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Mass Spectrometric Determination of Chemical Warfare Agents in Indoor Sample Media Typically Collected During Forensic Investigations

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Technical Report
DRDC Suffield TR 2005-097
October 2005

Canada

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AQ F06-05-3058

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Abstract

Terrorist use of chemical warfare agents against civilian targets could involve the targeting of enclosed populated spaces. DRDC Suffield, in collaboration with the Royal Canadian Mounted Police, identified a need for analytical methods for chemical warfare agent identification in media, including flooring, wall surfaces, office fabrics and paper products, that would typically be collected in an office environment during forensic investigations. Typical office environment media were spiked at the 4 to 20 µg/g level with either a complex munitions grade sample of tabun (GA) or with a standard containing the three nerve agents, sarin (GB), cyclohexyl methylphosphonofluoridate (GF), soman (GD) and the nerve agent simulant, triethyl phosphate (TEP), to evaluate the potentials of LC-ESI-MS and LC-ESI-MS/MS for forensic purposes. The spiked chemical warfare agents were recovered with varying efficiencies, but in all cases sufficient chemical warfare agent was recovered for identification purposes. In some instances the aqueous extracts contained numerous co-extracted sample components that complicated LC-ESI-MS analysis and hampered identification. These interferences were minimized during LC-ES-MS/MS analysis, where each of the chemical warfare agents was identified on the basis of acquired product ion mass spectra. MS data for all the spiked compounds in the nerve agent standard and the munitions grade tabun were acquired in the continuum mode with a resolution of 9000, which typically resulted in mass measurement errors of 0.001 Da or less. Application of the developed sample handling and analysis methodology is anticipated during forensic investigations where evidence of chemical warfare agent use is required for criminal prosecution or to assess remediation/restoration efforts following an incident.

Résumé

L'utilisation par les terroristes des agents de guerre chimiques contre des cibles civiles pourrait comprendre le ciblage d'espaces clos peuplés. RDDC Suffield a identifié, en collaboration avec la Gendarmerie Royale du Canada, un besoin en méthodes analytiques capables d'identifier des agents de guerre chimiques dans des milieux tels que les revêtements de sol, la surface des murs, les tissus des bureaux et les produits en papier, qui seraient normalement collectés dans des bureaux, durant les enquêtes judiciaires. Des milieux normaux de bureaux ont été ensemencés à un niveau de 4 à 20 µg/g soit avec un échantillon complexe de tabun, GA, (de qualité utilisée dans les munitions) GA soit avec un échantillon standard contenant les trois agents neurotoxiques sarin (GB), cyclohexyl methylphosphonofluoridate (GF), soman (Gd) et l'agent neurotoxique simulant, phosphate de triéthyle (TEP), pour évaluer le potentiel des techniques CPL-ESI-SM et CPL-ESI-SM/SM à des fins judiciaires. Les agents de guerre chimiques ont été récupérés plus ou moins efficacement mais dans tous les cas on en a récupéré suffisamment à ces fins. Dans certains cas, les extraits aqueux contenaient de nombreux composants d'échantillons co-extraits ce qui a compliqué les analyses CPL-ESI-SM et a gêné l'identification. Ces interférences ont été minimisées durant les analyses CPL-ESI-SM/SM, durant lesquelles chacun des agents de guerre chimique a été identifié sur la base du spectre de la masse ionique du produit acquis. Les données de la SM de tous les composés ensemencés dans l'agent neurotoxique standard et les échantillons de tabun (de qualité utilisée dans les munitions) ont été acquises en mode continu avec une résolution de 9000, ce qui résulte normalement en des erreurs des mesures de masse correspondant à 0.001 Da ou moins. On s'attend à ce que la méthodologie de la manipulation et des analyses des échantillons mis au point soit appliquée durant les enquêtes judiciaires pendant lesquelles les preuves d'utilisation d'agents de guerre chimiques sont requises pour les poursuites pénales ou pour évaluer les efforts en mesures correctives et en restauration qui font suite à l'incident.

Executive summary

Introduction: Terrorist use of chemical warfare agents against civilian targets could involve the targeting of enclosed populated spaces. DRDC Suffield, in collaboration with the Royal Canadian Mounted Police, identified a need for analytical methods for chemical warfare agent identification in media that would typically be collected in an office environment during forensic investigations. The present study focused on the development and application of sample handling and LC-ESI-MS/MS analytical methods for contaminated sample media that might be collected, including flooring, wall surfaces, office fabrics and paper products.

Results: Typical office environment media were spiked at the 4 to 20 µg/g level with either a complex munitions grade sample of tabun (GA) or with a standard containing the three nerve agents, sarin (GB), cyclohexyl methylphosphonofluoridate (GF), soman (GD) and the nerve agent simulant, triethyl phosphate (TEP), to evaluate the potentials of LC-ESI-MS and LC-ESI-MS/MS for forensic purposes. The spiked chemical warfare agents were recovered with varying efficiencies, but in all cases sufficient chemical warfare agent was recovered for identification purposes. In some instances the aqueous extracts contained numerous co-extracted sample components that complicated LC-ESI-MS analysis and hampered identification. These interferences were minimized during LC-ES-MS/MS analysis, where each of the chemical warfare agents was identified on the basis of acquired product ion mass spectra. MS data for all the spiked compounds in the nerve agent standard and the munitions grade tabun were acquired in the continuum mode with a resolution of 9000, which typically resulted in mass measurement errors of 0.001 Da or less.

Significance: Application of the developed sample handling and analysis methodology is anticipated during forensic investigations where evidence of chemical warfare agent use is required for criminal prosecution or to assess remediation/restoration efforts following an incident.

Future Plans: The reported method will be a valuable addition to the present methods for the identification of chemical warfare agents and their hydrolysis products in samples collected in support of counter-terrorism. Continued development and application of tandem mass spectrometry to samples containing chemical warfare agent contamination is anticipated with the recent availability of a high resolution tandem mass spectrometer at DRDC Suffield.

D'Agostino, P.A., Hancock, J.R., Chenier, C.L., 2005. Mass Spectrometric Determination of Chemical Warfare Agents in Indoor Sample Media Typically Collected during Forensic Investigations. DRDC Suffield TR 2005-097. Defence R&D Canada – Suffield.

Sommaire

Introduction : L'utilisation par les terroristes d'agents de guerre chimiques contre des cibles civiles pourrait comprendre le ciblage d'espaces clos peuplés. RDDC Suffield a identifié, en collaboration avec la Gendarmerie royale du Canada, un besoin en méthodes analytiques des agents de guerre chimiques qui seraient normalement collectés dans des milieux de bureaux durant les enquêtes judiciaires. L'étude actuelle est axée sur la mise au point et l'application de méthodes de manipulation d'échantillons et de méthodes analytiques CPL-ESI-SM et CPL-ESI-SM/SM pour des milieux d'échantillons contaminés, dont les revêtements de sol, la surface des murs, les tissus de bureaux et les produits en papier, qui pourraient être recueillis.

Résultats : Des milieux normaux de bureaux ont été ensemencés à un niveau de 4 à 20 µg/g soit avec un échantillon de tabun (GA) (de qualité utilisée dans les munitions) soit avec un échantillon standard contenant les trois agents neurotoxiques sarin (GB), cyclohexyl methylphosphonofluoridate (GF), soman (Gd) et l'agent neurotoxique simulant, phosphate de triéthyle (TEP), pour évaluer le potentiel de CPL-ESI-SM et CPL-ESI-SM/SM à des fins judiciaires. Les agents de guerre chimiques ont été récupérés plus ou moins efficacement mais dans tous les cas on en a récupéré suffisamment à ces fins. Dans certains cas, les extraits aqueux contenaient de nombreux composants d'échantillons co-extraits ce qui a compliqué les analyses CPL-ESI-SM et a gêné l'identification. Ces interférences ont été minimisées durant les analyses CPL-ESI-SM/SM, durant lesquelles chacun des agents de guerre chimique a été identifié sur la base du spectre de la masse ionique du produit acquis. Les données de la SM de tous les composés ensemencés dans l'agent neurotoxique standard et le tabun (de qualité utilisée dans les munitions) ont été acquises en mode continu avec une résolution de 9000, ce qui résulte normalement en des erreurs des mesures de masse correspondant à 0.001 Da ou moins.

La portée : On s'attend à ce que la méthodologie de la manipulation et des analyses des échantillons mis au point soit appliquée durant les enquêtes judiciaires pendant lesquelles les preuves d'utilisation d'agents de guerre chimiques sont requises pour les poursuites pénales ou pour évaluer les efforts en mesures correctives et en restauration qui font suite à l'incident.

Plans futurs : La méthode documentée sera un ajout important aux méthodes actuelles concernant l'identification des agents de guerre chimiques et leurs produits d'hydrolyse dans les échantillons recueillis pour soutenir la lutte contre le terrorisme. On s'attend à ce que continue la mise au point et l'application de la spectrométrie de masse en tandem aux échantillons contenant une contamination par des agents de guerre ceci grâce à la disponibilité récente des spectromètres de masse en tandem de haute résolution à RDDC Suffield.

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Introduction

Historically chemical warfare agent use has been associated with the use of these chemicals in a battlefield setting. Tear gas grenades were used in 1914 by the French at the outbreak of the First World War, but it was not until the Germans first used chlorine near Ypres in 1915 that the world entered the modern era of chemical warfare. Other chemical warfare agents such as phosgene and mustard were weaponized during the First World War and were used by both sides throughout the conflict. The use and development of chemical warfare agents 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 Italians against the Abyssinians (Ethiopia) during the 1936–1937 war and just prior to the Second World War, the Germans discovered and produced the first nerve agent, tabun. Tabun was weaponized by the Germans but neither side made use of their chemical weapons stocks. More effective nerve agents, such as VX, were developed in the 1950's, mustard was used in the Yemen Civil War (1963–1967) and allegations of chemical warfare agent use were reported in South East Asian conflicts. Nerve and mustard agents were used by Iraq in the 1980's war between Iran and Iraq, and were considered a real threat to United Nations armed forces during their action against Iraq in 1991. Widespread battlefield use of chemical warfare agents, long a concern of NATO Forces, ended rather abruptly with the ending of the Cold War.

The Chemical Weapons Convention has reduced the likelihood of battlefield chemical weapons use, but there remains a serious concern world-wide that other parties may make use of chemical warfare agents against civilian or military targets. Sarin, a well known nerve agent, was used by the Aum Shinrikyo sect in Japan in 1995 during an attack on the Tokyo underground transit system, during which a dozen people were killed and thousands more were injured. Public concern about the use of chemical warfare agents, or other weapons of mass destruction, reached a new peak following the terrorist events of September 11, 2001 and the subsequent delivery of anthrax-containing letters through the United States postal system. These events heightened security concerns within many countries and considerable resources were expended to improve all facets of response to the perceived terrorist threat.

Within Canada, one of the initiatives to counter terrorist use of weapons of mass destruction was the establishment of the Chemical, biological, radiological and nuclear Research and Technology Initiative (CRTI). This research-oriented organization originally formed three clusters to deal with the challenges associated with each of chemical, biological and nuclear use. Each cluster is made up of representatives from responsible Canadian government departments, with the Chemical Cluster being concerned with chemical warfare agents (and other toxic chemicals). The Royal Canadian Mounted Police (RCMP), in its lead forensics role, will triage samples and pass on suspect samples to the responsible laboratory within the Chemical Cluster. Samples suspected to contain chemical warfare agents would be sent to Defence Research and Development Canada (DRDC Suffield), the National laboratory tasked to analyse samples containing chemical warfare agents.

The development of analytical methods for the detection and identification of chemical warfare agents, their degradation products and related compounds has been thoroughly reviewed with different emphases on a number of occasions (1-9). Much of the analytical methods development was driven by the requirements of the military and their need to be able to detect and identify these compounds in typical battlefield samples. These methods focused largely on the determination of chemical warfare agents or their degradation products in environmental matrices such as soil (10-18), water (17, 19-22), air (23, 24), recovered munitions and munition blocks (12, 13, 25), decontamination solutions (26-28) and military clothing/gear (10, 12, 13). Newer methods based on solid phase microextraction (SPME) sampling followed by GC-MS analysis (29-34) and direct analysis by secondary ion mass spectrometry (35, 36) have been reported for environmental analyses, but most of the reported analytical methods are based on gas chromatography-mass spectrometry analysis of an extract of the collected medium (10-28). Organic extracts of chemical warfare agents may be analysed directly by GC-MS, but the hydrolysis products of chemical warfare agents usually require derivatization prior to GC-MS analysis (9, 15-18, 20, 22). More recently researchers have demonstrated the value of liquid chromatography-mass spectrometry as a complementary or replacement method for GC-MS, particularly for the confirmation of hydrolysis products of chemical warfare agents in aqueous extracts or samples (37-48), as the hydrolysis products may be analysed directly by LC-MS without the need for additional sample handling and derivatization. In addition, LC-MS has the added benefit that it may also be utilized for the determination of organophosphorus chemical warfare agents and related compounds in aqueous extracts of soil, water, snow and other samples (43-46, 49-50).

Methods for biological media, such as urine and blood, received increased attention following the use of mustard during the Iran-Iraq War (51), the Tokyo subway incident (52-54) and continues today given the concern over possible use of chemical warfare agents against civilian populations (7, 55-57). *J. Anal. Toxicology* recognized this research emphasis and a number of papers dealing with mass spectrometric determination of chemical warfare agents in biological matrices were published in a special issue (58-65).

Terrorist use of chemical warfare agent against civilian targets could involve the targeting of enclosed populated spaces. DRDC Suffield, in collaboration with the Royal Canadian Mounted Police, identified a need for analytical methods for chemical warfare agent identification in media that would typically be collected in an office environment during forensic investigations. The present study focused on the development and application of sample handling and LC-ESI-MS/MS analytical methods for contaminated sample media that might be collected, including flooring, wall surfaces, office fabrics and paper products.

Typical office environment media were spiked at the 4 to 20 µg/g level with either a complex munitions grade sample of tabun (GA) or with a standard containing three nerve agents, sarin (GB), cyclohexyl methylphosphonofluoridate (GF), soman (GD) and the nerve agent simulant, triethyl phosphate (TEP), to evaluate the potentials of LC-ESI-MS and LC-ESI-MS/MS for forensic purposes. Preliminary investigations with spiked office carpet (66, 67) were extended to include other office media including, latex painted drywall, office fabrics, photocopy paper and Dacron sampling swabs. Representative samples of each were spiked with chemical warfare agents and/or related compounds, extracted with water using ultrasonic vibration, centrifuged to reduce the presence of fines and analysed by LC-ESI-MS and LC-ESI-MS/MS. The spiked chemical warfare agents were recovered with varying efficiencies, but in all cases sufficient chemical warfare agent was recovered for identification purposes. In some instances the aqueous extracts contained numerous co-extracted sample components that complicated LC-ESI-MS analysis and hampered identification. These interferences were minimized during LC-ES-MS/MS analysis, where each of the chemical warfare agents was identified on the basis of acquired product ion mass spectra. MS data for all the spiked compounds in the nerve agent standard and the munitions grade tabun were acquired in the continuum mode with a resolution of 9000, which typically resulted in mass measurement errors of 0.001 Da or less.

Experimental

Office media samples

Samples of six different office media were selected for evaluation (typical sample weights given in parentheses).

1. 100% (solution dyed) nylon office carpet [A. Kulpe Karpet Ltd.], (1 g).
2. 20lb White photocopy paper [Xerographic], (0.25 g).
3. Fisherbrand sterile Dacron swab tip [Fisher Scientific], (0.1 g).
4. Latex painted drywall, (0.17 g).
5. 56% Nylon/44% polyester office fabric treated with Scotchguard [J. Ennis Fabrics], (0.23 g).
6. 100% Nylon office fabric treated with Teflon [J. Ennis Fabrics], (0.15 g).

Chemical warfare agent standards

Two different standards were used for spiking the office media listed above.

1. The standard containing GB, GD, GF and TEP was prepared at an individual concentration of 0.4 mg/mL level in dichloromethane. Triplicate samples of each of the media were spiked with a 5 μL aliquot of the mixture with the exception of the office carpet sample, which was spiked with a 10 μL aliquot.

2. The munitions grade tabun (GA) standard contained GA and a number of other sample components with the concentration of the sum of all the sample components being 1 mg/mL in dichloromethane. The office carpet and the photocopy paper were spiked with 25 μL and 10 μL aliquots of the munitions grade GA, respectively.

Sample handling

Weighed samples of the listed office media were placed in individual 20 mL screw capped glass scintillation vials (Wheaton). The samples were then spiked with either 5 μL (or 10 μL) aliquots of the standard containing GB, GD, GF and TEP or 10 μL (or 25 μL) aliquots of the munitions grade GA sample. The spiked samples were allowed to stand for 30 minutes. Spiked samples were ultrasonically extracted with 2 mL (photocopy paper, latex painted drywall, Dacron swab) or 5 mL (office carpet and both office fabrics) of water (Aldrich-Sigma) for 10 minutes. The water volume was sufficient to completely immerse the sample and a narrow glass vial of the correct height was inserted into the scintillation vial to ensure that the sample did not float. The vial was then subjected to ultrasonic vibration for 10 min. A 1 mL aliquot was removed and centrifuged at 14,000 rpm to remove any fines. A 750 µL portion of the centrifuged sample was placed in a 1.8 mL glass autosampler vial for analysis. The same procedures were followed for (unspiked) office media blanks.

Analysis

LC-ESI-MS and LC-ESI-MS/MS data were acquired using a Waters QTOF Ultima tandem mass spectrometer equipped with a Z-spray electrospray interface. The electrospray capillary was operated in the 1 to 3 kV range with a sampling cone voltage of 35 V. The collision energy was maintained at 5 V for LC-ESI-MS operation and was varied from 2 to 10 V (depending on the precusor 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 acquired from 40 to 700 Da (1 s with a 0.1 s interscan delay or 0.3 s with a 0.1 s interscan delay) and ESI-MS/MS (product ion mass spectra) data were acquired for the protonated molecular ions of the spiked compounds (1 s with a 0.1 s interscan delay). All data were acquired in the continuum mode with a resolution of 9000 (V-mode, 50% valley definition).

Chromatographic separations were performed with a Waters CapLC using a 5% to 75%B gradient over 30 minutes and a flow rate of 10 µL/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 MicroTech 150 mm x 0.32 mm i.d. fused-silica capillary columns packed with Zorbax C18 SB (5 µm particle size). The CapLC autosampler was used to introduce 1 µL samples of the aqueous extracts.

The retention times varied slightly throughout the course of the study due to the use of a new LC column (same packing material) for the latex painted drywall, Dacron swab and office fabrics sample extract analyses.

Results and discussion

Analytical methods need to be developed to ensure that suspect samples collected during forensic or other investigations can be analysed for the presence of chemical warfare agents in a timely manner. Increasingly researchers have utilized LC-MS as a complementary or replacement method for GC-MS, particularly for the confirmation of hydrolysis products of chemical warfare agents in aqueous extracts or samples (37-48). Recent publications have also demonstrated the application of LC-ESI-MS for the determination of organophosphorus chemical warfare agents and related compounds in water, snow and aqueous extracts (43-46, 49-50). Aqueous extracts of sample media offer a number of advantages and disadvantages for chemical warfare agent analysis. Advantages include the fact that aqueous extracts may be used to analyse both organophosphorus chemical warfare agents and their hydrolysis products during a single LC-MS analysis, that aqueous extracts tend to contain less co-extracted chemical interferences than organic extracts and that aqueous extracts do not generally dissolve or degrade the sample media as can be the case for organic solvent extracts. The principal disadvantage to aqueous extraction for the determination of organophosphorus chemical warfare agents is the possibility of chemical warfare agent hydrolysis during sample handling or on prolonged standing. Methods involving aqueous extraction followed by LC-ESI-MS and LC-ESI-MS/MS analysis of soil samples contaminated with organophosphorus chemical warfare agents and their hydrolysis products (43) and soil samples containing the hydrolysis products of munitions grade mustard (47) have been developed at DRDC Suffield. A similar approach for indoor office media that could be collected during a forensic investigation seemed worth investigating. The present study focused on the development and application of sample handling and LC-ESI-MS and LC-ESI-MS/MS analytical methods for contaminated indoor sample media that might be collected during a forensic investigation, including office flooring, wall surfaces, office fabrics and paper products.

Typical office environment media were spiked at the 4 to 20 µg/g level with a standard containing three nerve agents, sarin (GB), cyclohexyl methylphosphonofluoridate (GF), soman (GD) and the nerve agent simulant, triethyl phosphate (TEP), to evaluate the potential of LC-ESI-MS and LC-ESI-MS/MS for forensic purposes. Representative samples of the office media were spiked with the standard, extracted with water using ultrasonic vibration, centrifuged to reduce the presence of fines and analysed by both LC-ESI-MS and LC-ESI-MS/MS. The spiked compounds were recovered with varying efficiencies, but in all cases sufficient chemical warfare agent was recovered for identification purposes. Figure 1 to 6 illustrate the reconstructed-ion-current chromatograms for m/z 183, m/z 99, m/z 97 and the total-ion-current chromatogram obtained during LC-ESI-MS analysis of the aqueous extracts of the office carpet, photocopy paper, Dacon swab, latex painted drywall, and two office fabrics, spiked with this standard. The total-ion-current chromatogram provided a good indication of the complexity of the sample extract. Significant co-extracted components were observed in the office carpet extract (Figure 1), some were observed for the office fabrics and other sample extracts and very few were observed in the photocopy paper extract (Figure 2).

The reconstructed-ion-current chromatograms for m/z 97, the common product ion observed for alkyl methylphosphonic acids formed after hydrolysis of GB, GF and GD, were presented for each extract to give an indication of hydrolysis either during sample handling or on standing prior to analysis. Hydrolysis products were only noted during analysis of the photocopy paper extracts. Figure 7 illustrates typical product ion mass spectra for the three acids initially formed after hydrolysis of GB, GF and GD. At a collision energy of 4V all compounds exhibited the $[M+H]^+$ precusor and a significant product ion at m/z 97 due to loss of the alkene associated with the alkoxy group of GB, GF and GD. Hydrolysis products were not evident in the extracts of the other office media provided the analyses were completed within approximately an hour of sample handling. Over increasing hours some hydrolysis of the spiked chemical warfare agents was evident, consistent with earlier findings (44).

The reconstructed-ion-current chromatograms for m/z 183 ($[M+H]^+$ for TEP (and GD) and m/z 99 (a common product ion due to loss of the alkene associated with the alkoxy group of GB, GF and GD) were used to indicate the presence of the four spiked compounds. ESI-MS data were similar to previously published data acquired with a TOF mass spectrometer (50), with the principal differences being in the relative abundances of the sodiated and protonated acetonitrile adducts.

A semi-quantitative estimate of the recovery efficiency of each compound from each of the extracts was calculated by comparing the area under the reconstructed-ion-current chromatogram profiles for m/z 99+141 (GB), m/z 183 (TEP), m/z 99+181 (GF) and m/z 99+183 (GD) with data obtained during analysis of a standard solution. Table 1 lists the recovery estimates for the office media in triplicate. Recoveries varied with both media and compound, with TEP, a compound that resists hydrolysis, generally being recovered with the greatest efficiency. Recoveries were for the most part in the 50% to 85% range for the spiked chemical warfare agents. The lowest recoveries, about 25%, were for GF and GD from the latex painted drywall, a surface material that absorbs chemical warfare agents more easily than the chemically resistant paints used for military applications. At the lower recovery levels full, interpretable, mass spectra (ESI-MS or ESI-MS/MS) were readily acquired for all the spiked compounds leading to the conclusion that GB, TEP, GF and GD could be identified in contaminated office media of the type described at or below the µg/g level. Recovery variabilities of this order were also observed by Tornes, Opstad and Johnsen during a study investigating the recovery of GA, GB, GD, mustard and diisopropyl methylphosphonate from typical battlefield media including water, grass, soil, sand, protective suit materials and paper (10) and by D'Agostino, Hancock and Provost during the analysis of soils spiked with GB and GD (43).

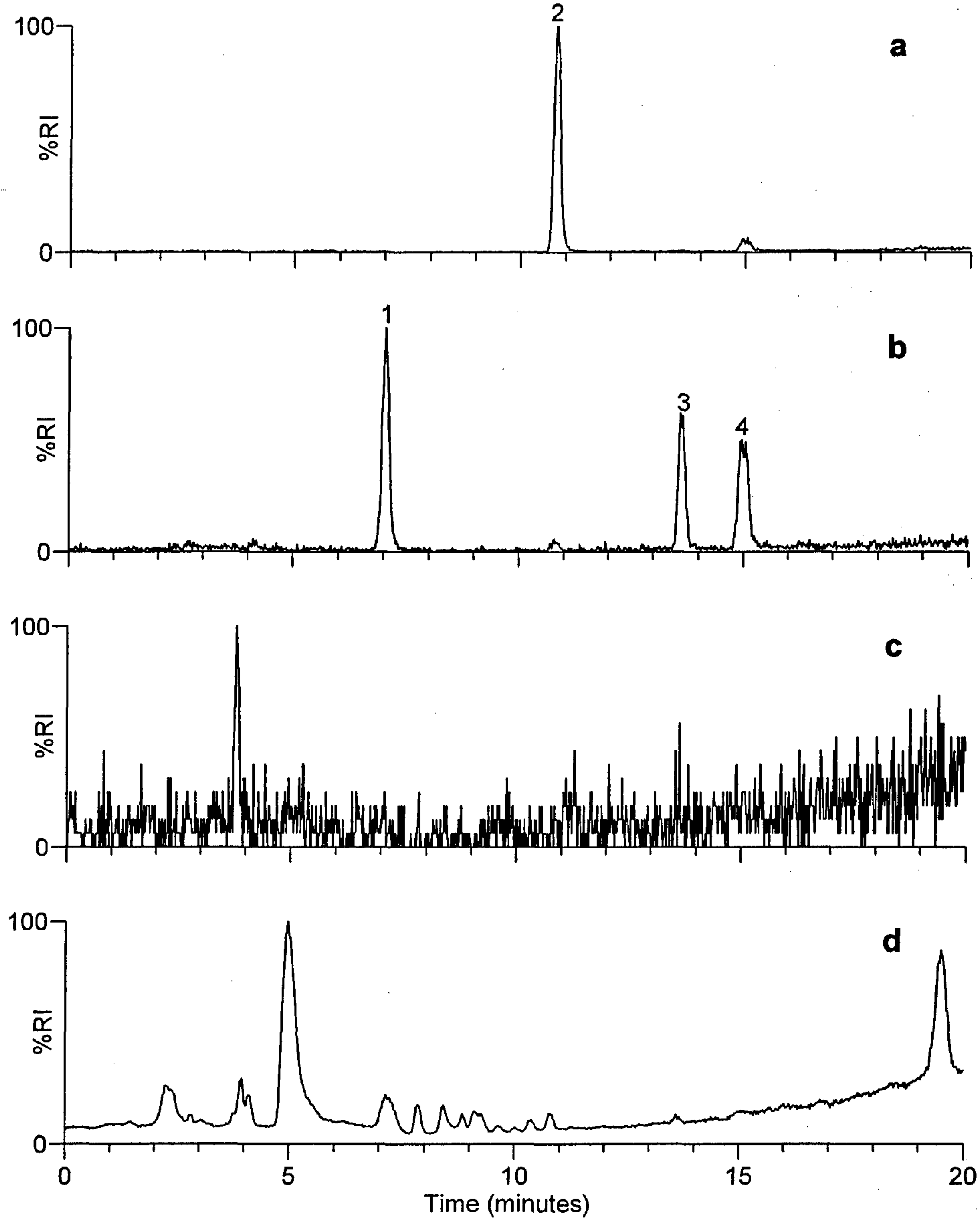

Figure 1. *Reconstructed-ion-current chromatograms for a) m/z 183, b) m/z 99, c) m/z 97 and d) total-ion-current chromatogram obtained during LC-ESI-MS analysis of an extract of an office carpet sample spiked at the 4 µg/g level with GB (1), TEP (2), GF (3) and GD (4).*

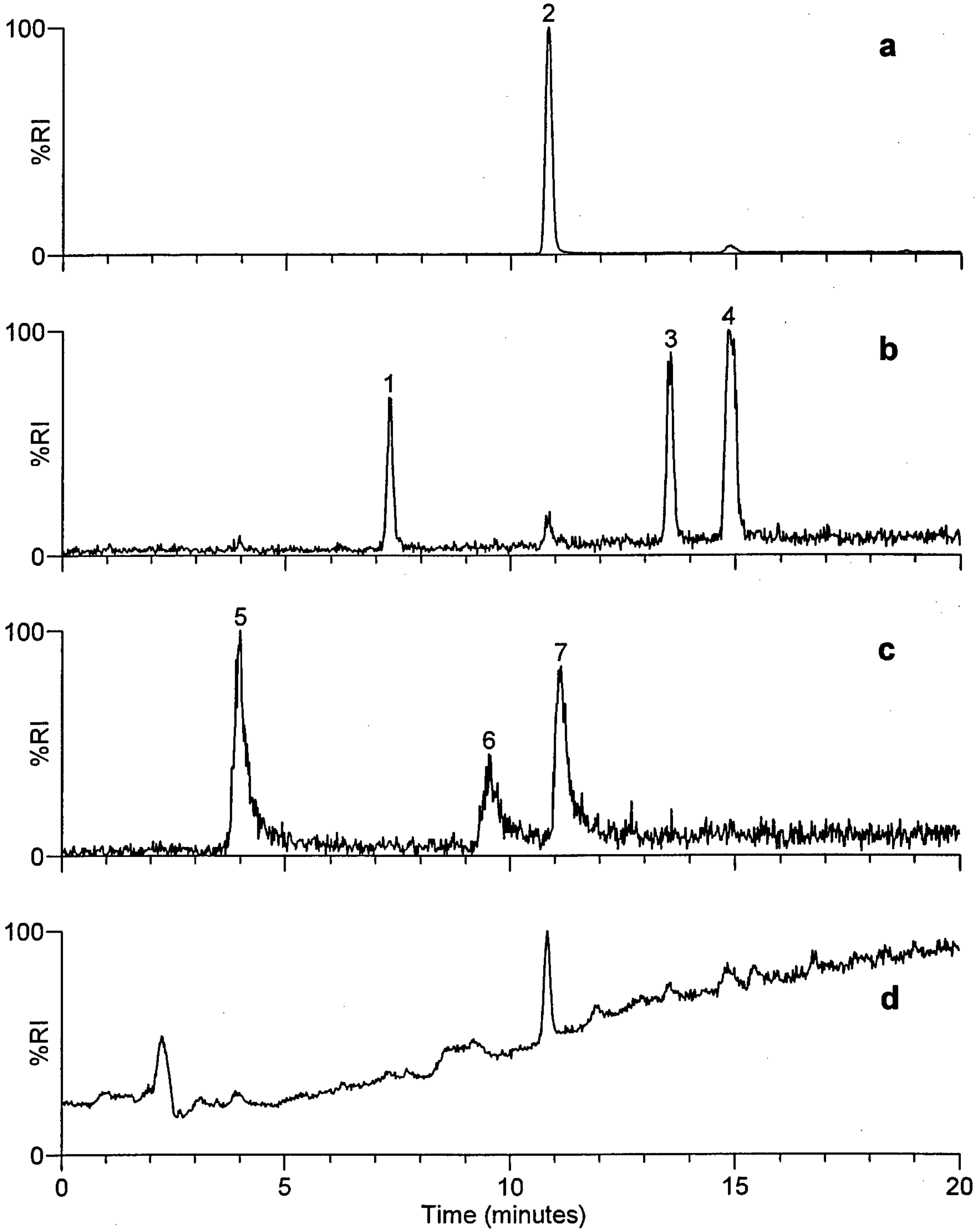

Figure 2. *Reconstructed-ion-current chromatograms for a) m/z 183, b) m/z 99, c) m/z 97 and d) total-ion-current chromatogram obtained during LC-ESI-MS analysis of an extract of a photocopy paper sample spiked at the 8 µg/g level with GB (1), TEP (2), GF (3) and GD (4). Isopropyl methylphosphonic acid (5), cyclohexyl methylphosphonic acid (6) and pinacolyl methylphosphonic acid (7), the hydrolysis products of GB, GF and GD, respectively, were also observed.*

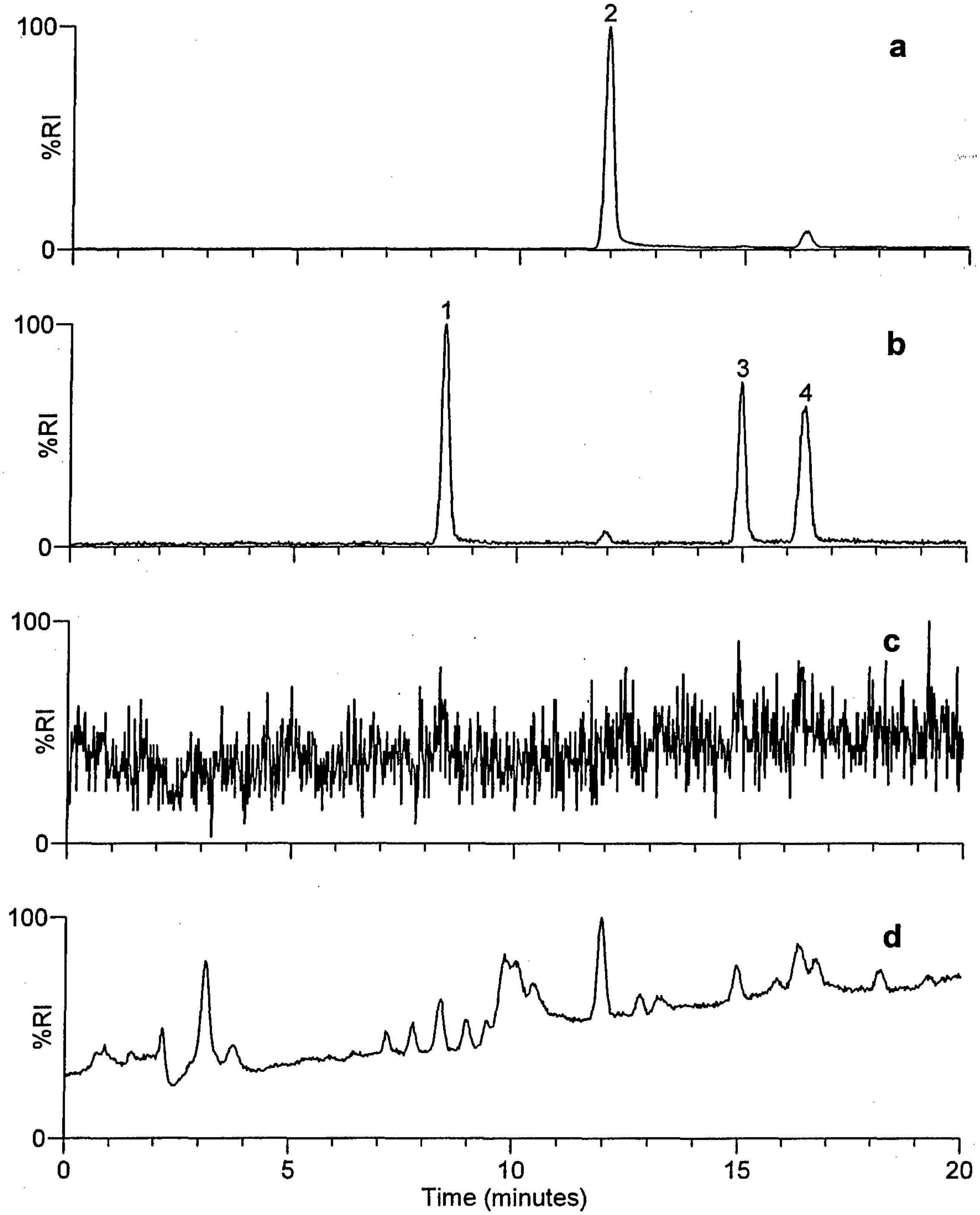

Figure 3. *Reconstructed-ion-current chromatograms for a) m/z 183, b) m/z 99, c) m/z 97 and d) total-ion-current chromatogram obtained during LC-ESI-MS analysis of an extract of a Dacron swab sample spiked at the 20 µg/g level with GB (1), TEP (2), GF (3) and GD (4).*

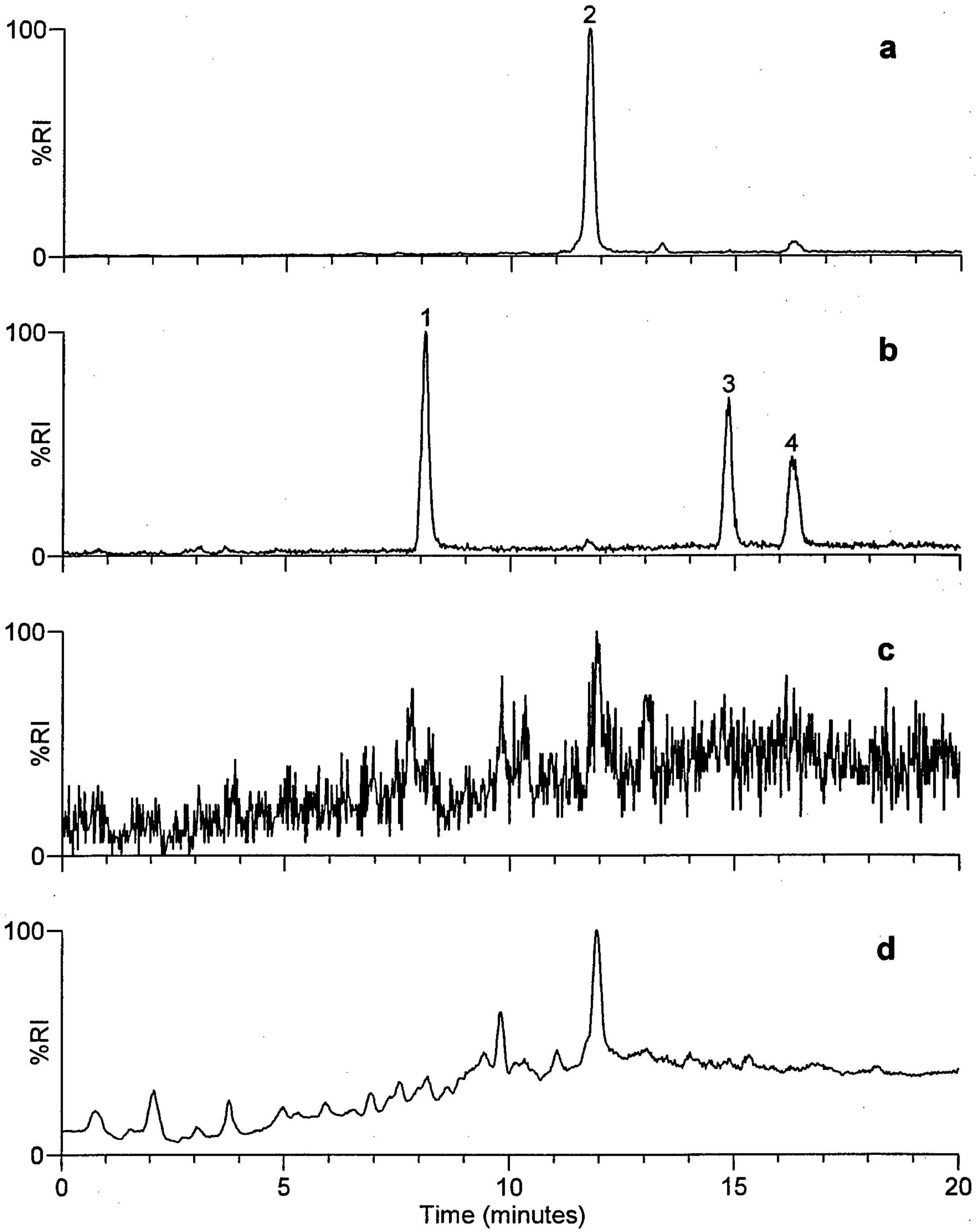

Figure 4. *Reconstructed-ion-current chromatograms for a) m/z 183, b) m/z 99, c) m/z 97 and d) total-ion-current chromatogram obtained during LC-ESI-MS analysis of an extract of a latex painted drywall sample spiked at the 12 μg/g level with GB (1), TEP (2), GF (3) and GD (4).*

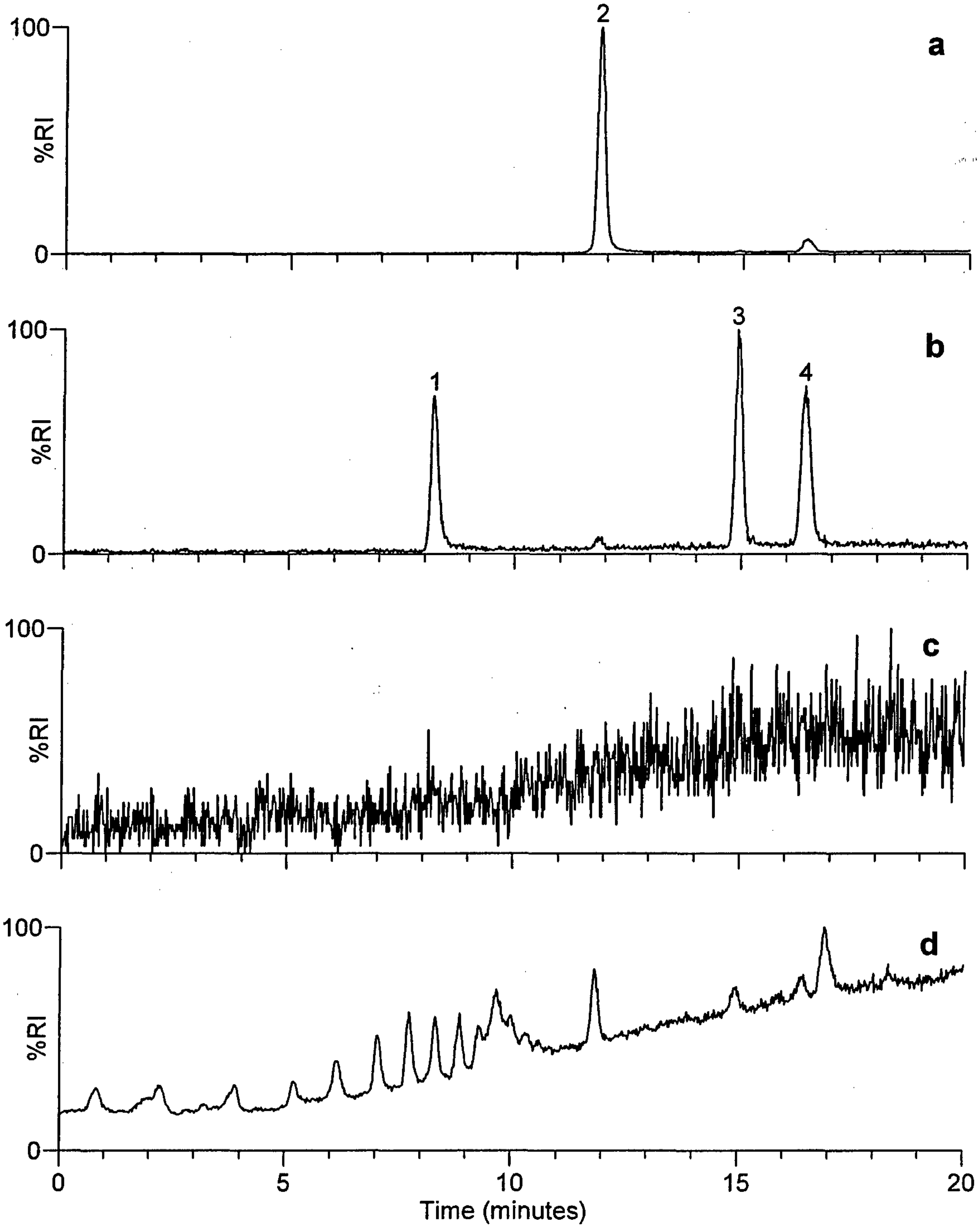

Figure 5. Reconstructed-ion-current chromatograms for a) m/z 183, b) m/z 99, c) m/z 97 and d) total-ion-current chromatogram obtained during LC-ESI-MS analysis of an extract of an office fabric (56% nylon/44% polyester) sample spiked at the 9 µg/g level with GB (1), TEP (2), GF (3) and GD (4).

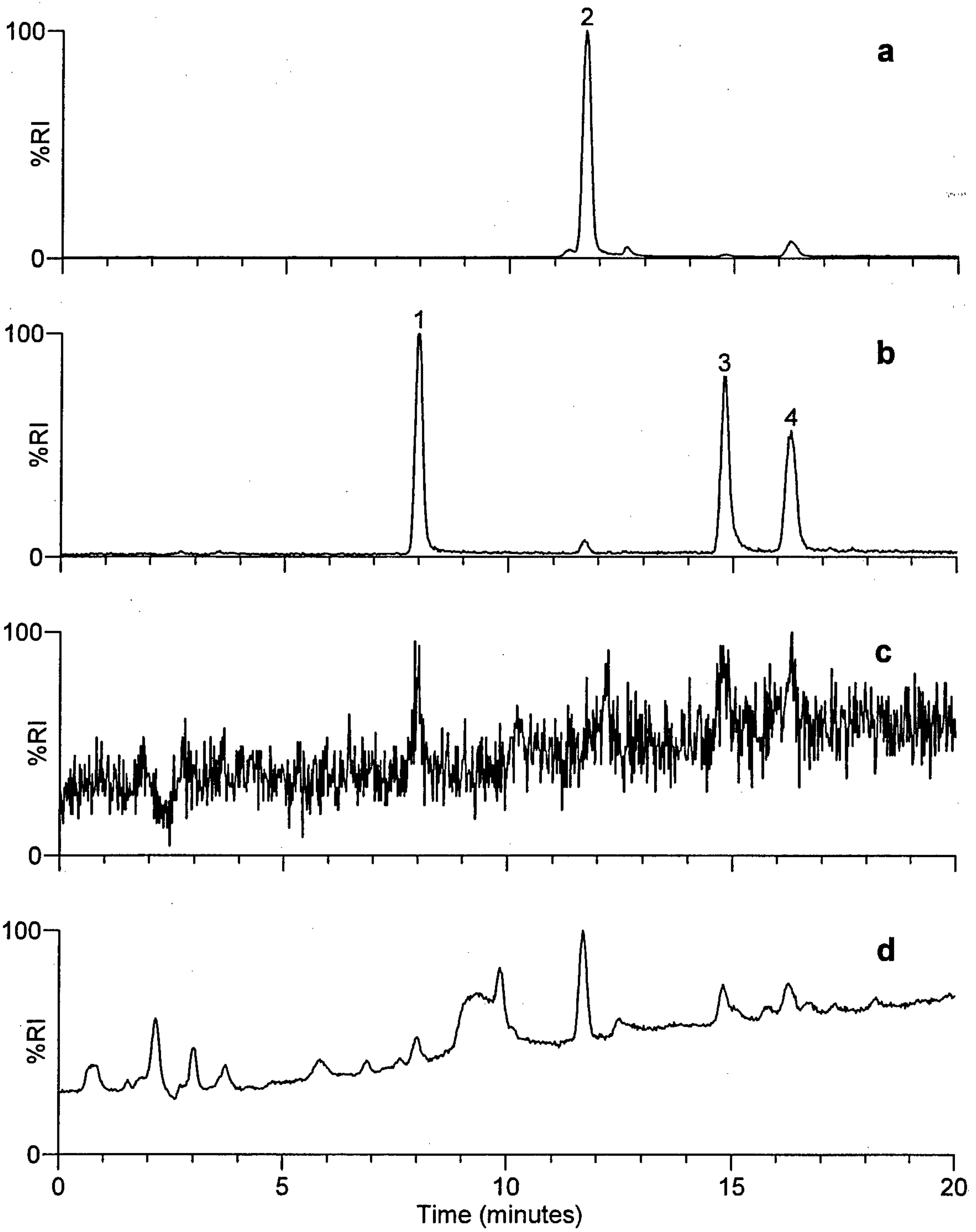

Figure 6. *Reconstructed-ion-current chromatograms for a) m/z 183, b) m/z 99, c) m/z 97 and d) total-ion-current chromatogram obtained during LC-ESI-MS analysis of an extract of an office fabric (100% nylon) sample spiked at the 13 μg/g level with GB (1), TEP (2), GF (3) and GD (4).*

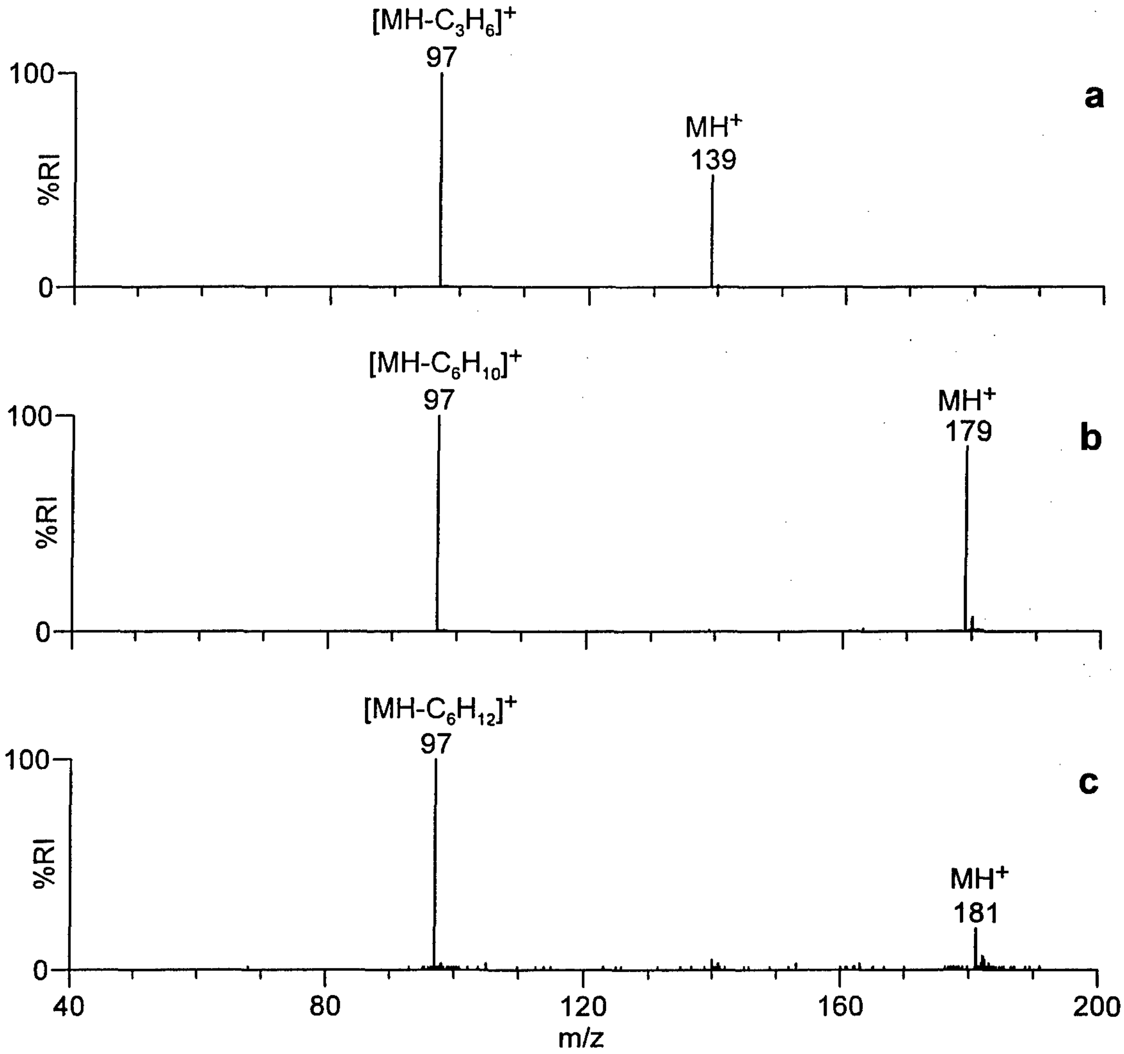

Figure 7. *Product ion mass spectra obtained for protonated molecular ions of a) isopropyl methylphosphonic acid (m/z 139), b) cyclohexyl methylphosphonic acid (m/z 179) and c) pinacolyl methylphosphonic acid (m/z 181) during LC-ESI-MS/MS analysis of an extract of a photocopy paper sample spiked at the 8 µg/g level with GB, TEP, GF and GD. (Collision energy: 4V).*

Table 1. Recovery of GB, TEP, GF and GD from office media (triplicate analyses).

Spiking Agent	Percentage Recovery from Office Media (Spiking Levels)					
	Office Carpet (4 µg/g)	Photocopy Paper (8 µg/g)	Dacron Swab (20 µg/g)	Latex Painted Drywall (12 µg/g)	56% Nylon / 44% Polyester Office Fabric (9 µg/g)	100% Office Fabric Nylon (13 µg/g)
GB	79 86 100	27 30 39	60 57 54	54 56 57	71 91 96	83 72 90
TEP	72 82 73	80 88 83	64 72 66	32 36 37	76 94 81	86 82 89
GF	43 48 49	23 30 37	56 57 47	20 22 26	69 85 75	69 65 76
GD	46 53 47	48 52 62	63 62 53	20 27 23	77 89 83	73 67 78

In some cases the aqueous extracts of the spiked office media contained numerous co-extracted sample components that complicated LC-ESI-MS analysis and hampered identification. These interferences were minimized during LC-ES-MS/MS analysis, where each of the chemical warfare agents was identified on the basis of acquired product ion mass spectra. Figure 8 illustrates a typical LC-ESI-MS total-ion-current chromatogram for the extract of a spiked office carpet sample. The GB was effectively masked by the other organic components and was not observable as a distinct chromatographic peak during LC-ESI-MS.

LC-ESI-MS/MS was used to resolve the GB from the other extract components. The chromatogram obtained for the product ions of m/z 141 ($[M+H]^+$ for GB) during LC-ESI-MS/MS resulted in a significantly less complex chromatogram where GB was completely resolved as a significant chromatographic component.

The selectivity afforded by ESI-MS/MS was evident following comparison of the mass spectra acquired during LC-ESI-MS and LC-ESI-MS/MS analysis of the spiked office carpet extract (Figure 9). During ESI-MS analysis, the characteristic ions for GB, at m/z 141 and m/z 99, due to $[M+H]^+$ and $[M+H-C_3H_6]^+$, respectively, were much less significant than the background ions. However the ESI-MS/MS mass spectrum for GB (collision energy 4V) contained only a precursor ion at m/z 141, due to $[M+H]^+$, and a product ion at m/z 99, due to loss of the alkene associated with the alkoxy group. Similar product ion mass spectra for the $[M+H]^+$ precursor ion of GF and GD were acquired with collision energies of 4V and 2V, respectively. Like GB, a common product ion was observed at m/z 99, due to loss of the alkene associated with the alkoxy group of GF and GD. GD also exhibited an additional product ion at m/z 85 due to $[C_6H_{13}]^+$ and an ion at m/z 117 due to $[m/z\ 99+H_2O]^+$. The $[M+H]^+$ precursor ion for TEP produced three product ions at m/z 155, m/z 127 and m/z 99, due to loss(es) of ethylene from the ethoxy substituents. A higher collision energy (8V to 10V) resulted in the best product ion mass spectra for identification purposes.

Terrorist use of chemical warfare agents may involve the use of crude or munitions grade chemical warfare agent that contains not only the chemical warfare agent but also related co-synthetic, degradation or other products. Extraction and identification of these additional sample components could be helpful in establishing a link between the agent used in the incident and a source, or provide an indication of synthetic route used to prepare the chemical warfare agent. A munitions grade sample of GA containing numerous related compounds (44) was selected to evaluate the applicability of the developed approach for the identification and characterization of related organophosphorus compounds. Two office media, office carpet and photocopy paper, were spiked with a munitions grade GA standard at the µg/g level (approximately 0.5 to 5 µg/g per sample component), extracted and analysed by LC-ESI-MS and LC-ESI-MS/MS. Recoveries ranged between 65% and 92% for GA and seven related organophosphorus compounds in the aqueous extracts, with GA being recovered with 65% efficiency from the photocopy paper and 75% efficiency from the office carpet. Some hydrolysis products of GA were observed in the photocopy paper extracts, consistent with earlier spiking results with GB, GF and GD with this medium.

Figure 10 illustrates LC-ESI-MS and LC-ESI-MS/MS chromatograms obtained during an analysis of aqueous extract of the office carpet spiked with munitions grade GA. Both ESI-MS and ESI-MS/MS data were obtained for each sample component, with the ESI-MS/MS data being acquired at collision energies that resulted in product ion mass spectra containing both the precursor $[M+H]^+$ ion and abundant, structurally informative product ions. Figure 11 illustrates typical product ion mass spectra for tabun and two other related organophosphorus sample components. High resolution data acquired for these and the other related compounds were acquired for identification purposes and have been compiled in Table 2. Errors associated with the mass measurement of the ions were generally less than 0.001 Da, supporting the proposed identities.

The developed method involving aqueous extraction followed by LC-ESI-MS and LC-ESI-MS/MS analysis was successfully applied to the analysis of six different indoor office media contaminated with common chemical warfare agents or a complex munitions grade GA sample. In all cases the spiked components were readily identified in the extracts on the basis of acquired high resolution ESI-MS and/or ESI-MS/MS data, making this method appropriate for these types of sample media and likely applicable to other media that could be collected in support of forensic investigations.

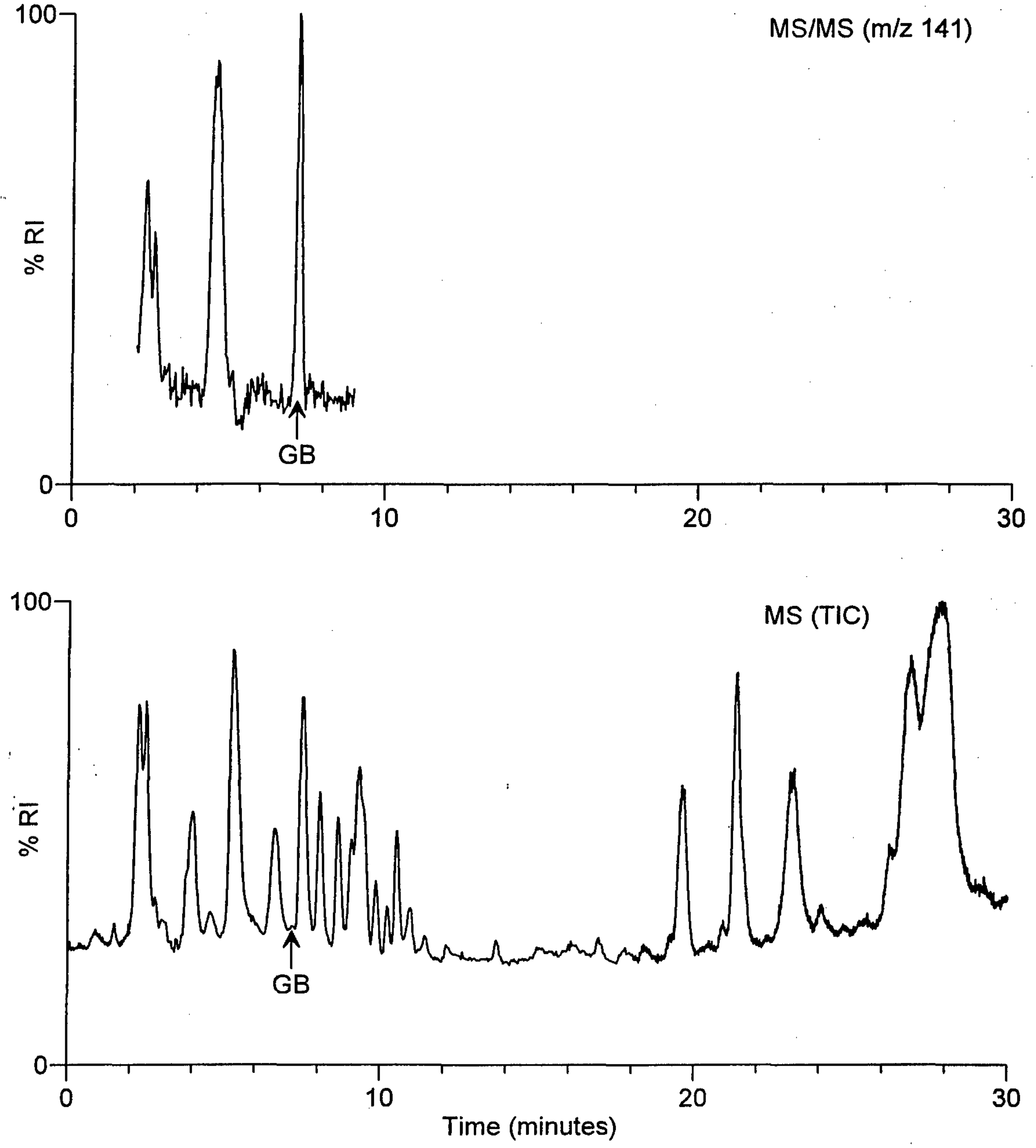

Figure 8. *LC-ESI-MS total-ion-current chromatogram (lower) and LC-ESI-MS/MS chromatogram for the product ions of m/z 141 (upper) obtained during analysis of an extract of a office carpet sample spiked at the 4 µg/g level with GB, TEP, GF and GD.*

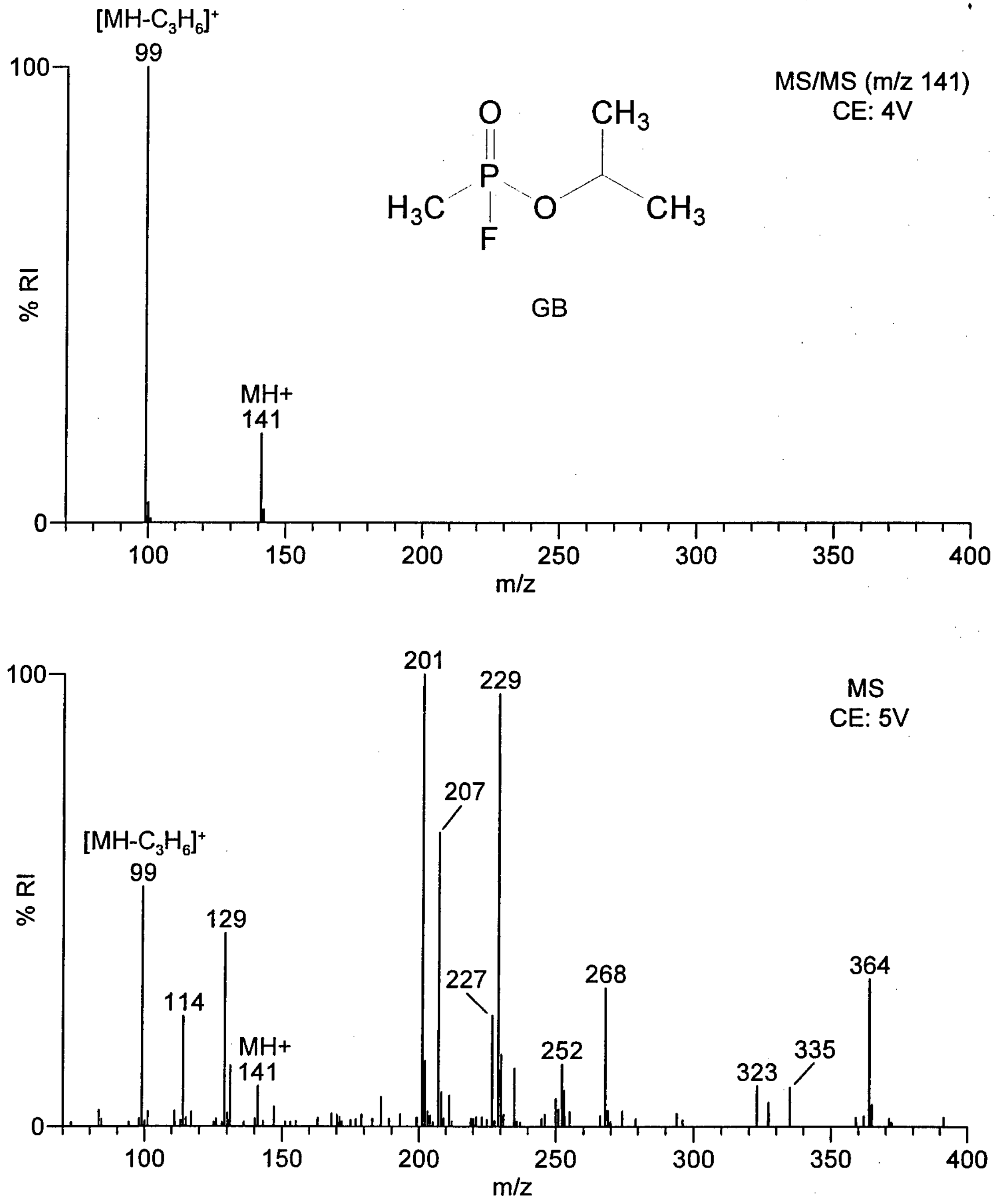

Figure 9. *ESI-MS (lower) and ESI-MS/MS (upper) data obtained at the retention time for GB. Data were obtained during analysis of an extract of an office carpet sample spiked at the 4 µg/g level with GB, TEP, GF and GD. (CE: Collision energy).*

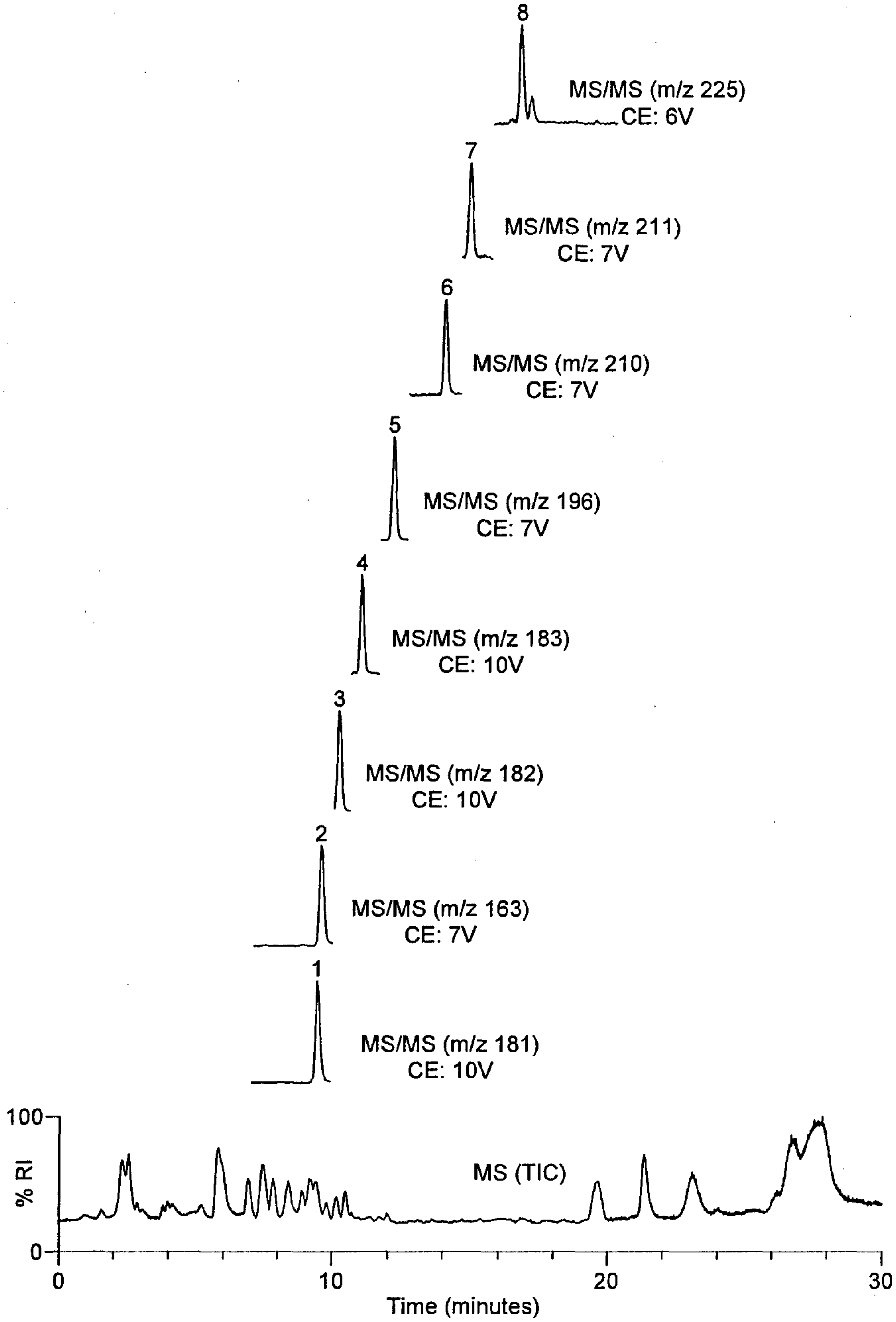

Figure 10. *LC-ESI-MS (lowest) and LC-ESI-MS/MS chromatograms (above) of an extract of an office carpet spiked with munitions grade GA (0.5 – 5 µg/g per component). Components 1 to 8 identified in Table 2. (CE: Collision energy).*

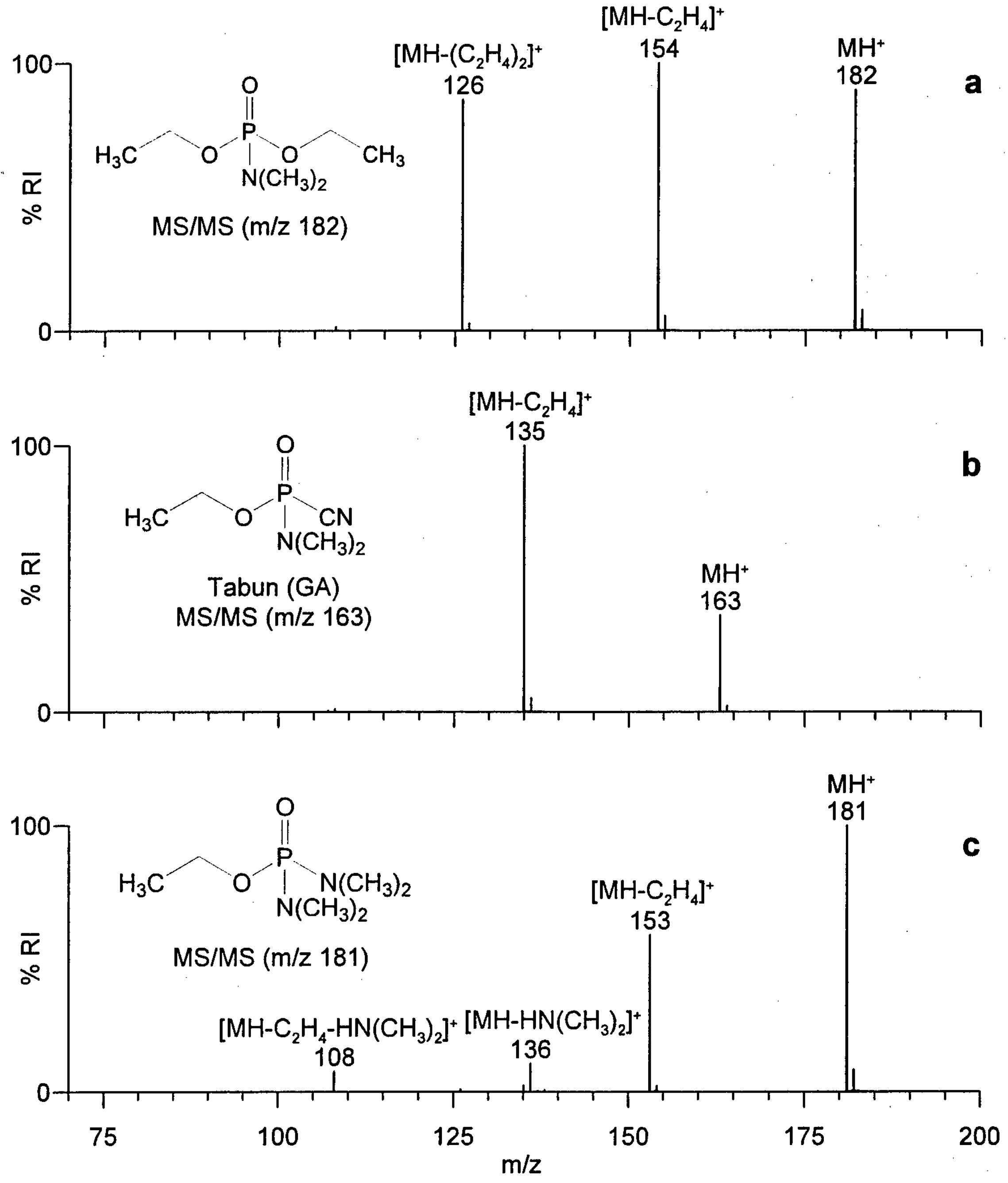

Figure 11. *Product ion mass spectra obtained for a) diethyl dimethylphosphoramidate (m/z 182, Collision energy: 10V), b) GA (m/z 163, Collision energy: 7V) and c) ethyl tetramethylphosphoramidate (m/z 181, Collision energy: 10V) during LC-ESI-MS/MS analysis of an office carpet sample spiked with munitions grade GA (0.5 to 5 μg/g per component).*

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

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
		$[MH-C_2H_4]^+$	153.0795	153.0793	0.0002
		$[MH-HN(CH_3)_2]^+$	136.0533	136.0527	0.0006
		$[MH- C_2H_4-HN(CH_3)_2]^+$	108.0215	108.0214	0.0001
2	Ethyl dimethyl-phosphoramidocyanidate (Tabun, GA)	MH^+	163.0628	163.0636	0.0008
		$[MH-C_2H_4]^+$	135.0316	135.0323	0.0007
3	Diethyl dimethylphosphoramidate	MH^+	182.0950	182.0946	0.0024
		$[MH-C_2H_4]^+$	154.0637	154.0633	0.0004
		$[MH-(C_2H_4)_2]^+$	126.0322	126.0320	0.0002
4	Triethyl phosphate	MH^+	183.0805	183.0786	0.0019
		$[MH-C_2H_4]^+$	155.0470	155.0473	0.0003
		$[MH-(C_2H_4)_2]^+$	127.0153	127.0160	0.0007
		$[MH-(C_2H_4)_3]^+$	98.9836	98.9847	0.0011
5	Ethyl isopropyl dimethylphosphoramidate	MH^+	196.1109	196.1102	0.0007
		$[MH-C_3H_6]^+$	154.0630	154.0633	0.0003
		$[MH-C_3H_6- C_2H_4]^+$	126.0327	126.0320	0.0007
6	Diisopropyl dimethylphosphoramidate	MH^+	210.1282	210.1259	0.0023
		$[MH-C_3H_6]^+$	168.0790	168.0789	0.0001
		$[MH-(C_3H_6)_2]^+$	126.0316	126.0320	0.0004
7	Diisopropyl ethyl phosphate	MH^+	211.1109	211.1099	0.0010
		$[MH-C_3H_6]^+$	169.0647	169.0629	0.0018
		$[MH-(C_3H_6)_2]^+$	127.0172	127.0160	0.0012
8	Triisopropyl phosphate	MH^+	225.1273	225.1255	0.0018
		$[MH-C_3H_6]^+$	183.0791	183.0786	0.0005
		$[MH-(C_3H_6)_2]^+$	141.0324	141.0316	0.0008
		$[MH-(C_3H_6)_3]^+$	98.9842	98.9847	0.0005

[1] Refer to Figure 10.

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

Conclusions

Terrorist use of chemical warfare agents against civilian targets could involve the targeting of enclosed populated spaces. DRDC Suffield, in collaboration with the Royal Canadian Mounted Police, identified a need for analytical methods for chemical warfare agent identification in media, including flooring, wall surfaces, office fabrics and paper products, that would typically be collected in an office environment during forensic investigations.

An analytical method involving aqueous ultrasonic extraction followed by LC-ESI-MS and LC-ESI-MS/MS analysis was developed and applied to the analysis of six different spiked indoor sample media. Aqueous extraction resulted in co-extraction of other chemicals from the office media samples, but was not destruction to any of the sample media studied. All office media were spiked at the low µg/g level with either a standard solution containing GB, GF, GD and TEP or a complex munitions grade GA sample. The recovery efficiencies from the six different media were generally in the 50% to 85% range, more than sufficient for the acquisition of full scanning ESI-MS and ESI-MS/MS high resolution data that could be used to confirm identification. In some instances the aqueous extracts contained numerous co-extracted sample components that complicated LC-ESI-MS analysis and hampered identification. These interferences were minimized during LC-ES-MS/MS analysis, where each of the chemical warfare agents was identified on the basis of acquired product ion mass spectra. MS data for all the spiked compounds in the nerve agent standard and the munitions grade GA were acquired in the continuum mode with a resolution of 9000, which typically resulted in mass measurement errors of 0.001 Da or less.

Application of the developed sample handling and analysis methodology is anticipated during future forensic investigations where evidence of chemical warfare agent use is required for criminal prosecution and to assess remediation/restoration efforts following an incident.

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DOCUMENT CONTROL DATA

(Security classification of title, body of abstract and indexing annotation must be entered when the overall document is classified)

1. ORIGINATOR (the name and address of the organization preparing the document. Organizations for who the document was prepared, e.g. Establishment sponsoring a contractor's report, or tasking agency, are entered in Section 8.) Defence R&D Canada – Suffield PO Box 4000, Station Main Medicine Hat, AB T1A 8K6	2. SECURITY CLASSIFICATION (overall security classification of the document, including special warning terms if applicable) Unclassified

3\. TITLE (the complete document title as indicated on the title page. Its classification should be indicated by the appropriate abbreviation (S, C or U) in parentheses after the title).

Mass Spectrometric Determination of Chemical Warfare Agents in Indoor Sample Media Typically Collected During Forensic Investigations (U)

4\. AUTHORS (Last name, first name, middle initial. If military, show rank, e.g. Doe, Maj. John E.)

D'Agostino, Paul A., Hancock, James R. and Chenier, Claude L.

5. DATE OF PUBLICATION (month and year of publication of document) October 2005	6a. NO. OF PAGES (total containing information, include Annexes, Appendices, etc) 40	6b. NO. OF REFS (total cited in document) 67

7\. DESCRIPTIVE NOTES (the category of the document, e.g. technical report, technical note or memorandum. If appropriate, enter the type of report, e.g. interim, progress, summary, annual or final. Give the inclusive dates when a specific reporting period is covered.)

Technical Report

8\. SPONSORING ACTIVITY (the name of the department project office or laboratory sponsoring the research and development. Include the address.)

9a. PROJECT OR GRANT NO. (If appropriate, the applicable research and development project or grant number under which the document was written. Please specify whether project or grant.)	9b. CONTRACT NO. (If appropriate, the applicable number under which the document was written.)
10a. ORIGINATOR'S DOCUMENT NUMBER (the official document number by which the document is identified by the originating activity. This number must be unique to this document.) DRDC Suffield TR 2005-097	10b. OTHER DOCUMENT NOs. (Any other numbers which may be assigned this document either by the originator or by the sponsor.)

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Terrorist use of chemical warfare agents against civilian targets could involve the targeting of enclosed populated spaces. DRDC Suffield, in collaboration with the Royal Canadian Mounted Police, identified a need for analytical methods for chemical warfare agent identification in media, including flooring, wall surfaces, office fabrics and paper products, that would typically be collected in an office environment during forensic investigations. Typical office environment media were spiked at the 4 to 20 µg/g level with either a complex munitions grade sample of tabun (GA) or with a standard containing the three nerve agents, sarin (GB), cyclohexyl methylphosphonofluoridate (GF), soman (GD) and the nerve agent simulant, triethyl phosphate (TEP), to evaluate the potentials of LC-ESI-MS and LC-ESI-MS/MS for forensic purposes. The spiked chemical warfare agents were recovered with varying efficiencies, but in all cases sufficient chemical warfare agent was recovered for identification purposes. In some instances the aqueous extracts contained numerous co-extracted sample components that complicated LC-ESI-MS analysis and hampered identification. These interferences were minimized during LC-ES-MS/MS analysis, where each of the chemical warfare agents was identified on the basis of acquired product ion mass spectra. MS data for all the spiked compounds in the nerve agent standard and the munitions grade tabun were acquired in the continuum mode with a resolution of 9000, which typically resulted in mass measurement errors of 0.001 Da or less. Application of the developed sample handling and analysis methodology is anticipated during forensic investigations where evidence of chemical warfare agent use is required for criminal prosecution or to assess remediation/restoration efforts following an incident.

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