including 1,4-thioxane and 1,4-dithiane, since these compounds were not ionized during LC-ESI-MS. However, more polar organosulfur compounds, such as thiodiglycol and longer chain diols, generally demonstrate improved peak shape during LC-ESI-MS analysis. Mustard was not detected by GC-MS in any dichloromethane extracts, but three of the soil sample extracts were found to contain mustard degradation products. LC-ESI-MS was used to provide an estimate of thiodiglycol concentration in the aqueous extracts and to characterize the longer chain diols. Thiodiglycol concentration was estimated to be present at the 200 $\mu g\ g^{-1}$ and 300 $\mu g\ g^{-1}$ level in soil samples **b** and **c**, respectively.

Total analysis times, including equilibration time between analyses, were similar for both techniques, typically requiring about 40 to 45 minutes between analyses. Peak widths for capillary column GC-MS separations were typically an order of magnitude better than for packed capillary LC-ESI-MS, offering the potential to resolve more sample components during a given analysis.

The relative sensitivity of LC-ESI-MS to GC-MS was estimated since the contribution of each sample component to the synthetic tabun sample used for comparison remains

unknown. Similar S/N ratios in the total-ion current were observed for the same trace sample components, estimated to be present in the low nanogram or sub-nanogram range.

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