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Review

Derivatisation reactions in the chromatographic analysis of chemical warfare agents and their degradation products

Robin M. Black*, Bob Muir
Dstl Porton Down, Salisbury, Wiltshire SP4 0JQ, UK

Abstract

The analysis of chemical warfare agents and their degradation products is an important component of verification of compliance with the Chemical Weapons Convention. Gas and liquid chromatography, particularly combined with mass spectrometry, are the major techniques used to detect and identify chemicals of concern to the Convention. The more polar analytes, and some of the more reactive or highly volatile agents, are usually derivatised to facilitate chromatography, and to impart properties beneficial for detection. This review focuses on derivatisation reactions used in the chromatographic analysis of chemical warfare agents, their degradation products and metabolites.

Keywords: Reviews; Derivatization, GC; Derivatization, LC; Mass spectrometry; Chemical warfare agents

Contents

*Corresponding author. Tel.: +44-1980-613-201; fax: +44-1980-613-834.
E-mail address: rmblack@dstl.gov.uk (R.M. Black).

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1. Introduction

1.1. Scope of the review

This paper reviews derivatisation reactions used in the gas and liquid chromatographic analysis of chemical warfare (CW) agents, their degradation products and metabolites. Some recent advances in derivatisation, which may find application in CW agent analysis, are also discussed. The term ''derivatisation'' is interpreted as the conversion of the analyte into another chemical species prior to chromatography or, in a small number of instances, prior to detection. Selected examples are included to illustrate typical applications. The review is not intended to be comprehensive in its coverage of applications. For more comprehensive reviews of the analysis of CW agents see Refs. [1–4]. An excellent overview of GC–MS analysis of CW agents and their degradation products has been given by Wils [5].

1.2. The requirement for analysis

The analysis of chemical warfare agents, their precursors and degradation products, is an important component of verification in support of the Chemical Weapons Convention (CWC) [6]. The CWC, which entered into force in 1997, prohibits the development, production, stockpiling and use of chemical weapons. It requires member states to destroy any existing chemical munitions and agent stockpiles. An important feature of the CWC is a system for verification of compliance, organised through the Technical Secretariat of the Convention's supervisory body, the Organisation for the Prohibition of Chemical Weapons (OPCW). The Director General of the OPCW has appointed designated laboratories to undertake analysis of samples that may arise from inspections of former CW production or storage sites, production sites for dual-use precursors or other discrete organic chemicals, and challenge inspections of suspected production sites. Designated

laboratories may also be asked to analyse samples in cases of allegations of CW use. Chemicals considered a potential risk to the Convention are listed in an Annex to the CWC under three Schedules, those in Schedule 1 posing the greatest risk. The requirement for analysis is the unequivocal identification of CW agents, their precursors and degradation products, in a variety of environmental matrices, at concentrations that may range from neat material down to parts per billion. The analysis of biomedical samples, for trace levels of biological markers of poisoning, is being addressed by a smaller number of laboratories [7].

The analysis of CW agents or simulants is also required for defensive research, for example, in support of trials of equipment used for physical protection, detection or decontamination. This may require sampling of vapours, liquids or complex residues. Finally, the remediation of land, associated with former CW production or storage sites, requires the trace analysis of CW agents and their degradation products in the environment [8].

1.3. The requirement for derivatisation

1.3.1. Gas chromatography

The major need for derivatisation is for the analysis of polar degradation products of CW agents. These analytes have insufficient volatility for GC analysis, may be thermally unstable, or have other chromatographic properties that give rise to peak tailing and poor detection limits. Examples are alkyl methylphosphonic acids and thiodiglycol, degradation products of nerve agents and sulphur mustard, respectively. In a few cases, derivatisation has been used to improve the chromatographic properties of the less volatile agents, for example, *O*-ethyl *S*-(2-diisopropylaminoethyl) methylphosphonothiolate (VX) and 3-quinuclidinyl benzilate (BZ).

Derivatisation is also used to reduce the reactivity and/or volatility of certain agents. Most CW agents are moderately reactive electrophilic species. Some are difficult to chromatograph because of interactions with column coatings or other free nucleophilic sites in the analytical system; the most important example is Lewisite 1. Some CW agents (or toxic chemicals specifically listed in the Annex on Chemicals of the CWC [6]) are reactive electrophilic gases at normal ambient temperatures; examples are phosgene and perfluoroisobutylene (PFIB). Derivatisation of these materials decreases both reactivity and volatility, thus facilitating chromatographic analysis.

Finally, derivatisation is used to enhance the selectivity or sensitivity of detection. The derivatisation of Lewisite with thiol reagents allows sulphur selective flame photometric detection (FPD). The conversion of phosphonic acids and thiodiglycol to perfluorinated derivatives enables detection by the very sensitive technique of negative ion chemical ionisation mass spectrometry (NICI–MS).

Although derivatisation may be employed to facilitate chromatographic separation of enantiomers, the alternative strategy of using chiral stationary phases has generally been used for resolving enantiomers of nerve agents [9].

1.3.2. Liquid chromatography

LC analysis of CW agents, and particularly their degradation products, is usually performed in order to avoid derivatisation, and the need to concentrate aqueous solutions to dryness. However, derivatisation has been used to enhance chromatographic properties or to facilitate detection, e.g., by UV, fluorescence or NICI–MS.

1.4. Some disadvantages of derivatisation

Derivatisation can be the major source of error in quantitative chromatographic analysis. Common problems are extraneous materials extracted from the matrix, including water, either suppressing derivatisation, or reacting with the derivatising agent to produce a complex background. Many derivatisations of polar analytes require concentration of aqueous solutions to dryness. Not only can this be the time-limiting factor in the analysis, it can also be a major source of error. Remaining traces of water may react with both the reagent and the derivative, and losses on evaporation may occur if the analyte, e.g., thiodiglycol, has a degree of volatility. Analyte isolation and clean-up must be appropriate to the derivatisation method used. A review of sample preparation procedures has been given by Kuitunen [10]. It has been argued that evidence for identification following derivatisation is not as strong as spectrometric characterisation of the intact agent or

degradation product. This may be true in certain cases, for example when methylation is used for derivatisation, and with some of the derivatives of phosgene. However, in most scenarios a suitable derivative provides acceptable evidence for the original analyte, whilst acknowledging that characterisation of the underivatised analyte, where possible, is preferable (or provides complementary evidence).

1.5. Considerations in the choice of derivatising agent

There have been a number of general reviews of derivatives for chromatography and reagents, for example Blau and Halket [11] and Taguchi [12]. Ideally, derivatisation reactions should proceed rapidly and selectively, with minimum energy input. Although rapid derivatisation is often observed, in reality high selectivity is achieved with few reagents. In most cases the functional groups for derivatisation are nucleophilic, and the derivatisation reagent is a reactive electrophile. As most extraneous materials in environmental and biological samples are also nucleophilic, selective derivatisation of the analyte is difficult to achieve. It is more easily achieved in the case of electrophilic analytes, as fewer electrophiles exist in the environment.

Derivatives should have good chromatographic properties and be chosen such that they have retention times well separated from interferents extracted from the sample matrix. Although the latter can often be achieved by varying GC conditions, it is useful to have a choice of more than one derivative. Derivatives should possess features that are advantageous for detection. Examples are: heteroelements that allow selective detection such as FPD and atomic emission detection (AED); structurally informative high mass ions in the EI mass spectrum for selective ion monitoring (SIM), preferably including a molecular or quasimolecular ion; electron capturing properties for NICI–MS or electron capture detection (ECD).

Other factors for consideration are that the reagent should preferably be commercially available, and present a low hazard in the laboratory, i.e., low toxicity, not too high volatility, stable to detonation, etc. The reagent and derivative should be robust, with good thermal stability and not excessively sensitive to traces of moisture, but this is difficult to achieve with silylation, which is the most versatile method of derivatisation.

2. Recent trends in derivatisation

Some recent advances, excluding silylation, have been reviewed by Wells [13]. Trends include the following: the increasing use of fluorinated derivatives as GC–NICI single stage and tandem MS have become more accessible; derivatisations with the analyte or reagent held on a solid support, often combining derivatisation with extraction; reagents that allow derivatisation of polar analytes directly in aqueous solution. Several new on-column methylating reagents have been reported.

The increasing use of fluorinated derivatives, e.g., pentafluorobenzyl esters, has led to a number of new reagents, although few appear to have gained widespread use. 4-(Trifluoromethyl)-2,3,5,6-tetrafluorobenzyl derivatives (prepared from the corresponding bromide) have been proposed as complementing pentafluorobenzyl derivatives for confirmation in environmental analysis, or where there are interferents with the latter [14]. On-column derivatisation of phenols has been reported with 3,5-bis(trifluoromethyl)benzyldimethylphenylammonium fluoride [15]. 4-Carboethoxyhexafluorobutyryl derivatives of fatty alcohols (prepared from the chloride using microwave heating) have been proposed as less volatile alternatives to heptafluorobutyryl esters [16]. Pentafluorobenzyl chloroformate [17] and octafluoropentyl chloroformate [18] have been used to derivatise alcohols, amino acids and other analytes. Perfluorooctanoyl chloride has been used to derivatise ethylene glycol [19].

One problem encountered in derivatisation can be the need to use a large excess of reagent to drive reactions to completion, which can lead to substantial chemical background or reduced column lifetime. Technology that has evolved from synthetic organic chemistry is derivatisation using analytes, reagents or catalysts held on a solid support. Ana-

lytical applications have been reviewed by Rosenfeld [20]. With supported analytes, the solid phase is usually the material used for solid-phase extraction (SPE), solid-phase microextraction (SPME) or solid adsorbent. Derivatisation is performed in situ, essentially combining it with extraction. Excess reagent can, in theory, be removed by washing. With supported reagents, excess reagent can be removed by mechanical means. Examples of supported analyte derivatisation that may find application in CW analysis are pentafluorobenzylation of acidic analytes in situ after capture on an ion-exchange resin [21] and the derivatisation of amino acids and peptides adsorbed onto silica using 9-fluorenylmethyl chloroformate [22] (possible applications in biomedical analysis using LC–MS). Examples of supported reagents or catalysts are a polymeric pentafluorobenzoylating reagent [23] and a polymer supported phase transfer catalyst for pentafluorobenzylation [24], e.g., of organophosphorus acids. Several examples of supported derivatisation chemistry in CW analysis are included in this review.

A common problem with highly polar analytes, particularly non-ionic ones, is isolation from an aqueous matrix prior to derivatisation. Liquid–liquid extraction and SPE may be inefficient, and concentration of aqueous solutions to dryness can be time consuming and a source of error. There has been considerable interest in derivatisation reactions that can be performed directly in aqueous media, producing derivatives that are readily extracted. Much attention has been focused on the use of chloroformates as derivatising agents; these have been reviewed by Hůsek [25]. One of the most effective is hexyl chloroformate, which has been used to derivatise polyhydroxy and polycarboxy analytes in aqueous solution [26], including ethylene glycol [27]. Pentafluorobenzyl chloroformate [17] and 2,2,3,3,4,4,5,5-octafluoropentyl chloroformate [18] have been used to form derivatives of amino, hydroxyl and carboxyl groups in aqueous solutions.

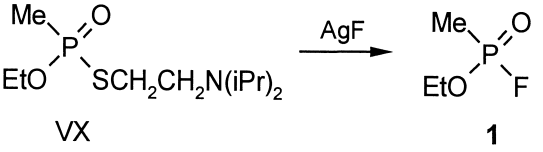
Some superior on-column (i.e., in the hot injection port) methylating reagents, plus an on-column reagent that produces bis(trifluoromethyl)benzyl derivatives, have been described by Wells and coworkers [13,15]; some of these are referred to in Section 3.3.1.

3. Derivatisation of nerve agents and their degradation products

3.1. Nerve agents

The nerve agents are the most potent and lethal of the stockpiled CW agents. They are electrophilic organophosphorus compounds that react with a nucleophilic serine residue in the active site of the enzyme acetylcholinesterase, thereby inhibiting the enzyme and paralysing nerve transmission. The nerve agents were developed just before, during and after World War II, but only in the last two decades have they been used. Recent use by terrorists in Japan has stimulated the development of a number of new or modified analytical methods for forensic analysis.

The nerve agents have sufficient volatility and thermal stability to be analysed satisfactorily by GC without derivatisation. However, in the case of low concentrations of the phosphonothiolate VX, interactions with adsorptive sites can lead to poor peak shapes, low signal-to-noise ratios and poor precision. This can be particularly troublesome in trialling of defensive equipment where quantitative sampling in air is required. One way of overcoming this is to convert the phosphonothiolate to a phosphonofluoridate **1**, a reaction first applied in semi-automated detectors for nerve agents. Fowler and Smith [28] described a method for sampling VX vapour at ng/m^3 levels in air by passing it through a filter impregnated with silver fluoride, and trapping the resultant ethyl methylphosphonofluoridate on Chromosorb 106 prior to thermal desorption and GC–FPD analysis. A similar derivatisation, using a short column of silver fluoride, was used for determining VX in benzene solution [29].



VX **1**

A conversion to phosphonofluoridates also provides a very sensitive procedure for the retrospective detection of human exposure to sarin and VX. The

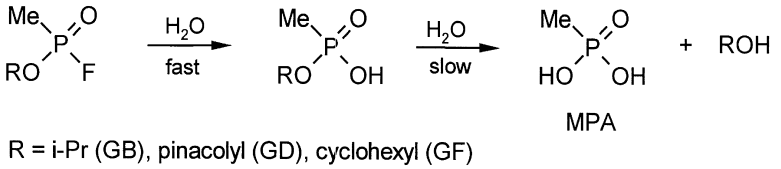


Fig. 1. Hydrolytic pathways for phosphonofluoridate nerve agents.

phosphonyl moiety covalently bound to inhibited blood butyrylcholinesterase is displaced as the phosphonofluoridate with potassium fluoride [30,31].

3.2. Degradation pathways for nerve agents

Nerve agents of the G series, isopropyl methylphosphonofluoridate (sarin, GB), pinacolyl methylphosphonofluoridate (soman, GD) and cyclohexyl methylphosphonofluoridate (cyclosarin, GF) hydrolyse in the environment to the corresponding alkyl methylphosphonic acid [32] (Fig. 1). Over time, the alkyl methylphosphonic acid undergoes a much slower hydrolysis to methylphosphonic acid (MPA). The alkyl methylphosphonic acids are also the major urinary metabolites of nerve agents [33].

The hydrolysis of VX and other V agents is more complex [34] (Fig. 2). VX contains hydrolytically labile P–S and P–O bonds, and cleavage of the S–C bond can also occur. Which route predominates depends on the pH and concentration. A 0.01 *M* solution in water produces ethyl methylphosphonic acid (EMPA) from P–S cleavage, and *S*-(2-diisopropylaminoethyl) methylphosphonothioate (EA 2192) from P–O cleavage in a ratio of ~6.5:1. Cleavage of the S–C bond to give ethyl methylphosphonothioic acid (EMPTA) is usually a minor pathway. EMPA undergoes slow further hydrolysis to MPA and ethanol; diisopropylaminoethanethiol is rapidly oxidised in air to the corresponding disulphide. The pathway of most concern with respect to the environment is cleavage of the P–O bond, because EA 2192 possesses high toxicity by systemic routes of administration.

MPA is the final degradation product of both series of nerve agents, being chemically resistant to further reaction, and as such is an important analyte (although it can also be a degradation product of fire retardent chemicals).

GA (tabun) is more labile than the other nerve agents. Two hydrolytic pathways occur (Fig. 3), with displacement of cyanide predominating at neutral

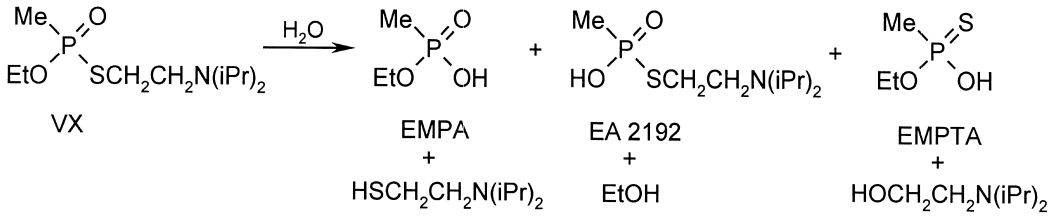


Fig. 2. Hydrolytic pathways for VX.

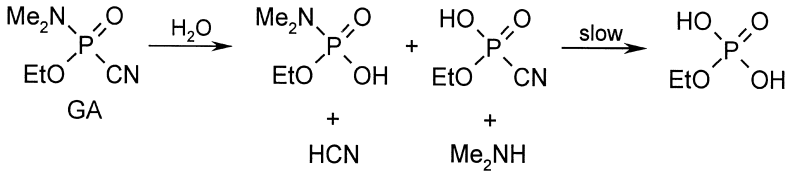


Fig. 3. Hydrolytic pathways for tabun.

and high pH, and displacement of the dimethylamino moiety predominating at low pH. The initial organophosphorus products from both pathways are further hydrolysed to ethyl phosphoric acid, which is also a degradation product of pesticides and other industrial chemicals, and eventually to inorganic phosphate.

3.3. GC analysis of phosphonic acids

Phosphonic acids have moderate to high polarity and low volatility. They have been converted to methyl, pentafluorobenzyl, trimethylsilyl and *tert*.-butyldimethylsilyl esters for GC analysis.

3.3.1. Methyl esters

Alkyl methylphosphonic acids, in a dry organic solvent such as methanol, are converted rapidly (15 min) at ambient temperature to their methyl esters (alkyl methyl methylphosphonates) using diazomethane (generated, for example, from *N*-methyl-*N*-nitroso-*N′*-nitroguanidine) [35]. Provided that a large excess of reagent is used, derivatisation yields can be >99%. The derivatives are not sensitive to traces of water and, if necessary, can be cleaned up by chromatography on silica. It is necessary to isolate the acids in a dry solution prior to derivatisation to avoid excessive consumption of the reagent. Incomplete derivatisation can lead to rapid column deterioration and contamination of the GC system.

The advantages of diazomethane are its high and selective reactivity with acidic analytes, and its high volatility allowing facile removal of excess reagent. Disadvantages are its toxicity, potential for detonation, and the fact that it needs to be prepared freshly for use. As derivatives, the methyl esters have less than ideal chromatographic properties. They can give rise to poor peak shapes, particularly dimethyl methylphosphonate derived from MPA, and have relatively short retention times which increase the chances of interference, e.g., distinction from early eluting background peaks of polluted waters was difficult [35]. Although most methyl esters give weak or non-existent molecular (M^+) ions in their EI mass spectra, they do give informative fragment ions. The base peak for alkyl methyl methylphosphonates is usually m/z 111, $[MeP(OMe)(OH)_2]^+$, from loss of C_nH_{2n-1} from the alkoxy substituent, e.g., C_3H_5 with isopropyl methyl methylphosphonate. Further loss of water gives a strong ion at m/z 93, $[MeP(O)OMe]^+$. Alkyl methyl ethylphosphonates give the corresponding fragment ions at 14 mass units higher. Secondary alkoxy substituents give high mass ions due to loss of alkene from the secondary carbon atom, e.g., loss of C_2H_4 with *sec*-butyl. Under methane CI conditions, the base peak is the MH^+ ion with major fragment ions similar to EI. For identification purposes, the combined spectra are generally highly informative. A disadvantage is that identification as a methyl ester is ambiguous as to whether the original analyte is the acid or ester. A disadvantage of methyl esters for trace analysis, in addition to their chromatographic properties, is that the highest mass ions are usually below m/z 200 and prone to interferences using SIM.

Few laboratories use methyl esters in OPCW Proficiency Tests, although they can be useful for identification for laboratories without access to LC–MS. Most laboratories use silylation. Driskell et al. [36] used methylation for the determination of phosphonic acid urinary metabolites. Urine was concentrated to dryness by azeotropic distillation of water with acetonitrile, followed by derivatisation with ethereal diazomethane (10 min, ambient temperature). The method was designed for high throughput rather than low limits of detection (4 ng/ml for alkyl methylphosphonic acids, 20 ng/ml for ethyl phosphoric acid).

Other reagents have been used to convert phosphonic acids to their methyl esters. (Trimethylsilyl)diazomethane, which is stable and commercially available as a hexane solution, has been used as a safer alternative to diazomethane for the methylation of phosphonic acids and EA 2192 [37]. Derivatisation is performed in the presence of methanol; in the presence of CD_3OD, a mixture of D_2 and D_3 deuterated methyl esters is obtained. Tørnes and Johnsen [38] reported derivatisation with trimethylphenylammonium hydroxide (TMPAH) [sold under the commercial name MethElut]. The phosphonic acids were retained from aqueous solution on an aminopropyl anion-exchange resin and eluted as an ion pair with the derivatising agent in methanol. Derivatisation occurs in the hot injection port of the GC, via thermal decomposition of the reagent. The limits of detection were 100 ng in 50-ml samples of water for both iPrMPA and pinacolyl MPA (PMPA).

Although the detection limits are rather modest, the reported advantage of the method is its simplicity in terms of sample manipulation. It was applied in the field to determine the degradation of sarin and soman under prevailing weather conditions. Sega et al. [39] applied a modification of this method to the determination of MPA, EMPA and iPrMPA in groundwater. The aminopropyl anion-exchange resin gave poor recoveries with ground water (which contains high concentrations of organic anions) and a strong quaternary amine-type anion-exchange resin was used. Detection limits were in the range 3–9 ng/ml from 50-ml samples.

Newer alternatives to TMPAH might be applicable for ''on-column'' derivatisation of phosphonic acids, though they may not be suitable for elution from the resin. Amijee et al. [40] compared a number of reagents that rely on methylation in the hot injection port. Although TMPAH is an efficient derivatising agent, it is caustic (pH~13), which leads to column deterioration, and it has low selectivity. An alternative is phenyltrimethylammonium fluoride, which has similar reactivity and is effectively neutral; a more selective methylating reagent is phenyltrimethylammonium acetate.

3.3.2. Silyl esters

The most widely used derivatisation of phosphonic acids is conversion to trimethylsilyl (TMS) or *tert.*-butyldimethylsilyl (TBDMS) esters. These can be analysed using selective detectors (e.g., FPD, NPD, AED, MS). A minor disadvantage is that excess derivatising agent can lead to silica deposits on FPD and NPD detectors. Conversion to TMS esters proceeds rapidly with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) or BSTFA + 1% trimethylsilyl chloride (TMSCl). Recommended reaction conditions are 60 °C for 30 min [41]. Creasy et al. [42] used BSTFA + 1% TMSCl in hexane, for 15 min at 60 °C, with derivatisation efficiencies in the range 80–100%. The TBDMS esters of phosphonic acids were first described by Purdon et al. [43]. Derivatisation conditions were evaluated using acetonitrile and toluene as solvents, and *N*-methyl-*N*-(*tert.*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) as reagent with and without 1% TBDMSCl as catalyst, and with TBDMSCl/imidazole in dimethylformamide. MTBSTFA with or without catalyst gave efficient conversions; TBDMSCl/imidazole gave lower yields plus a large number of by-products. Although derivatisation proceeded at ambient temperature, optimum conditions selected were MTBSTFA in acetonitrile at 60 °C for 1 h. Good chromatographic separation of the esters was achieved on a DB-5 column using splitless injection, provided the solvent toluene–acetonitrile ratio was at least 9:1. An alternative derivatising agent for TBDMS derivatives may be *tert.*-butyldimethylsilyl cyanide. This reagent derivatises the acids rapidly at ambient temperature (unpublished observations). Sng and Ng [44] have reported in situ derivatisation of phosphonic acids, thiodiglycol and benzilic acid with MTBSTFA vapour (15 min, 20 °C) after SPME on Carboxen-coated fibres.

No direct comparison of TBDMS and TMS derivatives has been reported, but the TBDMS derivatives are assumed to be more stable and less sensitive to traces of moisture than the TMS derivatives, as is the case with other analytes [11]. The TBDMS derivatives show good long-term stability. Derivatised iPrMPA and MPA, stored in their capped reaction vials at ambient temperature, showed no significant degradation over 6 days [43]. They could be further stored for at least 1 month in a freezer. Creasy et al. [42] reported that TMS esters of phosphonic acids were more stable than is generally believed. Derivatives prepared from soil and wipe samples spiked with phosphonic acids at 5 ppm, could still be detected after 5 months storage.

Although the mass spectral properties of TBDMS derivatives are often advantageous in comparison with TMS derivatives, with respect to the intensity of high mass ions, there is no major advantage in the case of phosphonic acids, other than inherently higher masses. In the EI spectra of alkyl methylphosphonic acids, molecular ions are very weak or absent. Both TMS [45] and TBDMS [43] esters of alkyl methylphosphonic acids give a base peak at m/z 153, assigned to $[M–C_nH_{2n}–Me]^+$ and $[M–C_nH_{2n}–Bu]^+$, respectively. A common ion at m/z 169 $[M–C_nH_{2n-1}]^+$ is observed with TMS derivatives. Higher mass ions with weak to moderate intensity are present at $[M–Me]^+$ (TMS) and [M–

Me]$^+$ and [M–Bu]$^+$ (TBDMS). With the di-esters of MPA, base peaks are observed at m/z 225 [M–Me]$^+$ (TMS) and 267 [M–Bu]$^+$ (TBDMS); higher homologues give similarly derived high mass ions. MPA can cause problems due to contamination of the GC system if derivatisation is incomplete. Purdon [43] reported good sensitivity for SIM of the two most intense ions of iPrMPA and MPA TBDMS derivatives (17 and 24 pg injected, respectively, signal-to-noise 2:1, using a quadrupole GC–MS system). The derivatives could be quantitated down to 300–500 pg under full scan MS conditions and 30–60 pg SIM. CI–MS, using methane, isobutane or ammonia as reagent gases provides moderate to intense protonated molecules MH$^+$, usually the base peak with isobutane and ammonia as reagent gases. Ammonia reagent gas, which has a high proton affinity, is particularly useful for selective detection in the presence of hydrocarbon oils, as the latter are not protonated using NH_3 CI–MS.

Derivatisation of phosphonic acids, particularly MPA, is notoriously sensitive to the presence of calcium and magnesium ions. This is a major problem for analysis in soil, and has resulted in laboratories failing to detect MPA in OPCW proficiency tests. The use of a cation-exchange resin (SCX) to remove metal ions from aqueous extracts is included in operating procedures recommended by the OPCW [41]. Kataoka et al. [46] provided quantitative data on the effects of divalent metal ions on the derivatisation of MPA, EMPA, iPrMPA and PMPA, and the improvements resulting from strong cation-exchange (Dowex 50W-X8) pre-treatment. MPA was most affected and iPrMPA and PMPA least affected. A further improvement was reported by the same group using the alternative strategy of capturing the phosphonic acids as their anions on a macroporous strong anion-exchange resin, and elution with 0.1 *M* HCl [47].

D'Agostino et al. [48] demonstrated that an extractive derivatisation of phosphonic acids is possible by allowing dry soil to stand with a solution of BSTFA and pyridine in dichloromethane for 1 h at 60 °C. Lemarie et al. [49] have recently reported that a similar procedure can be applied to wet soil with variable efficiency, depending on the soil type. EMPA and MPA (spiked into soil at 10 ppm) could be extracted as their TMS derivatives after treating wet soil mixed with sodium sulphate with BSTFA in dichloromethane (30 min, 60 °C). The procedure also worked with other analytes (thiodiglycol, ethyldiethanolamine and benzilic acid).

TMS and TBDMS derivatives have been widely used in the analysis of nerve agent degradation products. We routinely use TBDMS derivatives for OPCW proficiency tests and for environmental analysis. An example is shown in Fig. 4. Isopropyl methylphosphonic acid was detected as its TBDMS derivative in soil residues from a bomb crater, in a village alleged to have been subjected to a chemical attack some 4 years earlier [50]. The mass spectra of the TBDMS derivatives allow the monitoring of a number of fragment ions using SIM, or fragmentations using multiple reaction monitoring (MRM) under MS–MS conditions, thus providing a high level of confidence in the identification.

Creasy et al. [42] reported a quantitative method for MPA, alkyl MPAs and ethyl and isobutyl methylphosphonothioic acids in environmental samples, using a strong anion-exchange cartridge for SPE, TMS derivatives and AED detection. An advantage of AED for quantitation is that elemental response factors are independent of the compound type, though detection limits were modest (low ppm). Rohrbaugh and Sarver [45] reported a GC–MS–MS method using TMS derivatives for the selective detection of alkyl methylphosphonic acids in complex environmental matrices; detection limits were 200–500 pg injected, which are much higher than those normally obtained for TBDMS derivatives. Rohrbaugh [51] reported a procedure for the characterisation of VX hydrolysates (for a possible destruction process using an equimolar amount of water) in which the polar degradation products (mainly EMPA) were converted to their TMS derivatives, but still allowed unchanged VX to be detected. Conditions were optimised such that volatile components of the hydrolysate, such as diisopropylamine, were not obscured by peaks derived from BSTFA. Twenty-three compounds were identified in the treated mixture using EI and methane CI–MS, most of them as the original analyte. A disadvantage of the procedure was that EA 2192 was not derivatised because of its zwitterionic character. Hydrolysates in

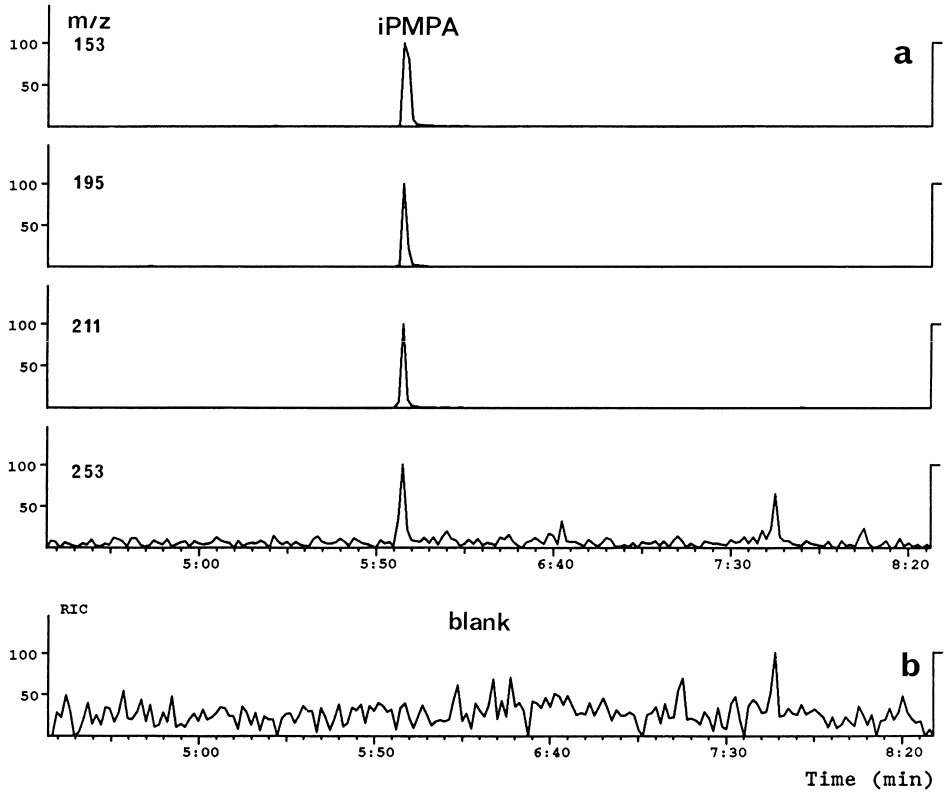


Fig. 4. (a) GC–MS–MS (CH_4 CI) MRM chromatograms, (m/z 253→211, 195, 153) showing the identification of iPrMPA (~80 ng/g) as its TBDMS derivative in a soil sample collected from a bomb crater, and (b) the reconstructed ion chromatogram from the preceding glassware blank.

which EA 2192 is an important component are better analysed using NMR or LC–MS [52].

Both TMS and TBDMS derivatives were used to confirm poisoning following the terrorist releases of sarin in Matsumoto (1994) and the Tokyo subway (1995), and an assassination with VX. iPrMPA was detected as its TBDMS derivative by GC–FPD in urine, collected up to the seventh day following exposure, from a casualty at Matsumoto, and MPA up to the third day [53]. EMPA was detected by GC–MS as its TBDMS derivative in the serum of a subject assassinated with VX [54]. iPrMPA was detected by GC–FPD as its TMS derivative in the urine of casualties of the Tokyo sarin incident [55]. In the latter case, the authors reported that addition of 10% TMSCl to BSTFA significantly improved derivatisation efficiency. Limits of detection for iPrMPA and EMPA were 25 ng/ml. Additional examples are given in Ref. [7].

3.3.3. Pentafluorobenzyl esters

Phosphonic acids are converted to their pentafluorobenzyl esters for applications where very low limits of detection are required, for example, the analysis of biomedical samples in cases of allegations of CW use. Although silyl derivatives were successfully used in the Japanese incidents, the first samples in most cases were collected within a few hours of the exposure. In cases of allegations of CW use, particularly in remote conflicts, samples may be collected up to weeks after the alleged event [7], and detection limits below 1 ng/ml are an advantage. Another factor is that, in cases of allegations of CW

use, a high throughput of samples is rarely an important requirement.

In contrast to methylation and silylation, pentafluorobenzylation of phosphonic acids, using pentafluorobenzyl bromide (PFBBr), is a relatively slow reaction. It requires more complex conditions, and a number of different procedures have been reported. In the first published study [35], the acids were treated with PFBBr in acetonitrile at 60 °C; conversion times for maximum peak area for isopropyl, and sec- and isobutyl methylphosphonic acids were between 200 and 400 min. The addition of a crown ether reduced reaction times by up to one-half, depending on the acid. Decomposition/dimerisation reactions of the reagent were noticeable. The authors subsequently reported that alkylation is best carried out using sodium or potassium salts of the acids [56]. The sodium salt was generated by treatment of the acid in tetrahydrofuran (preferred to acetone or dimethylformamide) with sodium hydride until the pH was 8–9 (avoiding hydrolysis of the alkoxy group). The salt was stirred with PFBBr and 18-crown-6 catalyst for 4 h at 45–50 °C.

Shih et al. [57] developed a method for urine and plasma analysis. A number of solvents for the derivatisation were investigated. Dichloromethane, ethyl acetate, acetone and acetonitrile all gave similar yields; dichloromethane was selected on the basis of ease of concentration. There was no significant difference in yields with reaction temperatures between 40 and 80 °C; 50 °C for 1 h was chosen as the optimum. The acids were first isolated from acidified urine or plasma by SPE on C_2 or C_{18}, and eluted with methanol. Sodium bicarbonate was added, the mixture concentrated to dryness, and derivatisation affected by treatment with PFBBr in dichloromethane using 18-crown-6 ether as catalyst. Fredriksson et al. [58] used a third set of conditions for the derivatisation. The acids were isolated by SPE on a SAX anion-exchange cartridge, eluted as the sodium salts with 0.3 *M* sodium bromide and concentrated to dryness. The residue was dissolved in acetonitrile, potassium carbonate added, and the mixture heated with PFBBr at 90 °C for 1 h. The derivatised acids were cleaned up on a Florisil cartridge. A low and variable recovery of the bis-PFB ester of MPA was noted. Derivatisation under phase transfer conditions, which have been used in organophosphorus pesticide analysis, was also explored but found to be inefficient for the more polar acids such as EMPA and iPrMPA. Miki et al. [59] have recently reported a modification, successfully adapted from pesticide residue analysis. Derivatisation was performed using a polymer-bound quaternary phosphonium phase transfer catalyst (tri-*n*-butylmethylphosphonium bromide). The reported advantage is that extraction, derivatisation and concentration are achieved simultaneously. Urine samples were passed through a Sep IC-AG cation-exchange cartridge, and the pH of the eluate adjusted to 4.5. Derivatisation was performed in a three-phase system in 15-ml glass vessels fitted with a condenser. The aqueous eluate, with added phosphate buffer, PFBBr in toluene, and polymer bound catalyst were vigorously stirred for 90 min at 85 °C. The derivatives were cleaned up on Florisil. This method avoids the need to concentrate the aqueous eluate containing the acids to dryness, and results in a lower inorganic salt content in concentrates. Detection limits were in the range 2.5–10 ng/ml using GC–MS–EI (SIM) and 0.06 ng/ml using GC–MS–NICI, although the chromatograms at 1 ng/ml showed a number of additional components.

All three of these procedures involve a number of manipulations and a heterogeneous derivatisation procedure, which are potential sources of error. In our laboratory we use a simplified, though still quite involved, procedure [60]. Acidified urine is extracted using a polymeric Oasis HLB cartridge, the acids eluted with acetonitrile, and derivatised directly in the eluate using PFBBr in the presence of potassium carbonate, at 90 °C for 1 h; the derivatives are cleaned up on Florisil as above. In conjunction with ion trap GC–MS–MS, this procedure gives very clean chromatograms and limits of detection below 1 ng/ml (Fig. 5), though extraction efficiency is low for EMPA.

The pentafluorobenzyl derivatives give sharp, symmetrical GC peaks, with longer retention times than methyl esters. They are usually intended specifically for use with NICI–MS. With positive EI, much of the ion current is concentrated in the non-specific ion m/z 181, $[C_6F_5CH_2]^+$. A moderately intense class specific ion is present at m/z 256, $[M–C_nH_{2n+1}–F]^+$, from loss of the alkyl group and a fluorine; higher mass compound specific ions are generally weak. Intense MH^+ ions are observed with

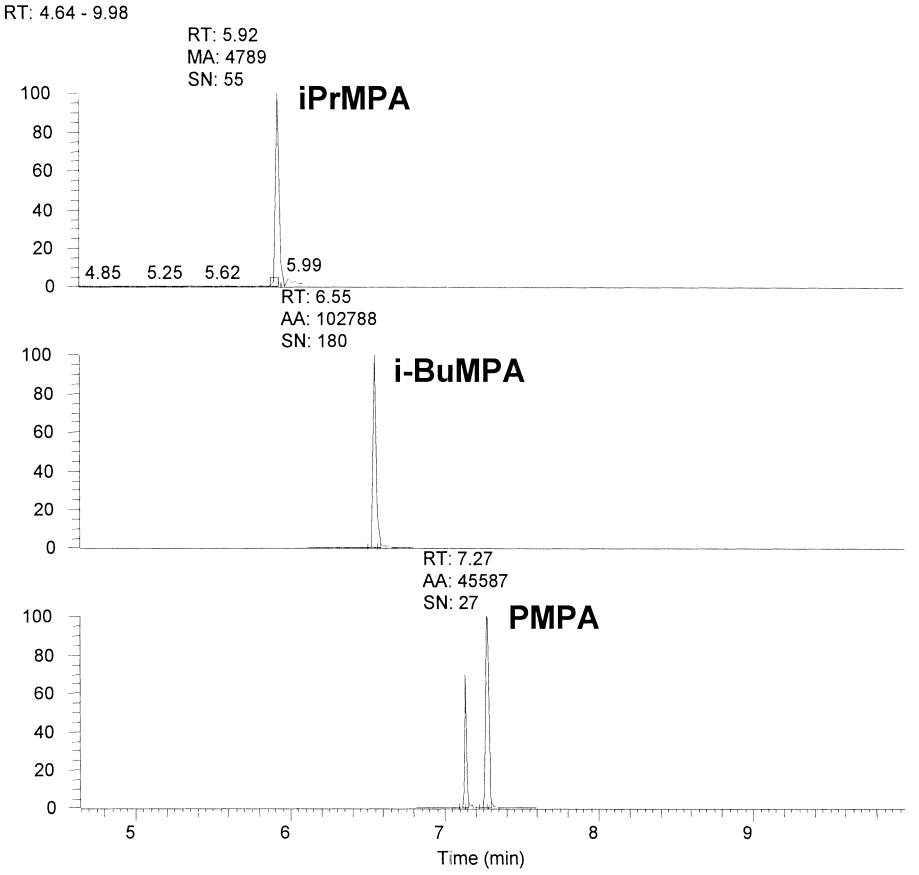


Fig. 5. GC–MS–MS (CH_4 NICI) MRM chromatograms showing the detection of iPMPA (m/z 137→95), i-butyl MPA (m/z 151→95) and PMPA (m/z 179→95), as their pentafluorobenzyl esters, in urine spiked with the acids at 1 ng/ml.

positive isobutane CI. With NICI, the base peak is $[M-181]^-$, from loss of the $C_6F_5CH_2$ moiety; the base peak is thus the anion of the acid. With virtually all of the ion current concentrated in this ion, very high sensitivity can be obtained using SIM. Signal to noise ratios are further enhanced using MS–MS, monitoring the transition $[M-C_6F_5CH_2]^- \rightarrow [M-C_6F_5CH_2-C_nH_{2n}]^-$. A disadvantage is that these are the only ions or fragmentations suitable for monitoring with most mass spectrometers.

Some of the newer fluorinated benzyl esters (Section 2) may find limited application in the analysis of phosphonic acids although it is unlikely that any will show major advantages. Flophemesyl (pentafluorophenyldimethylsilyl) derivatives [11], which have been used for steroids and fatty acids, have not been reported.

3.4. LC analysis of phosphonic acids

With the ready availability of atmospheric pressure ionisation on modern mass spectrometers, LC–MS–(MS) without derivatisation is the usual LC method for analysing phosphonic acids [61]. Bossle et al. [62] converted alkyl methylphosphonic acids to their *p*-bromophenacyl derivatives to facilitate UV detection. *p*-(9-Anthroyloxy)phenacyl derivatives have been used for fluorescence detection of alkylphosphonic acids [63]. Creasy [64] described an LC–MS method involving post-column derivatisation of the VX degradation product EA 2192 to its methyl ester using trimethylphenylammonium hydroxide. Derivatisation, which occurs in the APCI source, removes the zwitterionic character of the analyte and gave sensitivity at least comparable to

APCI or ESI without derivatisation. Other GC–MS and LC–MS methods for this analyte lacked robustness. MPA was similarly derivatised to its dimethyl ester, but the method offered no advantage.

3.5. Other polar degradation products or precursors

Hydrolysis products derived from substituents on phosphorus, or precursor alcohols, e.g., pinacolyl alcohol, 2-diisopropylaminoethanol, are derivatised by silylating agents but do not react with diazomethane. Some of these, e.g., pinacolyl alcohol, will chromatograph successfully without derivatisation. A degradation product that was used in a recent OPCW proficiency test is 2-diisopropylaminoethylsulphonic acid, $iPr_2NCH_2CH_2SO_3H$. This compound is formed during the decontamination of VX with bleach [34]. We derivatised this zwitterionic compound successfully with MTBSTFA (efficiency not determined), though it has been reported as difficult to silylate, as is the case for EA 2192. Diethylaminoethylsulphonic acid has been converted to its methyl ester with (trimethylsilyl)diazomethane [37].

4. Degradation products and metabolites of sulphur and nitrogen mustards

4.1. Sulphur and nitrogen mustards

Sulphur and nitrogen mustards are potent vesicants (blistering agents). They are reactive electrophiles and alkylate DNA and proteins in the body. Sulphur mustard was used on a massive scale in the later stages of World War I and was generally considered to be the most effective CW agent because of its combined effects on skin, eyes and lungs. Its use in more recent conflicts, and a serious legacy of abandoned or dumped munitions, has maintained a very active interest in the analysis of mustard and its degradation products. Nitrogen mustards have been of lesser concern. The intact agents are easily analysed by GC with a number of different detectors [1].

4.2. Degradation pathways

In the environment and in biological matrices, sulphur mustard is hydrolysed predominantly to the more polar and less volatile thiodiglycol (TDG) (Fig. 6) [8]. TDG may be oxidised in soil to the sulphoxide (TDGO), which is also a major urinary metabolite of sulphur mustard in the rat and human [65,66]; further oxidation to the sulphone is less commonly observed in the environment. In certain soil types, possibly through microbial assistance, thiodiglycolic acid has been observed [8] (and in a round robin exercise). Nitrogen mustards HN-3, HN-2 and HN-1 are hydrolysed to $N(CH_2CH_2OH)_3$, $MeN(CH_2CH_2OH)_2$ and $EtN(CH_2CH_2OH)_2$, respectively. Most attention has been focused on TDG analysis.

4.3. Thiodiglycol

TDG can be analysed by GC underivatised [67] but peak shapes are not ideal and derivatisation is required for analysis at concentrations $<\sim 1$ ppm. Two types of derivative have been used for TDG. The most commonly used are silyl ethers, either TMS or TBDMS. Pentafluorobenzoyl or heptafluorobutyryl esters have been used for biomedical sample analysis.

4.3.1. Silyl ethers

Conversion of TDG to its di-TMS derivative occurs rapidly at ambient temperature using either BSTFA or BSA in the presence of 1% TMSCl. Hexamethyldisilazane plus TMSCl has also been

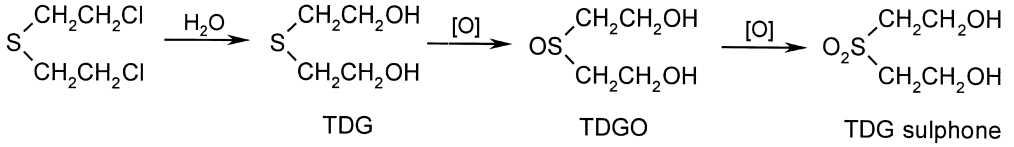


Fig. 6. Hydrolytic and oxidative degradation of sulphur mustard.

used [68,69], and rapid derivatisation is observed with the less commonly used reagent trimethylsilyl cyanide [70]. Derivatisation is normally performed after isolation in an anhydrous solvent but D'Agostino et al. [48] and Lemarie et al. [49] have reported extractive trimethylsilylation of TDG using BSTFA (see Section 3.3.2). Conversion to the di-TBDMS derivative requires more vigorous conditions using MTBSTFA + 1% TBDMSCl; generally 100 °C for 30 min or 1 h has been used. Tomkins and Sega [71] advocated 105 °C for 1 h to ensure complete derivatisation for the trace analysis of TDG in groundwater; Schoene et al. [72] used MTBSTFA in acetonitrile for 1 h at 80 °C.

Traces of water remaining after SPE extraction of TDG, or evaporative losses on prolonged concentration of aqueous solutions to dryness, can be major sources of error in the quantitative analysis of TDG using silylation. Tomkins and Sega [71] used Ambersorb 572 for SPE and reduced evaporative losses on concentration by adding pyridine to the eluant. We experienced similar problems of inconsistent apparent recoveries in developing a method using a polymeric cartridge for extraction.

Both silyl derivatives of TDG possess good chromatographic properties. With three heteroatoms present in TDG, the EI mass spectra, not surprisingly, show a large number of fragment ions. Under EI conditions, the di-TMS derivative gives no molecular ion, but does give a weak ion (~5%) at *m/z* 251 $[M-Me]^+$; other high mass ions are observed at *m/z* 191, 176, 161, 147, 133, 130, 116, formed from cleavages α and β to the various heteroatoms. The base peak is the reagent-derived *m/z* 73, $[Me_3Si]^+$. The quasi-molecular ions MH^+ and $[M+NH_4]^+$ are observed with ammonia CI, but the base peak is *m/z* 177, assigned to $[TMSOCH_2CH_2SCH_2CH_2]^+$, probably as an episulphonium ion. The TBDMS derivative similarly gives no M^+ in EI, but an intense or base peak at *m/z* 293, $[M-Bu]^+$, which is useful for SIM and quantitation (relative intensities vary with MS conditions; Tomkins and Sega [71] report *m/z* 293~40% relative abundance with a base peak *m/z* 73). Other intense high mass ions are present at *m/z* 233, 190, 189, 149, 148. With methane CI, a strong $[MH-CH_4]^+$ is observed at *m/z* 335, plus *m/z* 293 and a base peak at 219 that can be used for SIM [50]. D'Agostino and Provost [73] reported the TMS derivatives of TDG, and hydrolysis products of the longer chain mustards T and Q and other products (derivatised with BSTFA + 1% TMSCl, 60 °C, 20 min), in a hydrolysate of munitions grade sulphur mustard. Ammonia CI spectra provided MH^+ and $[M+NH_4]^+$ ions to aid identification, although intensities ranged from weak to 100%.

In our laboratory we use TBDMS or TMS derivatives to identify TDG in OPCW proficiency tests, and usually the TBDMS derivative for routine trace analysis, e.g., for land remediation. The TBDMS derivative is preferred for TDG alone, but the TMS is currently the derivative of choice if detection of the sulphoxide is also required (see Section 4.4). The TBDMS derivative was used to confirm the presence of TDG in soil collected from a bomb crater associated with an alleged CW attack [50].

For the analysis of TDG in blood or urine, lower limits of detection are normally required. Black and Read [74] developed a method that converted TDG to its bis-pentafluorobenzoyl derivative and used NICI–MS detection. Plasma or urine was extracted with ethyl acetate after absorption onto a Chem Elut tube (which provides a large surface area for extraction), and the extracts cleaned up on C_{18} or Florisil cartridges. After concentration, the dried residues were derivatised with pentafluorobenzoyl chloride in pyridine at ambient temperature for 5 min. Detection limits were 1 ng/ml. The NICI mass spectrum shows one significant ion, the molecular anion at *m/z* 510. With all of the ion current concentrated in this single high mass ion (plus isotope peaks), the method is inherently very sensitive, though with limitations for confirmatory analysis (a repeat analysis, e.g., on a second column would be required). Greater selectivity can be achieved using MS–MS conditions [75]. Product ions are observed in the collision-induced dissociation (CID) spectra at *m/z* 211 $[C_6F_5CO_2]^-$ and 167 $[C_6F_5]^-$, and, although these are non-informative ions, significantly cleaner chromatograms were obtained by monitoring the fragmentation *m/z* 510→167. For standards, 0.2 pg injected could easily be detected indicating a theoretical limit of detection of ~0.l ng/ml; however, the true detection limit in urine could not be determined because of very low background levels of TDG in normal urine (usually <1 ng/ml, source unknown). The derivatisation does not give a clean reaction product, and is only suitable for use with NICI–MS. Jakubowski

et al. [76] have analysed TDG in urine as its bis-heptafluorobutyryl (HFB) derivative, using GC–MS (EI). Urine, adjusted to pH 3–4, was concentrated to dryness, taken up in ethyl acetate, and derivatised with heptafluorobutyric anhydride at 60 °C for 1 h. Trifluoroacetic anhydride was also evaluated as a derivatising agent, but the trifluoroacetyl derivatives were less stable in solution. The HFB derivative produced analytically useful fragment ions at m/z 300 and 301 (70 and 50% relative abundance) resulting from loss of the $C_3F_7CO_2$ moiety. TDG in urine could be detected down to concentrations of 1 ng/ml. This method has since been used to detect TDG in the urine of a casualty accidentally exposed to sulphur mustard [77]. The HFB derivative also shows promise under positive CI conditions; the chromatogram obtained using positive ammonia CI was unusually clean [70].

Finally, Wils et al. [78] reported a procedure that converted TDG in urine back to sulphur mustard by treatment with concentrated HCl. The mustard was isolated from the headspace by adsorption onto Tenax. The method was used to detect TDG in urine from casualties of CW attacks, although it may also convert metabolites other than TDG to sulphur mustard. Control samples showed low concentrations of analyte (1–21 ng/ml).

No procedure has yet been reported that derivatises TDG directly in aqueous solution. It is possible that some of the new chloroformate derivatising agents may be able to meet this objective.

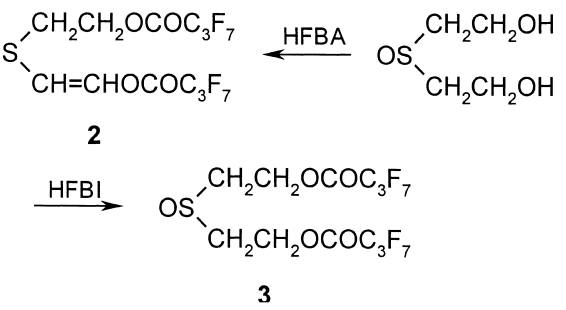
4.4. Thiodiglycol sulphoxide

TDGO is most conveniently analysed by LC–MS although limits of detection are modest (~10 ng/ml in clean water) [79] and not suitable for biomedical sample analysis. The trace analysis of TDGO using GC–MS has been reported only from the authors' laboratory. TDGO presents two challenges for analysis by GC. The first is isolation from the aqueous matrix, the second is derivatisation.

TDGO, because of the highly polarised nature of the S=O bond, is much more polar than TDG or TDG sulphone, and extraction from aqueous solution is difficult other than by concentration to dryness. Black and Read [80] developed a procedure that extracted TDGO with ethyl acetate–methanol (100:7) after absorption of urine onto a Chem Elut tube. The extracts were concentrated to dryness, cleaned up on Florisil, and derivatised with pentafluorobenzoyl chloride, as for TDG. It was observed that the derivative formed was the same as that from TDG, i.e., the sulphoxide function was reduced. This made it difficult to distinguish the sulphoxide from TDG at trace levels other than by selective extraction. Although the method gave a theoretical detection limit similar to that for TDG, many more extraneous peaks were observed in the chromatogram, no doubt resulting from the strength of the solvent required for extraction.

More recently we have investigated the derivatisation of TDGO with a number of reagents [70]. The derivatisation is much more complex than with TDG because the sulphoxide oxygen is an additional nucleophilic site for reaction. Three major types of derivative are formed, depending on the reagent and conditions. These result from simple derivatisation with preservation of the sulphoxide function, reduction to the corresponding TDG derivative, and Pummerer-type rearrangement [81] to derivatives of 1-hydroxy-TDG, which undergo elimination to olefinic products.

For example, the reaction of TDGO with heptafluorobutyric anhydride (HFBA) gave predominantly a derivative tentatively identified as **2**, formed by a Pummerer-type rearrangement and elimination (the latter may occur in the GC injector). A similar product was obtained with trifluoroacetic anhydride. In contrast, heptafluorobutyrylimidazole (HFBI), which has the advantage of not releasing acid during the reaction, gave the sulphoxide derivative **3** as the major product. Reduction to the TDG derivative was observed with heptafluorobutyryl chloride and trimethylsilyl cyanide.



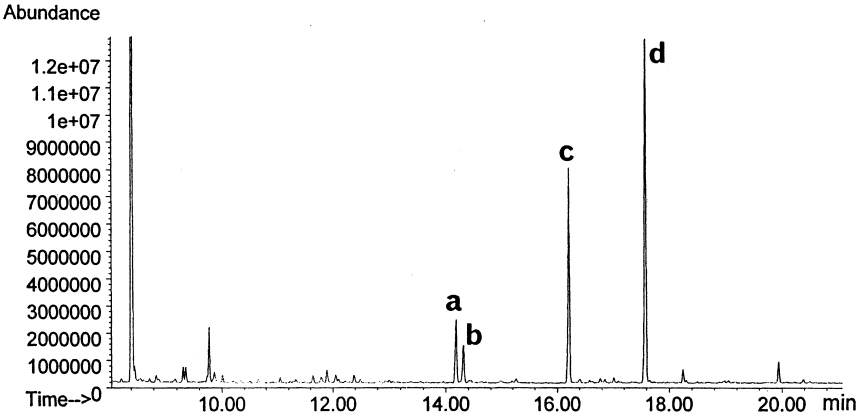
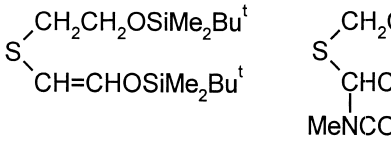


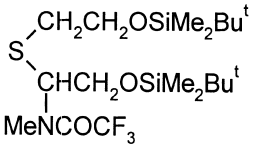
Fig. 7. GC–MS (EI) total ion chromatogram showing the formation of four derivatives, **a**–**d**, on derivatising TDGO with MTBSTFA.

The di-TMS derivative of TDGO is produced satisfactorily with the silylating reagents Tri-Sil BT (BSA + TMSCl 5:1) and with BSTFA. For this reason we use trimethylsilylation where the presence of TDGO is suspected. In contrast, MTBSTFA produces four derivatives (Fig. 7).

On the basis of GC–MS and GC–FTIR, products **a** and **b** (which gave similar spectra) were tentatively assigned as isomers (*cis* and *trans*) of the Pummerer derived products **4a,b**. Product **c** was tentatively identified as a Pummerer derived product **5** with addition of $MeNHCOCF_3$ from the reagent. Product **d** was tentatively identified as the tris-derivatised Pummerer product **6**.



4a,b



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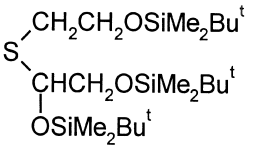

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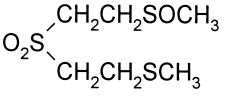
We are still investigating derivatisation of TDGO to determine which derivatisation procedure is best suited to biomedical sample analysis. One means of avoiding the problem is to treat urine with titanium trichloride, which selectively reduces the sulphoxide to TDG [80]. This reagent has been used in the analysis of DMSO, reducing it to dimethyl sulphide for GC analysis [82]. In combination with β-lyase metabolites (see Section 4.5), this provides a convenient procedure for analysing urine for mustard metabolites.

4.5. β-Lyase metabolites

Metabolites of sulphur mustard derived from the β-lyase pathway are observed in the rat and man. The two metabolites, **7** and **8**, identified in the rat, have one and two sulphoxide groups, respectively. These promote elimination reactions on hot surfaces in the GC–MS. Reduction with titanium trichloride [83] produces a single analyte **9**,



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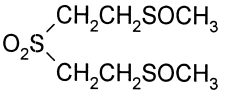

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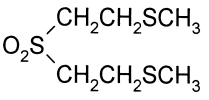

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which is efficiently extracted from urine on a C_8 SPE cartridge, and gives a sharp GC peak. Single stage GC–MS analysis, using ammonia chemical ionisa-

tion and SIM of m/z 232, $[M+NH_4]^+$ gave a detection limit of 2 ng/ml. GC–MS–MS analysis using MRM of m/z 232→75 $[MeSCH_2CH_2]^+$ reduced the detection limit to 0.1 ng/ml [75]. This improved limit of detection allowed the detection of β-lyase metabolites in the urine of seven CW casualties. MRM chromatograms are shown in Fig. 8.

4.6. Other biological indicators of poisoning requiring derivatisation

Sulphur mustard reacts with various nucleophilic acid residues in haemoglobin [7]. The amino acids are alkylated with the $-CH_2CH_2SCH_2CH_2OH$ moiety. Alkylated N-terminal valine and histidine have both been used to confirm poisoning in casualties after liberation from haemoglobin. The alkylated N-terminal valine is unique in that it can be selectively derivatised and released from the protein [84,85] using a procedure based on Edman degradation with pentafluorophenyl isothiocyanate, initially developed by Törnqvist et al. [86] for other alkylating agents. The liberated thiohydantoin derivative is then analysed by GC–MS, preferably after conversion to its heptafluorobutyryl derivative **10**.

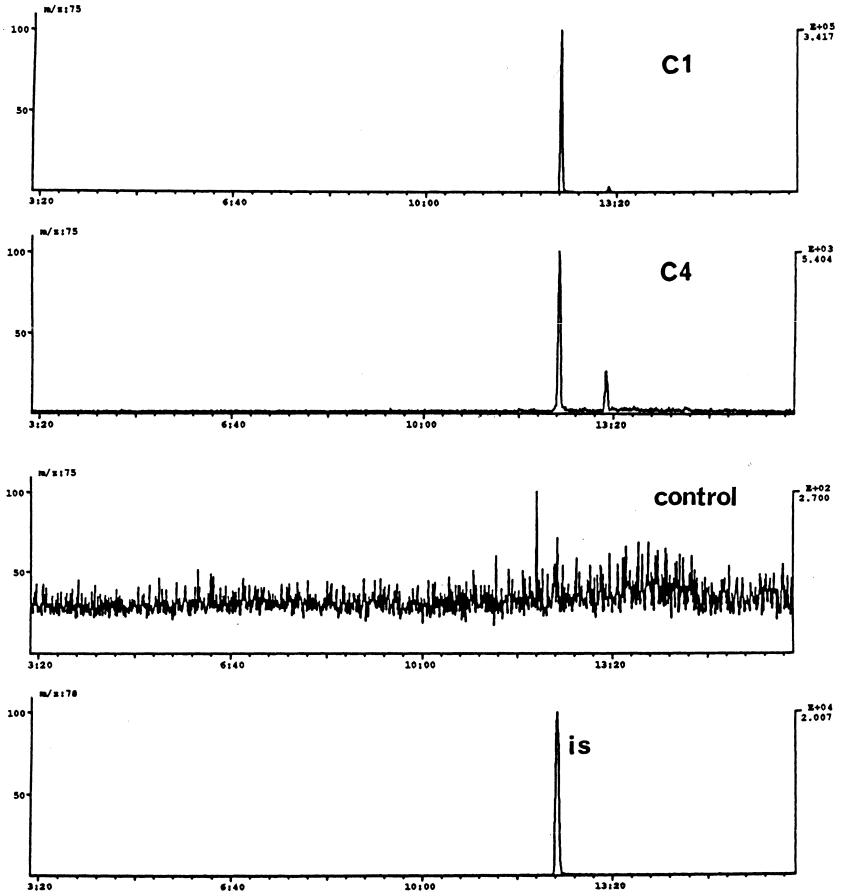
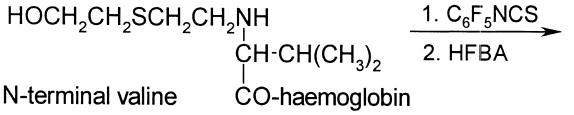
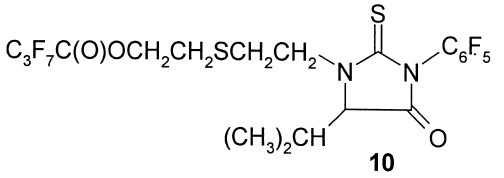


Fig. 8. GC–MS–MS (NH_3 CI) MRM chromatograms (m/z 232→75) showing the detection of β-lyase metabolites, after reduction with $TiCl_3$, in urine from two casualties of sulphur mustard poisoning, (a) subject C1, 220 ng/ml, (b) subject C4, 5 ng/ml, (c) control urine, and (d) the response to the deuterated internal standard (5 ng/ml, m/z 238→78).





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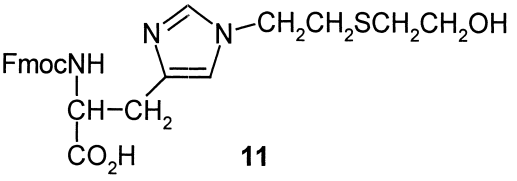
Histidine residues *N*-alkylated with sulphur mustard are more problematic as GC–MS of the alkylated amino acid (e.g., as its di-TBDMS derivative) lacks robustness. Electrospray LC–MS–MS of the fluorenylmethoxycarbonyl (Fmoc) derivative **11** has been used, after acidic digestion of haemoglobin [85,87].



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4.7. Hydrolysis products of nitrogen mustards

The hydrolysis products of nitrogen mustards, *N*-methyldiethanolamine, *N*-ethyldiethanolamine and triethanolamine, form TMS and TBDMS derivatives under conditions similar to those used for TDG; yields with triethanolamine are reported to be higher with TMS [10]. The EI mass spectra contain few major ions. The base peaks result from fragmentation β to the nitrogen atom, $[M–CH_2OSiMe_2R]^+$. Molecular ions are very weak or absent, but a weak to moderate ion is observed at $[M–Me]^+$; a moderately intense reagent-derived ion is observed at *m*/*z* 73, $[Me_3Si]^+$. Giachetti [88] used the tri-TMS derivative for the quantitative trace analysis of triethanolamine, collected on sorbent tubes during air sampling. Trimethylsilylimidazole + TMSCl was used for derivatisation and GC–MS–SIM for analysis. The LOD was 1–2 pg. Creasy [64] reported post-column derivatisation of triethanolamine and *N*-ethyldiethanolamine with benzoyl chloride for LC–APCI–MS analysis. Possible advantages of derivatisation are that the MH^+ ions are moved to a higher mass and the APCI mass spectra showed more fragment ions than with the underivatised compounds.

5. Organoarsenical agents

5.1. Lewisites 1 and 2

Like sulphur mustard, Lewisite is a potent vesicant but with greater volatility and a more rapid onset of action. Lewisite was first manufactured towards the end of World War I, but not in time to be used in that conflict. Weapon-grade Lewisite is composed of ~90% Lewisite 1, $ClCH=CHAsCl_2$, up to ~10% Lewisite 2, $(ClCH=CH)_2AsCl$, and <1% of Lewisite 3, $(ClCH=CH)_3As$, all predominantly as the trans isomer. Lewisite 3 imparts a geranium-like odour to Lewisite. In addition to being weaponised neat, Lewisite was mixed with sulphur mustard to depress the freezing point of the latter and to impart a faster onset of effects. There remains a legacy of former production sites and old munitions, which has spurred the development of a number of new analytical methods over the past decade.

5.2. Degradation pathways

Lewisites 1 and 2 react rapidly with water. Degradation of Lewisite 1 in the environment is dominated by hydrolysis, polymerisation, and partial oxidation from trivalent to pentavalent arsenic (Fig. 9). It initially forms chlorovinyl arsonous acid (CVAA) which undergoes a reversible dehydrative polymerisation to a polymeric oxide known as Lewisite oxide (CVAO). This material has very low solubility in water and precipitates. Some oxidation may occur in the environment to pentavalent arsonic acid derivatives. Lewisite 2 hydrolyses to bis(2-chlorovinyl)-arsenous acid, $(ClCH=CH)_2AsOH$, with some condensation to $(ClCH=CH)_2AsOAs(CH=CHCl)_2$.

5.3. GC analysis of Lewisites and their degradation products

Lewisite 1 is an exception among CW agents in that it is not readily analysed by GC. Although it can be chromatographed on a new GC column, the column rapidly deteriorates and analysis becomes

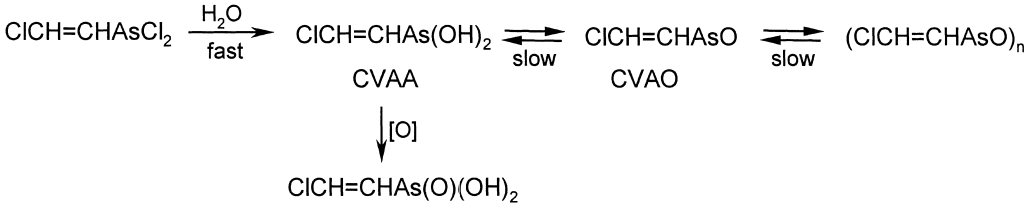
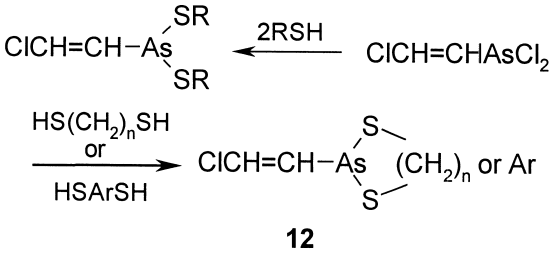


Fig. 9. Degradation pathways for Lewisite 1.

impracticable. Lewisite 2 can be chromatographed but is better derivatised. It is usually analysed in the presence of Lewisite 1 and is therefore similarly derivatised. Lewisite 3 presents no problems for GC and generally does not react with derivatising agents.

Trivalent arsenic forms stronger bonds with sulphur than it does with oxygen. Thiols are therefore used for derivatising Lewisites 1 and 2 and their hydrolysis products. The hydrolysis products are unusual in this respect in that they are usually derivatised with nucleophilic thiols rather than with an electrophilic reagent. Lewisites 1 and 2 react rapidly with mono and dithiols as shown. Dithiols form cyclic derivatives **12** with Lewisite 1, but with Lewisite 2, in dilute solution, a free thiol function remains.



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Dithiols are generally the preferred derivatising agents when analysis of only Lewisite 1 is required, but monothiols are preferred for the analysis of Lewisite 2. The main dithiols that are used to derivatise Lewisite 1 and CVAA are 1,2-ethanedithiol, 1,3-propanedithiol, 3,4-dimercaptotoluene (dimercaptol) and, to a lesser extent, 2,3-dimercaptopropanol (BAL, British Anti-Lewisite). Of the monothiols, thioglycolic acid methyl ester, ethanethiol, propanethiol and butanethiol have found the greatest application.

5.3.1. Dithiol derivatising agents

Haas [89] investigated the reactions of Lewisites 1 and 2 with a series of alkane ω dithiols (C_2–C_6, C_8), plus ethane and propanethiol. In competitive reactions between two dithiols (in acetone, over 30 min at ambient temperature), Lewisite 1 was derivatised almost exclusively with 1,2-ethanedithiol in the presence of higher homologues (1,3-, 1,4-, etc.), and with 1,3-propanedithiol in the presence of higher homologues (1,4-, 1,5-, etc.). This confirmed, as would be predicted, that the five membered 1,3,2-dithiarsenoline derivative is favoured thermodynamically (and on entropy grounds). The 1,2-ethanedithiol and 1,3-propanedithiol derivatives of Lewisite 1 were also formed selectively in competitive reactions with ethanethiol and propanethiol, respectively. Lewisite 2, which cannot form a cyclic derivative, showed little selectivity in competitive reactions. The derivatives were detected by GC–ECD, with limits of detection down to ~0.2 ng injected.

Chen et al. [90] used 3,4-dimercaptotoluene for analysing Lewisite 1 employing GC–MS and GC–AED detection. These authors reported some increase in peak area when the derivatisation of Lewisite was performed at 40 rather than 20 °C. The application of 3,4-dimercaptotoluene in an OPCW proficiency test has been described by Hooijschuur et al. [91]. In our laboratory we have used 3,4-dimercaptotoluene for derivatising Lewisite 1 and CVAA for many years. Its EI mass spectrum is shown in Fig. 10. One cautionary note is that 3,4-dimercaptotoluene can produce the Lewisite 1 derivative with Lewisite 2.

In one of the first applications of dithiol derivatisation, Fowler et al. [92] described the use of 1,2-ethanedithiol to derivatise CVAA in water. Derivatisation was achieved simply by adding the neat reagent to an aqueous solution containing CVAA, agitating for 15 s, and standing for 1 min at ambient temperature. Excess reagent, which can foul the GC syringe and tail into the CVAA GC peak, was

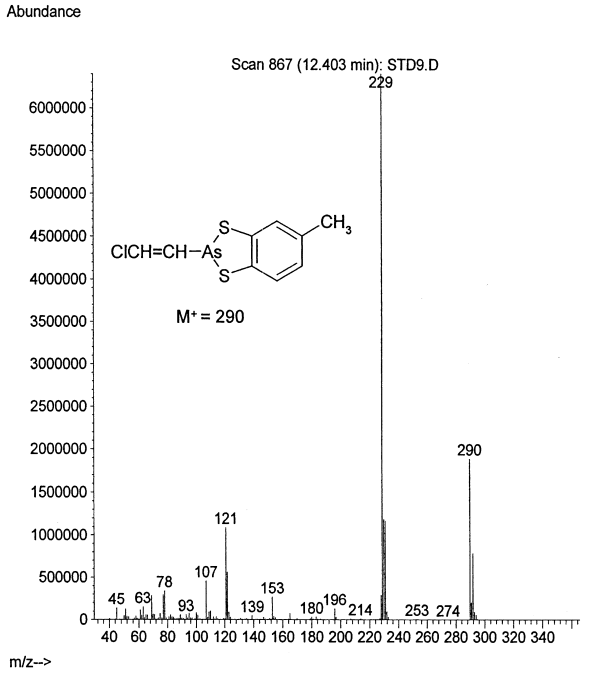


Fig. 10. EI mass spectrum of the derivative formed from Lewisite 1 and 3,4-dimercaptotoluene.

removed by precipitation with silver nitrate. The derivative was extracted into toluene and analysed by sulphur-specific FPD. Limits of detection were ~7 ng/ml for a 4-μl injection. Logan et al. [93] extended this method to the analysis of CVAA in urine. Urine was cleaned up by elution through C_{18} and the CVAA derivatised by adding ethanolic 1,2-ethanedithiol. Detection was by arsenic selective AED and by EI–MS using SIM. The EI mass spectrum of the 1,3,2-dithiarsenoline derivative shows a moderately intense M^+ ion at m/z 228 (plus Cl isotope peak), a more intense ion at m/z 200 (plus Cl isotope peak) $[M–C_2H_4]^+$, and a base peak at 167 $[AsS_2C_2H_4]^+$ suitable for SIM. CVAA was detected in the urine of guinea pigs by both detection methods up to 24 h after exposure to Lewisite (0.5 mg/kg).

Two papers have reported BAL as a dithiol reagent for CVAA. Zhou et al. [94] reported its use for analysing Lewisite hydrolysates using GC with microwave-induced plasma atomic emission detection (detection limit 0.1 μg/l). Fidder et al. [95] used BAL to derivatise free CVAA in blood and urine, and Lewisite residues bound to a cysteine residue in haemoglobin. Blood or urine was incubated with BAL at ambient temperature overnight, and the dithiarsenoline derivative extracted using C_{18} SPE. The free hydroxyl on the resulting cyclic derivative was then converted to its heptafluorobutyryl derivative with heptafluorobutyrylimidazole (50 °C, 1 h, toluene) (observable on the GC–MS chromatogram as a pair of diastereoisomers). This method was sensitive using GC–MS EI SIM monitoring the molecular ion m/z 454 (20 pg injected); NICI was problematic because of the absence of a pseudo-molecular ion and the dominance of the non-specific $[C_3F_7CO_2]^-$ ion.

Several papers have reported derivatisation combined with extraction. Szostek et al. [96] combined derivatisation of CVAA with SPME. A number of thiol reagents were compared, 1,2-ethane-, 1,3-propane- and 1,4-butane-dithiols, 3,4-dimercaptotoluene, thioglycolic acid methyl ester and BAL. CVAA prepared in 10 m*M* HCl was mixed with neat thiol for 1 min at ambient temperature before extraction using an SPME fibre. The thioglycolic acid methyl ester derivative was rejected because of decomposition in the hot injector required for SPME. 2,3-Dimercaptotoluene has insufficient water solubility for the SPME procedure. 1,2-Ethanedithiol and 1,3-propanedithiol were equally proficient, the latter being preferred because of the low purity of commercial ethanedithiol. Although increasing the reaction temperature had little effect on the derivatisation of CVAA, the conditions selected were 70 °C for 15 min which was optimal for $PhAsCl_2$. The LOD ($<6\times10^{-9}$ *M*) was improved by more than 2 orders of magnitude using SPME compared to conventional extraction with toluene. In a modification, pulsed FPD was used for detection [97]. Tomkins et al. [98] extended the SPME method to the quantitative analysis of soil samples associated with land remediation. They used less acidic conditions for extraction (ascorbic acid)–gas was evolved from the soil using hydrochloric acid; the ascorbic acid also acts as an antioxidant. It should be noted that acidic extraction is important for basic soils to prevent base promoted degradation of the ClCH=CH–As moiety, and to promote derivatisation. The 1,3-propanedithiol reagent was added to the ascorbic acid solution prior to extraction. These authors present a full discussion of the various conditions. LODs were in the range 140–300 ng/g soil using GC–FPD. The EI mass spectrum of the 1,3-propanedithiol derivative ex-

hibits a moderately intense M^+ at m/z 242/244, and ions at 181, 149 and 107 suitable for SIM. Wooten et al. [99] have very recently reported a method for the detection of CVAA in urine, using derivatisation with 1,3-propanediol, SPME for isolation and GC–MS. A very low limit of detection (7 pg/ml) was reported. In another modification Chaudot et al. [100] employed simultaneous extraction and 1,2-ethanedithiol derivatisation of CVAA from soil using supercritical fluid extraction (SFE) and pressurised solvent extraction. SFE (CO_2 + MeOH modifier) was the most efficient providing a detection limit 200 ng/g. For SFE, ethanedithiol was added to the soil matrix (stood for 10 min) before extraction, and for pressurised solvent extraction the reagent was added to the extracting solvent (ethyl acetate).

5.3.2. Monothiol derivatising agents

Schoene et al. [101] derivatised various organoarsenic chlorides, oxides and hydroxides with thioglycolic acid methyl ester. Derivatisation was performed under argon for 30 min at 70 °C in an ultrasonic bath, and the derivatives extracted with *n*-hexane. The EI mass spectrum of the Lewisite 1 derivative, $ClCH{=}CHAs(SCH_2CO_2Me)_2$ gave no M^+, but moderate to intense high mass ions at m/z 241, $[M{-}SCH_2CO_2Me]^+$, 180 (100%) $[AsSCH_2CO_2Me]^+$ and 107 $[AsS]^+$. An apparent disadvantage of this derivative is that it can undergo decomposition upon split-splitless injection into a hot (250 °C) injector, and Schoene used on column injection. Haas [102] compared the derivatisation of Lewisites 1 and 2 with 1,2-ethanedithiol, 1,3-propanedithiol and thiodiglycolic acid methyl and ethyl esters. All reactions appeared to be quantitative at 20 °C in acetone for 15 min. There was little difference in the detection limits (0.2–0.4 ng injected, GC–ECD) of the various derivatives, or between Lewisites 1 and 2, thiodiglycolic acid methyl ester being marginally the more sensitive for Lewisite 1. Split-splitless injection at 250–300 °C was used in this study without any reported decomposition of the thioglycolic ester derivatives. One possible advantage of the thioglycolic acid ester derivatives is that they have longer retention times than the short chain alkanethiols. From a recent study of *n*-alkanethiol derivatives, we selected butanethiol as the routine derivatising reagent for the combined analysis of Lewisites 1 and 2. They give derivatives with convenient retention times (~12–13 min), which exhibit molecular ions of moderate relative abundance at m/z 314 and 286, respectively, using EI. Butanethiol also has the advantage of being less volatile than ethane- and propanethiols. It is adsorbed, without breakthrough, by Tenax TA allowing on-tube derivatisation of Lewisites 1 and 2 in atmospheric sampling. The availability of a number of thiols for derivatisation of Lewisites allows facile variation of retention times if interferents are present in the chromatogram. Trifluoroethanethiol shows promise for selective detection using NICI–MS. It gave a detection limit of 20 pg on column with Lewisite 1, compared to ~500 pg with simple alkanethiols using EI–MS (unpublished observations).

5.3.3. Derivatisation of Lewisite acids with electrophilic reagents

The one disadvantage of derivatisation with nucleophilic thiol reagents is that it gives no information on speciation in the sample, and in certain applications, e.g., in decontamination reactions, it is desirable to distinguish Lewisites from their hydrolysis products. One means of distinguishing these species is to derivatise with an electrophilic reagent, which should only react with the hydrolysis products. Styrene oxide reacts with CVAA in aqueous solution to form a cyclic derivative (adapted from Muir et al. [103]) that can be extracted into dichloromethane and analysed by GC–MS–SIM. At present, the limit of detection is high (~10 μg/ml), but this should be adequate for levels usually found in OPCW proficiency tests. The acids from Lewisites 1 and 2 will also form TMS esters, although these do not appear to have been well characterised.

5.4. LC analysis of Lewisite acids

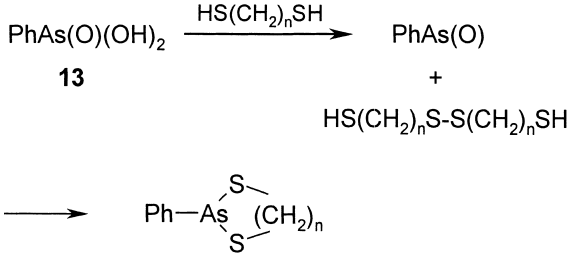
LC–MS analysis of trivalent acids derived from Lewisites is problematic, giving very poor signal-to-noise ratios [64]. LC–MS analysis is considerably improved after oxidation to the pentavalent arsonic acids, $(ClCH{=}CH)_2As(O)(OH)$ and $ClCH{=}CHAs(O)(OH)_2$ (unpublished observations). Creasy [64] used post-column derivatisation with 2-mercaptopyridine to improve the sensitivity (5–10 times) for CVAA. CVAA appears to add a single molecule of the reagent to give a sulphonium species

tentatively identified as ClCH=CHAs=S^+Py. As(III) oxide added two molecules of PySH.

5.5. GC of aromatic organoarsenicals

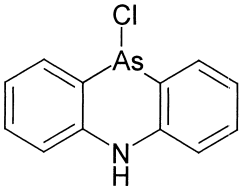
Although considered obsolete (and not listed in the CWC Schedules), aromatic arsenicals, phenylarsenic dichloride, $PhAsCl_2$ (PFIFFIKUS), diphenylarsenic chloride, Ph_2AsCl (Clark I), diphenylarsenic cyanide, Ph_2AsCN (Clark II) and phenylarsazine chloride (Adamsite), were produced in large amounts during World Wars I and II. The agents were disposed of after World War II, some at sea, others on land, and there remains a legacy of contaminated soil and water [104]. In the environment the compounds are subject to hydrolysis and oxidation.

Phenylarsenic dichloride, diphenylarsenic chloride and cyanide can be analysed without derivatisation by GC, though limits of detection for $PhAsCl_2$ are poor [104]. As environmental analysis invariably includes hydrolysis products, they are generally derivatised for analysis in a similar manner to Lewisites. Phenylarsenic dichloride and phenylarsenic oxide react rapidly with dithiols (C_2–C_6, C_8) at ambient temperature to yield cyclic derivatives similar to Lewisite [105]. 1,2-Ethane- and 1,3-propane dithiols react quantitatively to produce stable derivatives. Phenylarsonic acid, As(V), **13**, is derivatised after reduction to phenylarsenic oxide, As(III) by the reagent, which is oxidised to the disulphide. The derivatives formed with monothiols were not stable in acetone solution.

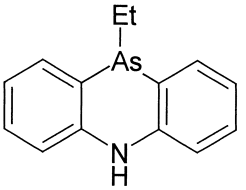


The reaction of diphenylarsenic chloride and diphenylarsenic cyanide with mono (C_2, C_3) and dithiols (C_2–C_6, C_8) showed little selectivity in competitive reactions, similar to Lewisite 2 [106]. Haas and co-workers [107] studied the reactions of mono and diphenyl arsenicals with ethane- and propanethiol, and the ethyl and methyl esters of thioglycolic acid (in acetone, 20 °C, 5 min). The LODs using GC–ECD detection (0.1–0.9 ng injected) were not substantially different for the various products, though the higher LODs were predictably observed with the dithiol derivatives of the diphenyl arsenicals. Haas and Krippendorf [104] reported the determination of mono- and diphenyl arsenicals in soil using GC–ECD. Soil was extracted with acetone and derivatised with ethane and propane mono and dithiols at ambient temperature for 30 min. Limits of detection were 0.1–0.6 ng. The availability of several thiols allowed manipulation of the retention time to eliminate matrix interferences.

Adamsite is the most difficult of the organoarsenicals to analyse. It has very high thermal stability and does not readily hydrolyse in the environment. Like most of the arsenicals, attempts to GC Adamsite leads to rapid column deterioration. Adamsite could not be derivatised with thioglycolic acid methyl ester [101] and no other thiol derivatives have been reported. Schoene et al. [108] developed two derivatisation reactions for Adamsite. In the first, it was reacted with bromine in acetic acid under reflux conditions to give 2,2′,4,4′,6,6′-hexabromodiphenylamine, confirmed by its EI mass spectrum which gave a strong M^+ and a base peak through loss of two bromines. The detection limit for a standard was ~0.4 ng injected using GC–AED, but the limit in soil was 80 ng/g due to chemical background. The derivatisation reaction was sensitive to variations in conditions and the mode of adding the bromine, other brominated products being formed. The alternative derivatisation involved pyrolytic ethylation with dimethylformamide diethylacetal and pyridine to give 10-ethyl-5,10-dihydrophenarsazine **14**. The reaction occurred in the hot (290 °C) GC injector, probably via a thermal dimerisation product of Adamsite. This derivative gave a simple EI spectrum consisting mainly of M^+ ~10%, a base peak at 242, and a fragment ion at 167. Using GC–AED and GC–MS–SIM, the method provided LODs around 3 ng/g soil. This second method was the preferred method. It is easier to perform, is less sensitive to matrix effects, and the first method is not unequivocal if diphenylamine were present in the matrix.



Adamsite



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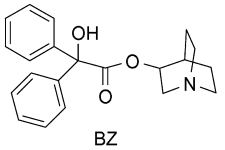
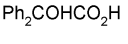
6. 3-Quinuclidinyl benzilate (BZ)

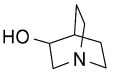
BZ is an incapacitating agent that acts on the central nervous system, by reversible blockade of muscarinic-type receptors in the cholinergic nervous system. Unlike the other CW agents discussed in this review, BZ does not form covalent bonds with nucleophilic sites in biological matrices. In a global context, it has attracted less concern than the nerve agents and vesicants, and this is reflected in the paucity of fully developed analytical methods for BZ and its degradation products. In the environment and in animals BZ is hydrolysed to benzilic acid and 3-quinuclidinol.



BZ



benzilic acid



3-quinuclidinol

BZ can be analysed by GC although retention times are long. On-column injection is preferred as BZ can decompose to benzophenone in a hot GC injector. 3-Quinuclidinol can also be analysed underivatised although signal-to-noise ratios are usually poor. LC–MS is our preferred method for these analytes [61]. BZ has been derivatised as its TMS derivative to improve chromatographic performance. Byrd et al. [109,110] described a GC–MS method for the determination of BZ and its hydrolysis products in urine. BZ and benzilic acid were isolated by C_{18} SPE after basification and acidification of urine, respectively. Quinuclidinol was isolated from urine at pH 6–7 using Florisil SPE. Concentrated residues were derivatised with *N*-methyl-*N*-trimethysilyltrifluoroacetamide (MSTFA) in acetonitrile. Three hours at 70 °C were required for complete conversion of BZ to its TMS derivative. The EI mass spectrum gives a very weak M^+, a base peak at m/z 255, $[Ph_2COTMS]^+$ which can be used for SIM, and a quinuclidinium ion at 110.

Derivatisation of benzilic acid and 3-quinuclidinol occurs in 15 min at ambient temperature with MSTFA in acetonitrile. The EI spectrum of benzilic acid–TMS shows no M^+, two weak characteristic ions at m/z 357, $[M–Me]^+$, and 329, $[M–CH_3CO]^+$, and a base peak at 255 suitable for SIM similar to BZ. Quinuclidinol–TMS gives a strong M^+ at m/z 199 suitable for SIM, plus a number of intense fragment ions. Both BZ–TMS and quinuclidinol–TMS tend to exhibit peak tailing on low polarity GC columns. The method could detect BZ at concentrations of 0.5 ng/ml and the hydrolysis products at 5 ng/ml, using large (20 ml) aliquots of urine for each analysis.

Benzilic acid also forms a TBDMS derivative using the general conditions (MTBSTFA, 1 h, 90 °C) used for screening in our laboratory for OPCW proficiency tests. Gu et al. [111] recently described the detection of benzilic acid in water using GC–MS–SIM after concentration and conversion to its methyl ester. Some decomposition of the derivative was observed in the hot GC inlet.

7. Highly volatile CW agents

7.1. Phosgene

Phosgene is known as a choking gas, causing a delayed fatal pulmonary oedema. It is a labile electrophile that reacts rapidly with nucleophiles such as hydroxyl, amino and sulphydryl groups, with some selectivity for nitrogen nucleophiles. It is assumed that reaction with nucleophilic sites on tissue macromolecules triggers the response that ultimately results in pulmonary oedema. Phosgene is both an industrial chemical and a CW agent, and is listed in Schedule 3 of the CWC as a dual use chemical [6]. Its major industrial uses include the manufacture of plastics, particularly polyurethanes and polycarbonates, insecticides and pharmaceuticals. It was used as a CW agent on a massive scale in World War I, accounting for approximately 80% of

the deaths resulting from chemical weapons.

GC determination of underivatised phosgene is possible by adsorption onto a suitable dual sorbent tube such as Tenax plus carbon molecular sieves [112], but has several shortcomings. Response factors were poor using ECD, other airborne chlorinated compounds may cause interference, and a long GC column is required. Like Lewisite, phosgene has two geminal displacable chlorines, and reacts with a range of mono- and bi-dentate nucleophiles, forming cyclic derivatives with the latter.

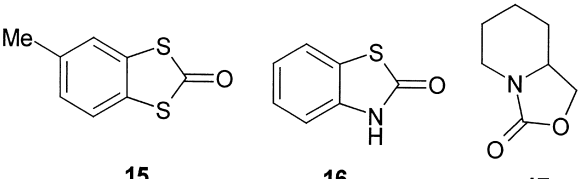
7.1.1. Derivatisation with mono-nucleophiles

Examples of derivatisation of phosgene with mono-nucleophiles are diethylamine [113], di-*n*-butylamine [114,115] and tryptamine [116]. Diethylamine was used as reagent, forming *N*,*N*,*N′*,*N′*-tetraethylurea, $Et_2NCONEt_2$, to determine unreacted phosgene in a chemical reaction mixture, but with a modest detection limit of 5 μg/ml in dichloromethane solution using GC–FID [113]. Schoene et al. [114] used a sorbent tube coated with di-*n*-butylamine to simultaneously trap and derivatise phosgene as $Bu_2NCONBu_2$. XAD-2 was coated with *n*-dibutylamine, and air (2 l) sampled from a test atmosphere at 100 ml/min. The derivatised phosgene was extracted by *n*-pentadecane/*n*-hexane, and analysis performed using GC–AED and GC–MS. The method suffered from breakthrough of the analytes onto a second sorbent tube, losses during prolonged storage, and was relatively insensitive (limits of detection in the lower ppm range). Hendershott [115] reported a similar method. Tryptamine has been used to derivatise phosgene in atmospheric samples [116] for analysis by LC. Air was drawn through an impinger filled with a solution of tryptamine in isooctane. Analysis of the resulting urea was performed by LC with fluorescence detection. Recoveries from phosgene containing air samples were around 89% with losses being attributed to hydrolysis of phosgene by trace amounts of water in the isooctane. At a sampling rate of 1 l/min, a detection limit of 0.04 mg/m^3 was achieved.

7.1.2. Derivatisation with bidentate nucleophiles

Examples of derivatisation with bidentate nucleophiles are 1-(2-pyridyl)-piperazine [117], 2-(hydroxymethyl)piperidine [118] and 2-aminophenol [119]. Conversely phosgene is used as a derivatising agent for bifunctional analytes [120]. Rando et al. [117] used Chromosorb coated with 1-(2-pyridyl)piperazine for dual use air sampling of phosgene and isocyanates. A sample flow-rate of 1 l/min for 20 min gave no breakthrough of phosgene. The derivatised phosgene was extracted from the Chromosorb with acetonitrile, and analysed by reversed-phase LC with UV detection. The detection limit was 5 ppb of phosgene in a 20-l air sample. Derivatised samples were found to be unstable with significant degradation after 1 week at 4 °C. 2-(Hydroxymethyl)piperidine coated onto XAD-2 can be used for sampling phosgene, formaldehyde and acrolein [118]. The cyclic derivative was extracted with toluene and analysed by GC–NPD. The stability of the derivate is good (full recovery after 19 days at ambient temperature). The limit of quantitation was 0.014 mg/m^3. 2-Aminophenol was used to convert phosgene into 2-benzoxazolinone for LC–UV determination of phosgene in polycarbonates used in the food industry [119].

Current research in our laboratory [121] has identified three bidentate nucleophilic reagents, dimercaptotoluene (used for Lewisite derivatisation), 2-aminothiophenol and 2-hydroxymethylpiperidine, suitable for on-tube (Tenax TA) derivatisation of phosgene with analysis by GC–MS–SIM. Each of these forms a five-membered cyclic derivative, **15**, **16** and **17**. Analysis can be performed using thermal desorption and GC–MS–SIM. The thermal desorption approach should be inherently more sensitive than extraction since no dilution of the sample occurs prior to analysis.



15 **16** **17**

7.2. Hydrogen cyanide

Hydrogen cyanide is known as a lethal ‘‘blood’’ gas. It reacts with trivalent iron of cytochrome oxidase, thereby inhibiting electron transport and starving the blood of oxygen. It can react with both

nucleophiles and electrophiles, depending on the conditions. It is metabolised to thiocyanate in the body. Like phosgene, hydrogen cyanide is a dual use chemical listed in Schedule 3 of the CWC [6]. Uses include electroplating, metal refining, and as a fumigant pesticide.

A variety of methods exist for the determination of cyanide in air. Spectroscopic methods usually involve the formation of a cyanide-containing complex after conversion to the cyanide ion by alkaline solution, e.g., the *N*-chlorosuccinimide–barbituric acid–pyridine system [122]; these will not be reviewed. Improved sensitivity and selectivity may be obtained using GC with a variety of detectors. Direct headspace analysis of blood using GC–NPD, without derivatisation, gave a modest detection limit of 14 μg/ml [123]. A more complex variation of this method involves the injection of blood headspace into a heated injection port packed with chloramine-T [124]. Hydrogen cyanide is halogenated to form cyanogen chloride, which is detected by GC–ECD. The detection limit for this method was 0.50 ng/ml. No derivatisation method for hydrogen cyanide in air with subsequent GC analysis appears to have been reported.

Derivatisation to pentafluorobenzyl cyanide has provided the most sensitive methods for detection of cyanide in aqueous solutions, including biomedical samples. Wu et al. [125] determined several anions as their pentafluorobenzyl derivatives, including cyanide. Derivatisation was accomplished with pentafluorobenzyl bromide in a water miscible solvent under basic conditions; thiocyanate was converted into bis(pentafluorobenzyl) sulphide. Analysis was by packed column GC–FID. Funazo et al. [126] reported pentafluorobenzyl *p*-toluenesulphonate as a new pentafluorobenzylating reagent but it was inefficient for cyanide. As is the case for pentafluorobenzylation of phosphonic acids, a number of modifications and improvements have been reported, including extractive alkylation using phase-transfer catalysis. Chen et al. [127] reported a two-phase derivatisation of anions in potassium borate buffer (pH 9.5) with pentafluorobenzyl bromide in dichloromethane, plus an immobilised phase transfer catalyst (Kryptofix 22 B polymer). Optimum conditions for cyanide using mechanical agitation were 3 h at 30 °C. Using GC–ECD, sub-nanomolar concentrations could be detected in waste water, urine and saliva. Blood is a more demanding matrix for analysis. Kage et al. [128] analysed cyanide and thiocyanate in blood using a two-phase pentafluorobenzylation procedure with tetradecyldimethylbenzylammonium chloride as phase-transfer agent. Blood was pre-treated with sodium sulphite and trichloroacetic acid to prevent oxidation of blood cyanide and precipitation of proteins. Derivatisation was performed in sodium borate buffer with ethyl acetate as the organic phase, at 55 °C for 30 min. Recoveries of around 80% for cyanide and thiocyanate resulted in detection limits of 0.01 and 0.003 μmol/ml blood, respectively, by GC–ECD. The mass spectrum of pentafluorobenzyl cyanide gave a molecular ion (m/z 207) as the base peak; pentafluorobenzylthiocyanate gave a weak M^+ and a base peak m/z 181, $[M–SCN]^+$. NICI–MS spectra do not appear to have been reported.

7.3. Cyanogen chloride

Cyanogen chloride, CNCl, is a highly volatile liquid which, like phosgene, causes pulmonary oedema possibly initiated from a reaction with nucleophilic sites on proteins. It is the third of the four dual-use chemicals listed in Schedule 3 of the CWC (the fourth is chloropicrin) [6]. Cyanogen chloride is also formed as a by-product of water disinfection, from the action of chlorinating agents on aliphatic amino acids in the presence of ammonium ion [129].

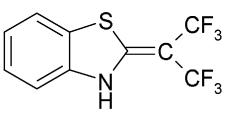
Atmospheric cyanogen chloride has been analysed without derivatisation, as for phosgene [112]. Together with phosgene, it has been sampled from test atmospheres onto XAD-2 sorbent coated with *n*-butylamine [114], again with the shortcomings discussed in Section 7.1. Cyanogen chloride in water has been determined by several techniques not involving derivatisation; these have been reviewed by Sclimenti et al. [129].

7.4. Perfluoroisobutene (PFIB)

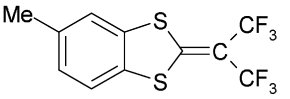
Like phosgene, PFIB is a reactive electrophile that produces a potentially fatal pulmonary oedema in man, probably through a reaction with nucleophilic sites on proteins. It is formed as a by-product in

tetrafluoroethylene production and in combustion of polymerised fluorocarbon compounds such as Teflon. Although it has not been used as a CW agent, it is specifically listed in Schedule 2 of the CWC as a toxic chemical of possible risk to the Convention [6].

PFIB (b.p. 6 °C) and similar fluorinated compounds may be sampled from the atmosphere using cryotrapping, e.g., Ref. [130] and chromatographed at sub-ambient temperatures. The direct use of Tenax or similar adsorbents is unsuitable, as they do not retain PFIB. To avoid cryotrapping procedures, a nucleophilic derivatising reagent can be used as with phosgene. PFIB will react with a host of nucleophiles [131] at ambient or sub-ambient temperatures. Like phosgene, PFIB has two halogens that can be displaced and forms stable cyclic derivatives with bidentate nucleophiles, e.g., **18** with 2-aminothiophenol [132]. Quick and Muir [121] have recently developed a method in which PFIB is passed through a Tenax TA tube loaded with 2-aminothiophenol and triethylamine. Analysis by thermal desorption GC–MS–SIM allowed sub-ppm quantitation. An alternative reagent is 3,4-dimercaptotoluene. PFIB reacts with both reagents to produce cyclic derivatives **18** and **19** which give molecular ions as base peaks in their EI mass spectra (*m*/*z* 285 and 316, respectively); these are advantageous for SIM.



18



19

8. Derivatisation as part of general screening procedures

The derivatisation procedure that is applicable to the broadest range of CW degradation products, precursor chemicals, and BZ, is silylation. Silylation, with BSTFA or MBTSTFA, is included in the operating procedures that are recommended for use in OPCW proficiency tests [41]. Scheduled chemicals and their degradation products that are derivatised include alkyl alkylphosphonic acids, alkyl alkylphosphonothioic acids, alkylphosphonic acids, alkyl phosphoric acids, dialkylaminoalcohols, pinacolyl alcohol, thiodiglycol, its sulphoxide and sulphone, hydrolysis products of nitrogen mustards, BZ, benzilic acid and 3-quinuclidinol. Derivatisation to TMS derivatives has marginally the broadest application, and with some analytes requires milder conditions than derivatisation to the bulkier TBDMS derivatives. Its major advantage over TBDMS is the derivatisation of thiodiglycol sulphoxide; it also gives greater yields with some aminoalcohols such as triethanolamine [10]. The advantages of TBDMS derivatisation are a presumed greater stability of the derivatives, greater robustness with respect to traces of moisture in the extract to be derivatised, and in some cases more intense informative high mass ions, particularly the $[M-57]^+$ ion. There is a preference for TMS derivatives among the laboratories that undertake OPCW proficiency tests, although in our laboratory we prefer to use TBDMS derivatives. The OPCW has selected TMS derivatisation for on-site analysis associated with inspections. Derivatisation to methyl esters is only appropriate for acidic analytes (phosphonic acids, benzilic acid). At least one laboratory uses methylation in addition to silylation in screening procedures, because methylation may be more efficient than silylation with some acids. In our experience, if LC–MS is available to complement GC–MS, this should not be necessary.

A separate derivatisation procedure is required for Lewisites I and II and their degradation products. 3,4-Dimercaptotoluene is the preferred reagent in most laboratories for Lewisite 1 and its degradation products, but 1,2-ethanedithiol and 1,3-propanedithiol are also efficient. It is advisable to also derivatise with a monothiol such as butanethiol if the presence of Lewisite 2 is suspected.

An illustration of the screening procedures used in the TNO Prins-Maurits Laboratory has been given by Hooijschuur et al. [91]. The virtual scenario for an OPCW proficiency test was an on-site challenge inspection of a small-scale facility suspected of producing non-declared CW agents. Samples for analysis were waste water, soil and a sample of organic liquid labelled as waste. Silylation was achieved using MSTFA at 70 °C for 30 min. In combination with LC–MS, ethylphosphonic, *N*-methyldiethanolamine and *N*-ethyldiethanolamine were identified as their TMS derivatives in the water sample, and methyl ethylphosphonic acid as its TMS derivative in the soil sample. Lewisite 1 was identified as its 3,4-dimercaptotoluene derivative in the organic liquid.

An additional example of the use of TMS derivatisation in a general screening procedure is given by Weimaster et al. [133], in which ethyl and diethylphosphoric acids (which are not scheduled chemicals) were detected in samples associated with a suspected CW incident. An example of the use of TBDMS derivatives in analyses associated with suspected use is given by Black et al. [50]. Isopropyl methylphosphonic acid, methylphosphonic acid and thiodiglycol were identified as their TBDMS derivatives in soil samples from bomb craters found in a village in Northern Iraq. This was the first time that residues from sarin had been detected following a suspected CW attack.

This review has focused on derivatisation reactions used in CW analysis. Although the procedures recommended for use are well tried and tested, it should be recognised that derivatisation can be the major source of error in an analysis and may introduce artefacts, particularly in an uncharacterised matrix. It is always preferable to use GC–MS with derivatisation, and LC–MS without derivatisation, as complementary techniques. A good example is a sulphoxide oxidation product of a mustard homologue reported by Hooijschuur et al. [91]; this compound was much more easily detected and identified using LC–MS than by GC–MS.

9. Conclusions

Robust derivatisation methods are available for most of the CW agents requiring derivatisation, and the more polar degradation products. New procedures continue to appear, particularly in biomedical sample analysis. One of the goals of new derivatisation procedures will be to remove the necessity to concentrate aqueous solutions to dryness, which is time consuming and a source of error. Advances can be expected in reagents that will derivatise directly in aqueous solution, or reagents that will derivatise in situ on SPE cartridges, SPME fibres or Tenax adsorbent tubes. For general screening purposes, silylation is likely to remain the procedure of choice for most polar degradation products. For biomedical sample analysis, in applications where low limits of detection are more important than sample throughput, increasing use of fluorinated derivatives in combination with NICI MS can be expected.

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References

[1] Z. Witkiewicz, M. Mazurek, J. Szulc, J. Chromatogr. 503 (1990) 293.
[2] Ch.E. Kientz, J. Chromatogr. A 818 (1998) 1.
[3] M. Mesilaakso, Chemical weapons chemical analysis, in: R.A. Meyers (Ed.), Encyclopedia of Analytical Chemistry, Wiley, Chichester, 2000, p. 899.
[4] E.W.J. Hooijschuur, Ch.E. Kientz, U.A.Th. Brinkman, J. Chromatogr. A 982 (2002) 177.
[5] E.R.J. Wils, in: R.A. Meyers (Ed.), Encyclopedia of Analytical Chemistry, Wiley, Chichester, 2000, p. 979.
[6] M. Mesilaakso, M. Rautio, in: R.A. Meyers (Ed.), Encyclopedia of Analytical Chemistry, Wiley, Chichester, 2000, p. 899.
[7] D. Noort, H. Benschop, R. Black, Arch. Toxicol. 184 (2002) 116.
[8] N.B. Munro, S.S. Talmage, G.D. Griffin, L.C. Waters, A.P. Watson, J.F. King, V. Hauschild, Environ. Health Perspect. 107 (1999) 933.
[9] R.M. Black, J.M. Harrison, in: F.R. Hartley (Ed.), The Chemistry of Organophosphorus Compounds, Vol. 4, Wiley, Chichester, 1996, p. 781.
[10] M.-L. Kuitunen, in: R.A. Meyers (Ed.), Encyclopedia of Analytical Chemistry, Wiley, Chichester, 2000, p. 1055.
[11] K. Blau, J. Halket (Eds.), Handbook of Derivatives for Chromatography, Wiley, Chichester, 1993.
[12] V.Y. Taguchi, in: R.E. Clement (Ed.), Gas Chromatography: Biochemical, Biomedical and Clinical Applications, Wiley, New York, 1990, p. 129.
[13] R.J. Wells, J. Chromatogr. A 843 (1999) 1.
[14] M. Saha, J. Saha, R.W. Giese, J. Chromatogr. 641 (1993) 400.
[15] M. Amijee, J. Cheung, R.J. Wells, J. Chromatogr. A 738 (1996) 57.
[16] A. Dasgupta, R. Macaulay, J. Chromatogr. A 695 (1995) 136.
[17] J.T. Simpson, D.S. Torok, S.P. Markey, Am. Soc. Mass Spectrom. 6 (1995) 525.
[18] V. Maurino, C. Minero, E. Pelizzetti, S. Angelino, M. Vincenti, Am. Soc. Mass Spectrom. 10 (1999) 1328.
[19] A. Dasgupta, W. Blackwell, J. Griego, S. Malik, J. Chromatogr. B 666 (1995) 63.
[20] J.M. Rosenfeld, J. Chromatogr. A 843 (1999) 19.
[21] M.D. Cummins, R.J. Wells, J. Chromatogr. B 785 (1997) 251.
[22] P. Campíns-Falcó, R. Herráez-Hernández, A. Sevillano-Cabeza, I. Trümpler, Anal. Chim. Acta 344 (1997) 125.
[23] K. Jedrzejczak, V.S. Gaind, Analyst 118 (1993) 1383.

[24] A. Miki, H. Tsuchihashi, H. Yamano, M. Yamashita, Anal. Chim. Acta 356 (1997) 165.
[25] P. Húsek, J. Chromatogr. B 717 (1998) 57.
[26] C. Minero, M. Vincenti, S. Lago, E. Pelizzetti, Fresenius J. Anal. Chem. 350 (1994) 403.
[27] C. Minero, M. Vincenti, E. Pelizzetti, Ann. Chim. 83 (1993) 511.
[28] W.K. Fowler, J.E. Smith, J. Chromatogr. 478 (1989) 51.
[29] D. Tingfa, Int. J. Environ. Anal. Chem. 27 (1986) 151.
[30] M. Polhuijs, J.P. Langenberg, H.P. Benschop, Toxicol. Appl. Pharmacol. 146 (1997) 156.
[31] E.M. Jakubowski, L.S. Heykamp, H.D. Durst, S.A. Thomson, Anal. Lett. 34 (2001) 727.
[32] A.F. Kingery, H.E. Allen, Toxicol. Environ. Chem. 47 (1995) 155.
[33] M.L. Reynolds, P.J. Little, B.F.G. Thomas, R.B. Bagley, B.R. Martin, Toxicol. Appl. Pharmacol. 80 (1985) 409.
[34] Y.-C. Yang, J.A. Baker, J.R. Ward, Chem. Rev. 92 (1992) 1729.
[35] J. Enqvist, M. Rautio (Eds.), B.2 Identification of Degradation Products of Potential Organophosphorus Warfare Agents. An Approach for the Standardisation of Techniques and Reference Data. The Ministry for Foreign Affairs of Finland, Helsinki, 1980.
[36] W.J. Driskell, M. Shih, L.L. Needham, D.B. Barr, J. Anal. Toxicol. 26 (2002) 6.
[37] M.D. Crenshaw, D.B. Cummings, in: Proc. ERDEC Sci. Conf. Chem. Biol. Def. Res. 1998, 1999, p. 715.
[38] J.Aa. Tørnes, B.A. Johnsen, J. Chromatogr. 467 (1989) 129.
[39] G.A. Sega, B.A. Tomkins, W.H. Griest, J. Chromatogr. A 790 (1997) 143.
[40] M. Amijee, J. Cheung, R.J. Wells, J. Chromatogr. A 738 (1996) 43.
[41] M. Rautio (Ed.), Recommended Operating Procedures for Sampling and Analysis in the Verification of Chemical Disarmament, The Ministry for Foreign Affairs of Finland, Helsinki, 1994.
[42] W.R. Creasy, A.A. Rodríguez, J.R. Stuff, R.W. Warren, J. Chromatogr. A 709 (1995) 333.
[43] J.G. Purdon, J.G. Pagotto, R.K. Miller, J. Chromatogr. 475 (1989) 261.
[44] M.T. Sng, W.F. Ng, J. Chromatogr. A 832 (1999) 173.
[45] D.K. Rohrbaugh, E.W. Sarver, J. Chromatogr. A 809 (1998) 141.
[46] M. Kataoka, N. Tsunoda, H. Ohta, K. Tsuge, H. Takesako, Y. Seto, J. Chromatogr. A 824 (1998) 211.
[47] M. Kataoka, K. Tsuge, Y. Seto, J. Chromatogr. A 879 (2000) 295.
[48] P.A. D'Agostino, L.R. Provost, J. Chromatogr. 645 (1993) 283.
[49] L. Lemarie, M.S. Sokolowski, C. Wickramage, in: Proceedings of the 3rd SISPAT International Symposium on Protection against Toxic Substances, Singapore, 2–6 December, 2002.
[50] R.M. Black, R.J. Clarke, R.W. Read, M.T.J. Reid, J. Chromatogr. A 662 (1994) 301.
[51] D.K. Rohrbaugh, J. Chromatogr. A 809 (1998) 131.
[52] W.R. Creasy, J. Mays, R. Deuvel, T.R. Connell, B.R. Williams, in: Proceedings of the 1996 Conference on Chemical and Biological Defense Research, 19–22 November 1996, Aberdeen Proving Ground, MD, 1997, p. 653.
[53] T. Nakajima, K. Sasaki, H. Ozawa, Y. Sekijima, H. Morita, Y. Fukushima, N. Yanagisawa, Arch. Toxicol. 72 (1998) 601.
[54] M. Katagi, M. Nishikawa, M. Tatsuno, H. Tsuchihashi, J. Chromatogr. B 689 (1997) 327.
[55] M. Minami, D.-M. Hui, M. Katsumata, H. Inagaki, C.A. Boulet, J. Chromatogr. B 695 (1997) 237.
[56] J. Enqvist, M. Rautio (Eds.), Trace Analysis of Chemical Warfare Agents. 1. An Approach to the Environmental Monitoring of Nerve Agents, The Ministry for Foreign Affairs of Finland, Helsinki, 1981.
[57] M.L. Shih, J.R. Smith, J.D. McMonagle, T.W. Dolzine, V.C. Gresham, Biol. Mass Spectrom. 20 (1991) 717.
[58] S.-Å. Fredriksson, L.-G. Hammarström, L. Henriksson, H.-Å. Lakso, J. Mass Spectrom. 30 (1995) 1133.
[59] A. Miki, M. Katagi, H. Tsuchihashi, M. Yamashita, J. Anal. Toxicol. 23 (1999) 86.
[60] R.M. Black, I. Morton, J. Riches (manuscript in preparation).
[61] R.M. Black, R.W. Read, in: R.A. Meyers (Ed.), Encyclopedia of Analytical Chemistry, Wiley, Chichester, 2000, p. 1007.
[62] P.C. Bossle, J.J. Martin, E.W. Sarver, H.Z. Sommer, J. Chromatogr. 267 (1983) 209.
[63] M.C. Roach, L.W. Ungar, R.N. Zare, L.M. Reiner, D.L. Pompliano, J.W. Frost, Anal. Chem. 59 (1987) 1056.
[64] W.R. Creasy, Am. Soc. Mass Spectrom. 10 (1999) 440.
[65] R.M. Black, K. Brewster, R.J. Clarke, J.L. Hambrook, J.M. Harrison, D.J. Howells, Xenobiotica 22 (1992) 405.
[66] R.M. Black, R.W. Read, Xenobiotica 25 (1995) 167.
[67] N.V. Beck, W.A. Carrick, D.B. Cooper, B. Muir, J. Chromatogr. A 907 (2001) 221.
[68] J. Enqvist, M. Rautio (Eds.), Systematic Identification of Chemical Warfare Agents. B.4 Identification of Precursors of Warfare Agents, Degradation Products of Non-Phosphorus Agents, and Some Potential Agents, The Ministry for Foreign Affairs of Finland, Helsinki, 1983.
[69] E.R.J. Wils, A.G. Hulst, Fresenius J. Anal. Chem. 321 (1985) 471.
[70] R.M. Black, I. Holden, M. Reid, in: Proceedings of the Seventh International Symposium on Protection Against Chemical and Biological Warfare Agents, Stockholm, June, 2001.
[71] B.A. Tomkins, G.A. Sega, J. Chromatogr. A 911 (2001) 85.
[72] K. Schoene, H.-J. Bruckert, J. Steinhanses, A. Krönig, Fresenuis J. Anal. Chem. 348 (1994) 364.
[73] P.A. D'Agostino, L.R. Provost, J. Chromatogr. 589 (1992) 287.
[74] R.M. Black, R.W. Read, J. Chromatogr. 449 (1988) 261.
[75] R.M. Black, R.W. Read, J. Chromatogr. B 665 (1995) 97.
[76] E.M. Jakubowski, C.L. Woodard, M.M. Mershon, T.W. Dolzine, J. Chromatogr. 528 (1990) 184.
[77] E.M. Jakubowski, F.R. Sidell, R.A. Evans, M.A. Carter, J.R. Keeler, J.D. McMonagle, A. Swift, J.R. Smith, T.W. Dolzine, Toxicol. Methods 10 (2000) 143.
[78] E.R.J. Wils, A.G. Hulst, J. van Laar, J. Anal. Toxicol. 12 (1988) 15.

[79] R.M. Black, R. W Read, J. Chromatogr. A 759 (1997) 79.
[80] R. M Black, R.W. Read, J. Chromatogr. 558 (1991) 393.
[81] T. Durst, in: Organic Sulphur Compounds, Comprehensive Organic Chemistry, Vol. 3, 1989, p. 121.
[82] M.C. Nishimura, P. Jacob, M.E. Cassel, L.H. Pitts, Drug Metab. Dispos. 17 (1989) 224.
[83] R.M. Black, R.J. Clarke, R.W. Read, J. Chromatogr. 558 (1991) 405.
[84] A. Fidder, D. Noort, A.L. de Jong, H.C. Trap, L.P.A. de Jong, H.P. Benschop, Chem. Res. Toxicol. 9 (1996) 788.
[85] R.M. Black, R.J. Clarke, J.M. Harrison, R.W. Read, Xenobiotica 27 (1997) 499.
[86] M. Törnqvist, J. Mowrer, S. Jensen, L. Ehrenberg, Anal. Biochem. 154 (1986) 255.
[87] D. Noort, A.G. Hulst, H.C. Trap, L.P.A. de Jong, H.P. Benschop, Arch. Toxicol. 71 (1997) 171.
[88] C. Giachetti, Chromatographia 48 (1998) 443.
[89] R. Haas, UWSF Z. Umweltchem. Ökotox. 10 (1998) 198.
[90] J. Chen, L. Zhou, G. Qu, Chin. J. Anal. Chem. 29 (2001) 1276.
[91] 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.
[92] W.K. Fowler, D.C. Stewart, D.S. Weinberg, E.W. Sarver, J. Chromatogr. 558 (1991) 235.
[93] T.P. Logan, J.R. Smith, E.M. Jakubowski, R.E. Nielson, Toxicol. Methods 9 (1999) 275.
[94] L. Zhou, G. Qu, H. Gu, Chin. J. Anal. Chem. 24 (1996) 125.
[95] A. Fidder, D. Noort, A.G. Hulst, L.P.A. de Jong, H.P. Benschop, Arch. Toxicol. 74 (2000) 207.
[96] B. Szostek, J. Aldstadt, J. Chromatogr. A 807 (1998) 253.
[97] D.R. Killelea, J.H. Aldstadt, J. Chromatogr. A 918 (2001) 169.
[98] B.A. Tomkins, G.A. Sega, C.-H. Ho, J. Chromatogr. A 909 (2001) 13.
[99] J.V. Wooten, D.L. Ashley, A.M. Calafat, J. Chromatogr. B 772 (2002) 147.
[100] X. Chaudot, A. Tambuté, M. Caude, J. Chromatogr. A 888 (2000) 327.
[101] K. Schoene, J. Steinhanses, H.-J. Bruckert, A. König, J. Chromatogr. 605 (1992) 257.
[102] R. Haas, Environ. Sci. Pollut. Res. 5 (1998) 2.
[103] B. Muir, J. Chromatogr. A 977 (2002) 251.
[104] R. Haas, A. Krippendorf, Environ. Sci. Pollut. Res. 4 (1997) 123.
[105] R. Haas, T.C. Schmidt, UWSF Z. Umweltchem. Ökotox. 9 (1997) 183.
[106] R. Haas, UWSF Z. Umweltchem. Ökotox. 9 (1997) 123.
[107] R. Haas, T.C. Schmidt, K. Steinbach, E. von Löw, Fresenius J. Anal. Chem. 361 (1998) 313.
[108] K. Schoene, H.-J. Bruckert, H. Jürling, J. Steinhanses, J. Chromatogr. A 719 (1996) 401.
[109] G.D. Byrd, L.T. Sniegoski, V.E. White, J. Res. Natl. Bur. Stand. (US) 93 (1988) 293.
[110] G.D. Byrd, L.T. Sniegoski, V.E. White, Report 1987 Order No. AD-A191746.
[111] H. Gu, G. Cai, L. Zhou, Y. Zhou, Chin. J. Anal. Chem. 22 (1994) 826.
[112] F.G. Lattin, U.D. Mehta, E.M. Jakubowski, L. Tinsley, D.G. Paul, in: Proc ERDEC Sci. Conf. Chem. Biol. Def. Res, 1994, p. 181.
[113] S.E. Massil, D. Ovadia, J. Chromatogr. 538 (1991) 435.
[114] K. Schoene, H.-J. Bruckert, J. Steinhanses, Fresenius J. Anal. Chem. 345 (1993) 688.
[115] J.P. Hendershott, Am. Ind. Hyg. Assoc. J. 47 (1986) 742.
[116] W.S. Wu, V.S. Gaind, Analyst 18 (1993) 1825.
[117] R.J. Rando, H.G. Poovey, S.-N. Chang, J. Liquid Chromatogr. 16 (1993) 3291.
[118] OSHA Analytical Methods Manual Part 1, Vol. 3, Method No. 61; Salt Lake City; available at: http://www.nioshdbs\oshameth\org061\061.htm.
[119] M. Gmeiner, P.G. Demertzis, R. Franz, Dtsche Lebensm. -Tundschau 94 (1998) 41.
[120] O. Gyllenhaal, J. Vessman, J. Chromatogr. 435 (1988) 259.
[121] S. Quick, B. Muir, Poster presentation, 2nd Mass Spectrometry Conference Applied to Chemical and Biological Warfare Agents, University of Warwick, 14–17 April 2002; manuscript in preparation.
[122] National Institute for Occupational Safety and Health (NIOSH) Manual of Analytical Methods, 1994. Method number 6010, issue 2: Hydrogen cyanide, US Dept. of Health and Human Services, Public Health Service, Cincinnati, OH.
[123] A.M. Calafat, S.B. Stanfill, J. Chromatogr. B 772 (2002) 131.
[124] H. Shiono, C. Maseda, A. Akane, K. Matsubara, Forensic Toxicol. 12 (1991) 50.
[125] H.L. Wu, S.H. Chen, S.-J. Lin, W.-R. Hwang, K. Funazo, M. Tanaka, T. Shono, J. Chromatogr. 269 (1983) 183.
[126] K. Funazo, M. Tanaka, K. Morita, M. Kamino, T. Shono, J. Chromatogr. 354 (1986) 259.
[127] S.-H.- Chen, S.-M. Wu, H.-S. Kou, H.-L. Wu, J. Anal. Toxicol. 18 (1994) 81.
[128] S. Kage, T. Nagata, K. Kudo, J. Chromatogr. B 675 (1996) 27.
[129] M.J. Sclimenti, C.J. Hwang, S.W. Krasner, in: Water Disinfection and Natural Organic Matter, American Chemical Society, 1996, p. 126.
[130] T. Noy, P. Fabian, R. Borchers, F. Janssen, C. Cramers, J. Rijks, J. Chromatogr. 393 (1987) 343.
[131] Y.B. Zeyfman, E.G. Ter-Gabrielyan, N.P. Gambaryan, I.L. Knunyants, Russ. Chem. Rev. 53 (1984) 256.
[132] C.M. Timperley, J. Fluorine Chem. 94 (1999) 37.
[133] J.F. Weimaster et al., J. Chem. Tech. Biotechnol. 64 (1995) 115.