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Analysis of degradation products of organophosphorus chemical warfare agents and related compounds by liquid chromatography–mass spectrometry using electrospray and atmospheric pressure chemical ionisation

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Abstract

A qualitative screening procedure was developed for the detection and identification of phosphonic acid hydrolysis products of organophosphorus chemical warfare agents, using liquid chromatography–mass spectrometry with electrospray ionisation (LC–ESP-MS). A mixed C_8/C_{18} reversed-phase column with gradient elution gave good chromatography for a series of phosphonic acids. Detection limits for aqueous solutions of standards were <50 ng/ml (<0.25 ng injected), providing an increase in sensitivity up to an order of magnitude compared with LC–MS using atmospheric pressure chemical ionisation (LC–APCI-MS). The methodology provides a rapid screening procedure for the hydrolysis products of nerve agents in aqueous samples and extracts. The application of LC–ESP/APCI-MS and LC–ESP/APCI-MS–MS to the identification of dialkyl esters of phosphonic acids is also described. Examples are given of the application of LC–MS to spiked water samples analysed during the course of proficiency testing. © 1998 Elsevier Science B.V.

Keywords: Organophosphorus compounds; Phosphonic acid; Chemical warfare agents

1. Introduction

The Chemical Weapons Convention (CWC) will enter into force in April 1997 and will ban the production, stockpiling and use of chemical weapons. An important aspect of the CWC is a verification regime that will investigate cases of suspected non-compliance. Part of this process will involve the analysis of chemical warfare (CW) agents, their precursors or their degradation products, in samples collected from suspected production or storage sites, or from the environment in cases of allegations of use. Most CW agents are volatile and relatively non-polar. They can be detected and identified unequivocally by gas chromatography–mass spectrometry (GC–MS) using electron and chemical ionisation techniques [1,2]. In cases where chemical background is high, or where concentrations of analyte are very low, GC–tandem mass spectrometry (GC–MS–MS) offers a more selective technique for increasing the confidence in an identification [3–6]. A characteristic of most CW agents is their reactivity towards nucleophiles, particularly with water present in the environment. The analysis of environmental and biological residues for hydrolysis products is therefore an important part of

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CW agent analysis. In a recent investigation, the hydrolysis products of the nerve agent sarin and the vesicant sulphur mustard were identified in soil samples and weapon fragments collected from the site of a chemical attack [6].

The hydrolysis products of CW agents are considerably less volatile and more polar than their precursor CW agents. In most cases they can be extracted from environmental residues with water following a Recommended Operating Procedure [1]. For GC–MS analysis, aqueous extracts are concentrated to dryness and the hydrolysis products are converted to trimethylsilyl (TMS) [1,7] or *tert.*-butyldimethylsilyl (*t*-BDMS) ethers or esters [6,8], or to methyl [9,10] or pentafluorobenzyl [11–13] esters in the case of phosphonic acids. These procedures require lengthy analysis times and, in the case of silyl derivatives, can result in a considerable variability in the apparent recovery when large amounts of extraneous materials are co-extracted. The presence of divalent metal ions such as calcium [6,14] may substantially interfere with the derivatisation of mono-alkylphosphonic acids such as methylphosphonic acid. LC–MS analysis offers a more direct method of analysis which may require no sample preparation, depending on the sample, the level of chemical background present and the limits of detection required. On-line and off-line preconcentration techniques can be used prior to LC–MS if lower limits of detection are required [15].

To date, few laboratories have applied LC or LC–MS to the analysis of CW agents and their degradation products, although the use of LC–MS is increasing as the costs and robustness of the technology become more acceptable. Kientz et al. [16,17] developed a screening method using microcolumn LC with flame photometric detection for the hydrolysis products of the nerve agents sarin, soman and VX, but the technique is not widely used. Wils and Hulst [18–20] applied LC–MS using thermospray ionisation (TSP) to the analysis of a broader range of CW related analytes including nerve agents, their hydrolysis products and hydrolysis products related to sulphur mustard, in aqueous samples such as river water. More recently Tørnes [21] has described loop injection and on-line LC–TSP-MS–MS to characterise some methylphosphonic acids. The application of LC–TSP-MS to spiked and real samples has been described by Creasy et al. [22] and by Weimaster et al. [23]. Although a useful technique, detection limits can be quite variable with TSP and thermally labile compounds may be degraded; occasional blockage of the TSP probe tip by non-volatile salts may give operational problems.

In recent years, atmospheric pressure ionisation techniques have become accepted as the most robust, sensitive and versatile for the LC–MS analysis of low and high molecular mass polar compounds [24–26]. These techniques are being increasingly applied to the analysis of water soluble pesticides [27–29]. Borrett et al. [30,31] have reported the use of positive and negative electrospray (ESP) ionisation, using loop injection, for the identification of degradation products of nerve agents and sulphur mustard. Kostiainen et al. [32] successfully applied ionspray mass spectrometry in combination with capillary zone electrophoresis (CZE) for the separation and identification of five nerve agent hydrolysis products. This technique offers high inherent sensitivity and a short analysis time but the limits of detection obtainable are limited by the small injections required. Other workers have demonstrated the potential of electrophoretic techniques for the analysis of phosphonic acids in combination with less specific methods of detection such as UV [33,34].

Our primary interest is the development of broadly applicable screening and identification techniques for a wide range of CW agents and their analogues as included in the lists of scheduled chemicals in the Annex to the CWC. These schedules include literally hundreds of possible analogues of nerve agents and their precursors, which produce a similar number of possible degradation products. Fig. 1 shows the general scheme of hydrolysis for nerve agents; the CWC schedules cover analogues where the P-alkyl group R may be methyl, ethyl, *n*- or isopropyl, and the O-alkyl R′ may be any alkyl or cycloalkyl group up to C_{10}. Our general approach is to use GC–MS

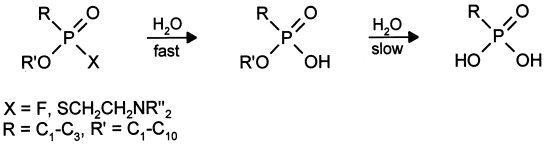

Fig. 1. The hydrolysis products of nerve agents.

for the screening and identification of CW agents and LC–MS/LC–MS–MS, in parallel with silylation/GC–MS, for the analysis of their degradation products. In a previous paper [35] we described a general screening procedure for the detection and identification of the hydrolysis products of CW agents and related compounds, using LC–MS with atmospheric pressure chemical ionisation (APCI). The procedure included ten hydrolysis products derived from organophosphorus nerve agents (O-alkyl alkylphosphonic acids and alkylphosphonic acids), plus hydrolysis products derived from sulphur mustard, nitrogen mustards and quinuclidinyl benzilate (BZ). Limits of detection were ≤0.2 ng injected (≤10 ng/ml water) for the neutral and basic analytes (e.g. thiodiglycol, triethanolamine) derived from sulphur and nitrogen mustards, but were significantly higher, in the range 0.2–8 ng injected (10–400 ng/ml in water) for the acidic hydrolysis products derived from the nerve agents. The least sensitivity was experienced with the most acidic and polar analytes such as ethyl- and methylphosphonic acids. Although this level of sensitivity has generally been found sufficient for use in proficiency tests, where samples are spiked at 1–10 ppm, it may not be sufficient in cases of allegations of CW use where concentrations may be <1 ppm. Previous use of atmospheric pressure ionisation techniques suggests that APCI is best suited to low molecular mass non-ionic analytes [27]. Electrospray/ionspray appear to be best suited to ionic and high molecular mass materials. Although not fully understood, ESP involves the nebulisation of a liquid in an electric field to produce an aerosol of highly charged droplets, followed by desorption of solvated analyte ions from the droplets after vaporisation of the solvent [26]. APCI also involves nebulisation but relies more on charge transfer in the gas phase to the mist issuing from the nebuliser. The alkylphosphonic acids, for which LC–APCI-MS was least sensitive, are those that are the most acidic and are predominantly deprotonated at pH>~2.5. Positive ion ESP at low pH, or negative ion ESP at higher pH, might therefore provide improved sensitivity. In this present paper we report improved conditions for the LC–MS analysis of phosphonic acids derived from nerve agents using positive ion ESP, and describe the broader application of ESP and APCI to the analysis of impurities that may be present in CW agents such as dialkyl alkylphosphonates.

2. Experimental

2.1. Materials

The phosphonic acids and dialkylphosphonates investigated, together with the abbreviations used, are shown in Table 1. Methyl, ethyl and *n*-propyl phosphonic acids were purchased from Aldrich (Gillingham, UK). Other compounds were synthesised in the Organic Chemistry Section, CBD and were >90% pure by NMR and LC–MS. Fisons (Loughborough, UK) HPLC-grade solvents were used; water was obtained from a Milli-Q system (Millipore).

2.2. LC conditions

A Hewlett–Packard LC system was used consist-

Table 1
Phosphonic acids and dialkyl phosphonates investigated

Name	Abbreviation
Phosphonic acids	
Methylphosphonic acid	MPA
Ethyl methylphosphonic acid	EMPA
n-Propyl methylphosphonic acid	PrMPA
Isopropyl methylphosphonic acid	iPrMPA
Isobutyl methylphosphonic acid	iBuMPA
Cyclohexyl methylphosphonic acid	CHMPA
Pinacolyl methylphosphonic acid	PinMPA
Ethylphosphonic acid	EPA
Methyl ethylphosphonic acid	MEPA
Ethyl ethylphosphonic acid	EEPA
n-Propyl ethylphosphonic acid	PrEPA
Isopropyl ethylphosphonic acid	iPrEPA
n-Propylphosphonic acid	PrPA
Methyl *n*-propylphosphonic acid	MPrPA
sec-Butyl *n*-propylphosphonic acid	sBuPrPA
Dialkyl alkylphosphonates	
Dimethyl methylphosphonate	DMMP
Ethylmethyl methylphosphonate	EMMP
Dimethyl ethylphosphonate	DMEP
Diethyl ethylphosphonate	DEEP
Diethyl-*n*-propylphosphonate	DEPP
Ethylmethoxyethyl *n*-propylphosphonate	
Ethylmethoxyethyl isopropylphosphonate	

ing of a Hewlett Packard model 1050 quaternary pump plus solvent conditioner. The system was fitted with a 250×2.1 mm I.D. Hichrom RPB column (Hichrom, Theale, UK), 5-μm particle size, C_8/C_{18} bonded silica, plus guard column. Gradient elution was used with solvents consisting of 0.1% formic acid or 0.05% TFA (trifluoroacetic acid) or 0.05 *M* ammonium formate, in water (solvent A) and in acetonitrile or methanol (solvent B). Chromatographic conditions were initially developed to separate a mixture of phosphonic acids consisting of MPA, EPA, EMPA, EEPA, PrPA, iPrMPA, CHMPA and PinMPA. Optimum ESP sensitivity was obtained with 0.1% formic acid in water (solvent A) and 0.1% formic acid in methanol (solvent B). The elution gradient was 5% B (0–5 min), 5% B to 80% B (5–15 min) and 80% B (15–22 min), at a flow-rate of 0.2 ml/min. Injections (5 μl) were made using a Rheodyne 9125 injector fitted with a 20-μl PEEK loop.

2.3. MS conditions

The column effluent was introduced into a Finnigan TSQ700 mass spectrometer via an atmospheric pressure ionisation source/interface, operated in either ESP or APCI mode. Conditions were investigated using spray voltages of 3–6 kV and interface capillary temperatures up to 250°C. For optimal analysis of phosphonic acids using ESP conditions were: spray voltage 5 kV, capillary temperature 200°C, Q0 offset −5 V, sheath gas N_2 at 410 kPa, auxiliary gas N_2 at a flow meter reading of 10. Capillary and tube lens voltages were optimised to give maximum response to *m/z* 181 ($[M+H]^+$) from PinMPA; this ensured that $[M+H]^+$ ions were prominent in the spectra. APCI conditions were as reported previously [35]: corona current 2 μA, vaporiser temperature 400°C, capillary temperature 150°C, Q0 offset −5 V, sheath gas nitrogen at a pressure of 410 kPa, auxiliary gas nitrogen at a flow-meter reading of 20.

2.4. Selected ion screening procedure

A selected ion screening procedure was developed based on the protonated molecules of the homologous series of acids with normal or branched chain O-alkyl substituents, i.e. *m/z* 97, 111, 125, 139, 153 and 181, plus *m/z* 179 for CHMPA; the ions *m/z* 97, 111 or 125 are also produced as $[RP(O)(OH)_2H]^+$ fragment ions (R=Me, Et, Pr) in most cases and serve as a second screening ion. The seven ions were monitored using a total scan time of 2 s, dwell time 0.28 s.

2.5. CID spectra

CID product ion spectra were obtained from the protonated molecules of the phosphonic acids using ESP or APCI under the LC conditions described above. Argon was used as collision gas at a pressure of 0.13 Pa (ESP) or 0.2 Pa (APCI), collision offset −20 V and Q0 offset −5 V (this reduced adduct ion formation with the solvent and ensured maximum $[M+H]^+$ entering Q1).

3. Results and discussion

3.1. Improved sensitivity using ESP

Under the gradient elution conditions employed previously [35], using a solvent of 0.05% TFA–water–CH_3CN, ESP was generally more sensitive than APCI by a factor of approximately 2. Subsequent variation of conditions was undertaken using ESP only. Changing the organic solvent from acetonitrile to methanol had little effect on sensitivity, although the intensities of adduct ions with solvent were reduced. The most significant improvement in sensitivity was obtained by substituting 0.1% formic acid for TFA as the acidic modifier. This gave a general increase in sensitivity by a factor of approximately 10. Zhou and Hamburger [36,37] have previously reported that TFA suppresses, and formic acid enhances, the formation of $[M+H]^+$ ions for a range of natural products when using ESP. These authors have given a detailed discussion of the factors involved [37]. The spectra obtained using MeOH as the organic solvent generally showed weaker solvent adducts compared with acetonitrile. MPA and O-alkyl methylphosphonic acids also gave weak solvent–fragment adducts at *m/z* 129 assumed to be $[MeP(O)(OH)_2H+MeOH]^+$. In addition to solvent adducts, adducts with Na^+ were generally observed.

Borret et al. [30] showed that phosphonic acids form a series of adducts with alkali metal ions. The use of reversed-phase LC in combination with MS reduces this problem in dirty samples because inorganic salts are not retained by the column, but divalent cations such as calcium are severely detrimental to the analysis of the simple alkylphosphonic acids and should be removed by elution through a cation exchange resin [1]. A preliminary investigation of negative ESP using 0.05 *M* ammonium formate as modifier at pH 5.9 gave strong $[M-H]^-$ ions; this solvent system enabled both positive and negative spectra to be obtained. However, sensitivity was less than with positive ESP using formic acid at pH 2.6 as modifier and the chromatographic separation was adversely affected. Borrett et al. [30] and Kostiainen et al. [32] have described the negative ion ESP spectra of several phosphonic acids.

3.2. Chromatography

A reconstructed ion chromatogram showing the separation and detection of 13 phosphonic acids (1 μg/ml in water) using selected ion monitoring, is shown in Fig. 2. Expanded selected ion chromatograms for *m/z* 97 and *m/z* 111, demonstrating the good signal-to-noise ratios for the lower alkyl homologues, are shown in Fig. 3. Peak shapes were generally good with minimal tailing. Injections of 5 μl or less gave the best resolution; larger volume injections gave enhanced detection limits but at the expense of resolution. Retention times, together with the major ions observed, are shown in Table 2. As was found using APCI, signal-to-noise ratios were lowest with the three most acidic and polar analytes, MPA, EPA and PrPA. Isomeric O-alkyl alkylphosphonic acids, e.g. MEPA–EMPA, EEPA–iPrMPA and iPrEPA–PrEPA were only partially resolved under the chromatographic conditions employed. The use of the method, under full scanning conditions, to detect EPA and iPrEPA in a dirty water sample spiked with each analyte at 10 ppm is shown in Fig. 4. Although less intense than iPrEPA, the peak for EPA gave a superior signal-to-noise ratio using ESP (S/N 15:1) in comparison with APCI (S/N 8:1) (also shown in Fig. 4). The analytes are clearly observed in the mass chromatogram for *m/z* 111 against a heavy background of polyethylene glycol-type contaminants as shown in the total ion chromatogram.

3.3. ESP spectra

The phosphonic acids generally gave intense [M+

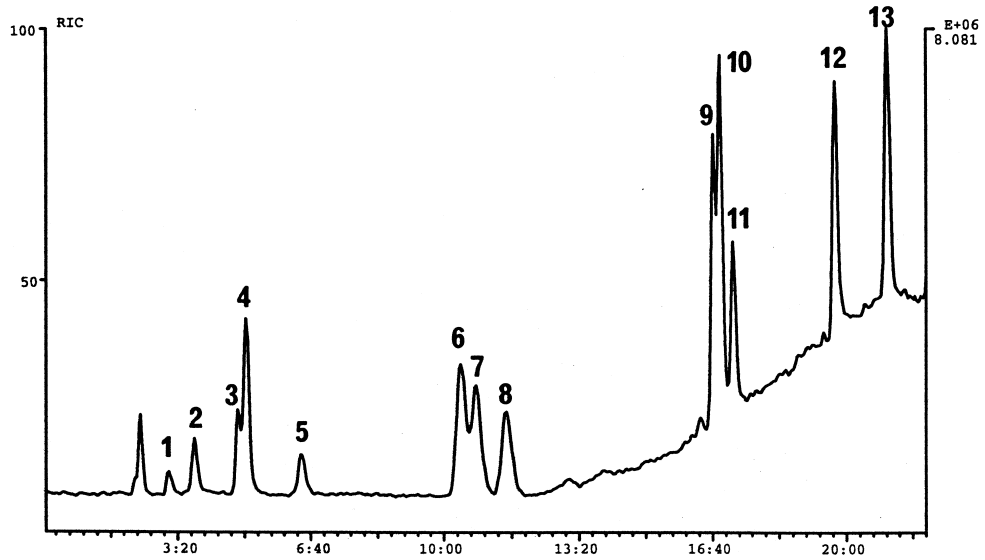

Fig. 2. LC–ESP-MS reconstructed ion chromatogram from a standard mixture of phosphonic acids (1 μg/ml of each): 1=MPA, 2=EPA, 3=MEPA, 4=EMPA, 5=PrPA, 6=EEPA, 7=iPrMPA, 8=PrMPA, 9=iPrEPA, 10=PrEPA, 11=iBuMPA, 12=CHMPA, 13=PinMPA.

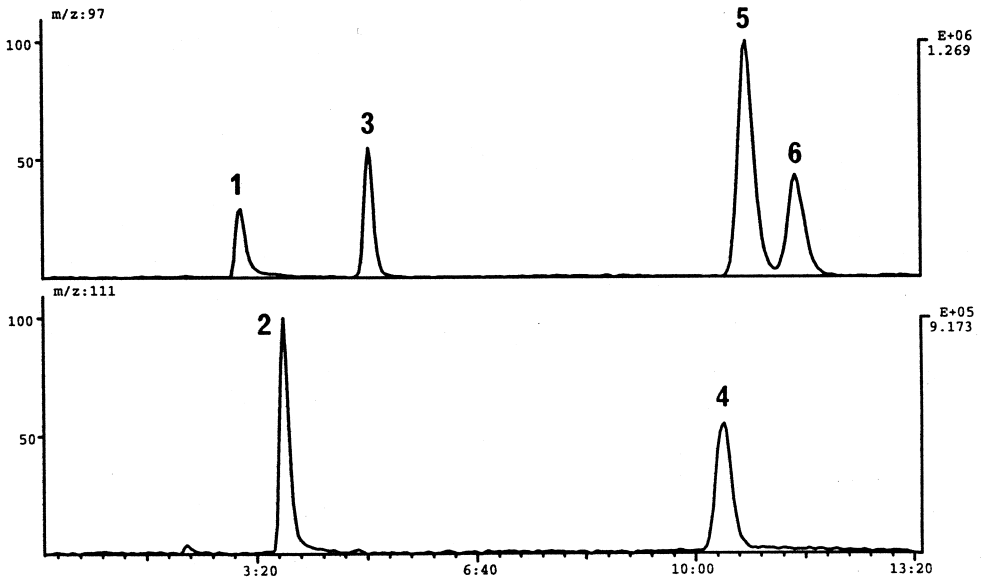

Fig. 3. LC–ESP-MS selected ion chromatograms showing the detection of lower alkyl homologues in a standard mixture of phosphonic acids (1 μg/ml of each): 1=MPA, 2=EPA, 3=MEPA, 4=EMPA, 5=PrPA, 6=EEPA.

$H]^+$ ions plus a major fragment ion $[RP(O)(OH)_2H]^+$ (R=Me, Et, Pr) in the case of O-alkyl alkylphosphonic acids. Adduct ions with water, sodium and methanol were observed in most cases, plus concentration dependent dimeric $[2M+H]^+$ ions (these vary from very weak to up to 50% abundance relative to the $[M+H]^+$ ion depending on the concentration). APCI and ESP spectra for iPrEPA are compared in Fig. 5. The ESP spectrum shows adduct ions with Na^+ (m/z 175) and methanol (m/z

Table 2
Retention times and ions observed for 14 phosphonic acids using LC–ESP-MS

Compound	Retention time (min.sec)	Ions observed (m/z)					
		$[2M+H]^+$ [a]	$[M+H+MeOH]^+$	$[M+Na]^+$	$[M+H+H_2O]^+$	$[M+H]^+$	Fragment ions
MPA	3.04	193	129	119	115	97	-
EPA	3.45	221	143	133	129	111	-
MEPA	4.54	249	157 (weak)	147	143	125	111 (weak)
EMPA	5.06	249	157 (weak)	147	143	125	97
PrPA	6.30	249	157	147	143	125	-
EEPA	10.32	277	-	161	157	139	97
MPrPA	10.44	277	171	161	157	139	-
iPrMPA	10.56	277	-	161	157	139	97
PrMPA	11.24	277	-	161	157	139	111
iPrEPA	16.43	305	185	175	-	153	111, 143
PrEPA	16.53	305	185	175	-	153	111, 143
iBuMPA	17.14	305	185	175	-	153	97, 129
CHMPA	19.41	357	211	201	-	179	97, 129
PinMPA	20.58	361	213	203	-	181	97, 129

[a] Concentration dependent.

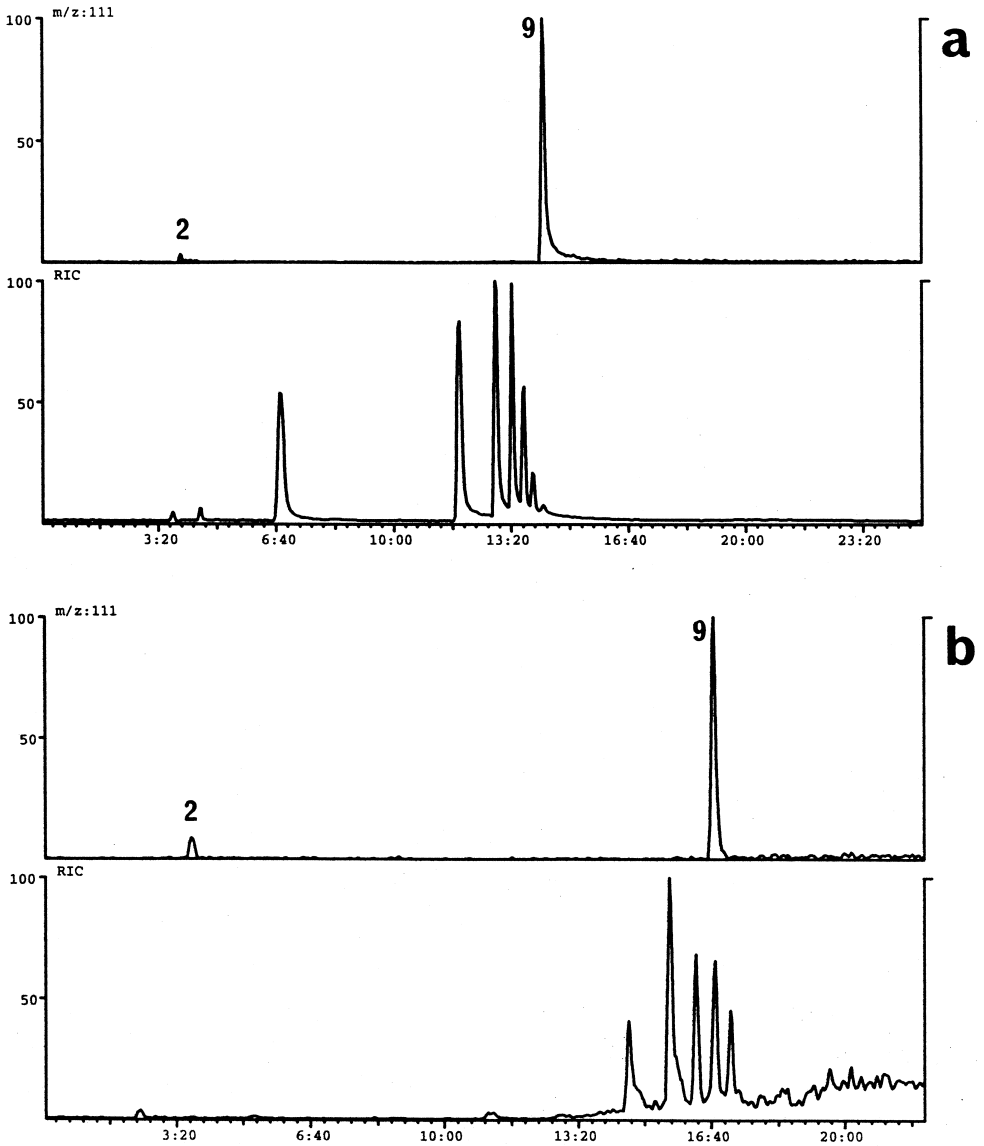

Fig. 4. Mass chromatograms (m/z 111) and total ion chromatograms, showing the detection of 2 (EPA) and 9 (iPrEPA), spiked into a sample of water (10 ppm) containing a heavy chemical background, (a) LC–APCI-MS, (b) LC–ESP-MS.

185), but both spectra are dominated by $[M+H]^+$ ions (m/z 153) and the fragment ion $[EtP(O)(OH)_2H]^+$ (m/z 111). The relative intensities of the adduct ions are very dependent on the source conditions and on the mobile phase composition at the time of elution and ionisation. It is therefore important that comparison of the spectra of unknown compounds with those of standards is performed under the same conditions. Alkylphosphonic acids gave intense (100%) $[M+H]^+$ ions plus adduct ions with water, Na^+ and methanol, but no major fragment ions. As reported previously using LC–APCI-MS [35], the fragment ions $[RP(O)(OH)_2H]^+$ can be used as the basis for a generic selected ion (or mass chromatographic) screening procedure for O-alkyl alkylphosphonic acids (except for O-methyl ana-

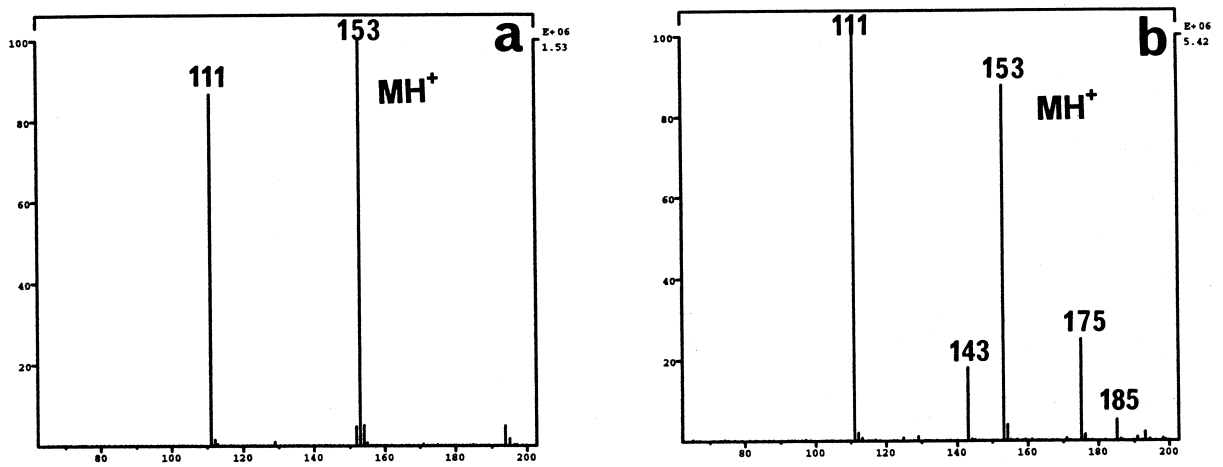

Fig. 5. LC–MS spectra of iPrEPA, (a) APCI and (b) ESP; ions at m/z 143 and 185 are adduct ions with MeOH, m/z 175 is $[M+Na]^+$.

logues) and alkylphosphonic acids, e.g. m/z 97 is common to methylphosphonic acids and m/z 111 for ethylphosphonic acids etc.

3.4. CID spectra

The major product ions together with their relative abundances, obtained by CID of the protonated molecules, are shown in Table 3. The O-alkyl alkylphosphonic acids gave common product ions $[RP(O)(OH)_2H]^+$ at m/z 97 (R=Me), 111 (R=Et) or 125 (R=Pr) in ESP and APCI spectra, with the exception of O-methyl analogues. These common product ions can be utilised for the application of LC–MS–MS to the trace analysis of phosphonic acids, with Q3 set to detect the common product ions and Q1 scanning for selected $[M+H]^+$ ions. Other fragment ions included loss of water from the $[RP(O)(OH)_2H]^+$ ions and, in the case of *n*-propylphosphonic acid, a weak ion at m/z 43 corresponding to the P-alkyl group. The CID spectra therefore provide a rapid means of provisionally

Table 3
CID product ion spectra of $[M+H]^+$ ions of phosphonic acids

Compound	Precursor ion m/z	Product ion spectra m/z (% abundance)
MPA	97	97 (100), 79 (28)
EPA	111	111 (100), 93 (30), 79 (2), 65 (5)
MEPA	125	125 (100), 107 (26), 93 (12), 79 (8), 65 (3)
EMPA	125	125 (5), 97 (100), 79 (2)
PrPA	125	125 (100), 107 (25), 65 (4), 43 (8)
EEPA	139	139 (26), 111 (100), 93 (6)
iPrMPA	139	139 (7), 97 (100), 79 (2)
PrMPA	139	139 (10), 97 (100), 79 (23), 43 (2)
iPrEPA	153	153 (3), 111 (100), 93 (1)
PrEPA	153	153 (2), 111 (100), 93 (3), 43 (2)
MPrPA	139	139 (100), 121 (16), 107 (11), 79 (3), 43 (7)
iBuMPA	153	153 (5), 97 (100), 57 (11)
CHMPA	179	97 (100), 83 (4)
PinMPA	181	181 (3), 97 (100), 85 (6)

Table 4
LC–ES-MS and CID product ion spectra of $[M+H]^+$ ions of dialkyl alkylphosphonates

Compound	$[M+H]^+$ m/z	$[M+Na]^+$ m/z	Fragment ions m/z	Product ion spectra of $[M+H]^+$ m/z (% abundance)
DMMP	125	147	111, 93 (weak)	125 (100), 93 (44)
EMMP	139	161	125, 111	139 (21), 111 (100), 93 (3)
DMEP	139	161	125	139 (100), 107 (29), 79 (2)
DEEP	167	189	139, 111	167 (35), 139 (55), 111 (100), 93 (3)
DEPrP	181	203	153, 125	181 (46), 153 (54), 139 (2), 125 (100), 111 (2)
iPr methoxyethyl MP[a]	197		155, 97	197 (5), 155 (100), 97 (51), 59 (57)
Et methoxyethyl iPrP[a]	211		133, 125 (w)	211 (23), 153 (100), 125 (99), 59 (33)

[a] APCI spectra.

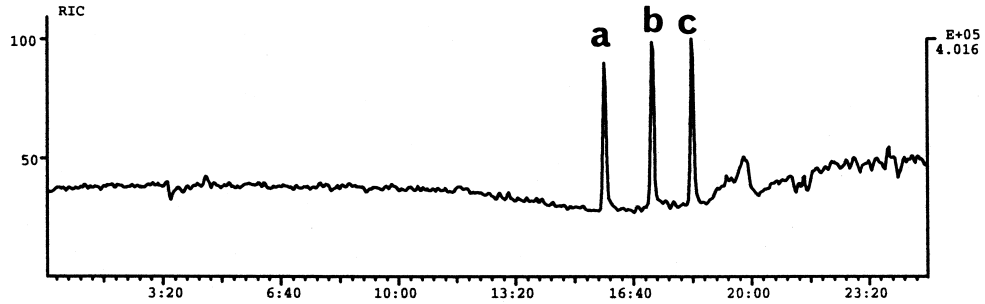

Fig. 6. LC–APCI-MS total ion chromatogram of an aqueous extract of a spiked soil sample showing the detection of three phosphonates (peaks a, b and c).

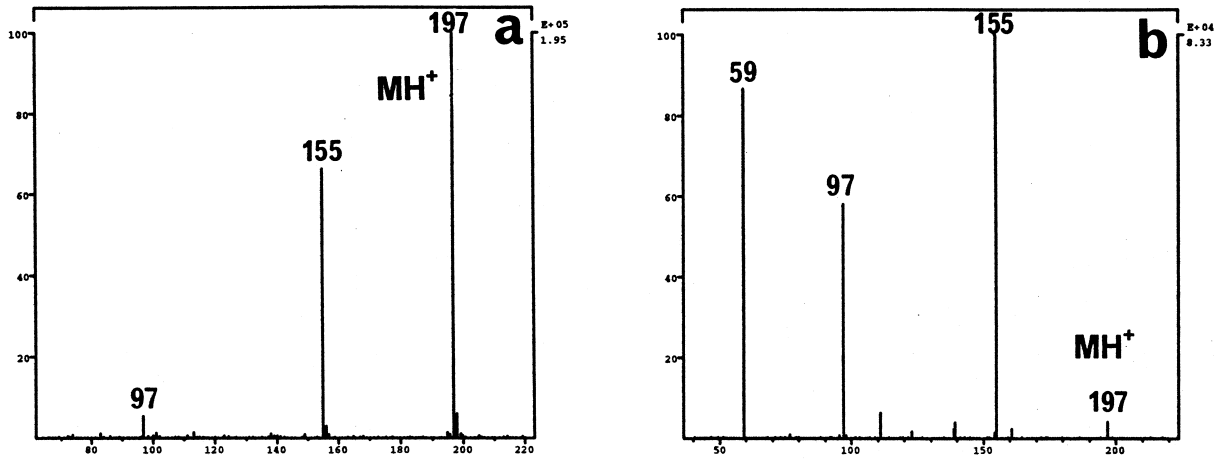

Fig. 7. (a) APCI and (b) APCI-CID spectra tentatively identifying peak b as propyl methoxyethyl methylphosphonate.

determining the alkyl group on phosphorus, although *n*-propyl is not obviously distinguished from isopropyl.

3.5. Dialkyl alkylphosphonates

The APCI and ESP spectra of dialkyl alkylphosphonates (Table 4) gave intense $[M+H]^+$ ions plus one or two major fragment ions. With the exception of methyl esters, the dialkyl alkylphosphonates showed neutral losses corresponding to loss of alkene with hydrogen transfer to P–O, e.g. $[M+H-28]^+$ for ethyl esters, $[M+H-42]^+$ for propyl esters etc. as was reported by Borrett et al. [30]. Methyl esters showed a fragment ion corresponding to loss of CH_4 from the protonated molecule. CID spectra of the protonated molecules are dominated by the product ions arising from similar losses in the case of ethyl ester and higher analogues and these ions provide a facile means of obtaining a tentative structure for the compounds. For example, diethyl methylphosphonate showed product ions corresponding to losses of one and two ethylene molecules. Methyl esters fragment by a different pathway, apparently by loss of methanol.

In a recent proficiency test three phosphonates

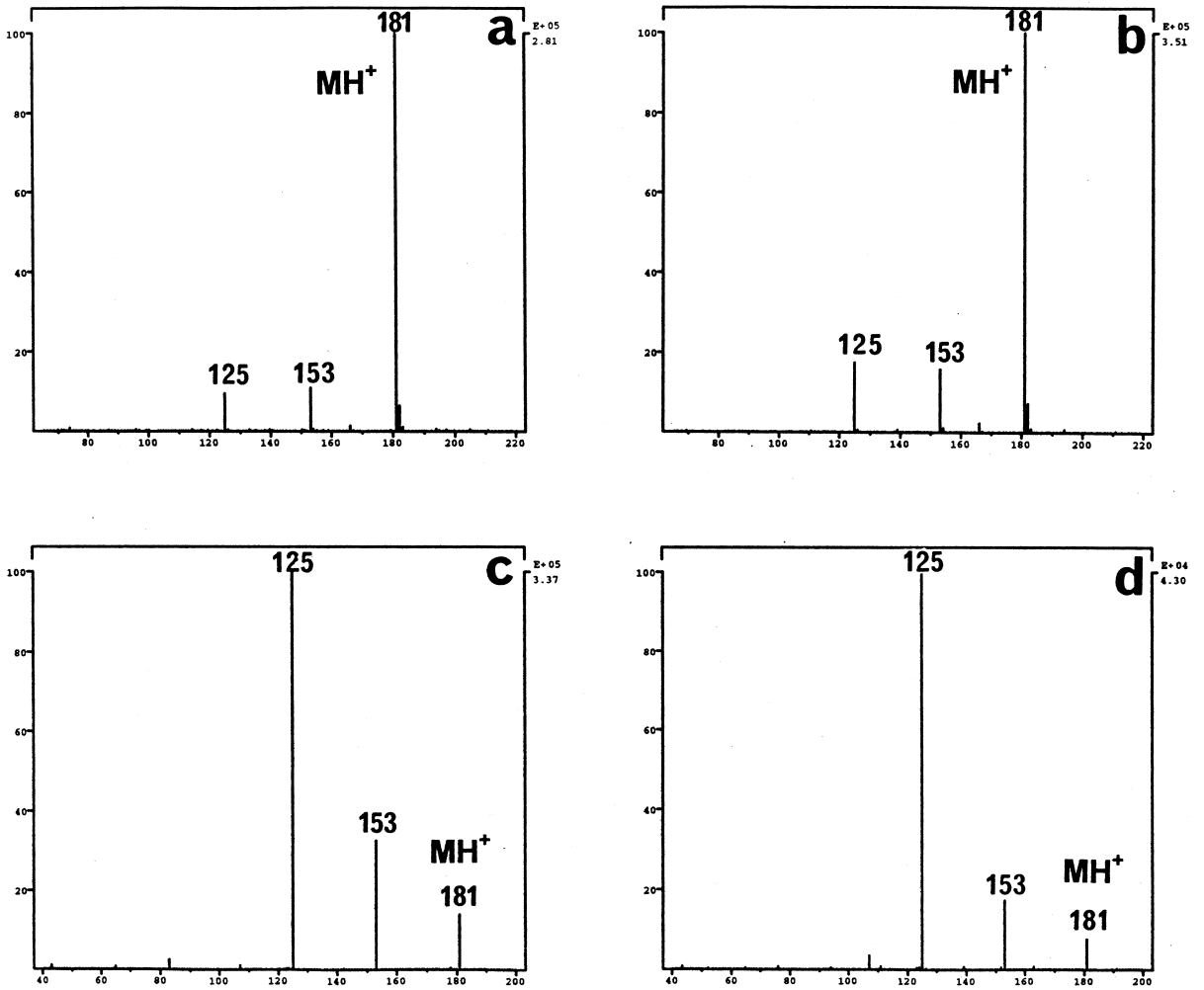

Fig. 8. APCI and APCI-CID spectra: (a) and (c) *sec*-butyl-*n*-propylphosphonic acid standard, (b) and (d) diethyl isopropylphosphonate from a spiked soil sample.

were spiked into a soil sample and appeared in the aqueous extract; a total ion chromatogram is shown in Fig. 6. Peak b in Fig. 6 gave a protonated molecule at m/z 197, which does not correspond to a member of the simple dialkyl alkylphosphonates (Fig. 7). A major fragment ion at m/z 155 (loss of 42 amu) indicated the presence of a propyl ester; a further loss of 58 suggested the presence of a $CH_3OCH_2CH_2$ ester (a butyl ester would be expected to lose C_4H_8). The CID spectrum (Fig. 7) showed major product ions at m/z 155 and 97, plus an unusually strong ion at m/z 59 which was assumed to be $[CH_3O{=}CHCH_3]^+$. The tentative identification of propyl methoxyethyl methylphosphonate was confirmed by comparison with a standard (the isopropyl isomer). Peak c was similarly tentatively identified as ethyl methoxyethyl propylphosphonic acid (see Table 4). Initial interpretation of APCI or ESP spectra must however be made with caution. Peak a in Fig. 6 possessed a retention time similar to that of *sec*-butyl *n*-propylphosphonic acid, and both its APCI and CID spectra were very similar (virtually identical) to that of the acid as shown in Fig. 8. The acid undergoes loss of ethylene from its *sec*-butyl substituent in addition to loss of butene. The analyte was in fact diethyl isopropylphosphonate which gives fragment ions at the same m/z values. For this reason we recommend that GC–MS with attempted derivatisation is used, in addition to LC–MS to confirm the identification of such compounds.

4. Conclusion

LC–MS is not used as a substitute for GC–MS in our laboratory but as a complementary technique. In terms of sensitivity and limits of detection in most matrices, GC–MS is superior for the nerve agent hydrolysis products, particularly when tandem mass spectrometry [6] or negative chemical ionisation of pentafluorobenzyl esters is used [11–13]. However, LC–MS provides a rapid screening of aqueous samples or extracts with minimal sample pretreatment, and a preliminary interpretation of fragmentation, in the case of unknowns, is much simpler than with derivatised compounds. As reported previously [35], for certain analytes such as thiodiglycol sulphoxide, LC–MS is superior to current GC–MS methods. LC–APCI-MS and LC–ESP-MS are expected to play an increasing role in the analysis of environmental samples for the hydrolysis products of chemical warfare agents.

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