Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 982 (2002) 177–200
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Review

Analytical separation techniques for the determination of chemical warfare agents

Edwin W.J. Hooijschuur[a,b,*], Charles E. Kientz[b], Udo A.Th. Brinkman[a]

[a]*Free University, Department of Analytical Chemistry and Applied Spectroscopy, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands*

[b]*TNO Prins Maurits Laboratory, P.O. Box 45, 2280 AA Rijswijk, The Netherlands*

Received 24 June 2002; received in revised form 9 September 2002; accepted 9 September 2002

Abstract

Today, the determination of chemical warfare agents (CWAs) is an important area of application in analytical chemistry. Chromatographic, capillary electrophoretic and mass spectrometric techniques are primarily used for the identification and quantification of a broad field of classical CWAs in environmental samples and neutralization masses, obtained after destruction of CWAs. This overview is illustrative for the state of the art and mainly focuses on the literature published since 1996.

Keywords: Reviews; Warfare agents

Contents

*Corresponding author. Present address: Pharma Bio-Research Group B.V., P.O. Box 200, 9470 AE Zuidlaren, The Netherlands. Tel.: +31-592-303-400; fax: +31-592-303-223.
E-mail address: ehooijschuur@pbr.nl (E.W.J. Hooijschuur).

0021-9673/02/$ – see front matter
PII: S0021-9673(02)01426-7

1. Introduction

Chemical warfare agents (CWAs) were first used on a large scale in World War I. Since then they have been employed several times in conflicts around the world. Classical CWA can be divided into several groups, the most lethal group being the nerve agents. Their name is derived from the major action of these chemicals on the nervous system. Nerve agents irreversibly react with the enzyme acetylcholinesterase in tissue fluid, which effects the accumulation of acetylcholine and continuous stimulation of the nervous system. Particularly, one nerve agent, sarin (isopropyl methylphosphonofluoridate), was in the news after its use against the population of the Kurdish village of Birjinni in 1988 and after terrorist attacks in Matsumoto city in 1994 and the Tokyo underground system in 1995. Although the terrorists used rather impure sarin and a primitive delivery system, it was effective enough to kill 12 people and injure more than 5000 others. Another well-known nerve agent, VX (*O*-ethyl *S*-2-diisopropylaminoethyl methylphosphonothiolate), which is among the most toxic substances ever produced by man, has a lethal concentration–time dose for 50% of the exposed individuals (LC_{T50}) of 10 mg min/m^3 as aerosolised agent. The second group, the vesicants, is used for casualty effects. These agents affect the eyes and lungs and blister the skin. Sulphur mustard (bis(2-chloroethyl)sulphide) was frequently used in World War I and in the second half of the 1980s in the Iran–Iraq war. The third group, blood agents, interfere with the oxygen transport capability of blood and may cause death by suffocation. Blood agents like hydrogen cyanide were used in World War I. The fourth group, incapacitating agents, has non-lethal physiological effects such as vomiting and/or mental effects. Although this categorisation is not exhaustive, the four categories mentioned cover the majority of classical CWAs. Next to this type of compound, there is a relatively large group of toxins and bioregulators which are regarded as possible chemical or biological warfare agents (BWAs), e.g. botulinium toxin, saxitoxin and ricin. The determination of BWAs generally requires special techniques; these will not be discussed in this paper.

The Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on their Destruction (the Chemical Weapons Convention; CWC) entered into force on 29 April, 1997 [1]. By June 2002, 145 countries had ratified or acceded the Convention. In the CWC, chemical weapons are defined as toxic chemicals and their precursors, except when intended for purposes not prohibited under the CWC; munitions, devices and equipment designed for releasing the chemical are also covered. A toxic chemical is defined as a chemical that through its chemical action on life processes can cause death, temporary incapacitation and/or permanent harm to humans or animals. A precursor is a chemical needed for the production of a toxic chemical, but can also be a degradation product of a toxic chemical. Generally, these compounds are considerably more polar and less volatile than their precursor CWA (see Fig. 1). The toxic chemicals and their precursors that have been identified for the application of verification measures are listed in Schedules 1–3 contained in the Annex on Chemicals of the CWC [1]. Altogether, thousands of chemicals are included in the Schedules, which contain mainly organic compounds with a wide variety of chemical and physical properties: neutral chemicals, acids, bases, volatiles and non-volatiles, with phosphorus, sulphur, fluorine

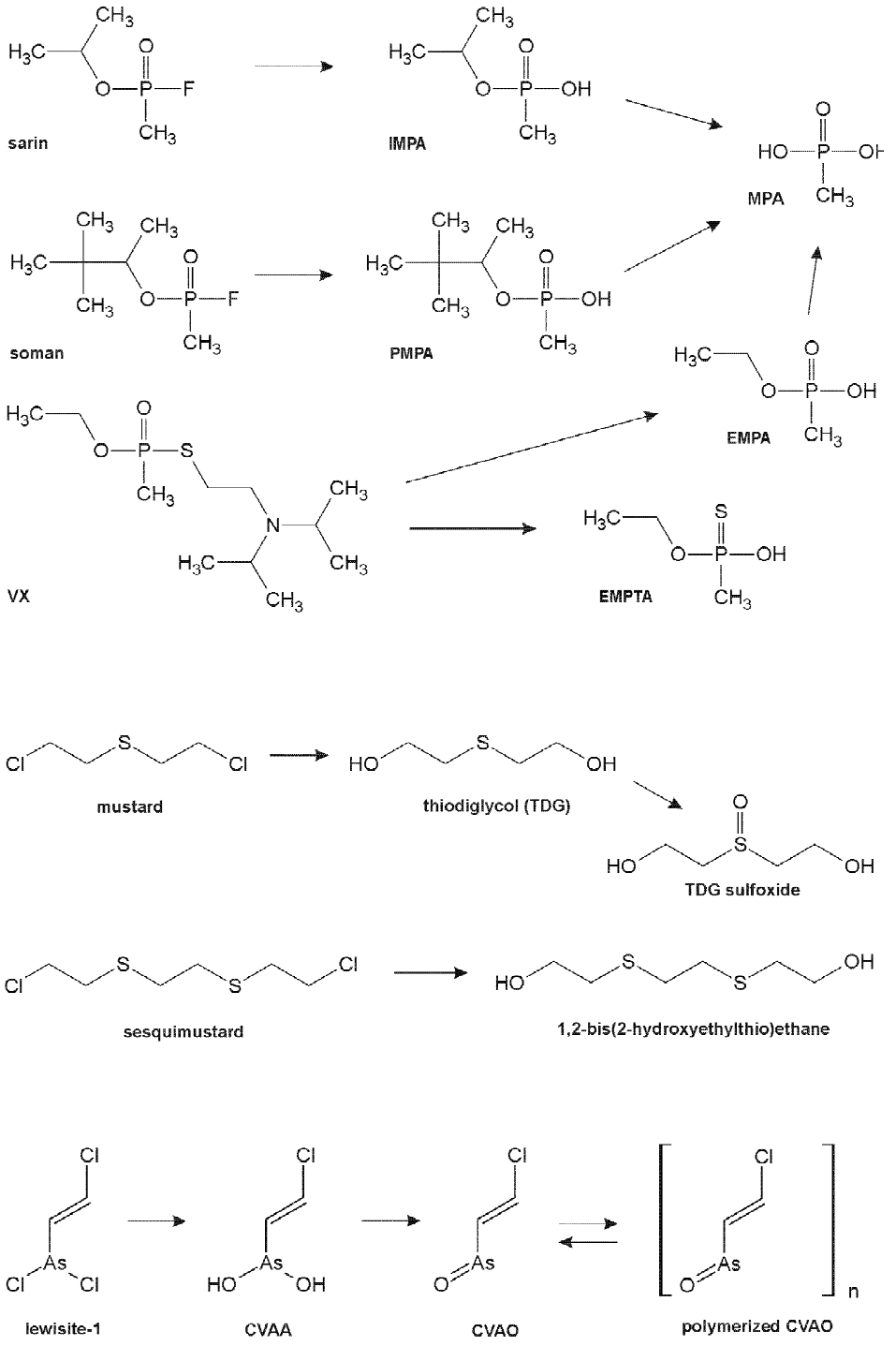

Fig. 1. Hydrolysis pathways of selected organophosphorus nerve agents, sulphur mustard, sesquimustard and lewisite-1. For acronyms, see text.

and/or chlorine heteroatoms frequently being part of the molecule. Since the CWC entered into force, many efforts have been made to develop and improve methods for the determination of CWAs and their precursors or degradation products, since such analyses may play a key role in the verification of the treaty as well as the monitoring of CWA destruction. The most frequently used methods for the unambiguous identification of CWAs and their precursor and breakdown products are based on gas chromatography (GC) in combination with mass spectrometry (GC–MS) and/or tandem mass spectrometry (GC–MS–MS), liquid chromatography (LC) coupled with MS(–MS), and nuclear magnetic resonance (NMR) spectrometry [2–5]. For screening purposes and quantitative determinations, GC, LC and capillary electrophoresis (CE) with mainly element-selective detectors are frequently used.

This paper reports on recent developments in the application of chromatographic, capillary electrophoretic and mass spectrometric techniques for the determination of classical CWAs in environmental samples and neutralization masses, obtained after destruction of CWAs. This overview is illustrative for the present state of the art and is not intended to be exhaustive.

2. Nerve agents

2.1. General

Nerve agents are rather volatile compounds and analysis by GC-based techniques is an obvious first choice, with GC–MS(–MS) playing the major role nowadays. However, in an aqueous environment, organophosphorus nerve agents readily hydrolyse to produce characteristic non-toxic compounds containing a C–P bond which is rare in nature. Relevant examples are given in Fig. 1. The most important breakdown products of nerve agents are alkyl alkylphosphonic acids which are specific for the original nerve agent. Therefore, detection of this type of compounds is a very important task for verification studies since the corresponding hydrolysis product can be used to indicate the presence of the nerve agent produced or used, which in itself may have been degraded completely.

2.2. Gas chromatography

GC is the most widely used chromatographic method for the determination of volatile compounds because of its high efficiency, the ease of operation and the possibilities for selective and sensitive detection. Nerve agents are rather volatile compounds with sufficient thermal stability and, in addition, contain a phosphorus atom which makes GC with flame photometric detection (FPD) or GC with nitrogen–phosphorus detection (NPD) very suitable combinations for selective detection and quantification. These robust techniques have been available since the 1970s and even though today often replaced by MS, they still are an important tool for screening purposes. A comprehensive review on chromatographic analysis of CWAs was published by Witkiewicz et al. in 1990 [6]. Next to FPD and NPD, also flame ionisation detection (FID) and photoionisation detection (PID) have been widely used to detect nerve agents. More recently, Fourier transform infrared spectroscopy (FTIR) [7–10] was used for the identification of nerve agent homologues and dialkyl methylphosphonates. For screening for the presence of CWAs, the use of retention indices (*RI*) is a suitable tool in GC [11,12]. However, it is widely accepted that unequivocal identification in relation to the CWC can only be based on spectrometric results and the increased availability of GC–MS and LC–MS has reduced the use of *RI* values as a method for identification significantly. Nevertheless, *RI* values support GC–MS identification and allow nerve agents with closely similar electron impact (EI) mass spectra to be distinguished. The largest collection of *RI* values of CWA-related chemicals is compiled in the Central OPCW Analytical Database [13].

Recent papers describe the use of GC–NPD and GC–FPD (P-mode) to test and optimise extraction methods for nerve agents and related compounds. Sarin, tabun (ethyl *N*,*N*-dimethyl phosphoramidocyanidate), soman (pinacolyl methylphosphonofluoridate) and VX were extracted from natural waters by means of solid-phase microextraction (SPME), which combines sampling, extraction, concentration and introduction into a single step, and quantified by GC–NPD and GC–MS [14]; this resulted in excellent limits of detection (LODs) of

0.05–1 ng/ml. Supercritical fluid extraction (SFE) followed by GC–FPD or GC–MS [15] was found to have some advantages over the more classical liquid solvent ultrasonication extraction [16] for nerve agents in alkyd-painted plates: SFE gave ca. 10% higher recoveries, was less time-consuming and used less organic solvent. The use of solid-phase extraction (SPE) followed by large-volume-injection (200 μl) GC–NPD has been studied for the sensitive determination of intact nerve agents such as sarin, soman, tabun, DFP (diisopropyl phosphorofluoridate) and VX in aqueous samples [17]. LODs at pg/ml levels could be achieved for all compounds using XAD-4 as sorbent and *n*-pentane–methanol (95:5, v/v) as extraction solvent.

In order to make the non-volatile degradation products of nerve agents amenable for GC-based analysis, several derivatization procedures have been recommended, e.g. trimethylsilylation [18], methylation [19], *tert*.-butyldimethylsilylation [20], pentafluorobenzylation [21] and treatment with trimethylphenyl ammonium hydroxide [22]. More recently, the alkylphosphonic acids, methylphosphonic acid (MPA), ethyl methylphosphonic acid (EMPA) and isopropyl methylphosphonic acid (IMPA) were extracted from 50-ml groundwater samples by SPE on a quaternary ammonium column, followed by both elution and derivatization with methanolic trimethylphenyl ammonium hydroxide. Subsequent analysis by GC–FPD resulted in LODs of 3–9 ng/ml [23].

A relatively new approach is the use of pulsed FPD (PFPD) as GC detection method [24]. The PFPD can separate the emission of carbon from those of sulphur and phosphorus in time, which causes enhanced selectivity and sensitivity. With PFPD, low detection limits of 180 fg/s (sulphur), 7 fg/s (phosphorus) and 2 pg/s (nitrogen) have been found. Amirav, who developed PFPD, proposed fast GC–PFPD to facilitate field analysis of a wide range of CWAs [25]. Fast GC is based on the use of special inlet systems with relatively short capillary columns operated at unusually high carrier gas flow rates [26,27]. With these systems, volatile organic compounds can be separated in seconds instead of minutes, with highly phosphorus- or sulphur-selective detection at LODs of 20–200 ng/m^3 for air samples. For the present types of application, GC–PFPD may well become a strong competitor to GC–FPD/NPD and even GC–atomic emission detection (AED), in particular because other elements (i.e. arsenic) can also be detected. The applicability of GC–AED for the determination of CWAs was studied by Stuff, Creasy and co-workers. They used GC–AED for the quantification of trimethylsilyl esters of alkyl methylphosphonic acids in environmental samples and found LODs of 10 μg/ml [28]. They also showed that with AED, one can calculate an approximate empirical formula for unknown VX-related compounds based on calibration using a standard solution of VX, which contains C, H, N, O, P and S [29]. This information, combined with GC–MS analysis led to the identification of three breakdown products of VX in an extract of a decontamination experiment, in which ozone treatment was used to clean a VX-contaminated surface of an aircraft or vehicle after exposure to the nerve agent. A disadvantage of the application of AED for samples with a high hydrocarbon content, e.g. diesel oil, is the quenching of the plasma, unless the hydrocarbon can be vented.

2.3. Gas chromatography–mass spectrometry

GC–MS and GC–MS–MS are the most popular techniques for the determination, and identification, of nerve agents and their degradation products in environmental samples and neutralization masses. GC–MS of nerve agents under EI conditions often results in extensive fragmentation, which may provide important structural information, while milder chemical ionisation (CI), typically using methane, isobutane or ammonia as reagent gas, is commonly used to provide molecular mass information.

Recently, Rohrbaugh published two papers dealing with GC–MS using EI and CI for the analysis of a reaction product, which was obtained after the destruction of VX with water [30], and a thermally degraded sample of VX [31], in which VX and 34 degradation products were found. A comparison of mass spectra obtained with GC–ion trap MS for VX under EI, methane CI and methanol CI conditions, which is shown in Fig. 2, demonstrates that methanol CI gave enhanced protonated molecular ion formation that could play a significant role in the identification of higher-molecular-mass degradation prod-

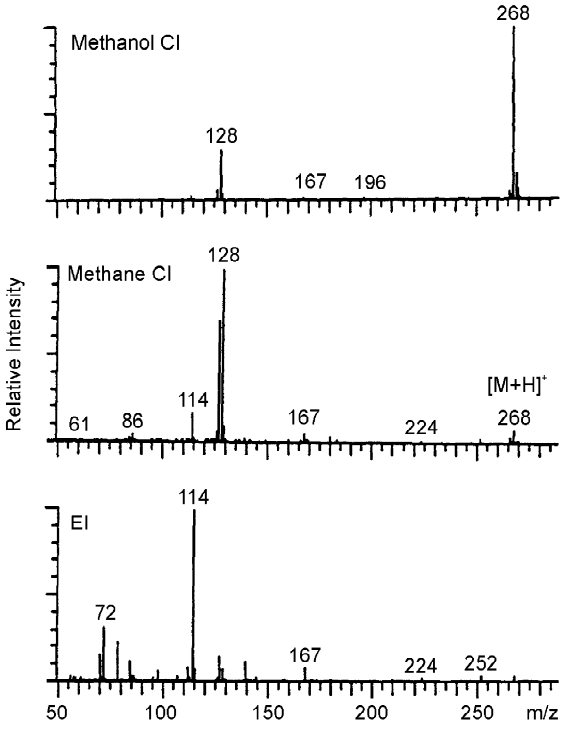

Fig. 2. EI, methane CI and methanol CI mass spectra of VX under ion-trap MS conditions [31].

ucts of VX. Furthermore, methanol CI significantly reduced fragmentation, which is favourable for sensitive detection. In addition, the use of methanol as a CI reagent is advantageous for field analysis by being less expensive, easier to transport and safer to use than traditional gaseous reagents, which require the use of cylinders. Stuff et al. validated a GC–MS method for the trace-level quantification of sarin in order to monitor the effectiveness of sarin destruction with ethanolamine [32]. Automated thermal desorption (ATD) was proposed by Carrick et al. instead of solvent desorption for the semi-quantitative GC–MS analysis of vapours containing a wide range of CWAs, which were sampled on Tenax TA [33]. The advantage of ATD over solvent desorption is that the whole sample is analysed, which provides increased sensitivity (LODs, 50 ng on tube), less sample preparation and the absence of interfering solvent peaks. However, the disadvantage is that, once analysed, no sample is left for re-analysis.

A drawback of GC–MS is the need for chemical derivatization of the more polar, non-volatile hydrolysis products of nerve agents, e.g. the alkylphosphonic acids, which is sometimes complicated and time-consuming, and may result in the formation of artefacts and limited recovery, or even non-detection. However, although LC–MS is in principle better suited to deal with these analytes, GC–MS is often required for their unambiguous identification. In general, the same derivatization procedures can be utilized as described above. GC–MS–MS was found to be a powerful tool for the screening of trimethylsilyl (TMS) esters of alkyl (R) methylphosphonic acids in complex matrices [34]. GC–EI-MS showed a base peak at m/z 153 for all five test compounds with the structure, $(H_3C)P(O)(OR)(O–Si(CH_3)_3$, which likely corresponds to the $[(H_3C)P(O)(OH)(O=Si(CH_3)_2)]^+$ ion resulting from the loss of the R group and a methyl group, and a common ion at m/z 169 (10–50% of base peak) resulting from the loss of the R group. Collision-induced dissociation (CID) of the m/z 153 precursor ion leads primarily to the structure $[HO=Si(CH_3)_2]^+$ at m/z 75, while CID of the m/z 169 fragments leads to ions at m/z 153 (base peak) and m/z 75 (25% of base peak). Next to the same common ions, methane CI-MS provides $[M+H]^+$ as the base peak, which is important for identification purposes. Monitoring of these specific fragmentations offers an elegant screening method for nearly all members of this compound class. However, derivatization is still required and LC- and CE-based techniques may therefore be considered as at least equally suitable screening tools.

Kataoka and co-workers studied the aqueous extraction of alkylphosphonic acids from various soil types, seawater and beverages, followed by *tert.*-butyldimethylsilylation (TBDMS) and GC–MS [35–37]. They observed severe problems caused by low extraction recoveries and, also, low derivatization recoveries. The yields of TBDMS derivatization were significantly decreased in the presence of calcium and magnesium ions in the samples, with the worst yields for the most hydrophilic compound, MPA. It was concluded that macroporous anion-exchange-resin pretreatment is an efficient method to eliminate matrix constituents present in aqueous soil extracts and sea water, which interfere with TBDMS derivatization [36], although the derivatization yields

were only 28–71% even then (compared with CE results obtained from the same samples without derivatization). Alkylphosphonic acids were retained on the resin and subsequently eluted with 0.1 *M* HCl. The eluate was neutralized with sodium hydrogen carbonate (pH ca. 7) followed by evaporation to dryness, derivatization and, finally, injection in the GC–MS system. LODs in the selected ion-monitoring (SIM) mode were ~0.2 μg/g of soil with a moderate within-day repeatability of ca. 20% (RSD; 3 μg/g soil, $n=5$). SPME was studied for the extraction of nerve agents from water [38], sarin from water and air [39] and for the extraction and in situ TBDMS derivatization of CWA degradation products [40], followed by GC–MS. An effective procedure was developed for the determination of MPA, EMPA and nPrPA (*n*-propylphosphonic acid), as well as TDG (thiodiglycol), which is the predominant degradation product of sulphur mustard, ethyl-2-hydroxyethyl sulphide and benzilic acid, which is a degradation product of BZ (3-quinuclidinyl benzilate). A Carboxen SPME fiber was exposed to the derivatization reagent for 5 min followed by 30 min extraction of acidified (pH 1.5) and salt-saturated aqueous samples. Subsequently, derivatization took place by exposure of the fiber to the reagent for 15 min; then, the fiber was injected into the GC–MS. The LODs were 10–100 ng/ml for the alkyl methylphosphonic acids. The method was successfully applied in a proficiency test organized by the OPCW. However, the present SPME method is not very convenient for quantification since the repeatability of ca. 10–35% (RSD, at 1 and 20 μg/ml water, $n=6$) is an indication of conditions which are difficult to control. Nevertheless, SPME is an ideal method for collecting samples in the field and transporting them to the laboratory for analysis.

2.4. Liquid chromatography

LC is a very suitable separation technique for the determination of the polar, non-volatile (alkyl) alkylphosphonic acids. Over the years, a variety of LC columns has been tested for their capability to separate these type of compounds. Ion chromatography as well as ion-pair LC are often performed on polymeric (PRP-1 and PRP-X100 [41], and AN300 [42]), alkyl-bonded silica [43] and, recently, porous graphitic carbon (PGC) [44] stationary phases. Detection is of special interest since alkylphosphonic acids do not possess chromophores or fluorophores, which makes UV and fluorescence detection impossible. However, the compounds show good ionisation and fragmentation in MS and MS–MS detection, which will be discussed in the next section. An alternative detection principle is evaporative light-scattering detection (ELSD) [44,45], which is considered a very convenient and universal detector for analytes which are less volatile than the eluent. The operation principle is based on (i) nebulisation of the eluent, (ii) evaporation of the eluent and (iii) scattering of light by the residual particles ideally constituting the analytes. Fig. 3 shows an LC–ELSD chromatogram of the separation of alkylphosphonic acids on a PGC-type Hypercarb S column, which is a strong reversed-phase packing material, using gradient elution at 0.2 ml/min. An acetonitrile gradient was used to elute the most hydrophobic compounds in the mixture. Unfortunately, LODs were not given for this method [44], but were 4 μg/ml when using ion-pair LC–ELSD [45]. Being a universal detector may also be considered a disadvantage since selectivity is required in many real-life applications. From that point of view, FPD, which was originally used for GC, is an ideal device for the detection of alkylphosphonic acids in the P-selective mode. In the late 1980s, Kientz et al. developed an on-line micro (μ) LC–FPD system [43]. Recently, the method was extended by Hooijschuur et al. to gradient elution μLC–FPD, which allows the separation of both lower and higher alkylphosphonic acids in a single run without extensive sample treatment [46]. Large-volume injections of 100 μl resulted in LODs of 6–800 ng/ml with acceptable repeatability. Gradient elution μLC–FPD was successfully applied during an OPCW proficiency test for the analysis of a water sample and an aqueous soil extract, which resulted in the detection of three relevant breakdown products of nerve agents [47].

All LC-based methods described so far can be used for screening, and some of them for quantification, purposes. However, in many cases identification of CWAs or their breakdown products is the major goal of a study. Therefore, LC–MS has a prominent position in the field of LC-based methods.

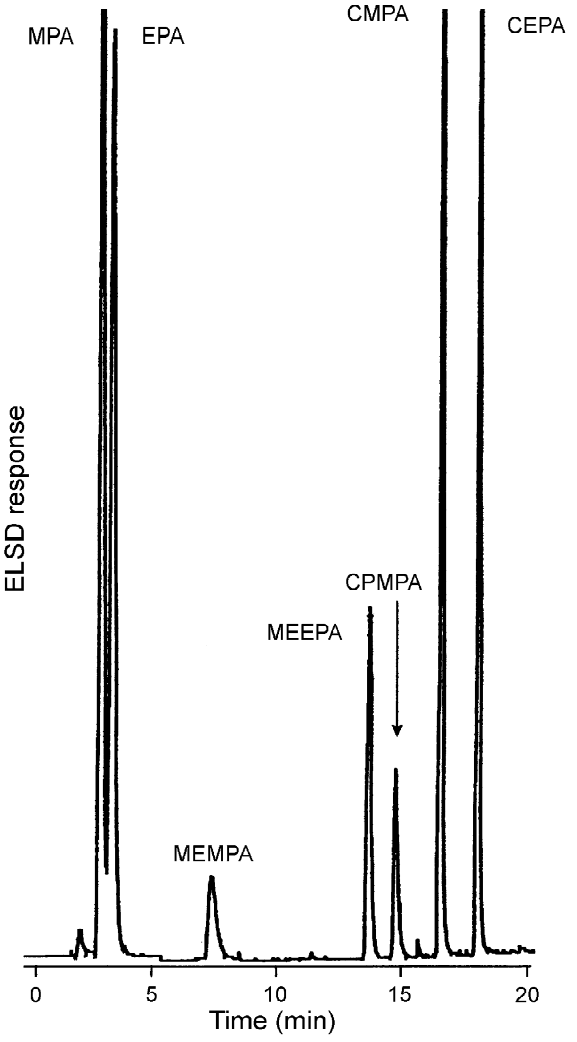

Fig. 3. LC–ELSD chromatogram of alkylphosphonic acids: MPA; EPA; MEMPA, (2-methoxyethyl) methylphosphonic acid; MEEPA, (2-methoxyethyl) ethylphosphonic acid; CPMPA, cyclopentyl methylphosphonic acid; CMPA, cyclohexyl methylphosphonic acid; CEPA, cyclohexyl ethylphosphonic acid. Column, 7 μm Hypercarb S (150×2.1 mm); gradient elution, 0–3 min, 0.1% trifluoroacetic acid in water; 3–18 min, up to 0.1% trifluoroacetic acid in acetonitrile [44].

2.5. Liquid chromatography–mass spectrometry

The first application of LC–MS, which featured a thermospray (TSP) interface for the determination of nerve agent breakdown products, was published in 1988 by Wils and Hulst [48] and followed by several other articles [49–51]. Wils and Hulst [49] demonstrated the differences in the spectra of VX obtained with EI, ammonia CI and TSP with an ammonium acetate buffer. They also showed that TSP mass spectra depend on the eluent composition. Due to the soft ionisation, TSP mass spectra are usually very simple and, consequently, less suitable for identification purposes. As a more powerful alternative, CID of the molecular ions, $[M+H]^+$ or $[M+NH_4]^+$, with subsequent recording of the product-ion spectra by MS–MS was presented by Tørnes [51] as a TSP-MS–MS method for the identification of alkyl methylphosphonic acids in aqueous samples. The use of MS–MS also improved the signal-to-noise ratios and resulted in LODs of 100 ng/ml in the full-scan mode. Generally speaking, with MS–MS, care should be taken that identification is based on a sufficient number of ions as well as matching intensity ratios recorded for reference compounds. However, LC–MS is continuously being developed and atmospheric pressure ionisation (API) techniques such as electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI) dominate the field today (cf. below). Since APCI and ESI, as well as TSP, are soft ionisation techniques, $[M+H]^+$ or $[M-H]^-$ fragments are predominant in the mass spectra of alkylphosphonic acids, which makes LC–MS extremely suitable for rapid screening of, and preliminary identification in, aqueous samples or extracts with minimal sample pretreatment, but less suitable than GC–MS in terms of unambiguous identification and sensitivity. Therefore, LC–MS is used as a complementary technique rather than a substitute to GC–MS.

Borrett and co-workers demonstrated the potential of ESI-MS–MS in the positive and negative ion mode for nerve agent breakdown products [52,53]. The positive ion ESI spectrum of MPA was somewhat complicated because of the formation of metal adducts next to the protonated molecular ion; the negative ion ESI spectrum was much cleaner and was dominated by the deprotonated molecular ion. The latter was also observed by Mercier and co-workers who replaced ELSD (cf. Section 2.4) by ESI-MS in the negative ion mode for the analysis of alkylphosphonic acids [44,45]. Black and Read published three papers [54–56] on LC–MS-based

screening procedures for hydrolysis products of CWAs using positive and negative APCI and ESI techniques. They applied LC–APCI-MS [54] for the screening of 19 hydrolysis products of nerve agents, sulphur and nitrogen mustards and BZ in a single run, using a C_8/C_{18} mixed-mode reversed-phase column and gradient elution with eluents of 0.05% TFA in water and acetonitrile. LODs in the positive SIM mode were in the range of $\leq$10–400 ng/ml, with intermediate sensitivity for the alkyl methylphosphonic acids (generally decreasing with increasing size of the alkyl groups) and worst sensitivity for the alkylphosphonic acids, viz. MPA and EPA (ethylphosphonic acid). In the next paper [55], improved conditions for the LC–ESI-MS analysis of phosphonic acids were reported. The most significant improvement of sensitivity was obtained by substituting 0.1% formic acid for TFA as the acidic modifier which resulted in LODs of $<$50 ng/ml ($<$0.25 ng injected). In the third paper [56], the LC conditions were modified (C_{18} column with ammonium formate–water–methanol gradient elution) which allowed positive- and negative-mode APCI and ESI. APCI was generally found to be more robust than ESI, probably due to variable adduct ion formation in the case of ESI, which depended on the sample composition and the experimental conditions. Fig. 4 shows an LC–APCI-MS selected ion chromatogram of a standard solution of (*O*-alkyl) alkylphosphonic acids and benzilic acid using negative-mode ionisation, which provided optimum sensitivity and selectivity for most of the acidic analytes with LODs of 10–100 ng/ml. In addition, in the negative ion mode, phosphonic acids give intense $[M-H]^-$ ions whereas dialkyl alkylphosphonates give no significant negative ions, which allows a ready distinction of phosphonic acids and isomeric phosphonates in flow injection analysis (FIA)–APCI-MS.

D'Agostino and co-workers showed the benefits of μLC–ESI-MS of aqueous samples containing intact nerve agents [57], and intact nerve agents and their degradation products [58,59] in a single run. Fig. 5 illustrates a typical μLC–ESI-MS chromatogram obtained for a degraded VX sample that had been stored in a glass container for about 15 years and which was dissolved in water to 1 mg/ml prior to analysis [58]. The molecular masses of 38 com-

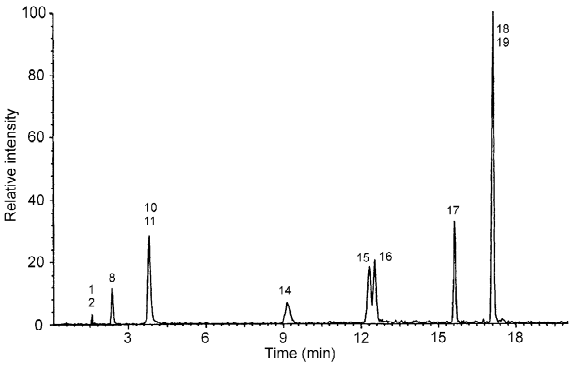

Fig. 4. LC–APCI-MS (negative ion mode) SIM chromatogram of phosphonic and benzilic acids (0.1 μg/ml): 1, MPA; 2, EPA; 8, EMPA; 10, IMPA; 11, EEPA (ethyl ethylphosphonic acid); 14, *sec*.-BuMPA; 15, *iso*-BuMPA; 16, *n*-BuMPA; 17, cyclohexyl MPA; 18, benzilic acid; 19, PMPA. Column, 5 μm Columbus C_{18} (150×2.0 mm); solvents, 0.02 *M* ammonium formate in water (A) and 0.02 *M* ammonium formate (B); gradient elution, 0–5 min, 5% solvent B; 5–15 min, up to 90% solvent B; 15–20 min 90% solvent B at 0.2 ml/min; SIM programme, 0–10 min 42 s, *m/z* 95, 109, 123, 137, 151; 10 min 44 s–20 min, *m/z* 95, 151, 177, 179, 183, 227 [56].

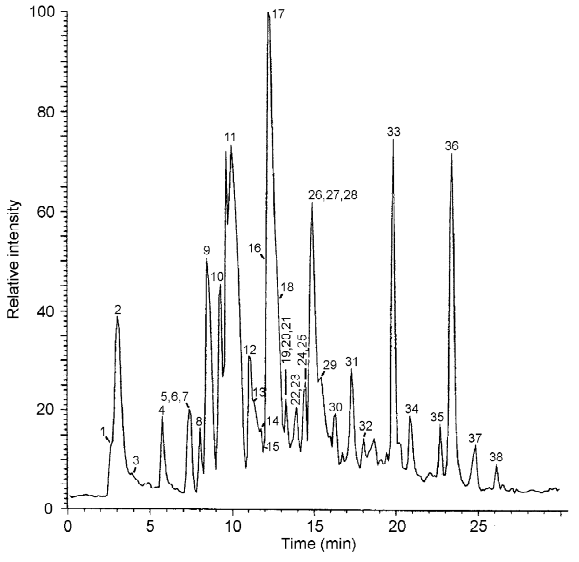

Fig. 5. μLC–ESI-MS total ion current (100–600 u) chromatogram of a degraded VX sample. Column, 5 μm Zorbax C_{18} SB (150×0.32 mm) fused-silica; solvents, 0.1% trifluoroacetic acid in water (A) and acetonitrile–water (95:5, v/v) (B); gradient elution, 1 to 75% solvent B over 30 min at 5 μl/min. Identified peaks are assigned in Table 1 [58].

Table 1
Compounds identified in degraded VX sample (peak no. corresponds with Fig. 5)

Peak no.	Compound name
2	EMPA
3	Diisopropylamine
9	Bis[2-(diisopropylamino)ethyl] sulphide
10	Diethyl dimethylpyrophosphonate
11	Bis[2-(diisopropylamino)ethyl] disulphide
12	1,8-Bis(diisopropylamino)-3,6-dithiaoctane
17	*O*-Ethyl *S*-[5-(diisopropylamino)-3-thiapentyl] methylphosphonothiolate
18	1,9-Bis(diisopropylamino)-3,4,7-trithianonane
27	*O*-Ethyl *S*-[(8,9-diisopropylamino)-3,6-dithiaoctyl] methylphosphonothiolate
28	1,12-Bis(diisopropylamino)-3,6,7,10-tetrathiadodecane
33	*N,N′*-Dicyclohexylurea
36	*N,N′*-Dicyclohexylthiourea

pounds were determined, with 23 compounds (cf. Table 1) being identified, or tentatively identified, after interpretation of the ESI-MS data obtained with a rather high cone voltage of 100 V, which promoted fragmentation. Next to VX-related compounds such as EMPA (peak no. 2) and *O*-ethyl *S*-[5-(diisopropylamino)-3-thiapentyl] methylphosphonothiolate (peak no. 17), also several ureas (peak nos. 33 and 36), compounds originating from VX stabilizers, were detected. A full-scan (50–500 u) LOD of 5 ng (1 μg/ml), based on the acquisition of an interpretable mass spectrum, was estimated. The same group also developed a procedure based on aqueous ultrasonic extraction combined with μLC–ESI-MS using a time-of-flight (TOF) instrument [60] for the determination of sarin, soman and their hydrolysis products, IMPA and PMPA, in contaminated soil. They compared it with an existing GC–MS-based method using dichloromethane extraction [61]. The methods were evaluated for three different soil types, red clay, tan sandy clay and brown sandy clay loam, at the 10 μg/g level. This is well below typical battlefield contamination levels which are estimated to be in the 100–1000 μg/g range, based on a contamination density of 1–10 g/m^2 (soil density about 1 g/cm^2 and a 1-cm sampling depth). As expected, the ultrasonic/LC-based method showed significantly better recoveries of the hydrolysis products than the dichloromethane/GC–MS procedure (GC–FID was used for recovery experiments). On the other hand, the recoveries of sarin and soman were comparable for both methods. This example clearly illustrates that aqueous extraction and LC–ESI-MS is to be preferred specifically when hydrolysis products have to be determined, which is often true. An additional example was presented recently: μLC–ESI-MS (TOF instrument) was used for the analysis of a snow sample that was accidentally contaminated with sarin during the destruction of chemical munitions [59]. Fig. 6 shows the full-scan spectra acquired for (b) sarin and its hydrolysis products, (a) IMPA, (c) diisopropyl methylphosphonate and (d) triisopropyl phosphate in the snow sample. In addition, 10 related compounds were detected, which all exhibit molecular ions that could be used to confirm the molecular mass. A rather high cone voltage of up to 70 V, which promotes fragmentation, was used to enhance the formation of important product ions which were used for identification.

Katagi et al. [62] showed that LC with continuous-flow frit fast atom bombardment (CF-FAB)-MS(–MS), which is generally regarded as an obsolete technique nowadays, is a sensitive analytical method for the determination of alkylphosphonic acids after *p*-bromophenacyl derivatization. The method involves enrichment of the derivatives on a trapping column, followed by backflushing of the analytes to a Capcell PAK UG C_{18} separation column with 5 m*M* ammonium acetate–acetonitrile (55:45, v/v, containing 0.1% glycerol) as eluent. Contrary to what the authors claim, it is not a very rapid method since it requires—next to derivatization—evaporation of 1-ml water samples to dryness, which is rather time-consuming. However, the method offers excellent full-scan product ion spectra of $[M+H]^+$

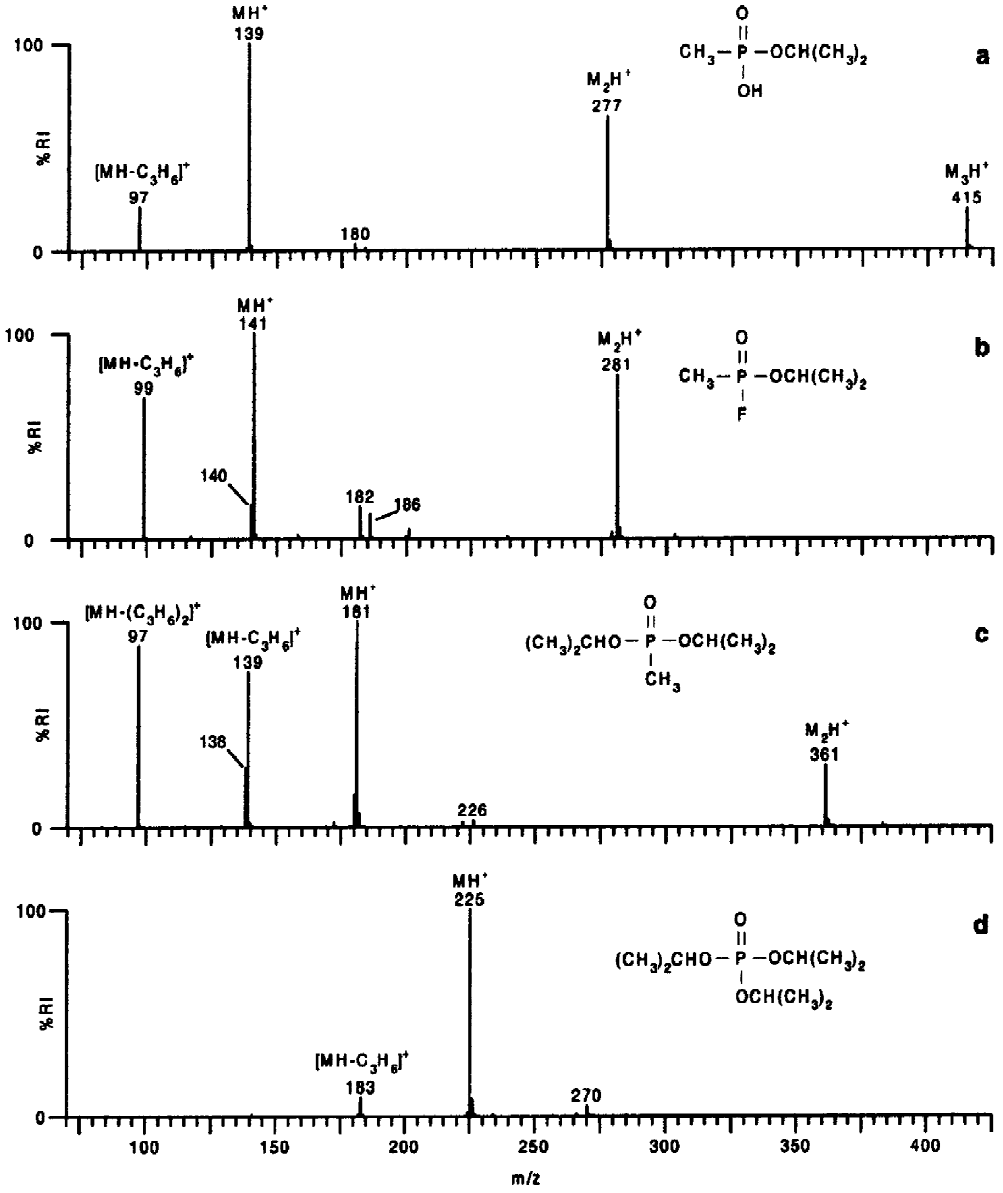

Fig. 6. ESI-MS–MS mass spectra of (a) IMPA, (b) sarin, (c) diisopropyl methylphosphonate and (d) triisopropyl phosphate, obtained during LC–MS of a contaminated melted snow sample which was diluted 1:10 with distilled water. See Fig. 5 for experimental details [59].

precursor ions in FIA (screening) as well as LC (confirmation) with LODs of 1–5 ng/ml and 1–20 ng/ml in river water, respectively.

Creasy studied LC–APCI-MS with post-column, in-source derivatization with trimethylphenylammonium hydroxide of several CWA-related compounds, including MPA and *S*-[2-(diisopropylamino)ethyl] methylphosphonothioic acid (DAEMPTA), which is a toxic minor hydrolysis product of VX, in order to increase sensitivity [63]. This procedure may be advantageous for LC–MS in some cases; however, significant disadvantages were

observed like ion suppression, interfering peaks in the mass spectra and source contamination caused by the reagent.

2.6. Capillary electrophoresis

In the past decade, CE has generated considerable interest because relatively simple and inexpensive instrumentation can be used to create fast and highly efficient separations. Indirect UV is the preferred detection mode for CE analysis of alkylphosphonic acids, which do not contain chromophoric groups [64–72]. Pianetti et al. were the first to study CE–indirect UV for the determination of the hydrolysis products of nerve agents [64]. The separation buffer was a 100 m*M* sodium borate solution containing 10 m*M* phenylphosphonic acid as a suitable UV-absorbing background electrolyte, and adjusted to pH 6.0. At this pH the electroosmotic flow (EOF) was high enough to allow the migration of the mainly monoionised alkylphosphonic acids to the cathode. The migration order was inversely related to the number of CH_2 groups and the total analysis time was 9 min. LODs of ca. 2 μg/ml and an acceptable linearity were obtained, but no real-life samples were analysed. Several groups used a reversed EOF achieved by the addition of an ''electroosmotic flow modifier'' to the background electrolyte [65–73]. In this co-electroosmotic mode, the EOF is in the same direction as the electrophoretic mobility and run times were generally less than 5 min. Higher separation efficiencies were achieved, but the LODs did not significantly improve. Nassar et al., who used the cationic surfactant, didodecyldimethylammonium hydroxide, to reverse the EOF [70–72], validated a method for the quantification of IMPA and PMPA in reaction masses [71]. Neutralization of sarin and soman was done with aqueous monoethanolamine, which resulted in the formation of monoethanolamine adducts next to the test analytes. After dilution with water, IMPA and PMPA were quantified in the linear range of 0.5–100 μg/ml with acceptable accuracy (86–99%) and precision (0.7–2.9%, $n=25$), and sufficient selectivity and with run times of less than 3 min. In another paper [72] electrokinetic injection was found to effect up to 100-fold improved LODs. For optimum results with environmental samples, clean-up over ion-exchange cartridges is necessary.

A highly sensitive CE–indirect UV method was proposed by Melanson et al. [69] who used the equation for LODs in indirect detection [74]:

$$C_{\mathrm{LOD}} = \frac{C_{\mathrm{P}}}{T_{\mathrm{R}} \cdot \mathrm{DR}} \quad (1)$$

where C_{P} is the concentration of the probe, T_{R} the transfer ratio (number of probe molecules displaced by one analyte molecule) and DR the dynamic reserve (ratio of background absorbance to noise). The optimum probe was again phenylphosphonic acid, but the optimum concentration was 1 m*M* as against 10 m*M* in earlier studies [64,70–72], which caused a 10-fold reduction of C_{P}, and correspondingly improved the sensitivity. Glutamic acid was used as buffering agent at its isoelectric point (pH 3.22). With zero net charge, an ampholite at its isoelectric point is an ideal buffering compound for indirect detection since it will not cause displacement of the probe and, thus, will keep T_{R} high, and does not add substantially to the conductivity. Coco(amidopropyl)ammoniumdimethylsulphobetaine (1 m*M*), a zwitterionic surfactant, was added to suppress the EOF with a separation voltage of −20 kV. The LODs of MPA were ca. 200 and 2 ng/ml after hydrodynamic and electrokinetic injection, respectively, with excellent migration time (RSD, 0.2%; $n=30$), and peak area (RSD <10%) repeatability. Unfortunately, no real-life applications were shown to prove the practicality of the approach. The same group presented indirect laser-induced fluorescence (LIF) detection for CE for the sensitive determination of alkylphosphonic acids [75]. MPA, EMPA, IMPA and PMPA were detected within 2 min using 50 μ*M* tetrakis(4-sulphophenyl)porphine as the probe. The LODs were 10 ng/ml for all compounds after hydrodynamic injection, which is 10-fold better than with the most sensitive indirect UV methods using the same injection mode [69,70].

To eliminate the problem of the non-selectivity of indirect UV or fluorescence detection, Robins and Wright used borate esterification of phosphonic acids and direct UV detection [76]. LODs in the order of nanograms were reported but no real-life samples were analysed. Conductivity detection was also proposed as an alternative for indirect UV detection

[70,73]. However, compared with indirect UV detection, sensitivity, efficiency and selectivity were all rather similar. Another approach for direct detection is to use a P-selective FPD coupled on-line with CE [47,77,78]. The same interface principle as originally used for μLC–FPD (cf. above) proved to be suitable also for CE–FPD. The CE system was ground via a make-up liquid, which was typically aqueous 1% formic acid, while the make-up flow also induced the necessary constant flow at the end of the CE capillary (ca. 10 μl/min) directed to the interface. The pressure was counterbalanced at the inlet of the capillary to prevent pressure-driven band broadening. If a combination of large-volume injection followed by electrophoretic matrix removal and sample stacking was used, alkylphosphonic acids could be determined extremely selectively at the low μg/ml level in water, aqueous extracts of soil and organic liquids. As an example, Fig. 7 shows CE–FPD electropherograms obtained after injection of (A) a test solution of alkylphosphonic acids, (B) a spiked water sample of unknown origin, after removal of cations by pressing the sample through an SCX column and (C) the corresponding blank. Two

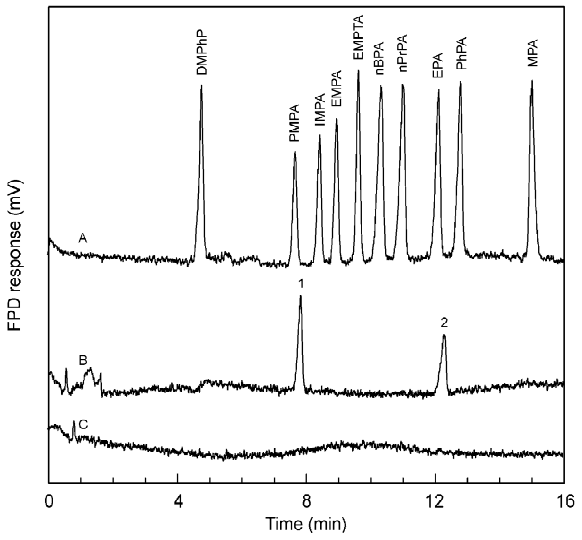

Fig. 7. CE–FPD (P-mode) electropherograms of (A) 12.3-nl injection of a reference solution (50–150 μg/ml); (B) 300-nl injection of suspected water sample; 1, iPrEPA; 2, EPA; (C) 300-nl injection of a blank. Separation buffer, 50 m*M* ammonium acetate (pH 9.0); voltage, +30 kV; make-up, 0.5% formic acid. Matrix removal of (B) and (C), −10 kV for 3.0 min [47].

distinct peaks, which were unambiguously identified by MS- and NMR-based techniques as (1) isopropyl ethylphosphonic acid (iPrEPA) and (2) EPA, showed up in the water but were absent from the blank. Recently, Jiang and Lucy proposed to use micellar electrokinetic chromatography (MEKC) with direct LIF detection for the determination of alkylphosphonic acids [79]. MPA, EPA and *n*-PrPA were derivatized with panacyl bromide in dry *N*,*N*-dimethylformamide, mixed with 400 m*M* NaCl dilution buffer to enhance high-salt stacking, and analysed using a 50 m*M* sodium cholate, 40% (v/v) acetonitrile and 50 m*M* borate run buffer. LODs were ca. 15 ng/ml.

The above CE procedures all allow rapid tentative identification without extensive sample preparation. However, CE–FPD provides additional selective (P) information on the presence of degradation products and precursors of nerve agents. The tentative identification, in its turn, can be employed to guide the method of sample pretreatment for GC–MS and NMR analysis, which remain important tools for identification purposes. On the other hand, identification of alkylphosphonic acids and their mono-esters can easily be performed by MS(–MS)-based detection as described above. Rather surprisingly, so far only two groups studied CE–ESI-MS–MS of nerve agent hydrolysis products [44,80,81]. Kostiainen et al. used CE–ESI-MS in the negative ion mode for a mixture of PMPA, IMPA, EMPA, EMPTA (ethyl methylthiophosphonic acid) and MPA, with a 20 m*M* ammonium acetate (pH 9.0) buffer and a 5-μl/min make-up flow of methanol [80]. All spectra showed a very abundant $[M-H]^-$ ion and little fragmentation at a moderate nozzle/skimmer voltage difference of 55 V. The LODs were 5 μg/ml when using 5.7 nl injection volumes. Mercier et al. proposed a 5 m*M* ammonium sorbate (pH 6.5) run buffer which allows simultaneous indirect UV and MS detection in the negative ion mode which results in LODs of 5 μg/ml (single MS) and 0.1 μg/ml (MS–MS) [81]. Sorbic acid, which is considered a non-volatile compound, could be used because a 5 μl/min make-up flow of pentanol caused sufficient dilution of the ca. 10 nl/min flow in the CE capillary and the molecular ion of sorbic acid, $[M-H]^-$ at m/z 111, did not interfere with any of the phosphonic acids studied, which were mainly

producing the $[M-H]^-$ molecular ions. CE–UV–MS(–MS) was applied to identify alkylphosphonic acids contained in a spiked tap water sample provided during an OPCW proficiency test. FIA mass spectra of the sample and corresponding blank water sample showed two possibly CWA-related compounds having signals at m/z 123 and m/z 207. Subsequent CE–UV–MS in the negative ion mode yielded two peaks in the UV and MS (SIM, m/z 123 and m/z 207) traces. Next, MS–MS was undertaken to identify both compounds. At a collision energy of 25 eV, the m/z 207 precursor ion gave a peak at m/z 95, which is a characteristic product ion of alkyl methylphosphonic acids. This compound was identified to be 2-ethylhexyl methylphosphonic acid. MS–MS of the m/z 123 precursor ion gave a peak at m/z 79, which is characteristic of $[PO_3]^-$. The 44 u difference corresponds with the loss of a C_3H_8 group. This compound was identified as isopropylphosphonic acid.

3. Mustard agents

3.1. General

The group of mustard agents includes sulphur mustards, viz. bis(2-chloroethyl)sulphide (sulphur mustard or HD), bis(2-chloroethylthio)ethane (sesquimustard or Q) and bis[(2-chloroethylthio)ethyl] ether (T), as well as nitrogen mustards, viz. bis(2-chloroethyl)ethylamine (HN-1), bis(2-chloroethyl)-methylamine (HN-2) and tris(2-chloroethyl)-amine (HN-3). Fig. 1 shows relevant examples of hydrolysis pathways of two mustard agents. The most important breakdown product of sulphur mustard is the polar, moderately volatile TDG, which is therefore an important target compound in verification studies, next to a large number of other less volatile breakdown products of other vesicants and impurities in munition-grade mustard formulations, which typically contain only 50–80% mustard with most of the remaining content being other (longer-chain) sulphur vesicants that would decompose to other products [82].

3.2. Gas chromatography

GC is the most widely used method for the determination of mustards because of their volatility and the possibilities for selective and sensitive detection. The main GC methods, which were developed in the 1970s and 1980s and can be found in a review by Witkiewicz et al. [6], are still important today for selective screening as well as quantification. Sulphur mustard can selectively be detected by FPD, but the sulphur-selective detector response is not linear and suffers from quenching by co-eluting hydrocarbons. An alternative would be to use sulphur chemiluminescence detection (SCD), which generally is one order more sensitive than FPD and provides a linear response with less quenching [83]. However, so far no paper has been published in connection with CWAs. Mustard agents and their degradation products can also be determined by GC–AED [29,84]. Mazurek and co-workers used GC–AED to identify yperite (sulphur mustard) residues in an yperite block recovered from the Baltic Sea. The C, S and Cl element chromatograms of one sample are shown in Fig. 8; about 50 compounds were detected, including sulphur mustard (peak 17 in Fig. 8B). AED detection enabled the calculation of approximate empirical formulae for 30 unknown mustard-related compounds based on calibration using a standard solution of sulphur mustard, which contains C, H, S, and Cl. Carbon was chosen as the reference element because the C-channel sensitivity makes determination reliable. The number of atoms of the other elements was calculated from:

$$(C/E_i) = \frac{A_{C_i}}{A_{E_i}} \cdot RRF_{C/E} \quad (2)$$

where C/E_i is the ratio (no. C-atoms/no. E-atoms) for compound i; A_{C_i} and A_{E_i} are the peak areas recorded in the C and E chromatograms, respectively, and $RRF_{C/E}$ is the C/E ratio response factor calculated from the reference standard. For the majority of the compounds the calculated molecular formulae were confirmed after identification by GC–MS. An interesting observation was the absence of TDG, which is the final hydrolysis product of sulphur mustard. Presumably, it was leached by the seawater. However, TDG may have been lost in the GC trace, because it is notorious for severe peak tailing and peak broadening (see below).

Conventional GC–FPD is useful for selective screening [85], quantification [86] and testing of new

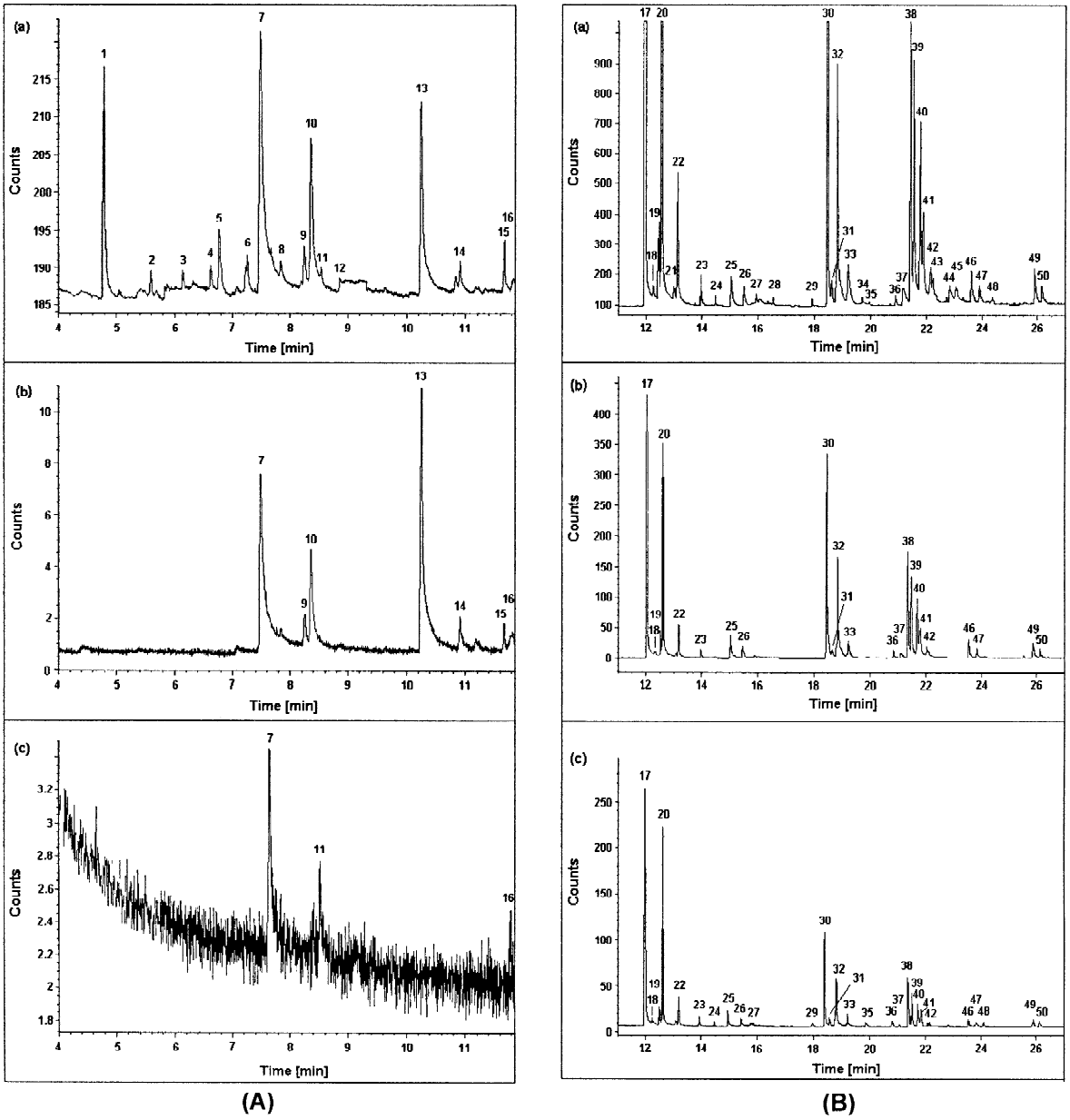

Fig. 8. Element chromatograms of (a) carbon, (b) sulphur and (c) chlorine, obtained by GC–AED of a sample from an yperite block. (A) First part of the chromatograms, split ratio 20:1; (B) second part of the chromatograms, split ratio 60:1. Column, HP-5 fused-silica (30 m×0.32 mm; d_f, 0.25 μm) coated with 95% methyl- and 5% phenyl-polysiloxane; carrier gas, He at 2 ml/min; injection volume, 1 μl. Temperature programme, 40 °C (3 min) at 10 °C/min to 280 °C (30 min hold) [84].

sample treatment procedures [86,87]. To quote an example, Tomkins et al. [86] used GC–FPD to study the extraction and degradation of sulphur mustard and by-products in soil and concrete from a facility used for the production of mustard. The authors applied pressurized liquid extraction (PLE) to rapidly extract the analytes using 10–30 ml of acetonitrile at elevated temperature (100 °C) and pressure (1500

p.s.i.; 1 p.s.i. = 6894.76 Pa). The LODs were ~5 μg/g soil or concrete, while the recovery typically exceeded 95% for the test compounds, which included 1,4-thioxane, 1,4-dithiane and TDG. The latter had to be derivatized to its *tert.*-butyldimethylsilyl derivative to achieve reliable GC performance with acceptable sensitivity. Therefore, the total analysis time for TDG was typically 3 h per sample and 1 h per sample for the other compounds. In another paper, which presents comparable results, the time-consuming derivatization of TDG was omitted [87]. Methanol–water (9:1) was found to be the best solvent for the extraction of TDG from three different types of soil. PLE provided improved recoveries compared with manual extraction.

A GC–NPD method was validated for the detection and quantification of the nitrogen mustards, HN-1 and HN-3, in air [88]. Headspace techniques using Tenax sorbent and thermal desorption and SPE using C_8 extraction disks and elution by ethyl acetate were compared. Both methods met the preset requirements for short-term measurements. The SPE method, which was also successfully used for longer-term measurements, has the advantage of the possibility of replicate analysis. In addition, SPE is not affected by changes in humidity which induced hydrolysis of the compounds on the Tenax tube.

3.3. Gas chromatography–mass spectrometry

While conventional GC detectors can be used to detect and quantify mustards, the use of MS(–MS) with EI or CI is indispensable for unambiguous identification. The use of these techniques for the determination of sulphur mustard and related compounds in soil [82] and aqueous samples [89] was introduced in the 1980s. In 1990–1995, several GC–MS applications for mustard and related compounds were reported, boosted by the use of mustard in the Iran–Iraq conflict (1980–1988). Wils et al. [90] used GC–MS to analyse mustard and related vesicants in rubber and paint samples with diesel fuel and aromatic white spirit to simulate a realistic background. The vesicants were isolated by extraction with dichloromethane or by dynamic headspace analysis at elevated temperature. Samples associated with a CWA incident involving sulphur mustard were studied by Black et al. [91]. The samples were obtained from a Kurdish village in the northern part of Iraq near the borders with Turkey and Iran and consisted of soil, bomb casing and sheep wool. GC–MS using headspace analysis, solvent extraction and thermal desorption methods successfully confirmed the presence of sulphur mustard and 21 related compounds.

More recently, a validated GC–MS method was reported for the trace-level quantification of mustard in order to monitor the effectiveness of mustard destruction with ethanolamine [32]. For mustard, 0.5 g KCl was added to 1.5 ml of the reaction mass, the mixture was vortexed, twice extracted with 1 ml hexane and, again, vortexed. Two μl of the hexane extract were injected (splitless) which resulted in an LOD of 30 ng/g in the SIM mode (*m*/*z* 109, 111, 158 and 160). ATD–GC–MS, which was described above, was tested for its use to determine sulphur mustard in air during an authentic CWA sampling and analysis trial [33]. The scenario simulated bomb craters caused by exploding sulphur mustard munitions. Pumped air samples were led (15 min at 1 l/min) through Tenax TA adsorbent tubes at a height of 0.1 m and a distance of 5 m downwind of the crater, which was contaminated with sulphur mustard. The tubes were sealed and taken to the laboratory. Sulphur mustard was positively identified by ATD–GC–full-scan MS (40–550 u).

Despite the high solubility of TDG in water and its moderate volatility, numerous GC–MS methods have been developed for the identification and quantification of this compound [89,92]. A nice example demonstrating the difficulty of extracting TDG from (ground)water samples followed by GC–MS was published recently [93]. Liquid–liquid extraction by desalting into ethyl acetate and dichloromethane was not successful. SPE using a tandem of C_{18}, to remove non-volatile interferences, and Amersorb 572, a carbonaceous sorbent which adsorbs TDG, followed by elution of the latter sorbent with dichloromethane and subsequent evaporation to dryness and derivatization with MTBSTFA was more attractive. The characteristic ions included the molecular ion $[M]^+$, $[M-CH_3]^+$ and the base peak at $[M-(CH_3)_3C]^+$. The average recovery was 23%, which illustrates the extraction problems. However, the recovery was consistent and reproducible, with an LOD of ca. 10 ng/ml. Alternatively, SPME using

a Carboxen fiber followed by in situ TBDMS derivatization (cf. Section 2.3), was used for GC–MS of TDG [40]; this gave an indifferent LOD of 200 ng/ml in deionised water. However, it should be noted that several attempts to reproduce this procedure in our laboratory were unsuccessful.

3.4. Liquid chromatography

The degradation products of sulphur mustards such as TDG, TDG sulphone and TDG sulphoxide, are rather polar, water-soluble compounds. Liquid separation technique-based analytical methods are therefore the first choice for the determination of such compounds. However, detection is rather problematic since these compounds do not contain suitable chromophores or fluorophores. LC–MS is the most straightforward approach and will be described in the next section. LC with electrochemical detection has been used for the direct detection of polar degradation and biodegradation products of sulphur mustard [94] and TDG in river water [95]. Recently, μLC–FPD (S-mode) using large-volume injections and peak compression by displacement with lower alcohols has successfully been used for the selective determination of TDG [96] and bis(2-hydroxyethylthio)alkanes, which are hydrolysis products of sulphur mustard homologues [97], in aqueous samples. Fig. 9 shows the μLC–UV chromatogram of an aqueous extract of a soil sample (trace a) and the corresponding μLC–FPD chromatograms, without and with addition of displacers (traces b and c, respectively), and a μLC–FPD chromatogram of the corresponding blank soil samples after addition of the displacers (trace d). The combined effect of peak compression and large-volume injection resulted in LODs of about 1 μg/g for all compounds. Gradient elution μLC–FPD in the S-mode (cf. Section 2.4) was successfully applied for the analysis of an aqueous soil extract [47]. One distinct peak was observed, which was identified by LC–MS–MS, GC–EI-MS of the TMS derivative and ^{1}H-NMR as 1,5-bis(2-hydroxyethylthio)pentane, a hydrolysis product of the mustard analogue, 1,5-bis(2-chloroethylthio)pentane.

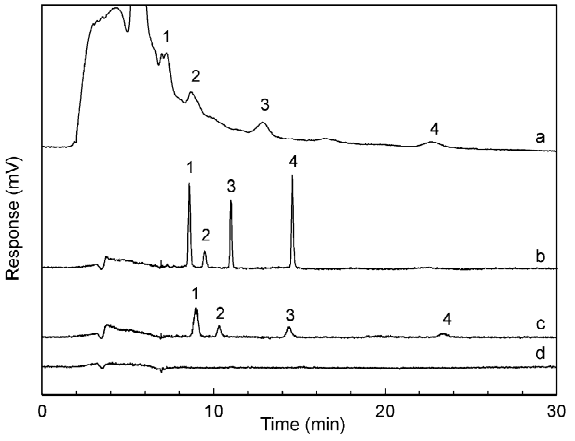

Fig. 9. μLC chromatograms of an aqueous extract of a 10-μg/g spiked soil sample. (a) Soil sample extract; UV, 200 nm; (b) soil sample extract; FPD; (c) soil sample extract with addition of 0.15% of 3-pentanol and 0.5% of 2-methyl-3-pentanol; FPD; (d) blank soil sample extract with addition of 0.15% of 3-pentanol and 0.5% of 2-methyl-3-pentanol; FPD. Peak designation: 1, bis(2-hydroxyethylthio)methane; 2, bis(2-hydroxyethylthio)ethane; 3, bis(2-hydroxyethylthio)propane; 4, bis(2-hydroxyethylthio)butane. Column, 5 μm LiChrosorb RP-18 (150×0.32 mm) fused-silica; injection volume, 20 μl; eluent, 10 m*M* acetate buffer pH 4.0–methanol (70:30, v/v) at 6 μl/min [97].

3.5. Liquid chromatography–mass spectrometry

LC–MS is an important tool for the determination, and identification, of mustard decomposition products. ESI-MS is frequently used, but it should be noted that mustard itself does not ionise during ESI-MS; therefore combined determination of mustard and its breakdown products is not possible by LC–ESI-MS. Thermospray [50,98], APCI [54] and ESI [53] interfaces have all been used to facilitate the introduction of mustard breakdown products into a mass spectrometer. The screening procedure reported by Read and Black and described in Section 2.5, was used to determine TDG, TDG sulphoxide, TDG sulphone and ethanolamine hydrolysis products of nitrogen mustard in the positive-ion ESI mode [54]. The ions monitored were m/z 105 ($[M+H-H_2O]^+$) and m/z 123 ($[M+H]^+$) for TDG, m/z 139 ($[M+H]^+$) for TDG sulphoxide, m/z 155 ($[M+H]^+$) and m/z 172 ($[M+NH_4]^+$) for TDG sulphone, m/z 120 for *N*-methyldiethanolamine, m/z 134 for *N*-ethyldiethanolamine, m/z 150 for triethanolamine, and, finally, m/z 146 for *N*,*N*-(diisopropyl)aminoethanol. The LODs were <0.01 μg/ml for all compounds. During several OPCW

proficiency tests, tentative identification was successfully obtained by this method for TDG and TDG sulphoxide in aqueous soil extracts. D'Agostino et al. used μLC–ESI-MS for the characterization of the principal products obtained after hydrolysis of HDQ and HDT munitions-grade mustard formulations, which predominately contain mustard (HD) and sesquimustard (Q), and mustard and bis[(2-chloroethylthio)ethyl] ether (T), respectively [99]. TDG and 10 related longer-chain diol, partially hydrolysed, and ether/thioether macrocyclic compounds were detected and identified following hydrolysis of the samples at an LOD level of ca. 2 ng/ml. Full-scan mass spectra were recorded with a sampling cone voltage that promoted fragmentation and resulted in both molecular-mass as well as product-ion information. The detected higher-mass diols were not observed during a prior GC–MS study [92], underscoring the importance of LC-based techniques. μLC–ESI-MS results were also reported for decomposition products of sulphur mustards using large-volume injection and peak compression [96,97]. Next to TDG, which showed the same mass spectra as reported by other workers [53,54,99], also mass spectra of longer-chain hydrolysis products, bis(2-hydroxyethylthio)alkanes and their oxidation products, were presented. The spectrum of bis(2-hydroxyethylthio)ethane, an important hydrolysis product of sesquimustard, showed molecular ions at m/z 205 ($[M+Na]^+$) and 183 ($[M+H]^+$), and fragment ions at m/z 165 ($[M+H-H_2O]^+$), 137 ($[M+H-C_2H_5OH]^+$) and 105 ($[M+H-HSC_2H_4OH]^+$, base peak), which is in agreement with earlier results [99]. The spectra of the longer-chain compounds showed corresponding ions. An interesting observation was the oxidation of these compounds in the presence of rather high concentrations of alcohols, added to obtain peak compression. This resulted in the formation of sulphoxides, which were identified by ESI-MS and ESI-MS–MS.

A different type of stable degradation products of mustard are sulphonium ions, which are formed via several intramolecular substitution reactions. These compounds, such as the 1-(2-chloroethyl)-1,4-dithianium ion, are formed in stored liquid mustard, precipitate from the solution and settle on the bottom of storage containers, which is often referred to as ''heel''. ESI-MS has successfully been applied for the direct detection of sulphonium ions formed during storage and hydrolysis of mustard [100]. Six synthesized mustard-related cyclic and open-chain sulphonium ions were readily detected at a concentration of 0.01 *M* with little fragmentation being observed. A heel sample dissolved in methanol was analysed by LC–ESI-MS; the mass spectra for the sample and a reference standard of the 1-(2-chloroethyl)-1,4-dithianium ion were similar, with the base peak at m/z 183 and the corresponding chlorine isotope at m/z 185. Conformation was obtained by MS–MS of the m/z 183 precursor ion, as is shown in Fig. 10. GC–MS of the heel sample revealed no sulphonium ions in the chromatogram because these compounds undergo thermal degradation to neutral species. Therefore it is believed that conventional GC–MS does not give a true picture of the composition of such samples but, on the other hand, LC–MS may also be problematic if it relies on partial extraction of the sample, which is biased towards small oligomers. LC–ESI-MS was also used successfully to monitor the formation and reaction of sulphonium ions during mustard hydrolysis in TDG–

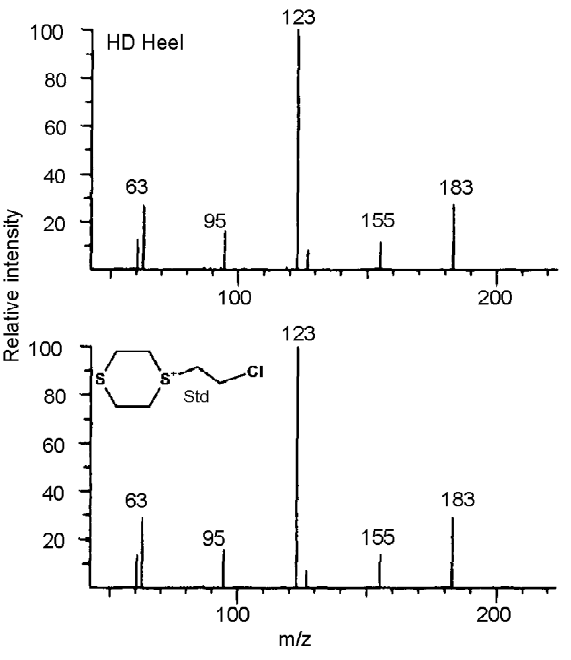

Fig. 10. ESI-MS–MS mass spectra of m/z 183 precursor ion of mustard heel (upper trace) and a 0.01 *M* standard solution of 1-(2-chloroethyl)-1,4-dithianium ion (lower trace) [100].

water mixtures, and may therefore play an important role for monitoring mustard destruction.

The analysis of alkanolamines, which are breakdown products of nitrogen mustards, is rather complicated because they are moderately volatile, decompose at elevated temperatures and are highly polar. Ion-exchange LC–MS(–MS) was presented as a suitable approach for the determination of some alkanolamines, including methyldiethanolamine (MDEA) and triethanolamine (TEA) which are breakdown products of HN-2 and HN-3, respectively, in wetland vegetation exposed to sour-gas contaminated groundwater [101]. All compounds were detected and identified based on (i) retention time, (ii) nominal mass of the $[M]^+$ ion in single MS and (iii) the $[M-H_2O]^+$ ion in the MS–MS spectra of the $[M]^+$ precursor ion, at an LOD of ca. 20 ng/g.

3.6. Capillary electrophoresis

The determination of mustard and related compounds by CE is not really straightforward since the analytes are generally not charged. However, Cheicante et al. [102,103] used MEKC–UV (200 nm) for the separation of sulphur mustard-related compounds such as TDG, TDG sulphoxide, 1,4-dithiane and 1,4-thioxane, and ethyl methylphosphonothioic acid, a breakdown product of VX, in standard solutions. Sodium dodecylsulphate was used as surfactant above its critical micelle concentration. Separation was achieved within 10 min, and LODs were 1–10 μg/ml. Since the detection wavelength of 200 nm is not selective at all, one may expect severe interferences if real-life samples are subjected to analysis.

4. Arsenic-containing agents

4.1. General

A number of arsenic-containing agents have been produced for use as CWAs. Chlorovinylarsines (lewisites) are complex mixtures of several compounds, which all occur as *cis* and *trans* isomers. Lewisite-1 (2-chlorovinylarsinous dichloride, L-1) is a vesicant agent: it reacts with the active sites of certain enzymes. Lewisite-2 [bis(2-chlorovinyl)arsinous chloride, L-2] and lewisite-3 [tris(2-chlorovinyl)arsine, L-3] are also toxic but less so than lewisite-1. Fig. 1 shows the breakdown route of lewisite-1, which hydrolyses rapidly to 2-chlorovinyl arsinous acid (CVAA), which in turn slowly degrades to lewisite oxide (CVAO) and, next, to a polymerised form of CVAO. Next to lewisites, phenylarsenic compounds have been produced as CWAs: diphenylarsine chloride (Clark-I), diphenylarsine cyanide (Clark-II) and phenarsazine chloride (Adamsite) are among the most important compounds.

4.2. Gas chromatography and gas chromatography–mass spectrometry

Because lewisite-1 readily hydrolyses in the presence of traces of water and its degradation products CVAA and CVAO are non-volatile, derivatization prior to GC is necessary for analysis at a low level (<10 ng per injection). A variety of derivatization agents, with most of them containing sulphur, have been proposed including alkyl thiols, thioglycolic acid alkyl ethers [104] and alkyl dithiols [105]. Fig. 11 shows the GC–EI-MS mass spectrum of lewisite-1, obtained after treatment of the organic liquid sample with 2,3-dimercaptotoluene (DMT): this treatment readily converts lewisite-1 into its lewisite–dimercaptotoluene adduct (see insert), a derivative which is amenable to GC [85]. The spectrum

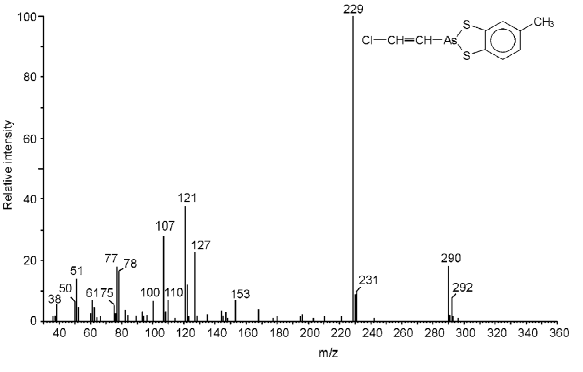

Fig. 11. GC–EI-MS mass spectrum of lewisite-1–DMT in an organic liquid sample. Fused silica capillary column (50 m×0.32 mm, d_f 0.25 μm) coated with CPSil8CB; carrier gas, He at 1.5 ml/min. Ion source temperature, 180 °C; electron energy, 70 eV; mass range m/z 20–500; cycle time, 1 s. Temperature programme: 120 °C (5 min) at 6 °C/min to 270 °C (5 min hold) [85].

shows the molecular ion signals at m/z 290 and 292 (in the 3:1 $^{35}Cl/^{37}Cl$ isotope ratio) and the base peak at m/z 229 due to $[M-CH{=}CHCl]^+$. The positive-ion isobutane GC–CI-MS mass spectrum displayed the corresponding $[M+H]^+$ signals at m/z 291 and 293, while the derivative also showed up in a GC–FPD (S-mode) chromatogram. Szostek and Aldstadt developed a rather rapid and convenient SPME–GC–MS method for the determination of CVAA, which can be derivatized by small alkyl dithiols such as 1,2-ethanedithiol (EDT) and 1,3-propanedithiol (PDT), into volatile and stable compounds [106]. After derivatization in acidic solution, the poly(dimethylsiloxane) SPME fiber was immersed for 10 min to reach the steady state. After 5 min desorption at 250 °C, GC–MS was performed. The mass spectra of CVAA–EDT and CVAA–PDT showed intense molecular ions at m/z 228 and 242, respectively, while loss of the chlorovinyl group resulted in ions at m/z 167 (CVAA–EDT) and 181 (CVAA–PDT). The LOD was 1 ng/ml, a more than 100-fold improvement compared with conventional solvent extraction methods.

Tomkins et al. modified the above procedure to enable the quantification of CVAO in soil [107]. CVAO—actually the sum of CVAA, CVAO and extractable polymerised CVAO in a given sample—was extracted and simultaneously derivatized from 2 g of neutral or basic soil using 10 ml 0.66% (w/v) ascorbic acid containing 100 μl/l of PDT. The CVAO–PDT derivative was sampled for 20 min by SPME, desorbed and determined by GC–FPD (S-mode) or GC–MS. LODs were 0.1–0.5 μg/g soil, with recoveries of, typically, 60%. As an alternative, simultaneous extraction and derivatization of CVAA with EDT from soil by SFE and PLE was studied and compared with ultrasonic extraction–derivatization [108]. SFE gave the highest recovery with an LOD of 0.2 μg/g soil (GC–FID was used for quantification), was the fastest extraction method and had the lowest solvent consumption. For all three methods, the extraction recovery decreased significantly upon ageing of the contaminated soil.

Next to lewisite-2 and lewisite-3 [104], other arsenic compounds developed as CWAs, e.g. Clark-I and Clark-II, were analysed by GC with electron-capture detection (ECD) without prior derivatization, or after derivatization with alkyl thiols and dithiols [109–111]. GC–AED is also suitable for the detection of arsenic-containing agents, viz. by using the arsenic and chlorine channels. A greyish sludge from a ton container used to store CWAs was analysed by GC–AED after extraction with hexane–chloroform (1:1, v/v) and subsequent derivatization with PDT [29]. Several compounds showing both arsenic and chlorine peaks were detected and, finally, lewisite-1, three isomers of lewisite-3 and two isomers of a dimeric form of lewisite-3 were identified by GC–MS. GC–AED analysis of the yperite block as described above, revealed one compound in the As-trace with the same retention time as Clark-I; its identity was confirmed by GC–MS [84]. Similar high selectivity can be obtained by GC–PFPD in the arsenic-selective mode, which may become an interesting alternative detection device for these types of CWAs [112].

4.3. Liquid chromatography and liquid chromatography–mass spectrometry

LC–MS of lewisites and their degradation products as an alternative to GC–MS has some problems since the ionisation efficiency of these compounds in ESI or APCI is very low. Therefore only a few LC–MS papers dealing with lewisite-related compounds were published in the literature. When using negative-ion APCI, at high analyte concentrations, arsenic(III) oxide gave ions at m/z 107 and 123; however, sensitivity was poor. Both negative-ion APCI and ESI also gave ions at m/z 305 ($[As_3O_5]^-$), 321 ($[As_3O_6]^-$), 412 ($[As_4O_7]^-$) and, possibly, larger clusters but, again, only at high concentrations. To improve the sensitivity, post-column in-source derivatization with 2-mercaptopyridine was used prior to LC–APCI-MS of CVAA and arsenic(III) oxide [63]. CVAA predominantly reacts with one 2-mercaptopyridinium ion ($[Cl–CH{=}CH–As–S{=}C_5H_4N]^+$), giving a mass spectrum with molecular ions, $[M+H]^+$, at m/z 246 and 248 (with correct $^{35}Cl/^{37}Cl$ isotope ratios). Under some conditions, a double derivative of CVAA was observed showing an $[M+H]^+$ ion at m/z 357.

LC–inductively coupled plasma MS enables the sensitive detection (LODs <0.1 ng/ml) of lewisite degradation products in environmental samples as

As(III) or As(V), but does not provide information for identification purposes [113]. Phenylarsine compounds, e.g. triphenylarsine compounds and Adamsite which could not be detected by GC–ECD (cf. above), were successfully analysed by LC with diode array UV detection and gradient elution on a C_{18} and a CN column [109]. The LODs were in the range of 0.25–7.5 μg/ml. The distinction between As(III) and As(V) compounds such as triphenylarsine and triphenylarsine oxide or phenylarsine oxide and phenylarsonic acid, was only possible by means of LC. However, there also were some problems. Clark-I and Clark-II reacted rapidly with water in the eluent to form diphenylarsine hydroxide and gave only one peak. Furthermore, some compounds eluted near the dead time on both columns (phenylarsonic acid), showed irreproducible retention (triphenylarsine oxide on the C_{18} column) or coeluted (Adamsite and diphenyl hydroxide on the CN column). Therefore, the choice of separation method depends on the analytical problem that has to be solved.

5. Miscellaneous agents

In addition to the main classes of compounds described above, a number of other compounds has been developed and produced for use as CWA. The incapacitant BZ (3-quinuclidinyl benzilate) can be determined as the intact molecule by GC–MS, but better results are obtained after derivatization of the hydroxyl group [114]. BZ was also detected at the 10-μg/ml level in a spiked soil sample by LC–APCI-MS [115]. The mass spectrum showed a distinct molecular ion peak, $[M+H]^+$, at m/z 338 with almost no fragmentation. The main degradation products of BZ, benzilic acid and 3-quinuclidinol, were included in the LC–APCI-MS screening procedure of Black and Read described above [54,56]. Optimum results for these analytes were obtained by negative- and positive-ion APCI, respectively, with LODs of <0.01 μg/ml in both instances in SIM, the monitoring ions being: m/z 128 ($[M+H]^+$) for 3-quinuclidinol, and m/z 227 $[M-H]^-$ and 183 $[M-CO_2H]^-$ for benzilic acid. Alternatively, SPME followed by in situ TBDMS derivatization (cf. Section 2.3), was used prior to GC–MS of benzilic acid [40]. The procedure was most successful for this compound because it was extracted under equilibrium conditions with a high recovery and with satisfactory RSDs of about 10%. The full-scan LOD was 1 ng/ml in deionised water.

Irritants such as tear gases and riot control agents, which cause incapacitating effects like irritation of the eyes and skin, respiratory difficulties and vomiting, are rather volatile agents. They are preferably analysed by GC–MS [33,116–118]. D'Agostino and Provost studied GC–EI-MS–MS for the identification of the irritants, *o*-chlorobenzylidenemalonitrile (CS), 2-chloroacetophenone (CN), dibenzoxazepine (CR) and 1-methoxycycloheptatriene (CH) [119]. The selectivity of the method was evaluated by the identification of CH in diesel exhaust, which is a complex sample containing an abundance of hydrocarbons. CH was completely masked by interfering peaks in the GC–MS total ion current chromatogram and could not be detected at a spiking level of 5 ng/ml in a concentrated dichloromethane Soxhlet extract of the sampled charcoal cartridge. Multiple-reaction monitoring in GC–MS–MS of the m/z 122 (M^+)→m/z 107 ($[M-CH_3]^+$)→m/z 92 ($[M-CH_2O]^+$) transitions resulted in an, expected, increase of selectivity and an LOD of 0.5 μg/ml for this sample.

6. Conclusions

The trace-level analysis of CWAs and their main degradation products and precursors receives much attention in the current literature. This attention is triggered by repeated reports on the actual or alleged use of CWAs, either in military operations or terrorist attacks. In addition, the emission of CWAs into the environment due to spillages or other accidents at storage facilities, causes much concern today. Furthermore, the activities started after the CWC entered into force in 1997, to destroy stocks of CWAs have boosted research in analytical methodology. Since CWAs and the degradation products have widely different characteristics as regards, e.g. volatility and polarity, frequently do not contain chromophoric groups and, in addition, have to be detected and identified in a great variety of samples, many different sample treatment and analytical separation and detection procedures are required. Moreover, de-

pending on the goal pursued—for example, investigation of possible use of CWAs on a battlefield or after a terrorist attack, monitoring the destruction of CWAs or cleaning of CWA storage facilities—either quantification or, and this is more often true, unambiguous identification is the main aim of the procedure. Since CWAs are almost invariably heteroatom-containing compounds, screening procedures often involve P-, S-, N- or As-mode FPD or AED, which in most instances show excellent selectivity. For identification and confirmation of identity, multiple techniques are used [85,120–125], with emphasis on GC and/or LC coupled with MS and MS–MS detection; in specific cases, NMR and FTIR also have a role to fulfil. Finally, it is encouraging to find that the results published in the recent literature and the outcome of the continually held OPCW proficiency tests clearly demonstrate progress with regard to the performance of the analytical procedures and the great care taken to avoid false-positive as well as false-negative results when interpreting the experimental data.

Acknowledgements

The authors would like to thank Mr. Eric Wils for proofreading of the manuscript and valuable suggestions.

References

[1] Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on their Destruction, Technical Secretariat of the Organisation for Prohibition of Chemical Weapons, The Hague, 1997, accessible through internet http://www.opcw.nl.
[2] R.M. Black, J.M. Harrison, in: F.R. Hartley (Ed.), The Chemistry of Organophosphorus Compounds, Wiley-Interscience, Chichester, 1996.
[3] Ch.E. Kientz, J. Chromatogr. A 814 (1998) 1.
[4] Chemical weapons chemicals analysis, in: M. Mesilaakso (Ed.), Encyclopedia of Analytical Chemistry, Wiley, New York, 2000.
[5] O. Kostiainen, Analysis of chemicals related to the chemical weapons convention, in: M.J. Bogusz (Ed.), Forensic Science, Elsevier, Amsterdam, 2000.
[6] Z. Witkiewicz, M. Mazurek, J. Szulc, J. Chromatogr. 503 (1990) 293.
[7] M.T. Söderström, R.A. Ketola, Fresenius J. Anal. Chem. 350 (1994) 162.
[8] M.T. Söderström, R.A. Ketola, O. Kostiainen, Fresenius J. Anal. Chem. 352 (1995) 550.
[9] H.D. Durst, J.R. Mays, J.L. Ruth, B.R. Williams, R.V. Duevel, Anal. Lett. 31 (1998) 1429.
[10] D.J. McGarvey, J.R. Stuff, B.R. Williams, H.D. Durst, Spectrosc. Lett. 33 (2000) 795.
[11] J.R. Hancock, P.R. Peters, J. Chromatogr. 538 (1991) 249.
[12] M. Kokko, J. Chromatogr. 630 (1993) 231.
[13] The Central OPCW Analytical Database, Technical Secretariat of the Organisation for Prohibition of Chemical Weapons, The Hague, 2001, Currently version 4.
[14] H.-Å. Lakso, W.F. Ng, Anal. Chem. 69 (1997) 1866.
[15] X. Chaudot, A. Tambuté, M. Caude, J. High Resolut. Chromatogr. 21 (1998) 457.
[16] M. Rautio (Ed.), Recommended Operating Procedures for the Sampling and Analysis in the Verification of Chemical Disarmament, Ministry for Foreign Affairs of Finland, Helsinki, 1994.
[17] C.E.A.M. Degenhardt-Langelaan, Ch.E. Kientz, J. Chromatogr. A 723 (1996) 210.
[18] D.J. Harvey, M.G. Horning, J. Chromatogr. 79 (1973) 65.
[19] A. Verweij, C.E.A.M. Degenhardt, H.L. Boter, Chemosphere 8 (1979) 115.
[20] J.G. Purdon, J.G. Pagatto, R.K. Miller, J. Chromatogr. 475 (1989) 261.
[21] M.L. Shih, J.D. Smith, J.D. McMonagle, T.W. Dolzine, V.C. Gresham, Biol. Mass Spectrom. 20 (1991) 717.
[22] J.A. Tørnes, B.A. Johnsen, J. Chromatogr. 467 (1989) 129.
[23] G.A. Sega, B.A. Tomkins, W.H. Griest, J. Chromatogr. A 790 (1997) 143.
[24] A. Amirav, H. Jing, Anal. Chem. 67 (1995) 3305.
[25] G. Frishman, A. Amirav, Field Anal. Chem. Technol. 4 (2000) 170.
[26] A. van Es, J. Janssen, C. Cramers, J. Rijks, J. High Resolut. Chromatogr. 11 (1988) 852.
[27] M. Klemp, A. Peters, R. Sacks, Environ. Sci. Technol. 28 (1994) 369A.
[28] W.R. Creasy, A.A. Rodriguez, J.R. Stuff, R.W. Warren, J. Chromatogr. A 709 (1995) 333.
[29] J.R. Stuff, W.R. Creasy, A.A. Rodriguez, H.D. Durst, J. Microcol. Sep. 11 (1999) 644.
[30] D.K. Rohrbaugh, J. Chromatogr. A 809 (1998) 131.
[31] D.K. Rohrbaugh, J. Chromatogr. A 893 (2000) 393.
[32] J.R. Stuff, R.L. Cheicante, K.M. Morrissey, H.D. Durst, J. Microcol. Sep. 12 (2000) 87.
[33] W.A. Carrick, D.B. Cooper, B. Muir, J. Chromatogr. A 925 (2001) 241.
[34] D.K. Rohrbaugh, E.W. Sarver, J. Chromatogr. A 809 (1998) 141.
[35] M. Kataoka, N. Tsunoda, H. Ohta, K. Tsuge, H. Takesako, Y. Seto, J. Chromatogr. A 824 (1998) 211.
[36] M. Kataoka, K. Tsuge, Y. Seto, J. Chromatogr. A 891 (2000) 295.
[37] M. Kataoka, K. Tsuge, H. Takesako, T. Hamazaki, Y. Seto, Environ. Sci. Technol. 35 (2001) 1823.

[38] W.F. Ng, H.-Å. Lakso, Anal. Chem. 69 (1997) 1866.
[39] J.F. Schneider, A.S. Boparai, L.L. Reed, J. Chromatogr. Sci. 39 (2001) 420.
[40] M.T. Sng, W.F. Ng, J. Chromatogr. A 832 (1999) 173.
[41] P.C. Bossle, D.J. Reutter, E.W. Sarver, J. Chromatogr. 407 (1987) 399.
[42] W.D. Vermillion, M.D. Crenshaw, J. Chromatogr. A 770 (1997) 253.
[43] Ch.E. Kientz, A. Verweij, H.L. Boter, A. Poppema, R.W. Frei, G.J. de Jong, U.A.Th. Brinkman, J. Chromatogr. 467 (1989) 385.
[44] J.-P. Mercier, Ph. Morin, M. Dreux, A. Tambuté, J. Chromatogr. A 849 (1999) 197.
[45] J.-P. Mercier, Ph. Morin, C. Elfakir, M. Lafosse, M. Dreux, A. Tambuté, J. Liq. Chromatogr. Relat. Technol. 22 (1999) 267.
[46] E.W.J. Hooijschuur, Ch.E. Kientz, U.A.Th. Brinkman, J. Chromatogr. A 907 (2001) 165.
[47] E.W.J. Hooijschuur, Ch.E. Kientz, U.A.Th. Brinkman, J. Chromatogr. A 928 (2001) 187.
[48] E.R.J. Wils, A.G. Hulst, J. Chromatogr. 454 (1988) 261.
[49] E.R.J. Wils, A.G. Hulst, J. Chromatogr. 523 (1990) 151.
[50] E.R.J. Wils, A.G. Hulst, Fresenius J. Anal. Chem. 342 (1992) 749.
[51] J.A. Tørnes, Rapid Commun. Mass Spectrom. 10 (1996) 878.
[52] V.T. Borrett, R. Colton, J.C. Traeger, Eur. Mass Spectrom. 1 (1995) 131.
[53] V.T. Borrett, R.J. Mathews, R. Colton, J.C. Traeger, Rapid Commun. Mass Spectrom. 10 (1996) 114.
[54] R.M. Black, R.W. Read, J. Chromatogr. A 759 (1997) 79.
[55] R.M. Black, R.W. Read, J. Chromatogr. A 794 (1998) 233.
[56] R.W. Read, R.M. Black, J. Chromatogr. A 862 (1999) 169.
[57] P.A. D'Agostino, J.R. Hancock, L.R. Provost, J. Chromatogr. A 840 (1999) 289.
[58] P.A. D'Agostino, J.R. Hancock, L.R. Provost, J. Chromatogr. A 837 (1999) 93.
[59] P.A. D'Agostino, C.L. Chenier, J.R. Hancock, J. Chromatogr. A 950 (2002) 149.
[60] P.A. D'Agostino, J.R. Hancock, L.R. Provost, J. Chromatogr. A 912 (2001) 291.
[61] P.A. D'Agostino, L.R. Provost, J. Chromatogr. 589 (1992) 287.
[62] M. Katagi, M. Tatsuno, M. Nishikawa, H. Tsuchihashi, J. Chromatogr. A 833 (1999) 169.
[63] W.R. Creasy, J. Am. Soc. Mass Spectrom. 10 (1999) 440.
[64] G.A. Pianetti, M. Taverna, A. Baillet, G. Mahuzier, D. Baylocq-Ferrier, J. Chromatogr. 630 (1993) 371.
[65] S.A. Oehrle, P.C. Bossle, J. Chromatogr. A 692 (1995) 247.
[66] J.-P. Mercier, Ph. Morin, M. Dreux, A. Tambuté, J. Chromatogr. A 741 (1996) 279.
[67] J.-P. Mercier, Ph. Morin, M. Dreux, A. Tambuté, J. Chromatogr. A 779 (1997) 245.
[68] K.-W. Lee, J.-Y. Jeon, K.-P. Lee, S.-H. Lee, B. Chiswell, Anal. Sci. 13 (1997) 247.
[69] J.E. Melanson, B.L.-Y. Wong, C.A. Boulet, C.A. Lucy, J. Chromatogr. A 920 (2001) 359.
[70] A.-E.F. Nassar, S.V. Lucas, W.R. Jones, L.D. Hoffland, Anal. Chem. 70 (1998) 1085.
[71] A.-E.F. Nassar, S.V. Lucas, C.A. Myler, W.R. Jones, M. Campisano, L.D. Hoffland, Anal. Chem. 70 (1998) 3598.
[72] A.-E.F. Nassar, S.V. Lucas, L.D. Hoffland, Anal. Chem. 71 (1999) 1285.
[73] T.E. Rosso, P.C. Bossle, J. Chromatogr. A 824 (1998) 125.
[74] P. Doble, M. Macka, P.R. Haddad, J. Chromatogr. A 804 (1998) 327.
[75] J.E. Melanson, C.A. Boulet, C.A. Lucy, Anal. Chem. 73 (2001) 1809.
[76] W.H. Robins, B.W. Wright, J. Chromatogr. A 680 (1994) 667.
[77] C.E. Sänger-van de Griend, Ch.E. Kientz, U.A.Th. Brinkman, J. Chromatogr. A 673 (1994) 299.
[78] Ch.E. Kientz, E.W.J. Hooijschuur, U.A.Th. Brinkman, J. Microcol. Sep. 9 (1997) 253.
[79] J. Jiang, C.A. Lucy, J. Chromatogr. A, in press.
[80] R. Kostiainen, A.P. Bruins, V.M.A. Häkkinen, J. Chromatogr. 634 (1993) 113.
[81] J.-P. Mercier, P. Chaimbault, Ph. Morin, M. Dreux, A. Tambuté, J. Chromatogr. A 825 (1998) 71.
[82] P.A. D'Agostino, L.R. Provost, Biomed. Environ. Mass Spectrom. 15 (1988) 553.
[83] R.L. Shearer, D.L. O'Neal, R. Rios, M.D. Baker, J. Chromatogr. Sci. 28 (1990) 24.
[84] M. Mazurek, Z. Witkiewicz, S. Popiel, M. Śliwakowski, J. Chromatogr. A 919 (2001) 133.
[85] E.W.J. Hooijschuur, A.G. Hulst, A.L. de Jong, L.P. de Reuver, S.H. van Krimpen, B.L.M. van Baar, E.R.J. Wils, Ch.E. Kientz, U.A.Th. Brinkman, Trends Anal. Chem. 21 (2002) 116.
[86] B.A. Tomkins, G.A. Sega, S.J. Macnaughton, Anal. Lett. 31 (1998) 1603.
[87] N.V. Beck, W.A. Carrick, D.B. Cooper, B. Muir, J. Chromatogr. A 907 (2001) 221.
[88] J.R. Stuff, R.L. Cheicante, H.D. Durst, J.L. Ruth, J. Chromatogr. A 849 (1999) 529.
[89] P.A. D'Agostino, L.R. Provost, A.S. Hansen, G.A. Luoma, Biomed. Environ. Mass Spectrom. 18 (1989) 484.
[90] E.R.J. Wils, A.G. Hulst, A.L. De Jong, J. Chromatogr. 625 (1992) 382.
[91] R.M. Black, R.J. Clark, D.B. Cooper, R.W. Read, D. Utley, J. Chromatogr. 637 (1993) 71.
[92] P.A. D'Agostino, L.R. Provost, J. Chromatogr. 645 (1993) 283.
[93] B.A. Tomkins, G.A. Sega, J. Chromatogr. A 911 (2001) 85.
[94] A.J. Clark, Anal. Proc. 30 (1993) 355.
[95] P.C. Bossle, S.F. Hallowell, D.J. Reutter, E.W. Sarver, J. Chromatogr. 330 (1985) 388.
[96] E.W.J. Hooijschuur, Ch.E. Kientz, U.A.Th. Brinkman, J. Chromatogr. A 849 (1999) 433.
[97] E.W.J. Hooijschuur, Ch.E. Kientz, A.G. Hulst, U.A.Th. Brinkman, Anal. Chem. 72 (2000) 1199.
[98] S. Munavalli, E.M. Jakubowski, H.D. Durst, J. Mass Spectrom. 30 (1995) 1716.
[99] P.A. D'Agostino, J.R. Hancock, L.R. Provost, J. Chromatogr. A 808 (1998) 177.

[100] D.K. Rohrbaugh, Y.-C. Yang, J. Mass Spectrom. 32 (1997) 1247.
[101] J.V. Headley, K.M. Peru, L.C. Dickson, Rapid Commun. Mass Spectrom. 13 (1999) 730.
[102] R.L. Cheicante, J.R. Stuff, H.D. Durst, J. Chromatogr. A 711 (1995) 347.
[103] R.L. Cheicante, J.R. Stuff, H.D. Durst, J. Capillary Electrophor. 2 (1995) 157.
[104] R. Haas, Environ. Sci. Pollut. Res. 5 (1998) 2.
[105] W.K. Fowler, D.C. Stewart, D.S. Weinberg, E.W. Sarver, J. Chromatogr. 558 (1991) 235.
[106] B. Szostek, J.H. Aldstadt, J. Chromatogr. A 807 (1998) 253.
[107] B.A. Tomkins, G.A. Sega, C.-H. Ho, J. Chromatogr. A 909 (2001) 13.
[108] X. Chaudot, A. Tambuté, M. Caude, J. Chromatogr. A 888 (2000) 327.
[109] R. Haas, A. Krippendorf, Environ. Sci. Pollut. Res. 4 (1997) 123.
[110] R. Haas, T.C. Schmidt, K. Steinbach, E. von Löw, Fresenius J. Anal. Chem. 361 (1998) 313.
[111] K. Schoene, H.J. Bruckert, H. Juerling, J. Steinhanses, J. Chromatogr. A 719 (1996) 401.
[112] D.R. Killelea, J.H. Aldstadt, J. Chromatogr. A 918 (2001) 169.
[113] D.A. Bass, J.S. Yeager, J.T. Kiely, J.S. Crain, L.M. Shem, H.J. O'Niel, M.J. Growdy, in: Proceedings of the 1995 Scientific Conference on Chemical and Biological Defense Research, US Army Edgewood Research Development and Engineering Center, 1995, p. 233.
[114] E.R.J. Wils, Chemical weapons chemicals analysis, in: M. Mesilaakso (Ed.), Encyclopedia of Analytical Chemistry, Wiley, New York, 2000, p. 987.
[115] R.M. Black, R.W. Read, Chemical weapons chemicals analysis, in: M. Mesilaakso (Ed.), Encyclopedia of Analytical Chemistry, Wiley, New York, 2000, p. 1013.
[116] E.R.J. Wils, A.G. Hulst, J. Chromatogr. 330 (1985) 379.
[117] K.E. Ferslew, R.H. Orcutt, A.N. Hagardorn, J. Forensic Sci. 31 (1986) 658.
[118] T.A. Kluchinsky, P.B. Savage, M.V. Sheely, R.J. Thomas, P.A. Smith, J. Microcol. Sep. 13 (2001) 186.
[119] P.A. D'Agostino, L.R. Provost, J. Chromatogr. A 695 (1995) 65.
[120] J.F. Weimaster, W.T. Beaudry, P.C. Bossle, M.W. Ellzy, G.L. Janes, D.W. Johnson, J.M. Lochner, S.G. Pleva, J.H. Reeder, D.K. Rohrbaugh, T.E. Rosso, L.J. Szafraniec, L.L. Szafraniec, T.G. Albro, W.R. Creasy, J.R. Stuff, P.B. Smith, I.R. Stewart, J. Chem. Technol. Biotechnol. 64 (1995) 115.
[121] M.T. Söderström, H. Björk, V.M.A. Häkkinen, O. Kostiainen, M.-L. Kuitunen, M. Rautio, J. Chromatogr. A 742 (1996) 191.
[122] W.R. Creasy, J.R. Stuff, B. Williams, K. Morrissey, J. Mays, R. Duevel, H.D. Durst, J. Chromatogr. A 774 (1997) 253.
[123] W.R. Creasy, M.D. Brickhouse, K.M. Morrissey, J.R. Stuff, R.L. Cheicante Jr., J. Ruth, J. Mays, B.R. Williams, R. O'Conner, H.D. Durst, Environ. Sci. Technol. 33 (1999) 2157.
[124] G.W. Wackner, P.W. Bartram, M.D. Brickhouse, T.R. Conell, W.R. Creasy, V.D. Henderson, J.W. Hovanec, K.M. Morrissey, J.R. Stuff, B.R. Williams, J. Chem. Soc. Perkin Trans. 2 (2000) 1267.
[125] M.D. Brickhouse, W.R. Creasy, B.R. Williams, K.M. Morrissey, R.J. O'Connor, H.D. Durst, J. Chromatogr. A 883 (2000) 185.