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Rapid screening procedures for the hydrolysis products of chemical warfare agents using positive and negative ion liquid chromatography–mass spectrometry with atmospheric pressure chemical ionisation

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Abstract

Qualitative screening procedures have been developed for the rapid detection and identification of the hydrolysis products of chemical warfare agents in aqueous samples and extracts, using liquid chromatography–mass spectrometry with positive and negative atmospheric pressure chemical ionisation (APCI). Previously reported screening procedures, which used positive APCI or electrospray ionisation (ESI), were modified by using LC conditions that allowed acquisition of positive and negative ion mass spectra. APCI was generally found to be more robust than ESI, probably due to variable adduct ion formation with ESI, depending on the condition of the sample and the system. Negative APCI provided selective detection of acidic analytes and allowed facile differentiation of alkyl alkylphosphonic acids from isomeric dialkyl alkylphosphonates. The combination of positive and negative APCI, using a C_{18} column and water–methanol mobile phase modified with ammonium formate, provides a rapid screening procedure for chemical warfare agent degradation products, with limits of detectability in the range 10–100 ng/ml. In the case of proficiency test samples, where analyte concentrations are in the range 1–10 ppm, introduction of the sample by infusion may provide an even faster preliminary screening procedure.

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

1. Introduction

Verification is an important component of monitoring compliance with the Chemical Weapons Convention. This process may 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 hydrolysed in the presence of water to more persistent degradation products; the analysis of environmental residues for hydrolysis products is therefore an important part of verification analysis [1,2].

The analysis of hydrolysis products in aqueous samples or extracts using gas chromatography–mass spectrometry (GC–MS) is time consuming, requiring

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concentration to dryness and derivatisation [1–4]; the presence of extraneous materials may interfere with derivatisation resulting in low apparent recoveries. Liquid chromatography (LC)–MS is being increasingly applied to the analysis of CW agent residues, initially using thermospray ionisation [5–7] and more recently using atmospheric pressure ionisation techniques [8–17]. In addition to advantages with regard to sample preparation, LC–MS may identify polar degradation products that are not seen using GC–MS analysis [8,16]. In two previous papers [8,9] we described a screening procedure for the detection and identification of the hydrolysis products of CW agents using LC–MS with atmospheric pressure chemical ionisation (APCI) [8], and the application of electrospray ionisation (ESI) to the analysis of phosphonic acid degradation products of nerve agents [9]. The APCI procedure included 10 hydrolysis products derived from organophosphorus nerve agents and their analogues (*O*-alkyl alkylphosphonic acids and alkylphosphonic acids) plus hydrolysis products derived from sulphur mustard, nitrogen mustards and quinuclidinyl benzilate (BZ). With maximum injection volumes of 20 μl, limits of detection using selected ion monitoring (SIM) were ≤0.2 ng injected (≤10 ng/ml water) for the neutral and basic analytes (e.g., thiodiglycol, triethanolamine), 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 nerve agents; least sensitivity was experienced with the most acidic and polar analytes such as methylphosphonic acid (MPA) and ethylphosphonic acid (EPA). The screening part of the procedure also poorly differentiated some dialkyl alkylphosphonates from isomeric alkyl alkylphosphonic acids. Some improvement in detection limits for phosphonic acids was obtained using positive electrospray ionisation [9], but this methodology was less effective for thiodiglycol and particularly for thiodiglycol sulphoxide. Further experience in our laboratory has indicated ESI to be less robust than APCI (as used on our current instrumentation), with quite variable signal-to-noise levels depending on the sample, apparently due to variable adduct ion formation. In this present paper we describe a modified procedure that allows LC–APCI-MS analysis to be performed rapidly in both positive and negative ion modes, under the same LC conditions. Introduction into the mass spectrometer using simple infusion into LC mobile phase is also described, as an even faster screening procedure for samples with low chemical backgrounds.

2. Methods

2.1. Chemicals

Ethyl, isopropyl, iso-, *n*- and *sec*.-butyl, pinacolyl and cyclohexyl MPA, ethyl EPA, thiodiglycol sulphoxide and thiodiglycol sulphone were synthesised in the Organic Chemistry Section, CBD and were >90% pure by nuclear magnetic resonance (NMR) and LC–MS. All other compounds were purchased from Aldrich (Gillingham, UK).

2.2. LC conditions

A Hewlett-Packard LC system was used consisting of a Hewlett-Packard Model 1050 quaternary pump plus solvent conditioner. The system was fitted with a 150×2.0 mm I.D. Columbus C_{18} bonded silica column (Phenomenex, Macclesfield, UK), 5 μm particle size.

Addition of a matched guard cartridge made no discernible difference to the chromatographic resolution. The mobile phase consisted of water (solvent A) and methanol (solvent B), each modified with 0.02 *M* ammonium formate. The elution gradient was 5% B (0–5 min), 5% B to 90% B (5–15 min) and 90% B (15–20 min), at a flow-rate of 0.2 ml/min. Injections (5–20 μl) were made using a Rheodyne 9125 injector fitted with a 50 μl polyether ether ketone (PEEK) loop. The same LC conditions were used for positive and negative APCI.

2.3. MS conditions

The column effluent was introduced into a Finnigan TSQ700 mass spectrometer via an atmospheric pressure ionisation source/interface operated in APCI mode. Capillary and tube lens voltages were optimised to give maximum response to *m/z* 181 $[M+H]^+$ from pinacolyl MPA for positive ion and *m/z* 179 $[M-H]^-$ for negative ion; this ensured that

$[M+H]^+$ or $[M-H]^-$ ions were prominent in the spectra. APCI conditions were as reported previously [8]: corona current 2 μA (positive ion) or 5 μA (negative ion), vaporiser temperature 400°C, capillary temperature 150°C, Q^0 offset ±5 V, sheath gas nitrogen at a pressure of 410 kPa, auxiliary gas nitrogen at a flow meter reading of 20. Negative ESI conditions employed were: spray voltage 4 kV, capillary temperature 200°C, Q^0 offset +5 V, sheath gas and auxiliary gas as above, optimisation as for APCI.

2.4. Acquisition of mass spectra using infusion

The aqueous sample was infused at 20 μl/min, using a Harvard syringe pump, into 5% MeOH–water–0.02 *M* ammonium formate at 0.1 ml/min. Positive and negative ion spectra were acquired using the same conditions as in 2.3 and averaged for 1 min to improve signal-to-noise.

2.5. Selected ion screening procedure

In the positive ion mode, SIM of hydrolysis products was based in most cases on the protonated molecules, and in some cases on ammonium adduct ions and significant fragment ions [8]. For phosphonic acids, 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 cyclohexyl MPA, were used; 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. Other ions monitored were as follows: thiodiglycol *m/z* 105 ($[M+H-H_2O]^+$) and 123 ($[M+H]^+$), thiodiglycol sulphoxide *m/z* 139 ($[M+H]^+$), thiodiglycol sulphone *m/z* 155 ($[M+H]^+$) and 172 ($[M+NH_4]^+$), *N*-methyldiethanolamine *m/z* 120, *N*-ethyldiethanolamine *m/z* 134, triethanolamine *m/z* 150, *N*,*N*-(diisopropyl)aminoethanol *m/z* 146, 3-quinuclidinol *m/z* 128 (all $[M+H]^+$), and benzilic acid *m/z* 183 ($[M+H-HCO_2H]^+$). In the negative ion mode, $[M-H]^-$ ions, *m/z* 95, 109, 123, 137, 151, 179, were monitored for the homologous series of phosphonic acids, plus *m/z* 177 for cyclohexyl MPA, and *m/z* 183 and 227 for benzilic acid. Retention times and ions monitored are shown in Table 1. In both modes, the chromatographic run was divided into time segments

Table 1
Retention times and ions monitored in selected ion screening procedure

Compound	Retention time (min:s)	Selected ions (*m/z*)	
		Positive ion	Negative ion
MPA	1:34	97	95
EPA	1:40	111	109
N-Methyldiethanolamine	2:07	120	
Thiodiglycol sulphoxide	2:07	139	
3-Quinuclidinol	2:09	128	
N-Ethyldiethanolamine	2:11	134	
Thiodiglycol sulphone	2:18	172, 155	
Ethyl MPA	2:25	125, 97	123, 95
Triethanolamine	2:38	150	
Isopropyl MPA	3:51	139, 97	137, 95
Ethyl EPA	3:51	139, 111	137, 109
N,*N*-Diisopropylaminoethanol	4:25	146	
Thiodiglycol	5:16	123, 105	
sec.-Butyl MPA	9:22	153, 97	151, 95
Isobutyl MPA	12:26	153, 97	151, 95
n-Butyl MPA	12:38	153, 97	151, 95
Cyclohexyl MPA	15:43	179, 97	177, 95
Benzilic acid	17:10	183	227, 183
Pinacolyl MPA	17:12	181, 97	179, 95

Table 2
Selected ion monitoring programme

Retention window	Selected ions monitored (m/z)	Dwell time[a] (s)
	Positive ion mode:	
0–3 min 22 s	97, 111, 120, 125, 128, 134, 139, 150, 155, 172	0.2
3 min 24 s–7 min 14 s	97, 105, 111, 123, 139, 146	0.33
7 min 16 s–14 min 13 s	97, 153	1.0
14 min 15 s–20 min	97, 179, 181, 183	0.5
	Negative ion mode:	
0–10 min 42 s	95, 109, 123, 137, 151	0.4
10 min 44 s–20 min	95, 151, 177, 179, 183, 227	0.33

[a] Total scan time 2 s in all cases.

(four for positive ion and two for negative ion) such that a maximum of 10 ions were scanned in any one segment (Table 2).

2.6. Collision induced dissociation (CID) spectra

LC–MS CID spectra were obtained as described previously [8]. For infused sample MS–MS, conditions were: collision gas argon 0.8 mTorr, collision energy −25 eV (positive ion) or +30 eV (negative ion), Q^0 offset ±5 V (1 Torr=133.322 Pa).

3. Results and discussion

3.1. LC separation

The C_{18} column used in this present study gave more robust and reproducible chromatography than the mixed C_8/C_{18} column used previously [8]. The use of methanol as organic modifier reduced adduct ion formation in comparison to acetonitrile. In order to obtain conditions suitable for the acquisition of positive and negative ion spectra, the selection of LC conditions was of necessity a compromise. Although the use of 0.05% trifluoroacetic acid [8] gave superior chromatography, this modifier does not allow the acquisition of negative ion spectra. To facilitate the latter, ammonium formate (0.02 *M*) was used as modifier, which allowed good sensitivity to be attained in both positive and negative ion mode. The chromatographic separation of standard analytes was adequate for the intended purpose; reconstructed ion chromatograms obtained in positive and negative ion modes from a standard mixture of analytes are shown in Figs. 1 and 2. Co-eluting analytes in the standard mixture, such as pinacolyl MPA and benzilic acid, were easily distinguished on the basis of selected ion chromatograms or mass chromatograms under full scanning conditions. Reduction of the ammonium formate concentration to 0.015 *M* resulted in less retention of some basic analytes, less efficient separation, and no detection of MPA and EPA at 0.1 μg/ml; an increase in the ammonium formate concentration to 0.025 *M* made little significant difference to either separation or sensitivity. Reduction of pH from approximately 6.4 to 4.8, by addition of formic acid, resulted in improved peak shape for *N*,*N*-(diisopropyl)aminoethanol but poorer peak shapes for other analytes, especially the ethanolamine hydrolysis products of nitrogen mustards. Other mobile phase modifiers were evaluated using the same gradient conditions. Ammonium acetate (0.02 *M*) gave very similar chromatography to that obtained with ammonium formate. Formic acid (0.1%), as used previously for positive ESI [9], gave the best separation of acidic analytes, but basic analytes, with the exception of *N*,*N*-(diisopropyl)aminoethanol, were poorly retained and co-eluted.

3.2. MS conditions

The initial MS conditions were as reported previously [8]. Some investigation of the effect of heated capillary and vaporiser temperatures, and source octapole (Q^0) offset, was carried out in positive ion mode. Reduction of vaporiser temperature from 400

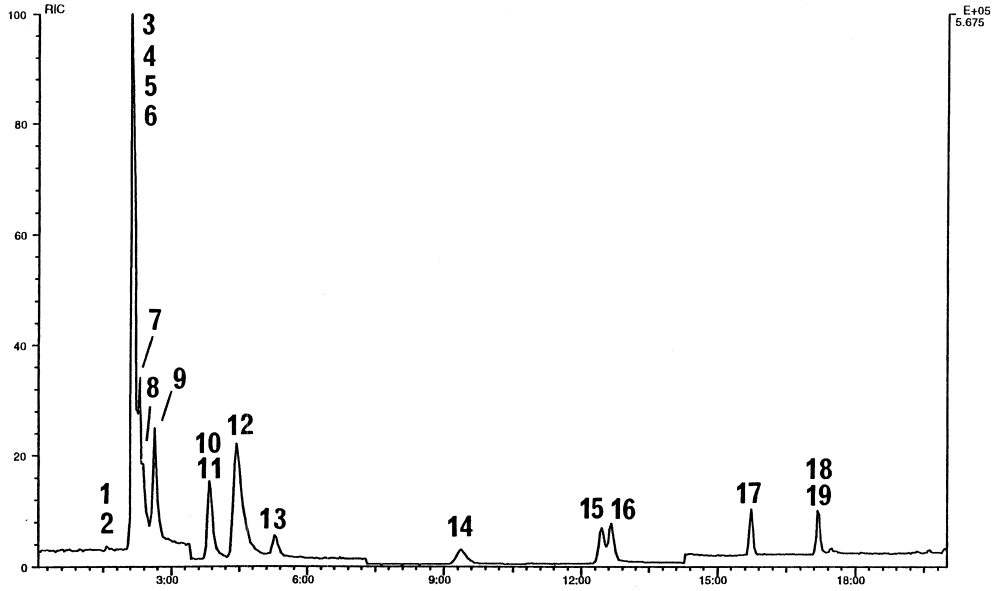

Fig. 1. LC–MS selected ion chromatograms (positive APCI) showing the detection of acidic, neutral and basic CW agent degradation products in water at 0.1 μg/ml: 1=MPA, 2=EPA, 3=*N*-methyldiethanolamine, 4=thiodiglycol sulphoxide, 5=3-quinuclidinol, 6=*N*-ethyl diethanolamine, 7=thiodiglycol sulphone, 8=ethyl MPA, 9=triethanolamine, 10=isopropyl MPA, 11=ethyl EPA, 12=*N*,*N*-(diisopropylamino)ethanol, 13=thiodiglycol, 14=*sec*.-butyl MPA, 15=isobutyl MPA, 16=*n*-butyl MPA, 17=cyclohexyl MPA, 18=benzilic acid, 19=pinacolyl MPA. Time scale in min.

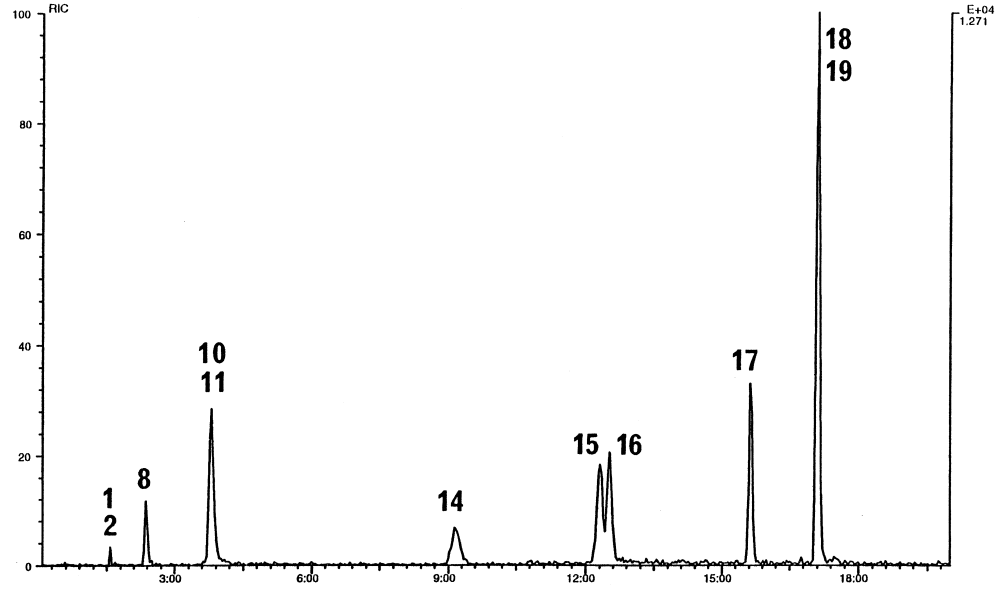

Fig. 2. LC–MS selected ion chromatograms (negative APCI) showing the selective detection of phosphonic and benzilic acids (0.1 μg/ml) in the same solution of standards as shown in Fig. 1. Time scale in min.

to 350°C had little effect whereas an increase to 450°C gave small sensitivity increases for the smaller phosphonic acids and thiodiglycol sulphoxide. Increased capillary temperature and larger Q^0 offset generally resulted in increased abundance of fragment ions; lower capillary temperatures than 150°C gave small increases in the intensity of $[M+H]^+$ and $[M+NH_4]^+$ ions. Comparison of chromatograms from a 0.1 μg/ml hydrolysis products mixture analysed with vaporiser 400°C, capillary 150°C (initial conditions) and vaporiser 450°C, capillary 130°C, in both positive and negative ion modes, showed few significant differences. The initial conditions [8] were therefore retained.

3.3. Negative and positive APCI using ammonium formate as modifier

Using positive ion APCI and SIM, and 20 μl injections, all analytes were detected at <0.01 μg/ml except MPA, EPA and benzilic acid, which were detected at approximately 0.1 μg/ml. Negative ion APCI or ESI provides selective detection of those acidic analytes, i.e., phosphonic acids and benzilic acid, that readily form anions (Fig. 2). Contrary to our expectations, negative ion APCI afforded improved signal-to-noise ratios and limits of detectability in comparison with negative ESI. The chromatograms obtained using negative APCI were cleaner than those obtained using ESI; signal-to-noise ratios were particularly good for pinacolyl and cyclohexyl MPA, for which positive ion mode gives a lower signal-to-noise in comparison to lower alkyl alkylphosphonic acids. Using SIM, all of the acidic analytes were detected at <0.01 μg/ml using 20 μl injections except for MPA and EPA, which could be detected at 0.1 μg/ml. Siegenthaler has also demonstrated the advantages of negative LC–ESI-MS, with in-source CID, for the detection and identification of phosphonic acids [12].

3.4. Infused APCI-MS

Infusion of a mixture of hydrolysis products showed that analytes were detected at 1 μg/ml in positive ion mode (Fig. 3), and most at 0.1 μg/ml (exceptions being pinacolyl and cyclohexyl MPA, and benzilic acid). Fig. 4 shows the detection of acidic analytes at 0.1 μg/ml in negative ion mode. Background ions again were generally reduced using

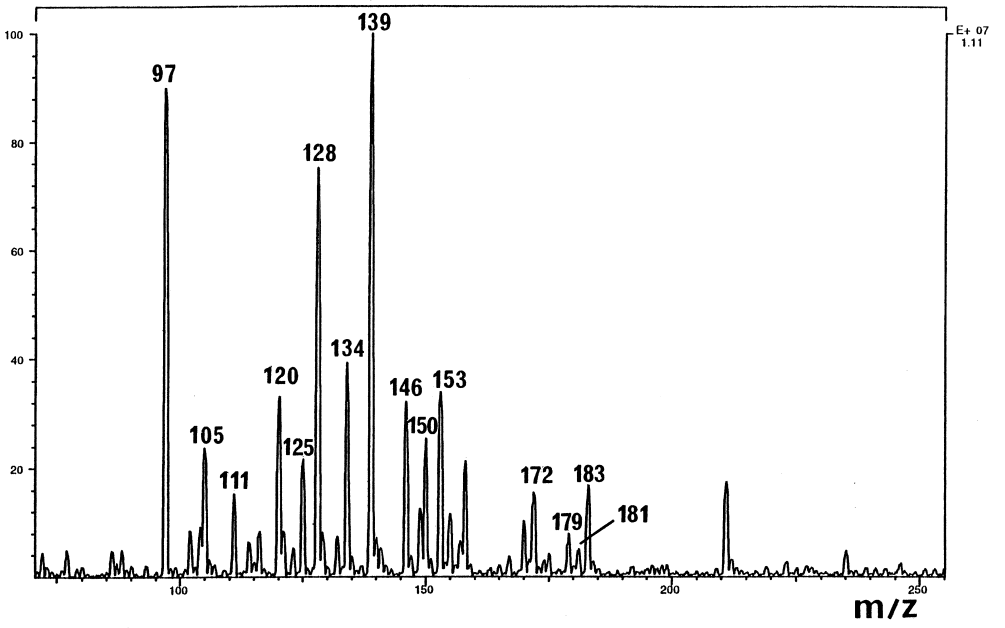

Fig. 3. Positive APCI spectrum, showing the detection of acidic, basic and neutral analytes in water at 1 μg/ml (infused at 20 μl/min into LC mobile phase at 0.1 ml/min, spectrum averaged over 1 min).

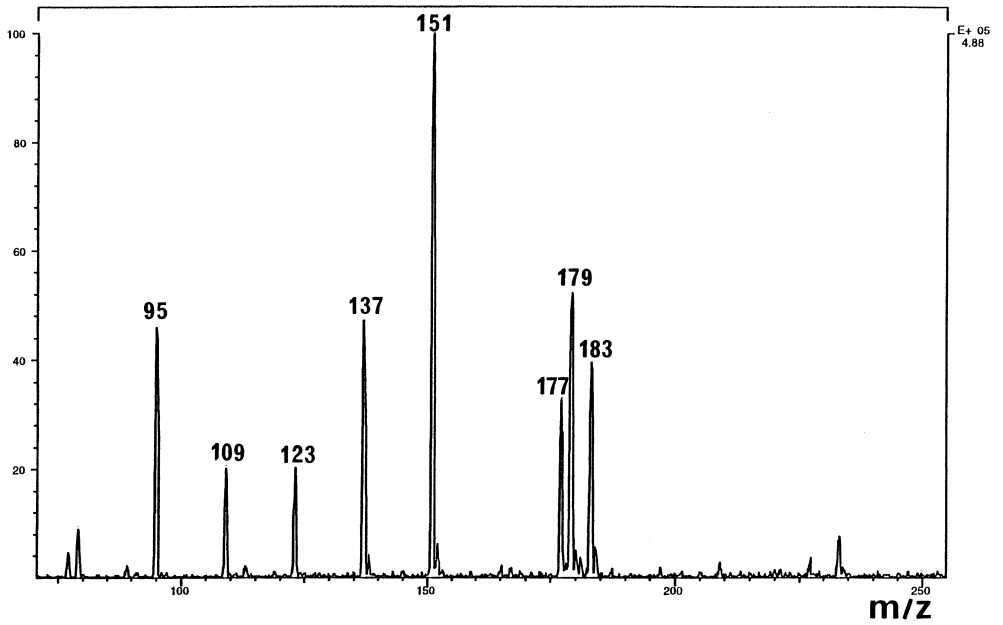

Fig. 4. Negative APCI, full scan, showing the selective detection of acidic analytes a mixture of acidic, basic and neutral analytes at 1 μg/ml (infused at 20 μl/min into LC mobile phase at 0.1 ml/min, spectrum averaged over 1 min).

APCI in comparison to ESI, resulting in cleaner spectra. Blank water generally showed a major ion at *m/z* 149 resulting from phthalate contamination. Interpretable product ion spectra could be acquired using this method at the 1 ppm level, although signal-to-noise levels were poor for negative ion CID spectra. The usefulness of the technique as a very rapid preliminary screening procedure would of course depend on the levels and nature of extraneous materials in the sample.

3.5. Dialkyl alkylphosphonates

The positive APCI spectra of dialkyl alkylphosphonates 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. using ESI [11]. Methyl esters showed a weak 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 esters and higher analogues, and these ions provide a facile means of obtaining a tentative structure for unknowns. For example, diethyl methylphosphonate showed product ions corresponding to neutral losses of one and two ethylene molecules. Methyl esters fragment by a different pathway, initially by loss of methanol [18]. In negative ion mode, phosphonic acids give intense $[M-H]^-$ ions whereas the phosphonates give no significant negative ions, thus readily distinguishing phosphonic acids from isomeric phosphonates. Fig. 5 illustrates the detection of four phosphonic acids in the presence of 10-times the concentration of two isomeric phosphonates.

3.6. Application of the procedure in proficiency tests

The procedure has been used successfully in the last two proficiency tests organised by the Organisation for the Prohibition of Chemical Weapons. Analytes tentatively identified using LC–MS were MPA, cyclohexyl MPA, di-(*n*-propylamino)ethanol, *n*-propyl *n*-propylphosphonic acid, *O*-*n*-propyl *n*-propylphosphonothioic acid (in spiked water sam-

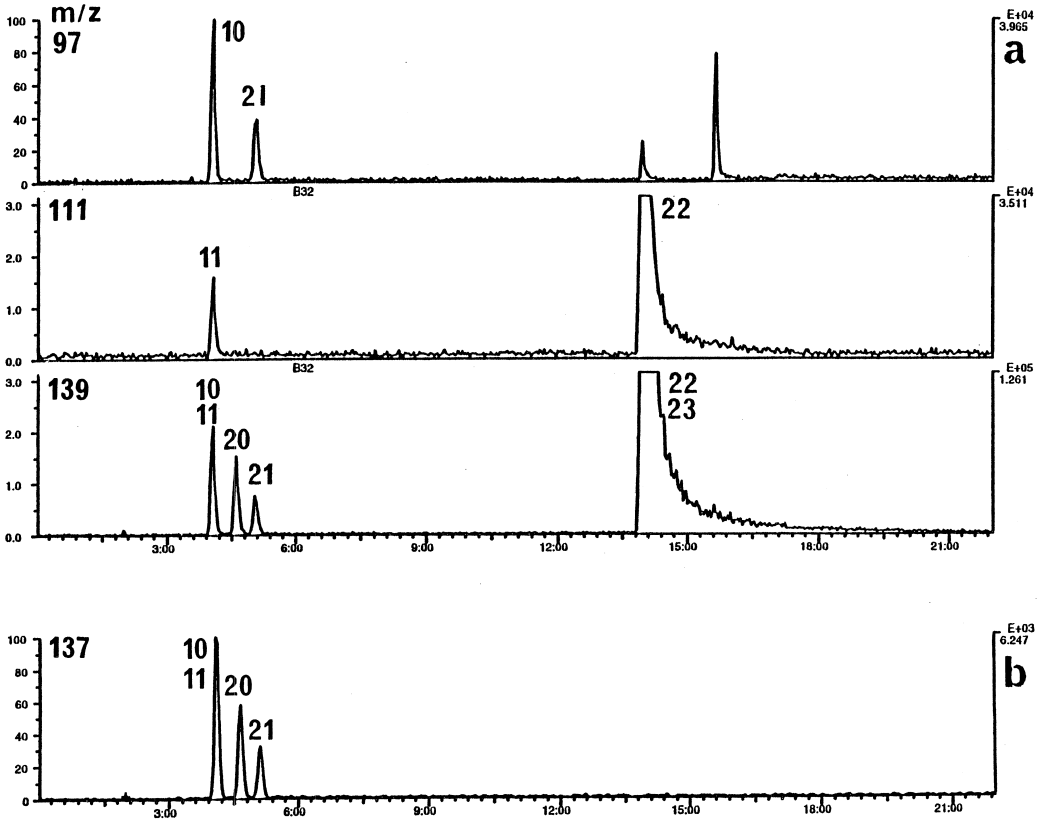

Fig. 5. LC–MS mass chromatograms (from full scan data) showing (a) positive APCI detection of four isomeric alkyl alkylphosphonic acids (1 μg/ml) and two isomeric dialkyl alkylphosphonates (10 μg/ml); (b) selective detection of the four phosphonic acids (1 μg/ml) using negative APCI: 10=isopropyl MPA, 11=ethyl EPA, 20=methyl *n*-propylphosphonic acid, 21=*n*-propyl MPA, 22=ethyl methyl methylphosphonate, 23=dimethyl ethylphosphonate. Time scales in min.

ples), MPA, ethyl MPA, ethyl 1-methoxy-2-propyl methylphosphonate (two diastereoisomers), thiodiglycol, thiodiglycol sulphoxide and 3-quinuclidinyl benzilate (in aqueous extracts of soil samples) and pinacolyl MPA (water extract of a wipe sample). In the case of analytes containing groups such as propyl, the initial screening does not identify the isomer (e.g., *n*- or isopropyl) unless standards are already available. If these are available then the isomers are usually differentiated on the basis of retention time, branched chain isomers such as isopropyl eluting earlier than *n*-alkyl isomers. Van Baar et al. [14] have recently reported a procedure for differentiating *P*-*n*-propyl isomers from *P*-isopropyl using tandem ESI. Clues to the identity of phosphonates containing alkoxy substituted side chains, such as ethyl 1-methoxy-2-propyl methylphosphonate, are obtained from the neutral losses observed in the positive APCI spectrum (e.g., loss of 72, C_4H_8O). Confirmation is made by comparison with synthetic standards, using LC–MS–MS and GC–MS as confirmatory techniques. A major advantage of the methodology is that when time is of the essence, a tentative identification can usually be made within 24 h of receipt of the sample. The procedure is also useful for the preliminary identification of unchanged agents, particularly BZ and V agents (these give strong $[M+H]^+$ ions), although these are also rapidly detected by GC–MS.

4. Conclusion

The combination of positive and negative ion

APCI, with LC using a water–methanol mobile phase modified with ammonium formate, provides a rapid screening procedure for a wide range of acidic, neutral and basic hydrolysis products of CW agents. Positive ionisation provides optimum sensitivity for the neutral and basic analytes, and negative ionisation optimum sensitivity and selectivity for most of the acidic analytes. By screening in both ionisation modes, combined with MS–MS for confirmation, the methodology provides an efficient procedure for the rapid detection and identification of hydrolysis products of CW agents in aqueous samples. LC–MS and related techniques are expected to play an increasing role in the analysis of environmental samples for the hydrolysis products of chemical warfare agents, as they are in the analysis of the more polar pesticides [19]. LC–MS is also applicable to the direct analysis of undegraded CW agents in aqueous matrices [5,17].

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