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Journal of Chromatography A, 759 (1997) 79–92

JOURNAL OF CHROMATOGRAPHY A

Application of liquid chromatography–atmospheric pressure chemical ionisation mass spectrometry, and tandem mass spectrometry, to the analysis and identification of degradation products of chemical warfare agents

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Received 4 June 1996; revised 3 September 1996; accepted 3 September 1996

Abstract

A qualitative screening procedure was developed for the detection of the hydrolysis and related products of chemical warfare agents using liquid chromatography–mass spectrometry with atmospheric pressure chemical ionisation. A mixed C_8/C_{18} reversed-phase column gave acceptable chromatography for the range of acidic, neutral and basic analytes. Detection limits for pure standards were less than 0.2 ng injected for the simple hydrolysis products of sulphur and nitrogen mustards, thiodiglycol sulphoxide, diisopropylaminoethanol and 3-quinuclidinol. Detection limits were in the range 0.2–8 ng injected for a series of alkylphosphonic acids and alkyl alkylphosphonic acids, and for benzilic acid. The methodology provides a rapid screening procedure for aqueous samples and extracts and was applied to the analysis of soil samples collected from bomb craters, and to spiked water and soil samples.

Keywords: Chemical warfare agents; Detection, LC; Alkylphosphonic acids; Ethanolamines; Thiodiglycol compounds; Quinuclidinol; Benzilic acid; Diisopropylaminoethanol

1. Introduction

The Chemical Weapons Convention was opened for signature in January 1993 and will enter into force after it has been ratified by 65 nations. An important aspect of investigating suspected cases of non-compliance with the Convention will be the detection and identification of chemical warfare (CW) agents, their precursors or their degradation products, in samples associated with challenge inspections of suspect production or storage facilities, or associated with allegations of use. The most widely applicable technique for the detection and identification of undegraded CW agents is gas chromatography–mass spectrometry (GC–MS) [1,2], used as a general screening procedure and for unequivocal identification by the application of electron and chemical ionisation techniques [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]. Many CW agents are chemically reactive electrophiles, whose toxicity is me-

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PII S0021-9673(96)00763-7

diated by reaction in vivo with key biological nucleophiles. This reactivity with nucleophiles is also a major mechanism for their degradation, e.g., by reaction with water present in the environment. The analysis of environmental and biological residues for products derived from hydrolysis is therefore an important component of 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 collected from the site of a chemical attack [6].

The hydrolysis products considered in this paper are those derived from nerve agents (alkyl alkylphosphonic acids, alkylphosphonic acids and diisopropylaminoethanol), sulphur mustard (thiodiglycol, thiodiglycol sulphoxide, thiodiglycol sulphone), nitrogen mustards (triethanolamine, N-ethyldiethanolamine, N-methyldiethanolamine) and quinuclidinyl benzilate, BZ (benzilic acid and 3-quinuclidinol). These compounds are considerably more polar and less volatile than their precursor CW agents. In most cases they are efficiently extracted from environmental residues with water following a Recommended Operating Procedure [2]. For GC–MS analysis, the aqueous extract is concentrated to dryness and the hydrolysis products converted to trimethylsilyl (TMS) [2,7,8] or *tert.*-butyldimethylsilyl (TBDMS) derivatives [6,9], or to methyl esters in the case of phosphonic acids [10,11] and benzilic acid [12]. Apart from introducing additional stages into the analysis, derivatisation, particularly to silyl derivatives, can result in a large variability in the apparent recovery, particularly in cases where large amounts of extraneous materials are co-extracted or, in the case of alkylphosphonic acids, in the presence of divalent metal ions such as calcium [6,13]. An obvious alternative to GC–MS is reversed-phase liquid chromatography–mass spectrometry (LC–MS), which allows the direct analysis of aqueous extracts or water samples with little or no sample preparation, depending on the concentration of analytes and extraneous materials present. The widespread use of LC–MS has been hindered by the lack of a robust universal LC–MS interface, problems of sensitivity, and the general cost and availability of the instrumentation. Recent advances in atmospheric pressure ionisation techniques [14], and their interfacing to quadrupole or ion trap MS systems, have overcome most of these disadvantages.

The four LC–MS sample introduction/ionisation techniques that have been most widely used for the analysis of polar pesticide residues are thermospray (TS), particle beam (PB) with electron ionisation (EI), atmospheric pressure chemical ionisation (APCI) and electrospray/ionspray (ES/IS) [15,16]. TS is well suited to most low-molecular-mass polar compounds but the sensitivity for different types of compound shows a wide variability and decomposition is a problem with thermally labile compounds. Because of its commercial availability for approximately 10 years, TS has been the most widely used LC–MS method for pesticide analysis [17,18]. PB with EI has the advantage that mass spectra resembling reference EI spectra are produced, but sensitivity for polar analytes is generally lower than with the other techniques, and there are limitations to the percentage of aqueous phase that can be accommodated at normal LC flow-rates [15]; it is not the best technique for water-soluble polar compounds [19]. The atmospheric pressure ionisation techniques APCI and ES/IS are proving to be the most sensitive and broadly applicable techniques for the analysis of polar compounds, APCI being more commonly applied to non-ionised compounds of low molecular mass [20]. APCI is reported to be less sensitive to changes in analyte structure in comparison with TS and PB and, like TS, it can be used with conventional-bore reversed-phase LC systems at normal flow-rates. The major limitation of TS, APCI and ES ionisation is that, under conditions providing optimum sensitivity, they usually produce protonated molecules or other adduct ions with little or no fragmentation; they are therefore good techniques for screening but have limitations for providing structural information or unequivocal "fingerprints" for confirmation of identification. Variation of the voltages between the spray nozzle and the skimmer prior to mass analysis can induce pre-analyser collision induced dissociation (CID), producing fragment ions for confirmation but, for an optimum signal-to-noise ratio, structural information is best obtained by using conventional tandem mass spectrometry (MS–MS). CID of the protonated molecule or high mass fragment ions provides reproducible product ion spectra that can be used for confirmation of identification, or for obtaining structural information in the case of unknowns [21].

Wils and Hulst [22–24] demonstrated the potential

of LC–TS-MS for the analysis of nerve agents, their hydrolysis products, and some hydrolysis products related to sulphur mustard, in aqueous samples such as river water. Kientz et al. [25,26] reported a screening method using microcolumn LC with flame photometric detection for nerve agents and their hydrolysis products. Kostiainen et al. [27] have demonstrated the application of capillary zone electrophoresis (CZE) in combination with IS-MS to the detection of nerve agent hydrolysis products and Creasy et al. [28] reported the use of TS-MS and CZE–MS in the analysis of samples prepared for an international collaborative exercise on the analysis of CW agents and their degradation products. During the preparation of this manuscript, Borrett et al. [29] reported the use of positive and negative ES, using loop injection, for the analysis of nerve agent and sulphur mustard degradation products. In this paper we report a general LC–MS screening procedure for the qualitative detection of the hydrolysis products of nerve agents, sulphur and nitrogen mustards and BZ, using LC–MS–MS for confirmation of identification. The procedure is illustrated by its application to chemical warfare residues collected from the Kurdish village of Birjinni, Iraq (previously analysed using GC–MS and GC–MS–MS [6]), and to samples that were analysed as part of a series of international collaborative exercises.

2. Experimental

2.1. Standards and solvents

The compounds (numbered in order of elution time) included in the screening procedure were: (**1**) methylphosphonic acid (MPA), (**2**) thiodiglycol sulphoxide, (**3**) triethanolamine, (**4**) N-methyldiethanolamine, (**5**) ethylphosphonic acid, (**6**) N-ethyldiethanolamine, (**7**) thiodiglycol sulphone, (**8**) 3-quinuclidinol, (**9**) ethyl MPA, (**10**) thiodiglycol, (**11**) *n*-propylphosphonic acid, (**12**) diisopropylaminoethanol, (**13**) ethyl ethylphosphonic acid, (**14**) isopropyl MPA, (**15**) *tert.*-butylphosphonic acid, (**16**) *n*-butylphosphonic acid, (**17**) cyclohexyl MPA, (**18**) pinacolyl MPA, (**19**) benzilic acid. Samples of **2**, **7**, **9**, **13**, **14**, **17** and **18** were synthesised in the Organic Chemistry Section, CBD and were ≥95% pure by NMR and LC-MS. Compounds **1**, **3**, **4**, **6**, **10–12**, **15**, **16** and **19** were purchased from Aldrich (Gillingham, UK). Fisons (Loughborough, UK) HPLC-grade solvents were used; water was obtained from a Milli-Q system (Millipore).

2.2. LC conditions

An LKB HPLC system was used consisting of two LKB 2150 pumps, a high pressure gradient mixing valve, an LKB 2156 solvent conditioner and an LKB 2152 controller. The system was fitted with a Hichrom RPB column (Hichrom, Theale, UK), 250×3.2 mm I.D., packed with a mixed C_8/C_{18} stationary phase of 5-μm particle size, plus guard column. The mobile phase consisted of 0.05% TFA (trifluoroacetic acid) in water (solvent A) and 0.05% TFA in acetonitrile (solvent B); a gradient elution was used from 95% A, 5% B for 5 min, to 20% A, 80% B from 5 to 20 min, and 20% A, 80% B from 20 to 25 min, at a flow-rate of 0.4 ml/min. Injections (20 μl or less) were made using a Rheodyne 9125 injector fitted with a 20 μl polyether ether ketone (PEEK) loop.

2.3. APCI-MS conditions

A Finnigan TSQ 700 triple stage quadrupole instrument was employed, fitted with a Finnigan atmospheric pressure ionisation source/LC interface.

2.3.1. Optimisation of APCI conditions

A standard solution containing a mixture of alkylphosphonic acids, alkyl methylphosphonic acids, thiodiglycol, thiodiglycol sulphoxide and thiodiglycol sulphone, was introduced via the LC system. The source parameters were varied between the following limits: corona current 2 to 8 μA, source octapole (Q0) offset voltage −5 to −20 V, vaporiser temperature 300 to 500°C, capillary temperature 100 to 250°C. Two sets of conditions were then adopted for use in screening procedures. The first set of conditions, applied as a general screening procedure for all of the analytes, used a corona current 2 μA, vaporiser temperature 400°C, capillary temperature 150°C and Q0 offset −5 V. The second set of conditions, applied as a screening procedure for phosphonic acids, used a corona current 2 μA, vaporiser temperature 400°C, capillary temperature 200°C, Q0 offset −20 V. In both cases, nitrogen was

used as sheath gas at a pressure of 410 kPa, and as the auxiliary gas at a flowmeter reading of 20. The electron multiplier voltage was 1000 or 1200 V. Instrument tuning parameters were optimised for each set of conditions.

2.3.2. Selected ion screening procedure

Nineteen hydrolysis products were included in the screening procedure. The selected ions monitored and retention times are shown in Table 1. A total of 14 ions was required to screen for the complete range of hydrolysis products. The mass spectrometer was programmed to monitor ions in three groups, according to retention times, as follows: *m/z* 97, 111, 120, 128, 134, 139, 150 and 155 (methylphosphonic acid to 3-quinuclidinol) from 0 to 5.4 min, dwell time 0.25 s each ion, total scan time 2 s; *m/z* 105, 111, 125, 139 and 146 (ethyl ethylphosphonic acid to *n*-butylphosphonic acid) from 5.4 min to 15 min, dwell time 0.4 s each ion, total scan time 2 s; *m/z* 179, 181 and 224 (cyclohexyl methylphosphonic acid to benzilic acid) from 15 min to 25 min, dwell time 0.67 s each ion, total scan time 2 s. For the alternative detection of phosphonic acids up to C_4, monitoring of only 4 ions was required: *m/z* 97, 111, 125 and 139, dwell time 0.5 s each ion, total scan time 2 s.

2.4. CID spectra

CID product ion spectra were obtained for all of the analytes under the general conditions described above (Section 2.3). Product ion spectra obtained from MH^+ or major fragment ions were recorded using loop flow injections into acetonitrile–water (50:50) plus 0.05% TFA, at a flow-rate of 0.2 ml/min. CID was obtained using argon as collision gas at a pressure of 0.2 Pa, collision offset −25 V and Q0 offset −5 V (this reduced adduct ion formation with the solvent and ensured maximum MH^+ entering Q1). Larger collision offset voltages were used in the case of 3-quinuclidinol in order to induce dissociation, the stable MH^+ ion *m/z* 128 giving little fragmentation even at −35 V. Benzilic acid gave *m/z* 182 as the only product ion at −25 V offset, and *m/z* 182 and 104 (due to further loss of benzene) at −35 V offset. Thiodiglycol sulphone *m/z* 155 required only −15 V offset for dissociation but the CID spectrum was weak.

Table 1
Retention times and selected ions monitored for 19 analytes

No.	Compound	Retention time (min)	Selected ion (*m/z*)
1	Methylphosphonic acid	3.71	97
2	Thiodiglycol sulphoxide	3.93	139
3	Triethanolamine	4.14	150
4	N-Methyldiethanolamine	4.18	120
5	Ethylphosphonic acid	4.39	111
6	N-Ethyldiethanolamine	4.44	134
7	Thiodiglycol sulphone	4.47	155
8	3-Quinuclidinol	5.00	128
9	Ethyl methylphosphonic acid	5.69	125
10	Thiodiglycol	6.84	105
11	*n*-Propylphosphonic acid	7.19	125
12	Diisopropylaminoethanol	8.45	146
13	Ethyl ethylphosphonic acid	11.07	139
14	Isopropyl methylphosphonic acid	11.51	139
15	*tert.*-Butylphosphonic acid	12.61	139
16	*n*-Butylphosphonic acid	13.35	139
17	Cyclohexyl methylphosphonic acid	16.37	179
18	Pinacolyl methylphosphonic acid	17.32	181
19	Benzilic acid	20.23	224

3. Results and discussion

3.1. Chromatography

The analysis of the complete range of hydrolysis products in a single screening procedure required a column capable of separating basic, acidic and neutral compounds whilst using the same mobile phase system. The mixed C_8/C_{18} column employed was initially developed to chromatograph basic compounds, without the need to use basic mobile phase modifiers, but it is also suitable for acidic analytes. This column provided acceptable chromatography for the complete range of CW agent hydrolysis products using a conventional water–acetonitrile–TFA mobile phase with gradient elution. A total ion current chromatogram obtained from a standard solution containing all of the analytes, and using the general screening conditions, is shown in Fig. 1. Some analytes, e.g., triethanolamine (**3**) and N-methyldiethanolamine (**4**), were not resolved under the conditions used but were easily distinguished by their mass chromatograms. A chromatogram obtained using the alternative APCI conditions, for detecting phosphonic acids only, is shown in Fig. 2 for the same standard mixture. Peak shapes were generally good with minimal tailing. Injections of 5 μl or less gave the best resolution; larger volume full loop injections gave enhanced detection limits but at the expense of resolution in the early part of the chromatogram. Some peak splitting occurred with larger injections, but was only a serious problem with N-ethyldiethanolamine (**6**) and, to a lesser degree, with 3-quinuclidinol. Retention times were reproducible within 10 s for all compounds and within 5 s for most. Replicate (n=6) 5 μl-injections of a 10 μg/ml solution of hydrolysis products gave peak heights reproducible within a R.S.D. of ≤20% for all of the compounds and ≤10% for most.

3.2. APCI spectra

Full scan APCI-MS and APCI-MS–MS spectra were obtained for all compounds. Under the conditions employed, APCI spectra of most neutral and basic analytes showed intense MH^+ ions with no or

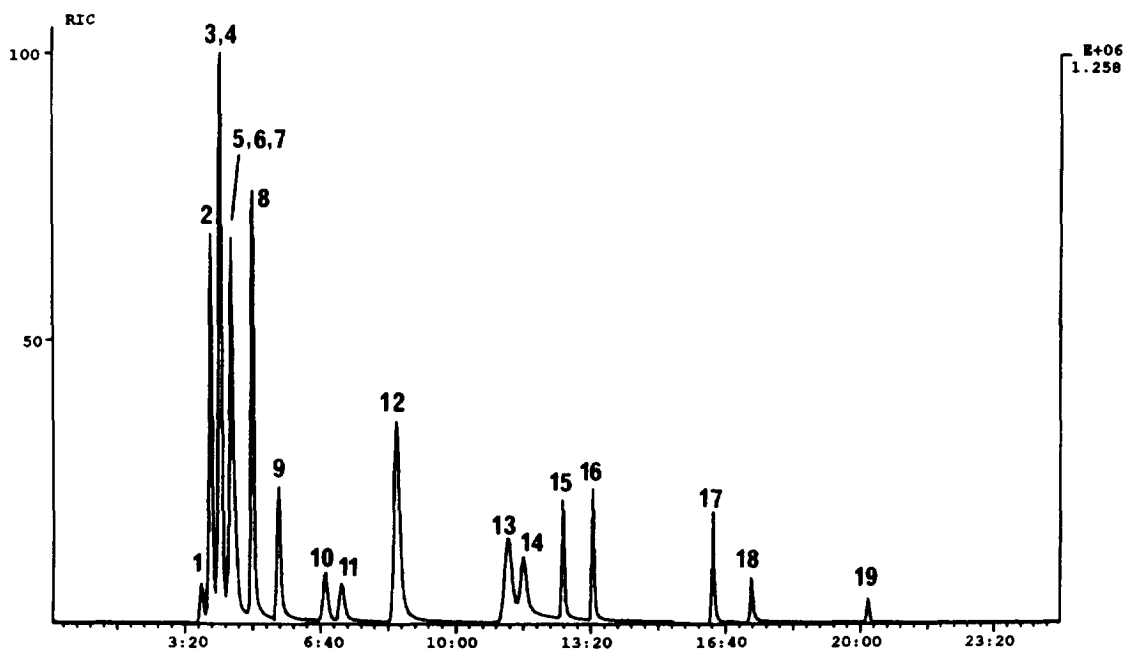

Fig. 1. Reconstructed ion current LC–MS chromatogram of a standard mixture of compounds (10 μg/ml each) (compounds are numbered as given in Section 2.1).

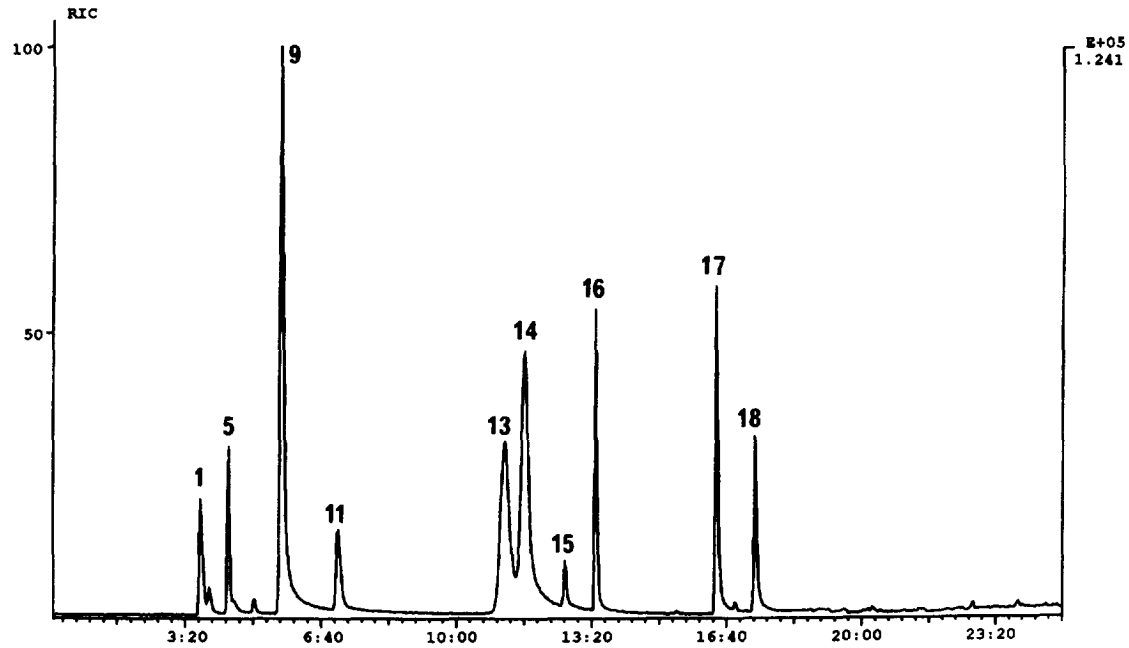

Fig. 2. Reconstructed ion current LC–MS chromatogram showing the selective detection of phosphonic acids in a standard mixture of analytes (10 μg/ml each) (compounds are as given in Section 2.1).

few significant fragment ions. Using the general screening conditions, alkyl alkylphosphonic acids, in addition to MH^+ ions, gave intense $[RP(O)(OH)_2H]^+$ fragment ions plus adduct ions with water and acetonitrile. Concentration dependent dimeric $[2M+H]^+$ ions were also observed. The relative intensities of the adduct ions depended 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%) MH^+ ions plus adduct ions with acetonitrile. Under the alternative APCI conditions for analysing phosphonic acids only, using a higher capillary temperature and Q0 offset, protonated molecules were not observed or were very weak for alkyl alkylphosphonic acids and the APCI spectra were dominated by the $[RP(O)(OH)_2H]^+$ fragment ions. These ions can be used as the basis for a generic selected ion (or mass chromatographic) screening procedure for alkyl alkylphosphonic acids and alkylphosphonic acids, e.g., using *m/z* 97 for methylphosphonic acids and *m/z* 111 for ethylphosphonic aicds. The two compounds that did not show intense MH^+ ions were thiodiglycol and benzilic acid. Thiodiglycol gave an intense ion at *m/z* 105 resulting from loss of water from MH^+; benzilic acid gave an intense ion *m/z* 224 presumed to be the adduct ion $Ph_2C{=}OH^+ + CH_3CN$. This appears to be formed by fragmentation rather than thermal degradation of benzilic acid to benzophenone because the latter did not give this adduct ion under similar conditions. The APCI mass spectra of thiodiglycol, thiodiglycol sulphoxide, triethanolamine and isopropyl MPA, obtained under LC–MS conditions, are shown in Fig. 3.

3.3. CID spectra

The major product ions together with their relative abundances, obtained by CID of the protonated molecules, or major fragment ions in the case of thiodiglycol and benzilic acid, are shown in Table 2. CID product ion spectra obtained from thiodiglycol, thiodiglycol sulphoxide, triethanolamine and isopropyl MPA are shown in Fig. 4. All of the compounds gave CID product ion spectra at a collision

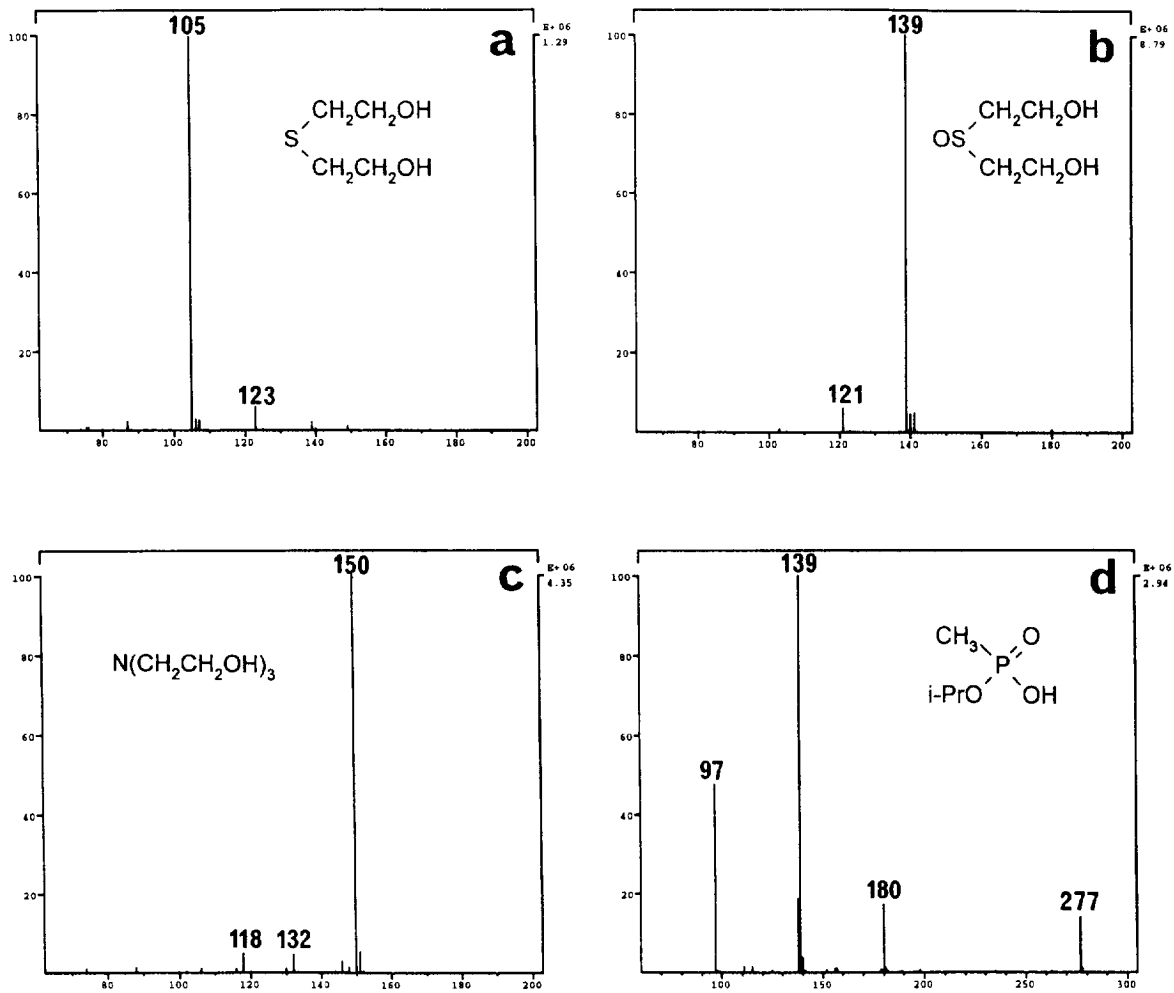

Fig. 3. APCI spectra: (a) thiodiglycol ($MH^+ = m/z$ 123), (b) thiodiglycol sulphoxide ($MH^+ = m/z$ 139), (c) triethanolamine ($MH^+ = m/z$ 150), (d) isopropyl MPA ($MH^+ = m/z$ 139, $2M+H^+ = m/z$ 277).

cell offset voltage of −25 V except for 3-quinuclidinol. The bicyclic protonated molecule of 3-quinuclidinol, m/z 128, gave little fragmentation even at an offset voltage of −35 V, and therefore LC–MS–MS under these conditions would not be a suitable method for confirmation of identification (although lack of fragmentation would be indicative of a stable cyclic structure).

The O-alkyl methylphosphonic acids gave a common product ion at m/z 97 in the APCI spectra, presumed to be $[MeP(O)(OH)_2H]^+$; O-alkyl ethylphosphonic acids gave the homologous product ion $[EtP(O)(OH)_2H]^+$ at m/z 111. 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 MH^+ ions. Using the alternative APCI conditions the common fragment ions, e.g., $[MeP(O)(OH)_2H]^+$, can be used as precursors transmitted by Q1 with Q3 detecting product ions m/z 79 and m/z 47.

3.4. Quantitation and limits of detection

In the verification analysis of CW agents, unequivocal identification is the most important re-

Table 2
CID spectra of MH^+ or high mass fragment ions

Compound No.	Precursor ion (*m/z*)	Product ion spectra,[a] *m/z* (%)
1	97	97 (34), 79 (100), 47 (6)
2	139	139 (24), 121 (3), 93 (22), 77 (100), 75 (21), 63 (45), 59 (21), 45 (6)
3	150	150 (52), 132 (50), 114 (36), 106 (8), 88 (87), 86 (6), 84 (10), 70 (100)
4	120	120 (12), 102 (40), 84 (3), 76 (5), 58 (100), 45 (21), 42 (3)
5	111	111 (57), 93 (100), 65 (60)
6	134	134 (61), 116 (100), 90 (9), 88 (14), 72 (60), 70 (14), 45 (10), 44 (12)
7	155	155 (100), 137 (36), 109 (29), 103 (9), 66 (4), 48 (4)
8	128	128 (100), 84 (4), 82 (10), 81 (4), 69 (4), 56 (10), 55 (7)
9	125	97 (100), 79 (46)
10	105	105 (40), 104 (5), 87 (11), 85 (3), 61 (91), 59 (13), 45 (100)
11	125	125 (70), 107 (100), 83 (5), 65 (30), 43 (89)
12	146	146 (22), 104 (100), 86 (46), 62 (98), 44 (62), 43 (16)
13	139	111 (100), 93 (47), 65 (5)
14	139	97 (100), 79 (19)
15	139	139 (10), 83 (17), 57 (100)
16	139	139 (54), 121 (100), 111 (5), 65 (20), 57 (80), 55 (27), 41 (10)
17	179	97 (100), 83 (6), 79 (3), 55 (3)
18	181	97 (100), 85 (3), 79 (4)
19	224	182 (61), 104 (100)

[a] Ions with relative intensity ≥3% of the base peak.

quirement. The procedure has therefore not been developed as a quantitative method. Limits of detection based on a *S/N* ratio of 3:1, estimated by 20-μl injections at concentrations down to 10 ng/ml, are shown in Table 3. Good sensitivity was shown for the analytes derived from the hydrolysis products of vesicants with the exception of thiodiglycol sulphone. However, our experience suggests that the latter is rarely an important degradation product of sulphur mustard, the most important ones being thiodiglycol and thiodiglycol sulphoxide. The method gave intermediate sensitivity for alkyl methylphosphonic acids, generally decreasing with increasing size of the alkyl groups, and was least sensitive for the alkylphosphonic acids. Methylphosphonic acid, and to a lesser extent ethylphosphonic acid, are the alkylphosphonic acids of importance as degradation products of CW agents. The nerve agent analogues derived from the higher alkylphosphonic acids are generally less toxic and only organophosphorus compounds with P-alkyl groups up to C_3 are specifically covered by the CWC. Two butyl homologues were included in the screening procedure because of the inclusion of *n*-butylphosphonic acid in a recent round robin exercise (see below).

It is not possible to compare the limits of detection obtainable with APCI and ES [27,29] from the data available. A possible disadvantage of ES-MS is the occurrence of multiple adduct ions with metal cations in spectra derived from untreated water samples or aqueous extracts of soil [29]. The absolute sensitivity (in terms of pg injected) of LC–APCI-MS is less than that of CZE–IS-MS [27] for alkyl MPAs, but this is offset by the much smaller injection volumes required for the latter. In terms of limits of detection in aqueous solution the CZE and LC methods appear to be comparable for the phosphonic acids. In comparison with GC–MS, using TBDMS or TMS derivatives, the LC–MS method was less sensitive for most analytes by 1–2 orders of magnitude although this may be partially offset by the higher recoveries obtained using direct LC–MS analysis (see below). The method is particularly useful for the analysis of thiodiglycol sulphoxide for which GC–MS is relatively less sensitive. It is likely that limits of detection could be lowered by introducing a preconcentration step, provided chemical background is low, or by using LC–MS–MS.

Parameters derived from calibration graphs of peak height vs. concentration for five analytes, made

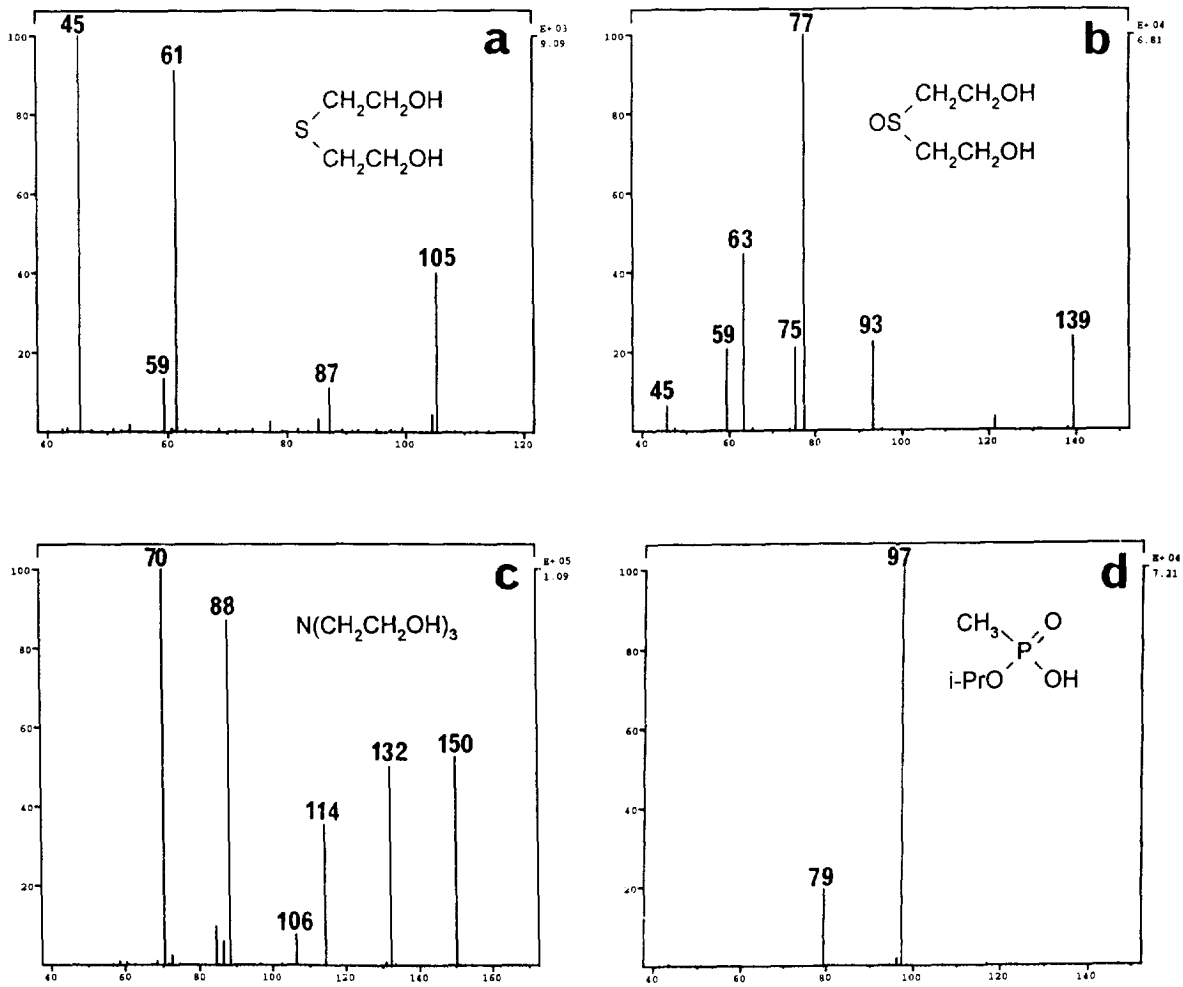

Fig. 4. CID spectra derived from (a) thiodiglycol, *m/z* 105, (b) thiodiglycol sulphoxide, *m/z* 139, (c) triethanolamine *m/z* 150, (d) isopropyl MPA, *m/z* 139.

Table 3
Limits of dectectability for standards

Compound No.	ng/ml	ng injected	Compound No.	ng/ml	ng injected
1	200	4	**11**	200	4
2	≤10	≤0.2	**12**	≤10	≤0.2
3	10	0.2	**13**	10	0.2
4	≤10	≤0.2	**14**	10	0.2
5	400	8	**15**	100	2
6	≤10	≤0.2	**16**	200	4
7	300	6	**17**	50	1
8	≤10	≤0.2	**18**	50	1
9	10	0.2	**19**	20	0.4
10	≤10	≤0.2			

Table 4
Linear regresion data for selected analytes

Compound No.	Slope	Intercept	Correlation coefficient[a]
1	152±33	−145±31	0.983
14	196±67	−56±43	0.995
10	148±13	−1.34±7.99	0.999
2	680±101	−132±50.9	0.998
8	828±166	1.71±149	0.996

[a] Calculated over the calibration range 0.05–10 μg/ml (0.5–10 μg/ml for MPA).

up in water at 6 concentrations from 0.05–10 μg/ml (4 concentrations from 0.5–10 μg/ml for MPA) are shown in Table 4. Acceptable linearity (for a qualitative screening procedure) over the range 0.1–10 μg/ml was observed for **2**, **8**, **10** and **14**. MPA (**1**) did not give a linear calibration, resulting in a pronounced concave curve at low concentrations. This analyte is very prone to binding to metal ions and probably to a variety of polar sites present in the LC–MS system.

The method is designed primarily for use as a general qualitative screening procedure for the hydrolysis products of CW agents, and as a confirmatory method for most of these analytes using LC–MS–MS. The analytes vary considerably in terms of their physicochemical properties. MPA has a pK_a of around 2 and is highly polar; thiodiglycol is a neutral compound whilst the hydrolysis products of nitrogen mustards, diisopropylaminoethanol and 3-quinuclidinol are strongly basic. If optimum sensitivity is required then a separate screen for the phosphonic acids would be preferable, possibly using electrospray ionisation rather than APCI and using a column whose properties are optimised for acidic analytes.

3.5. Applications to the analysis of environmental residues

In a previous paper [6] we reported the identification of the hydrolysis products of the vesicant sulphur mustard and the nerve agent sarin in soil samples collected from four bomb craters found in the Kurdish village of Birjinni in Northern Iraq. The analyses were performed using GC–MS and GC–MS–MS, with derivatisation of thiodiglycol and phosphonic acids to their TBDMS derivatives. Two of these samples have been further analysed using the LC–MS method reported above. Soil sample 4C was previously found to contain thiodiglycol (approximately 3 ppm) using GC–MS. Thiodiglycol (**10**) and its sulphoxide (**2**) were readily detected in an aqueous extract of the sample using the LC screening method. Mass chromatograms of the ions *m/z* 105 and *m/z* 139, constructed from an LC–MS analysis performed in full scan mode, are shown in Fig. 5. Confirmation of the identification of **2** was obtained from the LC–MS–MS spectrum, shown in Fig. 6. LC–MS provided good sensitivity for the sulphoxide which may be an important degradation product of sulphur mustard in some types of soil. In a recent round robin exercise [30], in which loamy soil was spiked with **10**, the latter was degraded to below detectable levels but **2** (along with thiodiglycolic acid, 1,4-dithiane and 1,4-dithiane sulphoxide) was one of the identified degradation products. Thiodiglycol sulphoxide is also an important urinary excretion product of sulphur mustard although its use as a biological marker of poisoning is limited by the presence of very low concentrations in normal human urine [31]. Thiodiglycol sulphoxide is less easily detected using GC–MS than is thiodiglycol. Under the normal conditions used for converting thiodiglycol to its TBDMS derivative [6], the sulphoxide forms a complex mixture of products. A TMS derivative of the sulphoxide is formed under normal conditions using BSA and TMSCl as derivatising reagents although detection limits are generally higher than those obtained for thiodiglycol by a factor approaching 5. Derivatisation of thiodiglycol sulphoxide using pentafluorobenzoyl chloride produces the bis(pentafluorobenzoyl) derivative of

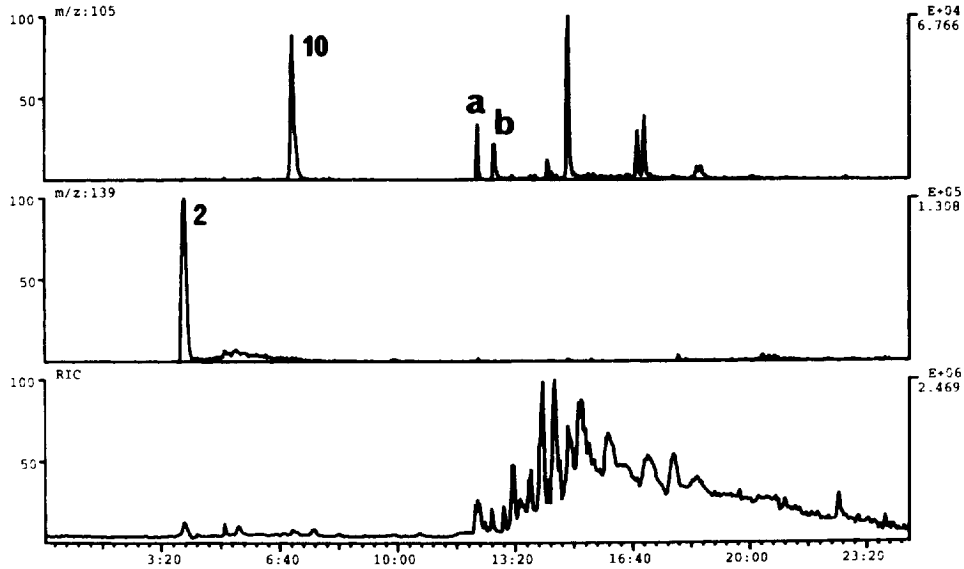

Fig. 5. (a) Mass chromatograms showing the detection of thiodiglycol (**10**) and thiodiglycol sulphoxide (**2**) in a water extract of soil sample 4C (peaks labelled a and b are additional contaminants derived from sulphur mustard); (b) total ion current chromatogram (most of the material eluting after 13 min was derived from the explosive tetryl).

thiodiglycol [31]. Two of the additional components observable in the mass chromatogram for *m/z* 105, labelled a and b in Fig. 5, were tentatively identified as the mustard derived products $HO(CH_2CH_2S)_3CH_2CH_2OH$ and $HOCH_2CH_2SCH_2CH_2S(O)CH_2CH_2OH$, respectively. The LC–APCI–MS spectrum of the former (Fig. 6) shows the presence of a ^{34}S isotope ion corresponding to 3 S atoms, and loss of water from the protonated molecule; *m/z* 105 ($[CH_2CH_2SCH_2CH_2OH]+$) and *m/z* 165 (loss of SCH_2CH_2OH+H) were the major product ions formed under CID conditions.

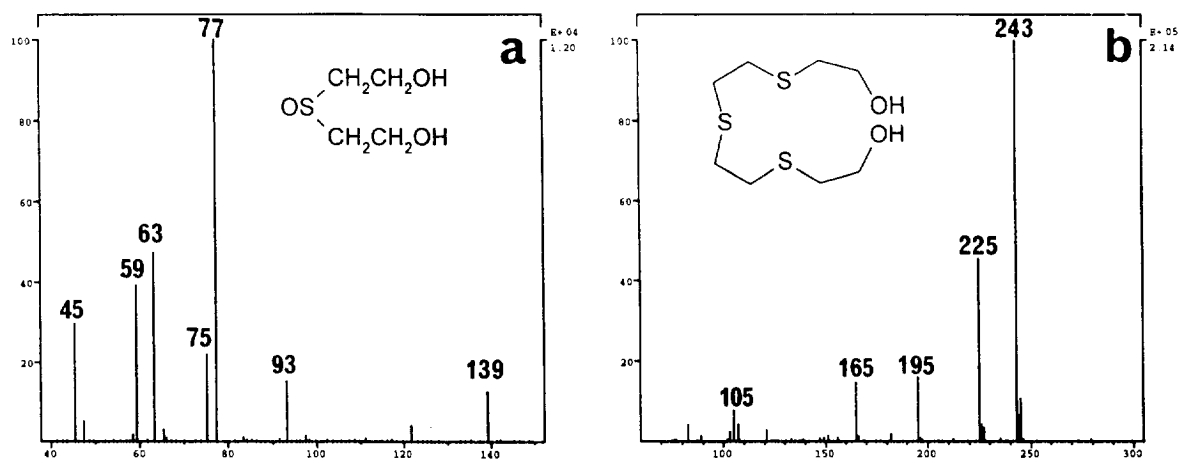

Fig. 6. (a) CID spectrum confirming the identification of thiodiglycol sulphoxide in soil sample 4C; (b) APCI spectrum of additional component derived from sulphur mustard.

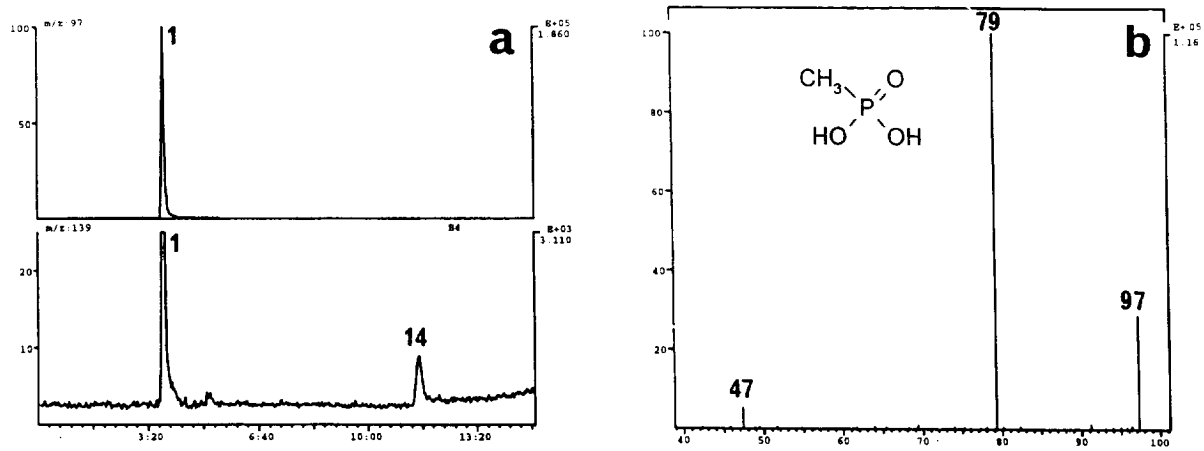

Fig. 7. (a) Mass chromatograms showing the detection of (1) MPA (*m/z* 97) and (14) isopropyl MPA (*m/z* 139) in a water extract of soil sample 4J; (b) CID spectrum confirming the identification of MPA.

Soil sample 4J was previously found to contain MPA (40 ppm) and isopropyl MPA (30 ppb). The chromatogram of a water extract (Fig. 7) shows the facile detection of MPA (1); a product ion spectrum of *m/z* 97 provided confirmation. Isopropyl MPA (4) was present at very low concentrations in the extract and GC–MS with selected ion monitoring or GC–MS–MS using selected reaction monitoring would be required for confirmation at these concentrations [6] (some further hydrolysis of isopropyl MPA to MPA may have occurred in the sample during storage since the original GC–MS analysis).

3.6. Application to the analysis of water and soil samples in round robin exercises

The LC–MS methodology has been used for screening purposes, and for confirmation of identification, in a series of international collaborative (round robin) exercises. Examples of analytes that have been identified in aqueous extracts of spiked soil or in water samples are **1**, **2**, **6**, **7**, **16**, cyclohexyl ethylphosphonic acid and cyclopentyl methylphosphonic acid. Fig. 8 shows selected ion chromatograms demonstrating the detection of **1** and **16** in an untreated water sample spiked at the 10 ppm level. Although **16** was not an expected analyte the acquisition of CID spectra helped to deduce the structure; for example the absence of *m/z* 97 and a very weak ion at *m/z* 111 excluded the possibility of the compound being a methyl- or ethylphosphonic acid derivative. The intense ion at *m/z* 57 provided good evidence for the compound being a butylphosphonic acid. The MS–MS spectrum provided confirmation of identity by comparison with a standard, and readily distinguished the *n*-butyl isomer (**16**) from the *tert.*-butyl isomer (**15**) as shown in Fig. 8 (standards for the other isomers were not available).

3.7. LC–MS vs. GC–MS

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. However, LC–MS allows for a more rapid screening of aqueous samples or aqueous extracts with minimal sample pretreatment, and without the need for concentration to dryness and derivatisation which can be adversely affected where large amounts of extraneous materials are present in an extract. For certain analytes, such as thiodiglycol sulphoxide, it is superior to current GC–MS methods as illustrated by the analysis of bomb crater and round robin samples. LC–MS has the additional advantage that it facilitates the identification of additional components, such as the two polar products identified in soil sample 4C, that would be less easily identified in a derivatised extract using GC–MS. It is expected that LC–MS will play an increasing role in the analysis of environmental

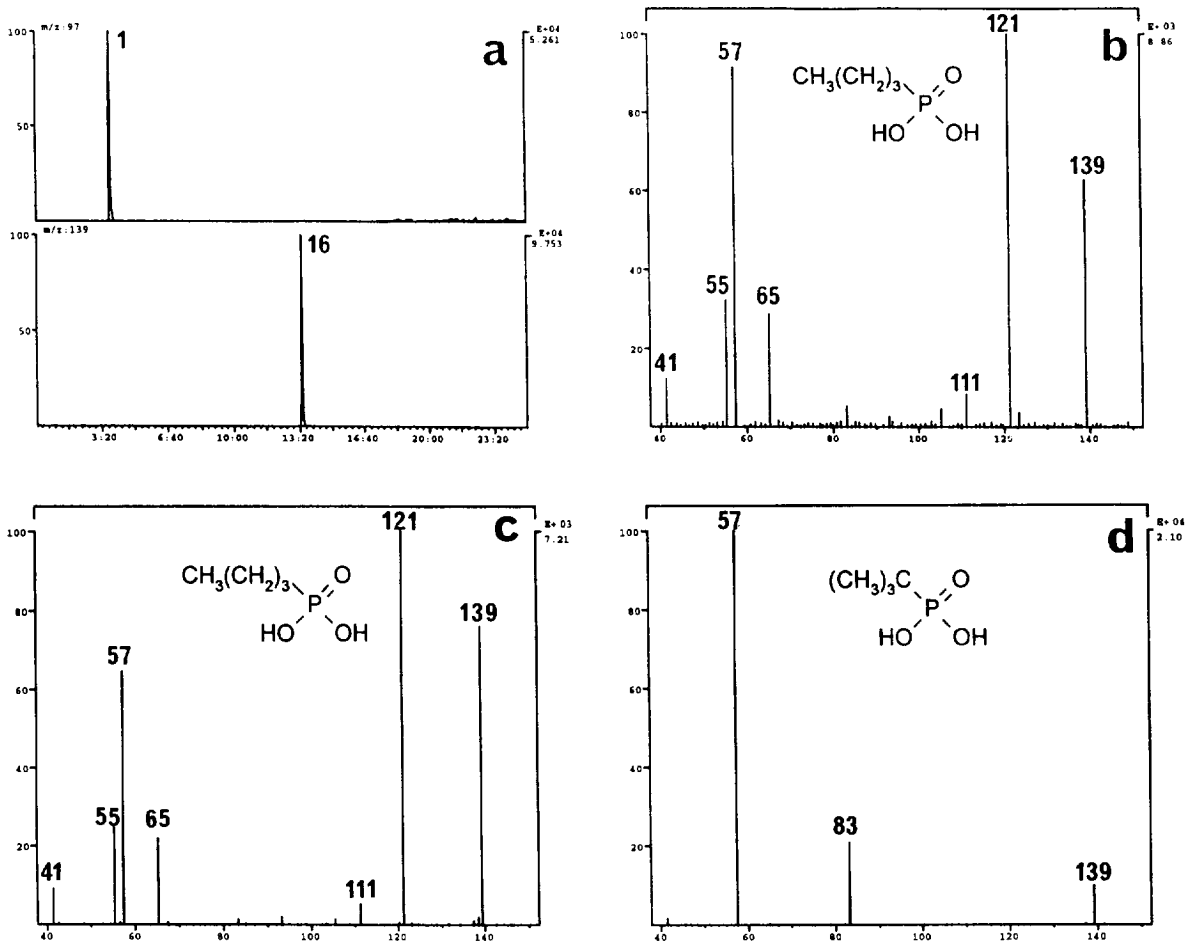

Fig. 8. (a) Mass chromatograms showing the detection of (1) MPA (m/z 97) and (16) n-butylphosphonic acid (m/z 139) in a spiked water sample; (b), (c) and (d) CID spectra of n-butylphosphonic acid in the sample, n-butylphosphonic acid standard and *tert.*-butylphosphonic acid standard, respectively.

samples for the hydrolysis products of chemical warfare agents; GC–MS is likely to remain the primary method for the identification of intact chemical warfare agents.

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