Review

Recent advances and applications of LC-MS for the analysis of chemical warfare agents and their degradation products – A review

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ABSTRACT

More than 165 States Parties have ratified the Chemical Weapons Convention and agreed not to develop, produce, stockpile, transfer or use chemical weapons and to destroy their own chemical weapons and production facilities. The Chemical Weapons Convention 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. Heightened concerns over possible terrorist use, continued interest within the defence community and the requirements for a verifiable Chemical Weapons Convention, have all driven the development and application of liquid chromatography-mass spectrometry methods for chemical warfare agents. Considerable research effort has been expended over the past several years due to increased security concerns and this review covers recent advances and applications of liquid chromatography-mass spectrometry for the detection, characterization and confirmation of chemical warfare agents and their degradation products. Potential areas for new research efforts have also be identified.

KEYWORDS: Liquid chromatography, Mass spectrometry, Atmospheric pressure ionization, Chemical warfare agents, Degradation products, Review

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INTRODUCTION

The use of chemical warfare agents in modern warfare dates back to 1914 when the French first used tear gas grenades at the outbreak of the First World War, but it was not until the Germans first used chlorine near Ypres in 1915 that the world entered the present era of chemical warfare. Other chemical warfare agents, such as phosgene and mustard, were weaponized during the First World War and both sides used these and other chemical warfare agents during the conflict. Just prior to the Second World War, the Germans discovered and produced the first nerve agent, tabun. Tabun was weaponized by the Germans but neither side made use of their chemical weapons stocks. More toxic nerve agents including VX were developed in the 1950's and chemical warfare agent use was reported in South East Asian conficts. Nerve and mustard agents were used by Iraq in the 1980's war between Iran and Iraq and were considered a real threat to coalition armed forces during their action against Iraq in 1991. Most recently, sarin was released by the Aum Shinrikyo cult in the Tokyo underground transit system (1995) resulting in thousands seeking medical attention and twelve deaths.

The most significant chemical warfare agents in terms of military capacity and past use are the nerve and blister agents. Figure 1 illustrates the structures of some common chemical warfare agents and degradation products associated with their hydrolysis.

The Convention on the Prohibition of the Development, Production, Stockpiling and Use of

Nerve Agents [CAS number]

Sarin (GB)
[107-44-8]
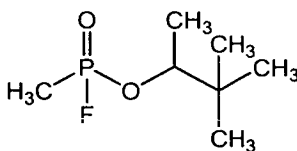
Soman (GD)
[96-64-0]
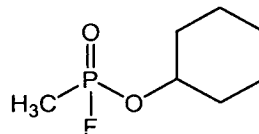
Cyclohexyl Sarin (GF)
[329-99-7]
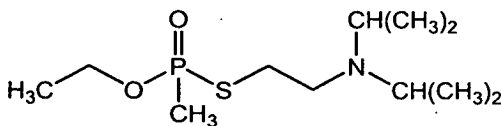
VX
[50782-69-9]
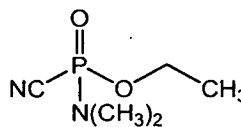
Tabun (GA)
[77-81-6]

Blister Agents [CAS number]
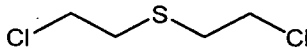
Mustard (H)
[505-60-2]

Lewisite (L)
[541-25-3]
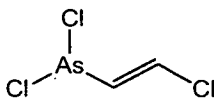
Nitrogen Mustard (HN-3)
[555-77-1]

Sesquimustard (Q)
[3563-36-8]

Hydrolysis Products [CAS number]
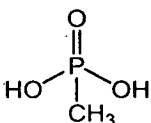
Methylphosphonic
acid [993-13-5]
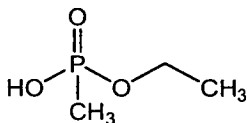
Ethyl methylphosphonic
acid [1832-53-7]
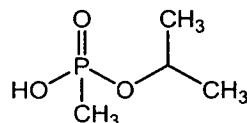
Isopropyl methylphosphonic
acid [1832-54-8]
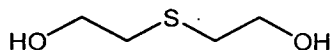
Thiodiglycol
[111-48-8]

Figure 1: Common chemical warfare agents and related hydrolysis products.

Chemical Weapons and their Destruction (commonly referred to as the Chemical Weapons Convention or CWC) was opened to signature in 1993, with the treaty coming into force on 29 April 1997. More than 165 State Parties have ratified the CWC and thereby agreed not to develop, produce, stockpile, transfer or use chemical weapons and also agreed to destroy their stocks of chemical weapons and production facilities.

Recent concerns over possible terrorist use, continued interest by 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 support forensic investigations. Considerable research effort has been expended over the past several years due to increased security concerns and this review covers the recent advances and applications of liquid chromatography-mass spectrometry (LC-MS) for the detection, characterization and confirmation of chemical warfare agents and their degradation products. Potential areas for new research efforts will also be identified.

IDENTIFICATION METHODS

Chemical warfare agents have often been referred to as warfare gases and, the military phrase "gas, gas, gas" has become synonymous with attack by chemical warfare agents. In fact, most chemical warfare agents exist as liquids at ambient temperatures and depending on their volatility may pose either an inhalation or contact hazard, or both. This physical characteristic has made the analysis of chemical warfare agents amenable to the analytical techniques commonly employed for most environmental analyses, namely gas chromatography (GC) and liquid chromatography (LC) with a variety of detectors including mass spectrometry (MS) and Fourier transform infrared (FTIR) spectroscopy. Synthetic or relatively pure samples not requiring chromatographic separation are also frequently characterized by nuclear magnetic resonance (NMR).

Samples containing chemical warfare agents typically contain multiple components that are best charact-

erized following chromatographic separation. Gas chromatography 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 [1-3]. GC-MS analysis methods form the cornerstone of the Technical Secretariat of The Organization for the Prohibition of Chemical Weapons (OPCW) recommended analytical procedures and have been used extensively during designated laboratory proficiency testing [4]. Electron impact mass spectrometric data and spectrometric or spectroscopic data from a second analytical technique (e.g., LC-MS, FTIR or NMR) have typically been acquired to meet OPCW identification requirements, as the OPCW demands that identified compounds must be confirmed by at least two different spectrometric or spectroscopic methods.

Both the nerve and blister agents undergo hydrolysis in the environment [5] and methods are required for the detection and confirmation of these compounds as well. These compounds are significant as they would not be routinely detected in environmental samples and their identification strongly suggests the prior presence of chemical warfare agents. Many degradation products of chemical warfare agents, especially those formed following hydrolysis of the nerve agents, are much less volatile than the parent compounds and must be derivatized prior to GC analysis. A number of derivatization reagents, leading to the formation of pentfluorobenzyl, methyl, or silyl esters have been investigated to allow GC-MS analysis of hydrolysis products of chemical warfare agents [6]. The most commonly targeted degradation compounds include the alkyl methylphosphonic acids and methylphosphonic acid associated with nerve agent hydrolysis and the primary hydrolysis product of mustard, thiodiglycol.

Wils and Hulst were the first to demonstrate the use of LC-MS for the direct analysis of nerve agent hydrolysis products [7] and VX [8], using thermospray ionization, a technique that has now been superseded by atmospheric pressure ionization(API). Increasingly researchers have developed API based LC-MS methods (e.g., electrospray (ESI), ionspray (IS) and atmospheric pressure chemical ionization (APCI)) as

complementary or replacement methods for the characterization of chemical warfare agents and/or their degradation products. A number of LC-MS methods have been reported for the confirmation of these compounds, with this technique being used most frequently during the analysis of aqueous samples or extracts. Review papers on LC-MS analytical methods for chemical warfare agents and related compounds have been published by both Black and Read [9] and Hooijschuur, Kientz and Brinkman [3], with the more recent review focusing on the chromatographic separation of chemical warfare agents over the 1996 to 2001 time period [3]. In a more targeted review, Noort, Benschop and Black [10] reviewed LC-MS and other methods dealing with biomonitoring of exposure to chemical warfare agents. In many cases LC-MS proved to be an attractive alternative to GC-MS for aqueous analyses, as both the organophosphorus chemical warfare agents and their hydrolysis products could be analysed directly without the need for additional sample handling and derivatization steps associated with GC-MS analysis [6].

LC-MS ANALYSES

LC-MS has become firmly entrenched as a key method for the identification of chemical warfare agents, their hydrolysis products and related compounds in a variety of different sample types. It has been used most commonly for the analysis of aqueous samples, including biological fluids and aqueous extracts of sample media such as soil. Table 1 lists recently reported methods by sample media and includes the mode of ionization, LC conditions and compounds analysed.

Table 1: LC-MS analyses of chemicals related to the CWC.

Media	Ref	Ionization	Chromatography	Compounds Analysed
Water	11	ESI (PI)	- Mixed C_8/C_{18} (250 X 2.1 mm) column. - MeOH/water (0.1% formic acid) gradient at 200 µL/min.	- Alkyl methylphosphonic acids, alkyl ethylphosphonic acids, alkyl alkylphosphonic acids and dialkyl alkylphosphonates.
Water	12	ESI (PI)	- C_{18} (150 X 0.32 mm) column. - ACN/water (0.1% trifluoro-acetic acid) gradient at 5 µL/min.	- Munitions grade mustard hydrolysis products including thiodiglcol and longer chain diols.
Water	13	APCI (PI)	- C_{18} (250 X 2.1 mm) column. - water (0.001 to 0.05 M ammonium acetate) isocratic at 250 µL/min.	- Hydrolysis products of VX, lewisite and nitrogen mustard using post column derivatization.
Water	14	ESI (PI)	- C_{18} (150 X 0.32 mm) column. - ACN/water (0.1% trifluoroacetic acid) gradient at 5 µL/min.	- VX and numerous VX degradation products and related compounds.
Water	15	ESI (PI)	- C_{18} (150 X 0.32 mm) column. - ACN/water (0.1% trifluoroacetic acid) gradient at 5 µL/min.	- Nerve agents, sarin, soman, tabun and cyclohexyl methylphosphonofluoridate.

Table 1 Contd.

Water	16	Fast atom bombardment (PI)	- C_{18} (150 X 1.5 mm) column. - ACN/water (0.005 M ammonium acetate) isocratic at 100 µL/min.	- Derivatized (*p*-bromophenacyl) alkyl phosphonic acids.
Water	17	IS (NI)	- PGC (150 X 2.1 mm) column. - ACN/water (trifluoroacetic acid) gradient/isocratic at 200 µL/min.	- Alkylphosphonic acids, alkyl methylphosphonic acids and alkyl ethylphosphonic acids.
Water	18	IS (NI)	- PGC (150 X 2.1 mm) column. - ACN/water (trifluoroacetic acid) gradient/isocratic at 200 µL/min.	- Alkylphosphonic acids.
Water	19	APCI (PI/NI)	- C_{18} (250 X 2.0 mm) column. - MeOH/water (0.02M ammonium formate) gradient at 200 µL/min.	- Hydrolysis products of ten nerve agents, mustard, nitrogen mustard and quinuclidinyl benzilate (BZ)
Water	20	ESI (PI)	- C_{18} (150 X 0.32 mm) column. - MeOH/water (0.2% formic acid) isocratic at 6 µL/min.	-Thiodiglycol and other hydrolysis products of sulfur mustards.
Water	21	ESI (PI)	- C_{18} (150 X 0.32 mm) column. - ACN/water (0.1% trifluoroacetic acid) gradient at 16 µL/min.	Nerve agents, sarin, soman, tabun and cyclohexyl methylphosphonofluoridate and their hydrolysis products.
Water	22	ESI (PI)	- C_{18} (150 X 0.32 mm) column. - ACN/water (0.1% trifluoroacetic acid) gradient at 16 µL/min.	- Sarin and its degradation products and numerous related compounds.
Water	23	ESI (NI)	- C_{18} (150 X 2.1 mm) column. - MeOH/water (0.01M ammonium formate) gradient at 200 µL/min.	- Alkyl methylphosphonic acids.
Soil	20	ESI (PI)	- C_{18} (150 X 0.32 mm) column. - MeOH/water (0.2% formic acid) isocratic at 6 µL/min.	-Thiodiglycol and other hydrolysis products of sulfur mustards.
Soil	21, 24	ESI (PI)	- C_{18} (150 X 0.32 mm) column. - ACN/water (0.1% trifluoroacetic acid) gradient at 16 µL/min.	Nerve agents, sarin and soman and their hydrolysis products.
Soil	23	ESI (NI)	- C_{18} (150 X 2.1 mm) column. - MeOH/water (0.01M ammonium formate) gradient at 200 µL/min.	- Alkyl methylphosphonic acids.

Table 1 Contd.

Soil	25,26	ESI (PI)	- C$_{18}$ (150 X 0.32 mm) column. - ACN/water (0.1% trifluoroacetic acid) gradient at 10 µL/min.	- Thiodiglycol and longer chain diols associated with hydrolysis of munitions grade mustard.
Munition	27	APCI (PI)	- C$_{18}$ (100 X 2.1 mm) column. - ACN/water (0.05M ammonium acetate) gradient at 250 µL/min.	- Phosphorothioates and related compounds.
Munition	21	ESI (PI)	- C$_{18}$ (150 X 0.32 mm) column. - ACN/water (0.1% trifluoroacetic acid) gradient at 16 µL/min.	- Tabun, its hydrolysis product and numerous related compounds.
Synthetic	25	ESI (PI)	- C$_{18}$ (150 X 0.32 mm) column. - ACN/water (0.1% trifluoroacetic acid) gradient at 10 µL/min.	- Tabun and numerous tabun related compounds
Synthetic	28	ESI (PI)	- C$_{18}$ (250 X 2.0 mm) column. - MeOH/water (0.02M ammonium formate) gradient at 200 µL/min.	- Hydrolysis and oxidation products of two longer chain sulfur vesicants (Q and T).
Urine	29	ESI (PI)	- C$_{18}$ (250 X 2.0 mm) column. - MeOH/water (0.02M ammonium formate) gradient at 200 µL/min.	- Beta-lyase metabolites of sulfur mustard.
Urine	30	ESI (PI/NI)	- C$_{18}$ (150 X 2.0 mm) column. - ACN/water (0.05% formic acid) gradient at 200 µL/min.	- Sulfur mustard metabolite 1,1 '-sulfonylbis[2-S-(N-acetylcysteinyl)ethane].
Serum	31	ESI (PI/NI)	- PRP-X100 (200 X 0.32 mm) column. - ACN/water (0.5% formic acid) isocratic at 20 µL/min.	- Isopropyl methylphosphonic acid.
Serum	16	Fast atom bombardment (PI)	- C$_{18}$ (150 X 1.5 mm) column. - ACN/water (0.005 M ammonium acetate) isocratic at 100 µL/min.	- Derivatized (p-bromophenacyl) alkyl phosphonic acids.
Serum	32	ESI (PI)	- C$_{18}$ (150 X 0.30 mm) column. - ACN/water (0.2% formic acid) gradient at 6 µL/min.	- Albumin/sulfur mustard adducts
Plasma	33	APCI (PI)	- OD-H (250 X 4.6 mm) column. - Hexane/isopropanol isocratic at 800 µL/min.	- Enantiomers of VX

Many of the LC-MS applications listed in Table 1 involve the analysis of degradation products of chemical warfare agents as these compounds can be analysed directly using an API method of ionization. Both positive ion (PI) and negative ion (NI) modes have been used with advantages in selectivity [23] or sensitivity [19] generally being cited as the reasons for the choice. More universal screening of the wide range of possible degradation products associated with chemicals scheduled by the CWC would likely involve a PI screening procedure or one that targets a number of key compounds using both PI and NI modes depending on the compound [19]. Figure 2 illustrates LC-APCI-MS selected ion monitoring (SIM) chromatograms in both PI and NI mode for nineteen acidic, neutral and basic CW agent degradation products in water using the developed
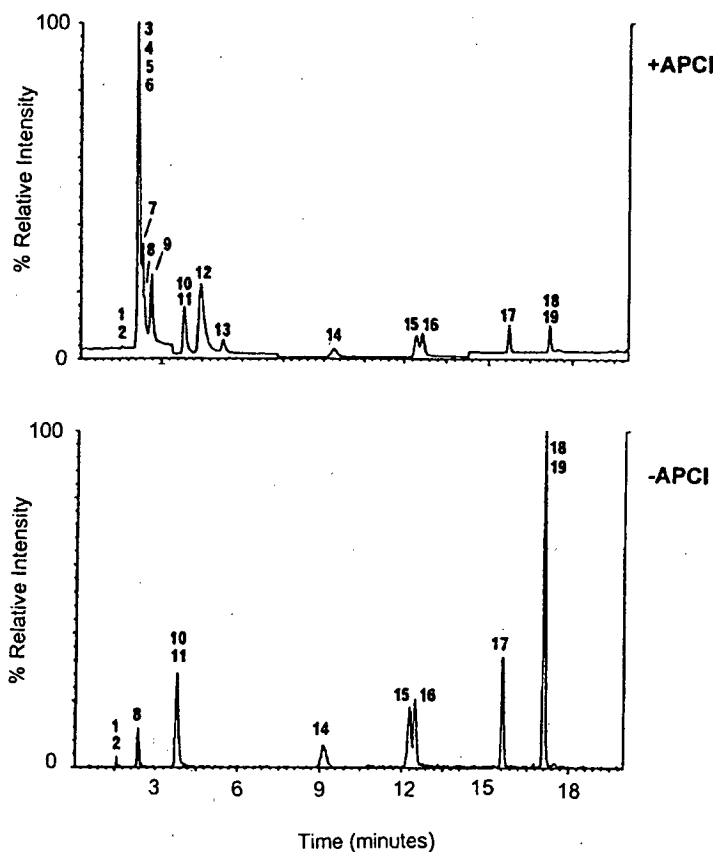

Figure 2: LC-MS selected ion current chromatograms, PI APCI (upper) showing the detection of 19 degradation products (each 0.1 µg/mL in water), and NI APCI (lower) showing the selective detection of phosphonic acids and benzilic acid in the mixture. 1. methylphosphonic acid, 2. ethylphosphonic acid, 3. N-methyldiethanolamine, 4. thiodiglycolsulfoxide, 5. 3-quinuclidinol, 6. N-ethyldiethanolamine, 7. thiodiglycol sulfone, 8. ethyl methylphosphonic acid, 9. triethanolamine, 10. isopropy methylphosphonic acid, 11. ethyl ethylphosphonic acid, 12. N,N-diisopropylaminoethanol, 13. thiodiglycol, 14. sec.-butyl methylphosphonic acid, 15. isobutyl methylphosphonic acid, 16. n-butyl methylphosphonic acid, 17. cyclohexyl methylphosphonic acid, 18. benzilic acid and 19. pinacolyl methylphosphonic acid [19]. (Copyright © (1999) with permission from Elsevier).

screening procedure.

ESI, APCI and IS have all been utilized for the analysis of chemical warfare agents and their degradation products, with ESI being preferred for the lower flow rates associated with packed capillary LC separation [20, 21, 26]. APCI and IS methods have usually been associated with the use of larger bore LC columns and higher flow rates (e.g., 200 µL/min) and were found to be less susceptible to variable adduct ion formation than ESI [19]. However, regardless of the API technique or instrument used, the acquired mass spectra remain similar in ion content, exhibiting adduct ions (e.g., $[M+H]^+$ or $[M+Na]^+$) that may be used to determine molecular mass and characteristic product ions (e.g., $[M+H-H_2O]^+$ or $[M+H-C_nH_{2n}]^+$) indicative of the compound's structure. Relative intensities of the characteristic ions will vary depending on instrumental conditions [21].

Most recent publications have focused on the direct analysis of hydrolysis products related to the more common chemical warfare agents, including, thiodiglycol and longer chain diols that form following hydrolysis of mustard and munitions grade mustard formulations [12, 19, 20, 25, 26, 28] and alkyl methylphosphonic acids and methyl phosphonic acid that form following hydrolysis of the nerve agents [11, 13, 14, 17-19, 21-24, 31]. Additional degradation products related to nitrogen mustard, quinuclidinyl benzilate and less common nerve agents included in the CWC schedules have been included in several reports, often associated with preparing for or actual OPCW proficiency testing [4, 11, 17, 19]. Derivatization was employed in several instances to enhance sensitivity [13, 16], but for the most part derivatization has only been used to facilitate GC-MS analyses [6].

LC-ESI-MS has the additional advantage that it may also be used for the identification of organophosphorus chemical warfare agents and related compounds that are often present in samples or sample extracts due to degradation or synthetic procedure [14, 15, 21, 22, 24, 25, 33]. Identification and characterization of these related sample components remains an area of interest as the identification of these compounds may provide source or synthetic clues during an investigation. However it should be noted that the CW agent,

mustard, does not ionize during LC-ESI-MS and its determination in samples should be done by GC-MS [25] or by another spectrometric or spectroscopic technique.

Most LC separations have been performed on C_{18} LC columns with acetonitrile (ACN) or methanol (MeOH)/water gradients using trifluoroacetic acid, formic acid or ammonium formate modifiers. Trifluoroacetic acid generally provides the best chromatographic resolution [19] and this modifier has been used frequently for lower flow rate analyses [21, 22, 26]. Trifluoroacetic acid will suppress analyte signal at higher flow rates and may not be the best choice for NI work as it could compete with the analyte during API-MS. Ammonium formate was selected by Black and Read [19] as a buffer comprise for a broad screening procedure where both PI and NI data were acquired. Gradient separations with C_{18} provided the flexibility to analyse a variety of analytes from small, polar hydrolysis products, such as thiodiglycol and methylphosphonic acid, to larger, less polar compounds such as VX and related compounds [14]. The major problem associated with use of C_{18} columns remains the poor retention for the smaller, polar hydrolysis products. Improvements have been observed with the use of porous graphitic carbon (PGC) or other columns [17,18].

Chiral separations can be quite valuable for toxicological studies or during the development of antidotal therapies. Smith reported the first chiral separation of the two enantiomers of VX using a Chiralcel OD-H column and a hexane/isopropanol mobile phase. Both enantiomers were completely resolved during the isocratic separation [33]. Figure 3 illustrates the LC-MS total-ion-current chromatogram obtained for the chiral separation of a VX standard and typical ESI-MS data acquired during the analysis.

DETECTION LIMITS

Detection limits being reported for LC-MS have been approaching those possible with GC-MS. Newer instruments have made it routinely possible to detect compounds in full scanning mode in the 0.5 to 5 ng range and at levels about two orders of magnitude better during SIM. Reported detection limits for the determination of
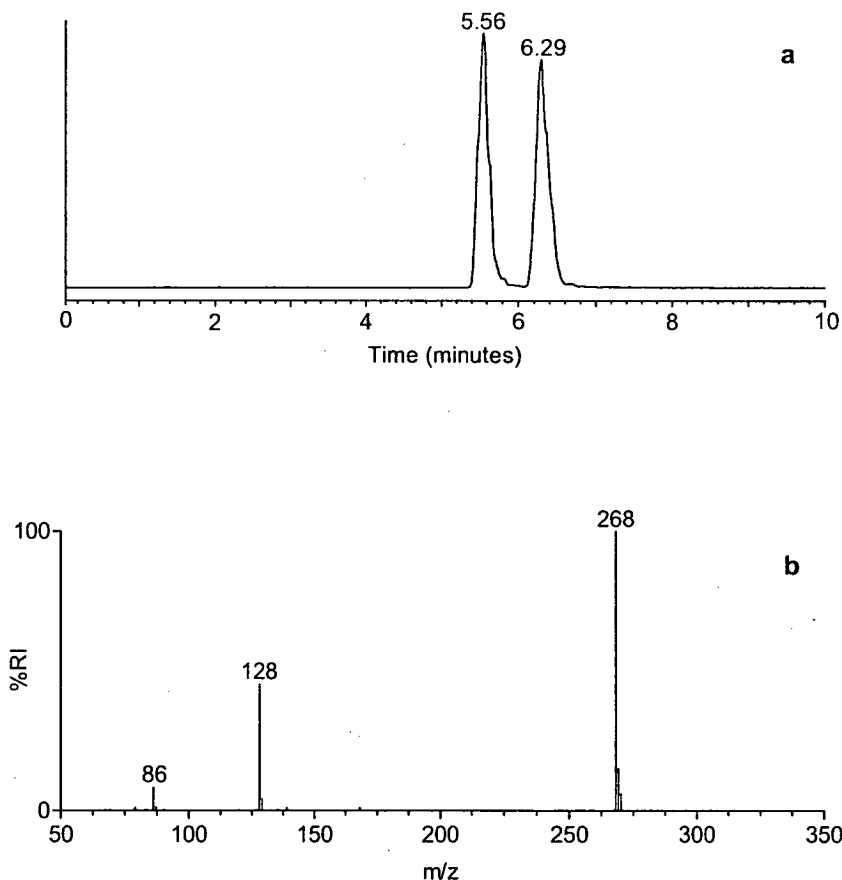
Figure 3: a) LC-MS TIC of a VX standard in hexane and b) APCI mass spectrum for VX enantiomer at 5.56 min using a fragmentor voltage of 100V (mass spectrum for VX enantiomer at 6.29 min was identical) [33]. (Copyright © (2004) with permission from Preston Publications).

chemical warfare agents hydrolysis products using LC-API-MS have been found to be quite compound dependent. In a early paper, Black and Read used SIM and detected neutral and basic compounds in the < 200 pg range (or < 10ng/mL water), but some acidic compounds, like methylphosphonic acid and thiodiglycol, exhibited detection limits of up to 8 ng (or 400 ng/mL) [11]. Later improvements to the above method, including the use of both PI and NI, dropped detection limits by a factor of four at the higher end [19]. A similar method, using LC-ESI-MS (NI), resulted in SIM detection limits that ranged from 250 pg to 5 ng, with methylphosphonic acid being at the upper limit [23]. Figure 4 illustrates a typical LC-ESI-MS (NI) separation for the six alkyl methylphosphonic acids investigated at the 5 μg/mL level.

D'Agostino et al. determined an LC-ESI-MS detection limit for triethyl phosphate, a compound resistant to hydrolysis but similar to chemical warfare agents. This compound could be detected at 50 pg under full scanning conditions [21]. Detection limits may be improved by employing larger volume injections with peak compression.
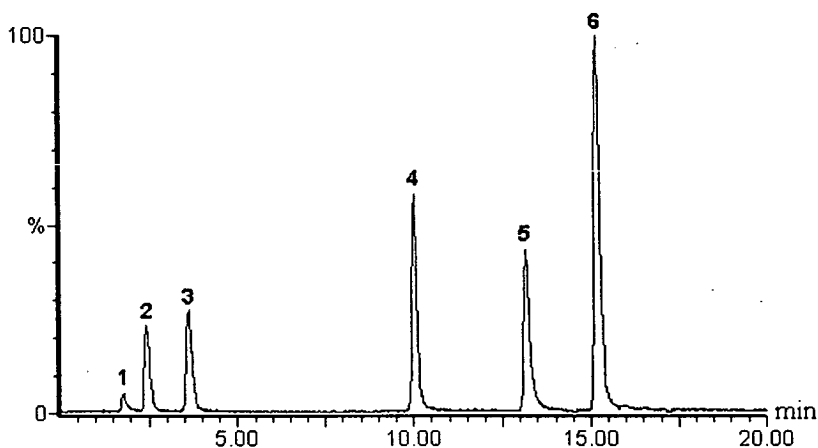
Figure 4: LC-ESI-MS SIM chromatogram of six alkyl methylphosphonic acids (5 μg/mL, ions monitored: m/z 95, 123, 137. 151, 177, 179). 1. methylphosphonic acid, 2. ethyl methylphosphonic acid, 3. isopropyl methylphosphonic acid, 4. *i*-butyl methylphosphonic acid, 5. cyclohexyl methylphosphonic acid and, 6. pinacolyl methylphosphonic acid [23]. (Copyright © (2004) with permission from Elsevier).

Hooijschuur et al. reported full scanning detection limits of 500 to 800 ng/mL with this technique for longer chain diols associated with mustard hydrolysis. Finally some of the best absolute detection limits were reported during LC-ESI-MS/MS in the multiple reaction monitoring (MRM) mode. Noort et al. reported an absolute detection limit of 2 pg for isopropyl methylphosphonic acid [31] and 4 pg for a mustard adduct [32].

STRUCTURAL ELUCIDATION

API-MS and API-MS/MS have been used for a number of structural elucidation studies, frequently without the need for LC separation. The OPCW requires specific identification of P-alkyl substituents and to this end van Baar, Hulst and Wils were able to differentiate iso- and n-propylphosphonic acids using tandem mass spectrometry [34]. This method was extended by Cooper et al. to include numerous dialkyl propylphosphonates and alkyl propylphosphonochloridates [35]. Figure 5 illustrates the mass spectrometric differentiation of two isomers, diethyl isopropylphosphonate from diethyl n-propylphosphonate, during APCI- MS/MS analysis of the products of m/z 125 ([MH-(C_2H_4)_2]^+)

using an ion trap mass spectrometer. D'Agostino, Hancock and Provost demonstrated the applicability of in-source fragmentation for identification purposes during LC-ESI-MS analysis of a complex sample of degraded VX [14], as well as for other samples [12, 15, 21, 22, 25]. Doubly charged ions, observed for bis [2-(diisopropylamino)ethyl] sulfide and other longer chain bis(diisopropylamino)thioalkanes, at lower sampling cone voltages were completely resolved with moderate resolution and could be easily assigned based on the half mass spacing in the [M+2H]^{2+} isotopic cluster. Higher sampling cone voltages resulted in the production of numerous characteristic product ions that were used to help identify more than 25 VX related compounds [14]. Bell et al. investigated similar compounds and detailed extensively the reactions and fragmentation pathways of two isomeric O-alkyl S-(2-dialkylamino)ethyl methylphosphonothiolates using an ion trap equipped with an ESI source [36]. Interestingly, they were able to produce a number of product ions from the [M+Na]^+ ion of a VX related pyrophosphate using this method. Additional ESI studies have also been performed on isotopically labelled dimethyl methylphosphonate
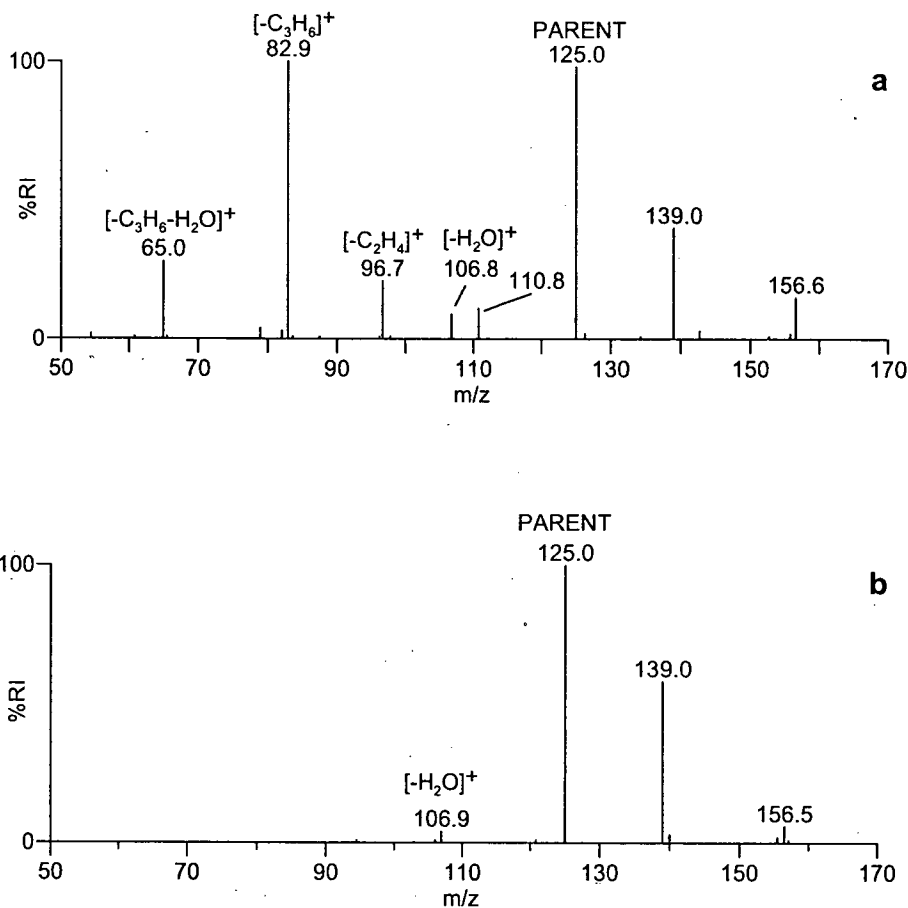
Figure 5: APCI-MS/MS product ion spectra of m/z 125, $[MH-(C_2H_4)_2]^+$, for diethyl isopropylphosphonic acid (upper) and diethyl *n*-propylphosphonic acid (lower) [35]. (Copyright © (2004) with permission from Elsevier).

ions [37] and *N,N*-dialkylaminoethanols [38].

Higher resolution data for chemical warfare agents and related compounds, important for the identification of previously uncharacterized compounds, was greatly aided by the introduction of instruments with time-of-flight (TOF) mass analysers. These instruments may be used to acquire full scanning higher resolution data(typically 5000 to 17000 resolution, 50% valley) for sample components without the signal loss associated with magnetic sector instrumentation. D'Agostino et al.

demonstrated the ultility of high resolution LC-ESI-MS and LC-ESI-MS/MS data for the identification of sarin related compounds in snow [22], during the identification of numerous tabun impurities in a synthetic sample [25], and for the determination of longer chain diols in soil samples collected from a former mustard storage site [26]. Figure 6 illustrates a packed capillary LC-ESI-MS/MS separation of an aqueous extract of a soil sample collected from a former mustard storage site. The ESI-MS/MS data obtained during high resolution
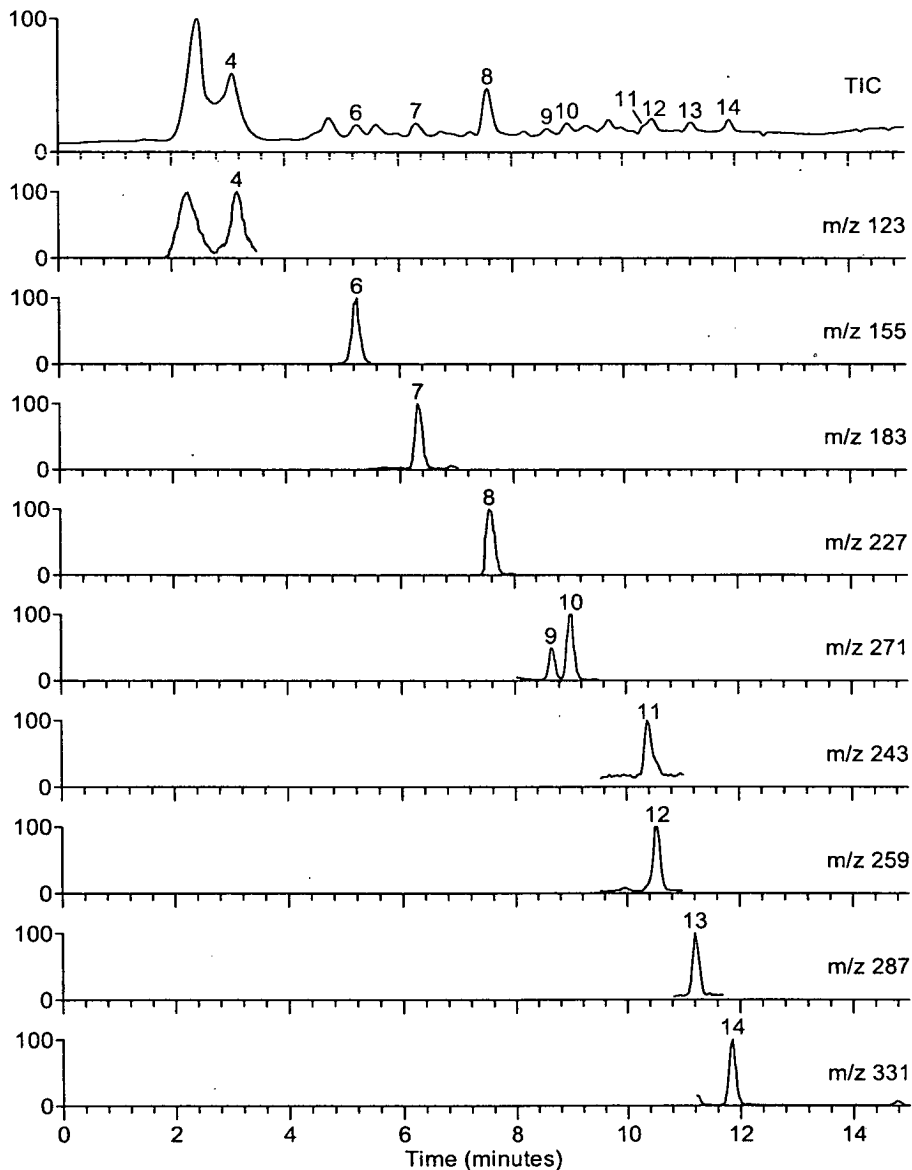
Figure 6: Packed capillary LC-ESI-MS and LC-ESI-MS/MS chromatograms obtained for an aqueous extract of a soil sample taken from a former mustard storage site. The top chromatogram illustrates the total-ion-current (40 to 700 Da) obtained during LC-ESI-MS analysis. The lower nine chromatograms illustrate the LC-ESI-MS/MS chromatograms that were obtained during a single analysis, with the mass of the selected precursor ion (MH⁺) changing with time. Several numbered sample components are identified Table 2 [26]. (Copyright © (2004) with permission from Elsevier).

analysis of thiodiglycol and three other longer chain diols associated with munitions grade mustard hydrolysis are illustrated in Figure 7. Errors associated with the mass measurements were generally <0.001 Da (Table 2). Liu et al. also recently demonstrated the use of high resolution LC-ESI-MS in NI mode for the confirmation of spiked alkyl methylphosphonic acids in water and soil samples [23].
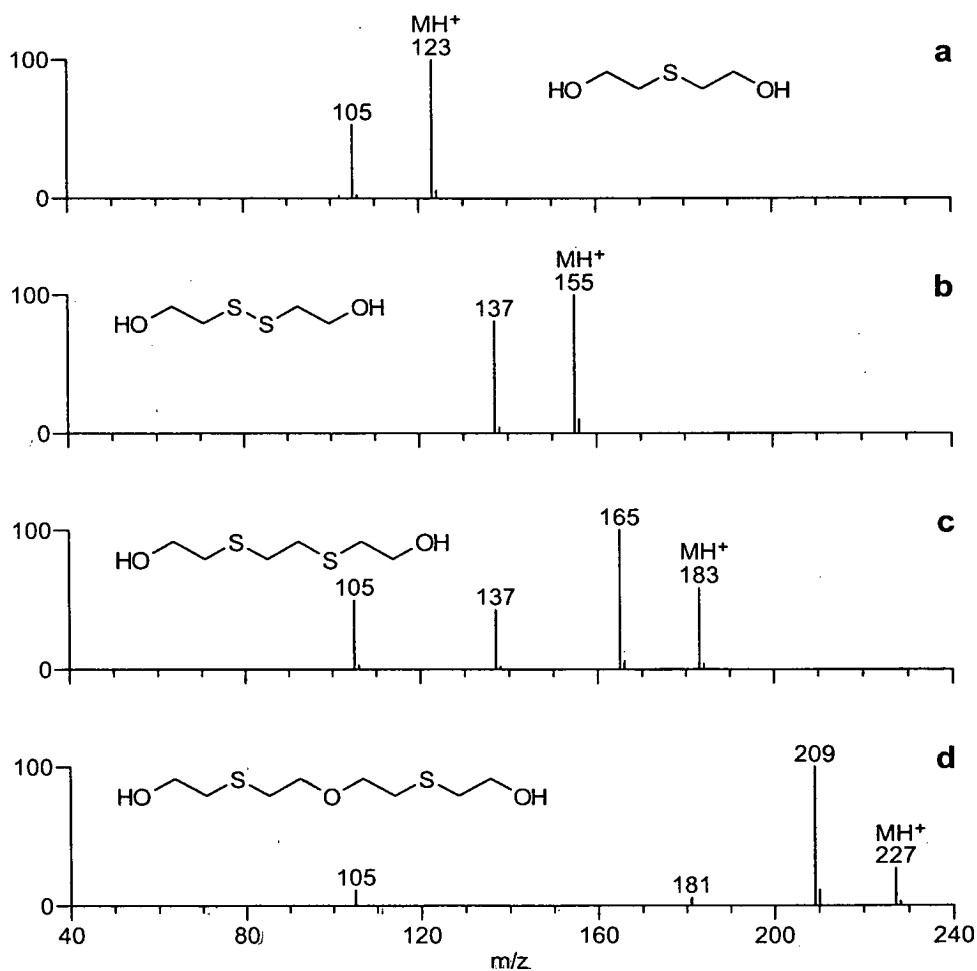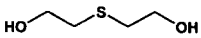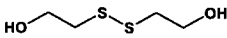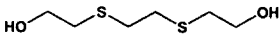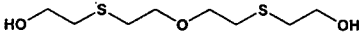

Figure 7: ESI-MS/MS product mass spectra obtained for the MH$^+$ ion of a) thiodiglycol (collision energy: 2 volts), b) bis(hydroxyethyl)disulfide (collision energy: 2 volts), c) 3,6-dithia-1,8-octanediol (collision energy: 4 volts) and d) 6-oxa-3,9-dithia-1,11-undecanediol (collision energy: 5 volts) during LC-ESI-MS/MS analysis of an aqueous extract of a soil sample taken from a former mustard storage site [26]. (Copyright © (2004) with permission from Elsevier).

Table 2: High resolution LC-ESI-MS/MS identification of munitions grade mustard hydrolysis products in an aqueous extract of a soil sample taken from a former mustard storage site.

Peak #[1]	Compound Name	Ion Identity	Observed Mass (Da)[2]	Theoretical Mass (Da)	Error (Da)
1	Thiodiglycol	MH^+	123.0480	123.0480	0.0000
		$(MH-H_2O)^+$	105.0377	105.0374	0.0003
	HO⌒⌒S⌒⌒OH				
2	Bis(hydroxyethyl)-disulfide	MH^+	155.0197	155.0200	0.0003
		$(MH-H_2O)^+$	137.0098	137.0095	0.0003
	HO⌒⌒S⌒S⌒⌒OH				
3	3,6-Dithia-1,8-octanediol	MH^+	183.0518	183.0513	0.0005
		$(MH-H_2O)^+$	165.0412	165.0408	0.0004
		$(MH-HOC_2H_5)^+$	137.0101	137.0095	0.0006
		$(MH-HOC_2H_4SH)^+$	105.0373	150.0374	0.0001
	HO⌒⌒S⌒⌒S⌒⌒OH				
4	6-Oxa-3,9-dithia-1,11-undecanediol	MH^+	227.0777	227.0775	0.0002
		$(MH-H_2O)^+$	209.0668	209.0670	0.0002
		$(MH-HOC_2H_5)^+$	181.0360	181.0357	0.0003
		$(MH-HOC_2H_4SC_2H_4OH)^+$	105.0373	105.0374	0.0001
	HO⌒⌒S⌒⌒O⌒⌒S⌒⌒OH				

[1] Refer to Figure 6.
[2] Average of (typically) 4 to 7 full scans (40 to 350 Da) across the chromatographic peak.

RELATED ANALYSES

Capillary electrophoresis (CE) was used as the means of separation during the first analysis of chemical warfare agent degradation products using API-MS [39]. This means of separation is generally not as robust or as easy to interface to API-MS instruments as LC and has not been used as frequently as LC for the separation of chemical warfare agents and related compounds. Nasser et al. used CE with indirect UV for trace determinations in soil and water [40], while Mercier et al. investigated CE-MS for the determination of phosphonic acids in aqueous samples on several occasions [17, 18, 41].

Ion mobility spectrometry (IMS), a technology used in hand-held military detectors for chemical warfare agents, has shown promise for very rapid separation of chemical warfare agent degradation products [42] and simulants [43] in an electrospray ionization atmospheric pressure ion mobility orthogonal reflectron time-of-flight mass spectrometer. Sodiated and protonated adducts were obtained for a number of phosphonic acids, thiodiglycol, and 1,4-dithiane [42]. The observance of ESI-MS data for 1,4-dithiane dissolved in water/methanol (5% acetic acid) was unusual, as other researchers have been unsuccessful in producing ESI-MS data from relatively non-polar

compounds such as mustard, 1,4-oxathiane, and 1,4-dithiane [25].

Smith and Shih [44] compared ESI and APCI data obtained for common chemical warfare agent hydrolysis products to that obtained by particle beam MS using flow injection analysis. Most of the compounds investigated did not produce a molecular ion, but the particle beam MS data did contain sufficient fragmentation ions to identify each of the target compounds.

API-MS/MS using flow injection analysis has been applied in biomonitoring applications [10, 45] where chromatography was not required for identification. Similarly, target compounds in proficiency testing samples, could be characterized using ESI-MS/MS without the need for chromatographic separation [4], although analysts must be aware of the potential for ion suppression by more dominant sample components.

The CWC also includes in its schedules candidate toxins that were reviewed in the past [9] but were not reviewed at this time. This would be better treated as a separate subject given the explosive growth in biological and proteomic applications of mass spectrometry. Finally, LC-MS methods for toxic industrial chemicals, including pesticides [46], which might be used in a terrorist scenario or as agents of expediency, were also not included in this review.

FUTURE STUDY

Several possible areas of future study were identified during this review. To date, most of the focus on LC-API-MS applications for chemical warfare agents has focused on environmental sample such as soil or water. These types of samples would be significant for a battlefield scenario but may not be as relevant following a terrorist event. The rapid analysis of biological samples will be extremely important in this situation from both a forensic (prosecution) perspective and to determine exposure within a population. Read and Black [29, 30] and Noort et al. [32] have recently reported specific, sensitive LC-MS methods that could be used. PI and NI ESI-MS data were obtained by Read and Black for 1,1'-sulfonylbis[2-S-(N-acetylcysteinyl)ethane, a mustard metabolite that was detected in human urine following exposure. An isotope dilution GC-

MS/MS method has also been developed at the Center for Disease Control which could be applied to the detection and identification of chemical warfare agent degradation products[47]. In general, the difficulties associated with complex biological fluids and tissues remain a concern.

The forensic media collected at the scene of a terrorist attack could well involve the collection of samples from within an enclosed space, such as an office building. This has been an area of interest within D'Agostino's group, which has collaborated with the Royal Canadian Mounted Police in a study investigating media that might be collected during a forensic investigation involving the use of chemical warfare agents. A variety of sample media, including flooring, wall surfaces, office fabrics, window coverings and paper products or packaging, have been considered for investigation. Chemical warfare agents were spiked onto these media, recovered and positively identified in all cases. In some cases LC-ESI-MS/MS analysis was preferred to reduce chemical interference [48]. Figure 8 illustrates LC-ESI-MS and LC-ESI-MS/MS chromatograms acquired for an aqueous extract of an office carpet sample spiked at the 0.5 to 5 µg/g level with a complex munitions grade tabun sample. Figure 9 illustrates the ESI-MS/MS data acquired for three of the identified compounds, including tabun. ESI-MS/MS data, compiled in Table 3, were acquired under high resolution conditions to facilitate component identification. Other potential terrorist scenarios should be identified with a focus on potential sampling media and appropriate means of identification.

Improvements could also be made in the chromatographic resolution of the lower molecular mass, more polar hydrolysis products of chemical warfare agents, as they are generally poorly retained during most analyses. Matrix problems and ion suppression have also been indicated in some papers and noted during the analysis of aqueous samples from a military site, but this issue has not been addressed.

Sensitivity improvements could be achieved by using lower bore columns operating with flows in the nanolitre/minute range. Separation times could also be reduced with the use of new higher pressure LC systems that use LC columns with smaller particle sizes (e.g., Ultra Performance LC).
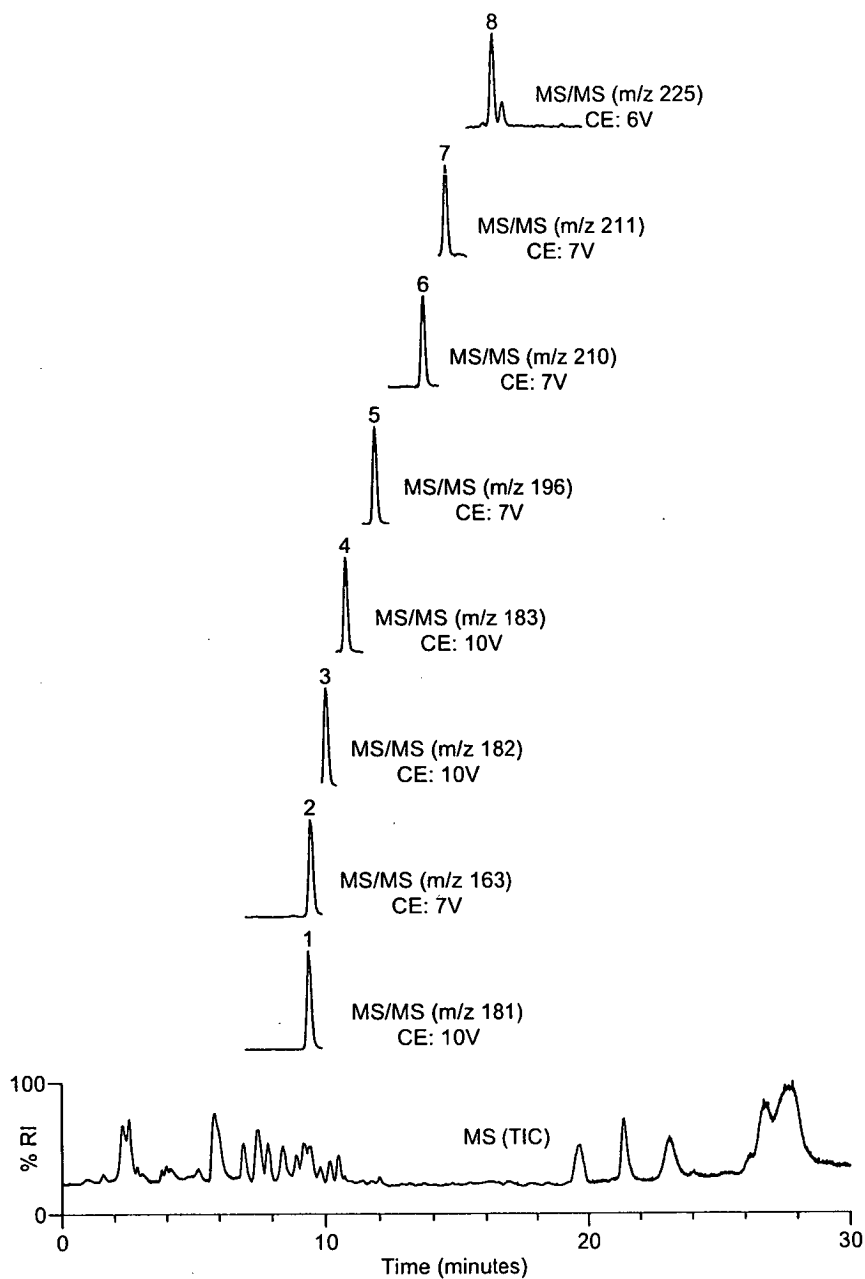
Figure 8: LC-ESI-MS (lowest) and LC-ESI-MS/MS chromatograms (above) for office carpet spiked with munitions grade tabun (0.5 – 5 µg/g). Components 1 to 8 identified in Table 3.
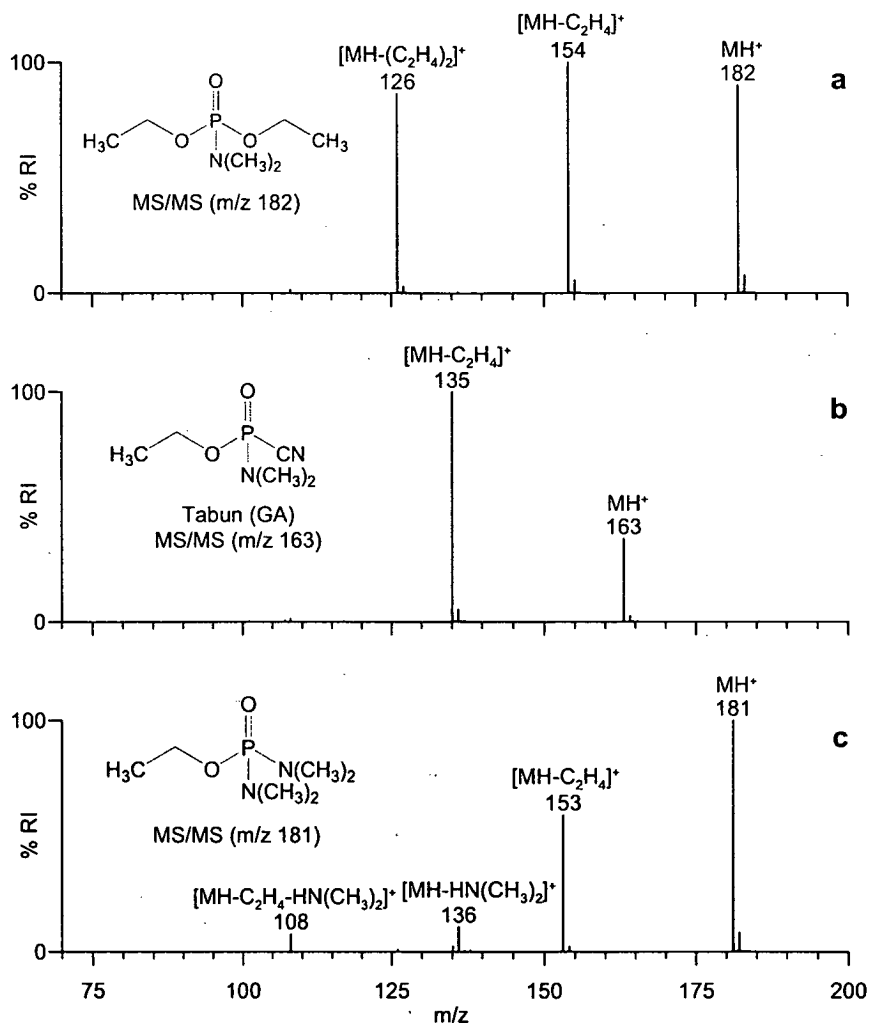
Figure 9: Product ion mass spectra obtained for a) diethyl dimethylphosphoramidate, b) tabun and c) ethyl tetramethylphosphoramidate.

An ESI-MS library containing data for about 60 chemical warfare agents, hydrolysis products and related compounds has been created and is available on the Internet [49]. It contains both higher and lower sampling voltage mass spectra with the former containing more product ion information. During most ESI-MS analyses, the molecular mass will be evident, simplifying identification and limiting the number of possible matches in the mass spectral database. While ESI-MS data is not as amenable to database searching as EI-MS data, spectra obtained with different instruments generally exhibit the same ions, albeit with differences in their relative intensities. Fits may not be as good EI-MS, but use and creation of API-MS databases will aid future analyses, particularly in cases where standards are unavailable.

Table 3. ESI-MS/MS data acquired for munition grade tabun components identified in a spiked office carpet extract.

Peak Number[1]	Compound Name	Ion	Observed Mass (Da)[2]	Theoretical Mass (Da)	Error (Da)
1	Ethyl tetramethyl-phosphoramidate	MH^+	181.1108	181.1106	0.0002
		$[MH-C_2H_4]^+$	153.0795	153.0793	0.0002
		$[MH-HN(CH_3)_2]^+$	136.0533	136.0527	0.0006
		$[MH-C_2H_4-HN(CH_3)_2]^+$	108.0215	108.0214	0.0001
2	Ethyl dimethylphosphoramido-cyanidate (Tabun, GA)	MH^+	163.0628	163.0636	0.0008
		$[MH-C_2H_4]^+$	135.0316	135.0323	0.0007
3	Diethyl dimethylphosphor-amidate	MH^+	182.0950	182.0946	0.0024
		$[MH-C_2H_4]^+$	154.0637	154.0633	0.0004
		$[MH-(C_2H_4)_2]^+$	126.0322	126.0320	0.0002
4	Triethyl phosphate	MH^+	183.0805	183.0786	0.0019
		$[MH-C_2H_4]^+$	155.0470	155.0473	0.0003
		$[MH-(C_2H_4)_2]^+$	127.0153	127.0160	0.0007
		$[MH-(C_2H_4)_3]^+$	98.9836	98.9847	0.0011
5	Ethyl isopropyl dimethylphosphor-amidate	MH^+	196.1109	196.1102	0.0007
		$[MH-C_3H_6]^+$	154.0630	154.0633	0.0003
		$[MH-C_3H_6-C_2H_4]^+$	126.0327	126.0320	0.0007
6	Diisopropyl dimethylphosphor-amidate	MH^+	210.1282	210.1259	0.0023
		$[MH-C_3H_6]^+$	168.0790	168.0789	0.0001
		$[MH-(C_3H_6)_2]^+$	126.0316	126.0320	0.0004
7	Diisopropyl ethyl phosphate	MH^+	211.1109	211.1099	0.0010
		$[MH-C_3H_6]^+$	169.0647	169.0629	0.0018
		$[MH-(C_3H_6)_2]^+$	127.0172	127.0160	0.0012
8	Triisopropyl phosphate	MH^+	225.1273	225.1255	0.0018
		$[MH-C_3H_6]^+$	183.0791	183.0786	0.0005
		$[MH-(C_3H_6)_2]^+$	141.0324	141.0316	0.0008
		$[MH-(C_3H_6)_3]^+$	98.9842	98.9847	0.0005

[1] Refer to Figure 9 for peak numbering and collision energies (CV) used during LC-ESI-MS/MS.

[2] Average of scans across the chromatographic peak (lock mass used).

LC-API-MS has not been used in a field portable role like GC-MS, but the versatile nature of this approach has researchers interested in developing field portable instrumentation [50]. A number of efforts are underway to develop instrumentation based on API-MS since this technique has the potential to rapidly detect and identify chemical warfare agents and their degradation products as well as selected toxic industrial chemical, toxins and biological warfare agent biomarkers.

CONCLUSION

As a result of the current security environment, there has been a renewed emphasis on the development of methods for the analysis of chemical warfare agents, their degradation products and related compounds. Analysts have increasingly included LC-MS as an important technique for the confirmation of presence of these compounds in aqueous samples, including biological fluids and aqueous extracts of sample media. LC-MS has and will continue to be used regularly in applications including OPCW proficiency testing, environmental testing and remediation, forensic investigations and biomonitoring. This technique may become an important technology in future field portable detection and identification strategies.

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