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Analysis of Chemical Warfare Agents by LC-MS: Third Chemical Cluster CRTI Training Exercise

P. A. D'Agostino, C. L. Chenier and C. R. Jackson Lepage

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Author

Original signed by Paul A. D'Agostino

Paul A. D'Agostino

Approved by

Original signed by Chris Weickert

Chris Weickert

H/DIS

Approved for release by

Original signed by Paul D'Agostino

Paul D'Agostino

DRP Chair

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Abstract

The Chemical Cluster, one of five clusters created by the Chemical, Biological, Radiological and Nuclear and Explosive Research and Technology Initiative (CRTI), was established to help Canada prepare for, prevent and respond to terrorist events. This working group, made up of representatives from Canadian government departments, has identified a number of chemicals of concern and assigned laboratories with appropriate expertise to provide the analytical support necessary to confirm these compounds in suspect samples. The Royal Canadian Mounted Police (RCMP), in its lead forensics role, will attempt to tentatively identify the chemical(s) of concern and pass on the samples to the responsible laboratory within the Chemical Cluster. Samples containing large amounts of relatively pure chemical warfare agents should trigger a response with one of the chemical monitoring devices (e.g., Chemical Agent Monitor) used by the RCMP to triage samples. Defence R&D Canada Suffield has been tasked to analyse samples suspected to contain chemical warfare agents for the Chemical Cluster and would receive this type of suspect sample. There remains a possibility that samples with a lower level of chemical warfare agent contamination might inadvertently find their way into a laboratory tasked with another type of analysis. To manage this possibility, the laboratories receiving these types of samples should have an analytical screening capability to allow for the tentative identification of chemical warfare agents in samples and sample extracts. This report summarizes the third chemical warfare agent training exercise in sample preparation and analysis by liquid chromatography-mass spectrometry (LC-MS) given by DRDC Suffield to other Chemical Cluster laboratories.

Résumé

La grappe de la chimie, l'une des cinq grappes créées par l'Initiative de recherche et de technologie chimique, biologique, radiologique, nucléaire et explosive (IRTC), a été établie pour aider le Canada à se préparer aux événements terroristes, les éviter et y répondre. Ce groupe de travail, composé de représentants de ministères gouvernementaux canadiens, a identifié un certain nombre de produits chimiques inquiétants; il a désigné des laboratoires ayant l'expertise appropriée pour fournir le soutien analytique requis pour confirmer les composants de ces échantillons suspects. La Gendarmerie royale du Canada (GRC) qui a le rôle de leader médico-légal tentera d'identifier les produits chimiques inquiétants et transmettra les échantillons au laboratoire responsable parmi la grappe de la chimie. Les échantillons contenant de grandes quantités d'agents de guerre chimiques relativement purs devraient déclencher une réponse sur l'un des appareils de surveillance chimique (par ex. : le détecteur d'agent chimique) utilisés par la GRC pour trier les échantillons. R & D pour la défense Canada Suffield, ayant pour mission d'analyser les échantillons suspects de contenir des agents de guerre chimiques pour la grappe chimique, recevrait ce type d'échantillon suspect. Il est encore possible que les échantillons ayant une quantité plus faible de contamination d'agent de guerre chimique puissent cheminer par inadvertance dans un laboratoire devant effectuer un autre type d'analyse. Pour gérer cette possibilité, les laboratoires recevant ces types d'échantillons devraient posséder une capacité de criblage analytique permettant de tenter l'identification d'agents de guerre chimiques dans les échantillons et extraits d'échantillons. Ce rapport fait le sommaire du troisième exercice de formation traitant de la préparation d'échantillons et d'analyses au moyen de couplage de chromatographie en phase liquide et spectrométrie de masse (CPL-SM) d'agents de guerre chimiques donnés d'autres laboratoires de la grappe chimique par RDDC Suffield.

Executive summary

Introduction: Concerns over possible terrorist use, continued interest by the defence community and the requirements of a verifiable Chemical Weapons Convention (CWC), have driven the development and application of analytical methods for the detection, characterization and confirmation of chemical warfare agents. The Chemical Cluster working group within the Chemical, Biological, Radiological & Nuclear and Explosive Research and Technology Initiative (CRTI) has identified a number of chemicals of concern and assigned laboratories with appropriate expertise to provide the analytical support necessary to confirm these compounds in suspect samples. The Royal Canadian Mounted Police (RCMP), in its lead forensics role, will attempt to tentatively identify the chemical(s) of concern and pass on the samples to the responsible laboratory within the Chemical Cluster. Samples containing large amounts of relatively pure chemical warfare agents should trigger a response with one of the chemical monitoring devices (e.g., Chemical Agent Monitor) used by the RCMP to triage samples. Defence R&D Canada & Suffield (DRDC Suffield) has been tasked to analyse samples suspected to contain chemical warfare agents for the Chemical Cluster and would receive this type of suspect sample. There remains a possibility that samples with a lower level of chemical warfare agent contamination might inadvertently find their way into a laboratory tasked with another type of analysis. To manage this possibility, the laboratories receiving these types of samples should have an analytical screening capability to allow for the tentative identification of chemical warfare agents in samples and sample extracts. This report summarizes the third chemical warfare agent training exercise in sample preparation and analysis by liquid chromatography-mass spectrometry (LC-MS) given by DRDC Suffield to other Chemical Cluster laboratories.

Results: The analytical exercise participants successfully analysed a chemical warfare agent test mixture by LC-MS, interpreted the acquired mass spectra and correctly identified the unknown chemical warfare agents spiked into nylon fabric and liquid samples. Chemical warfare agents were identified on the basis of both a LC retention time and electrospray ionization (ESI) mass spectrometric match with authentic reference standards (or library data).

The analytical participants were briefed on both safety considerations and chemical warfare agent detection devices. Detection devices, including the Chemical Agent Monitor, were demonstrated and sampling kits were available for examination.

Significance: Each of the analytical exercise participants conducts sample handling and analysis for a variety of target compounds for their government departments (RCMP, Health Canada, Environment Canada). If their sample handling methods co-extracted chemical warfare agents the analysts would be able to identify the common chemical warfare agents provided the LC-MS analyses were conducted under full scanning ESI-MS conditions.

Future Plans: This analytical training exercise may be provided to additional government partners to further their ability to respond to the chemical/biological/nuclear threat.

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Sommaire

Introduction: Les inquiétudes au sujet de l'utilisation terroriste, l'intérêt constant que porte la communauté de la défense et les besoins relatifs à la Convention sur les armes chimiques (CAC) ont abouti au développement et à l'application de méthodes pour la détection, la caractérisation et la confirmation d'agents de guerre chimiques. Le groupe de travail de la grappe de la chimie appartenant à l'Initiative de recherche et de technologie chimique, biologique, radiologique, nucléaire et explosive (IRTC) a identifié un certain nombre de produits chimiques inquiétants; il a désigné des laboratoires ayant l'expertise appropriée pour fournir le soutien analytique requis pour confirmer les composants de ces échantillons suspects. La Gendarmerie royale du Canada (GRC) qui a le rôle de leader multidisciplinaire tentera d'identifier les produits chimiques inquiétants et transmettra les échantillons au laboratoire responsable parmi la grappe de la chimie. Les échantillons contenant de grandes quantités d'agents de guerre chimiques relativement purs devraient déclencher une réponse sur l'un des appareils de surveillance chimique (par ex. : le détecteur d'agent chimique) utilisés par la GRC pour trier les échantillons. R & D pour la défense Canada Suffield, ayant pour mission d'analyser les échantillons suspects de contenir des agents de guerre chimiques pour la grappe chimique, recevrait ce type d'échantillon suspect. Il est encore possible que les échantillons ayant une quantité plus faible de contamination d'agent de guerre chimique puissent cheminer par inadvertance dans un laboratoire devant effectuer un autre type d'analyse. Pour gérer cette possibilité les laboratoires recevant ces types d'échantillons devraient posséder une capacité de criblage analytique permettant de tenter l'identification d'agents de guerre chimiques dans les échantillons et extraits d'échantillons. Ce rapport fait le sommaire du troisième exercice de formation traitant de la préparation d'échantillons et d'analyses au moyen de couplage de chromatographie en phase liquide et spectrométrie de masse (CPL-SM) d'agents de guerre chimiques donnés à d'autres laboratoires de la grappe chimique par RDDC Suffield.

Résultats: Les participants aux exercices analytiques ont réussi à analyser un mélange d'essai d'agents de guerre chimiques par CPL-SM, à interpréter le spectre de masse acquis et à identifier correctement les agents de guerre chimiques inconnus ensemenés dans des échantillons de tissus de nylon et de liquides. Les agents de guerre chimiques ont été identifiés à la fois sur la base de la durée de la rétention CPL et de la correspondance spectrométrique par la méthode ESI-MS avec des normes de référence authentiques (ou les bibliothèques de données).

On a informé les participants aux analyses à la fois au sujet des facteurs de sécurité et des appareils de détection d'agents de guerre chimiques. On a démontré les appareils de détection, dont le détecteur d'agent chimique (DAC), et les trousse d'échantillons ont été mises à la disposition de l'étude.

Portée des résultats: Chaque participant aux exercices analytiques effectue une manipulation et analyse d'échantillons d'une variété de composés ciblés par son ministère (GRC, Santé Canada, Environnement Canada). Si chaque méthode de manipulation des échantillons parvient à extraire conjointement des agents de guerre chimiques, les analystes devraient être

capables d'identifier les agents de guerre chimiques communs dans la mesure où les analyses sont effectuées dans les conditions complètes de balayage ESI-MS.

Perspectives d'avenir: Cette formation d'exercices analytiques pourrait être fournie à d'autres partenaires gouvernementaux pour améliorer la capacité de réponse aux menaces chimiques, biologiques et nucléaires.

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Introduction

CRTI training - Identification of chemical warfare agents

The Chemical Cluster, one of five clusters created by the Chemical, Biological, Radiological and Nuclear and Explosive Research and Technology Initiative (CRTI), was established to help Canada prepare for, prevent and respond to terrorist events. This working group, made up of representatives from Canadian government departments, has identified a number of chemicals of concern and assigned laboratories with appropriate expertise to provide the analytical support necessary to confirm these compounds in suspect samples. The Royal Canadian Mounted Police (RCMP), in its lead forensics role, will attempt to tentatively identify the chemical(s) of concern and pass on the samples to the responsible laboratory within the Chemical Cluster. Samples containing large amounts of relatively pure chemical warfare agents should trigger a response with one of the chemical monitoring devices (e.g., Chemical Agent Monitor) used by the RCMP to triage samples. Defence R&D Canada Suffield (DRDC Suffield) has been tasked to analyse samples suspected to contain chemical warfare agents for the Chemical Cluster and would receive this type of suspect sample. There remains a possibility that samples with a lower level of chemical warfare agent contamination might inadvertently find their way into a laboratory tasked with another type of analysis. To manage this possibility, the laboratories receiving these types of samples should have an analytical screening capability to allow for the tentative identification of chemical warfare agents in samples or sample extracts.

DRDC Suffield provided a three-day chemical warfare agent training course in sample preparation and analysis by LC-MS. Four analysts with hands-on LC-MS (or GC-MS) experience from laboratories within the Chemical Cluster were provided with both lectures and chemical warfare agent training designed to aid in the tentative identification of chemical warfare agents in collected samples.

- Exercise Outline:
1. Lectures on sample handling and analysis of chemical warfare agents by GC-MS and LC-MS.
 2. Analysis of chemical warfare agent standards by LC-MS. Interpretation of MS data.
 3. Sample handling and analysis of samples contaminated at the $\mu\text{g/g}$ level (part per million) with an unknown chemical warfare agent(s). Interpretation of LC-MS data.
 4. Lecture on technologies for field detection of chemical warfare agents.

Historical background

Chemical warfare agents are toxic chemicals controlled by the Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and their Destruction (commonly referred to as the Chemical Weapons Convention or CWC). Poisonous or toxic compounds have been utilized in an effort to gain military superiority throughout history but it is only during the past century that chemical warfare agents have been produced and used on a large scale. Lachrymator (tear agents) grenades were used by the French and German armies at the outbreak of the First World War, but it was not until the German army used chlorine near Ypres in 1915 that the world entered the modern era of chemical warfare. Other chemical warfare agents including phosgene and mustard were weaponized during the First World War and were used by both sides throughout the conflict. Widespread use of these weapons resulted in more than a million chemical weapons casualties, with attributable deaths exceeding 100,000.

Chemical warfare agent use continued following the First World War despite the signing of the 1925 Geneva Protocol, which banned the first use of chemical weapons. Mustard was used by the Italian army against Ethiopia during the 1936-1937 war and chemical weapons were used in China by the Japanese armed forces during the Second World War. Germany discovered tabun and sarin, two highly toxic organophosphorus compounds, in the late 1930s and produced substantial stocks of the former during the Second World War. The major powers established extensive weapons stockpiles and aside from their use in China and a number of other isolated incidents, chemical weapons were not employed in that conflict. Following the Second World War most of the chemical warfare agent stocks were burned or dumped at sea, the latter method of disposal resulting in a continuing environmental hazard in the Baltic Sea and other areas.

VX, the most toxic of the standard nerve agents, was discovered and weaponized by the United States in the 1950s, while similar efforts in the former Soviet Union resulted in the discovery of a somewhat more toxic isomer of VX which was also weaponized. Nerve and mustard agents were used repeatedly by Iraq in the Gulf War (1980-1988), with Iranian casualties reported to be in the tens of thousands. The willingness of the Iraqi regime to use chemical weapons in the war against Iran and against the indigenous Kurdish population was recognized by United Nations armed forces during the Gulf War (1990-1991) and considered a real threat to the coalition forces during the liberation of Kuwait. Following the Gulf War, the United Nations established a special commission (UNSCOM) to uncover and oversee the destruction of Iraqi chemical and biological stocks and related delivery systems. Most recently, sarin was released by the Aum Shinrikyo cult in the Tokyo underground transit system resulting in thousands seeking medical attention and twelve deaths.

On April 29, 1997, the CWC collected sufficient signatures for the treaty to come into force. To date more than 180 Member States have ratified the CWC and agreed not to develop, produce, stockpile, transfer or use chemical weapons and agreed to destroy their own chemical weapons and production facilities. A strong compliance monitoring regime involving site inspections was built into the CWC to ensure that the treaty remains verifiable.

The Organisation for the Prohibition of Chemical Weapons, or OPCW, based in the Hague has responsibility for implementation of the treaty. Routine OPCW inspections have taken place at declared sites, including small-scale production, storage and destruction sites, and challenge inspections may take place at sites suspected of non-compliance. Proliferation of chemical weapons and their use will hopefully decrease over the coming years as the OPCW proceeds towards its goal of world-wide chemical weapons destruction.

The al-Qaeda terrorist attacks of September 2001 and subsequent Capitol Hill anthrax letters heightened concern worldwide over the possible use of chemical or biological weapons by terrorist groups. Concerns over terrorist use of chemical weapons within the homeland security and defence communities and the requirements of a verifiable CWC have all driven the development and application of analytical methods for the detection and identification of chemical warfare agents. Analytical techniques play an important role in this process, as sampling and analysis will be conducted to ensure treaty compliance, to investigate allegations of use and to verify the use of these weapons for forensic purposes.

Chemical warfare agent categories

Chemical warfare agents have been classified into a number of categories based on their effect on humans. Categories include nerve, blister, choking, vomiting, blood, tear and incapacitating, with the nerve and blister agent categories being most significant in terms of military utility and past use. For these reasons, the analytical methods developed for these compounds will be emphasized over the other groups. The choking, blood and vomiting agents, generally considered obsolescent chemical agents, were employed during the First World War. The tear agents were used during the Vietnam War by the United States, but their primary use today is in riot control and training.

Table 1 lists the most common chemical warfare agents, with their Chemical Abstracts registry numbers. It has been estimated that more than 10,000 compounds are controlled under the CWC, although in practical terms the actual number of chemical warfare agents, precursors and degradation products that are contained in the OPCW database is in the hundreds. The structures of some of the more common nerve and blister agents and their hydrolysis products are illustrated in Figure 1.

Table 1. Common chemical warfare agents

a) Nerve (reacts irreversibly with acetylcholinesterase which results in acetylcholine accumulation, continual stimulation of the body's nervous system and eventual death)

<u>Full Name (Trivial Name(s))</u>	<u>CAS No.</u>
O-Isopropyl methylphosphonofluoridate (sarin, GB)	107-44-8
O-Pinacolyl methylphosphonofluoridate (soman, GD)	96-64-0
O-Cyclohexyl methylphosphonofluoridate (GF)	329-99-7
O-Ethyl <i>N,N</i> -dimethylphosphoramidocyanidate (tabun, GA)	77-81-6
O-Ethyl <i>S</i> -2-diisopropylaminoethyl methylphosphonothiolate (VX)	50782-69-9

b) Blister (affects the lungs, eyes and produces skin blistering)

<u>Full Name (Trivial Name(s))</u>	<u>CAS No.</u>
Bis(2-chloroethyl)sulfide (mustard, H)	505-60-2
1,2-Bis(2-chloroethylthio)ethane (sesquimustard, Q)	3563-36-8
Bis(2-chloroethylthioethyl)ether (T)	63918-89-8
Tris(2-chloroethyl)amine (HN-3)	555-77-1
2-Chlorovinylidichloroarsine (lewisite, L)	541-25-3

c) Choking (affects respiratory tract and lungs)

<u>Full Name (Trivial Name(s))</u>	<u>CAS No.</u>
Chlorine	7782-50-5
Phosgene (CG)	75-44-5

d) Vomiting (causes acute pain, nausea and vomiting)

<u>Full Name (Trivial Name(s))</u>	<u>CAS No.</u>
Diphenylarsinous chloride (DA)	712-48-1
10-Chloro-5,10-dihydrophenarsazine (adamsite, DM)	578-94-9
Diphenylarsinous cyanide (DC)	23525-22-6

e) Blood (prevents transfer of oxygen to the body's tissues)

<u>Full Name (Trivial Name(s))</u>	<u>CAS No.</u>
Hydrogen cyanide (HCN, AC)	74-90-8

f) Tear (causes tearing and irritation of the skin)

<u>Full Name (Trivial Name(s))</u>	<u>CAS No.</u>
[(2-chlorophenyl)methylene]propanedinitrile (CS)	2698-41-1
2-Chloro-1-phenylethanone (CN)	532-27-4
Dibenz[<i>b,f</i>][1,4]oxazepin (CR)	257-07-8

g) Incapacitating (prevents normal activity by producing mental or physiological effects)

<u>Full Name (Trivial Name(s))</u>	<u>CAS No.</u>
3-Quinuclidinyl benzilate (BZ)	6581-06-2

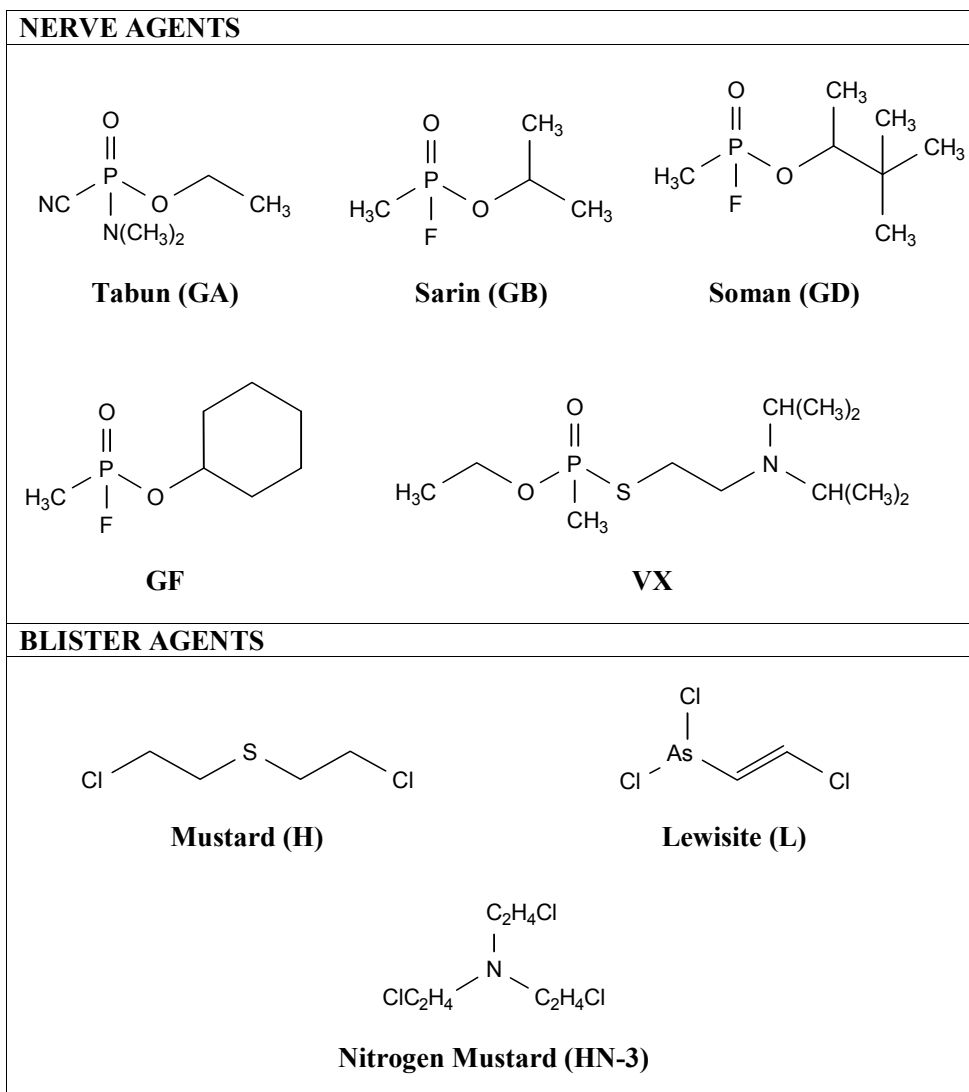


Figure 1. Structures of common chemical warfare agents.

Identification methods

Chemical warfare agents have often been referred to as warfare gases and, the military phrase "gas, gas, gas" has become synonymous with attack by chemical warfare agents. In fact, many chemical warfare agents exist as liquids at ambient temperatures but have varying degrees of volatility and pose both a vapor hazard as well as a liquid contact hazard. This physical characteristic has made the analysis of chemical warfare agents amenable to the analytical techniques commonly employed for most environmental analyses, namely gas chromatography (GC) and liquid chromatography (LC) with a variety of detectors including

mass spectrometry (MS). Synthetic or relatively pure samples not requiring chromatographic separation are also frequently characterized by nuclear magnetic resonance (NMR) or Fourier transform infrared (FTIR) spectroscopy.

The OPCW inspectorate, an important end user of analytical techniques for chemical warfare agents, requires the use of two or more spectrometric techniques and the availability of authentic reference standards for the unambiguous identification of controlled compounds. For this reason, the combined use of GC-FTIR has received increased attention as newer technologies have led to detection limits approaching those routinely reported during GC-MS analysis. For analyses involving low levels of chemical warfare agents in the presence of high levels of interfering chemical background, tandem mass spectrometry (MS/MS) is often employed.

Chromatography

Samples contaminated with chemical warfare agents typically contain multiple components that are best characterized following chromatographic separation. These samples generally fall into one of the following general categories; a) munitions or munition fragments (e.g., neat liquid or artillery shell casing), b) environmental (e.g., soil, water, vegetation or air samples), c) man-made materials (e.g., painted surfaces or rubber) and d) biological media (e.g., blood or urine). The ease of analysis depends on the amount of sample preparation required to obtain a suitable sample or extract for chromatographic analysis. In the simplest case where neat liquid can be obtained, the sample requires dilution with a suitable solvent prior to analysis. Environmental and other samples generally require (at a minimum) solvent extraction and concentration prior to analysis.

The most frequently employed analytical separation method for the screening of samples contaminated with chemical warfare agents is capillary column GC. Separation of chemical warfare agents may be achieved with many of the commercially available fused silica columns coated with polysiloxane or other films and retention index data relative to n-alkanes and alkylbis(trifluoromethyl)phosphine sulfides (M-series) have been reported for many chemical warfare agents and related compounds. In general, the best separations have been achieved with moderately polar films such as (86%)-dimethyl-(14%)-cyanopropylphenyl-polysiloxane. Chiral stationary phases have also been developed for the resolution of stereoisomers of several chiral nerve agents, most notably soman. The use of multiple columns of differing polarity during one analysis has been successfully employed during chemical warfare agent analysis and the term "retention spectrometry" was coined to describe this technique.

GC detectors commonly applied to pesticide residue analysis have also been applied to the screening of samples for chemical warfare agents with detection limits typically being in the nanogram to picogram range. Flame ionization detection (FID) is routinely used for preliminary analyses as this technique provides a good indication of the complexity of a sample extract. Figure 2 illustrates typical GC-FID chromatographic separations obtained for three different munitions-grade mustard formulations, HT, HS and HQ, each of which contain mustard and a number of related longer chain blister agents. The longer chain blister agents,

sesquimustard (Q) and bis[(2-chloroethylthio)-ethyl]ether (T) were present in all three samples along with a number of other related compounds that may provide synthetic procedure or source information.

The need for higher specificity and sensitivity has led to the application of element specific detectors such as flame photometric detection (FPD), thermionic detection (TID), atomic emission (AED) and electron capture detection (ECD). The simultaneous use of FID with one or more element specific detectors has also been demonstrated during dual or tri channel GC analysis using conventional and thermal desorption sample introduction. While data obtained with these detectors may provide strong collaborative evidence for the presence of chemical warfare agents, they cannot be used for full confirmation. Use of GC with one or more spectrometric technique such as MS is required to confirm the presence of chemical warfare agents.

Both the nerve and blister agents undergo hydrolysis in the environment and methods are required for retrospective detection and confirmation of these compounds. These compounds are significant as they would not be routinely detected in environmental samples and their identification strongly suggest the prior presence of chemical warfare agents. The degradation products of the chemical warfare agents, in particular the nerve agents, are non-volatile hydrolysis products that must be derivatized prior to GC analysis. A variety of derivatization reagents, leading to the formation of pentafluorobenzyl, methyl, *tert*-butyldimethylsilyl and trimethylsilyl ethers (or esters), have been investigated to allow GC analysis of organophosphorus acids related to the nerve agents (e.g., alkyl methylphosphonic acids and methylphosphonic acid). Increasingly, liquid chromatography-electrospray-mass spectrometry (LC-ESI-MS), is being used for these types of analyses, as electrospray mass spectrometric data may be used to identify chemical warfare agents, their degradation products and related compounds in aqueous samples or extracts without the need for additional sample handling and derivatization steps.

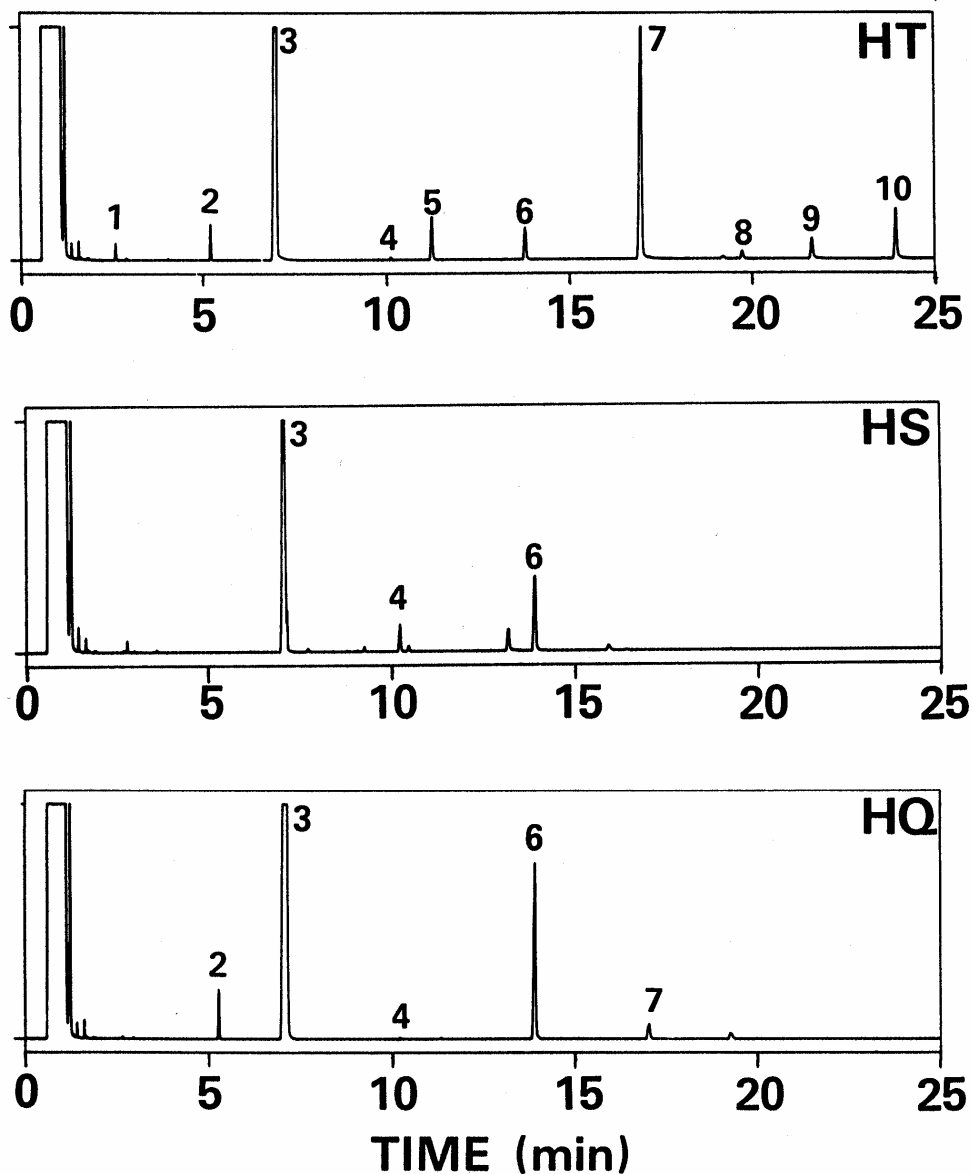


Figure 2. Capillary column GC-FID chromatograms of three munitions-grade mustard samples; HT (top), HS (middle) and HQ (bottom). Identified compounds include: 1. 1,4-thioxane, 2. 1,4-dithiane, 3. mustard (H), 4. bis(2-chloroethyl)disulfide, 5. 2-chloroethyl (2-chloroethoxy)ethyl sulfide, 6. sesqui-mustard (Q), 7. bis(2-chloroethylthioethyl)ether (T), 8. 1,14-dichloro-3,9-dithia-6,12-dioxatetradecane, 9. 1,14-dichloro-3,6,12-trithia-9-oxatetradecane and 10. 1,16-dichloro-3,9,15-trithia-6,12-dioxaheptadecane. (GC conditions: 15 m x 0.32 mm ID J&W DB-1; 50°C (2 min) 10°C/min 280°C (5 min)).

Mass spectrometry

Mass spectrometry is the method of choice for the detection and characterization of chemical warfare agents, their precursors, degradation products and related compounds. Extensive use has been made of GC-MS and the mass spectra of numerous chemical warfare agents and related compounds have been published, with the most common chemical warfare agent mass spectra being available in the OPCW, commercial or defence community databases.

Most of the MS data has been obtained under electron impact (EI) ionization conditions. However many of the chemical warfare agents, in particular the organophosphorus nerve agents and the longer chain blister agents related to mustard, do not provide molecular ion information under EI-MS. This hinders confirmation of these chemical warfare agents and makes identification of novel chemical warfare agents or related impurities difficult. For this reason, considerable effort has been devoted to the use of chemical ionization (CI) as a complementary ionization technique. This milder form of ionization generally affords molecular ion information for the chemical warfare agents and has been used extensively for the identification of related compounds or impurities in chemical warfare agent munition samples and environmental sample extracts. The characterization of these related compounds is important during analyses since this data may provide an indication of the origin of the sample, the synthetic process utilized or the degree of sample degradation.

Isobutane, ethylene and methane gases were initially demonstrated as suitable CI gases for the acquisition of organophosphorus nerve agent molecular ion information. More recently, the efficacy of ammonia CI-MS for organophosphorus nerve agents and related compounds was demonstrated and many laboratories now employ this complementary confirmation technique. Ammonia CI not only offers abundant molecular ion data but also affords a high degree of specificity as less basic sample components are not ionized by the ammonium ion. Additional structural data may be obtained through the use of deuterated ammonia CI, as this technique provides hydrogen/deuterium exchange data that indicates the presence of exchangeable hydrogen(s) in CI fragmentation ions. Finally, for full confirmation, the acquired EI and CI mass spectrometric data should be compared to authentic reference data obtained under identical experimental conditions.

Capillary column GC-MS/MS offers the analyst the potential for highly specific, sensitive detection of chemical warfare agents as this technique significantly reduces the chemical noise associated with complex biological or environmental sample extracts. The specificity of product scanning with moderate sector resolution, as well as the specificity of ammonia CI, were demonstrated with a hybrid tandem mass spectrometer during analysis of painted panel samples circulated during an international round robin verification exercise.

The painted panel extract was contaminated with numerous hydrocarbons and only two of the three longer chain blister agents, sesquimustard (Q) and bis(2-chloroethylthioethyl)ether (T), could be identified during capillary column GC-MS (EI) analysis (Figure 3a). The arrow indicates the chromatographic retention time of the third blister agent, 2-chloroethyl (2-chloroethoxy)ethyl sulfide (O). The specificity of ammonia CI (Figure 3b) was clearly demonstrated during this analysis. All three longer chain blister agents were identified in the presence of high levels of interfering hydrocarbons, as the hydrocarbons were not sufficiently

basic to ionize. Similarly, it was possible to use the high resolution of hybrid tandem mass spectrometry to discriminate between ions at m/z 123 arising from the longer chain blister agents from those ions at m/z 123 arising from the hydrocarbon background. The resultant GC-MS/MS chromatogram (Figure 3c), where only m/z 123 ions due to the blister agents were transmitted into the collisional activated dissociation cell of the MS, was virtually free of chemical noise and all three components were detected. The three longer chain blister agents were well resolved with the J&W DB-1701 capillary column, with all three components exhibiting similar product spectra during GC-MS/MS analysis.

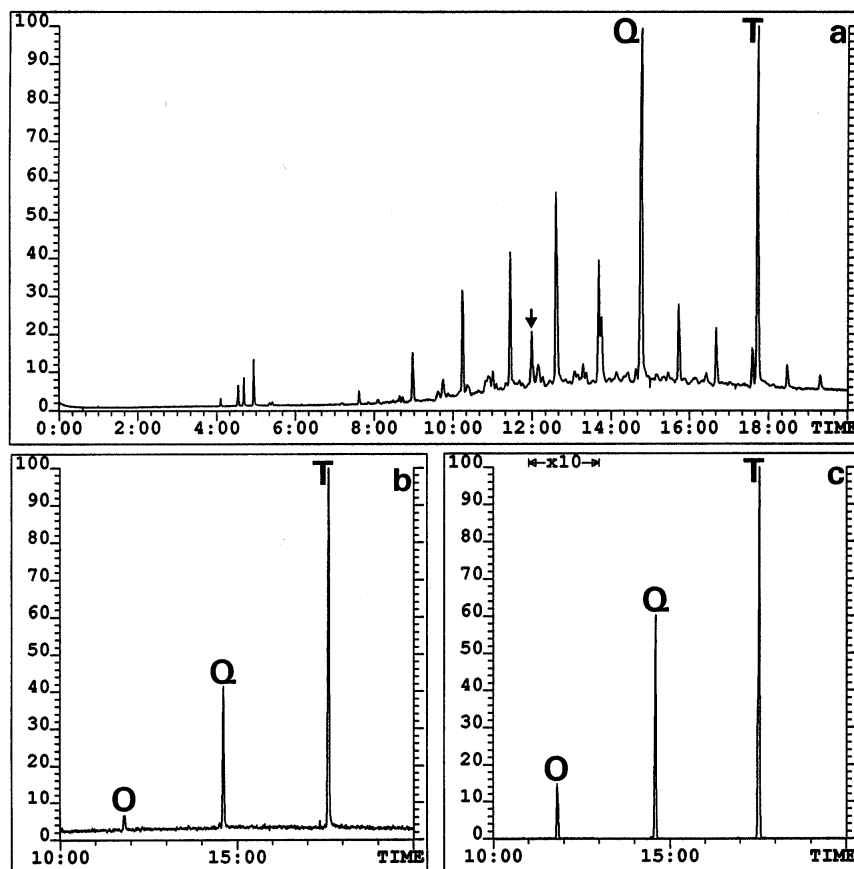


Figure 3. Capillary column a) GC-MS (EI), b) GC-MS (ammonia CI) and c) GC-MS/MS (EI) chromatograms obtained during analysis of international round robin painted panel extracts. Sequimustard (Q) and bis(2-chloroethylthioethyl)ether (T) were detected during EI analysis. The downward arrow in a) indicates the retention time of 2-chloroethyl (2-chloroethoxy)ethyl sulfide (O). This compound was masked by the sample matrix during EI analysis and was only detected following b) ammonia CI and c) MS/MS analysis. (GC conditions: 15 m x 0.32 mm ID J&W DB-1701, 40°C (2 min) 10°C/min 280°C (5 min), X-axis: time (minutes)).

Both the nerve and blister agents undergo hydrolysis in the environment and methods are required for retrospective detection and confirmation of these hydrolysis products. Hydrolysis products are significant as they are generally compounds that would not be routinely detected in environmental samples and their presence strongly suggest the prior presence of chemical warfare agents. The degradation products of the chemical warfare agents, in particular the nerve agents, are non-volatile hydrolysis products that must be derivatized prior to GC analysis. Alternatively aqueous samples or extracts may be analyzed by LC-MS, negating the need for additional sample handling steps and derivatization.

Atmospheric pressure ionization (e.g., ESI, ionspray and atmospheric pressure CI) techniques have enabled the direct mass spectrometric analysis of the hydrolysis products of chemical warfare agents. LC-ESI-MS methods have been used for the direct analysis of chemical warfare agent hydrolysis products in a number of studies and have recently been used for the analysis of nerve agents. These new methods complement existing GC-MS methods for the analysis of chemical warfare agents and their hydrolysis products and LC-ESI-MS methods have replaced some GC-MS methods used for the analysis of contaminated aqueous samples or extracts.

Mustard and longer chain blister agents hydrolyze to their corresponding diols, with thiodiglycol being the product formed following hydrolysis of mustard. Figure 4a illustrates a typical LC-ESI-MS chromatogram obtained for the aqueous extract of a soil sample taken from a former mustard storage site. The soil sample extract contained several diols including thiodiglycol (Figure 4b) and 6-oxa-3,9-dithia-1,11-undecanediol (Figure 4c), the hydrolysis products of blister agents mustard and bis(2-chloroethylthioethyl)ether, respectively. ESI-MS data for both compounds contained protonated molecular ions that could be used to confirm molecular mass and characteristic lower mass product ions.

Figure 5 illustrates the LC-ESI-MS chromatogram for a complex munitions-grade tabun sample. Tabun and a number of related compounds were identified based on their acquired ESI-MS data. The mass spectra contained $(M+H)^+$, $(M+H+ACN)^+$ ions and/or protonated dimers that could be used to confirm the molecular mass of each compound. Structural information was provided by inducing product ion formation in either the ESI interface or the quadrupole collisional cell of a MS/MS instrument. Product ions due to alkene loss from the alkoxy substituents, and the acetonitrile (ACN) adduct associated with these product ions, were generally observed. Figure 6 illustrates typical ESI-MS data obtained for tabun and three other nerve agents.

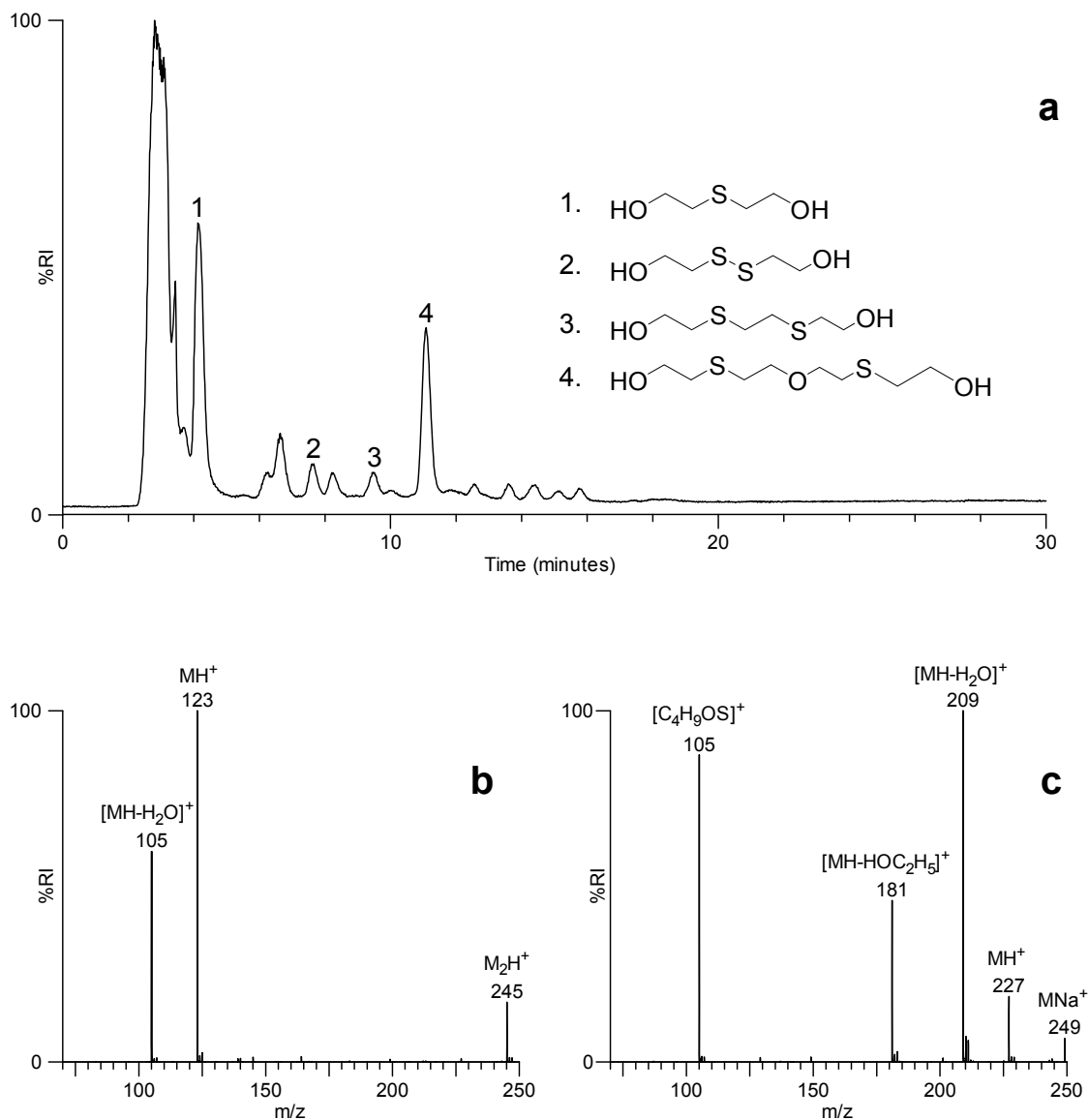


Figure 4. a) Packed capillary LC-ESI-MS chromatogram obtained for the water extract of a soil sample obtained from a former mustard site. ESI-MS data obtained for b) thiodiglycol (sampling cone voltage: 20 V) and c) 6-oxa-3,9-dithia-1,11-undecanediol (sampling cone voltage: 30 V). (LC conditions: 150 mm x 0.32 mm i.d. C_{18} , acetonitrile/water gradient).

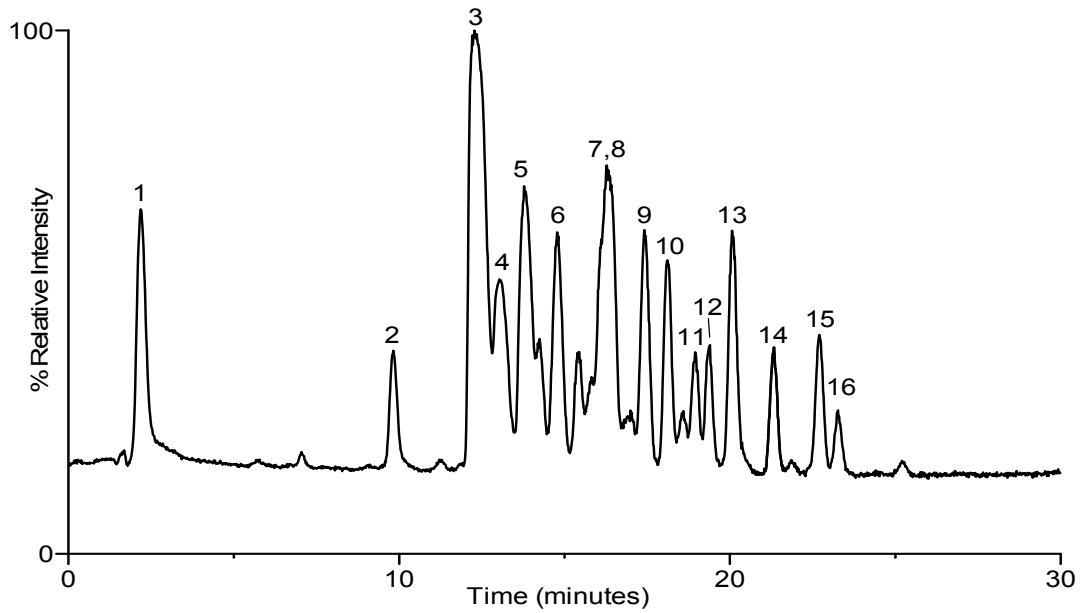


Figure 5. Packed capillary LC-ESI-MS chromatogram obtained for 0.1 mg/mL munitions-grade tabun sample. Tabun (peak number 3) and fifteen related organophosphorus compounds were identified by ESI-MS. (LC conditions: 150 mm x 0.32 mm i.d. C₁₈, acetonitrile/water gradient).

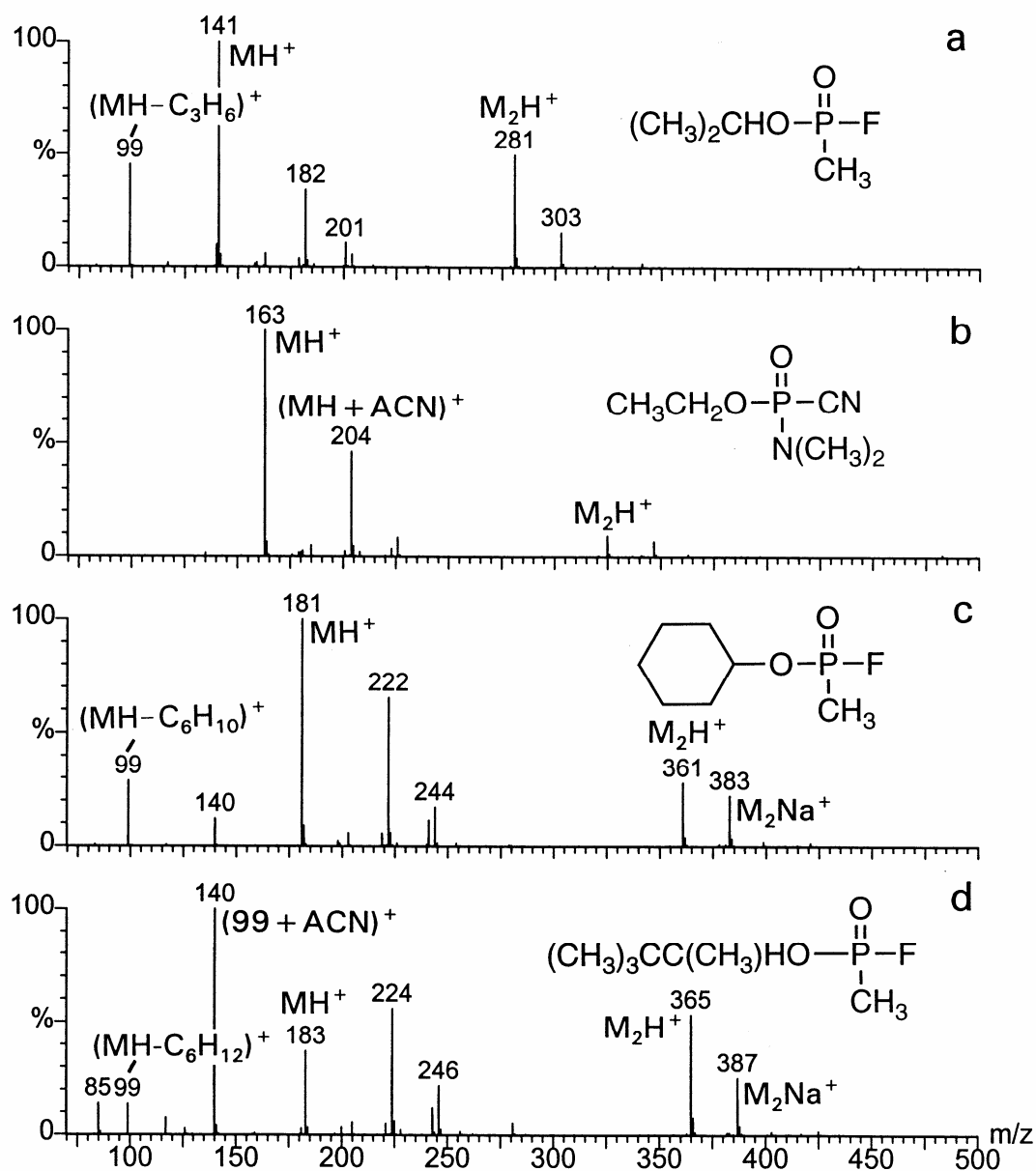


Figure 6. ESI-MS data obtained for a) sarin (GB), b) tabun (GA), c) cyclohexyl methylphosphonofluoridate (GF) and d) soman (GD) with a sampling cone voltage of 20 V.

Acquisition of high resolution data for chemical warfare agents and related compounds was greatly aided by the introduction of instruments with time-of-flight (TOF) mass analysers. These instruments, while lacking the dynamic range and sensitivity associated with triple quadrupole instruments, may be used to acquire high resolution, full scanning data (typically 5000 to 17000 resolution, 50% valley) for unknown sample components without the signal

losses typically associated with magnetic sector instrumentation. The utility of high resolution LC-ESI-MS and LC-ESI-MS/MS data has been demonstrated during the identification of numerous tabun impurities in a synthetic sample, for the identification of sarin related compounds in snow, and for the determination of longer chain diols in soil samples collected from a former mustard storage site.

This approach was used during a recent investigation involving typical forensic media which might be collected at the scene of an indoor terrorist attack. A variety of indoor sample media, including flooring, wall surfaces, office fabrics, window coverings and paper products or packaging, were spiked with chemical warfare agents to assess the applicability of aqueous extraction and LC-ESI-MS and LC-ESI-MS/MS analysis for the identification of chemical warfare agents. The spiked chemical warfare agents were recovered and positively identified by ESI-MS with efficiencies that varied with media. Figure 7 illustrates LC-ESI-MS and LC-ESI-MS/MS chromatograms acquired during a single analysis of an aqueous extract of an office carpet sample spiked at the 0.5 to 5 $\mu\text{g/g}$ level with a complex munitions grade tabun sample. The crude chemical warfare agent mixture was included in this study since being able to identify the related organophosphorus compounds in an extract may aid in establishing source information during a forensic investigation. All ESI-MS/MS data acquired during this study were acquired using a QToF instrument under high resolution conditions to enable identification of the tabun components (Table 2).

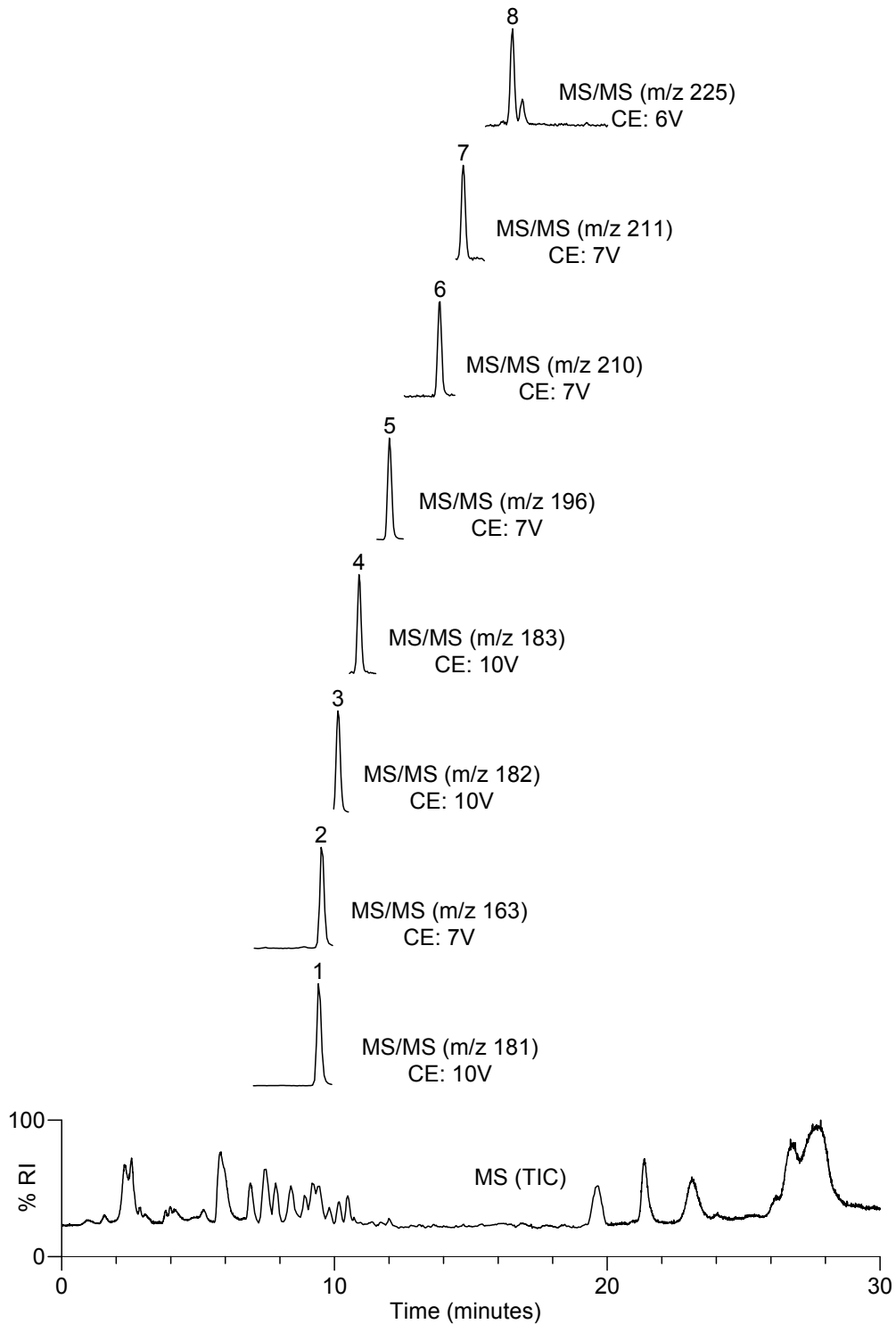


Figure 7. LC-ESI-MS (lowest) and LC-ESI-MS/MS chromatograms (above) of an extract of an office carpet spiked with munitions grade GA (0.5 □ 5 µg/g per component). Components 1 to 8 identified in Table 2. CE: Collision energy.

Table 2. ESI-MS/MS data acquired for munitions grade tabun components identified in an extract of a spiked office carpet sample.

Peak Number ¹	Compound Name	Ion	Observed Mass (Da) ²	Theoretical Mass (Da)	Error (Da)
1	Ethyl tetramethylphosphorodiamidate	MH ⁺	181.1108	181.1106	0.0002
		[MH-C ₂ H ₄] ⁺	153.0795	153.0793	0.0002
		[MH-HN(CH ₃) ₂] ⁺	136.0533	136.0527	0.0006
		[MH-C ₂ H ₄ -HN(CH ₃) ₂] ⁺	108.0215	108.0214	0.0001
2	Ethyl dimethyl-phosphoramidocyanidate (Tabun, GA)	MH ⁺	163.0628	163.0636	0.0008
		[MH-C ₂ H ₄] ⁺	135.0316	135.0323	0.0007
3	Diethyl dimethylphosphoramidate	MH ⁺	182.0950	182.0946	0.0004
		[MH-C ₂ H ₄] ⁺	154.0637	154.0633	0.0004
		[MH-(C ₂ H ₄) ₂] ⁺	126.0322	126.0320	0.0002
4	Triethyl phosphate	MH ⁺	183.0805	183.0786	0.0019
		[MH-C ₂ H ₄] ⁺	155.0470	155.0473	0.0003
		[MH-(C ₂ H ₄) ₂] ⁺	127.0153	127.0160	0.0007
5	Ethyl isopropyl dimethylphosphoramidate	[MH-(C ₂ H ₄) ₃] ⁺	98.9836	98.9847	0.0011
		MH ⁺	196.1109	196.1102	0.0007
		[MH-C ₃ H ₆] ⁺	154.0630	154.0633	0.0003
6	Diisopropyl dimethylphosphoramidate	[MH-C ₃ H ₆ -C ₂ H ₄] ⁺	126.0327	126.0320	0.0007
		MH ⁺	210.1282	210.1259	0.0023
		[MH-C ₃ H ₆] ⁺	168.0790	168.0789	0.0001
7	Diisopropyl ethyl phosphate	[MH-(C ₃ H ₆) ₂] ⁺	126.0316	126.0320	0.0004
		MH ⁺	211.1109	211.1099	0.0010
		[MH-C ₃ H ₆] ⁺	169.0647	169.0629	0.0018
8	Triisopropyl phosphate	[MH-(C ₃ H ₆) ₂] ⁺	127.0172	127.0160	0.0012
		MH ⁺	225.1273	225.1255	0.0018
		[MH-C ₃ H ₆] ⁺	183.0791	183.0786	0.0005
		[MH-(C ₃ H ₆) ₂] ⁺	141.0324	141.0316	0.0008
		[MH-(C ₃ H ₆) ₃] ⁺	98.9842	98.9847	0.0005

¹ Refer to Figure 7.

² Average of scans across the chromatographic peak (lock mass used).

Direct analysis of contaminated media would provide the analyst with a more rapid analysis without the need for sample preparation. Direct analysis of pinacolyl methylphosphonic acid, 2-chloroethyl sulfide, VX, nitrogen mustards and mustard on surfaces, including soil and concrete, using static secondary ion mass spectrometry (SIMS) and tandem mass spectrometry have been performed in the PI or NI mode. Although not as precise as other analytical methods, this technique enables detection in a fraction of the time normally associated with conventional methods requiring extraction and analysis.

Rapid analysis in real time developments have continued with the development of the direct analysis in real time (DART) source and the development and application of desorption electrospray ionization (DESI) for direct sample ionization and analysis. The DART source has been used to directly analyse VX on concrete with a TOF instrument, while the DESI technique has been used at DRDC Suffield to directly determine a number of chemical warfare agents from exposed solid phase microextraction (SPME) fibers.

Considerable effort has been expended on the development of field portable MS and GC-MS instruments, as this technique holds the greatest promise for the confirmation of chemical warfare agents under field situations. The OPCW has field portable GC-MS instrumentation that may be taken on-site to confirm the presence of chemical warfare agents. An atmospheric pressure MS/MS has also been developed and evaluated for real-time detection of nerve agents in air. Alternatively, air samples may be collected on solid phase microextraction (SPME) fibers or on Tenax tubes that may be thermally desorbed into an on-site GC-MS instrument. Finally, rapid separation and detection of chemical warfare agents has recently been demonstrated with ESI-ion mobility spectrometry (IMS)-MS. IMS is commonly used in military devices (e.g., Chemical Agent Monitor) for rapid field detection and this approach could lead to the development of instrumentation for the analysis of aqueous samples.

Field detection

The development of field detection methods for chemical warfare agents was driven by specific military requirements, with a variety of detection devices and other chemical warfare agent defence equipment having been produced for military applications. Most of the effort in this area resulted from the perceived threat during the Cold War era and although this threat has decreased dramatically, interest in chemical detection equipment persists because of world-wide chemical weapons proliferation. During the 1990-1991 Gulf War chemical detection equipment was deployed in the operational theatre and similar equipment was used to support the United Nations Special Commission during the destruction of Iraqi chemical weapons. Equipment of this type has been used by the OPCW and has increasingly been put in the hands of First Responders to prepare for possible chemical warfare agent terrorism incidents.

Most equipment or devices used for field detection of chemical warfare agents by the military falls into one of several general categories. Tests making use of chemical reagents have been used extensively, with commonly used examples being 3-way detection paper, Detehit nerve agent detector strips, Dräger tubes and the M256A1 Chemical Agent Detection Kit. Table 3 lists examples of selected chemical detection equipment by country and indicates the principle of detection and capabilities of each system.

Table 3. Selected Military Chemical Warfare Agent Detection Devices

<u>Country</u>	<u>Device Name and Capabilities</u>
Canada	Chemical Agent Detection System (CADS II) Early warning system that controls a network of Chemical Agent Monitors (see U.K.) for real time detection of nerve and blister agents
	M256A1 Chemical Agent Detection Kit Wet chemistry detection of nerve, blister, choking and blood agent vapours
	3-Way Detector Paper Solubility-based detection of nerve and blister agent liquids
Czech Rep.	Detehit Nerve-Agent Detector Immobilized-enzyme detection of nerve agent liquid or vapours
Finland	ChemPro □ Hand-held Chemical Detector Alarm for the ion mobility spectrometric detection of classical chemical warfare agents
France	PROENGIN Portable Chemical Contamination Monitor (AP2C, TIMs and AP4C) Hand-held flame photometric detection of nerve and blister agents The AP4C and TIMs detector variants provide additional detection for several toxic industrial chemicals (TICs)
Germany	MM-1 Mobile Mass Spectrometer Quadrupole mass spectrometric detection of chemical warfare agents
	Rapid Alarm and Identification Devices □ (RAID-1, RAID-M-100, RAID-S and RAID-XP) Ion mobility spectrometric detection of nerve and blister agents and selected TICs. The RAID-XP device incorporates radiological detection
Switzerland	IMS 2000 CW Agent Detector Ion mobility spectrometric detection of nerve and blister agents
U.K.	Chemical Agent Monitor (CAM), ECAM, GID-3, and LCD Detectors Ion mobility spectrometry based monitors for the detection of nerve and blister agents
U.S.A	MINICAMS Gas chromatographic detection of nerve and blister agents.
	M21 Remote Sensing Chemical Agent Alarm (RSCAAL) Passive infrared detection of chemical warfare agents

The development of point detectors improved the response capability of military forces and most of the commonly deployed devices have been based on rapid, sensitive detection of chemical warfare agents at a particular location. The Chemical Agent Monitor (CAM), first fielded in the 1980s as a hand-held alarm, was adopted by many countries for military field detection. Improved ion mobility spectrometry (IMS) devices have been produced and put into service in a variety of detection roles. Newer IMS based devices include the GID-3, the Lightweight Chemical Detector (LCD), the Enhanced Chemical Agent Monitor (ECAM), the RAID detector series and the ChemPro Hand-held Chemical Agent Detector. Competition to IMS based point detectors comes primarily from devices based on flame photometric detection (FPD), with the AP2C, AP4C and TIMs devices being widely used for chemical warfare agent detection due to their reliability. Use of only one technology for field detection is generally not recommended and most military forces and first responders will approach detection with a suite of options, with many opting to employ two or more detection technologies (e.g., IMS and FPD backed up with reagent based tests) to improve certainty of detection. Standoff detection, based on active and passive IR and other techniques, while desirable, has not been developed to a point of wide-spread adoption.

Identification of the chemical warfare agent in the field has become increasingly important and equipment that can provide this information in real time or near real time has been developed. The MiniCAMS, based on solid adsorbent sampling, gas chromatographic separation and FPD detection was developed for sensitive detection of chemical warfare agents at storage, demilitarization and other sites. Increasingly, mass spectrometric based instruments have been developed for identification purposes, with a number of military and environmental field portable instruments having been developed. Mass spectrometric instruments developed include the MM-1 Mobile Mass Spectrometer (deployed in the German Fuchs recon vehicle), the Block II Chemical and Biological Mass Spectrometer and the Viking and Bruker portable GC-MS systems. Sampling and analysis in a field situation remains an integral part of the OPCW strategy and the scientists involved in verifying the CWC currently use specialized field portable GC-MS instrumentation.

Many nations concerned about possible terrorist attacks have equipped their First Responders with military detection equipment used for chemical warfare agent identification and taken steps to ensure that laboratory networks are available to support forensic, remediation and other purposes. Detection networks utilizing ion mobility spectrometry and other means of detection and transportable laboratories containing analytical equipment have been developed and these may be deployed to sites such as the Olympics or Heads-of-States meetings where there is a perceived risk.

Safety and disposal

Chemical warfare agents are extremely hazardous and lethal compounds. They should only be used in designated laboratories by personnel trained in safe-handling and decontamination procedures and with immediate access to medical support. Safety and standard operating procedures must be developed and approved before any chemical warfare agents are handled. Chemical warfare agents can only be safely handled in laboratory chemical hoods with a minimum face velocity of 100 linear feet per minute equipped with emission control devices

that limit exhaust concentration to below 0.0001 mg/m³. Personnel handling chemical warfare agents should wear rubber gloves, lab coats, and full-faceshields and keep a respirator (gas mask) within easy reach. Sufficient decontaminant to destroy all chemical warfare agents being handled must be on hand before commencing operations. Additionally, sufficient quantities of nerve-agent antidote (HI-6) and reactive skin decontamination lotion (RSDL), necessary to treat all involved personnel, must be on hand prior to handling neat chemical warfare agents.

Blister and nerve agents can be destroyed using methanolic solutions of sodium or potassium hydroxide. Decontaminated chemical warfare agents must be disposed of in an environmentally approved method according to local legislation.

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Experimental

Sample and sample handling

The exercise participants analysed a test mixture containing chemical warfare agents and two samples (nylon office fabric and liquid) where the contamination was unknown. All samples and blanks used in the exercise were prepared by the DRDC Suffield Analytical Laboratory.

The chemical warfare agent test mixture used for quality control purposes contained sarin (GB), triethyl phosphate (TEP), cyclohexyl methylphosphonofluoridate (GF) and soman (GD) at a concentration of 0.002 mg/mL (prepared in water and analysed immediately to reduce the possibility of hydrolysis).

Spiked nylon office fabric samples were prepared by adding 5 μ L of 2 mg/mL GF (in dichloromethane) to 0.12 g of fabric. The samples were allowed to stand for 10 minutes prior to sample handling and analysis by the participants. Each spiked fabric sample was ultrasonically extracted for 10 minutes with water (3 mL) in a 20 mL scintillation vial. An aliquot of the water layer (1 mL) was removed and centrifuged at 14000 rpm to remove fines. A portion of this extract (0.5 mL) was removed and stored in a screw-capped Teflon-lined 1.8 mL sample vial prior to liquid chromatography electrospray mass spectrometry (LC-ESI-MS) analysis (analysed immediately to reduce the possibility of hydrolysis). Office fabric blanks were treated in a similar manner.

A liquid sample, typical of what might be collected from a suspicious laboratory during a forensic investigation, was analysed directly by LC-MS for the presence of chemical warfare agents (and related compounds). The aqueous sample contained munitions grade tabun at 0.005 mg/mL.

Desorption electrospray ionization mass spectrometry (DESI-MS) was also evaluated for the rapid analysis of SPME fiber samples. Nylon office fabric (0.12 g), placed in a 20 mL headspace sampling vial, was spiked with 5 μ L of 2 mg/mL GF (identical to LC-MS fabric sample). The dichloromethane was allowed to evaporate and SPME sampling was conducted on the headspace above the spiked fabric contained in the sampling vial for 5 min at 40 °C. Supelco (Bellefonte, PA, USA) (65 micron film thickness) polydimethylsiloxane/divinyl benzene (PDMS/DVB) SPME fibers were used for headspace sampling.

Instrumental analysis

Mass spectrometric data were acquired in the laboratory using a Waters (Milford, MA, USA) Q-ToF Ultima tandem mass spectrometer equipped with a Z-spray electrospray interface. The electrospray capillary was operated in the 1.5 to 3 kV range with a sampling cone voltage of 35 V. The collision energy was generally maintained at 5 V for LC-ESI-MS operation and was varied from 3 to 10 V (depending on the precursor ion selected) for LC-ESI-MS/MS

operation. Argon was continually flowing into the collision cell at 9 psi during both LC-ESI-MS and LC-ESI-MS/MS operation. Nitrogen desolvation gas was introduced into the interface (80°C) at a flow rate of 300 L/h and nitrogen cone gas was introduced at a flow rate of 50 L/h. ESI-MS data were typically acquired from 70 to 700 Da and ESI-MS/MS (product ion mass spectra) data were acquired for the protonated molecular ions of the spiked compounds (0.3 to 1 s). All data were acquired in the continuum mode with a resolution of 8000 (V-mode, 50% valley definition).

DESI-MS and DESI-MS/MS data were obtained under similar conditions using the Waters Q-ToF Ultima tandem mass spectrometer. A laboratory stand was used to hold and position the SPME manual holder so that the fiber could be introduced into the ESI plume. The plexiglass sleeve on the Z-spray interface contained a septum port to facilitate the safe introduction of SPME fibers contaminated with chemical warfare agents. The LC solvents, 50:50 acetonitrile/water (0.1% trifluoroacetic acid), were sprayed at 10 μ L/min during DESI analyses.

LC-MS separations were performed with an Agilent 1100 capillary LC (Palo Alto, CA, USA) using a 5% to 75%B gradient over 5 minutes and a flow rate of 10 μ L/min. The following solvent compositions were prepared for the mobile phase: Solvent A (0.1% trifluoroacetic acid in water) and Solvent B (acetonitrile). All LC separations were performed with Agilent 50 mm x 0.3 mm i.d. fused-silica capillary columns packed with Zorbax SB C18 (1.8 μ m particle size). An autosampler was used to introduce 1 μ L aqueous injections.

Table 4 summarizes the analysis schedule for the exercise.

Table 4. CW agent analysis schedule for the training course

Sample	Collision Energy	Remarks
1. TEP (0.5 ng/μL in water)	5 V	QA/QC standard. First run of the day. Check S/N and interpret data.
2. TEP (0.5 ng/μL in water)	5 V	Check S/N and interpret data.
3. TEP (0.5 ng/μL in water)	10 V	Generation of product ions and interpret data.
4. Water blank	5 V	Carryover of TEP?
5. CW agents (GB/TEP/GF/GD) (2 ng/μL in water)	5 V	Interpret data.
6. CW agents (GB/TEP/GF/GD) (2 ng/μL in water)	8 V	Interpret data.
7. CW agents (GB/TEP/GF/GD) (2 ng/μL in water)	vary	MS/MS on selected compounds.
8. Water and sample blanks		Carryover of agents?
9. Unknown #1 blank (spiked office fabric aqueous extract)	5 V	Complexity?
10. Unknown #1 spike (spiked office fabric aqueous extract)	5 V	CW agent (s) present?
11. Water and sample blanks	5 V	Carryover of agents?
12. Unknown #2 (aqueous sample)	5 V	Identify CW agent(s). Other compounds?
13. Unknown #2 (aqueous sample)	vary	Identify CW agent(s). Other compounds? MS/MS?
14. Water and SPME blanks	5 V	Carryover of agents?
15. DESI-MS (SPME of spiked office fabric sample)	vary	Identify CW agent(s).

Results and discussion

LC-MS analysis of test mixture

A test mixture containing three common chemical warfare agents (GB, GD and GF) and the simulant TEP at the 0.002 mg/mL level was initially analysed to assess the quality of the LC-MS data being generated, to provide an opportunity for handling of a dilute solutions containing chemical warfare agents, and to provide an opportunity to interpret the resultant mass spectra. Figure 8 illustrates a typical LC-MS chromatogram obtained for a 1 μ L injection of the chemical warfare agent test mixture. Each sample component (2 ng) was readily resolved and ESI mass spectra for each sample component were acquired, interpreted and compared to library mass spectra contained in the DRDC Suffield ESI-MS database. Figure 9 illustrates typical ESI mass spectra acquired for GB, TEP GF and GD.

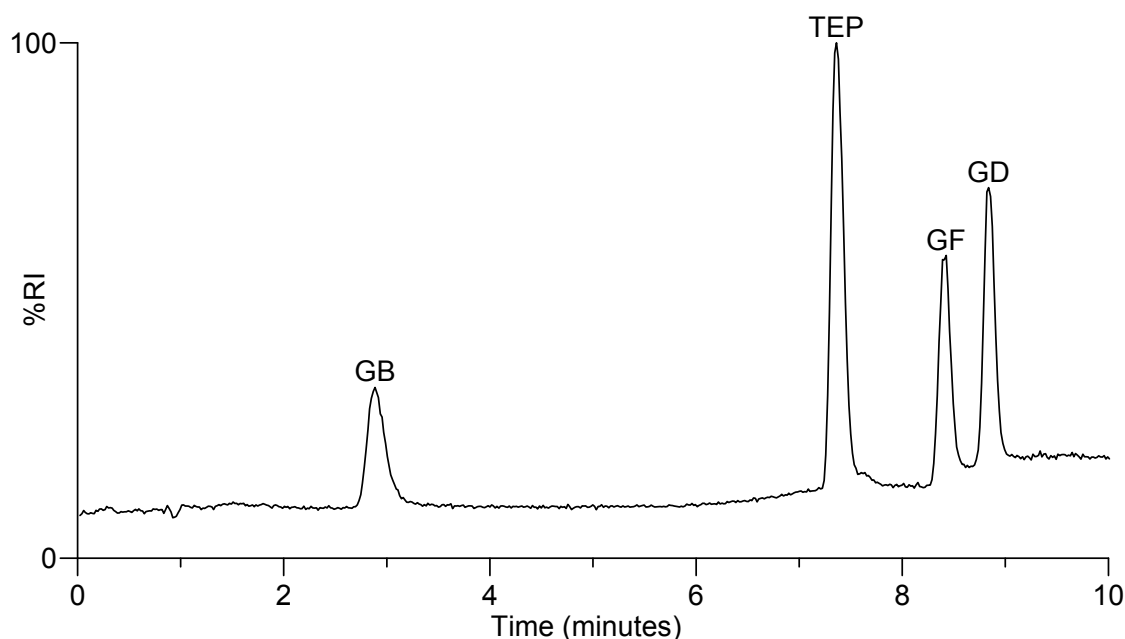


Figure 8. LC-MS total-ion-current chromatogram of chemical agent test mixture containing 2 ng of sarin (GB), triethyl phosphate (TEP), cyclohexyl methylphosphonofluoridate (GF) and soman (GD).

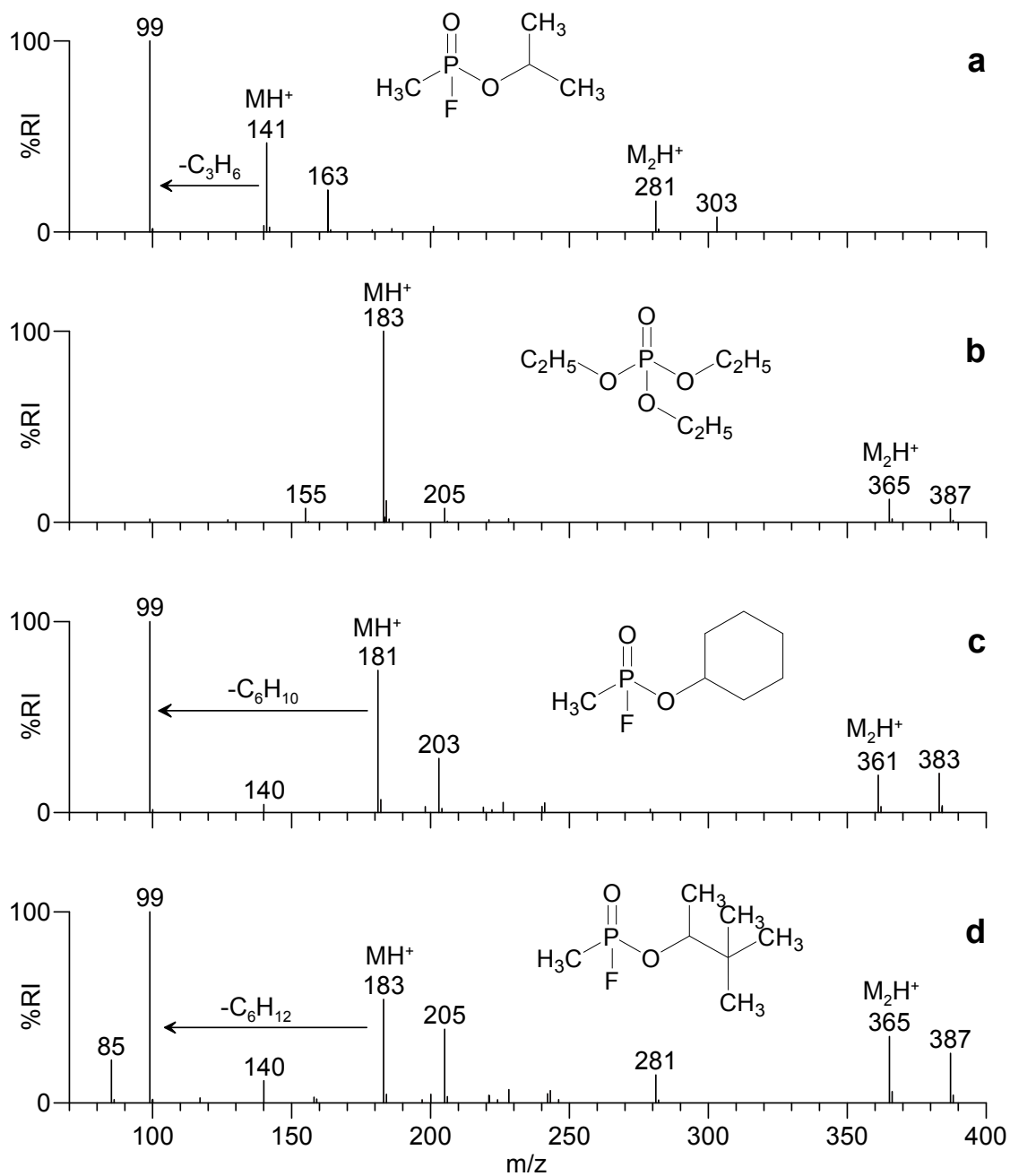


Figure 9. ESI-MS data obtained for a) GB, b) TEP, c) GF and d) GD (collision energy: 5 V).

LC-MS analysis of nylon fabric sample

A spiked nylon fabric sample and blank was provided as an unknown for LC-MS analysis with the following note.

Unknown 1: Nylon Fabric Sample

Evidence collected from an office chair fabric where the occupant experienced symptoms that may be consistent with chemical warfare agent exposure (suspect fabric).

Similar chairs were found in other offices and a sample of "clean" fabric was taken from an office a good distance away (clean fabric).

Was the fabric in the suspect case exposed to chemical warfare agent?

The spiked sample and its corresponding blank were extracted with water using the method described in the Experimental. Sample extracts (1 μL) were analysed by LC-MS and the acquired mass spectra were interpreted and compared to library spectra contained in the DRDC Suffield ESI-MS database. Figure 10 illustrates a typical LC-MS chromatograms obtained for the aqueous extract of the nylon fabric blank and nylon fabric sample spiked with GF. The aqueous extracts contained multiple sample components but the GF was readily identified by comparison of both the LC retention time and ESI mass spectral data (Figure 11) with reference data acquired for GF under identical experimental conditions.

LC-MS analysis of liquid sample

A liquid sample, typical of what might be collected during a forensic investigation was provided with the following note.

Unknown 2: Liquid Sample

Liquid evidence collected from an possible clandestine chemical warfare synthetic laboratory.

Identify the chemical warfare agents and any other related compounds?

The aqueous sample was analysed directly by LC-MS for the presence of chemical warfare agents (and related compounds). Figure 12 illustrates the acquired LC-MS chromatogram, containing munitions grade tabun (GA) at the 0.005 mg/mL level. Tabun and a number of other related organophosphorus compounds (Figure 13) were identified by interpreting the acquired mass spectrometric data or by comparison of acquired data with that contained in the DRDC Suffield ESI-MS database supplied to the participants.

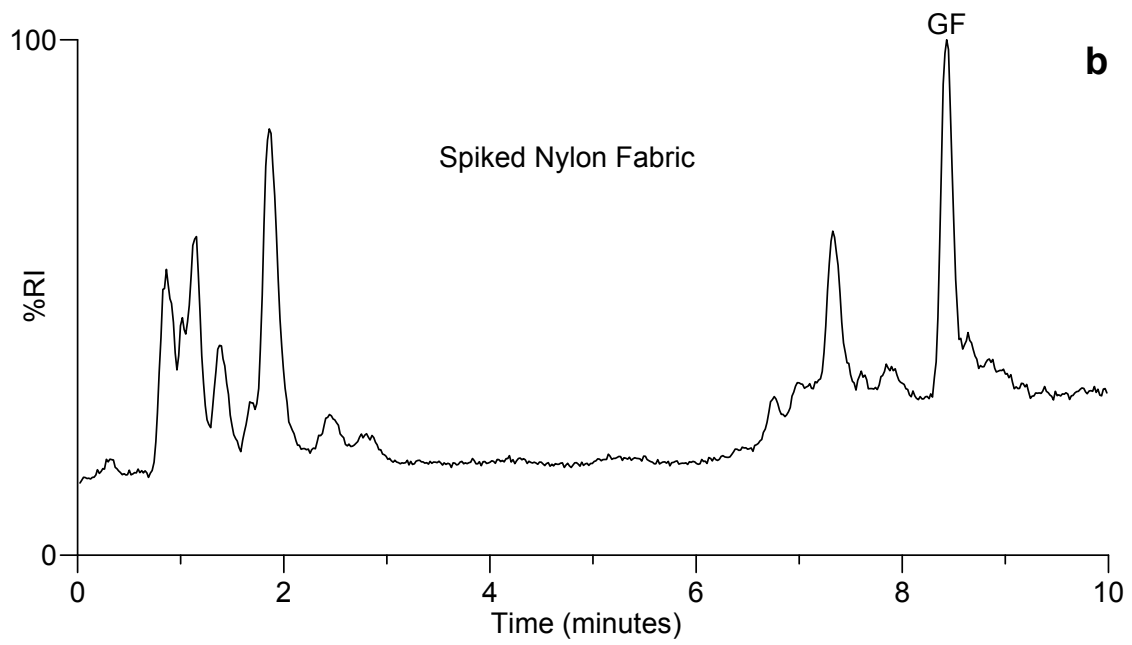
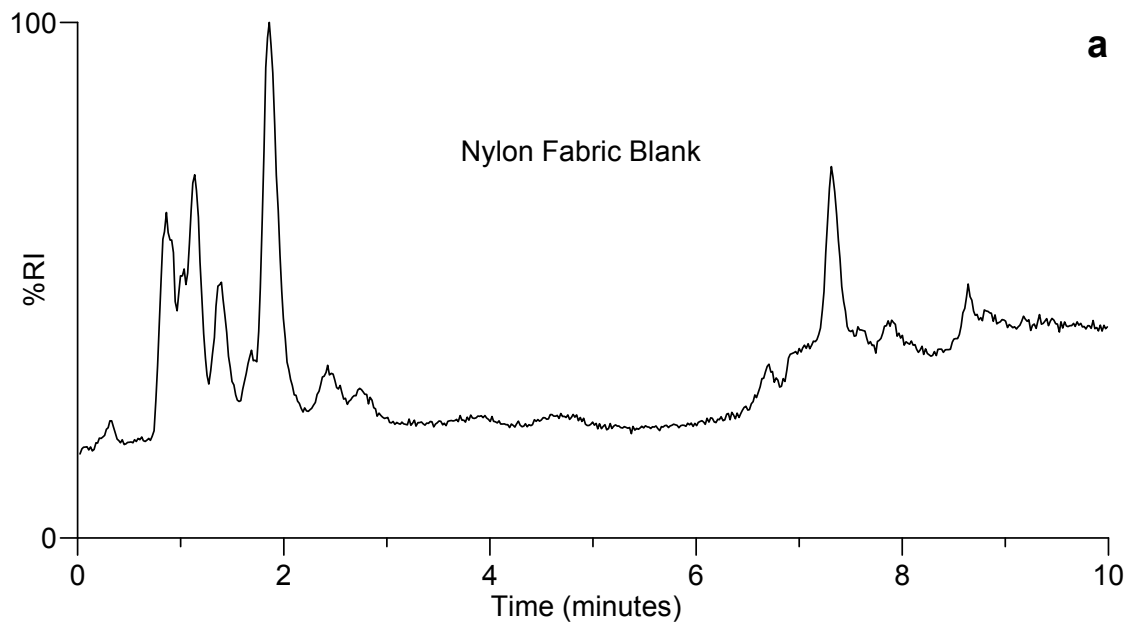


Figure 10. LC-MS total-ion-current chromatogram of a) aqueous extract of nylon fabric blank and b) nylon fabric spiked with GF at the 80 $\mu\text{g/g}$ level.

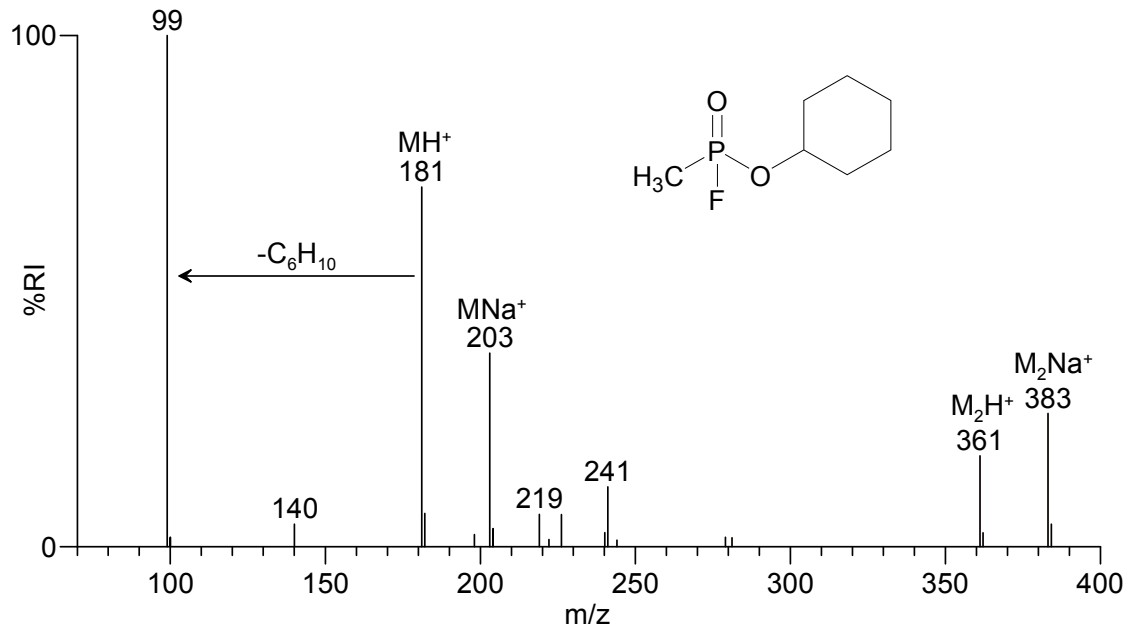


Figure 11. ESI-MS data obtained for GF contained in the aqueous extract of the spiked nylon fabric sample (refer to Figure 10).

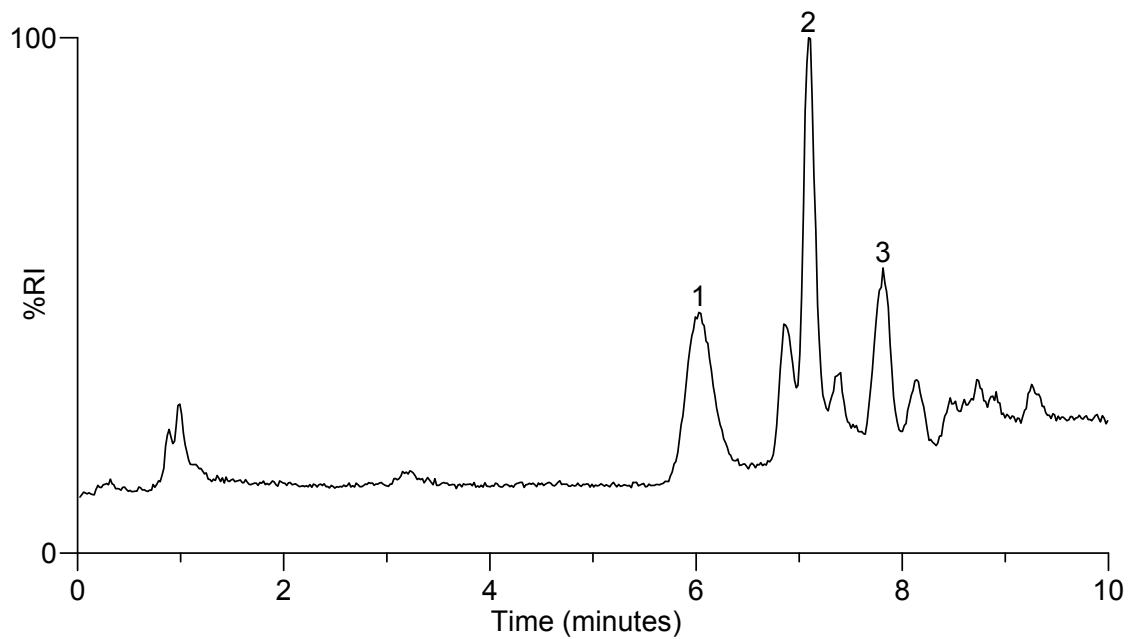


Figure 12. LC-MS total-ion-current chromatogram of unknown liquid sample.

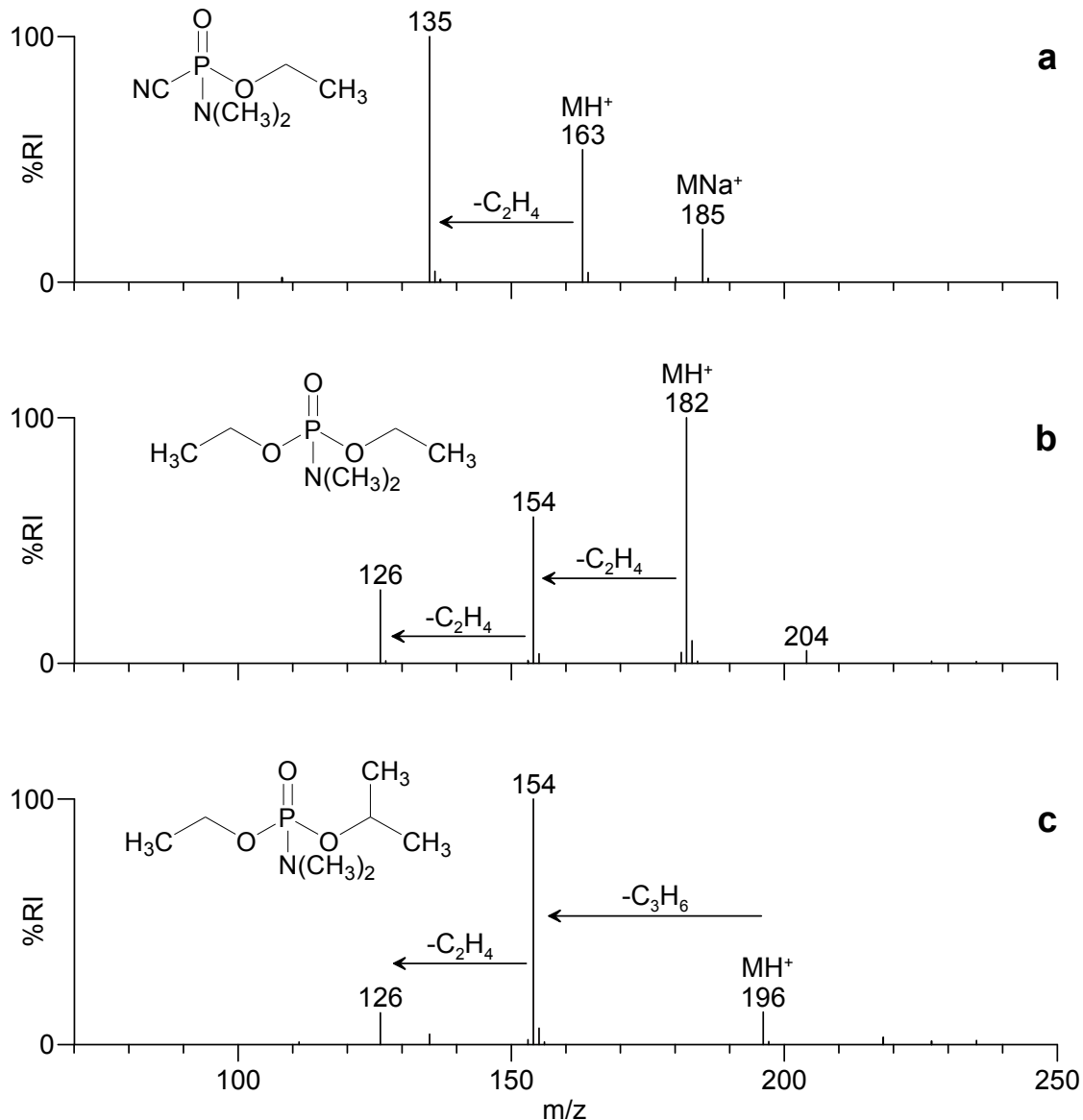


Figure 13. Typical ESI-MS data obtained for the unknown liquid sample. a) Tabun, b) diethyl dimethylphosphoramidate, c) ethyl tetramethylphosphorodiamidate and several other components were detected in the unknown liquid sample spiked at the 5 µg/ml level with munitions grade tabun.

DESI-MS analysis of nylon fabric sample

A spiked nylon fabric sample (identical to the LC-MS unknown) was provided to demonstrate the applicability of headspace SPME sampling and DESI-MS and DESI-MS/MS analysis for chemical warfare agents. This novel approach to sample handling and analysis is emerging as an important analytical method at DRDC Suffield as it significantly reduces sample handling and analysis time. The headspace above the spiked nylon fabric sample and its corresponding

blank were sampled using the method described in the Experimental. SPME fibers were analysed directly by DESI-MS and DESI-MS/MS and the acquired data were interpreted and compared to library spectra. Figure 14 illustrates typical DESI-MS/MS chromatograms obtained for m/z 181 ($[M+H]^+$ ion for GF) for the nylon fabric blank and nylon fabric sample spiked with GF. DESI-MS/MS data (product ions of m/z 181) were obtained for GF sampled onto the solid phase microextraction fiber using a collision energy of 4 V (Figure 15).

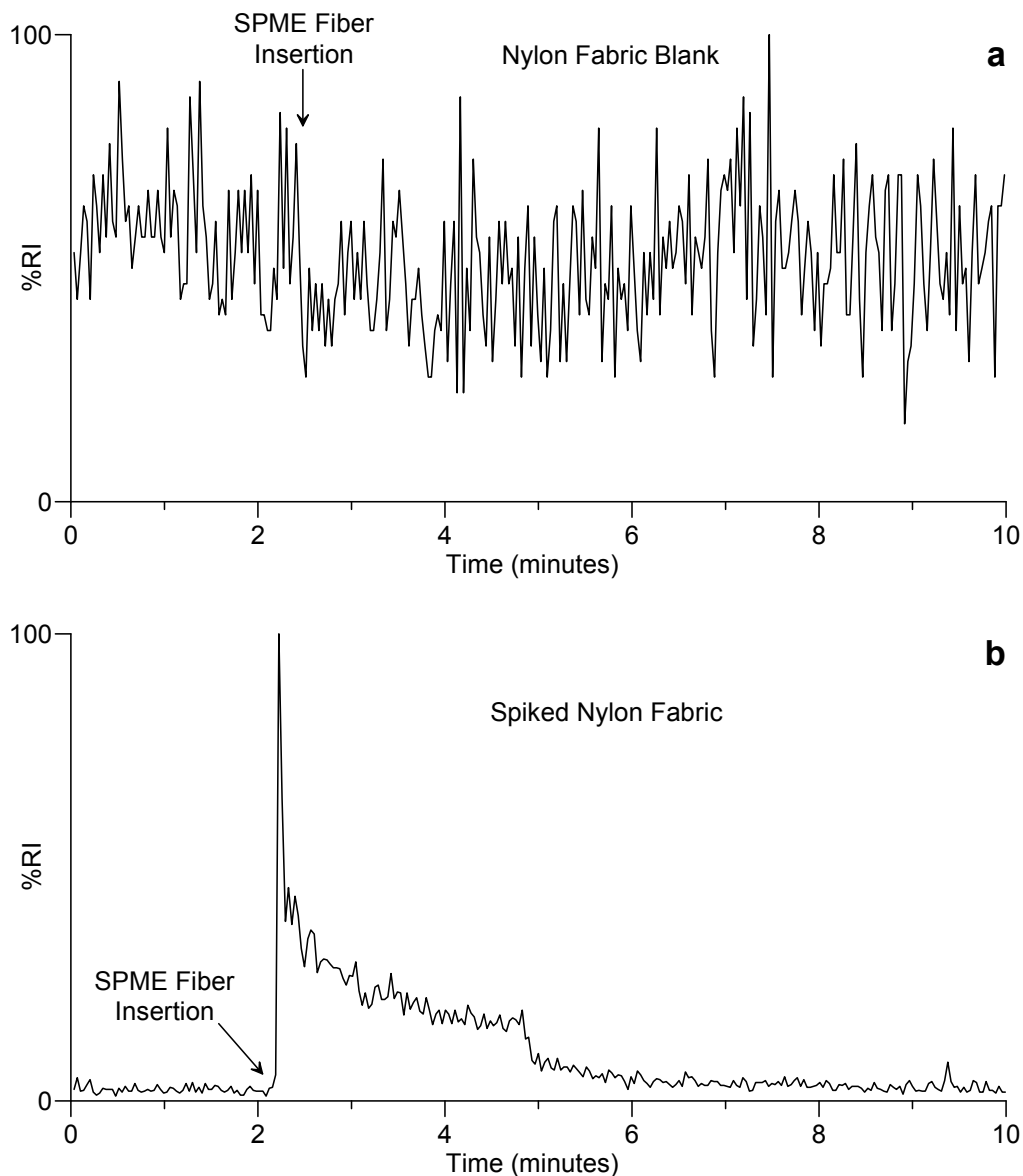


Figure 14. DESI-MS/MS (m/z 181) for the a) nylon fabric blank and b) nylon fabric spiked at the $80 \mu\text{g/g}$ level with GF following headspace sampling (5 minutes at 50°C) with a solid phase microextraction fiber.

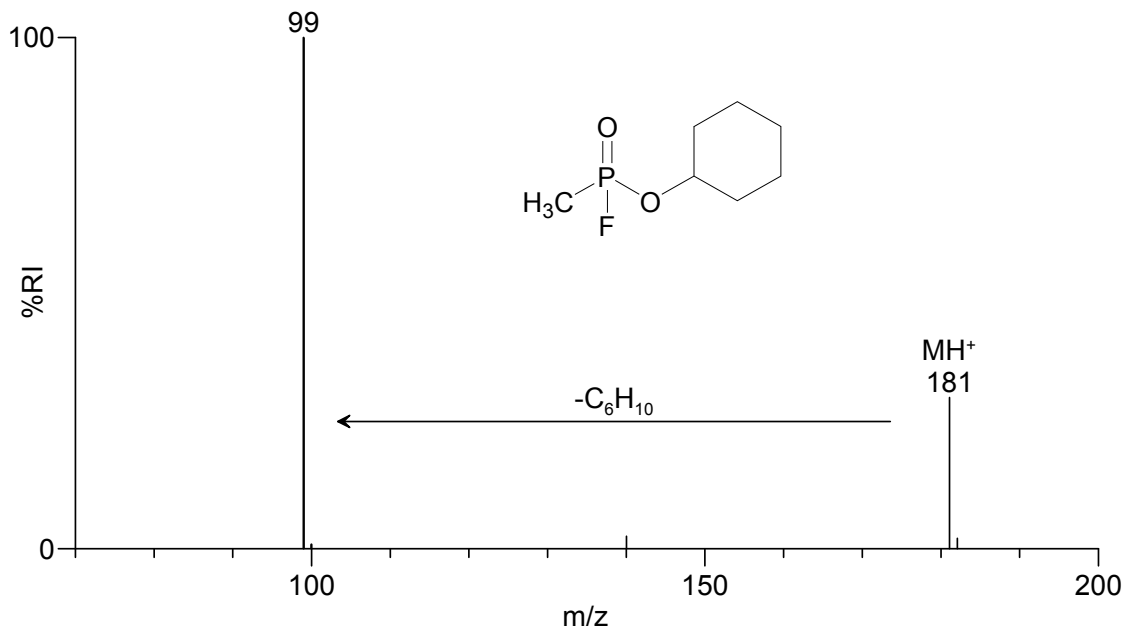


Figure 15. DESI-MS/MS data obtained for GF sampled onto the solid phase microextraction fiber (collision energy: 4 V).

Chemical warfare agent detection devices used by the Canadian Forces student workshop

The Canadian Forces (CF) have six in-service devices for the detection of chemical warfare chemical warfare agents. These devices are based on a number of different chemical principles ranging from chemical solubility to ion-mobility spectrometry and detect the presence of a range of nerve, blister, blood and choking chemical warfare agents.

3-Way Paper is a dye-impregnated paper that can detect the presence of nerve and blister agents in their liquid state. Dye-solubility is the principle behind 3-Way Paper and a positive response to agent would be indicated by the appearance of a spot with the corresponding dye color. G-series nerve agents are visualized by a yellow colour change, V-series nerve agents by a green colour change and blister agents by a red colour change. Available in booklet form with a legend on the cover for colour comparison, 3-Way Paper is produced and marketed by Anachemia Canada Inc. Its ease of use and low cost make the 3-Way Paper an economical tool in the detection of chemical warfare agents.

The Nerve Agent Vapour Detector (NAVD) is a small clear plastic ticket with two paper sections, one impregnated with acetylcholinesterase and the other impregnated with a colorless dye that reacts with active acetylcholinesterase to form a blue complex. Once the paper sections are wetted and exposed to the suspect atmosphere, they are pressed together. In the absence of nerve agent, a blue-coloured spot develops but if nerve agent is present, the

enzyme is inhibited and no blue spot appears. The NAVD is highly selective but it is limited to nerve agent detection and does not indicate which nerve agent is present. Its 5.5 x 2.5 x 0.2 cm size makes it the smallest detection device in-service with the CF. It is manufactured and distributed by Anachemia Canada Inc.

The M256A1 kit was developed in an attempt to combine the detection of many chemical warfare agents in a single device. This kit, produced by Anachemia Canada Inc., contains a hard-plastic carrying case, 12 sampler-detectors and detailed instruction cards that are attached to the M256A1 case. Each sampler-detector incorporates an enzyme impregnated paper spot for nerve agent detection, as described for the NAVD; a test spot, and accompanying heater assembly, for blister agents such as mustard (H) and phosgene oxime (CX); a tablet to identify the presence of Lewisite (L); and finally a test spot for blood agents such as cyanogen chloride (CK) and hydrogen cyanide (AC). Small chemical-filled ampoules are broken to allow chemical combinations to flow through plastic channels and wet the appropriate test spots prior to a 10 minute vapour exposure. Instructions for use are also provided on each sampler-detector's protective foil wrap. This detector system is small, inexpensive and allows users to determine whether their immediate environment is safe enough to remove their protective gear. It is manufactured and distributed by Anachemia Canada Inc.

The Chemical Agent Monitor (CAM) has been an integral part of the Canadian Forces chemical warfare agent detection equipment since 1986. The CAM was initially developed and produced by Graseby Dynamics Ltd. in the UK. Graseby Dynamics Ltd. has since been incorporated into Smiths Detection who now holds the rights to produce and market the complete line of CAM products. The CAM uses ion-mobility spectrometry (IMS) to detect nerve and blister agent vapours. Air samples are drawn into the nozzle (i.e. probe) and pass through a silicon membrane before coming into contact with acetone vapour, provided by the sieve breather assembly, circulating in the CAM. The green arrows in Figure 14 illustrate the internal airflow pattern. The acetone and agent molecules are ionized by the Ni^{63} radioactive source to form low-mobility ion clusters. The gating grid is opened to allow the ion clusters to travel towards the ion collector plate, which maintains positive or negative polarity depending on the operator's choice of G or H mode. This process is repeated many times per second and the time it takes for the clusters to reach the collector plate, referenced to an internal reactant ion peak drift, is compared to known times for agent ion clusters. If the measured ion mobilities correspond to known agent ion mobilities, a bar graph response is produced on the LCD graphical display. The amount of ions measured is relative to the concentration of agent in the sampled vapour and the number of bars visualized, from one to eight, reflects the estimated concentration. This method of detection provides some selectivity for chemical warfare agents by monitoring only those times in the IMS spectrum where nerve or blister agent ion clusters appear. This programmed selectivity can prevent the user from observing high concentrations of toxic chemicals not included in the manufacturer's software. Newer versions of the CAM, which include the CAM2Plus and the ECAM, still detect the classical chemical warfare agents (H-series, G-series and VX) but are also programmed to include the blood and choking agents AC, phosgene (CG) and chlorine (Cl_2). While in operation, the CAM samples continuously and responds to low levels of agent in one to five seconds. The size (1.9 kg) and cost of a CAM is significantly larger than the three other personal chemical warfare agent detectors used by the CF but none of these provide such rapid detection as the CAM.

CAM (SECTION OF INNER LAYOUT)

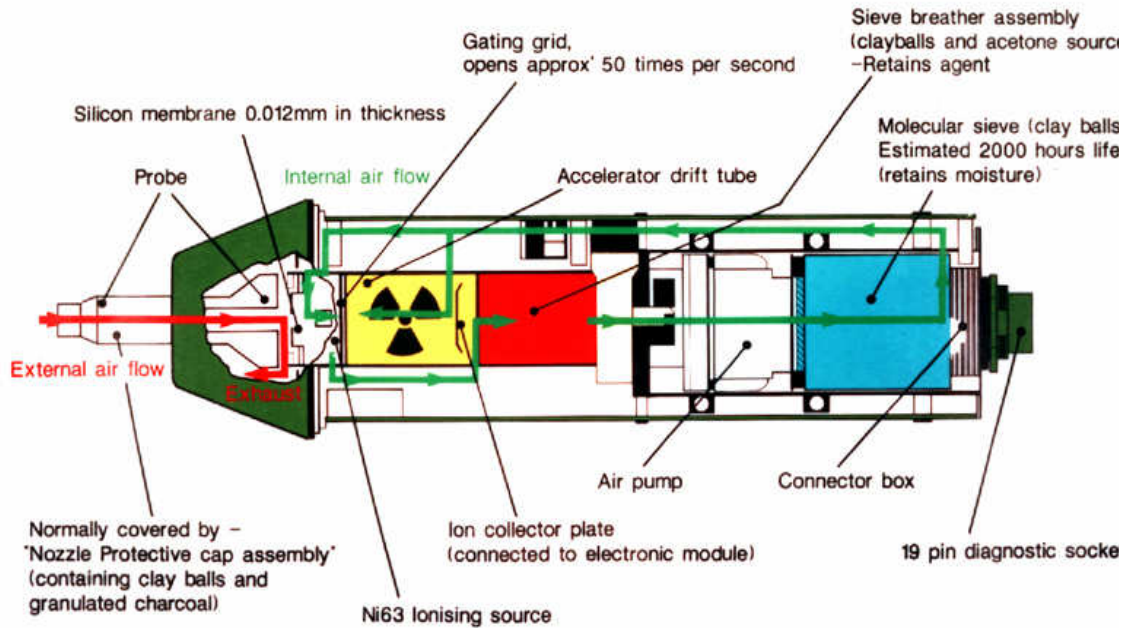


Figure 16. General schematic for the Chemical Agent Monitor (CAM).

A system for simultaneous remote monitoring of G and H agents was required by the CF during the Iraq War in 1990-91 and DRDC Suffield developed the CADS II to fulfill that requirement. This networked system uses two CAMs per sensor station, one operating in G mode with the other in H mode. A solar cell with back-up battery provides power at each station and a central control unit controls the network of stations, each of which may be deployed up to 3000 meters from the central control unit.

The only detection system currently in-service with the CF that is specifically engineered for simultaneous G and H monitoring, is the GID-3, an IMS-based detector now being manufactured by Smiths Detection. The unique feature of the GID-3 is the dual ion drift tubes and collector plates that allow for both positive and negative ions to be measured simultaneously. This system is fitted with a much larger battery than the CAM and is well suited for unmanned operation or vehicle mounting. The GID-3 has a bar graph for visual alert, audible alarms and can be remotely monitored through a networked warning system.

Recently the CF awarded a contract to VisionTec to provide and support the AP4C detector, produced by Proengin in France, as the newest piece of chemical detection equipment to be put in-service with the CF. This device is based on flame photometry and will eventually

replace the CAM as the hand-held chemical detector for the CF. The CF is currently evaluating other commercially available chemical warfare agent detection equipment to increase their capability in personal detection. Some of the equipment of interest include the LCD detector series produced by Smiths Detection in the UK and the ChemPro[®] manufactured by Environics Oy in Finland along with other devices which meet the CF's light-weight personal detection requirements. DRDC Suffield also has research underway to examine and extend the use of solid-phase micro-extraction (SPME) techniques in conjunction with field-portable GC-MS for the rapid detection and identification of chemical warfare agents and toxic industrial chemicals (TICs) that may be encountered by the CF and civilian first-responders.

Conclusions

Each of the analytical exercise participants conducts sample handling and analysis for a variety of target compounds for their government departments (RCMP, Health Canada, Environment Canada). If their sample handling methods co-extracted chemical warfare agents the analysts would be able to identify the common chemical warfare agents, provided LC-MS analyses were conducted under full scanning conditions.

The analytical exercise participants successfully analysed a chemical warfare agent test mixture by LC-MS, interpreted the acquired mass spectra and correctly identified the unknown chemical warfare agents spiked onto nylon fabric samples and a liquid sample during LC-MS analysis. Chemical warfare agents were identified on the basis of both a LC retention time and ESI mass spectrometric match with authentic reference standards (or library data).

DESI-MS was also demonstrated as an emerging technique for the analysis of chemical warfare agents. The headspace above nylon fabric samples spiked with GF was sampled and successfully analysed by DESI-MS and DESI-MS/MS.

The analytical participants were briefed on both safety considerations and chemical warfare agent detection devices. Detection devices, including the Chemical Agent Monitor, were demonstrated and handled by the participants and several sampling kits were opened up for examination.

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The Chemical Cluster, one of five clusters created by the Chemical, Biological, Radiological Nuclear and Explosive Research and Technology Initiative (CRTI), was established to help Canada prepare for, prevent and respond to terrorist events. This working group, made up of representatives from Canadian government departments, has identified a number of chemicals of concern and assigned laboratories with appropriate expertise to provide the analytical support necessary to confirm these compounds in suspect samples. The Royal Canadian Mounted Police (RCMP), in its lead forensics role, will attempt to tentatively identify the chemical(s) of concern and pass on the samples to the responsible laboratory within the Chemical Cluster. Samples containing large amounts of relatively pure chemical warfare agents should trigger a response with one of the chemical monitoring devices (e.g., Chemical Agent Monitor) used by the RCMP to triage samples. Defence R&D Canada Suffield has been tasked to analyse samples suspected to contain chemical warfare agents for the Chemical Cluster and would receive this type of suspect sample. There remains a possibility that samples with a lower level of chemical warfare agent contamination might inadvertently find their way into a laboratory tasked with another type of analysis. To manage this possibility, the laboratories receiving these types of samples should have an analytical screening capability to allow for the tentative identification of chemical warfare agents in samples and sample extracts. This report summarizes the third chemical warfare agent training exercise in sample preparation and analysis by liquid chromatography-mass spectrometry (LC-MS) given by DRDC Suffield to other Chemical Cluster laboratories.

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Liquid chromatography, mass spectrometry, chemical warfare agents, review

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