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Technical Memorandum
DRDC Suffield TM 2011-001
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Canada

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Abstract

A Synapt HDMS quadrupole time-of-flight tandem mass spectrometer was used for the first time to acquire both ion mobility spectrometry (IMS) and tandem mass spectrometry (MS^n where n = 2 or 3) data for chemical defence compounds following liquid chromatographic (LC) sample introduction. LC-MS screening was used to establish retention time windows for significant sample components prior to the acquisition of LC-IMS-MS^n data. Two organophosphorus chemical warfare agent simulants, triethyl phosphate and tributyl phosphate, were initially used to develop a broad spectrum analytical approach for multi-component sample characterization by LC-IMS-MS^n. Unique ion mobility profiles and high resolution MS^n data, often referred to as time-aligned parallel (TAP) fragmentation data, enabled confirmation of both simulants during a single analysis. The LC-IMS-MS^n methodology was then applied to multi-component chemical warfare agent samples that had undergone hydrolysis. Tabun (GA) and O-ethyl S-[2-(diisopropylamino)ethyl] methylphosphonothiolate (VX) hydrolysis products and a number of related compounds were confirmed at the nanogram level. Compounds were differentiated on the basis of their acquired IMS profiles and full scanning, high resolution MS^n data. MS^n data for each compound contained evidence of the $[M+H]^+$ ion and up to nine characteristic product ions. Application of the developed methodology is anticipated for the confirmation of individual sample components in multi-component chemical warfare agent mixtures requiring broad spectrum analysis or for the acquisition of spectrometric data that would aid in the identification of previously uncharacterized sample components.

Résumé

On a utilisé pour la première fois un système de spectrométrie de masse en tandem Synapt HDMS, à quadrupôle à temps de vol, pour acquérir des données de spectrométrie de mobilité ionique (SMI) et des données de spectrométrie de masse en tandem (SM^n avec n = 2 ou 3) sur des composés ayant trait à la défense chimique, après injection préalable de l'échantillon dans un système de chromatographie en phase liquide (CL). On a réalisé une évaluation rapide par CL/SM pour établir des fenêtres de temps de rétention pour des composants importants des échantillons avant l'acquisition de données par CL/SMI/SM^n. On a d'abord utilisé deux agents organophosphorés simulant des agents de guerre chimique, du phosphate de triéthyle et du phosphate de tributyle, pour développer une approche d'analyse à large spectre pour la caractérisation d'échantillons à plusieurs composants par CL/SMI/SM^n. Des profils de mobilité ionique uniques et des données de SM^n haute résolution, souvent référencées comme données de fragmentation parallèle alignées dans le temps (TAP), ont permis la confirmation de la présence des deux agents de simulation lors d'une même analyse. La technique de CL/SMI/SM^n a ensuite été utilisée pour des échantillons contenant plusieurs agents de guerre chimique ayant subi une hydrolyse. La présence de produits d'hydrolyse du tabun (GA) et du méthylphosphonothiolate de *O*-éthyle et de *S*-[2-(diisopropylamino)éthyle] (VX) et d'un certain nombre de composés connexes a été confirmée au niveau du nanogramme. Les composés ont été identifiés sur la base des profils de SMI obtenus et de données de SM^n recueillies en haute résolution et à plein balayage. Les données de SM^n pour chaque composé renfermaient des preuves de la présence de l'ion $[M+H]^+$ et de celle de jusqu'à neuf ions produits caractéristiques. On prévoit appliquer la

technique développée à la confirmation de la présence de composants individuels dans des échantillons de mélanges d'agents de guerre chimique nécessitant une analyse à large spectre ou à l'acquisition de données spectrométrique qui pourrait permettre l'identification de composants d'échantillons non caractérisés jusqu'à présent.

Executive summary

Liquid Chromatographic Electrospray Ionization Mass Spectrometric Characterization of Simulants, Hydrolysed Tabun and VX Using Ion Mobility and Tandem Mass Spectrometric Data

Paul A. D'Agostino and Claude L. Chenier, DRDC Suffield TM 2011-001, Defence R&D Canada – Suffield, February 2011.

Introduction: DRDC Suffield recently reported a desorption electrospray ionization (DESI) application of the Synapt HDMS that enabled the rapid acquisition of both ion mobility spectrometry (IMS) and tandem mass spectrometry (MS^n, where n = 2 or 3) data. Individual chemical warfare agents, collected on solid phase microextraction fibers, were differentiated on the basis of their IMS profiles and MS^n data. A DESI-IMS-MS^n screening approach was also developed and applied to the analysis of Dacron sampling swabs, office furniture fabric or cardboard spiked with sarin, soman, tabun or cyclosarin. For the analysis of complex multi-component samples, a chromatographic technique such as liquid chromatography (LC) would be preferred over DESI sample introduction as this technique enables separation of sample components prior to spectrometric characterization. LC-IMS-MS^n was evaluated in this study for broad spectrum chraracterization and confirmation of individual sample components in a chemical warfare agent simulant mixture and two hydrolysed chemical warfare agent samples.

Results: Two organophosphorus chemical warfare agent simulants, triethyl phosphate and tributyl phosphate, were initially used to develop a broad spectrum analytical approach for multi-component sample characterization by LC-IMS-MS^n. Unique ion mobility profiles and high resolution MS^n data, often referred to as time-aligned parallel (TAP) fragmentation data, enabled confirmation of both simulants during a single analysis. The LC-IMS-MS^n methodology was then applied to multi-component chemical warfare agent samples that had undergone hydrolysis. Tabun or O-ethyl S-[2-(diisopropylamino)ethyl] methylphosphonothiolate (VX) hydrolysis products and a number of related compounds were confirmed at the nanogram level. Compounds were differentiated on the basis of their acquired IMS profiles and full scanning, high resolution MS^n data. MS^n data contained evidence of the $[M+H]^+$ ion and up to nine product ions.

Significance: Confirmation of a chemical warfare agent requires the acquisition of data from at least two different spectrometric techniques and comparison of the acquired data with reference data or to that obtained for authentic reference compound(s). This usually requires more time due to the need for multiple spectrometric analyses. In the present study we were able to demonstrate the acquisition of both ion mobility spectrometry and tandem mass spectrometry data for chemical defence compounds during a single LC-IMS-MS^n analysis.

Future plans: Application of the developed methodology is anticipated for the confirmation of individual sample components in multi-component chemical warfare agent mixtures requiring broad spectrum analysis or for the acquisition of spectrometric data that would aid in the identification of unknowns. Strong consideration is now being given to acquiring IMS and MS^n data for available chemical defence compounds at DRDC Suffield and the establishment of a database.

Sommaire

Caractérisation d'agents de simulation par chromatographie en phase liquide couplée à la spectrométrie de masse avec ionisation par électronébulisation, données sur le tabun et le VX hydrolyses obtenues par spectrométrie de masse en tandem et spectrométrie de mobilité ionique

Paul A. D'Agostino et Claude L. Chenier, RDDC Suffield TM 2011-001, R&D pour la défense Canada – Suffield, février 2011.

Introduction : RDDC Suffield a récemment rapporté une application de désorption/ionisation par électronébulisation (DESI) avec le système Synapt HDMS qui permet l'acquisition rapide de données de spectrométrie de mobilité ionique (SMI) et de spectrométrie de masse en tandem (SM^n avec n = 2 ou 3). Des agents de guerre chimique individuels, collectés sur des fibres de microextraction en phase solide, ont été différenciés sur la base de leurs profils de SMI et de données de SM^n. Une approche d'évaluation rapide par DESI-SMI/SM^n a aussi été développée et appliquée à l'analyse de tampons d'échantillonnage en Dacron, de tissu d'ameublement de bureau ou de carton dopés avec du sarin, du soman, du tabun ou du cyclosarin. Pour l'analyse d'échantillons complexes à plusieurs composants, une technique chromatographique telle que la chromatographie en phase liquide (CL) serait préférable à l'introduction des échantillons dans un dispositif DESI, car cette technique permet la séparation des composants de l'échantillon avant leur caractérisation spectrométrique. Pour la présente étude, on a évalué la technique de CL/SMI/SM^n pour la caractérisation à large spectre et la confirmation des composants individuels d'un échantillon de mélange d'agents simulant des agents de guerre chimique et de deux échantillons d'agents de guerre chimique hydrolysés.

Résultats : On a d'abord utilisé deux agents organophosphorés simulant des agents de guerre chimique, le phosphate de triéthyle et le phosphate de tributyle, pour développer une approche d'analyse à large spectre pour la caractérisation d'échantillons à plusieurs composants par CL/SMI/SM^n. Des profils de mobilité ionique uniques et des données de SM^n haute résolution, souvent référencées comme données de fragmentation parallèle alignées dans le temps (TAP), ont permis la confirmation de la présence des deux agents de simulation lors d'une même analyse. La technique de CL/SMI/SM^n a ensuite été utilisée pour des échantillons contenant plusieurs agents de guerre chimique ayant subi une hydrolyse. La présence de produits d'hydrolyse du tabun (GA) et du méthylphosphonothiolate de *O*-éthyle et de *S*-[2-(diisopropylamino)éthyle] (VX) et d'un certain nombre de composés connexes a été confirmée au niveau du nanogramme. Les composés ont été identifiés sur la base des profils de SMI obtenus et de données de SM^n haute résolution et plein balayage. Les données de SM^n pour chaque composé renfermaient des preuves de la présence de l'ion $[M+H]^+$ et de celle de jusqu'à neuf ions produits caractéristiques.

Importance : La confirmation de la présence d'un agent de guerre chimique requiert l'acquisition de données au moyen d'au moins deux techniques spectrométriques différentes et la comparaison des données acquises à des données de référence ou à des données obtenues avec des composés de référence authentiques. Ceci requiert habituellement plus de temps en raison de la nécessité de plusieurs analyses spectrométriques. Pour la présente

étude, on a été en mesure de montrer la possibilité d'acquérir des données de spectrométrie de mobilité ionique et des données de spectrométrie de masse en tandem sur des composés ayant trait à la défense chimique lors d'une seule analyse par CL/SMI/SM^n.

Travaux futurs : On prévoit appliquer la technique développée à la confirmation de la présence de composants individuels dans des échantillons de mélanges d'agents de guerre chimique nécessitant une analyse à large spectre ou à l'acquisition de données spectrométrique qui pourrait permettre l'identification de composants inconnus. On songe fortement maintenant à acquérir des données de SMI et de SM^n sur les composés ayant trait à la défense chimique et disponibles à RDDC Suffield et à établir une base de données.

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Introduction

Analytical methods for the detection and identification of chemical warfare agents, their degradation products and related compounds has been thoroughly reviewed over the past decade with several different emphases [1-10]. Aqueous samples or extracts containing organophosphorus chemical warfare agents, their hydrolysis products and related compounds, may be analysed directly by liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS) [3, 7-10] without the need for additional sample handling and derivatization steps [6]. DRDC Suffield has undertaken a number of LC-ESI-MS studies in the past including the analysis of tabun and related compounds in synthetic and munitions grade samples [11-13], the analysis of sarin in snow samples [14, 15] and the analysis of VX and its degradation products in an aged sample [16, 17]. A large number of sample components were characterized following LC-ESI-MS analysis of chemical warfare agent samples at DRDC Suffield and this analytical technique forms a cornerstone in the overall detection and identification strategy employed for laboratory-based chemical warfare agent identification.

Ion mobility spectrometry (IMS) has been used successfully within the defence and public security communities for the detection of a variety of compounds including explosives and chemical warfare agents, with the hand-held Chemical Agent Monitor (CAM) being one of the more recognized IMS devices [18]. Waters Corporation recently developed the Synapt HDMS, a tandem mass spectrometer containing an ion mobility cell in the Triwave collision region between the quadrupole and time-of-flight mass analysers. This novel instrument was installed at DRDC Suffield, giving analysts the potential to acquire both ion mobility and tandem mass spectrometric data during a single experiment. Most reported applications with this relative new instrument have dealt largely with the analysis of larger biomolecules, even though IMS has historically been used largely for the analysis of smaller molecules [18].

IMS has been interfaced prior to the mass analyser on a variety of mass spectrometers, with Hill and co-workers reporting application of IMS-MS for the rapid separation and analysis of chemical warfare agent simulants and degradation products [19-24]. High field asymmetric waveform IMS-MS was utilized for the analysis of chemical warfare agents spiked into food products [25] and most recently DRDC Suffield reported a desorption electrospray ionization (DESI) application of the Synapt HDMS [26, 27]. Incorporation of the IMS cell within the Triwave collision cell of the Synapt HDMS allowed acquisition of both IMS data and MS^n (n=2 or 3) data following DESI sample introduction. Data acquired in this manner, referred to as time-aligned parallel (TAP) fragmentation data, were used for the first time for the rapid confirmation of chemical warfare agents [26]. Individual chemical warfare agents were differentiated on the basis of their IMS profiles and acquired full scanning, high resolution MS^n data. MS^n data contained evidence of the $[M+H]^+$ ion and at least three other characteristic product ions. A screening approach was also developed for the analysis of sarin, soman, tabun and cyclosarin. Individual chemical warfare agents were spiked onto Dacron sampling swabs, office furniture fabric or cardboard and successfully screened for the presence of all four chemical warfare agents during a single DESI-IMS-MS^n analysis [27].

As the number of sample components requiring characterization increases, the usefulness of batch introduction techniques such as DESI decreases. Chromatographic sample introduction techniques, including liquid chromatography, would typically be employed to enable separation of sample components prior to spectrometric characterization. A Synapt HDMS instrument was used for the first time to acquire both IMS and MS^n (n = 2 or 3) data for chemical defence compounds following LC sample introduction. Samples were initially screened by LC-MS under low collision energy conditions that resulted in formation of significant $[M+H]^+$ ions for the sample components. Retention time windows, associated with observed $[M+H]^+$ ions during LC-MS screening, were established for the acquisition of LC-IMS-MS^n data for individual sample components. A mixture of two simulants, triethyl phosphate and tributyl phosphate, were initially utilized for the evaluation of LC-IMS-MS^n for compound confirmation. Application of the developed approach to more complex samples containing multiple sample compounds related to hydrolysed tabun or VX resulted in acquisition of both IMS and MS^n during each LC-IMS-MS^n analysis. Moderate collision energy settings were employed to ensure the observation of both the $[M+H]^+$ ion and several significant product ions for each sample component.

Experimental

Samples

Samples were obtained from the Canadian National Single Small Scale Facility at DRDC Suffield.

a) Triethyl phosphate (TEP) and tributyl phosphate (TBP) in water/acetonitrile (1:1) at a concentration of 0.5 ng/µL and 5 ng/ µL, respectively.

b) Hydrolysed munitions grade tabun sample in water at a concentration of 10 ng/µL (multi-component sample).

c) Hydrolysed aged VX sample in water at a concentration of 20 ng/µL (multi-component sample).

Instrumental

LC-IMS-MS data were acquired using a Synapt HDMS tandem mass spectrometers (Waters, Manchester, UK) equipped with a Z-spray electrospray interface. The electrospray capillary was operated at 3 kV. Nitrogen desolvation gas (100 ^{o}C) was introduced into the interface (80 ^{o}C) at a flow rate of 300 L/h and nitrogen nebulizer gas was introduced at a flow rate of 50 L/h. The sampling cone was set at 15V and the collision energies in the trap and transfer regions varied between 7 and 15 eV. Helium (35 mL/min) was used in the ion mobility region and argon (4.5 mL/min) was used in the trap region during LC-IMS-MS data acquistion. MS^n data were acquired from m/z 70 to m/z 300 for the protonated molecular ion (or product ion) for the simulants and from m/z 40 to m/z 300 for the protonated molecular ion (or product ion) for the hydrolysed tabun and VX components. Data were acquired in the continuum mode with a resolution of 8000 (V-mode, 50% valley definition) and a scan rate of 0.5 s/scan. A low mass resolution setting of 12 (equivalent to a 1 m/z window) was used for quadrupole ion selection.

LC separations were performed with an Agilent 1100 capillary LC (Palo Alto, CA, USA) using a 5% to 75%B gradient over 5 minutes (chemical warfare agent simulants) or 15 minutes (hydrolysed tabun and hydrolysed VX) 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 Agilent 50 mm x 0.3 mm i.d. fused-silica capillary columns packed with Zorbax SB C18 (1.8 µm particle size). An autosampler was used for all (1 µL) injections.

Results and Discussion

The Synapt HDMS, with its unique collision cell, offers a high degree of flexibility, enabling analysts to perform a variety of MS^n (typically n = 2 or 3) experiments by making use of the ESI source region and Triwave collision cell. Organophosphorus chemical warfare agents typically ionize in the ESI source giving rise to abundant $[M+H]^+$ ions (and other adduct ions) at lower sampling cone voltages. If desired, product ions may be produced with increasing sampling cone voltages. Ions formed in the ESI source may be passed through the quadrupole mass analyser or a particular ion may be selected by the quadrupole mass analyser for subsequent investigation. The quadrupole mass analyser can mass select with varying window widths and a one m/z window was used to reduce chemical noise during data acquisition. Mass selected $[M+H]^+$ ions (or product ions) may then undergo fragmentation in the trap collision region prior to the IMS cell in the Triwave collision cell. During a typical experiment, moderate trap collision energy settings that maintained significant $[M+H]^+$ intensity but also resulted in the formation of one or more product ions were used. The $[M+H]^+$ ion and product ions exiting the trap collision region were rapidly separated (9 msec) in the IMS cell on the basis of their ion mobility, resulting in the acquisition of a characteristic ion mobility profile for each compound. Finally the ion mobility separated ions were fragmented in the transfer collision region using moderate energy setting that resulted in the acquisition of MS^n data for the $[M+H]^+$ ion and the product ions separated in the IMS cell. Acquisition of IMS and MS^n data in this manner has been referred to as time-aligned parallel (TAP) fragmentation. Elemental compositions were confirmed by high resolution.

DESI-IMS-MS^n was recently demonstrated for the rapid confirmation of organophposphorus chemical warfare agents sampled onto SPME fibers [26, 27]. Within minutes an ion mobility profile for the organophosphorus chemical warfare agent and up to six MS^n full scanning, high resolution mass spectra were acquired. MS^n data contained the $[M+H]^+$ ion for the organophosphorus chemical warfare agent and at least three other characteristic product ions that could be used to confirm the presence of each compound.

DESI, a batch sample introduction technique, worked well for the rapid identification of target compounds in a relatively simple sample. More chemically complex samples generally require a separation step, such as liquid chromatography, prior to spectrometric characterization and identification of the sample components.

During the present study LC-IMS-MS^n was initially evaluated for the acquisition of IMS and MS^n data for a simple mixture of simulants. The developed approach was then applied to more complex, hydrolysed tabun and VX samples containing multiple sample components.

LC-IMS-MS^n analysis of simulants

A simple mixture of two organophosphorus chemical warfare agent simulants, triethyl phosphate and tributyl phosphate was used to investigate the usefulness of LC-IMS-MS^n for confirmation purposes. The mixture was initially screened by LC-MS to determine an appropriate retention time window for each of the simulants and to confirm the presence of the $[M+H]^+$ ions at m/z 183

for triethyl phosphate and m/z 267 for tributyl phosphate. The established retention time windows were then used for quadrupole mass selection of each $[M+H]^+$ ion during LC-IMS-MS^n analyses. Each of the selected $[M+H]^+$ ions was fragmented in the trap region with a moderate collision energy of 10 eV. The $[M+H]^+$ ion and generated product ions were separated in the drift region by ion mobility, giving rise to a characteristic ion mobility profile for each simulant. Ion mobility separated ions were then fragmented in the transfer collision region with a moderate collision energy of 10 eV. The resulting high resolution MS^n (n = 2 or 3) spectra for each of the ion mobility separated ions formed a set of spectra that uniquely characterized the simulant.

Figure 1 illustrates the LC-IMS-MS^n separation of triethyl phosphate and tributyl phosphate. A retention time window of approximately one minute was used during $[M+H]^+$ ion selection.

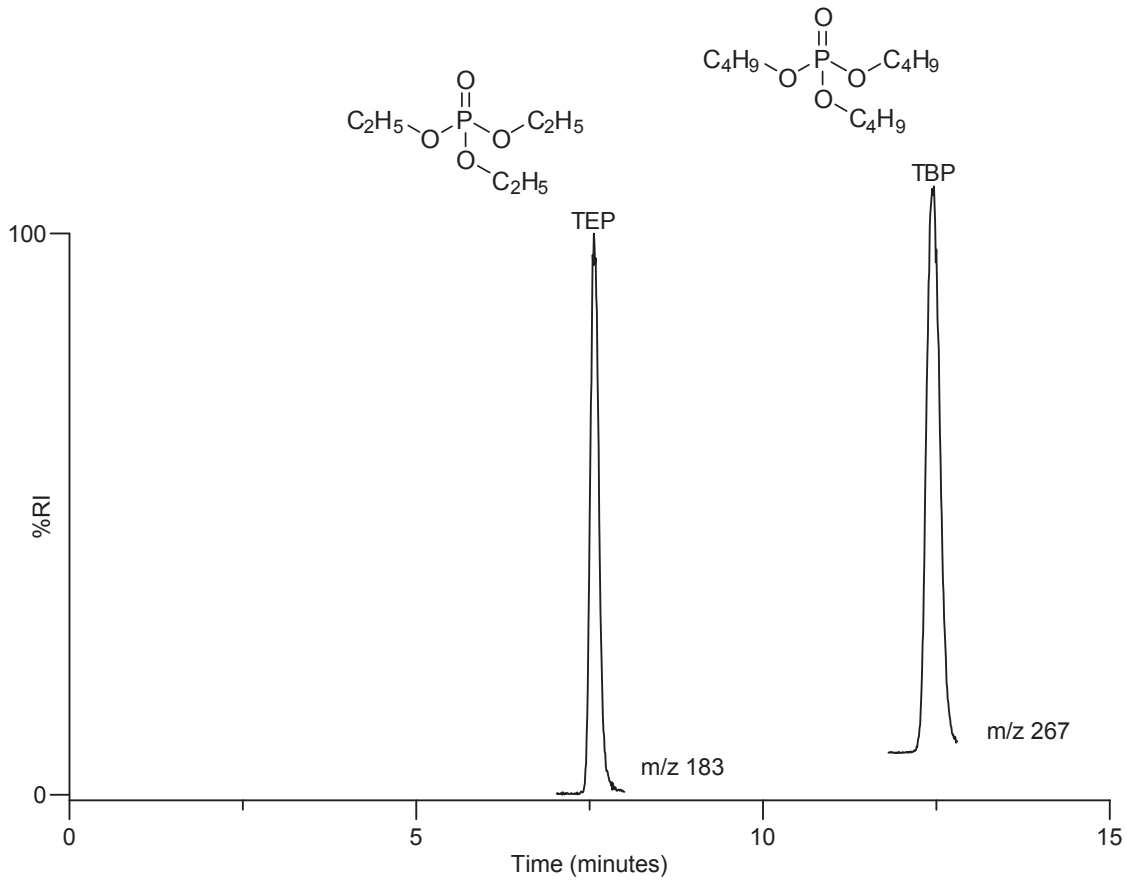

Figure 1: LC-IMS-MS^n separation of triethyl phosphate (TEP) and tributyl phosphate (TBP).

Figure 2 illustrates the unique ion mobility profiles obtained for triethyl phosphate and tributyl phosphate. The $[M+H]^+$ ion and three product ions, due to sequential loss of the alkene associated

with the alkoxy group for each simulant, were separated in the drift region by IMS over a 9 msec timeframe. Reproducibility was consistent with previously reported data, within ± 0.045 msec (equivalent to 1 bin number), over the course of the study [26].

Figures 3 and 4 illustrate the MS^n data acquired for triethyl phosphate and tributyl phosphate, respectively, and possible ion structures consistent with the observed MS^n data using trap and transfer collision energy settings of 10 eV. The $[M+H]^+$ ion and four product ions were observed in the MS^n data collected for each simulant. The trap collision energy setting of 10 eV resulted in a reasonable distribution of ions for IMS separation. If required, a higher transfer collision energy setting (up to about 20 eV) could be selected to enhance product ion formation [26]. Errors associated with mass measurement were generally < 2 mDa, typical of those reported during prior experiments [28].

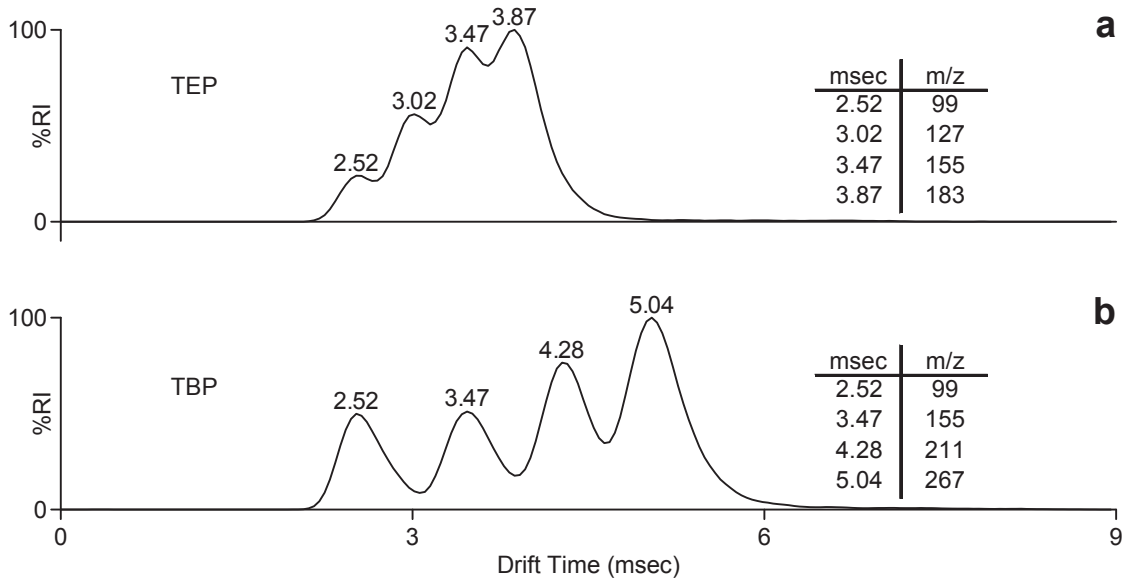

Figure 2: Ion mobility profiles obtained for a) triethyl phosphate and b) tributyl phosphate during LC-IMS-MS^n analysis. The $[M+H]^+$ ion selected by the quadrupole mass analyser and the product ions generated in the trap region of the collision cell were separated by IMS.

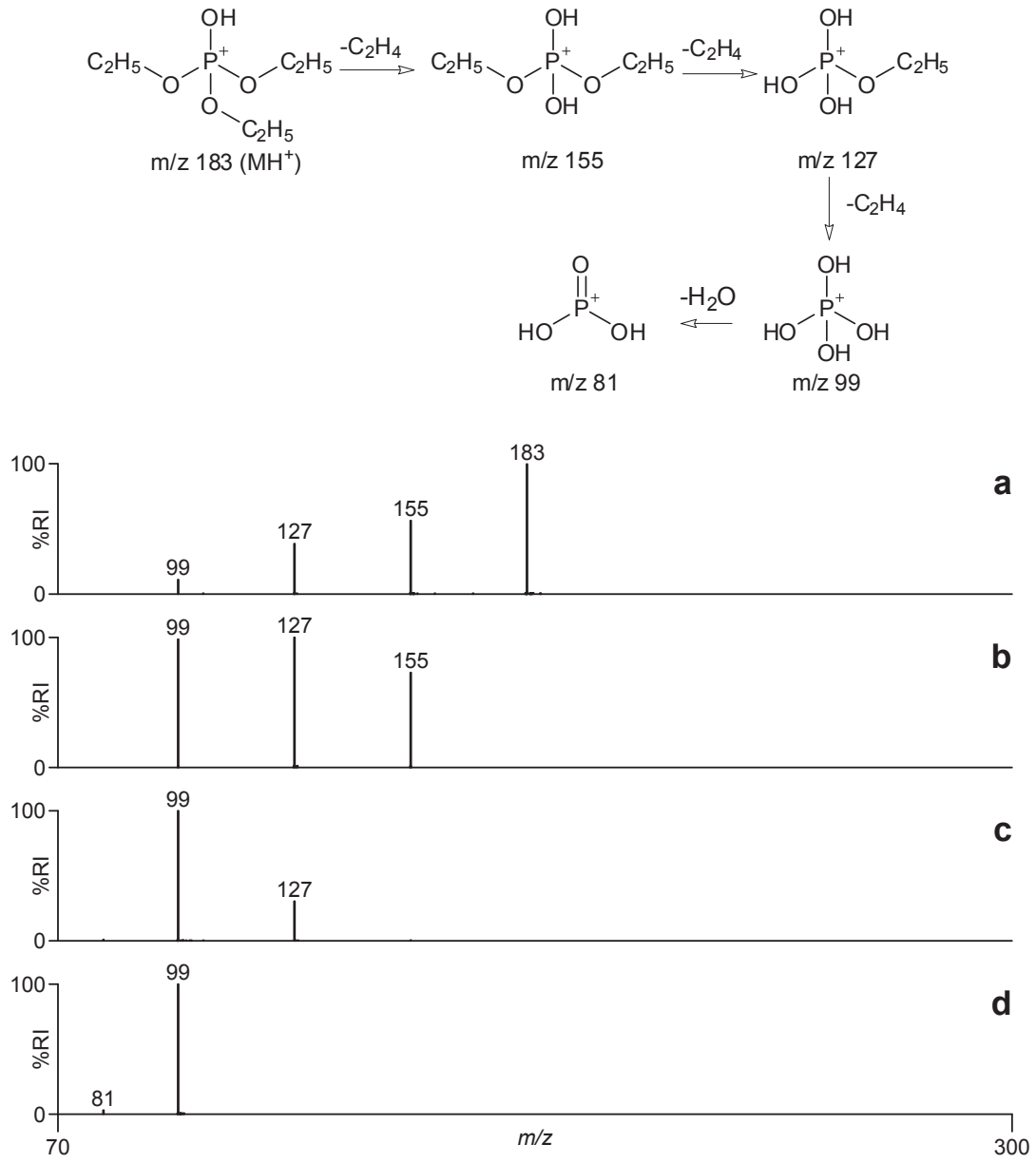

Figure 3: MS^2 spectrum for the $[M+H]^+$ ion at a) m/z 183 and MS^3 spectra for the product ions at b) m/z 155, c) m/z 127 and d) m/z 99 observed during LC-IMS-MS^n analysis of triethyl phosphate (collision energies: trap 10 eV, transfer 10 eV) .

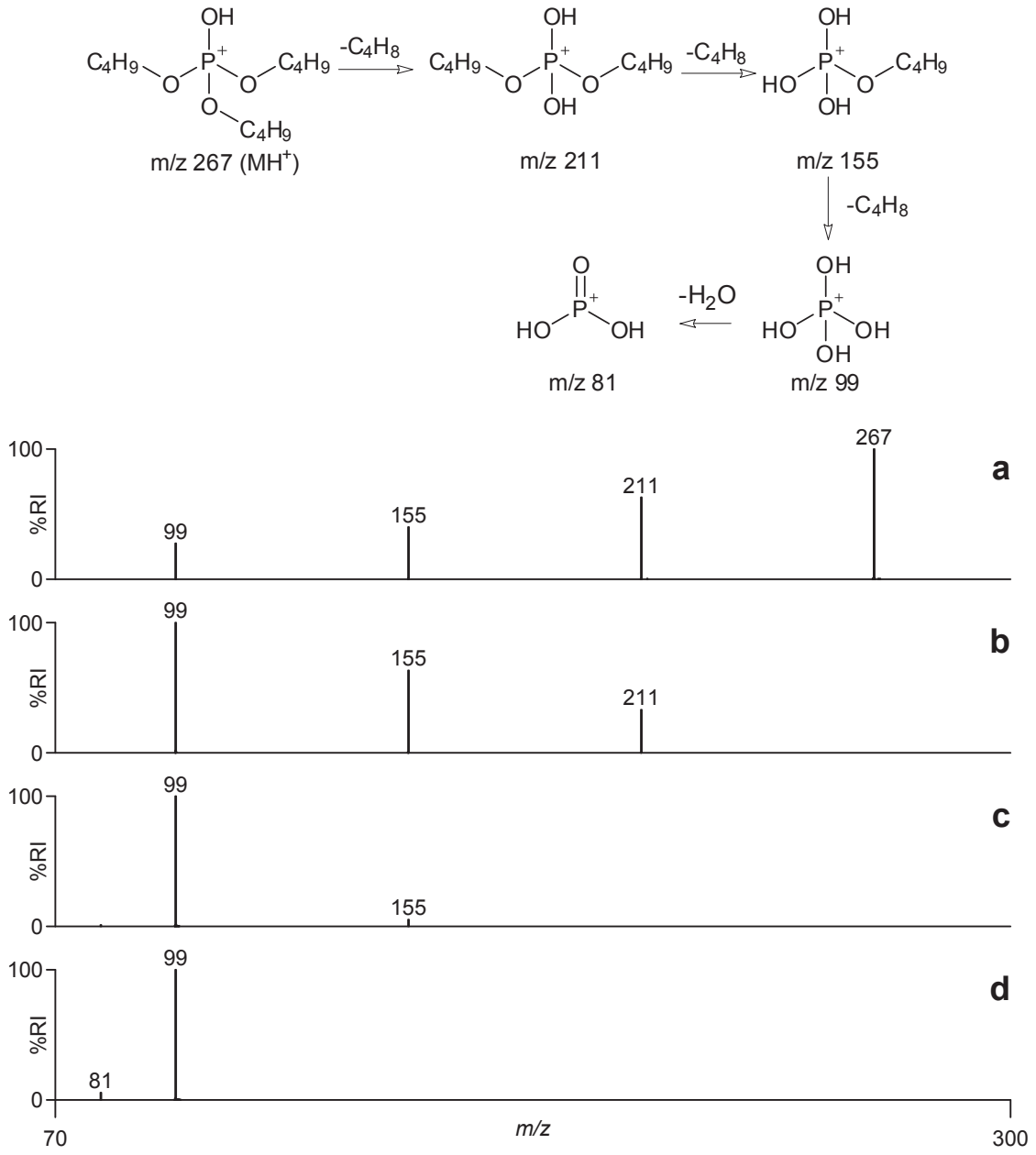

Figure 4: MS^2 spectrum for the $[M+H]^+$ ion at a) m/z 267 and MS^3 spectra for the product ions at b) m/z 211, c) m/z 155 and d) m/z 99 observed during LC-IMS-MS^n analysis of tributyl phosphate (collision energies: trap 10 eV, transfer 10 eV).

LC-IMS-MS^n analysis of hydrolysed tabun

Characterization and identification of sample components in an extract or sample is often referred to as broad spectrum analysis. The LC-IMS-MS^n approach was used in this manner to characterize and identify significant sample components in a hydrolysed munitions grade tabun

sample. LC-MS analysis, under low collision energy conditions that favoured $[M+H]^+$ formation, indicated the presence of eight significant sample components. Retention time windows were assigned for quadrupole mass selection of the observed $[M+H]^+$ ions and trap and transfer collision energy settings in the 7 eV to 12 eV range were selected for each sample component based on the degree of product ion formation observed during screening. A trap collision energy setting that yielded both the $[M+H]^+$ ion and one or more significant product ion was selected along with a transfer energy setting that promoted a reasonable degree of product ion formation (while still maintaining the presence of the precursor ion). Figure 5 illustrates the LC-IMS-MS^n separation of the sample components in the hydrolysed tabun sample, with the corresponding m/z value used for $[M+H]^+$ selection. A hydrolysis product of tabun, ethyl dimethylphosphoramidic acid, five monophosphorus compounds and two pyrophosphates (anhydrides) associated with tabun were observed [11-13]. One of the identified pyrophosphates, bis(ethyl dimethylphosphoramidic) anhydride, contains two asymmetric phosphorus atoms, which resulted in the detection of two chromatographic peaks. The pair of diastereoisomers exhibited mass spectra that appeared to be identical.

Figure 6 illustrates the eight ion mobility profiles acquired during LC-IMS-MS^n analysis of the hydrolysed tabun sample. Some similarity in ion mobility profile was observed for compounds exhibiting similar m/z values for both the $[M+H]^+$ ions and product ions. This was most evident for diethyl dimethylphosphoramidate and ethyl tetramethylphosphorodiamidate, two monophosphorous compounds differing in mass by 1 Da. Drift times at the apex were identical within experimental error and only the relative intensities varied slightly, due to differences in ion structure. The differences in ion mobility profile alone might not be convincing enough for identification, but when assessed with the decidedly different MS^n data (and LC retention time) acquired during the same LC-IMS-MS^n analysis, a strong argument can made for confirmation of both compounds (refer to Figures 8 and 9 for MS^n data).

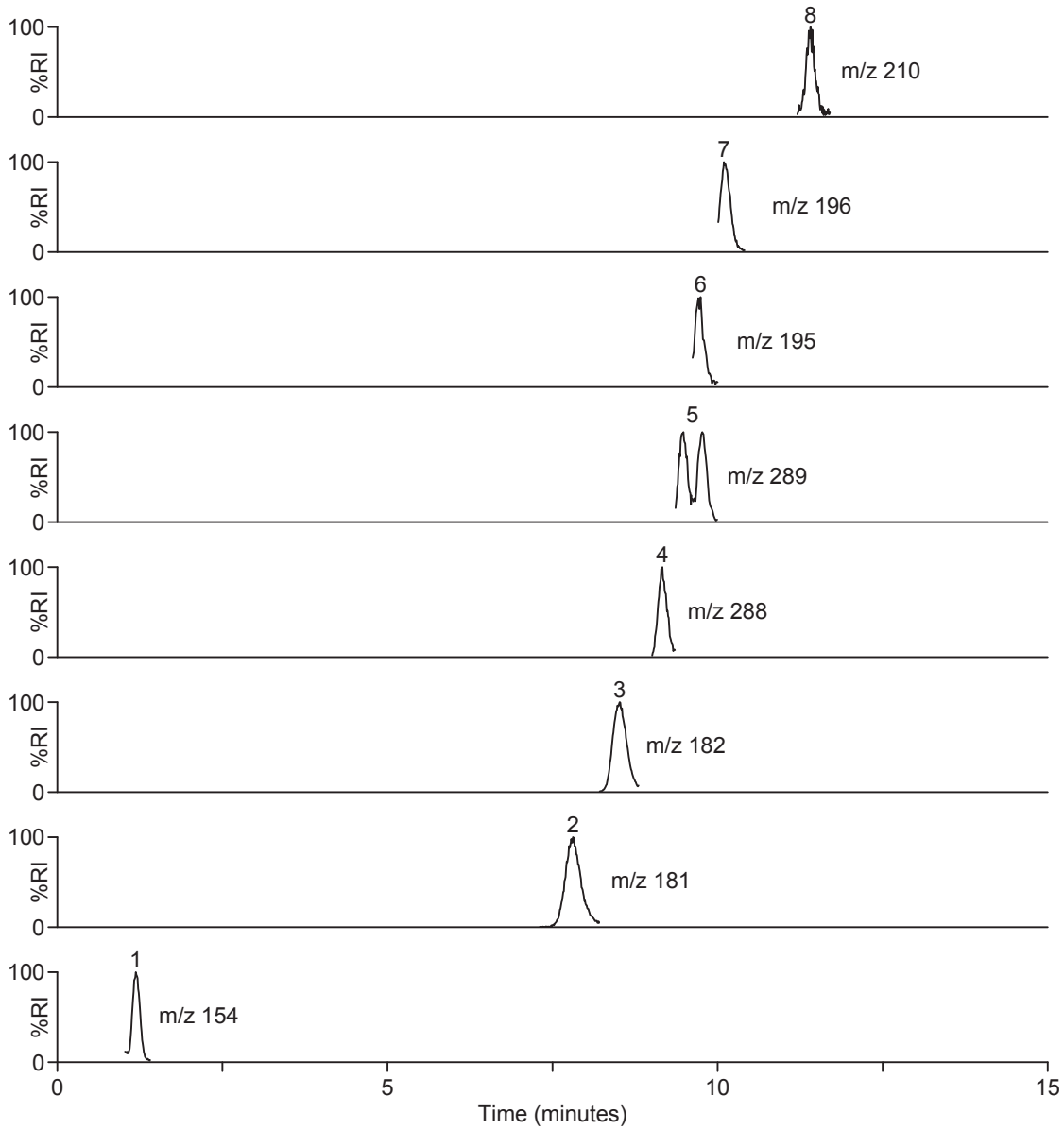

Figure 5: LC-IMS-MS^n separation of components in a hydrolysed tabun sample (10 ng/µL). 1. ethyl dimethylphosphoramidic acid, 2. ethyl tetramethylphosphorodiamidate, 3. diethyl dimethylphosphoramidate, 4. bis(ethyl dimethylphosphoramidic) anhydride, 5. ethyl dimethylphosphoramidic tetramethylphosphorodiamidic anhydride, 6. isopropyl tetramethylphosphorodiamidate, 7. ethyl isopropyl dimethylphosphoramidate, 8. diisopropyl dimethylphosphoramidate,.

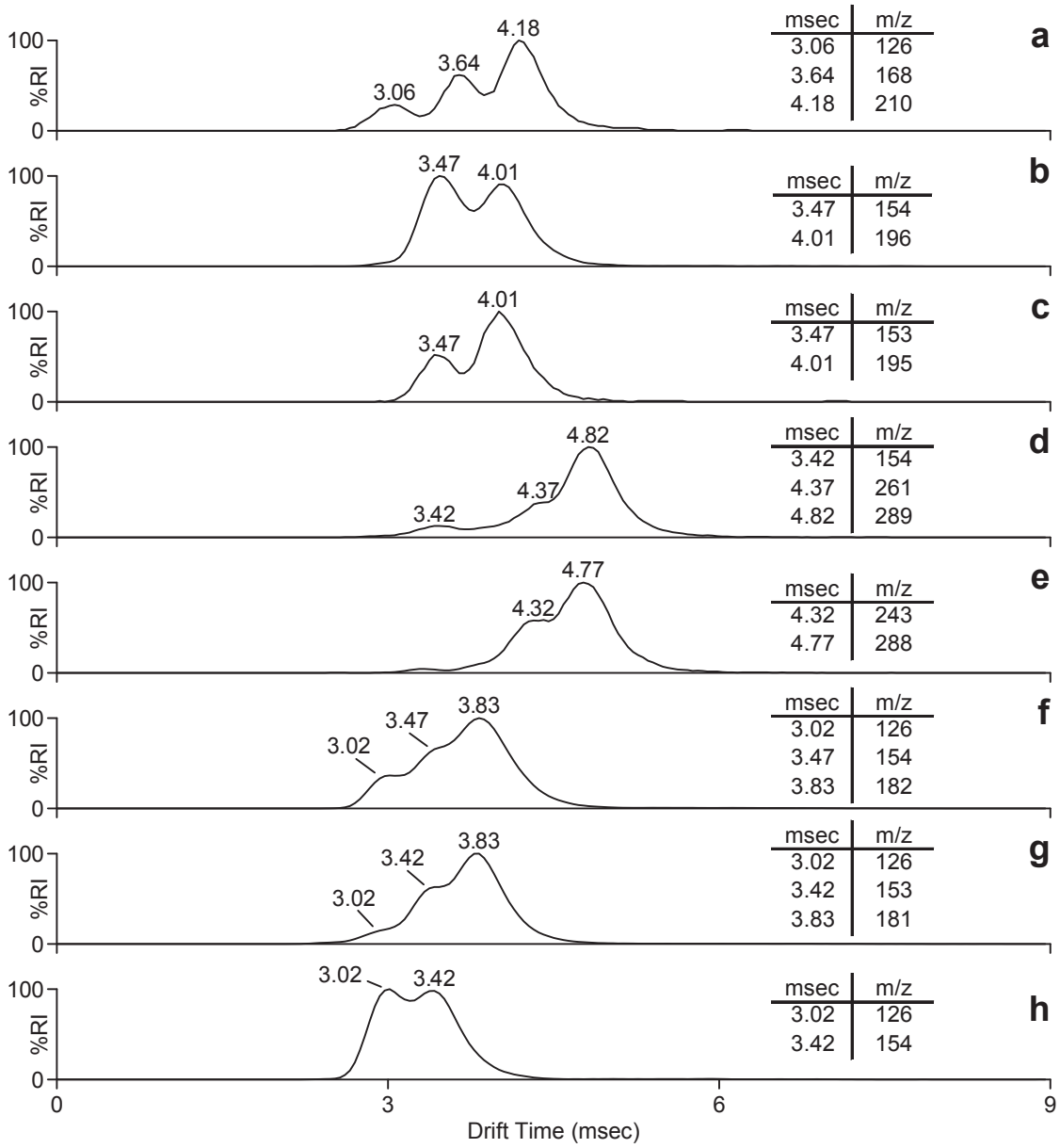

Figure 6: Ion mobility profiles obtained for a) diisopropyl dimethylphosphoramidate, b) ethyl isopropyl dimethylphosphoramidate, c) isopropyl tetramethylphosphorodiamidate, d) bis(ethyl dimethylphosphoramidic) anhydride, e) ethyl dimethylphosphoramidic tetramethylphosphoro-diamidic anhydride, f) diethyl dimethylphosphoramidate, g) ethyl tetramethylphosphorodiamidate and h) ethyl dimethylphosphoramidic acid during LC-IMS-MS^n analysis of a hydrolysed tabun sample. The [M+H]$^+$ ion selected by the quadrupole mass analyser and the product ions generated in the trap region of the collision cell were separated by IMS.

Figure 7 to 14 illustrate the MS^n data acquired during LC-IMS-MS^n for each of the eight significant sample components in the hydrolysed tabun sample. Possible fragmentation pathways and ion structures, based on the observed product ions in the acquired mass spectra have been

presented above the acquired MS^n data. MS^n data were acquired under high resolution conditions (8000 resolution, 50% valley definition) and in all cases the measured mass of the observed ion was consistent with the theoretical mass of the proposed ion.

For the hydrolysis product of tabun, ethyl dimethylphosphoramidic acid (Figure 7), and several of the monophosphorous compounds related to tabun (Figures 9, 12, 13 and 14), product ion formation was relatively simple. Losses of the alkene associated with the alkoxy group was generally observed along with the loss of water for several compounds. The pyrophosphates and ethyl tetramethylphosphorodiamidate MS^n data (Figures 8, 10 and 11) were acquired with higher collision energies, which generally resulted in the formation of additional product ions at lower mass. Similar findings were observed for organophosphorous chemical warfare agents during prior DESI-IMS-MS^n investigations [26, 27].

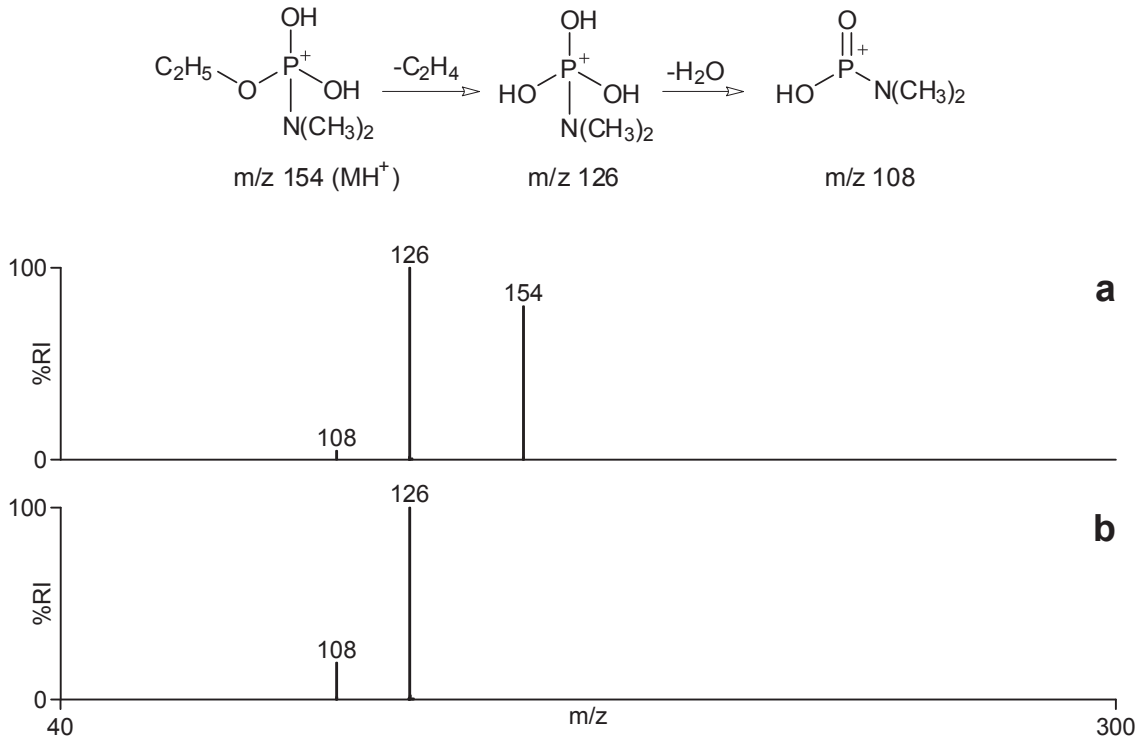

Figure 7: MS^2 spectrum for the $[M+H]^+$ ion at a) m/z 154 and MS^3 spectra for the product ion at b) m/z 126 observed for ethyl dimethylphosphoramidic acid during LC-IMS-MS^n analysis of a hydrolysed tabun sample (collision energies: trap 10 eV, transfer 10 eV).

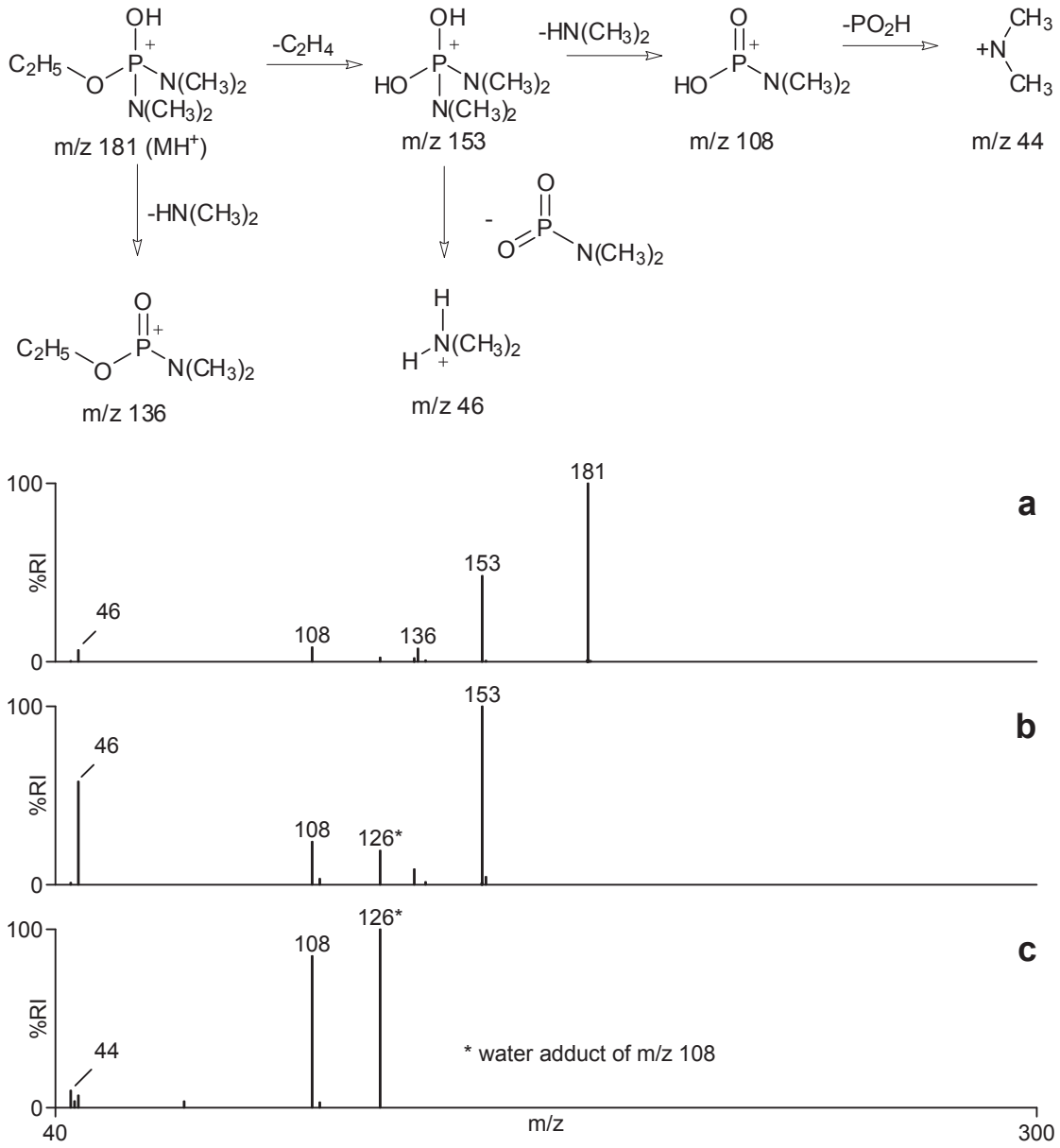

Figure 8: MS^2 spectrum for the $[M+H]^+$ ion at a) m/z 181 and MS^3 spectra for the product ions at b) m/z 153 and c) m/z 126 observed for ethyl tetramethylphosphorodiamidate during LC-IMS-MS^n analysis of a hydrolysed tabun sample (collision energies: trap 12 eV, transfer 12 eV).

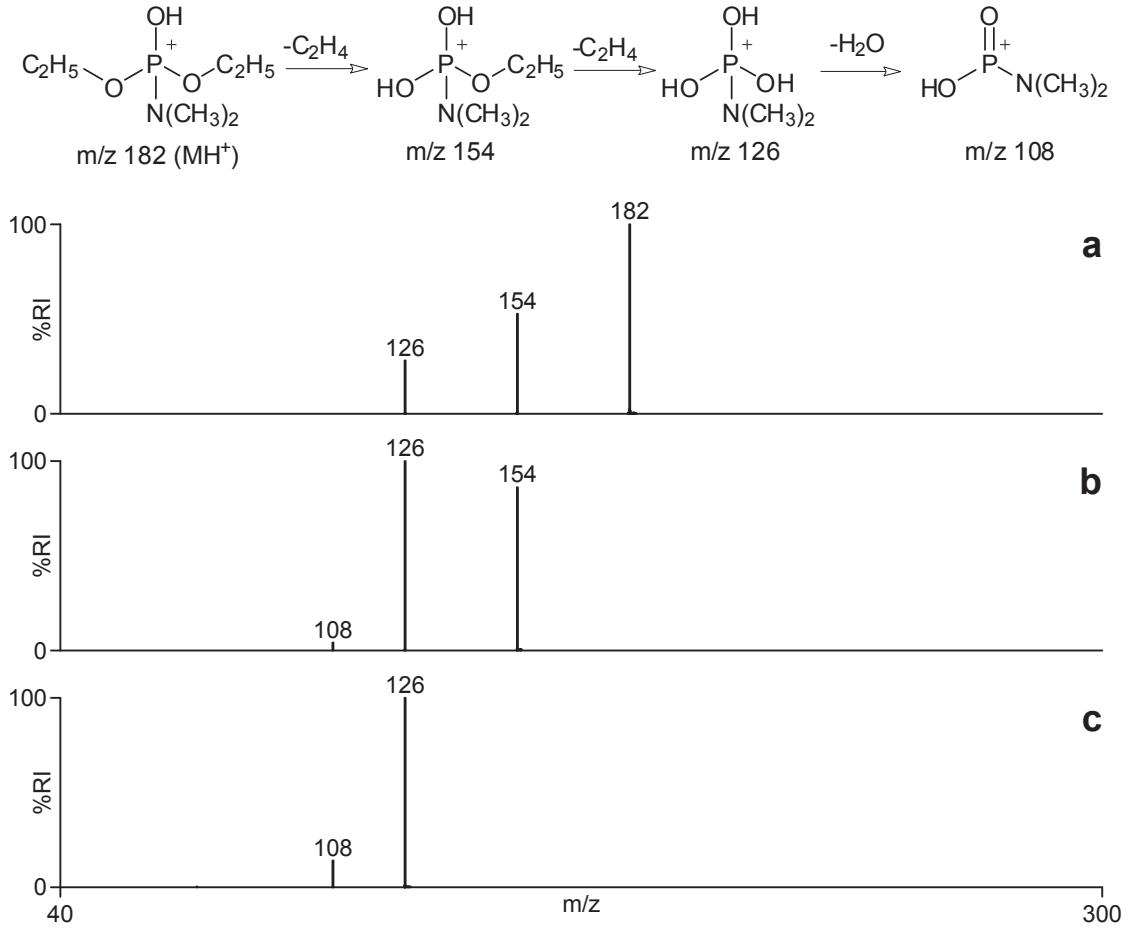

Figure 9: MS^2 spectrum for the $[M+H]^+$ ion at a) m/z 182 and MS^3 spectra for the product ions at b) m/z 154 and c) m/z 126 observed for diethyl dimethylphosphoramidate during LC-IMS-MS^n analysis of a hydrolysed tabun sample (collision energies: trap 10 eV, transfer 10 eV).

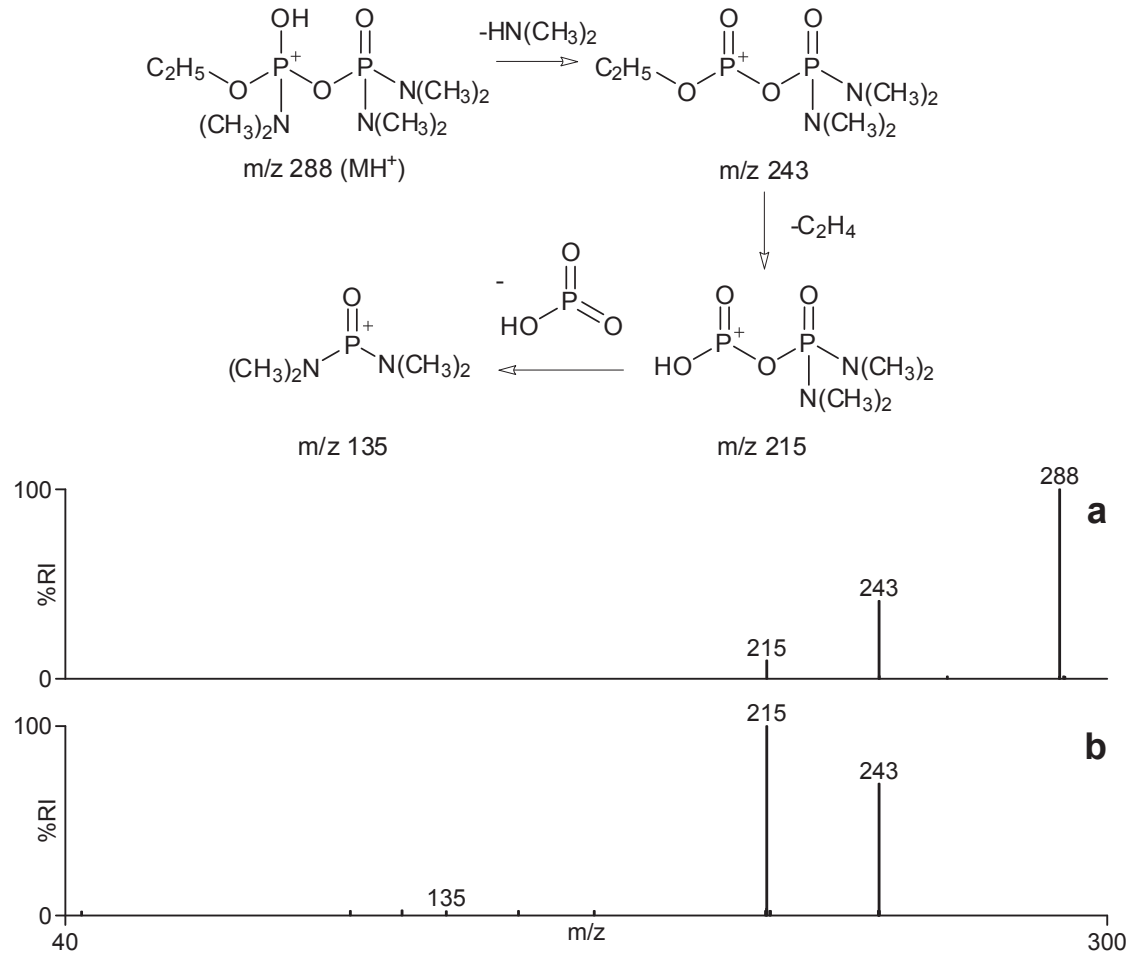

Figure 10: MS^2 spectrum for the $[M+H]^+$ ion at a) m/z 288 and MS^3 spectra for the product ion at b) m/z 243 observed for ethyl dimethylphosphoramidic tetramethylphosphorodiamidic anhydride during LC-IMS-MS^n analysis of a hydrolysed tabun sample (collision energies: trap 12 eV, transfer 12 eV)

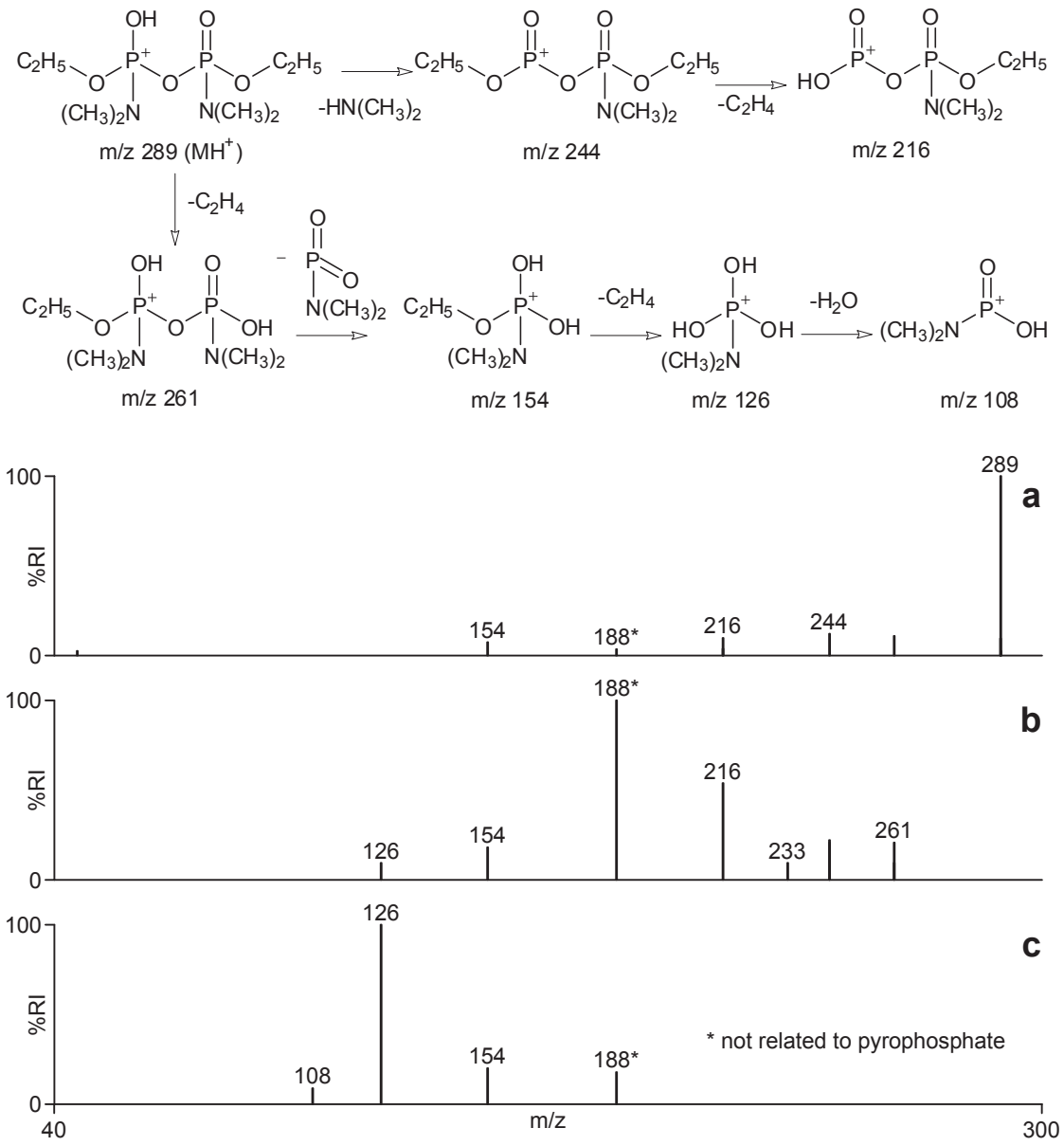

Figure 11: MS^2 spectrum for the $[M+H]^+$ ion at a) m/z 289 and MS^3 spectra for the product ions at b) m/z 261 and c) m/z 154 observed for bis(ethyl dimethylphosphoramidic) anhydride during LC-IMS-MS^n analysis of a hydrolysed tabun sample (collision energies: trap 12 eV, transfer 12 eV).

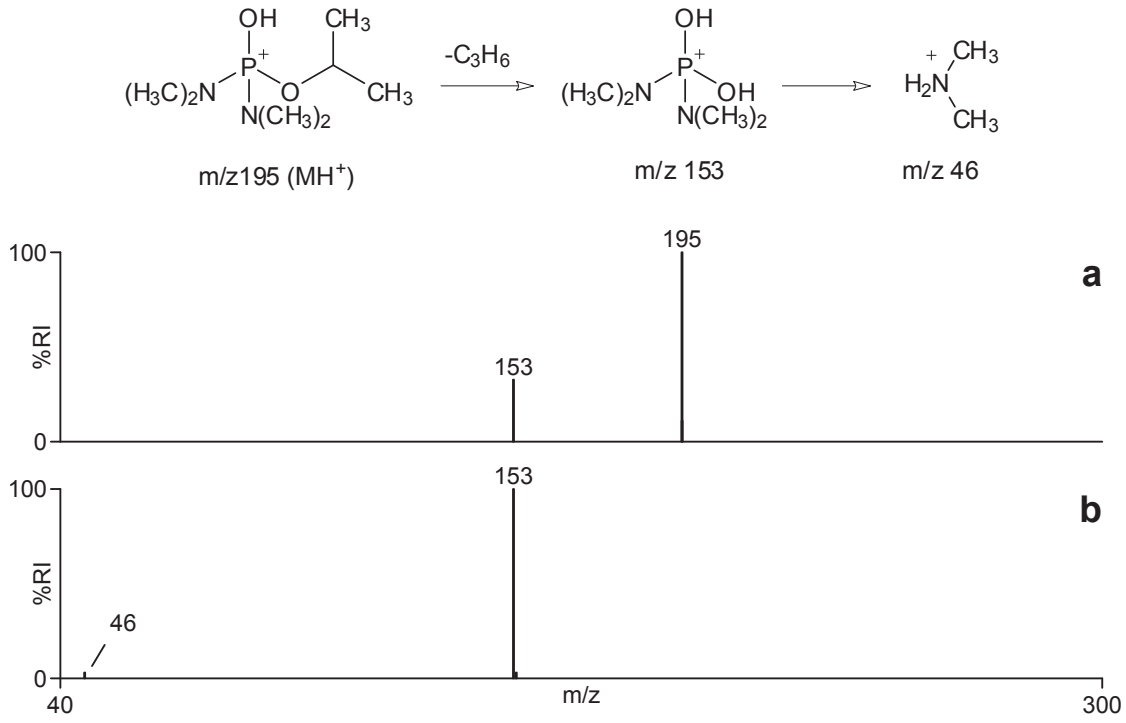

Figure 12: MS^2 spectrum for the $[M+H]^+$ ion at a) m/z 195 and MS^3 spectra for the product ion at b) m/z 153 observed for isopropyl tetramethylphosphorodiamidate during LC-IMS-MS^n analysis of a hydrolysed tabun sample (collision energies: trap 7 eV, transfer 7 eV).

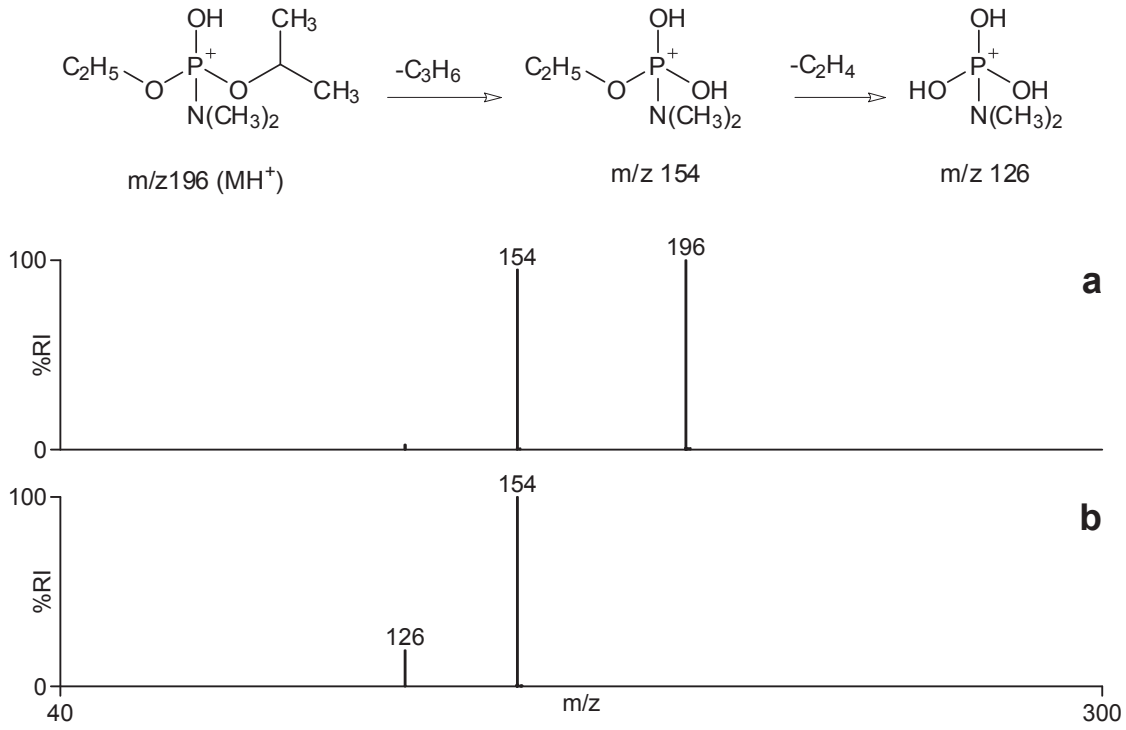

Figure 13: MS^2 spectrum for the $[M+H]^+$ ion at a) m/z 196 and MS^3 spectra for the product ion at b) m/z 154 observed for ethyl isopropyl dimethylphosphoramidate during LC-IMS-MS^n analysis of a hydrolysed tabun sample (collision energies: trap 7 eV, transfer 7 eV).

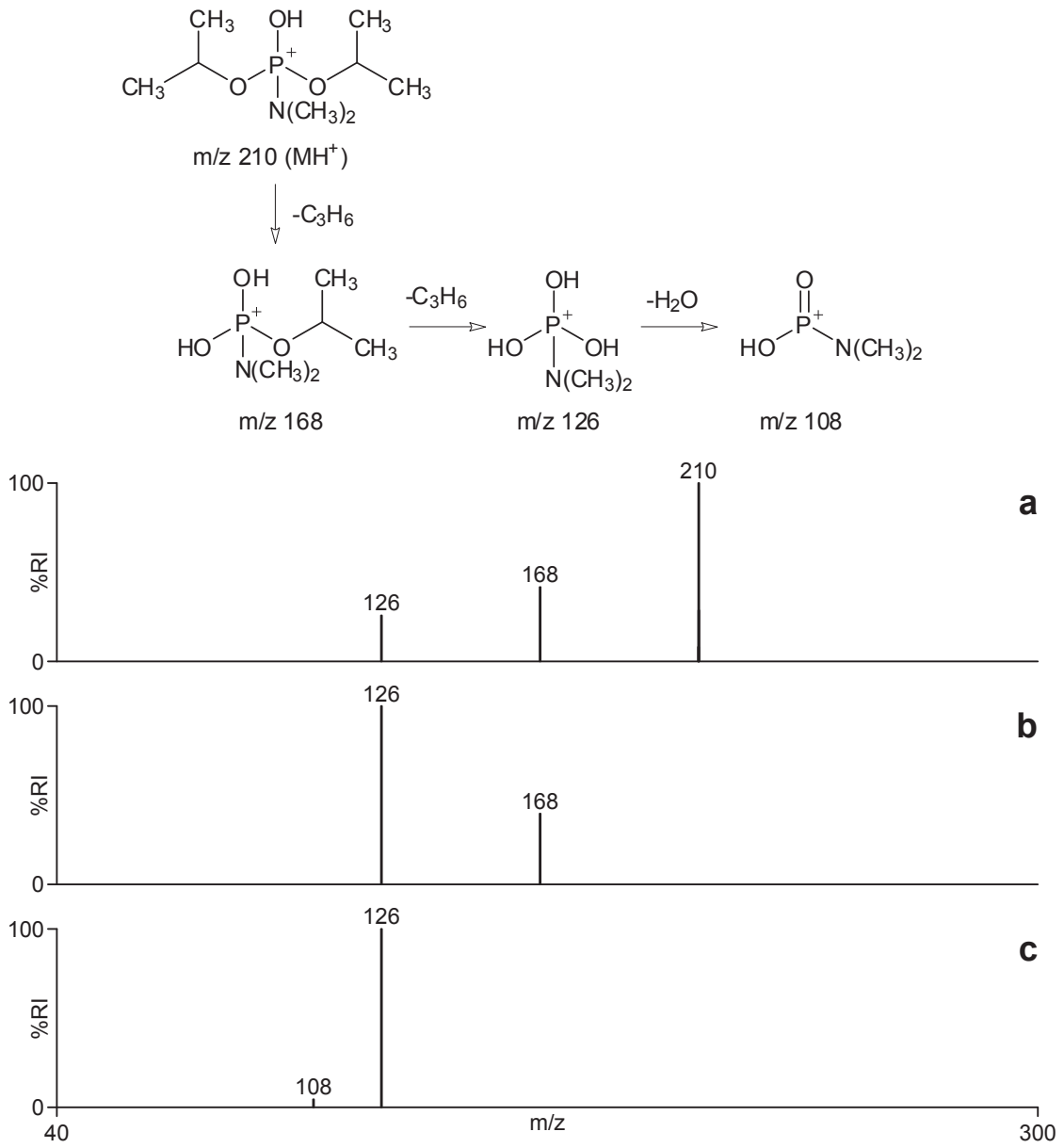

Figure 14: MS^2 spectrum for the $[M+H]^+$ ion at a) m/z 210 and MS^3 spectra for the product ions at b) m/z 168 and c) m/z 126 observed for diisopropyl dimethylphosphoramidate during LC-IMS-MS^n analysis of a hydrolysed tabun sample (collision energies: trap 7 eV, transfer 7 eV).

LC-IMS-MS^n analysis of hydrolysed VX

The broad spectrum approach was also applied to a hydrolysed VX sample containing VX related sample components. LC-MS analysis, under low collision energy conditions that favoured $[M+H]^+$ formation, indicated the presence of five significant sample components. Retention time windows were assigned for quadrupole mass selection of the observed $[M+H]^+$ ions and trap and

transfer collision energy settings in the 8 eV to 15 eV range were selected for each sample component based on the degree of product ion formation observed during screening. A trap collision energy setting that yielded both the $[M+H]^+$ ion and one or more significant product ion was selected along with a transfer energy setting that promoted a reasonable degree of product ion formation (while still maintaining the presence of the precursor ion). Figure 15 illustrates the LC-IMS-MS^n separation of the sample components in the hydrolysed VX sample, with the corresponding m/z value used for $[M+H]^+$ selection. The primary hydrolysis product of VX, ethyl methylphosphonic acid and four other compounds associated with VX (diisopropylamine, bis[2-(diisopropylamino)ethyl] sulfide, diethyl methylphosphonate and bis[2-(diisopropylamino)ethyl] disulfide, associated with VX observed [16, 17]) were identified.

Figure 16 illustrates the five ion mobility profiles acquired during LC-IMS-MS^n analysis of the hydrolysed VX sample. The ion mobility profiles of the five compounds were unique and this data, together with the acquired high resolution MS^n data, enabled confirmation of these compounds.

Figure 17 to 21 illustrate the MS^n data acquired during LC-IMS-MS^n for each of the five significant sample components in the hydrolysed VX sample. Possible fragmentation pathways and ion structures, based on the observed product ions in acquired mass spectra, have been presented above the acquired MS^n data. MS^n Data were acquired under high resolution conditions (8000 resolution, 50% valley definition) and in all cases the measured mass of the observed ion was consistent with the theoretical mass of the proposed ion.

For the hydrolysis product of VX, ethyl methylphosphonic acid (Figure 17), and diethyl methylphosphonate (Figure 20), product ion formation was relatively simple. Loss of the alkene associated with each alkoxy group was observed along with the loss of water. Diisopropylamine (Figure 18) exhibited a significant product ion at m/z 60 due to loss of C_3H_6 and several lower mass ions. Higher trap and transfer collision energies (15 eV) were used to fragment bis[2-(diisopropylamino)ethyl] sulphide (Figure 19) and bis[2-(diisopropylamino)ethyl] disulfide (Figure 21), two longer chain compounds often associated with VX degradation [16, 17]. The two compounds were easily differentiated on the basis of their MS^n data acquired for the $[M+H]^+$ ion and the first significant product ion at m/z 188 (bis[2-(diisopropylamino)ethyl] sulphide) and m/z 220 (bis[2-(diisopropylamino)ethyl] disulfide). A common product ion at m/z 160 produced similar MS^3 data for both compounds. Possible ion structures for ten ions observed in the MS^n data were rationalized for these compounds.

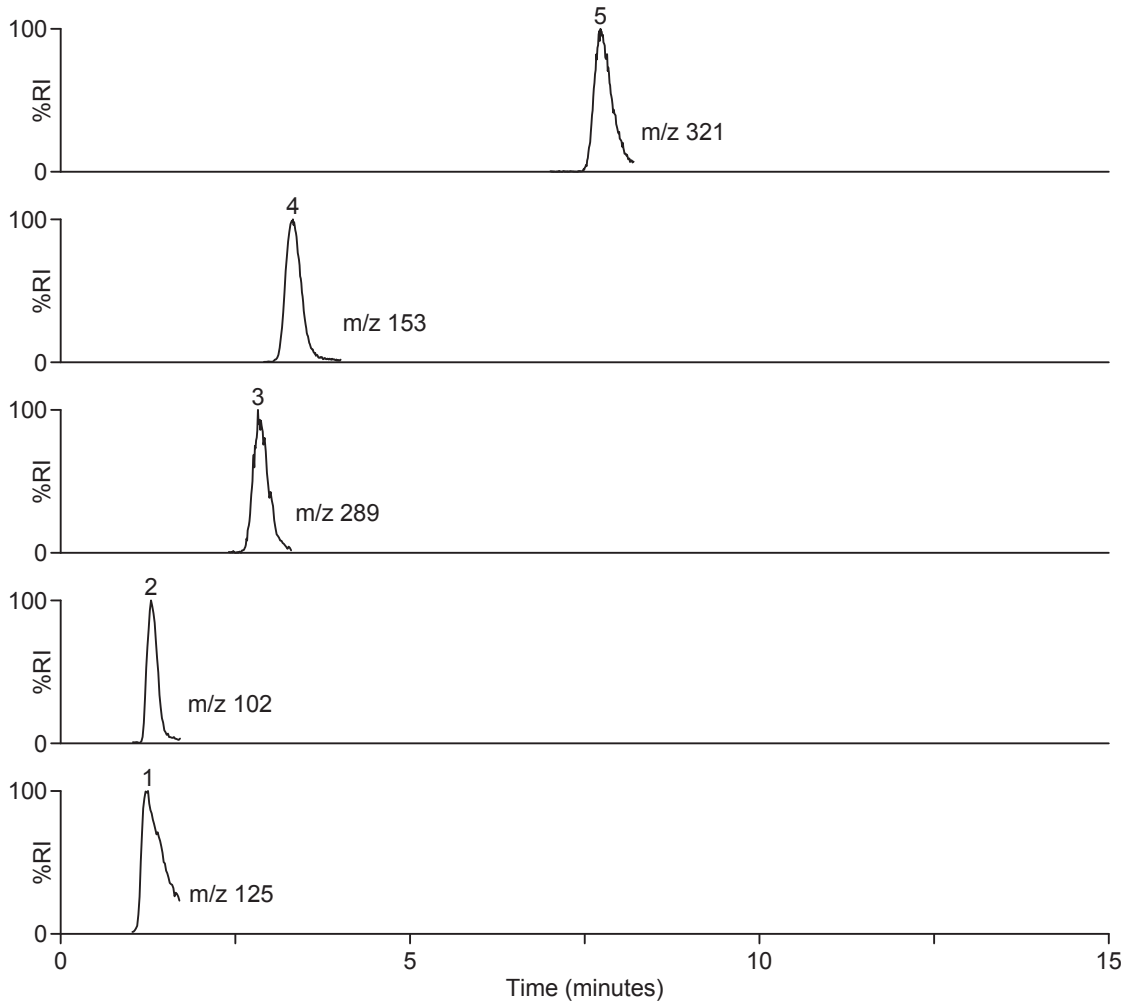

Figure 15: LC-IMS-MS^n separation of components in a hydrolysed VX sample (20 ng/µL). 1. ethyl methylphosphonic acid, 2. diisopropylamine , 3. bis[2-(diisopropylamino)ethyl] sulfide, 4. diethyl methylphosphonate, 5. bis[2-(diisopropylamino)ethyl] disulfide.

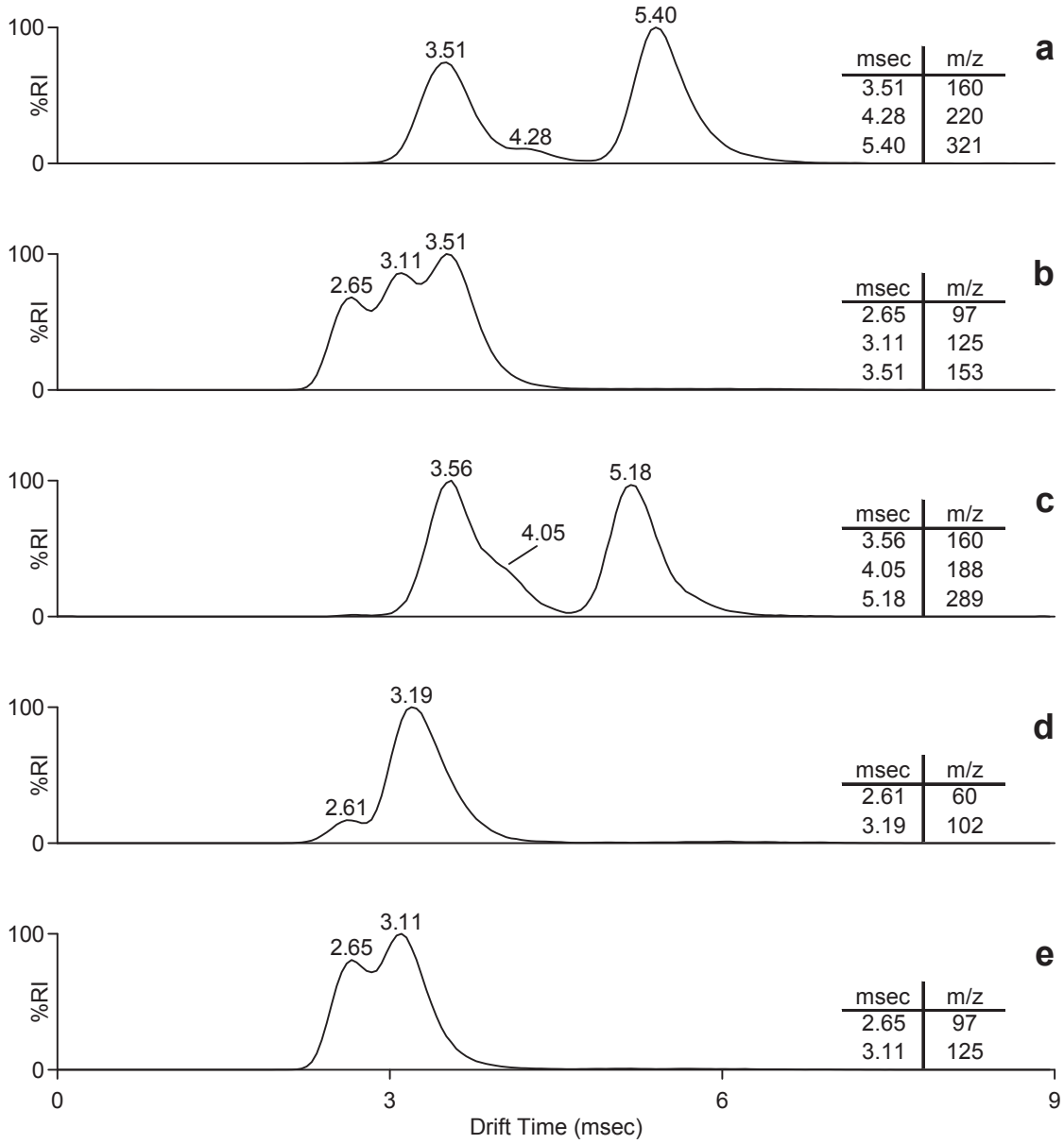

Figure 16: Ion mobility profiles obtained for a) bis[2-(diisopropylamino)ethyl] disulphide, b) diethyl methylphosphonate, c) bis[2-(diisopropylamino)ethyl] sulfide, d) diisopropylamine and e) ethyl methylphosphonic acid during LC-IMS-MS^n analysis of a hydrolysed VX sample. The $[M+H]^+$ ion selected by the quadrupole mass analyser and the product ions generated in the trap region of the collision cell were separated by IMS.

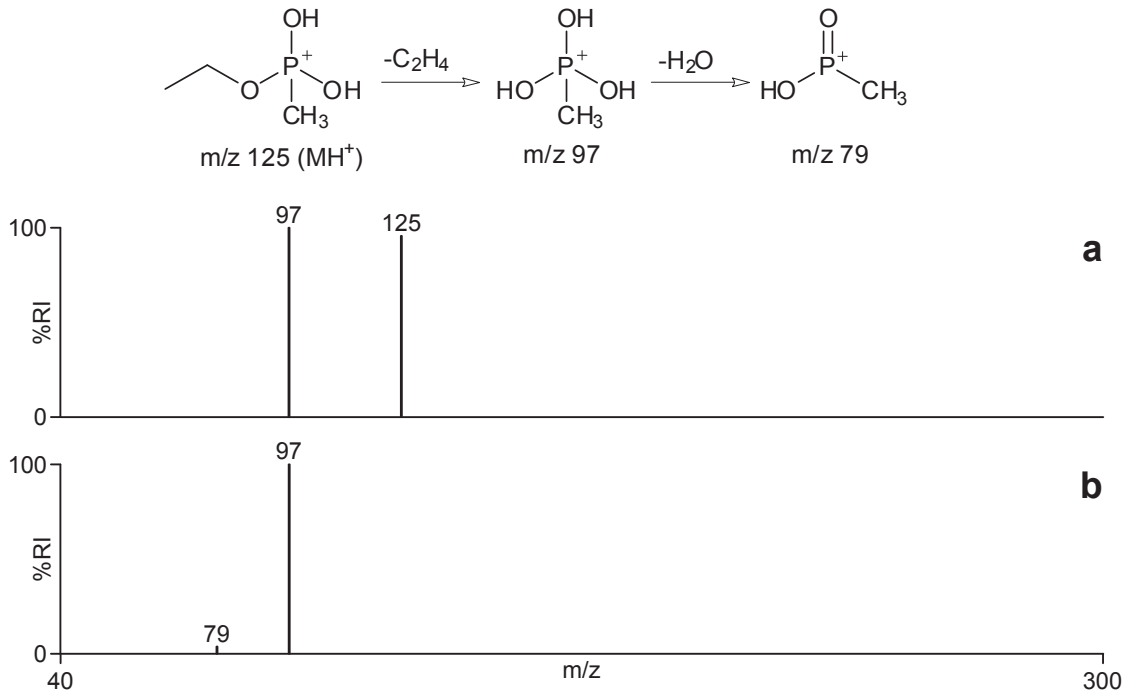

Figure 17: MS^2 spectrum for the $[M+H]^+$ ion at a) m/z 125 and MS^3 spectra for the product ion at b) m/z 97 observed for ethyl methylphosphonic acid during LC-IMS-MS^n analysis of a hydrolysed VX sample (collision energies: trap 8 eV, transfer 8 eV).

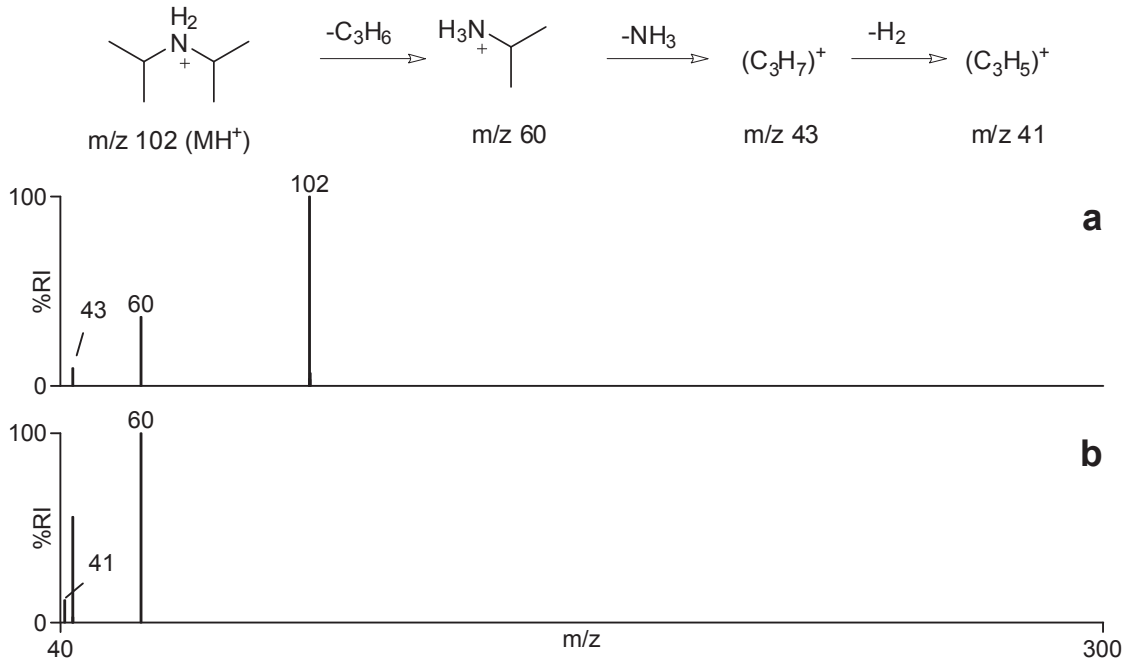

Figure 18: MS^2 spectrum for the $[M+H]^+$ ion at a) m/z 102 and MS^3 spectra for the product ion at b) m/z 60 observed for diisopropylamine during LC-IMS-MS^n analysis of a hydrolysed VX sample (collision energies: trap 10 eV, transfer 10 eV).

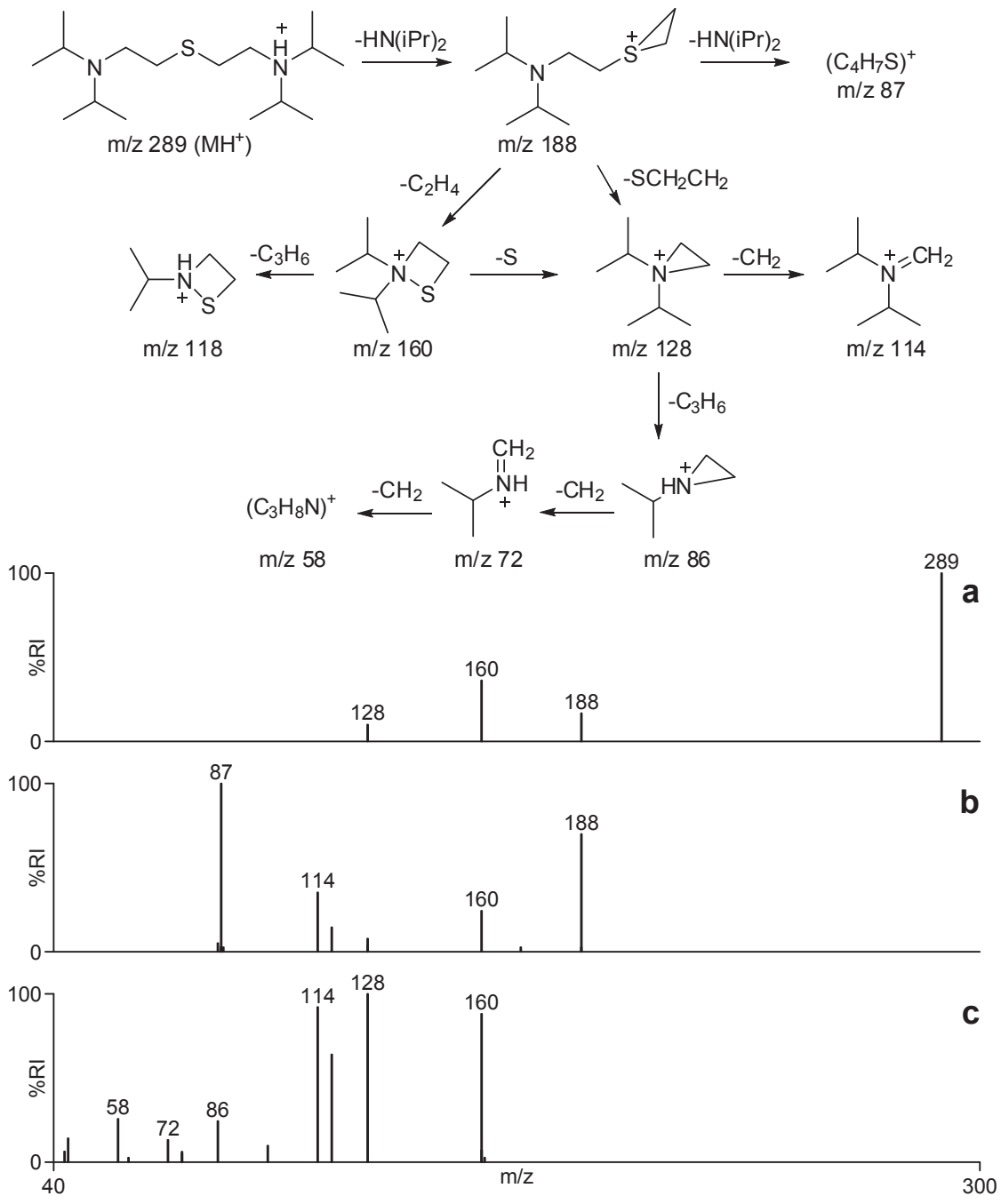

Figure 19: MS^2 spectrum for the $[M+H]^+$ ion at a) m/z 289 and MS^3 spectra for the product ions at b) m/z 188 and c) m/z 160 observed for bis[2-(diisopropylamino)ethyl] sulfide during LC-IMS-MS^n analysis of a hydrolysed VX sample (collision energies: trap 15 eV, transfer 15 eV).

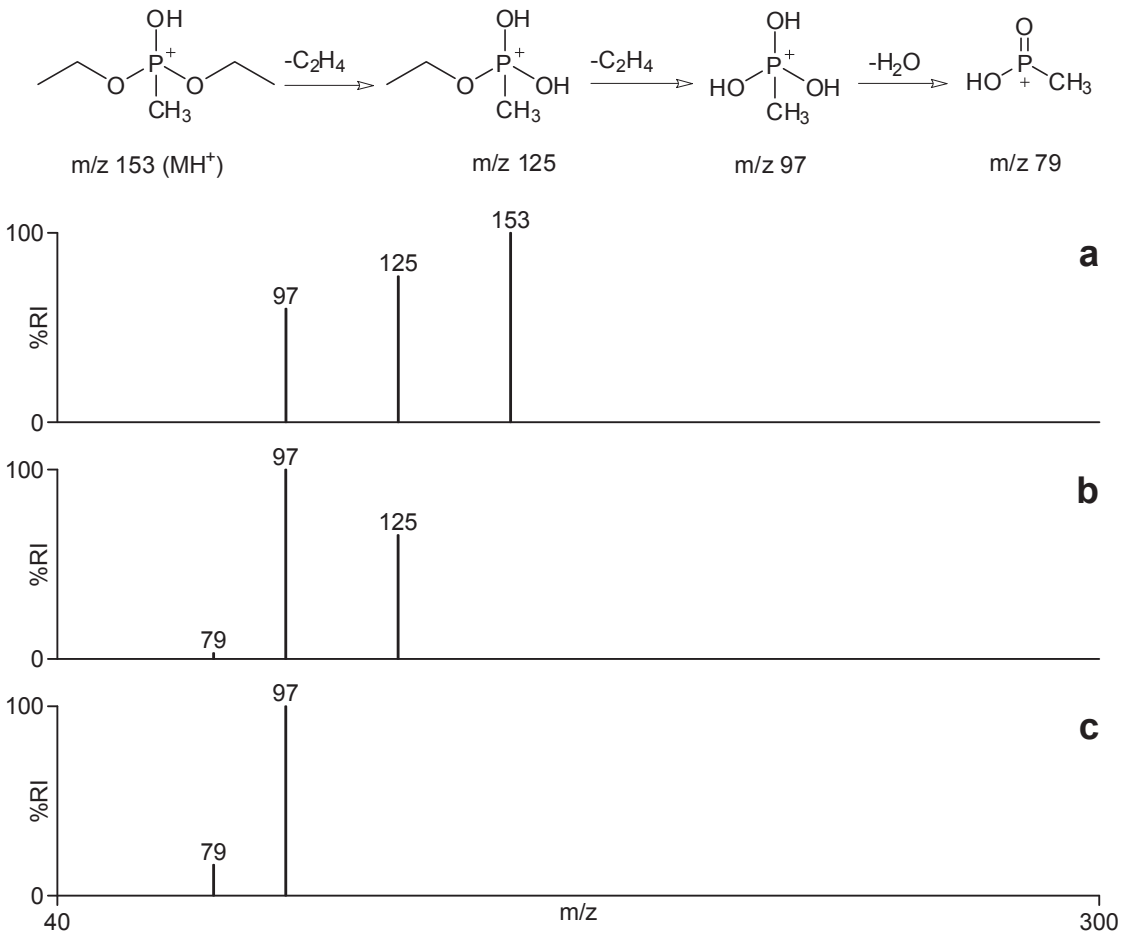

Figure 20: MS^2 spectrum for the $[M+H]^+$ ion at a) m/z 153 and MS^3 spectra for the product ions at b) m/z 125 and c) m/z 97 observed for diethyl methylphosphonate during LC-IMS-MS^n analysis of a hydrolysed VX sample (collision energies: trap 10 eV, transfer 10 eV).

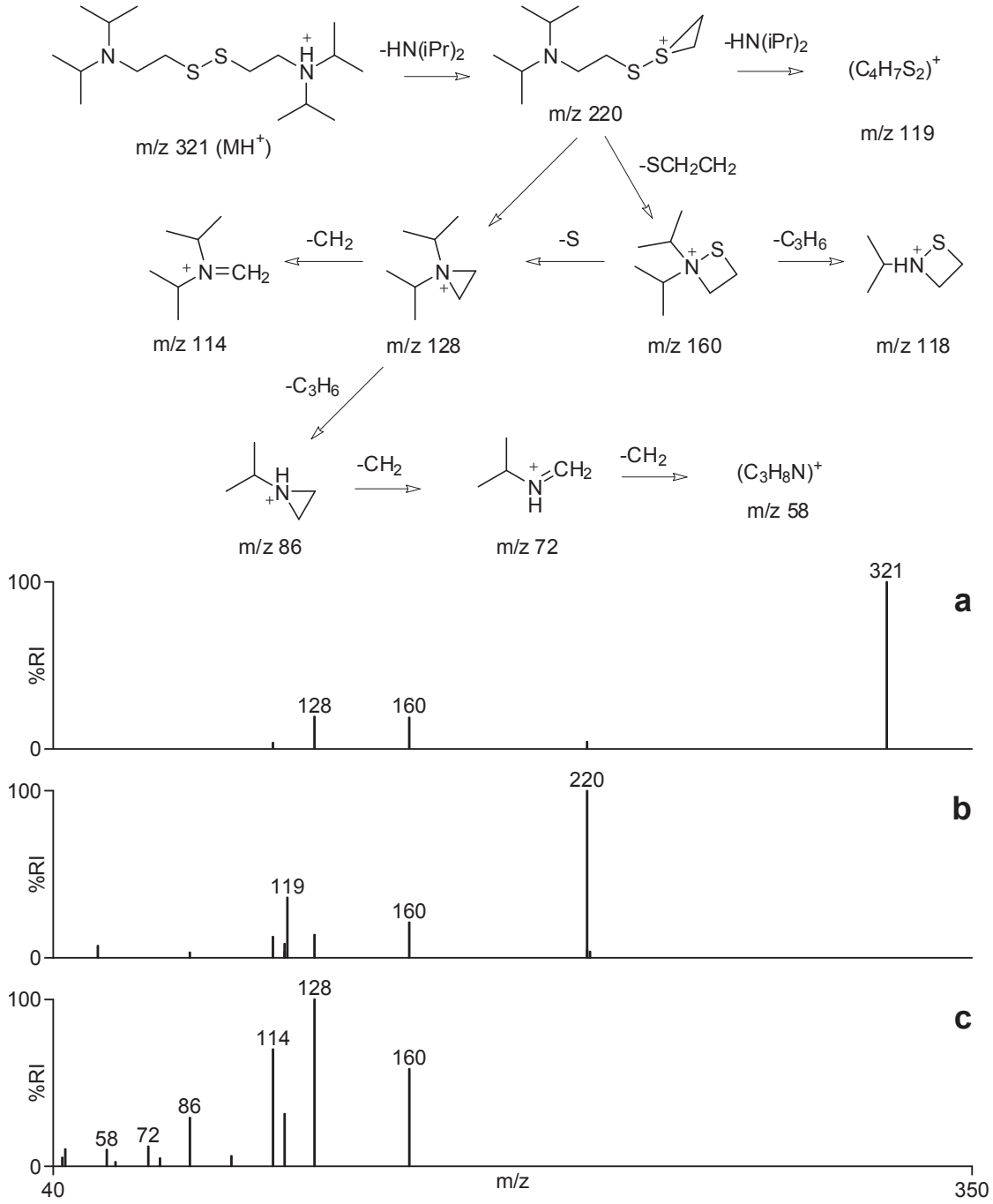

Figure 21: MS^2 spectrum for the $[M+H]^+$ ion at a) m/z 321 and MS^3 spectra for the product ions at b) m/z 220 and c) m/z 160 observed for bis[2-(diisopropylamino)ethyl] disulfide during LC-IMS-MS^n analysis of a hydrolysed VX sample (collision energies: trap 15 eV, transfer 15 eV).

Chemical warfare agents

The ion mobility profiles of five organophosphorus chemical warfare agents were reported in two prior publications with a bin number time scale [26, 27], where 200 bins was equivalent to 9 msec. Use of the msec time scale would be preferred for comparative purposes and the Figure illustrating the ion mobility profiles for the five organophosphorus chemical warfare agents has been reproduced in Figure 22 with a msec time scale. Most importantly, the ion mobility profiles for the organophosphorus chemical warfare agents were different from those acquired in the present study dealing with simulants and hydrolysed tabun and VX, which suggests an application for IMS during chemical identification.

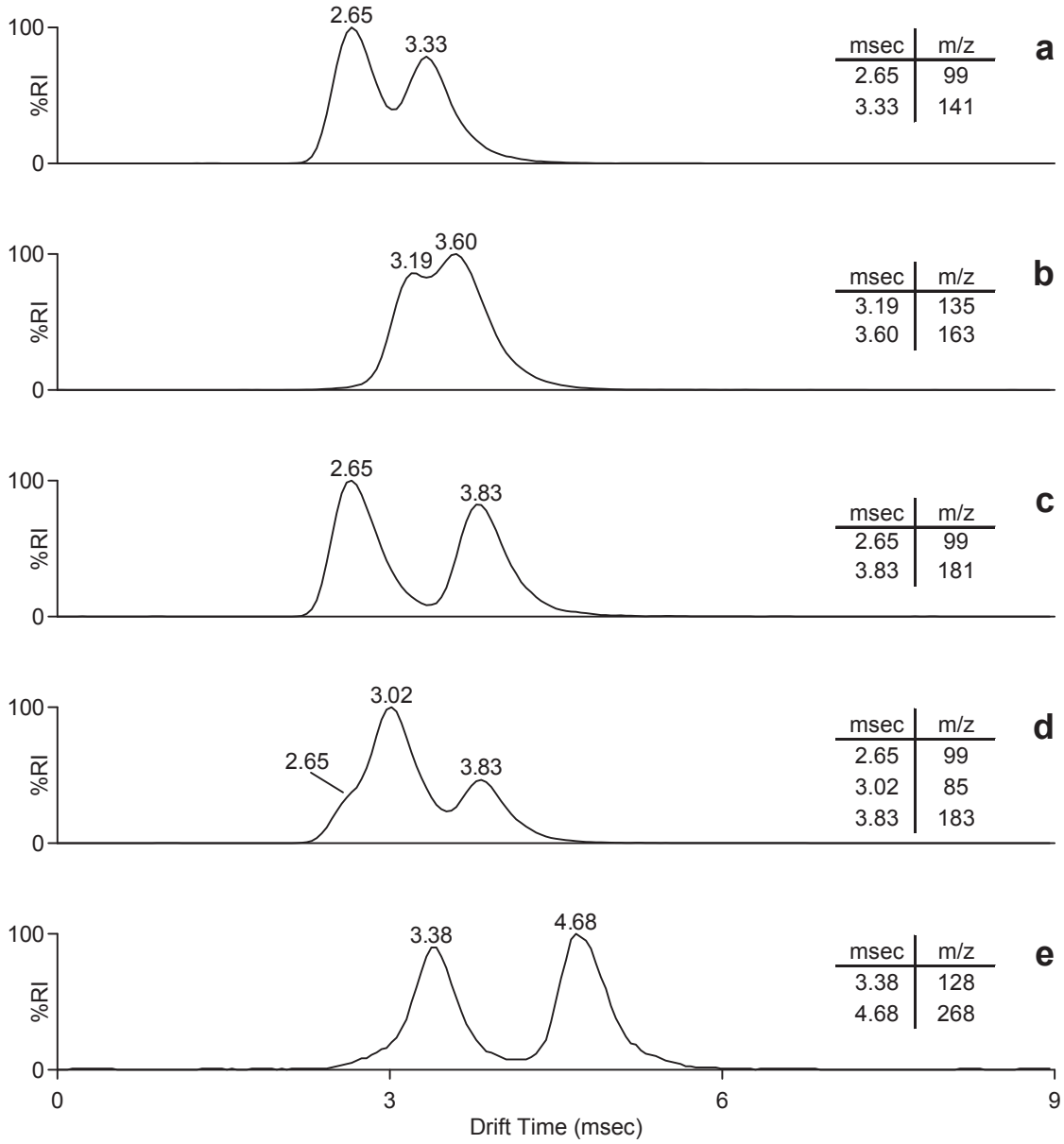

Figure 22: Ion mobility profiles obtained for sarin (GB), tabun (GA), cyclosarin (GF), soman (GD) and VX following DESI-IMS- MS^n analysis of SPME fibers exposed to the headspace above 5 µg each compound. The $[M+H]^+$ ion selected by the quadrupole analyser and one or more product ions, generated in the trap region of the collision cell, were separated by IMS [26, 27].

Instrumental considerations

An argument can be made that similar MS^n data could be acquired with a conventional quadrupole time-of-flight instrument provided that product ion formation can occur in the ESI source region. With some instruments, including the Synapt HDMS, the sampling cone voltage can be increased to produce the same product ions that are generated by increasing the trap collision energy in the Triwave collision cell. However, in this case, the quadruole mass analyser would also need to cycle through the m/z values of the $[M+H]^+$ and product ions. MS^2 data could then be generated for the $[M+H]^+$ ion and MS^3 data for product ions formed for a given compound in the ESI source region. Depending on ESI source design, sampling cone fragmentation may not be effective, as was the case with our prior instrument, a Waters Ultima quadrupole time-of-flight instrument. It was possible to induce product ion formation with the RF1 setting, but with considerably less flexibility. A compromise RF1 setting (not compound specific) which would result in more or less product ion formation than was considered optimal had to be used, since the RF1 value could not be tailored to a specific compound with a specific retention time.

The flexibility of the Synapt HDMS with its Triwave collision cell allows the analyst the option of target compound analysis, as was demonstrated during DESI-IMS-MS^n of organophosphorus chemical warfare agents [26, 27],or broad spectrum analysis for compound identification, as was demonstrated in the LC-IMS-MS^n examples. Once conditions have been defined for a given compound a target method appropriate for repetitive analyses could be established. Both IMS and MS^n (n = 2 or 3) data for a compound were acquired when the $[M+H]^+$ ion for a compound is selected with the quadrupole mass analyser during this study. However, if required, MS^4 data may also be acquired with the Synapt HDMS by mass selecting a product ion that has been formed in the ESI source. This was not exercised in the present study but could and has been performed at DRDC Suffield by simply increasing the sampling cone voltage until the desired product ion has been formed in sufficient abundance. A quadrupole selected product ion could fragment in the trap collision region, the ions generated could then be separated by IMS and each of the separated ions could fragment in the transfer collision region. This could not be performed with a conventional quadrupole time-of-flight instrument.

Conclusions

Confirmation of chemical warfare agents requires the acquisition of data from at least two spectrometric techniques. Normally this would require multiple analyses, often using a different instrument. A Synapt HDMS quadrupole time-of-flight tandem mass spectrometer, recently installed at DRDC Suffield, may be used to acquire both ion mobility spectrometric (IMS) and tandem mass spectrometric (MS^n where n = 2 or 3) data and this capability was recently reported during the desoption electrospray ionization (DESI) analysis of solid phase microextraction fibers. This analytical approach was extended in the present study to more complex, multi-component samples where separation by liquid chromatography was beneficial. The organophosphorus chemical warfare agent simulants, triethyl phosphate and tributyl phosphate, were initially used to develop a broad spectrum analytical approach for multi-component sample characterization by LC-IMS-MS^n. Unique ion mobility profiles and high resolution MS^n data, often referred to as time-aligned parallel (TAP) fragmentation data, enabled confirmation of both simulants during a single analysis. The LC-IMS-MS^n methodology was then applied to multi-component chemical warfare agent samples that had undergone hydrolysis. Tabun or O-ethyl S-[2-(diisopropylamino)ethyl] methylphosphonothiolate (VX) hydrolysis products and a number of related compounds were confirmed at the nanogram level. Individual compounds were differentiated on the basis of their acquired IMS profiles and full scanning, high resolution MS^n data. MS^n data for each compound contained evidence of the $[M+H]^+$ ion and up to nine characteristic product ions.

The developed methodology may be applied to the confirmation of individual sample components in multi-component chemical warfare agent mixtures requiring broad spectrum analysis or for the acquisition of spectrometric data that would aid in the identification of unknown sample components.

IMS and MS^n data has been acquired for five organophosphorus chemical warfare agents, two organophosphorus simulants, two chemical warfare agent hydrolysis products and twelve related compounds in this and the prior DESI-IMS-MS^n study. The potential of this analytical approach for either target compound or broad spectrum analysis warrants continued investigation. Strong consideration is now being given to acquiring IMS and MS^n data for available chemical defence compounds at DRDC Suffield and the establishment of a database.

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Liquid Chromatographic Electrospray Ionization Mass Spectrometric Characterization of Simulants, Hydrolysed Tabun and VX Using Ion Mobility and Tandem Mass Spectrometric Data

4. AUTHORS (last name, followed by initials – ranks, titles, etc. not to be used)

D'Agostino, P. A.; Chenier, C. L.

5. DATE OF PUBLICATION (Month and year of publication of document.)	6a. NO. OF PAGES (Total containing information, including Annexes, Appendices, etc.)	6b. NO. OF REFS (Total cited in document.)
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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.)	10b. OTHER DOCUMENT NO(s). (Any other numbers which may be assigned this document either by the originator or by the sponsor.)
DRDC Suffield TM 2011-001	

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A Synapt HDMS quadrupole time-of-flight tandem mass spectrometer was used for the first time to acquire both ion mobility spectrometry (IMS) and tandem mass spectrometry (MS^n where n = 2 or 3) data for chemical defence compounds following liquid chromatographic (LC) sample introduction. LC-MS screening was used to establish retention time windows for significant sample components prior to the acquisition of LC-IMS-MS^n data. Two organophosphorus chemical warfare agent simulants, triethyl phosphate and tributyl phosphate, were initially used to develop a broad spectrum analytical approach for multi-component sample characterization by LC-IMS-MS^n. Unique ion mobility profiles and high resolution MS^n data, often referred to as time-aligned parallel (TAP) fragmentation data, enabled confirmation of both simulants during a single analysis. The LC-IMS-MS^n methodology was then applied to multi-component chemical warfare agent samples that had undergone hydrolysis. Tabun (GA) and O-ethyl S-[2-(diisopropylamino)ethyl] methylphosphonothiolate (VX) hydrolysis products and a number of related compounds were confirmed at the nanogram level. Compounds were differentiated on the basis of their acquired IMS profiles and full scanning, high resolution MS^n data. MS^n data for each compound contained evidence of the $[M+H]^+$ ion and up to nine characteristic product ions. Application of the developed methodology is anticipated for the confirmation of individual sample components in multi-component chemical warfare agent mixtures requiring broad spectrum analysis or for the acquisition of spectrometric data that would aid in the identification of previously uncharacterized sample components.

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Liquid Chromatography; Tandem Mass Spectrometry; Ion Mobility Spectrometry; Chemical Warfare Agents; Tabun; VX; Simulants

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