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Application of gas chromatography–mass spectrometry and gas chromatography–tandem mass spectrometry to the analysis of chemical warfare samples, found to contain residues of the nerve agent sarin, sulphur mustard and their degradation products

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Abstract

Samples of clothing, grave debris, soil and munition fragments, collected from the Kurdish village of Birjinni, were analysed by GC–MS with selected ion monitoring (SIM) for traces of chemical warfare agents and their degradation products. Positive analyses were confirmed, where possible, by full scan mass spectra, or at low concentrations by additional GC–MS-SIM analysis using chemical ionisation, by higher resolution GC–MS-SIM, and by GC–tandem mass spectrometry using multiple reaction monitoring. Sulphur mustard and/or thiodiglycol were detected in six soil samples; isopropyl methylphosphonic acid and methylphosphonic acid, the hydrolysis products of the nerve agent sarin, were detected in six different soil samples. Trace amounts of intact sarin were detected on a painted metal fragment associated with one of these soil samples. The results demonstrate the application of different GC–MS and GC–MS–MS techniques to the unequivocal identification of chemical warfare agent residues in the environment at concentrations ranging from low ppb to ppm (w/w). They also provide the first documented unequivocal identification of nerve agent residues in environmental samples collected after a chemical attack.

1. Introduction

The prime requirement in analytical investigations of the use of chemical warfare (CW) agents is for the unequivocal detection and identification of the agents concerned and/or their degradation products [1]. The recent use of chemical warfare agents in the Iran–Iraq conflict has been well documented [2–4] and corroborated or confirmed by the chemical analysis of samples retrieved from bomb craters and unexploded weapons [2–4], and from human casualties [5–7]. Subsequent allegations of CW use against the Kurdish community within Iraq were substantiated by the analysis of samples obtained from a ruptured munition collected from the mountainous region of Northern Iraq [8]. In all but one of these incidents, positive analyses have revealed the presence of sulphur mustard and/or its degradation products such as thiodiglycol and 1,4-dithiane. Most of the positive environmental samples were obtained soon after the event, from bomb craters or unexploded munitions, and contained relatively high concentrations of agent or degradation

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products enabling unequivocal identification to be obtained by full-scan GC–MS and other less sensitive techniques such as NMR. In a single case [2], a virtually neat sample of the nerve agent tabun (GA) was given to UN investigators, reported as being the fill of an unexploded munition, although the more widespread use of nerve agents was strongly suspected during the Iraq–Iran war and against Kurdish communities in northern Iraq during 1987 and 1988 [4].

In October 1992, the Chemical and Biological Defense Establishment (CBDE) was asked to analyse a number of samples which had been collected in June 1992 from the Kurdish village of Birjinni, in the mountainous region of northern Iraq, by a team of forensic scientists from the Physicians for Human Rights and Middle East Watch Organisations [1]. The village was reported to have been subjected to a chemical attack on August 25th, 1988, almost four years earlier. Four villagers were reported to have died after the attack; surviving casualties reported feelings of suffocation, temporary blindness, muscular paralysis and spasms. The investigating team collected samples of clothing, soil and insect pupae from the exhumed skeletal remains of two of the victims, an old man and a young boy. Additional samples of soil and metal fragments were collected from four out of twelve bomb craters present in or near the village.

In this paper we report full details of the analytical methods employed to identify nerve agent and sulphur mustard residues in these samples. The results demonstrate the utility of different GC–MS and GC–MS–MS techniques for the unequivocal identification of CW agent residues in authentic environmental samples at concentrations ranging from low ppb to ppm (w/w).

2. Experimental

2.1. Samples

The samples, sealed inside plastic bags tagged with police forensic labels, were transported from Iraq, unopened, at ambient temperature via Boston (USA), and received at CBDE on October 5th, 1992. A fully documented audit trail was maintained from collection to analysis. After receipt, the samples were stored at 4°C prior to analysis. The samples consisted of the following: *sample 1*, a shirt plus intrathoracic contents (soil, insect pupae) from burial 1; *sample 2*, trousers from burial 2; *sample 3*, a shirt and vest from burial 2; *samples 4A–4L*, dry, stony, light brown soil samples collected from four bomb craters; some metal fragments, believed to be from munitions, were associated with some of these soil samples (4D, 4H, 4I, 4J). The fragment associated with soil sample 4H consisted of a twisted piece of double skinned metal (iron), *ca.* 7×5 cm, mass 35 g, coated with green paint.

Each sample was divided into appropriate subsamples for analysis and assigned unique CBDE identification numbers. Small sections were cut from the shirts, vest and trousers for analysis. Soil containing tiny pupae was removed from sample 1 and analysed separately. Fragments of metal present in soil samples 4D, 4H, 4I and 4J were analysed separately from the soil.

2.2. Standards and solvents

Samples of 1,1-thiobis(2-chloroethane) (sulphur mustard), O-ethyl N,N-dimethylphosphoramidocyanidate (tabun, GA), isopropyl methylphosphonofluoridate (sarin, GB), pinacolyl methylphosphonofluoridate (soman, GD), cyclohexyl methylphosphonofluoridate (GF), O-ethyl S-2-diisopropylaminoethyl methylphosphonothiolate (VX), isopropyl methylphosphonic acid (iPMPA), pinacolyl methylphosphonic acid, cyclohexyl methylphosphonic acid and ethyl methylphosphonic acid were synthesised in the Organic Chemistry Section, CBDE and were 99% pure by NMR and GC–MS or MS. Thiodiglycol (TDG) (99%) was purchased from Aldrich (Gillingham, UK) and methylphosphonic acid (MPA) (98%) from Lancaster Synthesis (Morecambe, UK). Fisons (Lough borough, UK) Distol-grade solvents were used. Water was distilled and purified using a Milli-Q system (Millipore, Bedford, MA, USA).

2.3. General analytical procedures

The samples were extracted with dichloromethane for the analysis of intact CW agents and with water for the analysis of hydrolysis products. For screening purposes the dichloromethane extracts were analysed specifically for sulphur mustard and nerve agents (tabun, sarin, soman, GF, VX) using GC–MS with selected ion monitoring (SIM) employing electron ionisation (EI). The aqueous extracts were derivatised and analysed specifically for TDG, the hydrolysis product of sulphur mustard, and for MPA and alkyl methylphosphonic acids, the hydrolysis products of nerve agents (except tabun). Additional analysis by full-scanning GC–MS was used to identify other significant volatile contaminants present. Positive analyses were confirmed following the procedures for unambiguous identification suggested in the series of Blue Books for the identification of CW agents [9], *i.e.* by full-scan EI and chemical ionisation (CI) mass spectra where obtainable, or by higher-resolution GC–MS-SIM (EI) and GC–tandem mass spectrometry (GC–MS–MS) to confirm low-resolution GC–MS-SIM data. In the single case of sarin detection, the analysis was confirmed by GC–MS–MS using both EI and ammonia CI on two GC columns of differing polarity. Quality control checks were run at least daily and glassware blanks were run before each sample.

2.4. Sample preparation

Soil samples

Screening for intact agents and other volatiles. Samples were allowed to reach ambient temperature before opening. Aliquots of soil (1–5 g, according to the size of the sample) were weighed into 3-ml vials or 15-ml tubes (for the larger aliquots) fitted with PTFE-lined screw caps. The soil was extracted by tumbling for 1 h with dichloromethane (2 or 5 ml). After standing for 2–3 min, 1-ml aliquots of the dichloromethane extracts were transferred to screw capped vials for GC–MS analysis and concentrated, if required, to small volume under nitrogen at 40°C.

Screening for hydrolysis products. Fresh aliquots (1–4 g) of soil were weighed into 15-ml tubes with PTFE-lined screw caps. Water (2 or 5 ml) was added and the soil extracted by tumbling for 1 h. After centrifuging at 1600 rpm (270 *g*) for 30 min, 1- or 2-ml aliquots of extract were transferred to 1-ml vials and concentrated to dryness using a Gyrovap centrifugal evaporator (Howe, Banbury, UK) at 60°C. To improve recovery of MPA, the extractions with water were repeated on fresh aliquots but were eluted through Dowex 50W-X8 cation-exchange resin (H^+ form, 200–400 mesh, 40–80 μm) (Fluka, Gillingham, UK) [500 mg, pre-washed with deionised water (2 × 2 ml), 0.01 *M* hydrochloric acid (2 × 2 ml) and deionised water (1 ml)] contained in a 3-ml Bond Elut reservoir.

Extractions for confirmation of initial screening results. Fresh aliquots (1–3 g) of soil were weighed into 15-ml tubes and extracted with dichloromethane (3 ml) as above. After standing for 2–3 min the dichloromethane extract was filtered through a 0.45-μm PVDF filter (Gelman, Ann Arbor, MI, USA) into 4-ml screw-capped vials. Residual dichloromethane was removed from the soil by evaporation in a centrifugal evaporator at 35°C. The soil was then extracted with water (3 ml) as above. Extracts were centrifuged at 1600 rpm for 30 min and as much of the aqueous fraction as possible filtered through 0.45-μm PVDF filters into 4-ml screw capped vials. Aliquots (1 ml) were transferred to 1-ml vials and concentrated to dryness in a centrifugal evaporator at 60°C.

2.4.2. Metal fragments

Samples were weighed into suitably sized small beakers or screw capped jars and dichloromethane added just to cover the sample. Beakers were covered with sealing film and placed in an ultrasonic bath for 1 h. Aliquots (2 ml) of the extracts were removed, filtered through 0.45-μm PVDF filters into 4-ml screw-capped vials and concentrated if required under nitrogen. Residual dichloromethane was removed from the fragments by evaporation under nitrogen at 40°C. Water was then added just to cover the fragment and extraction carried out as above.

Aliquots (1 or 2 ml) of extract were filtered through 0.45-μm PVDF filters into 1-ml vials and concentrated to dryness in a centrifugal evaporator at 60°C.

2.4.3. Clothing samples

Aliquots of cloth (1–3 g) were weighed into 24-ml Wheaton vials with PTFE-lined screw caps. The samples were extracted by tumbling for 1 h with dichloromethane (10 ml). As much of the dichloromethane extract as possible was filtered through 0.45-μm PVDF filters into 4-ml screw capped vials. Residual dichloromethane was removed from the samples by evaporation in a centrifugal evaporator at 35°C. The samples were then extracted by tumbling for 1 h with water (10 ml). After centrifuging at 1000 rpm (100 *g*) for 10 min, aliquots (1 or 2 ml) of the aqueous layer were filtered through 0.45-μm filters into 1-ml vials and concentrated to dryness in a centrifugal evaporator at 60°C. Larger samples of cloth (4–10 g) were similarly extracted with water (50 ml) and 25-ml aliquots concentrated to dryness using a rotary evaporator. Residues were transferred to 1-ml vials in 1-ml volumes of water and concentrated to dryness as above.

2.4.4. Derivatisation of dried aqueous extracts

Acetonitrile (50 μl) and N-methyl-N-(*tert.*-butyldimethylsilyl)-trifluoroacetamide–1% *tert.*-butyldimethylchlorosilane (Fluka) (50 μl) were added to the dried residues and the samples heated in a heating block for 1 h at 60°C [10].

GC–MS, SIM and full scan, of dichloromethane extracts

Dichloromethane extracts were screened using a Finnigan MAT TSQ 700 mass spectrometer (Finnigan MAT, Hemel Hempstead, UK) interfaced to a Varian 3400 gas chromatograph. The gas chromatograph was fitted with a 30 m × 0.25 mm I.D. DB-5 (J & W) bonded phase column, film thickness 0.25 μm, inserted directly into the ion source, with a 1 m × 0.25 mm I.D. retention gap (phenyl methyl deactivated). Helium at 103 kPa was used as carrier gas. The oven was heated at 40°C for 0.5 min, from 40 to 300°C at 15°C/min, and held at 300°C for 2 min. Aliquots (1 μl) were injected through a Varian SPI injector, heated at 35°C for 0.5 min, from 35 to 250°C in 1.5 min, and held at 250°C for 30 min. The transfer line was heated at 300°C. The TSQ 700 was operated in Q1MS mode using EI and SIM. Electron energy was 70 eV, emission current 400 μA, electron multiplier 1500 V, conversion dynode − 5 kV and source temperature 150°C. Ions monitored were *m/z* 99 (sarin, soman, GF), 109 (mustard), 114 (VX), 125 (sarin), 126 (soman), 133 (tabun), 158 (mustard) and 162 (tabun). Dwell time was 0.125 s each ion, total scan time 1 s.

Confirmation of sulphur mustard, 1,4-dithiane, 1,4-thioxane and tetryl, by full-scan GC–MS, was performed under similar GC conditions but employing a 25 m × 0.22 mm I.D. BP5 column (SGE, Milton Keynes, UK), film thickness 0.25 μm, using 1-μl splitless injections, injector temperature 220°C, split delay 0.5 min. The mass spectrometer was scanned from *m/z* 40–400 at 1 s per scan using EI as above, or using methane CI with electron energy 150 eV, source pressure 930 Pa (gauge reading), source temperature 150°C.

For confirmation of sarin by SIM, GC conditions were as above. Using EI, ions monitored were *m/z* 81, 99, 125, dwell time 0.33 s each ion, total scan time 1 s. Using ammonia CI (source conditions as for methane CI), ions monitored were *m/z* 141 (MH^+), 158 ($M + NH_4^+$), dwell time 0.5 s each ion, total scan time 1 s.

2.6. GC–MS, SIM and full scan, of water extracts

Water extracts were screened for the *tert.*-butyldimethylsilyl (TBDMS) derivatives of the hydrolysis products of sulphur mustard, sarin, soman, GF and VX, using a Finnigan MAT 4600 GC–MS system operated in the selected ion mode. The gas chromatograph was fitted with a 25 m × 0.22 mm I.D. BP5 column, film thickness 0.25 μm, with 50 cm × 0.25 mm retention gap (phenyl methyl deactivated). Helium at 103 kPa was used as carrier gas. The oven was heated at 90°C for 1 min, from 90 to 250°C at 10°C/min,

and held at 250°C for 1 min. Splitless injections (1 μl) were used, injector temperature 275°C, split delay 0.5 min. The GC–MS interface was heated at 250°C. EI at 70 eV was employed, emission current 300 μA, electron multiplier 1500 V, source temperature 150°C. Ions monitored were *m/z* 153 (MPA, iPMPA, pinacolyl MPA, cyclohexyl MPA, ethyl MPA), 267 (MPA), 293 (TDG). Dwell time was 0.3 s each ion, total scan time 1 s.

Confirmation by full-scan GC–MS was performed under similar conditions. The mass spectrometer was scanned from *m/z* 40–400 at 1 s per scan using EI as above, or using methane CI with electron energy 150 eV, source pressure 107 Pa, source temperature 120°C.

2.7. GC–MS-SIM (CI)

Confirmation of TDG, MPA and iPMPA, where concentrations were too low to obtain satisfactory full-scan data, was performed using methane CI as above with SIM of five ions per analyte. Ions monitored were TDG-$(TBDMS)_2$, *m/z* 219, 293, 294, 335, 336; MPA-$(TBDMS)_2$, *m/z* 267, 309, 310, 325, 326; iPMPA-TBDMS, *m/z* 153, 195, 211, 239, 253. Dwell time was 0.16 s each ion, total scan time 1 s.

2.8. Higher-resolution GC–MS-SIM

Confirmation using higher-resolution (HR) SIM was performed using a VG Autospec Q instrument (VG Analytical, Wythenshawe, UK) interfaced to a Hewlett-Packard 5890 series II gas chromatograph. The gas chromatograph was fitted with a 25 m × 0.22 mm I.D. BP5 column, film thickness 0.25 μm, or with a 25 m × 0.25 μm I.D. Ultra 2 column (Hewlett-Packard), film thickness 0.25 μm. For confirmation of the TBDMS derivatives of TDG, MPA and iPMPA, the oven was heated at 90°C for 0.5 min, from 90 to 260°C at 10°C/min and held at 260°C for 2 min. Splitless injections (1 μl) were used, injector temperature 250°C, split delay 0.5 min. For confirmation of sarin the oven was heated at 35°C for 2 min, from 35 to 300°C at 15°C/min, injection as above, split delay 2 min. The mass spectrometer was operated at 5000 resolution using EI, source temperature 220°C. Ions monitored were TDG-$(TBDMS)_2$, *m/z* 233.1395 ($C_{11}H_{25}OSSi$), 293.1427 ($C_{12}H_{29}O_2SSi_2$), *m/z* 242.9856 (perfluorokerosene) was used as mass lock; MPA-$(TBDMS)_2$, *m/z* 195.0063 ($C_4H_{12}O_3PSi_2$), 267.1002 ($C_9H_{24}O_3PSi_2$), 309.1471 ($C_{12}H_{30}O_3PSi_2$); iPMPA-TBDMS, *m/z* 153.0137 ($C_3H_{10}O_3PSi$), 195.0606 ($C_6H_{16}O_3PSi$), 237.1076 ($C_9H_{22}O_3PSi$), *m/z* 218.98562 (perfluorokerosene) was used as mass lock; sarin, *m/z* 99.0011 (CH_5O_2FP), 125.0168 ($C_3H_7O_2FP$), *m/z* 118.9920 (perfluorokerosene) was used as mass lock. Dwell time was 80 ms per ion, 20 ms delay.

2.9. GC–MS–MS multiple reaction monitoring

A Finnigan MAT TSQ 700 instrument interfaced to a Varian 3400 gas chromatograph was employed, operated in the multiple reaction monitoring (MRM) mode. For the confirmation of TDG, MPA and iPMPA, the GC was fitted with a 25 m × 0.22 mm I.D. BP-5 column, film thickness 0.25 μm; GC conditions were as in the screening procedure except that 1-μl splitless injections were used, injector temperature 220°C, 0.5 min split delay. Methane CI was employed, source conditions as above. Collision-activated decomposition (CAD) was performed using argon as collision gas at 0.13 Pa, collision offset − 20 eV. Reactions monitored were TDG-$(TBDMS)_2$, *m/z* 335→231, 189, 159, 147; MPA-$(TBDMS)_2$, *m/z* 325→309, 267; iPMPA-TBDMS, *m/z* 253→211, 195, 153. Dwell times were 0.2 s (TDG), 0.33 s (MPA) and 0.25 s (iPMPA) each ion, including undissociated parent ion, total scan time 1 s.

For the confirmation of sarin, GC–MS–MS analysis was performed under four different conditions. GC separation was performed using a non-polar BPX5 column (dimensions and conditions as for BP5), or using a polar 15 m × 0.25 mm I.D. DBWAX column, film thickness 0.5 μm, with 50 cm × 0.25 mm I.D. retention gap (phenyl methyl deactivated). For the latter the oven was heated at 40°C for 0.5 min, from 40 to 240°C at 10°C/min, and held at 240°C for 1 min.

Splitless injections (1 μl) were used, injector temperature 220°C, split delay 0.5 min. The transfer line was heated at 240°C. EI, ammonia CI [11] and CAD conditions were as described above. Reactions monitored were EI, *m/z* 125→99, 81; CI, *m/z* 158→141, 99. Dwell time was 0.33 s each ion including undissociated parent ion, total scan time 1 s.

2.10. Quantitation

No attempt was made at accurate quantitation; soil samples were considerably heterogeneous. Crude estimation of the amounts of analyte extracted from soil samples was performed by comparing peak areas of selected ions used in the GC–MS-SIM (EI) screening procedure [TDG-$(TBDMS)_2$, *m/z* 293; MPA-$(TBDMS)_2$, *m/z* 267; iPMPA-TBDMS, *m/z* 153; sulphur mustard, *m/z* 109] with the peak areas from appropriate standards (0.5 or 1 ng injected). In those cases where sulphur mustard was detected in the dichloromethane extract, TDG-$(TBDMS)_2$ was determined in the water extracts, using GC–MS-SIM (CI), *m/z* 293, after sequential extraction of the sample with dichloromethane (to remove sulphur mustard) and water. Sarin on fragment 4H(M) was estimated using the transition *m/z* 125→99 in the GC–MS–MS (EI) procedure.

2.11. Quality control

Glassware blanks (*i.e.* solvent taken through the entire extraction and derivatisation procedure) were run prior to the extraction of each sample.

Blanks, either the glassware blank for the next sample or a solvent or reagent blank, were analysed after every positive sample or standard to ensure that no cross-contamination occurred. System performance was checked by the analysis of standards at least daily or every 3–5 samples. Any problems identified were rectified and standards repeated before continuing with the analysis of samples. For the analysis of intact agents, samples of sulphur mustard, tabun, sarin, soman, GF and VX (100 pg or 1 ng of each) were injected in dichloromethane. For the analysis of hydrolysis products, TBDMS derivatives of TDG, iPMPA and MPA (0.5 or 1 ng of each) were injected in acetonitrile.

3. Results

3.1. Standards

Representative reconstructed ion chromatograms (*i.e.* summations of SIM chromatograms) for standard solutions of sulphur mustard, nerve agents and their derivatised hydrolysis products, as used for screening purposes, are shown in Fig. 1. Limits of detection were estimated as being in the range 10–50 ng extracted per sample based on a *S/N* ratio of *ca.* 3:1, with the exception of VX for which the screening procedure is less sensitive due to its poor chromatographic properties. Representative GC–MS-SIM (CI), GC–HRMS-SIM and GC–MS–MS-MRM chromatograms are shown below for the sample analyses. CAD spectra for the TBDMS derivatives of TDG, MPA and iPMPA, and for sarin, used for the selection of product ion monitoring for GC–MS–MS analysis, are shown in Fig. 2.

3.2. Grave samples 1, 2 and 3

No traces of nerve agents, sulphur mustard or their hydrolysis products were detected in any of the samples from the graves. Increasing the amounts of clothing extracted was not beneficial since the relatively large chemical background present increased proportionately. A repeat of the aqueous extraction with removal of cations by ion exchange also gave negative results for MPA.

3.3. Soil and metal fragments 4A–4L

The results for soil samples 4A–4L plus associated metal fragments are shown in Table 1.

Soil samples 4A, 4B and 4D

Soil samples 4A, 4B and 4D contained sulphur mustard at concentrations (0.6–10 ppm) suffi-

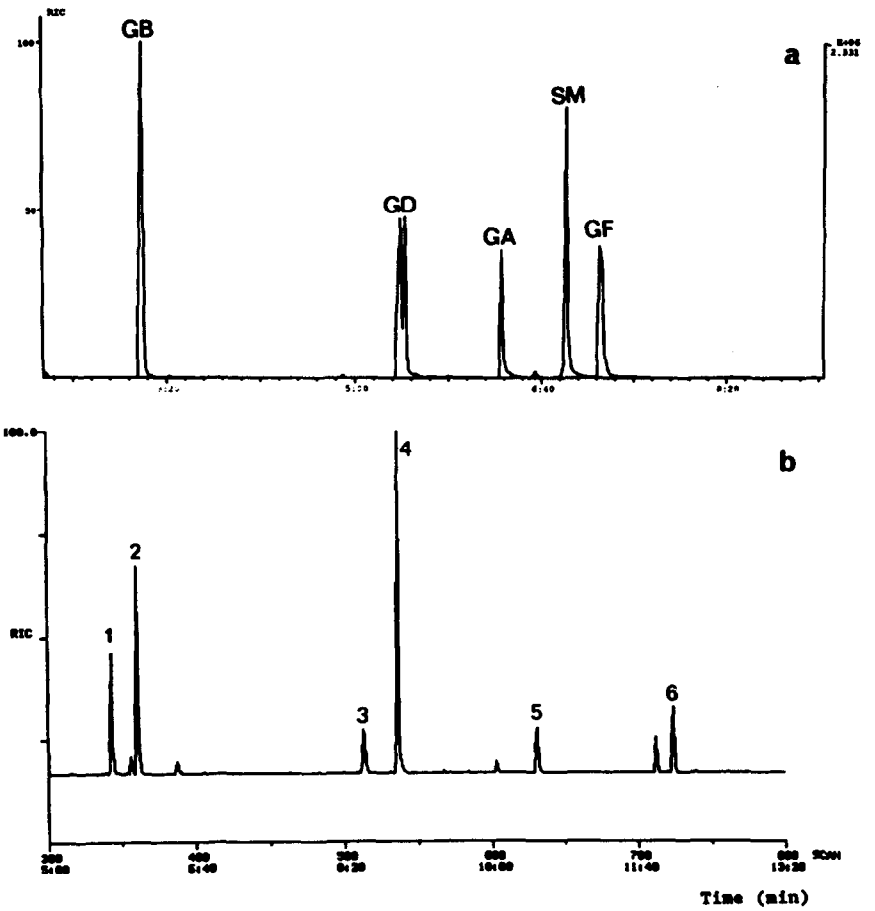

Fig. 1. Reconstructed ion current chromatograms for (a) nerve agents GA, GB, GD, GF and sulphur mustard (SM) (1 ng injected) and (b) TBDMS derivatives of (1) ethyl MPA, (2) iPMPA, (3) pinacolyl MPA, (4) MPA, (5) cyclohexyl MPA and (6) TDG (500 pg injected).

cient to obtain full-scan data. The dichloromethane extracts of samples 4A, 4B and 4D gave qualitatively similar total ion current (TIC) chromatograms; that from sample 4A is shown in Fig. 3 together with a full-scan EI mass spectrum obtained for sulphur mustard. In addition to sulphur mustard, small amounts of related cyclic degradation products, 1,4-thioxane and 1,4-dithiane, were present. The major component of the dichloromethane extracts was the explosive tetryl (N-methyl-N,2,4,6-tetranitroaniline), which chromatographs as the thermal decomposition product N-methyl-2,4,6-trinitroaniline [12], apparent as a very intense peak in Fig. 3. Two related products were also tentatively identified, 2,4,6-trinitroaniline (or possibly the N-nitro derivative) and 2,4,6-trinitrohydrazine (or possibly 2,4,6-trinitro-1,3-benzenediamine). One major peak remained unidentified in the extracts although it did not appear to be a CW-related material. TDG was detected at sub-ppm levels in the aqueous extracts of these samples, and on the metal fragment associated with soil sample 4D, and confirmed by GC–MS-SIM and GC–MS–MS data as shown in Table 1.

Soil samples 4C, 4E and 4F

No sulphur mustard was detected in the dichloromethane extracts of samples 4C, 4E and 4F, but its hydrolysis product TDG was detected

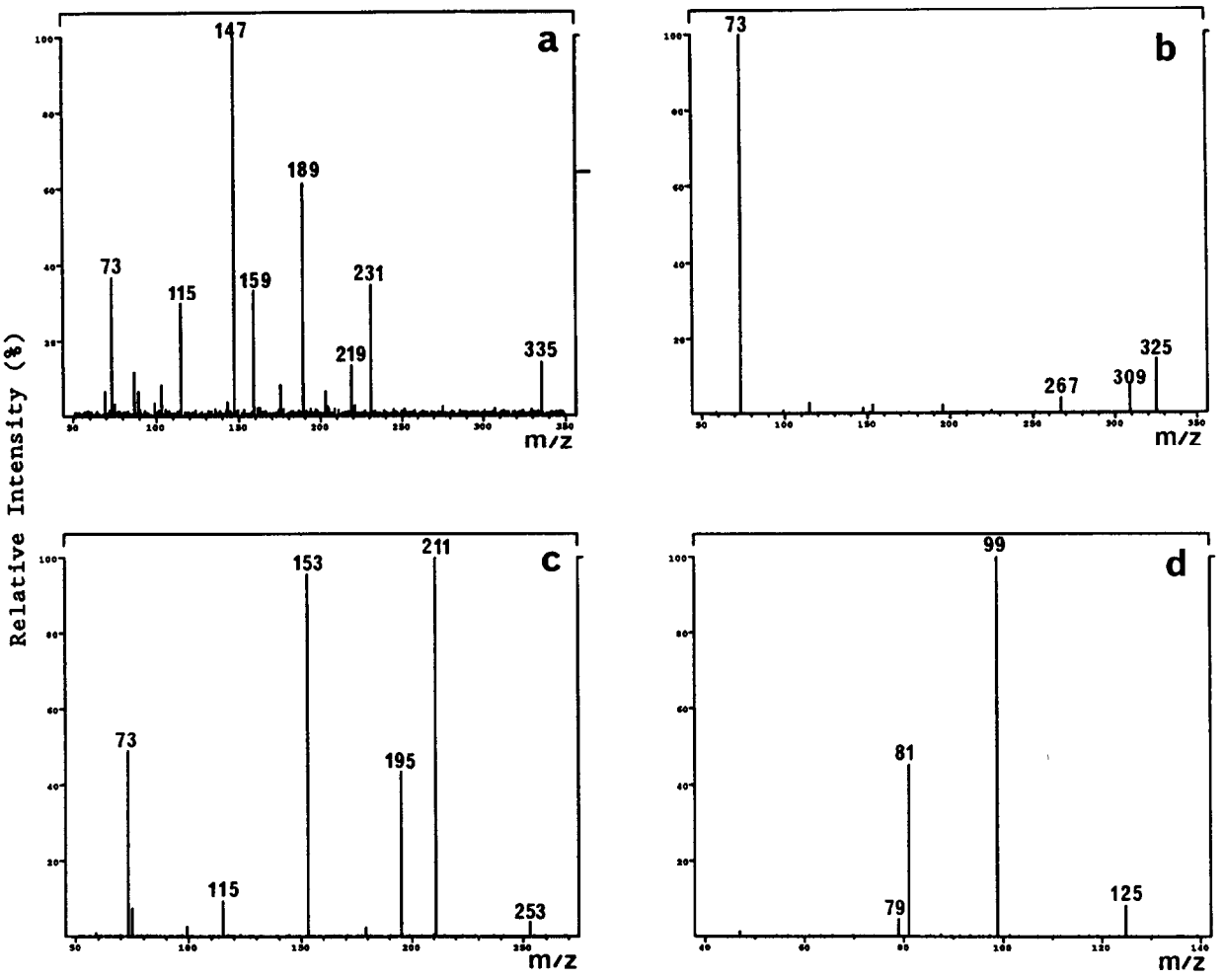

Fig. 2. CAD spectra of (a) TDG-$(TBDMS)_2$, *m/z* 335, (b) MPA-$(TBDMS)_2$, *m/z* 325, (c) iPMPA-TBDMS, *m/z* 253 (all CH_4 CI) and (d) GB, *m/z* 125 (EI).

in the aqueous extracts. It was confirmed by full-scan EI and CI spectra of the TBDMS derivative at the higher concentrations (3 ppm) present in sample 4C, shown in Fig. 4. Samples 4E and 4F contained low-ppb concentrations which were confirmed by GC–MS-SIM (CI) monitoring five ions, by higher-resolution GC–MS-SIM (EI), and by GC–MS–MS. The data for sample 4E, at concentrations estimated as 20 ppb, are shown in Fig. 5.

Soil samples 4G, 4H, 4I, 4J, 4K and 4L

Soil samples 4G, 4H, 4I, 4J, 4K and 4L, plus the metal fragments associated with samples 4H, 4I and 4J, were each found to contain iPMPA and MPA using the SIM screening procedure. Using simple aqueous extraction neither were extracted at levels sufficient to obtain good quality full-scan spectra of the TBDMS derivatives, and neither were observable above baseline noise in full-scan total ion current chromatograms. Confirmation of both MPA and iPMPA was initially obtained using GC–MS-SIM (CI) monitoring five ions, by higher-resolution GC–MS-SIM and by GC–MS–MS. Data for iPMPA in sample 4G are shown in Figs. 6, 7 and 8 together with the glassware blank for iPMPA under GC–MS–MS conditions. Following a re-

Table 1
Analysis of soil samples 4A–L

Sample	Number[a]	SIM screen	Confirmed by	Approximate concentration[b]
Soil	4A	Mustard	Full-scan EI, CI[c]	3 ppm
		TDG	CI-SIM, HR-SIM, MS–MS	200 ppb
Soil	4B[d]	Mustard	Full-scan EI, CI[c]	10 ppm
		TDG	CI-SIM, HR-SIM, MS–MS	300 ppb
Soil	4C	TDG	Full-scan EI, CI	3 ppm
Soil	4D	Mustard	Full-scan EI, CI[c]	600 ppb
		TDG	CI-SIM, HR-SIM, MS–MS	100 ppb
Metal	4D(M)	TDG	CI-SIM, HR-SIM, MS–MS	
Soil	4E	TDG	CI-SIM, HR-SIM, MS–MS	20 ppb
Soil	4F	TDG	CI-SIM, HR-SIM, MS–MS	100 ppb
Soil	4G[d]	iPMPA	CI-SIM, HR-SIM, MS–MS	80 ppb
		MPA	CI-SIM, HR-SIM, MS–MS	60 ppb[e]
Soil	4H	iPMPA	HR-SIM	6 ppb
		MPA	Full-scan EI, CI[e]	600 ppb[e]
Metal	4H(M)	iPMPA	CI-SIM, HR-SIM, MS–MS	
		MPA	CI-SIM, HR-SIM, MS–MS	
		GB	CI-SIM, HR-SIM, MS–MS[f]	
Soil	4I	iPMPA	CI-SIM, HR-SIM, MS–MS	100 ppb
		MPA	Full-scan EI, CI[e]	40 ppm[e]
Metal	4I(M)	iPMPA	CI-SIM, HR-SIM, MS–MS	
		MPA	CI-SIM, HR-SIM, MS–MS	
Soil	4J	iPMPA	CI-SIM, HR-SIM, MS–MS	30 ppb
		MPA	Full-scan EI, CI[e]	60 ppm[e]
Metal	4J(M)	iPMPA	CI-SIM, HR-SIM, MS–MS	
		MPA	CI-SIM, HR-SIM, MS–MS	
Soil	4K	iPMPA	CI-SIM, HR-SIM	200 ppb
		MPA	Full-scan EI, CI[e]	4 ppm[e]
Soil	4L	iPMPA	CI-SIM, HR-SIM	50 ppb
		MPA	Full-scan EI, CI[e]	7 ppm[e]

Abbreviations: CI-SIM = GC–MS-selected ion monitoring, methane CI, five ions; HR-SIM = GC–MS-selected ion monitoring, resolution 5000, EI, two or three ions; MS–MS = GC–MS–MS multiple reaction monitoring, two or three product ions.
[a] Numbers as received; additional CBDE numbers were assigned.
[b] Estimated to 1 significant figure.
[c] Additional volatiles 1,4-dithiane, 1,4-oxathiane, tetryl plus degradation products of tetryl, also identified by full-scan GC–MS.
[d] Mostly stones.
[e] After ion-exchange clean up.
[f] Using EI and ammonia CI on non-polar and polar GC columns.

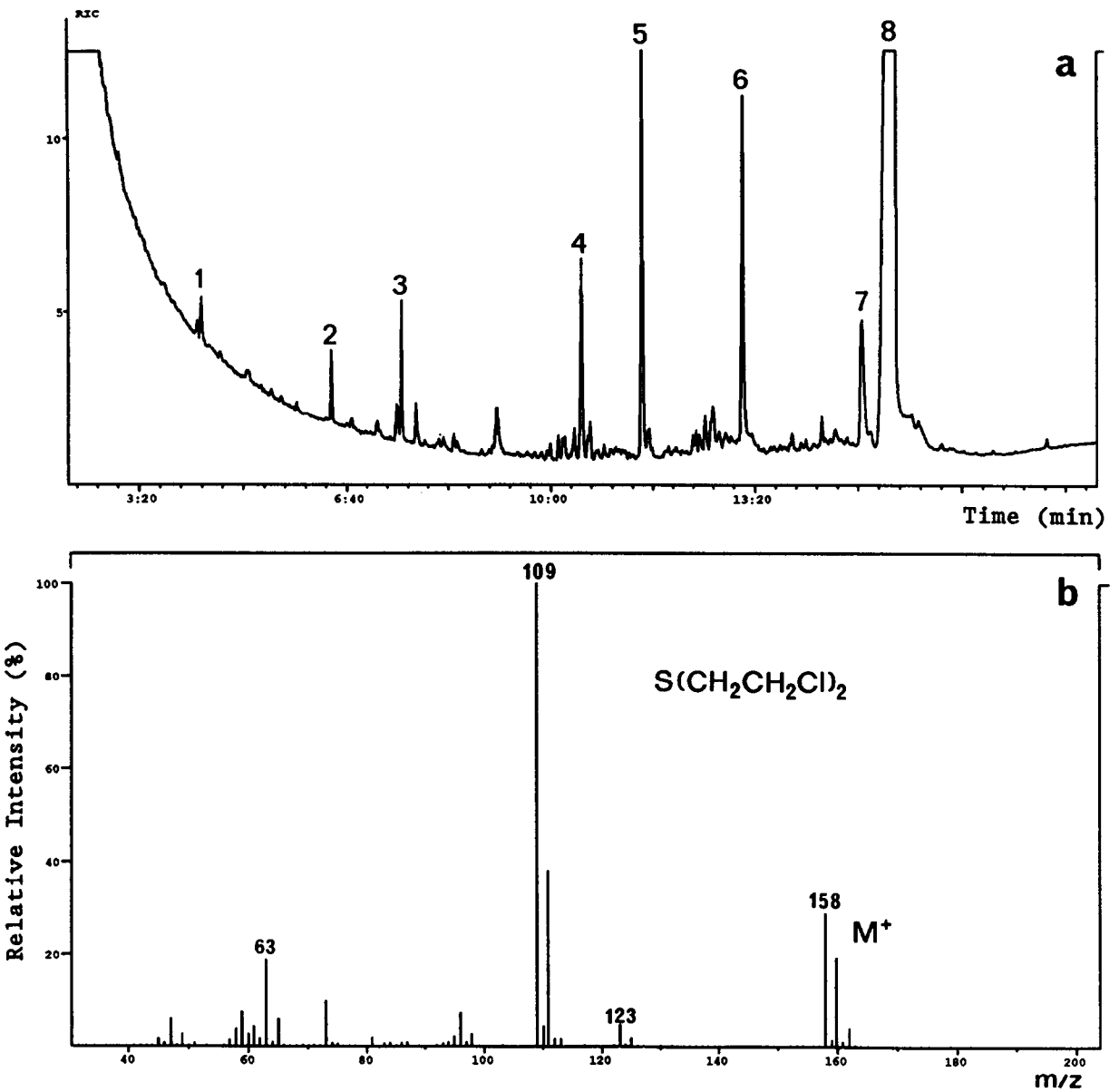

Fig. 3. (a) Total ion current chromatogram from the dichloromethane extract of soil sample 4A showing the presence of (1) 1,4-thioxane, (2) 1,4-dithiane, (3) sulphur mustard, (4) 2,6-bis(1,1-*tert.*-butyl)-2,5-cyclohexadiene-1,4-dione, (5) unidentified, (6) 2,4,6-trinitrophenylhydrazine (tentative identification), (7) 2,4,6-trinitroaniline and (8) tetryl. (b) Full-scan EI spectrum confirming the identification of sulphur mustard.

cent round robin exercise it was reported [13] that recoveries of MPA could be increased by passage of the aqueous extract through an acidic cation-exchange resin prior to derivatisation, which removes metal ions, possibly calcium which binds strongly to MPA. When this modified procedure was applied to these samples the concentrations of MPA found in the extracts increased substantially (up to 1000-fold) and good quality EI and CI full-scan data were

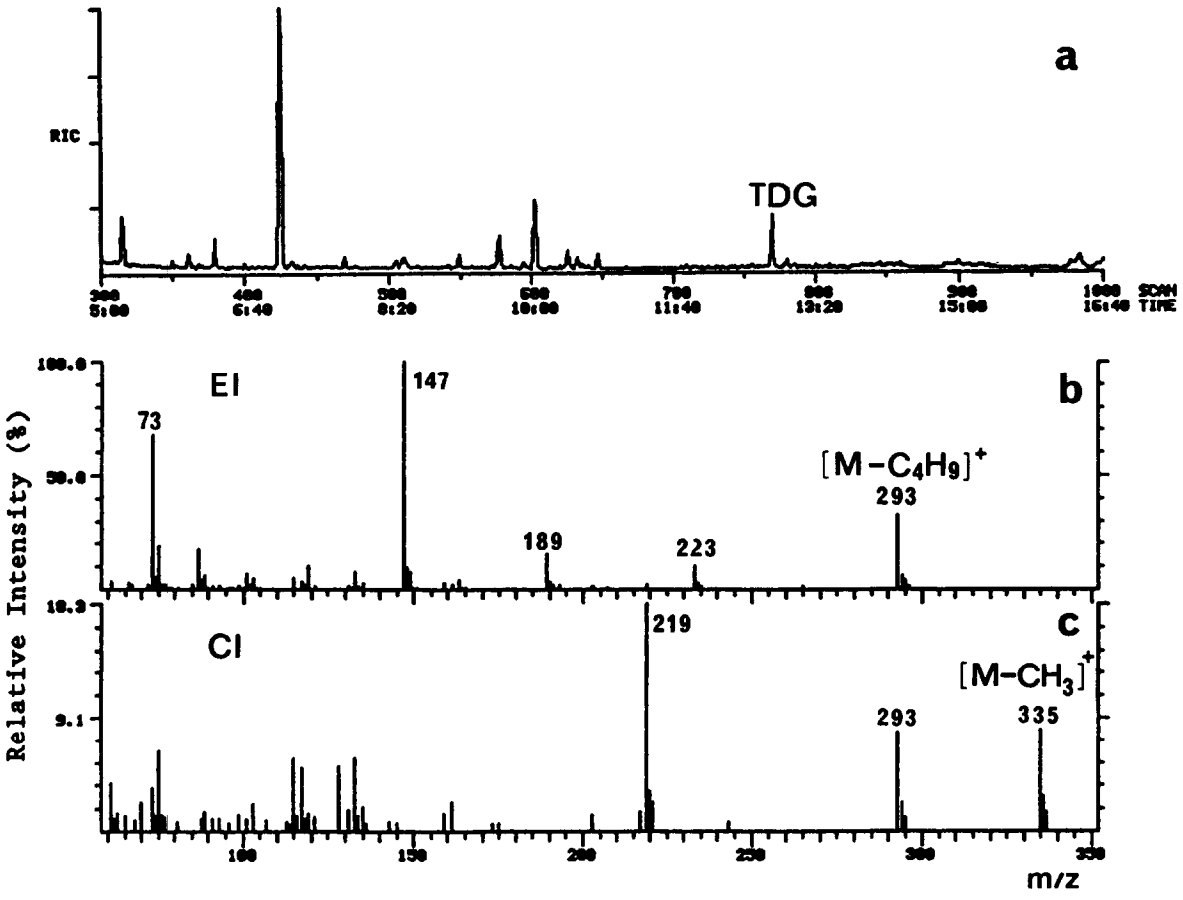

Fig. 4. (a) Total ion current chromatogram from the aqueous extract of soil sample 4C, with (b) EI and (c) CH_4 CI full-scan spectra confirming the identification of TDG-$(TBDMS)_2$.

obtained except for soil sample G which consisted mainly of stones. The total ion current chromatogram and EI and CI mass spectra of MPA-$(TBDMS)_2$, obtained from the aqueous extract of sample 4J after ion-exchange clean up and derivatisation, are shown in Fig. 9. Elution through the cation-exchange resin made little difference to the recoveries of iPMPA which remained in the extracts at concentrations below those required to obtain good quality full-scan data.

Metal fragment 4H(M)

In the case of a single metal fragment, associated with soil sample 4H, a relatively weak response at the retention time of sarin was observable in the selected ion screening procedure. After further concentration of the extract, additional evidence for the presence of sarin was obtained by GC–MS-SIM using both EI and ammonia CI [11] and by higher-resolution GC–MS-SIM (Fig. 10). To provide unequivocal evidence for the unexpected identification of intact sarin the sample was further analysed by GC–MS–MS, using both EI and ammonia CI, and employing two columns of widely differing polarity (methyl–5% phenyl silicone and Carbowax). Responses corresponding to those of a standard were obtained under each experimental condition. Chromatographic peak shape and S/N ratios on two or three product ions were superior using the polar

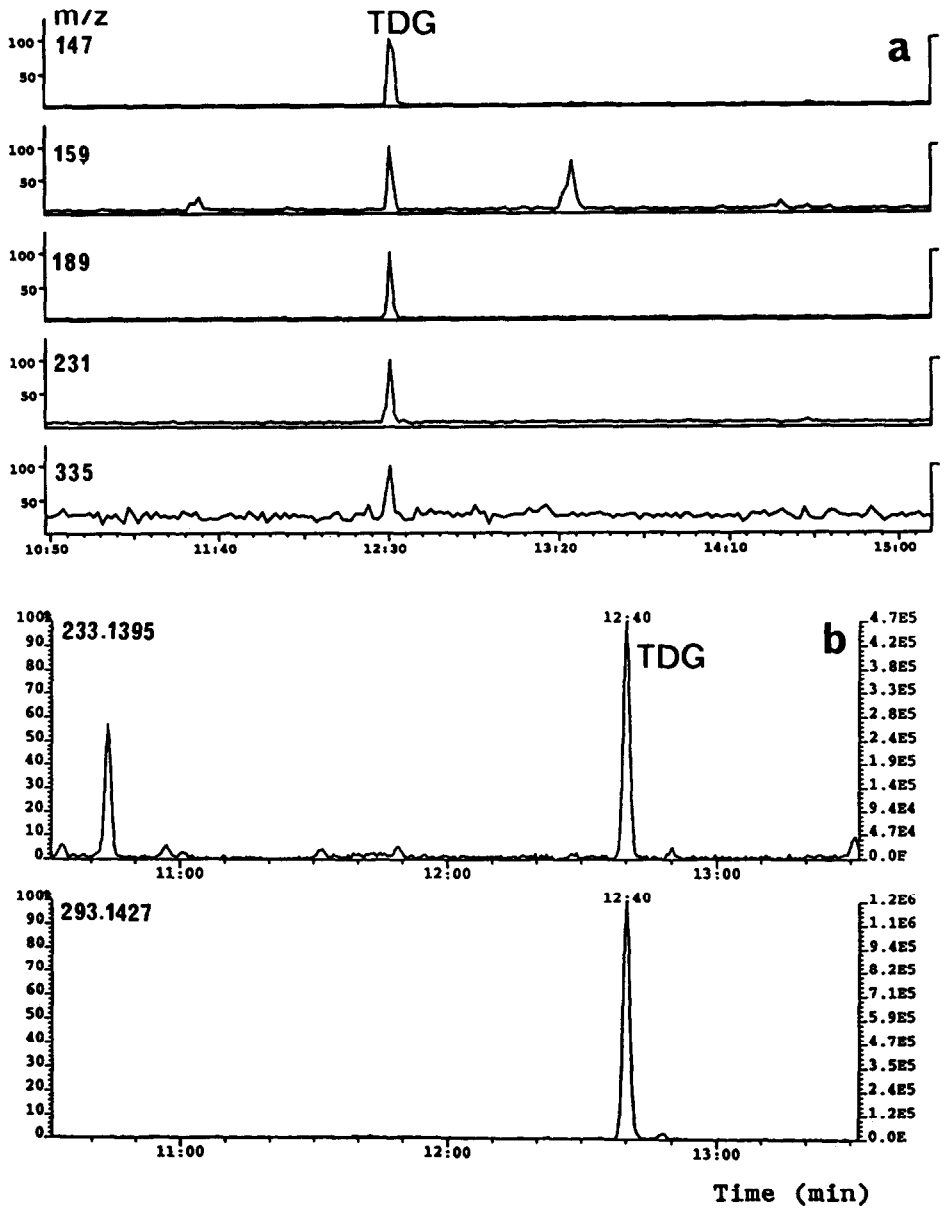

Fig. 5. (a) GC–MS–MS-MRM chromatograms (CH_4 CI) and (b) high-resolution SIM (EI) chromatograms confirming the identification of TDG-$(TBDMS)_2$ in the aqueous extract of soil sample 4E.

DBWAX column as shown in Fig. 11. MRM chromatograms, obtained using EI with a BP5 column and ammonia CI with a DBWAX column, are shown in Fig. 12. The total amount of sarin extracted from the 35-g fragment was estimated as 170 ng by GC–MS–MS. Both MPA and iPMPA were detected in the aqueous extract of the fragment; the GC–MS–MS response for iPMPA extracted from the metal fragment is shown in Fig. 13.

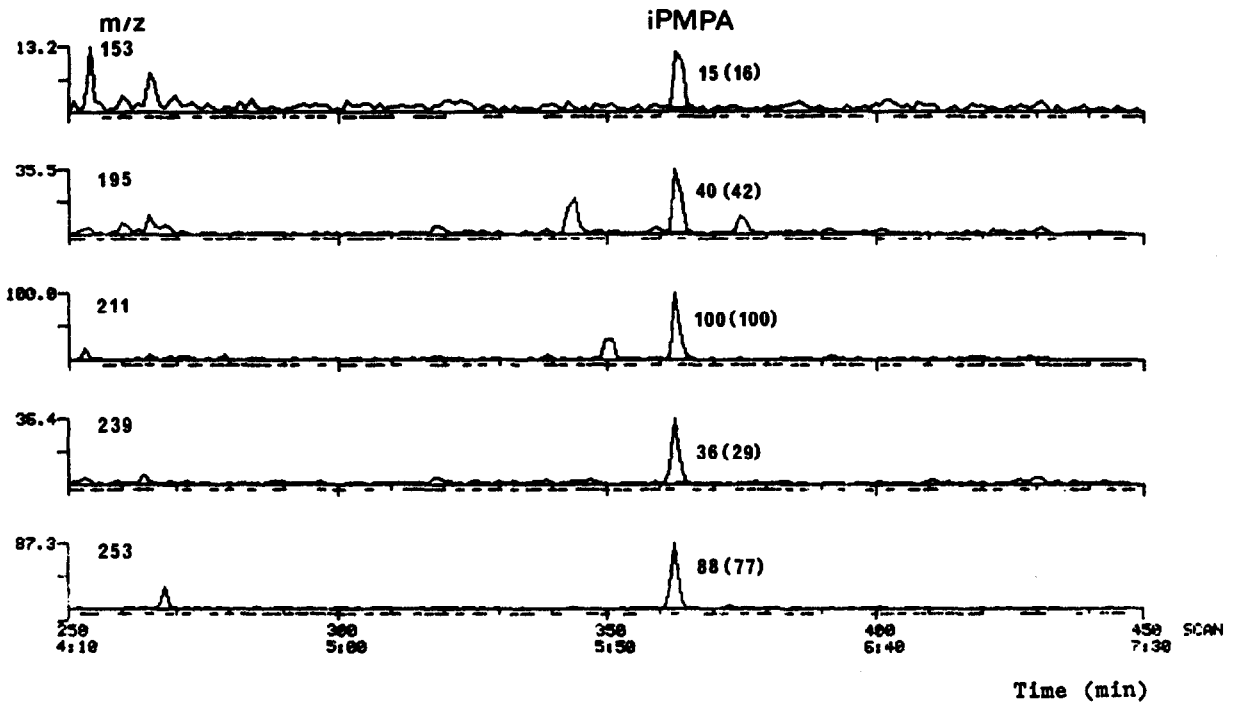

Fig. 6. Selected ion current chromatograms (CH_4 CI) confirming the identification of iPMPA-TBDMS in the aqueous extract of soil sample 4G. Relative peak intensities are shown together with those obtained from a standard in parentheses.

4. Discussion

The results demonstrate the utility of the different GC–MS and GC–MS–MS techniques which can be applied to forensic-type analyses to detect and unambiguously identify environmental contaminants at concentrations ranging from ppm to low ppb. At ppm levels, good quality full-scan mass spectra are usually obtainable and these provide the most desirable data where possible. At lower concentrations, where the more sensitive technique of GC–MS-SIM is required for detection, the combination of GC retention time (± 2 s) with the response for three characteristic ions, with ratios within 15–20% of those for a standard, have generally been accepted as proof of identification, the chances of a second compound responding in exactly the same manner having being calculated as extremely low [14]. Ion ratios for GC–MS-SIM using methane CI were generally reproducible within 20% limits when compared to standards at similar concentrations, but the relative intensities of the two most intense ions for iPMPA-TBDMS derivative were sometimes reversed at low concentrations. However, as recommended [9], the additional use of higher resolution GC–MS-SIM and/or GC–MS–MS-MRM adds an additional degree of certainty to the analysis and makes confirmation unequivocal within reasonable limits of probability. For samples such as the metal fragment, found to contain trace levels of sarin against a fairly noisy background, GC–MS–MS proved invaluable for improving S/N ratios and the specificity required for an unambiguous identification. The additional use of GC columns of widely differing polarity in combination with GC–MS–MS reinforced the results for sarin. The combination of EI, DBWAX column and GC–MS–MS appears to offer an excellent system for the confirmation of sarin. GC–MS–MS was also particularly useful

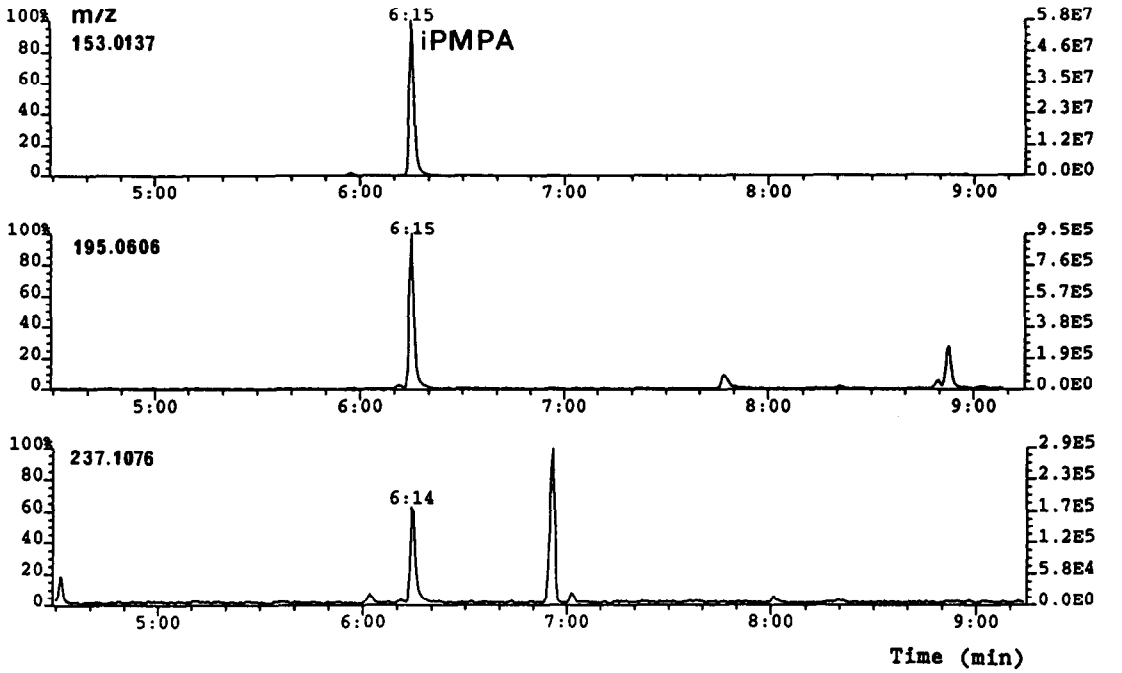

Fig. 7. Selected ion current chromatograms (EI) at 5000 resolution confirming the identification of iPMPA-TBDMS in soil sample 4G.

for the confirmation of iPMPA-TBDMS and gave superior S/N ratios and cleaner traces than higher-resolution SIM (Figs. 7 and 8). Similarly GC–MS–MS was useful for confirmation of TDG and MPA TBDMS derivatives although a disadvantage under the MS conditions used was that most of the product ion current from the protonated molecular ion of MPA-$(TBDMS)_2$ was concentrated in the non-characteristic ion m/z 73, which is derived from the TBDMS moiety. D'Agostino and co-workers [15–17] have demonstrated the advantages of GC–MS–MS using ammonia CI for the detection of sarin spiked into air and concrete samples, and other laboratories have reported exploratory work applying MS–MS techniques to CW agent detection [18–20]. In cases where identification was still in doubt the use of LC–MS [21,22] or an alternative derivative for GC–MS may be useful, *e.g.* pentafluorobenzoyl [23], heptafluorobutyryl [24] or trimethylsilyl [25] for thiodiglycol, and methyl [26,27], pentafluorobenzyl [26,28] or trimethylsilyl esters [25] for methylphosphonic acids. We prefer to use TBDMS derivatives for screening for TDG and methylphosphonic acids because of their ease of preparation, stability and mass spectral properties [10]. They have the added advantage over methyl or pentafluorobenzyl esters that TDG can be screened for simultaneously with methylphosphonic acids.

The absence of any traces of nerve agents, mustard or their hydrolysis products in the samples collected from the graves was not unexpected. Any agent which may have originally been present would most likely have been well dispersed and therefore more likely to be degraded and leached from the clothing and soil, although the results on the soil samples suggest that MPA is fairly persistent in soil. In contrast, all of the soil samples 4A–4L and their associated metal fragments, collected from bomb craters, gave positive results for sulphur mustard, TDG or sarin hydrolysis products. Information obtained following completion of the initial analyses indicated that the results correlated well with the sampling of different bomb craters. Soil

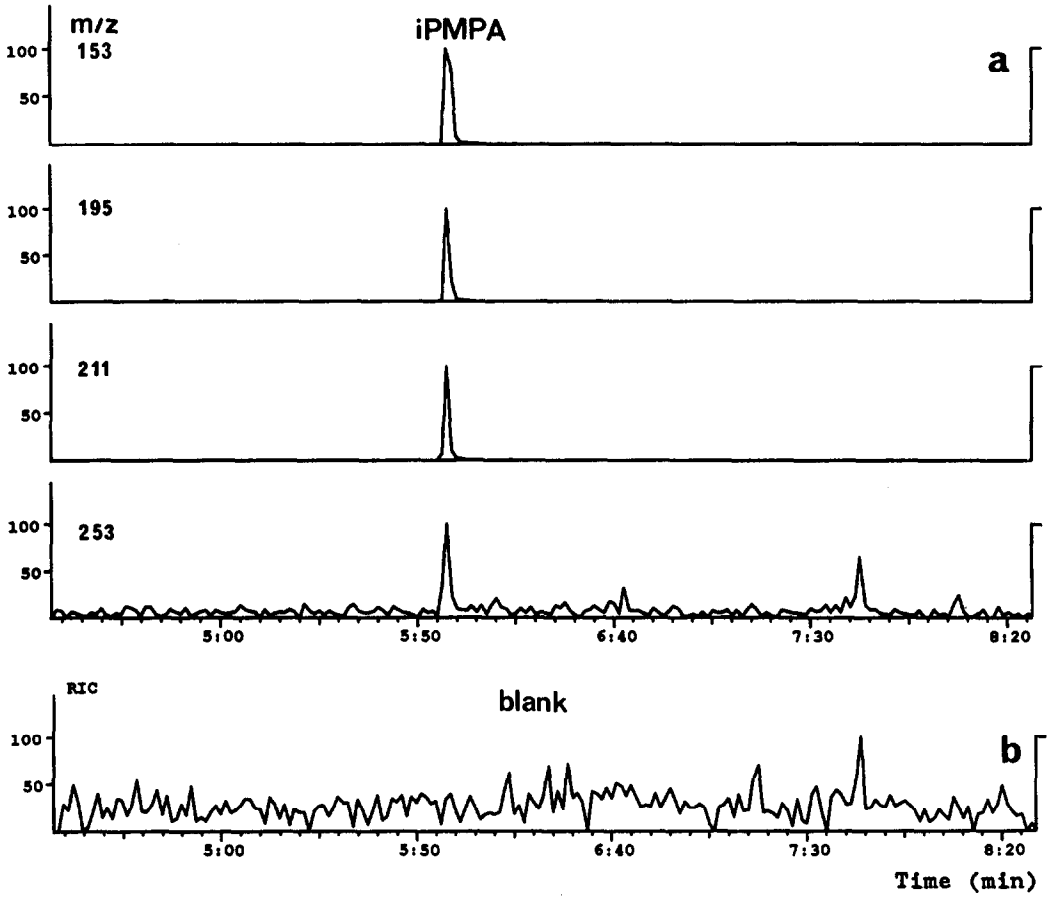

Fig. 8. GC–MS–MS-MRM chromatograms (CH_4 CI), (a) confirming the identification of iPMPA-TBDMS in soil sample 4G and (b) the reconstructed ion current trace from the preceding glassware blank.

samples 4A, 4B, 4C, and 4D, 4E, 4F, which contained sulphur mustard and/or TDG, were obtained from the first two craters sampled. Soil samples 4G, 4H, 4I, and 4J, 4K, 4L, which contained the hydrolysis products of sarin, were obtained from the third and fourth craters sampled. Samples 4A, 4D, 4G and 4J were collected from the centres of the respective craters, samples 4B, 4E, 4H and 4K from the southern edges and samples 4C, 4F, 4I and 4L from the northern edges of the craters.

In addition to sulphur mustard, samples 4A, 4B and 4D contained 1,4-dithiane, 1,4-thioxane and the explosive tetryl. These same compounds were found in the residues from a ruptured bomb, collected from the Kurdish region of northern Iraq, analysed at CBDE in 1988 [8]. The relatively high concentrations of tetryl present in these three samples caused some column degradation during the analysis of concentrated extracts. Samples 4G–4L, and the accompanying metal fragments in the case of samples 4H, 4I and 4J, all contained iPMPA and MPA, the hydrolysis products of sarin, at variable concentrations in the ppb (iPMPA) to ppm (MPA) range. iPMPA is the more important of these hydrolysis products since it is the initial product of rapid hydrolysis which confirms that the agent used was GB. MPA, which is formed by further hydrolysis of iPMPA, is the end product of hydrolysis of other organophosphorus nerve agents (*e.g.* soman, GF, VX). It may also arise

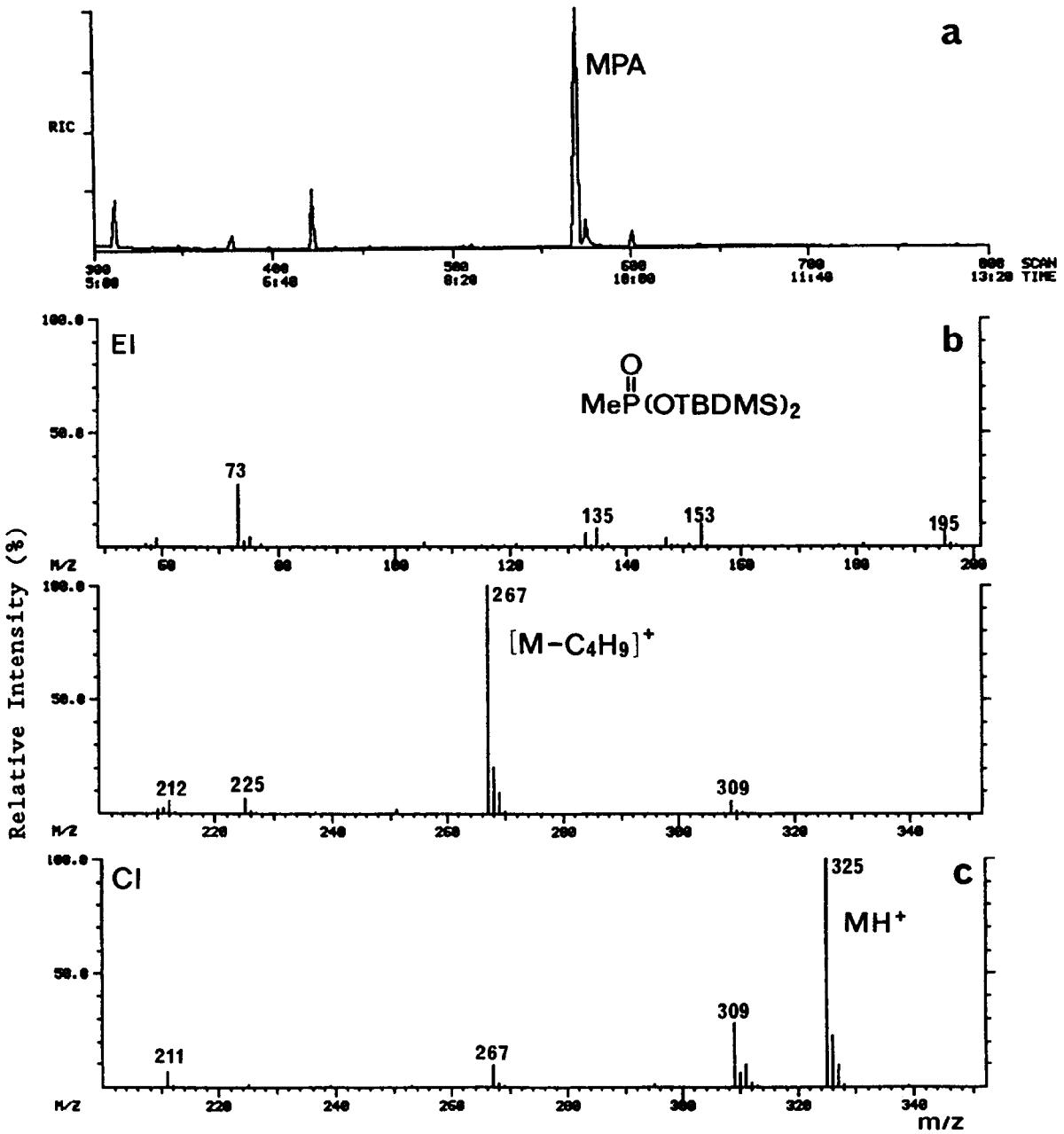

Fig. 9. (a) Total ion current chromatogram from the aqueous extract of soil sample 4J after passage through cation-exchange resin, with (b) EI and (c) CH_4 CI full scan spectra confirming the identification of MPA-$(TBDMS)_2$.

from the hydrolysis of other compounds such as fire retardants and one or two pesticides containing P–CH_3, although its presence in soil is most likely to be associated with CW use. No intact sarin was detected in any of the soil samples. In contrast to sulphur mustard, sarin is miscible with water and complete hydrolysis in a soil environment would be expected. Hydrolysis

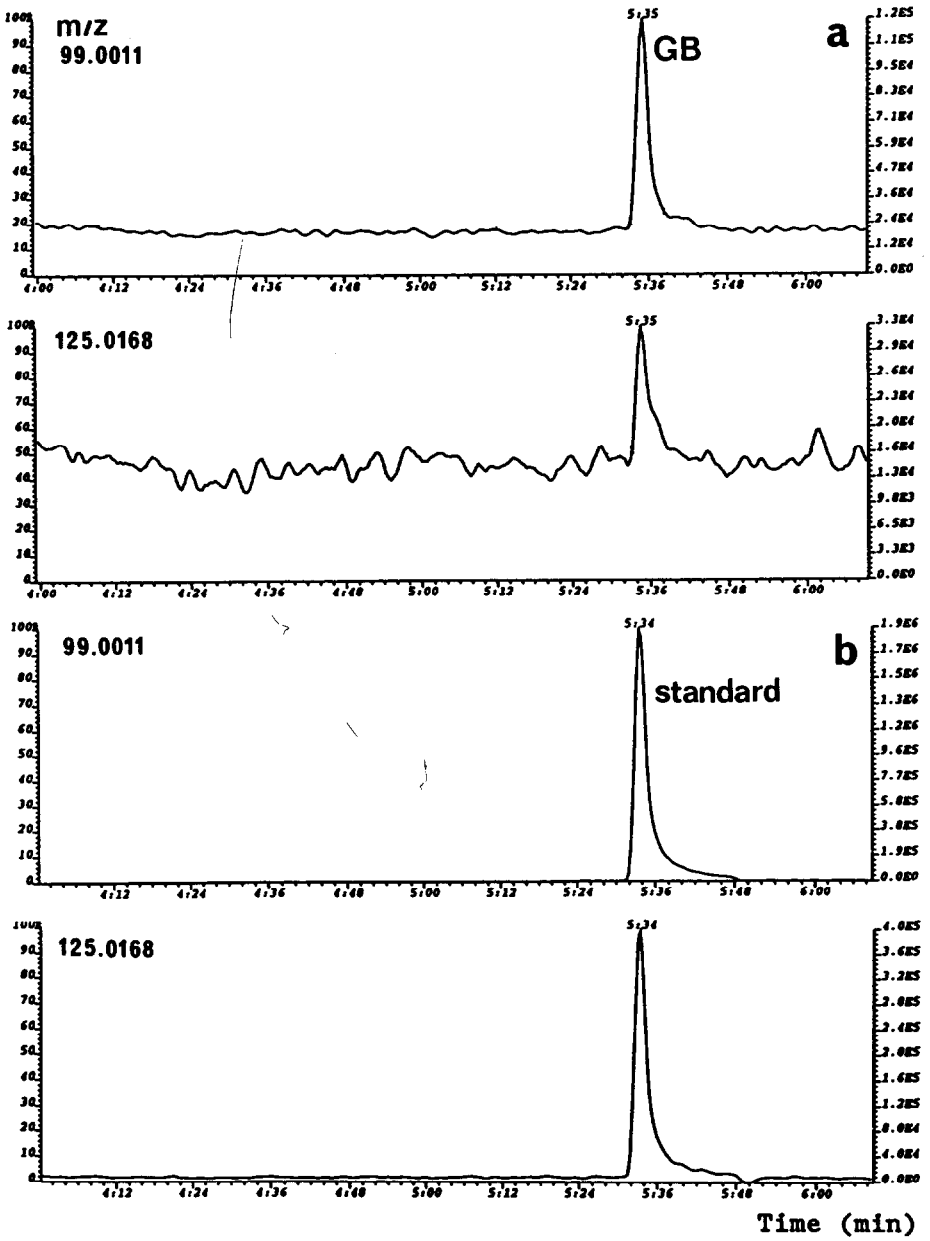

Fig. 10. Selected ion current chromatograms (EI) at 5000 resolution showing (a) the detection of GB in the dichloromethane extract of metal fragment soil 4H(M) and (b) the response from a standard (1 ng injected).

products alone were detected in nine of the twelve soil samples and these results once again emphasise the importance of analysing for hydrolysis products of CW agents in environmental samples.

The presence of sarin on the metal fragment after four years in the environment was unexpected. The GC–MS–MS data were unequivocal and clean glassware blanks were obtained for all of the analyses. This particular sample was unique in that the metal was coated with a military-type green paint. Sarin is readily

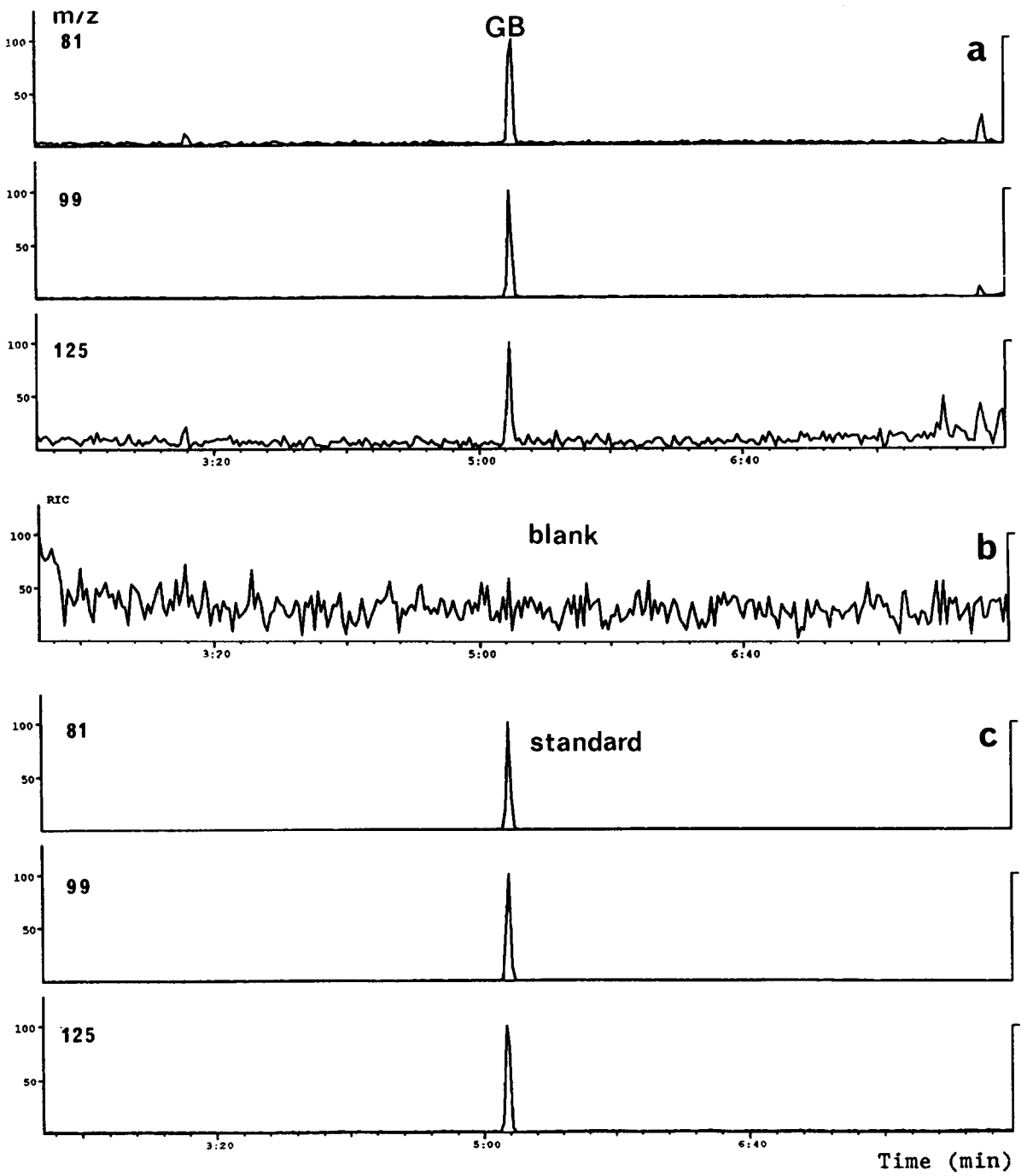

Fig. 11. GC–MS–MS-MRM chromatograms (EI, DBWAX polar column), (a) confirming the identification of GB in dichloromethane extract of metal fragment 4H(M), (b) the reconstructed ion current trace for the glassware blank and (c) the response from a standard (1 ng injected).

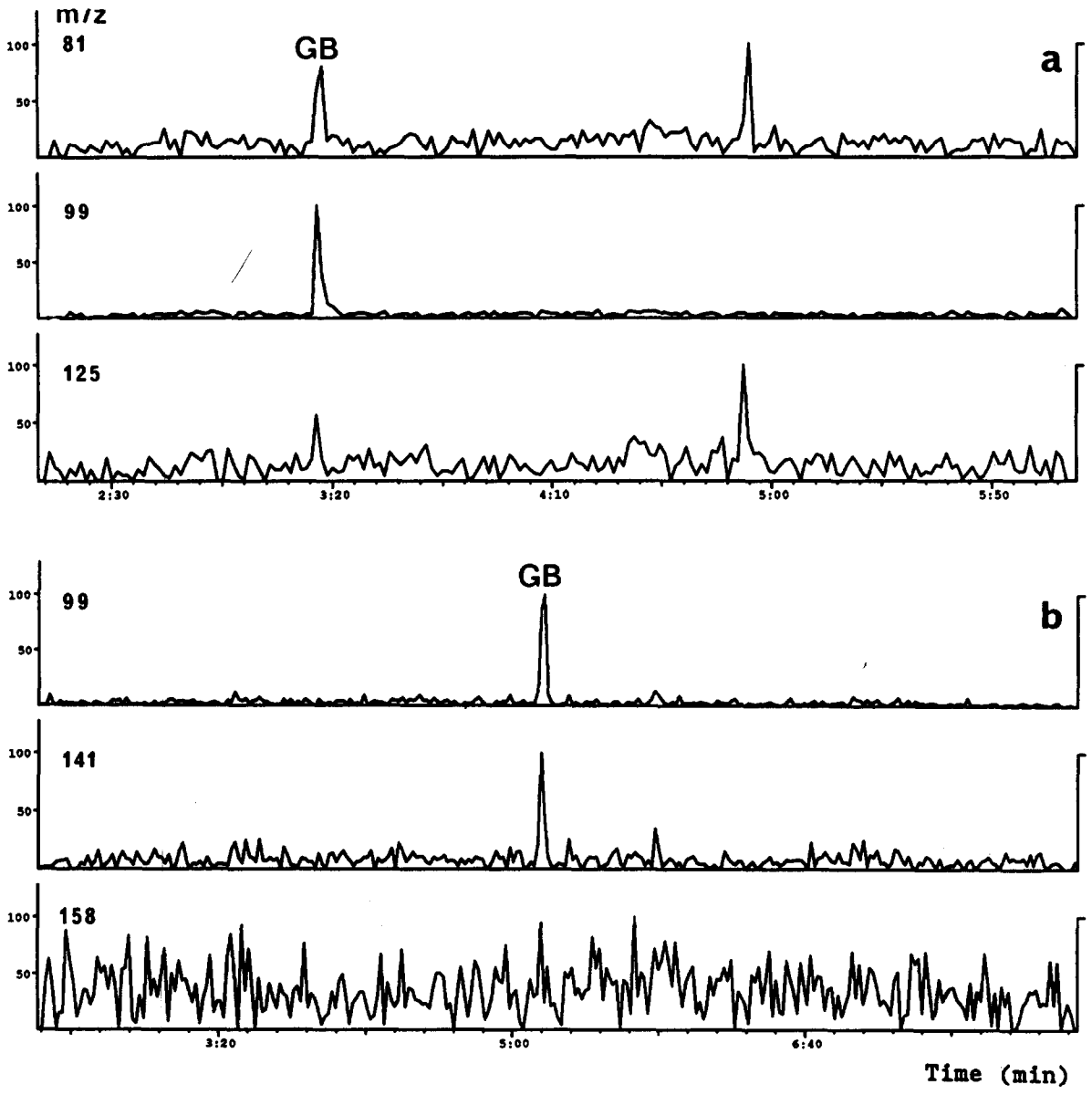

Fig. 12. GC–MS–MS-MRM chromatograms confirming the identification of GB in the dichloromethane extract of metal fragment 4H(M). (a) EI, BPX5 non-polar column; (b) NH_3 CI, DBWAX column.

absorbed into paints where it would be protected from moisture. A less likely explanation is that the sarin had been protected from environmental degradation by penetration of the double skinned layer of metal of which the fragment was composed. The detection of intact sarin on the metal fragment after four years in the environment suggests that paint may be a useful material to analyse in future investigations of alleged CW use. To our knowledge, these analyses provide the first example of an unequivocal confirmation of nerve agent residues in environmental samples collected after an alleged use of chemical weapons. It is particularly encouraging for the

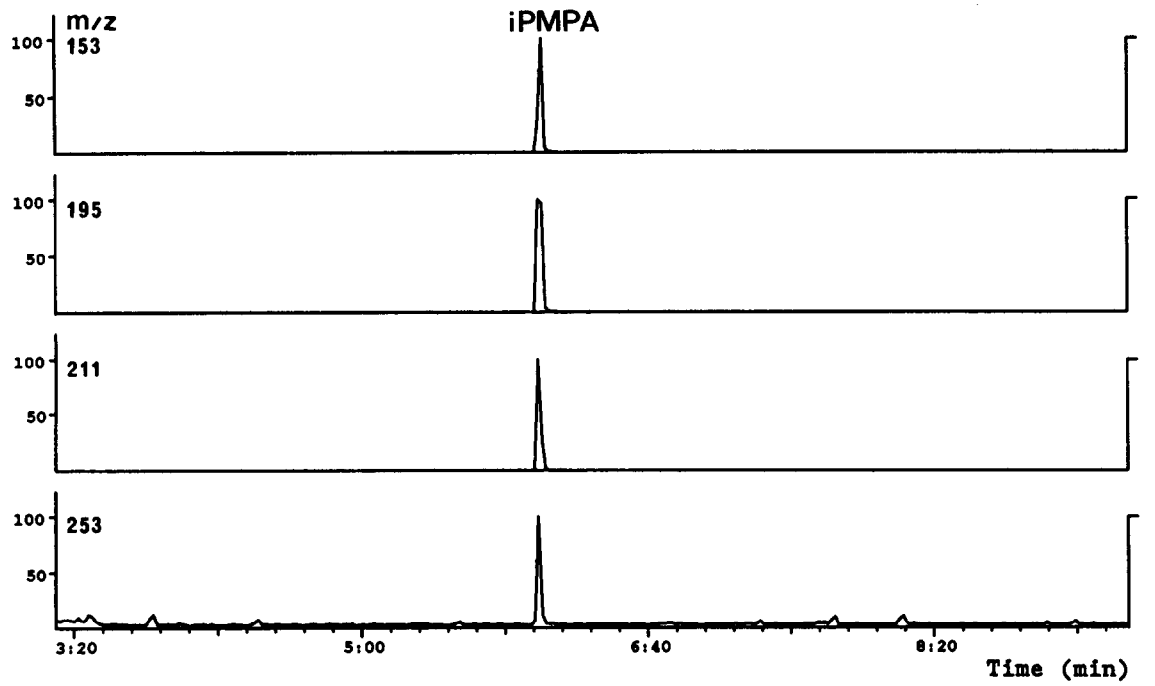

Fig. 13. GC–MS–MS-MRM chromatograms (CH_4 CI) confirming the identification of iPMPA-TBDMS in the aqueous extract of metal fragment 4H(M).

Chemical Weapons Convention that the use of both sulphur mustard and the much less persistent nerve agent sarin may be confirmed several years after the event provided that samples are collected from a point of high initial contamination.

5. Acknowledgements

The grave samples were collected by a forensic team under the leadership of Dr. Clyde Snow of the Physicians for Human Rights Organisation. The samples from bomb craters were collected by the team archeologist Dr. Jim Briscoe. Liason with CBDE was organised by Dr. Alistair Hay, Leeds University.

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