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Identification of chemical-weapons-related compounds in decontamination solutions and other matrices by multiple chromatographic techniques

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Abstract

Several applications have arisen for the high confidence identification of chemical weapons agents and related compounds in toxic waste or other complex matrices. Three examples of analysis of agents or byproducts are presented: GA (ethyl N,N-dimethylphosphoramidocyanidate, or tabun) and synthetic byproducts in an complex organic liquid solution; HD [bis(2-chloroethyl)sulfide, or mustard gas] in a decontamination solution; and VX (S,2-diisopropylaminoethyl ethyl methylphosphonothioate) and a toxic hydrolysis product (S,2-diisopropylaminoethyl methylphosphonothioic acid, also known as EA-2192). Multiple chromatographic methods were used to unequivocally identify low concentrations of agent with high confidence, including results from gas chromatographic analysis with mass spectral detection (with electron impact or chemical ionization), infrared detection, atomic emission detection and flame photometric detection, as well as liquid chromatography–tandem mass spectrometry.

Keywords: Warfare agents

1. Introduction

The Chemical Weapons Convention [1] (CWC) and USA laws create several mandates that involve trace analysis of chemical weapons (CW) agents. One application is CWC treaty verification. For this purpose, the detection of trace levels of CW agents is required in a variety of environmental matrices, including soil, water or wipes. Agent may also have to be identified with high confidence in complex industrial process mixtures, which could potentially contain chemically related pesticides or other products. Methods have been published for this purpose [2]. These methods have been tested in a series of round robin exercises or inter-laboratory comparison tests in laboratories of participating nations, sponsored by the Provisional Technical Secretariat (PTS) of the Organization for the Prohibition of Chemical Weapons, The Hague, Netherlands. Some results of these exercises have been published by various laboratories [3–7]. Results from sample analysis of actual inspections for verification of CW bans have also been published [8].

Destruction methods are under study for chemical neutralization of stockpiled and nonstockpiled chemical weapons [9,10]. These processes use liquid-phase chemical reactions with a decontamination

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reagent, or other processes, to chemically destroy the agent by converting it to products of lower toxicity [11–13].

One of the primary requirements for the certification of a neutralization process is the demonstration that CW agents have been completely destroyed in the reaction mass that is produced. For neutralization processes, any residual agent must be detected in a complex liquid reaction mass which includes high concentrations of reaction products and byproducts. This matrix can introduce many interferences. In some cases, detection limits as low as 20 ppb are desirable, although these regulatory requirements have not necessarily been established. A low residual agent concentration is required by state or federal regulatory agencies to permit the treatment of the reactor waste products with fewer restrictions on handling, transportation and treatment.

The liquid samples in decontamination solutions, in matrices such as caustic water solutions, bleach or organic liquids, can be difficult to prepare using conventional methods such as solvent extraction or solid-phase extraction. Even after preparation, the solutions contain a variety of compounds that are related to the CW agents themselves. Weapons-grade agents have relatively low purity and can contain many types of byproducts and starting materials that were generated when the agent was manufactured. The agents may also have been stored for decades in steel containers, giving ample time for slow reactions to produce other compounds. After the mixture is neutralized, the related compounds may remain in solution or may react with the decontamination reagent. The decontamination products of the CW agents are present at very high concentrations. Thus, the final decontamination mixture is a very complex combination of compounds related to the CW agent, some at high concentrations and many more at trace levels. To make the mixture even more complex, the feedstock can sometimes consist of mixtures of weapons-grade agents.

The analytical techniques for identifying the CW agents in these solutions must be robust enough to discriminate between the agent itself and the myriad of related compounds. In some cases, straightforward techniques such as gas chromatography with mass spectral detection are adequate. However, it is necessary to do careful methods development and methods validation for every method and every matrix that must be analyzed before the analytical method can be released for routine sample analysis.

This publication discusses three case studies that demonstrate the advantages of using multiple chromatographic or spectroscopic methods for identifying targeted CW compounds in complex matrices with a high degree of confidence. The case studies illustrate difficult analytical problems that can be associated with decontamination or other CW agent analysis. A study of GA led to a requirement for a method for analysis of trace byproducts in a complex matrix using gas chromatography (GC)–tandem mass spectrometry (MS–MS). Analysis of HD using GC with atomic emission detection (AED) and MS detection was used to identify a similar interference and validate an analytical method. Detection of VX and an ionic hydrolysis product shows the necessity of using multiple analytical techniques, including GC with MS and infrared detection (IRD) and liquid chromatography (LC)–MS–MS.

2. Experimental

A variety of spectroscopic techniques and instrumentation were available for these studies at the Edgewood Research, Development, and Engineering Center (ERDEC), Aberdeen Proving Ground-Edgewood Area, MD, USA. The following instruments were used in this study: Hewlett-Packard 5890 Series II+ gas chromatograph; Hewlett-Packard 1090M high-performance liquid chromatograph; Hewlett-Packard 5971 or 5972 mass-selective detector with electron impact ionization (EI) or with chemical ionization (CI); Hewlett-Packard 5989A MS Engine with Analytica of Branford atmosphere pressure chemical ionisation (APCI) source; Finnigan TSQ-7000 tandem mass spectrometer with APCI source or EI/CI source; Hewlett-Packard dual flame photometric detection (FPD) system; Bio-Rad (formerly Hewlett-Packard) 5965B infrared detector; and Hewlett-Packard 5921A AED system.

A variety of standard compounds were used as reference materials. Some were purchased commercially, but agent standards were obtained from the ERDEC Certified Army Standard Analytical Reference Material (CASARM) program. The com-

Table 1
Identities and sources of standard materials

Abbreviation	Compound name	CAS number	Source
GA	Ethyl N,N-dimethylphosphoramidocyanidate	77-81-6	CASARM
DMPDC	N,N,-Dimethylphosphoramidic dichloride	683-85-2	In house, ERDEC
DEDMPA	O,O-diethyl N,N-dimethylphosphoramidate	2404-03-7	In house, ERDEC
TEP	Triethylphosphate	78-40-0	Aldrich
EBDMPA	O-Ethyl Bis-(N,N-dimethyl) phosphoramidate	2404-65-1	In house, ERDEC
EDMPACl	O-Ethyl N,N-dimethyl phosphoramidic chloride	2510-93-2	In house, ERDEC
BDMPAC	Bis-(N,N-dimethyl)phosphoramidocyanidate	14445-60-4	In house, ERDEC
VX	S,2-Diisopropylaminoethyl ethyl methylphosphonothioate	50782-69-9	CASARM
EA-2192	S,2-diisopropylaminoethyl methylphosphonothioic acid	73207-98-4	CASARM
HD	Bis(2-chloroethyl)sulfide	505-60-2	CASARM

pounds, abbreviations, CAS numbers and source of standards are listed in Table 1.

3. Results and discussion

3.1. GA byproducts

The CW agent GA has been reported to contain a significant number of impurities [14–17]. This is a result of the way GA is synthesized [14]. The impurities can vary from sample to sample depending on the synthetic or purification methods, so the presence of these impurities may provide information about the synthesis of the GA if found in a treaty-related inspection [18], or they may be present in GA decontamination solutions. Some of the compounds are relatively easy to identify using techniques such as GC–FPD or GC–MS. However, some are chemically very similar, and a considerable amount of effort is needed to develop a method for resolving and identifying all of them. For example, analyzing two particular impurities required the development of a GC–MS–MS method. This example illustrates the range of techniques that may be needed for high confidence, sensitive identifications of analytes.

The methods development was performed on a sample provided by the PTS as part of an Interlaboratory Comparison Test. Analysis of the sample, which was in a chlorobenzene solvent matrix, using phosphorus GC–FPD showed that this sample had three major peaks and several low-concentration, phosphorus-containing compounds. Using a HP-5 GC column, peaks for two of the three major components coeluted, but they could be identified by GC–MS via comparison to an EI library as GA, N,N,-dimethylphosphoramidic dichloride (DMPDC) and O,O-diethyl N,N-dimethylphosphoramidate (DEDMPA).

A number of chromatographic and spectroscopic techniques were used to identify the three major peaks, as is the usual practice for this type of exercise [4–7]. The GC conditions were optimized. Analysis on an HP-5 compared to an HP-1301 column showed that on the HP-5 column, the GA, DEDMPA and a trace analyte coeluted, so further analysis was done using a HP-1301 column. The identifications were confirmed by using GC–CI-MS to obtain $M+H^+$ ion signals. GA and DEDMPA were identified by GC–IRD from interpretation of IR peaks and by comparisons to the IR spectra of standards. DMPDC co-eluted with interference peaks and the solvent tail, so it was impossible to obtain a clean IR spectrum of the compound. The elements in the P-containing compounds were identified by GC–AED, although the presence of Cl in DMPDC was difficult to confirm by GC–AED because of carryover of the huge Cl signal from the chlorobenzene solvent. The three compounds were also confirmed by one- and two-dimensional NMR by our laboratory [19] and others [20]. The chromatographic and spectroscopic results were compared to standards for each of the three compounds.

As a result of the comparison of these complementary techniques, the identifications of the three main peaks were established with extremely high confi-

dence, in spite of the significant signals from background interferences. Quantitation of the compounds gave concentrations of 50–100 ppm.

Identification of the trace impurities was considerably more difficult. They could not be positively identified in the sample using GC–MS. Analysis of a CASARM GA standard with GC–MS with EI and CI indicated potential identifications as GA synthetic byproducts. GC–MS–MS with CI was used to identify the minor peaks in the sample from interpretation of the MS–MS fragmentation spectra in comparison to the anticipated synthetic byproducts, and from comparison to the impurity peaks in the GA standard. Four of the trace analytes in the sample were assigned as triethylphosphate (TEP), O-ethyl bis-(N,N-dimethyl) phosphoramidate, O-ethyl N,N-dimethyl phosphoramidic chloride and bis-(N,N-dimethyl)phosphoramidocyanidate.

The conditions that were used for the GC–MS–MS detection using chemical ionization are as follows: Hewlett-Packard 5980 Series II+ (GC); Finnigan TSQ-7000 (MS); He carrier gas at a flow-rate of 1.0 ml/min at 35°C with electronic pressure programming; GC temperature ramp of 35°C (for 1 min) to 280°C at 15°C/min; GC column, Hewlett-Packard HP-1301, 30 m×0.25 mm I.D., 0.25 μm film thickness; splitless injection volume of 1 μl using manual injection; CI gas of methane at 2500 mTorr; collision gas of argon at 2.5 mTorr; with the Q1 quadrupole fixed at the parent $M+H^+$ mass; and the Q3 quadrupole either scanned or fixed at a selected fragment mass for multiple reaction monitoring (1 Torr=133.322 Pa).

A full scan MS–MS fragmentation spectrum of each parent ion, using the GA standard and the other standards that were available, was obtained in order to find the major fragment peaks. Once the major MS–MS fragment ions and the retention times for each of the compounds were identified, a multiple reaction monitoring (MRM) sequence was set up on the Finnigan TSQ-7000. This sequence set both the parent ($M+H^+$) ion on the first quadrupole, and alternated between at least three fragment ions on the third quadrupole during an appropriate GC retention time window. This approach does not provide quite as much confidence for identifications as scan mass spectra, but it provides reasonably confident identifications in a complex matrix and is more sensitive for a scanning quadrupole instrument. The retention times and MRM ions for the analytes are given in Table 2. The GA standard, other standards and the organic liquid sample were analyzed using the same conditions.

The most difficult of the minor analytes to identify in the sample by this method was TEP. Examination of the GC–CI-MS chromatogram indicated that TEP could be resolved from GA and DEDMPA on an HP-1301 column, although it still eluted quite near DEDMPA. Unfortunately, DEDMPA has a molecular mass of 181 u, giving a ^{13}C isotope peak for the $M+H^+$ ion at 183 u, the same $M+H^+$ ion mass as TEP. In addition, the ^{13}C ion has isotopically labelled fragment ions at 155 and 127, as does TEP. The GC retention time showed some peak shifting and broadening in the complex matrix compared to a standard, so a retention time match was not completely conclusive. Since DEDMPA was present in concentrations of 100–1000 times higher than TEP

Table 2
GC retention times, parent ions and fragment ions for analysis of compounds related to GA

Compound abbreviation	GC retention time (min)	Parent ion $(M+H)^+$ (m/z)	Fragment ions (m/z)
GA	8.3	163	135, 126, 117, 108
DMPDC	7.3	162	144, 126, 108
DEDMPA	8.13	182	154, 126, 108
TEP	8.2	183	155, 127, 99
EBDMPA	8.87	181	153, 126, 46
EDMPACl	7.95	172	144, 126, 108
BDMPAC	9.82	162	135, 117, 44
[^{13}C]DEDMPA[a]	8.13	183	155, 127, 126

[a] See Section 3.1 for explanation of this analyte.

in the organic sample, the signal for TEP was difficult to confirm conclusively, even though TEP gave the largest signal of the trace analytes.

A reasonable identification for TEP was made by adding the 126 u fragment ion to the MRM sequence. This provided one fragment at 99 u that arose from TEP but not from [^{13}C]DEDMPA, and one ion at 126 u from [^{13}C]DEDMPA that was not present for TEP. Fig. 1 shows the MRM traces for each fragment ion channel in the top panel, which clearly show that peaks for the two different compounds are present. A trace for a TEP standard is shown for comparison in the bottom panel.

This example shows the hierarchy of analytical methods that can be used to characterize a sample, depending on the matrix, the analyte concentration, and the sensitivity that is required. Some of the compounds were routinely identified using techniques such as GC–FPD, using GC retention time matches, or GC–MS, using electron impact mass spectral library searches. Some of the compounds coeluted, which could interfere with both of these methods. Better confidence was obtained by using GC columns with different phases or thicknesses to resolve the peaks and by using alternative detectors, such as AED or IRD. However, some of the analytes were chemically very similar and were present in low (<1 μg/ml) concentrations, and additional effort was needed to develop a GC–MS–MS method for identifying them. Finally, the positive identification of TEP required additional validation to demonstrate that the GC–MS–MS method was adequate for positive identification of TEP in the presence of DEDMPA. Each of these extra steps required additional labor and more expensive instrumentation.

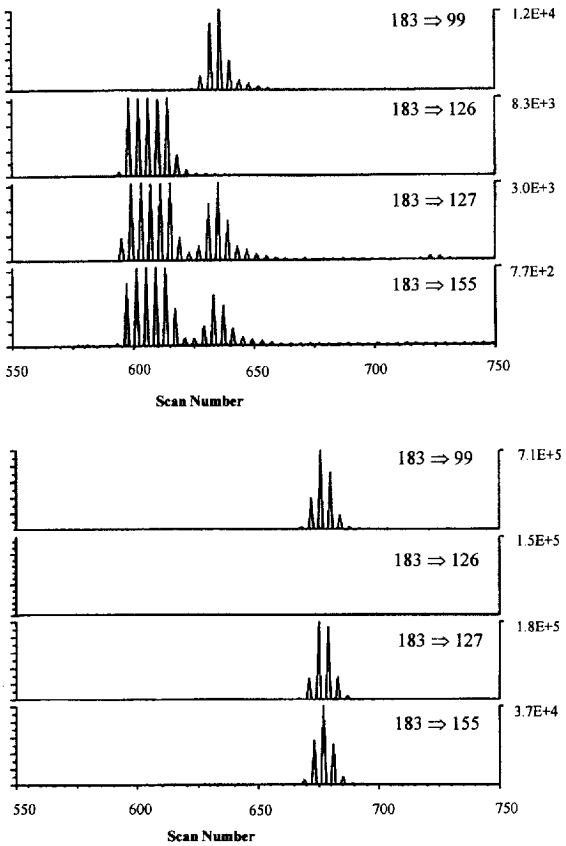

Fig. 1. Multiple reaction monitoring traces for the M+H$^+$ parent ions at 183 u of TEP to the fragment ion channel at 99, 126, 127 and 155 u. Top panel: organic liquid sample which contains both TEP and [^{13}C]DEDMPA; bottom panel: TEP standard. The jagged appearance of the plot is an artifact of the Finnigan software for MRM plots, so each triangle represents a single data point.

Obviously, it would be preferable to use only the simplest and least expensive analytical technique for a given application, but in a complex matrix, the difficulty of the analysis can depend to a critical degree on accidental interferences and on the required sensitivity. These factors can greatly affect the cost of the determinations.

3.2. Interference with HD in a decontamination solution

Studies are in progress in our laboratory concerning the reactions of CW agents in basic monoethanolamine (MEA) as one type of chemistry for CW neutralization. As part of the study of HD (distilled sulfur mustard agent) reactions in MEA, a method was developed to detect residual HD in the reaction mass. Care had to be taken to resolve an interfering compound in the decontamination products that could potentially be mistaken for HD on both the GC–AED and the GC–MS.

The reaction was performed by the addition of HD to a 10 times by mass excess of a mixture of 10% aqueous NaOH and MEA. Analysis of a terminal sample of the reaction mass using GC–AED was interpreted to indicate the presence of several ppm of HD, using detection by the S and Cl emission

channels and comparison of the retention time to a standard solution. Analysis of the same samples by GC–MS in single ion monitoring (SIM) mode by two different groups with monitoring of the major fragment ions of HD at mass 109, 111, 158 and 160 produced a peak in the total ion chromatogram (TIC) at a corresponding retention time as the GC–AED. The peak was still observed from analysis of the reaction mass several days after the reaction was completed.

The analyses showed the presence of a significant peak near the HD retention time which was interpreted to be HD, due to the agreement of the GC–AED and GC–MS results. Of course, the assumption was made that the product did not contain any other compound that was similar enough to meet these criteria, since such a compound had not been reported previously. However, given the high reactivity of HD in basic MEA, it seemed unlikely that HD could survive in the reaction mass for an extended time, so it was clear that reexamination of the method was needed.

The analytical method was modified to improve the GC resolution, and the sample was reanalyzed with the mass spectrometer in scan mode. The analysis conditions were as follows: Hewlett-Packard 5980 Series II+ (GC); Hewlett-Packard 5921 (AED); Hewlett-Packard 5972 (MS); He carrier gas with a flow-rate of 35 cm/s with electronic pressure programming; GC ramp of 50°C (hold for 3 min) to 150°C at 10°C/min, and to 280°C at 70°C/min; column, Hewlett-Packard HP-5, 30 m×0.25 mm I.D., 0.25 μm film thickness; 1 μl splitless injection; MS ionization in electron impact ionization mode with 70 eV electrons; MS scan mode from 20 to 350 u with 1.3 scans/s; MS SIM mode for ions of *m/z* 109, 111, 158 and 160, with a dwell time of 100 μs/ion; and AED at 181 nm for the S line and 479 nm for the Cl line. Sample preparation was done by adding excess KCl to 10 ml of reaction mass, followed by extraction of the saturated solution with 2×2 ml of hexane.

By examining the scanned mass spectra and SIM ion ratios, it was immediately obvious that an interference accounted for the apparent presence of HD. The mass spectra for the suspect peak had a significant ion at 156 as well as 158. Analysis by GC–CI-MS confirmed the molecular mass was 156 u for two ^{35}Cl atoms, with two corresponding ^{37}Cl isotope peaks at 158 and 160 u, respectively. Reexamination of the previous SIM results showed that the ion ratios did not correspond to HD. Fig. 2 shows the SIM trace of a unspiked sample and a HD-spiked sample, including the extracted ion chromatograms for each SIM ion. Fig. 3 shows a comparison of the scan mass spectra with EI ionization for the interference and HD. Although the interference has a considerably different scan spectrum, including large peaks at 121 and 156 u which are not present in the HD spectrum, the concentration is high enough that the 158 peak gives a significant signal in SIM mode compared to the low-concentration (50 ng/ml) HD spike.

Since the molecular mass of HD is 158, a compound with molecular mass of 156 with an S and two Cl atoms suggests either a vinyl analog of HD or a cyclic species. Since the linear vinyl compound is expected to be reactive with MEA, the most likely

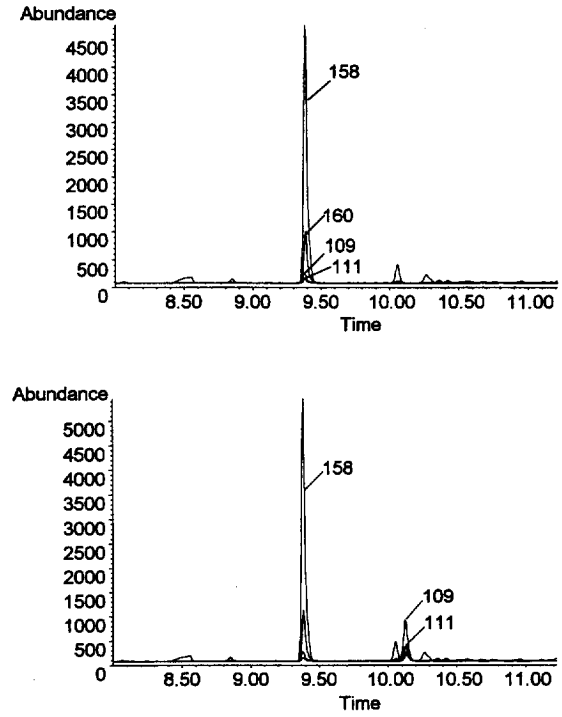

Fig. 2. The SIM trace of a MEA decontamination sample of HD. Top trace: decontamination solution; bottom trace: the same solution spiked with HD. The extracted ion chromatograms for each SIM ion are labeled with the mass.

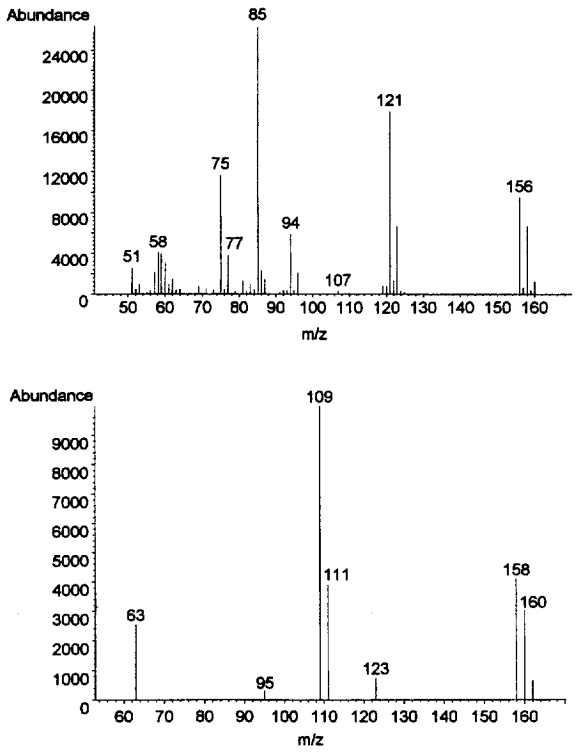

Fig. 3. A comparison of the scan mass spectra with EI ionization for the interference and HD. Top panel: interference tentatively assigned as dichlorotetrahydrothiophene or an isomer; bottom panel: library spectrum of HD.

candidate for the interference is a sulfur heterocycle, dichlorotetrahydrothiophene. Unfortunately, the concentration of this compound was too low to obtain an infrared spectrum to confirm the identification or to assign an isomer, and no standards have been made.

In order to improve the HD detection method to avoid misidentifying this interference in routine analysis, it is essential to specify ion ratios as well as SIM TIC peaks. For HD, the ratio of 111 to 109 was 0.38, of 158 to 109 was 0.28, and of 160 to 109 was 0.2. The recovery of HD using a hexane extract from a spiked solution was found to be 73%, and the HD detection limit was 50 ng/ml.

3.3. Identification of VX and EA-2192

The nerve agent VX can be decontaminated with a variety of reagents. In some reactions in the presence of hydroxide, a hydrolysis product of VX can form: S,2-diisopropylaminoethyl methylphosphonothioic acid (known as EA-2192 in the ERDEC numbering system). This hydrolysis product is nearly as toxic as VX [12].

An important part of the certification of a decontamination procedure is the analytical measurements to demonstrate that VX has been destroyed in the reaction conditions that were used. It may also be necessary to detect EA-2192. Several methods were studied for analysis of VX and EA-2192, including LC–MS–MS and derivatization with GC–MS and GC–IRD.

Analysis of VX by GC methods is fairly routine. VX has a tertiary amine group which is protonated at low pH, but it can be solvent extracted for GC analysis from aqueous or polar solvents at high pH with a solvent such as methylene chloride. The analysis is more difficult in decontamination solutions because of the presence of decontamination products, but VX can be detected by GC–EI-MS by careful control of the GC conditions to optimize the resolution for a particular matrix. The specificity can be improved by using GC–CI-MS for VX detection. Chemical ionization with methane provides for the formation of the $M+H^+$ ion at mass 268.2 u, which has less interference. Additional selectivity can be obtained with MS–MS. The most abundant fragment ion of VX is at 128 u, and this fragment ion channel is sufficient to provide good selectivity and sensitivity for VX. Operation in single reaction monitoring (SRM) mode provides good sensitivity and eliminates VX interferences for most matrices.

EA-2192 is more difficult to analyze than VX. This compound cannot be extracted from polar solvents and is not volatile, so it cannot be analyzed directly via GC methods. EA-2192 has the property of being a zwitterion in aqueous solution, since it has both a phosphonothioic acid group and a tertiary amine group. At neutral pH ranges, both of these groups carry a charge, and one of the groups is charged at all pH values.

EA-2192 can be analyzed by LC–MS using either a reversed-phase or a normal-phase (weak anion-exchange) separation. The use of LC–MS–MS improves the selectivity and sensitivity. Using APCI ionization, the $M+H^+$ ion is formed, which can be fragmented to a predominant 128 u ion. A detection limit of 50–100 ppb can typically be achieved in

standards and some types of decontamination solutions.

Both VX and EA-2192 can be detected in the same LC–MS–MS run using the reversed-phase separation method with a gradient [21]. Fig. 4 shows a chromatogram for a mixed standard of 100 ng/ml VX and 1000 ng/ml of EA-2192. The following conditions were used: Hewlett-Packard 1090M (HPLC); Finnigan TSQ-7000 with APCI source; column, ODS Hypersil, 100×2.1 mm; flow-rate, 0.4 ml/min; and injection volume, 10–25 μl. The gradient conditions are: (A=100% aqueous 0.05 *M* ammonium acetate, B=acetonitrile), initial, 100% A; 2 min, 100% A; 3 min, 60% A, 40% B; 5 min, 40% A, 60% B; 15 min, 100% B; 20 min, 100% A (to reinitialize column). The gradient conditions were optimized for minimum run times. The mass spectrometer conditions are: APCI heater, 400°C; corona current, 5 μA; capillary temperature, 200°C; MS–MS mode, multiple reaction monitoring, positive ion detection; MS–MS ions for VX, 268 u (parent) to 128 u (fragment); MS–MS ions for EA-2192, 240 u (parent) to 128 u (fragment); confirmation channel for VX, 268 u (parent) to 167 u (fragment); confirmation channel for EA-2192, 240 u (parent) to 162 u (fragment); collision energy (COFF), −20 V; scan time, 0.5 s/scan; collision gas, argon at 2.5 mTorr; multiplier, 1700 V (or determined by autotune). Typically, the confirmation ion channels are not needed, and they decrease the sensitivity of the method by about a factor of ten. The sample preparation consisted of mixing the caustic reaction mass with glacial acetic acid to neutralize the base (typically 1 part acid to 1 part sample) and diluting by mass to about 1:5 in aqueous 0.1 *M* ammonium acetate buffer to be compatible with the LC mobile phase. This dilution is necessary since the EA-2192 chromatography is very sensitive to the sample solvent.

Another approach to the analysis of EA-2192 is to chemically derivatize the compound so it is volatile for GC analysis. This capability is particularly important for the analysis of samples that may be generated in the field, since most current fieldable instruments are based on GC analyses. Attempts to derivatize EA-2192 to form the trimethylsilyl (TMS) or acetyl derivatives using appropriate derivatizing reagents have not been successful. TMS derivatives of alkyl methylphosphonic acids and alkyl methylphosphonothioic acids can be made routinely [22], but the TMS derivative of EA-2192 has not been observed.

It is possible to form the methyl derivative of EA-2192 (the methyl analog of VX) by derivatizing

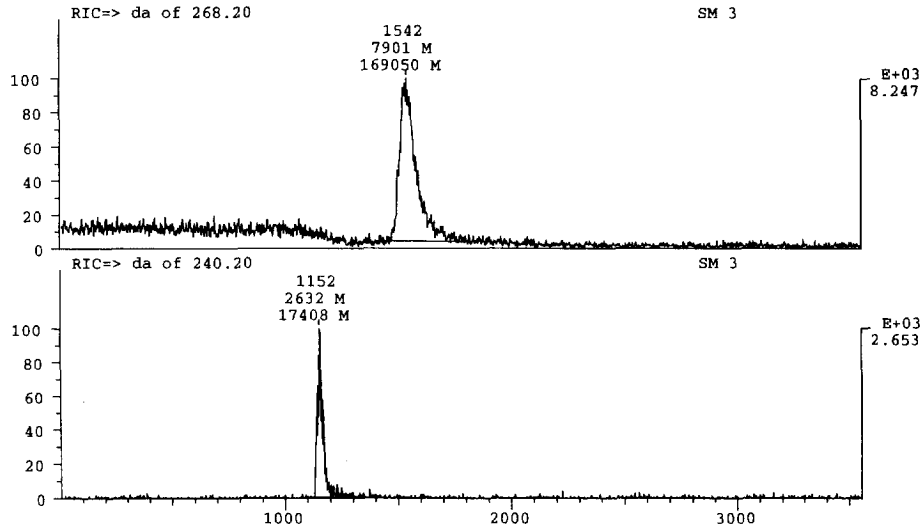

Fig. 4. LC–MS–MS chromatogram for a mixed standard of 100 ng/ml VX and 1000 ng/ml of EA-2192. Top panel: MS–MS fragment channel for VX using 268 to 128 u fragmentation; bottom panel: MS–MS fragment channel for EA-2192 using the 240 to 128 u fragmentation.

with trimethylphenylammonium hydroxide (TMPAH). This reagent has been reported for the derivatization of alkyl methylphosphonic acids [23,24] and other acids [25,26] in hot GC injection ports. It has been proposed that the TMPAH cation forms an ion pair with the acid anion in solution, and the ion pair decomposes in the hot injector port to form the methyl derivative [23].

The formation of the methyl derivative of EA-2192 was confirmed with several different detectors, including GC–MS, GC–FPD and GC–IRD. The GC–FPD shows a peak for both P and S. Fig. 5 shows the GC–MS spectra using chemical ionization comparing VX to the methyl derivative of EA-2192. As expected, the $M+H^+$ ions are observed at 268 u for VX and at 254 u for the EA-2192 derivative. Analogous fragmentation to the $(M+H-16)^+$ and the 114 and 128 u peaks is observed.

Fig. 6 shows a comparison of the GC–IRD spectra of the EA-2192 derivative and VX. These spectra are very similar except for a shift in the bands below 1100 cm^{-1}. In the case of VX, the very strong band at 1040 cm^{-1} and a second moderately strong band at 947 cm^{-1} are indicative of the P–O–

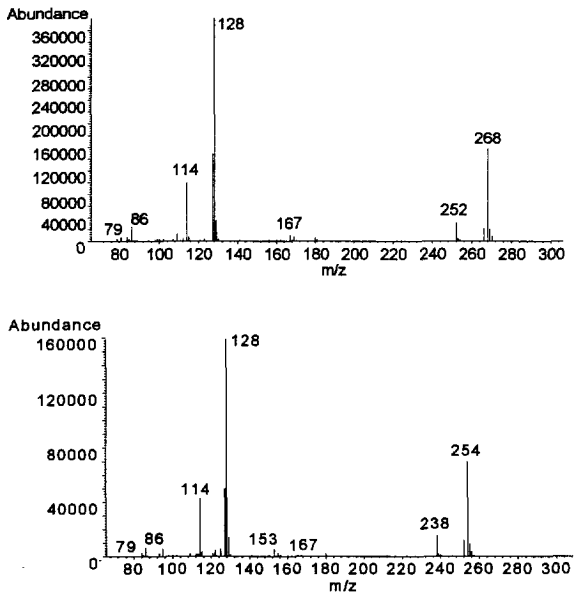

Fig. 5. GC–MS mass spectra using chemical ionization comparing VX (top panel) to the methyl derivative of EA-2192 (bottom panel), made using TMPAH. The $M+H^+$ ions are 268 and 254 u, respectively.

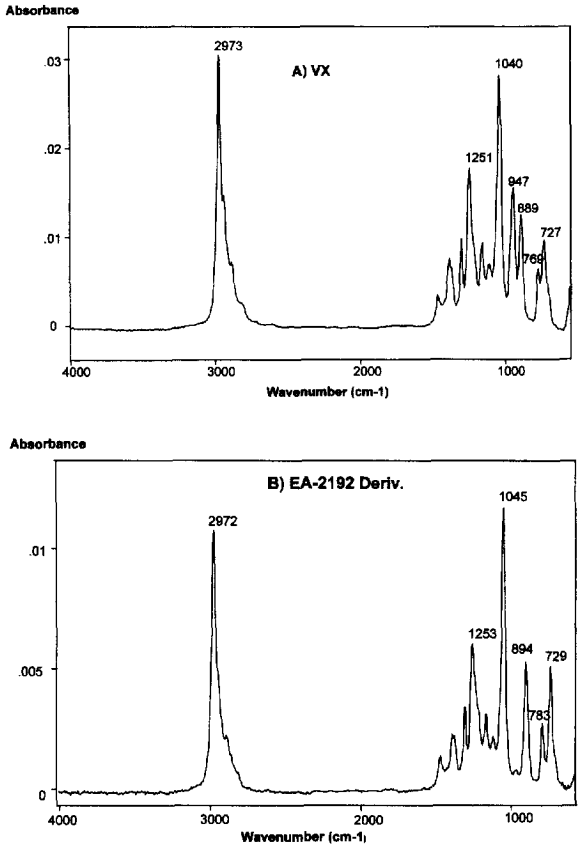

Fig. 6. A comparison of the GC–IRD spectra of (A) VX to (B) the methyl derivative of EA-2192 made using TMPAH. These spectra are very similar except for a shift in the bands below 1100 cm^{-1} due to the substitution of a methyl group for the ethyl group in VX (see Section 3.3). The vapor phase spectra were taken in a light pipe Fourier transform IR over the range of 600–4000 cm^{-1} at a rate of 1.5 spectra/s and co-addition of 4 spectra. Resolution is 8 cm^{-1}.

ethyl group. The shift in the strongest band to 1045 cm^{-1} in the derivative and the disappearance of the 947 cm^{-1} band indicate the substitution of a methyl group in the derivative for the ethyl group in VX. These spectra confirm that the methyl derivative of EA-2192 is being generated by this method.

The use of TMPAH as a methylating agent is an effective way for derivatizing EA-2192 to form a volatile product, as demonstrated by the spectral data. It provides a feasible approach for analyzing this nonvolatile compound. However, routine sample analysis using this reagent, particularly for decon-

tamination solutions, will require a considerable amount of methods characterization. Since TMPAH produces a derivative in the GC injection port, it is necessary to determine whether memory effects from residual nonvolatile analyte are a problem. Optimal reagent concentrations must be determined to improve sensitivity. In neutralization solutions, interferences may be a problem. Further work is in progress to detect EA-2192 in decontamination solutions by modifying the solid-phase extraction method of Tørnes and Johnsen [23].

4. Conclusions

Several new applications have arisen for high confidence identification and quantitation of chemical weapons agents and other related compounds. Detection of agents is needed for evaluation of the safety of samples and for regulatory requirements to insure agent destruction. Analytical methods are needed for complex matrices such as environmental samples, industrial process mixtures and decontamination solutions. Examples involving the analyses of GA and its byproducts in a complex solution, HD and an interference in a decontamination solution, and a hydrolysis product of VX were given.

To meet the requirements of methods development and validation for particular matrices, multiple chromatographic methods are important to unequivocally identify low concentrations of agent with high confidence. Chromatographic or spectroscopic methods with various detectors, including GC–MS, GC–MS–MS, GC–AED, GC–IRD, NMR and LC–MS, provide independent information to provide higher confidence and eliminate false positive results.

Analysis of CW agents in various matrices shows that interference from related compounds can obscure an analyte signal due to coelution, as was observed for the GA byproducts in the organic liquid samples, requiring a GC–MS–MS method to completely characterize the sample. Interferences can also produce signals that can give false positive results, as was observed in the HD decontamination solution, although HD was resolved chromatographically and could be identified to a detection limit of 50 ng/ml in a decontamination solution by using GC–MS and GC–AED. Some compounds, such as EA-2192, can be inherently difficult to detect. EA-2192 was analyzed using LC–MS–MS methods to 100 ng/ml detection limits, and it was successfully derivatized using trimethylphenyl ammonium hydroxide for GC detection. Each matrix must be carefully examined for interferences or other complications in order to develop routine analytical methods. The cost and effort that is required to develop the method depends critically on both the nature of the analyte and the complexity of the matrix.

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References

[1] Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and their Destruction, United States Control and Disarmament Agency, Washington, DC, 1993.
[2] M. Rautio (Editor), Recommended Operating Procedures for Sampling and Analysis in the Verification of Chemical Disarmament, The Ministry for Foreign Affairs of Finland, Helsinki, 1990–1994.
[3] E.R.J. Wils, A.G. Hulst, P.E.J. Verweil, S.H. van Krimpen and A. Niederhauser, Fresenius. J. Anal. Chem., 343 (1992) 297–303.
[4] P.A. D'Agostino and L.R. Provost, J. Chromatogr., 645 (1993) 283–292.
[5] M. Mesilaakso and E.-L. Tolppa, Anal. Chem., 68 (1996) 2313–2318.
[6] M.T. Söderström, H. Björk, V.M.A. Häkkinen, O. Kostiainen, M.-L. Kuitunen and M. Rautio, J. Chromatogr. A, 742 (1996) 191–203.
[7] A. Rodriguez, E. Gonzales, B. Williams, M. Brickhouse, J. Stuff and L. Hoffland, presented at Pittcon '96 (The Pittsburgh Conference), Chicago, IL, 3–8 March, 1996.
[8] R.M. Black, R.J. Clarke, R.W. Read and M.T.J. Reid, J. Chromatogr. A, 662 (1994) 301–321.

[9] NATO Advanced Research Workshop, Destruction of Chemical Weapons: Report of the NATO Advanced Research Workshop on Destruction of Military Toxic Waste, Naaldwijk, Netherlands, 22–27 May, 1994. Available on the World Wide Web at http://www.opcw.nl/chemhaz/arwnaal1.htm

[10] US Army Materiel Systems Analysis Activity Summary Report, Technical and Economic Analysis Comparing Alternative Chemical Demilitarization Technologies to the Baseline, Vol. 1, Aberdeen Proving Ground, MD, July 1996. Available on the World Wide Web at http://www-pmcd.apgea.army.mil/alttech/frameset.html

[11] J. Epstein, J.J. Callahan and V.E. Bauer, Phosphorus, 4 (1974) 157–63.

[12] Y.-C. Yang, L.L. Szafraniec, W.T. Beaudry and D.K. Rohrbaugh, J. Am. Chem. Soc., 112 (1990) 6621–6627.

[13] Y.-C. Yang, Chem. Ind., 1 May (1995) 334–338.

[14] P.A. D'Agostino, A.S. Hansen, P.A. Lockwood and L.R. Provost, J. Chromatogr., 347 (1985) 257–266.

[15] P.A. D'Agostino, L.R. Provost and K.M. Looye, J. Chromatogr., 465 (1989) 271–283.

[16] P.A. D'Agostino and L.R. Provost, J. Chromatogr., 598 (1992) 89–95.

[17] M. Sokolowski and A. Szymanska, Proceedings of the 1995 Edgewood Scientific Conference on Chemical and Biological Defense Research, 14–17 November 1995, Edgewood Research, Development and Engineering Center, Aberdeen Proving Ground, Edgewood Area, MD, pp. 149–155.

[18] S. Budiansky, Nature (London), 308 (1984) 483.

[19] M.D. Brickhouse, K. RaghuVeer and H.D. Durst, in preparation.

[20] C. Albaret, D. Loeillet and P.L. Fortier, presented at the 1996 ERDEC Scientific Conference on Chemical and Biological Defense Research, 19–22 November 1996, Edgewood Research, Development and Engineering Center, Aberdeen Proving Ground, Edgewood Area, MD.

[21] W.R. Creasy, J. Mays, R. Duevel, T.R. Connell and B.R. Williams, presented at the 1996 ERDEC Scientific Conference on Chemical and Biological Defense Research, 19–22 November 1996, Edgewood Research, Development and Engineering Center, Aberdeen Proving Ground, Edgewood Area, MD. (available on request as EAI Report 97-2/96/001F from the authors).

[22] W.R. Creasy, A.A. Rodriguez, J.R. Stuff and R.W. Warren, J. Chromatogr. A, 709 (1995) 333–344.

[23] J.A. Tørnes and B.A. Johnsen, J. Chromatogr., 467 (1989) 129–138.

[24] T.G. Albro, Evaluation of Trimethylphenylammonium Hydroxide (TMPAH) as a Derivatizing Agent for the Methylation of Methylphosphonic Acid (MPA), EAI Report 14/93/002D2, Abingdon, MD, 1993, 6 pp. Available on request from the authors.

[25] E.W. Robb and J.J. Westbrook, III, Anal. Chem., 35 (1963) 1644–47.

[26] G.W. Stevenson, Anal. Chem., 38 (1966) 1948–49.