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Comparison of LC-ESI-MS and GC-MS for the Analysis of a Synthetic Tabun Sample

P.A. D'Agostino, J.R. Hancock and C.L. Chenier
Defence R&D Canada – Suffield

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Author

Paul A. D'Agostino

Approved by

Jacques Lavigne

H/CBDS

Approved for release by

Robin Clewley

DRP Chair

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Abstract

Packed capillary LC-ESI-MS and capillary column GC-MS were compared for the analysis of a synthetic tabun sample as each method has advantages for the analysis of samples containing chemical warfare agents, their hydrolysis products and related compounds. Twelve sample components were identified during LC-ESI-MS analysis of the sample, while only ten were detected by GC-MS. The two less volatile compounds not detected by GC-MS contained hydroxyl substitution and would only be detected by GC-MS following derivatization and a second GC-MS analysis. The total analysis times, including equilibration times between analyses, were similar, typically requiring about 40 to 45 minutes. Peak widths for capillary column GC-MS separations were typically an order of magnitude better than packed capillary LC-ESI-MS, offering the potential to resolve more sample components during a given analysis. The relative sensitivity of the methods was estimated since the exact contribution of each sample component to the mixture used for comparison remains unknown. Interpretable full mass spectra and similar S/N ratios in the total-ion-current were observed for the trace sample components, estimated to be present in the low nanogram or subnanogram range, using both methods.

Résumé

On a comparé la spectrométrie de masse CPL-IPE-SM à micro-capillaires à la spectrométrie de masse à colonnes capillaires CPG-SM pour analyser un échantillon de tabun synthétique, chacune des méthodes ayant des avantages pour les analyses des échantillons contenant des agents de guerre chimique, les produits de leur hydrolyse et autres composés. Douze constituants d'échantillons ont été identifiés durant l'analyse CPL-IPE-SM de l'échantillon alors que dix seulement ont été identifiés par CPG-SM. Les deux composés moins volatiles non détectés par CPG-SM contenaient une substitution d'hydroxyle et ne pouvaient être détectés que par CPG-SM après la dérivatisation et une seconde analyse CPG-SM. Les durées totales d'analyses, y compris les durées d'équilibration entre les analyses étaient similaires et nécessitaient typiquement 40 à 45 minutes. Les largeurs de pic pour les séparations de colonnes capillaires CPG-SM étaient typiquement d'un meilleur ordre de grandeur que les capillaires concentrés CPL-IPE-SM, offrant la possibilité de résoudre plus de constituants d'échantillons durant une analyse donnée. La sensibilité relative des méthodes a été estimée puisque la contribution exacte de chaque composant d'échantillon, au mélange, demeure inconnue. Des spectres de masse interprétables et des rapports similaires signal/bruit dans le courant ionique total ont été observés pour les composés des échantillons traceurs, estimés être présents dans des quantités variant de quelques nanogrammes à moins d'un nanogramme, en utilisant les deux méthodes.

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Executive summary

Introduction: The Canadian Forces (CF) may be called on to perform peacekeeping or battlefield operations in regions of the world where there is a significant threat of chemical/biological (CB) warfare agent use. To operate effectively in these theatres the CF must be able to identify the CB agent used. Mass spectrometry (MS) is a powerful analytical technique for the identification of both known and unknown compounds and DRDC Suffield is currently investigating this instrumental technique in fulfillment of CF detection and identification requirements.

Results: Packed capillary LC-ESI-MS and capillary column GC-MS were compared for the analysis of a synthetic tabun sample as each method has advantages for the analysis of samples containing chemical warfare agents, their hydrolysis products and related compounds. Twelve sample components were identified during LC-ESI-MS analysis, while only ten were detected by GC-MS. The two less volatile compounds not detected by GC-MS contained hydroxyl substitution and would only be detected by GC-MS following derivatization and a second analysis. Derivatization was not required during LC-ESI-MS analysis, a definite advantage for this technique over GC-MS for the reporting of mixtures containing chemical warfare agents, related compounds and lower volatility, hydrolysis products.

Total analysis times, including equilibration times between analyses, were similar, typically requiring about 40 to 45 minutes. Peak widths for capillary column GC-MS separations were typically an order of magnitude better than packed capillary LC-ESI-MS, offering the potential to resolve more sample components during a given analysis.

The relative sensitivity of packed capillary LC-ESI-MS to capillary column GC-MS was estimated since the exact contribution of each sample component to the mixture used for comparison remains unknown. Interpretable full mass spectra and similar S/N ratios in the total-ion-current were observed for the trace sample components, estimated to be present in the low nanogram or subnanogram range using both methods.

Significance: The CF may be deployed in regions of the world where there is a significant threat of chemical/biological warfare agent use. Identification of the CB agent is of importance since the results of such analyses would contribute to the development of strategic and political positions regarding future Canadian military operations and would facilitate the dissemination of technical advice to in-theatre field commanders and medical personnel.

Future Plans: The reported methods would be valuable for the identification of organophosphorus chemical warfare agents and their hydrolysis products in samples collected by the Canadian Forces or in support of Chemical Weapons Convention challenge inspections.

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Sommaire

Introduction: Les Forces canadiennes (FC) peuvent être appelées à entreprendre des opérations de maintien de la paix ou de champ de bataille dans des régions du monde où existe une menace importante d'utilisation d'agents chimiques et biologiques de guerre (CB). Pour être capable d'opérer efficacement dans ces théâtres, les FC doivent être capables d'identifier les agents CB utilisés. La spectrométrie de masse (SM) est une technique analytique puissante pour l'identification des composés connus et inconnus et RDDC Suffield examine actuellement cette technique qui est instrumentale pour satisfaire aux besoins de détection et d'identification des FC.

Résultats: On a comparé la spectrométrie de masse CPL-IPE-SM à micro-capillaires à la spectrométrie de masse à CPG-SM pour analyser un échantillon de tabun synthétique, chacune des méthodes ayant des avantages pour les analyses des échantillons contenant des agents de guerre chimique, les produits de leur hydrolyse et autres composés. Douze constituants d'échantillons ont été identifiés durant l'analyse CPL-IPE-SM de l'échantillon alors que dix seulement ont été identifiés par CPG-SM. Les deux composés moins volatiles non détectés par CPG-SM contenaient une substitution d'hydroxyle et ne pouvaient être détectés que par CPG-SM après la dérivatisation et une seconde analyse. La dérivatisation n'a pas été requise durant l'analyse CPL-IPE-SM, ce qui est l'avantage essentiel de cette technique par rapport à CPG-SM en ce qui concerne l'analyse des mélanges contenant des agents de guerre chimiques, les produits d'hydrolyse de moindre volatilité et autres composés.

Les durées totales d'analyses, y compris les durées d'équilibration entre les analyses étaient similaires et nécessitaient typiquement 40 à 45 minutes. Les largeurs de pic pour les séparations de colonnes capillaires CPG-SM étaient typiquement d'un meilleur ordre de grandeur que les capillaires concentrés CPL-IPE-SM, offrant la possibilité de résoudre plus de constituants d'échantillons durant une analyse donnée.

La sensibilité relative des méthodes a été estimée puisque la contribution exacte de chaque composant d'échantillon, au mélange, demeure inconnue. Des spectres de masse interprétables et des rapports similaires signal/bruit dans le courant ionique total ont été observés pour les composés des échantillons traceurs, estimés être présents dans des quantités variant de quelques nanogrammes à moins d'un nanogramme, en utilisant les deux méthodes.

La portée des résultats : Les FC peuvent être déployées dans des régions du monde où existe une menace importante d'utilisation d'agents de guerre chimiques et biologiques. L'identification des agents CB est importante puisque les résultats de telles analyses contribuent au développement de positions stratégiques et politiques en ce qui concerne les futures opérations militaires canadiennes et devraient faciliter la dissémination de conseils techniques aux commandants dans les théâtres d'opérations ainsi qu'au personnel médical.

Plans futurs : Les méthodes documentées seraient précieuses pour l'identification des agents de guerre chimiques organophosphorés et leurs produits d'hydrolyse recueillis en échantillons par les Forces canadiennes ou en soutien aux inspections par mise en demeure de la Convention sur les armes chimiques.

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Table of contents

Abstract i

Résumé i

Executive summary iii

Sommaire iv

Table of contents v

List of figures vi

List of tables vi

Introduction 1

Experimental 3

- Sample and sample handling 3
- Instrumental analysis 3

Results and Discussion 4

- Chromatographic Separations 4
- Mass Spectometric Data 5
- Sensitivity 15

Conclusions 16

References 17

List of figures

Figure 1. Typical a) GC-MS and b) LC-ESI-MS total-ion-current chromatograms acquired for the synthetic tabun sample. Sample components are identified in Table 1.....5

Figure 2. ESI-MS data acquired for ethyl phosphoric tetramethylphosphorodiamidic anhydride during LC-ESI-MS analysis with sampling cone voltages of a) 20 volts and b) 40 volts. 8

Figure 3. ESI-MS data acquired for octamethyltetramidotriphosphoric acid (or isomer) during LC-ESI-MS analysis with sampling cone voltages of a) 20 volts and b) 50 volts.....9

Figure 4. ESI-MS data acquired for hexamethylphosphorotriamide during LC-ESI-MS analysis with sampling cone voltages of a) 20 volts and b) 30 volts.....10

Figure 5. ESI-MS data acquired for bis(tetramethylphosphorodiamidic) anhydride during LC-ESI-MS analysis with sampling cone voltages of a) 20 volts and b) 50 volts.....11

Figure 6. ESI-MS data acquired for ethyl dimethylphosphoramidic tetramethylphosphorodiamidic anhydride during LC-ESI-MS analysis with sampling cone voltages of a) 20 volts and b) 40 volts.....12

Figure 7. ESI-MS data acquired for diethyl phosphoric tetramethylphosphorodiamidic anhydride during LC-ESI-MS analysis with sampling cone voltages of a) 20 volts and b) 40 volts. 13

Figure 8. ESI-MS data acquired for bis(ethyl dimethylphosphoramidic) anhydride during LC-ESI-MS analysis with sampling cone voltages of a) 20 volts and b) 40 volts.....14

List of tables

Table 1. Compounds identified in tabun synthetic sample by LC-ESI-MS.....6

Introduction

More than 140 State Parties, including Canada, have ratified the Chemical Weapons Convention (CWC) and agreed not to develop, produce, stockpile, transfer or use chemical weapons and to destroy their own chemical weapons and production facilities. The CWC has reduced the likelihood of chemical weapons use by State Parties, but there remains a serious concern that other parties may make use of these weapons against civilian or military targets. DRDC Suffield maintains a chemical warfare agent synthetic capability within the Canadian National Single Small-Scale Facility and provides the Canadian Forces and other National interests with a laboratory based identification capability for chemical warfare agents, degradation products and related compounds. Analyses of this type requires the use of sensitive, specific analytical methods, particularly when unambiguous proof is required for the presence of chemical warfare agents. These analytical demands are being actively addressed by DRDC Suffield through the development and application of new analytical methods for the detection and identification of chemical warfare agents in a variety of samples.

Gas chromatography (GC) has been used extensively for the separation and identification of chemical warfare agents, with gas chromatography-mass spectrometry (GC-MS) being used frequently by DRDC Suffield and other researchers for the characterization of these compounds (1,2). GC-MS, while suitable for the direct analysis of organophosphorus chemical warfare agent in organic extracts, is usually not preferred for the direct analysis of aqueous samples or extracts since these samples normally require additional sample handling steps and derivatization prior to analysis.

Liquid chromatography-electrospray-mass spectrometry (LC-ESI-MS) is being used increasingly, as ESI-MS data may be used to directly identify chemical warfare agents, degradation products and related compounds in collected aqueous samples or extracts. Researchers have developed atmospheric pressure ionization (e.g., electrospray (ESI), ionspray and atmospheric pressure chemical ionization) methods for the characterization of polar pesticides (3), organophosphate esters (4), and chemical warfare agents and/or their degradation products (5-24). These ionization modes have been interfaced to liquid chromatography and capillary electrophoresis (CE), with LC-MS (9-12, 14, 15, 18-24) and CE-MS (5, 13) methods being reported for the identification of lower volatility chemical warfare agent hydrolysis products. Use of this analytical technique has been recently extended to include the identification of chemical warfare agents as well. In the past three years a number of LC-ESI-MS papers have been published on the identification of organophosphorus chemical warfare agents and their hydrolysis products in aqueous (or snow) samples (14-17, 20, 23, 24) and aqueous extracts of contaminated soil samples (21, 22) during a single analysis.

Electrospray mass spectra from DRDC Suffield investigations (14-17, 20-24) were acquired with a resolution of 5000 (50% valley definition) in the continuum mode at several sampling cone voltages with a time-of-flight mass spectrometer. At lower sampling cone voltages (typically 20 volts) the mass spectra were dominated by protonated, sodiated and protonated acetonitrile adducts and/or their dimers that could be used to confirm the molecular mass of

each compound. Structural information was obtained by inducing product ion formation in the ESI interface at higher sampling cone voltages (typically 30 volts or more). Representative data obtained at both lower and higher sampling cone voltages for each compound were selected for entry into the DRDC Suffield ESI-MS Database as part of a database collaboration between Canada (DRDC Suffield) and The Netherlands (TNO Prins Maurits Laboratory) (25).

Both GC-MS and LC-ESI-MS have been utilized on a regular basis to support the DRDC Suffield chemical warfare agent synthetic capability. The relative merits of GC-MS and LC-ESI-MS have been discussed (25) but a direct comparison of these two analytical approaches for the same sample has not been previously reported. During this study a synthetic, multi-component tabun sample, containing twelve tabun related compounds, was analysed by both capillary column GC-MS and packed capillary LC-ESI-MS to allow comparison of the sensitivity, speed and selectivity of these two important identification methods.

Experimental

Sample and sample handling

GC-MS was compared to LC-ESI-MS for the analysis of chemical defence compounds with a multi-component tabun synthetic sample that had been submitted to the DRDC Suffield Analytical Laboratory for purity analysis by mass spectrometry. The synthetic procedure failed to produce tabun. However a number of other phosphate and pyrophosphate compounds similar in structure to tabun were formed in the reaction vessel. An initial portion (5 µL) of the synthetic tabun sample, used for GC-MS investigations, was diluted 6000 to 1 with dichloromethane (30 mL). A second portion (5 µL) was taken to dryness and diluted 6000 to 1 with water (30 mL) and used for LC-ESI-MS analyses.

Instrumental analysis

The tabun sample taken up in dichloromethane was analysed by GC-MS (Agilent 5973N under EI conditions: 70 eV, 0.035 mA, 230°C) using a 15m x 0.25mm ID J&W DB-35MS capillary column and the following temperature program: 40°C (2 min) 10°C/min 280°C (5 min). All injections (1 µL) were cool on-column at 43°C. The mass spectrometer was operated in full scanning mode and scanned from 40 to 400 Da at 2.08 scans/sec (unit resolution).

LC-ESI-MS data were acquired for the tabun sample taken up in water using a Micromass LCT time-of-flight mass spectrometer equipped with the Z-spray electrospray interface. The electrospray capillary was operated at 3.2 kV with sampling cone voltages in the 20 to 50 volts range. Nitrogen desolvation gas was introduced into the interface (80 °C) at a flow rate of 480 L/h. Nitrogen nebulizer gas was introduced at a flow rate of 66 L/h. ESI-MS data were acquired from 70 to 700 Da (1 sec) in the continuum mode with a resolution of 5000 (50% valley definition).

LC separations were performed with a MicroTech 150 mm x 0.32 mm i.d fused-silica capillary column packed with Zorbax C_{18} SB (5 µm particle size). The sample was introduced onto the column with a Rheodyne 8125 manual injector equipped with a 5 µL sample loop. The following solvent compositions were prepared for the mobile phase: Solvent A (0.1% trifluoroacetic acid (TFA) in water) and Solvent B (0.1% TFA in acetonitrile/water, 95:5). Chromatographic separations were performed with an Applied Biosystems model 140B dual syringe pump using a 1% to 40%B gradient over 30 minutes. In order to minimize dead volume effects and ensure reproducible mixing, the mobile phase was delivered at 200µL/min and split prior to the injector such that the flow through the column was 16 µL/min.

Results and Discussion

DRDC Suffield has analysed tabun samples on a number of occasions and published several GC-MS and LC-ESI-MS papers containing acquired mass spectra. An initial paper (26) containing the EI and ammonia chemical ionization (CI) mass spectra for tabun and five related compounds was followed several years later by a comprehensive paper, containing the EI and ammonia CI mass spectra of tabun and twenty related compounds found during GC-MS analysis of a munitions grade tabun sample (27). A similar munitions grade tabun sample was analysed by LC-ESI-MS using an Autospec-Q mass spectrometer (20) and more recently with a LCT time-of-flight mass spectrometer. Nineteen phosphates and pyrophosphates were characterized by ESI-MS using both higher and lower sampling cone voltages. The ESI-MS data acquired with the LCT were similar in content to the Autospec-Q data and have been incorporated into the DRDC Suffield ESI-MS Database (25).

The synthesized tabun sample used in this comparative study was initially screened by GC-MS and LC-ESI-MS for purity purposes. Tabun was not detected, but in its place were twelve related compounds, including six compounds that had not been previously characterized during prior studies (20, 26, 27) in tabun containing samples. This sample was therefore selected for a comparison of capillary column GC-MS to packed capillary LC-ESI-MS, with a secondary goal being the interpretation and inclusion of the new ESI-MS data into the DRDC Suffield ESI-MS Database.

Chromatographic Separations

Figure 1 illustrates the GC-MS and LC-ESI-MS chromatograms obtained for a 6000 to 1 dilution of the synthetic tabun sample. Twelve sample components were identified during LC-ESI-MS analysis, while only ten were detected by GC-MS. The two compounds not detected by GC-MS contained hydroxyl substitution and would only be detected by GC-MS following derivatization. Both low volatility compounds, ethyl phosphoric tetramethylphosphoro-diamidic anhydride and octmethyltetramidotriphosphoric acid were detected along with the ten more volatile phosphate and pyrophosphates during a single analysis by LC-ESI-MS analysis. Derivatization was not required, a definite advantage for LC-ESI-MS over GC-MS for the reporting of mixtures containing low volatility compounds.

LC-ESI-MS analyses typically take from 30 to 45 minutes with a 15 minute solvent equilibration between analyses. In this particular example a 30 minute gradient program was employed. GC-MS analysis times were comparable with a analysis time of 31 minutes and up to 10 minutes to recycle the GC oven between analyses. The principal advantage of GC versus LC separation was in the efficiency of separation. GC chromatographic peak widths were typically an order of magnitude narrower, making it possible to potentially resolve a greater number of sample components.

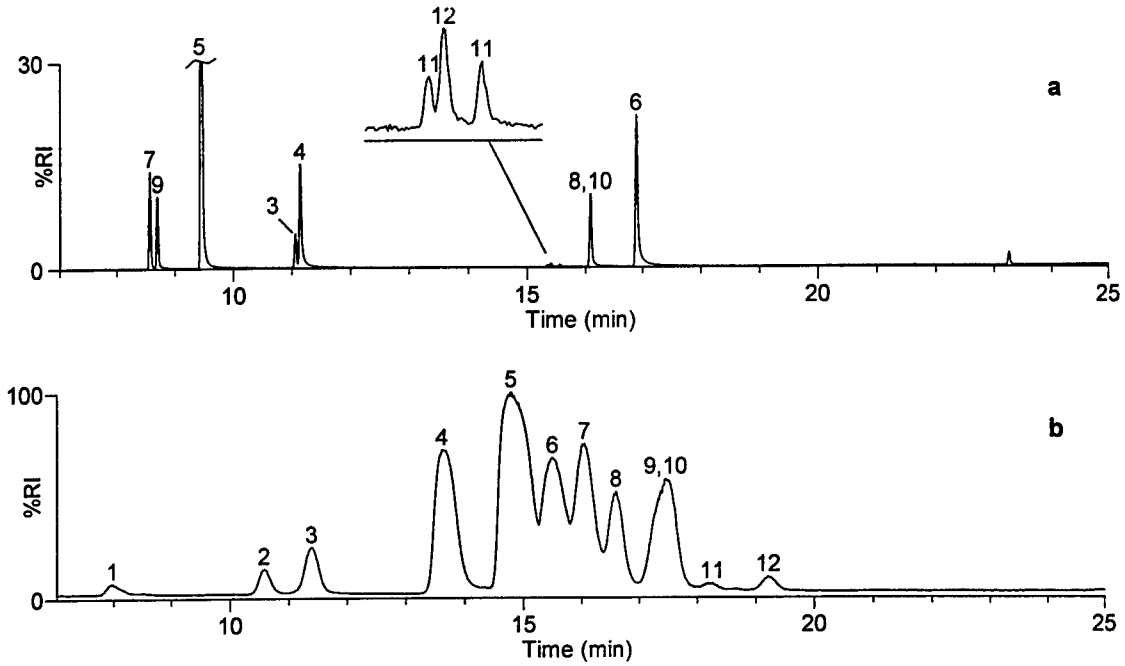


Figure 1. *Typical a) GC-MS and b) LC-ESI-MS total-ion-current chromatograms acquired for the synthetic tabun sample. Sample components are identified in Table 1.*

Mass Spectometric Data

EI-MS data acquired for the compounds observed during GC-MS analysis were consistent with EI data contained in the NIST database supplied with the Agilent 5973N data system and/or the DRDC Suffield EI Database. Six of the compounds (chromatographic peak numbers 3, 5, 7, 9, 11 and 12 listed in Table 1) had been identified during prior LC-ESI-MS analyses (20), with the remaining compounds being candidates for inclusion in the DRDC Suffield ESI-MS Database.

Table 1 lists the identities of the twelve compounds based on the interpretation of the high resolution data obtained during LC-ESI-MS analysis with sampling cone voltages of 24 volts (lower sampling cone voltage for protonated molecular ion mass determinations) and 40 volts (higher sampling cone voltage for product ion mass determinations). Protonated molecular ion mass determinations (mean ± SD) were based on five measurements while product ion mass determinations (mean ± SD) were based on three measurements. The acquired masses for the protonated molecular ion and product ions compared favorably to the theoretical values listed in Table 1, with most errors being less than 0.005 Da.

Table 1. Compounds identified in tabun synthetic sample by LC-ESI-MS.

Peak Number	Compound Name	Ion	Observed Mass (Da) (mean ± SD)	Theoretical Mass (Da)	Average Error (Da)
1	Ethyl phosphoric tetramethylphosphorodiamidic anhydride	MH^+	261.0741 ± 0.0012	261.0769	0.0028
		$[MH-C_2H_4]^+$	233.0464 ± 0.0019	233.0456	0.0008
		$[(Me_2N)_2P(OH)_2]^+$	153.0791 ± 0.0013	153.0793	0.0002
		$[(Me_2N)_2PO]^+$	135.0695 ± 0.0010	135.0687	0.0008
2	Octamethyltetramido-triphosphoric acid (or isomer)	MH^+	367.0979 ± 0.0017	367.1065	0.0086
		$[MH-HNMe_2]^+$	322.0473 ± 0.0019	322.0487	0.0014
3	Tetramethylphosphorodiamidic cyanide	MH^+	162.0979 ± 0.0017	162.0796	0.0017
4	Hexamethylphosphorotriamide	MH^+	180.1244 ± 0.0010	180.1266	0.0022
		$[(Me_2N)_2PO]^+$	135.0681 ± 0.0004	135.0687	0.0006
5	Ethyl tetramethylphosphoramidate	MH^+	181.1081 ± 0.0014	181.1106	0.0025
		$[MH-C_2H_4]^+$	153.0779 ± 0.0002	153.0793	0.0014
6	Bis(tetramethylphosphoro-diamidic) anhydride	MH^+	287.1362 ± 0.0014	287.1402	0.0040
		$[MH-HNMe_2]^+$	242.0815 ± 0.0037	242.0823	0.0008
7	Diethyl dimethylphosphoramidate	MH^+	182.0924 ± 0.0013	182.0946	0.0022
		$[MH-C_2H_4]^+$	154.0635 ± 0.0002	154.0633	0.0002
		$[MH-(C_2H_4)_2]^+$	126.0307 ± 0.0002	126.0320	0.0013
8	Ethyl dimethylphosphoramidic tetramethylphosphorodiamidic anhydride	MH^+	288.1212 ± 0.0012	288.1242	0.0030
		$[MH-HNMe_2]^+$	243.0647 ± 0.0004	243.0664	0.0017
		$[MH-HNMe_2-C_2H_4]^+$	215.0359 ± 0.0008	215.0351	0.0008
9	Triethyl phosphate	MH^+	183.0776 ± 0.0008	183.0786	0.0010
		$[MH-C_2H_4]^+$	155.0469 ± 0.0005	155.0473	0.0004
		$[MH-(C_2H_4)_2]^+$	127.0155 ± 0.0001	127.0160	0.0005
		$[MH-(C_2H_4)_3]^+$	98.9848 ± 0.0003	98.9847	0.0001
10	Diethyl phosphoric tetramethylphosphorodiamidic anhydride	MH^+	289.1049 ± 0.0011	289.1082	0.0033
		$[MH-C_2H_4]^+$	261.0790 ± 0.0006	261.0769	0.0021
		$[MH-HNMe_2]^+$	244.0554 ± 0.0020	244.0504	0.0050
		$[MH-(C_2H_4)_2]^+$	233.0489 ± 0.0007	233.0456	0.0033
		$[(Me_2N)_2P(OH)_2]^+$	153.0826 ± 0.0012	153.0793	0.0033
		$[(Me_2N)_2PO]^+$	135.0705 ± 0.0009	135.0687	0.0018
11	Bis(ethyl dimethylphosphoramidic) anhydride	MH^+	289.1064 ± 0.0011	289.1082	0.0018
		$[MH-C_2H_4]^+$	261.0753 ± 0.0015	261.0769	0.0016
		$[MH-HNMe_2]^+$	244.0517 ± 0.0021	244.0504	0.0013
		$[(Me_2N)(EtO)P(OH)_2]^+$	154.0648 ± 0.0018	154.0633	0.0015
		$[(Me_2N)P(OH)_3]^+$	126.0374 ± 0.0019	126.0320	0.0054
12	Diethyl phosphoric ethyl dimethylphosphoramidic anhydride	MH^+	290.0884 ± 0.0013	290.0922	0.0038
		$[MH-C_2H_4]^+$	262.0613 ± 0.0013	262.0609	0.0004
		$[MH-(C_2H_4)_2]^+$	234.0307 ± 0.0014	234.0296	0.0011
		$[MH-(C_2H_4)_2]^+$	206.0003 ± 0.0032	205.9983	0.0020
		$[(EtO)_2P(OH)_2]^+$	155.0500 ± 0.0006	155.0473	0.0027
		$[(EtO)P(OH)_3]^+$	127.0184 ± 0.0016	127.0160	0.0024

Figures 2 and 3 illustrate typical ESI-MS data obtained with both a higher (40 or 50 volts) and lower (20 volts) sampling cone voltage for ethyl phosphoric tetramethylphosphorodiamidic anhydride and octmethyltetramidotriphosphoric acid, respectively. A product ion due to loss of C_2H_4 from the ethoxy substituent, as well as two lower mass products at m/z 153 and m/z 135, due to loss of $O_2POC_2H_5$ and $(HO)_2(O)POC_2H_5$ from the protonated molecular ion at m/z 261, were observed for ethyl phosphoric tetramethylphosphorodiamidic anhydride. Octamethyltetramidotriphosphoric acid produced product ions at m/z 322 and m/z 215 due to loss of $HN(CH_3)_2$ and $(HO)OP[N(CH_3)]_2$ from the protonated molecular ion at m/z 367. A minor ion at m/z 153, of the same structure as that observed for ethyl phosphoric tetramethylphosphorodiamidic anhydride, was also detected.

The ESI-MS data for the phosphates in the tabun synthetic sample have been reported previously with the exception of the data for hexamethylphosphorotriamide (Figure 4). Hexamethylphosphorotriamide contained both protonated molecular (m/z 180) and diamer (m/z 359) ions that were used to confirm molecular mass as well a product ion at m/z 135 due to the loss of $HN(CH_3)_2$ from the protonated molecular ion (at a higher sampling cone voltage of 30 volts). The sampling cone voltage was increased to as high as 50 volts with no additional product ion formation.

A number of pyrophosphates were also observed in the sample. The ESI-MS data acquired for bis(tetramethylphosphorodiamidic) anhydride (Figure 5) contained a protonated molecular ion at m/z 287 with a lower sampling cone voltage (20 volts) and a number of product ions with a sampling cone voltage of 50 volts. A significant product ion at m/z 242, due to loss of $HN(CH_3)_2$ from the protonated molecular ion, and two lower mass product ions at m/z 153 and m/z 135, characteristic of an organophosphorus compound with a $[HN(CH_3)_2]_2[R]P{=}O$ substructure, were also observed (25). Similar data were also observed for ethyl dimethylphosphoramidic tetramethylphosphorodiamidic anhydride (Figure 6), with product ions at m/z 243 and m/z 215, resulting from sequential loss of $HN(CH_3)_2$ and C_2H_4 from the protonated molecular ion at m/z 288. The characteristic lower mass product ions at m/z 153 and m/z 135 (low relative intensity) confirmed the presence of an organophosphorous compound with a $[HN(CH_3)_2]_2[R]P{=}O$ substructure.

Two additional pyrophosphates with identical elemental composition, diethyl phosphoric tetramethylphosphorodiamidic anhydride (Figure 7) and bis(ethyl dimethylphosphoramidic) anhydride (Figure 8), could not be differentiated by GC-MS and exhibited identical ESI mass spectra dominated by a protonated molecular ion at m/z 289 with a sampling cone voltage of 20 volts. Both anhydrides contained two ethoxy and two dimethylamine substituents based on the higher mass product ions observed at m/z 261, m/z 244 and m/z 233. Elemental assignments for a number of the product ions observed during accurate mass measurement have been summarized in Table 1. Differentiation of the two possible isomers was possible on the basis of the characteristic lower mass ions observed with a sampling cone voltage of 40 volts. Bis(ethyl dimethylphosphoramidic) anhydride exhibited ions at m/z 126 and m/z 154 indicating the presence of both ethoxy and dimethyl amine substitution at each phosphorus atom, while the product ions at m/z 135 and m/z 153 for diethyl phosphoric tetramethylphosphorodiamidic anhydride indicated that one phosphorus contained both dimethylamine substituents.

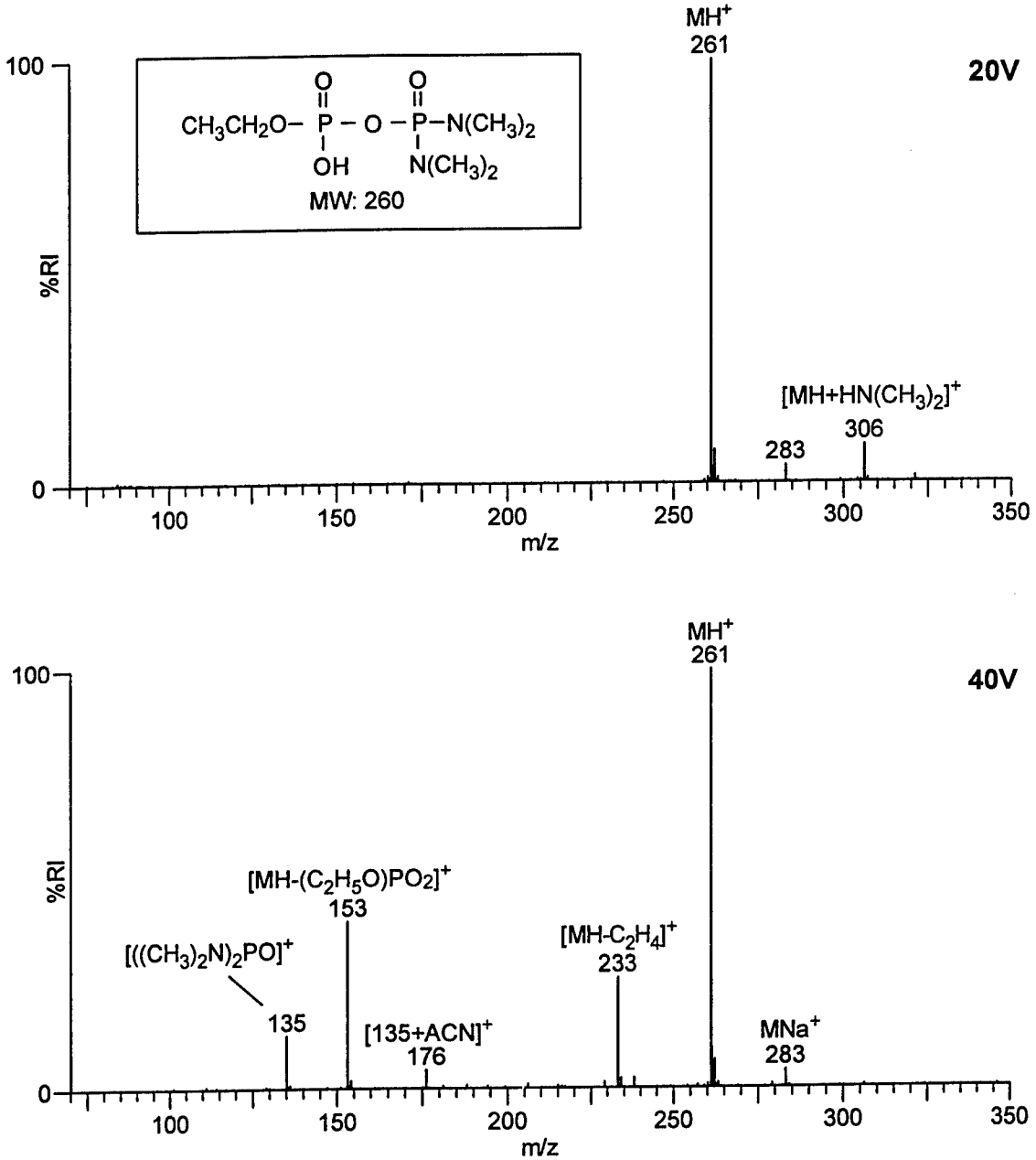


Figure 2. *ESI-MS data acquired for ethyl phosphoric tetramethylphosphorodiamidic anhydride during LC-ESI-MS analysis with sampling cone voltages of a) 20 volts and b) 40 volts.*

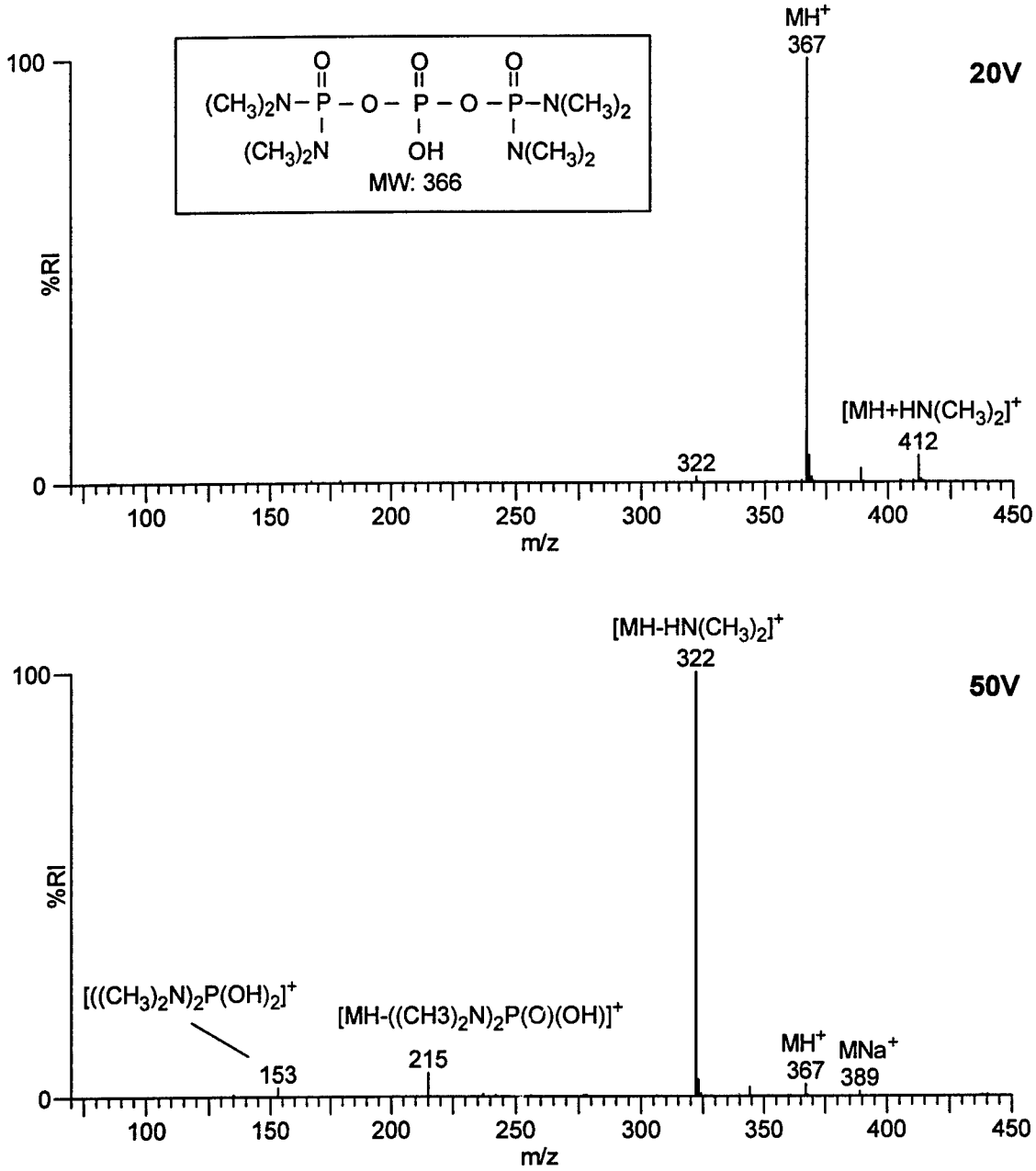


Figure 3. *ESI-MS data acquired for octamethyltetramidotriphosphoric acid (or isomer) during LC-ESI-MS analysis with sampling cone voltages of a) 20 volts and b) 50 volts.*

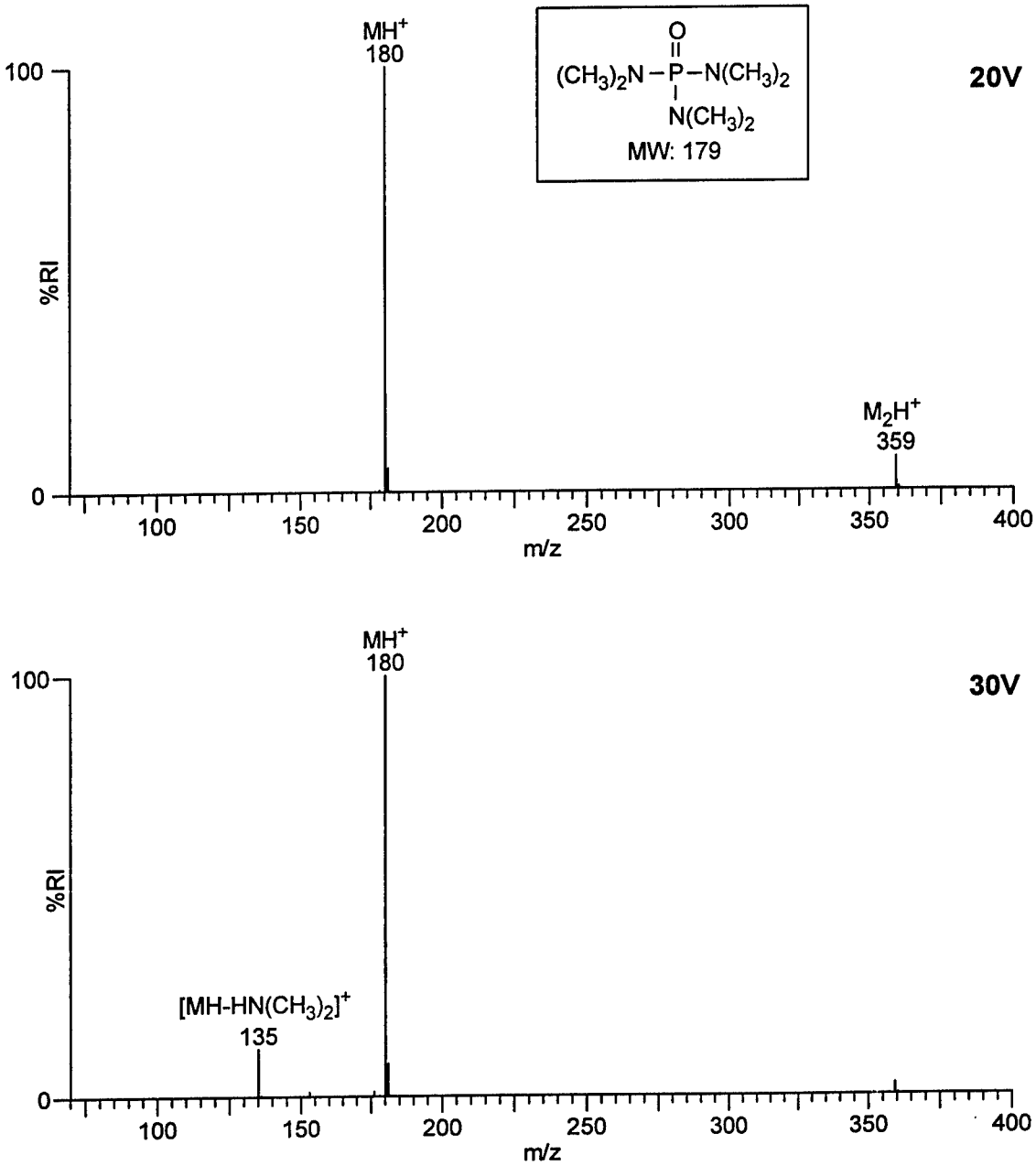
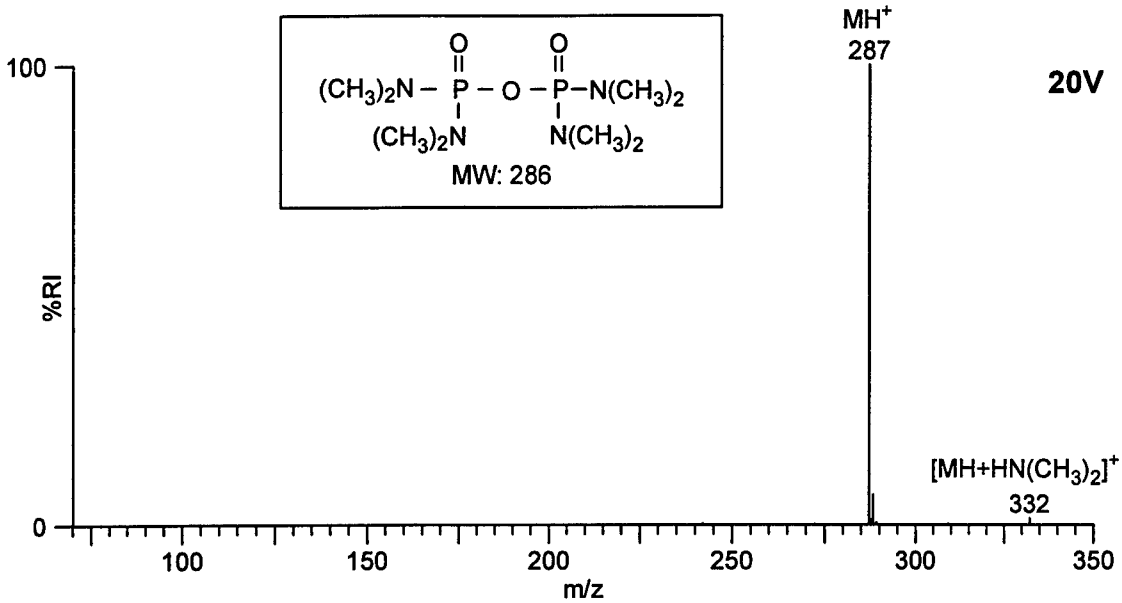


Figure 4. *ESI-MS data acquired for hexamethylphosphorotriamide during LC-ESI-MS analysis with sampling cone voltages of a) 20 volts and b) 30 volts.*



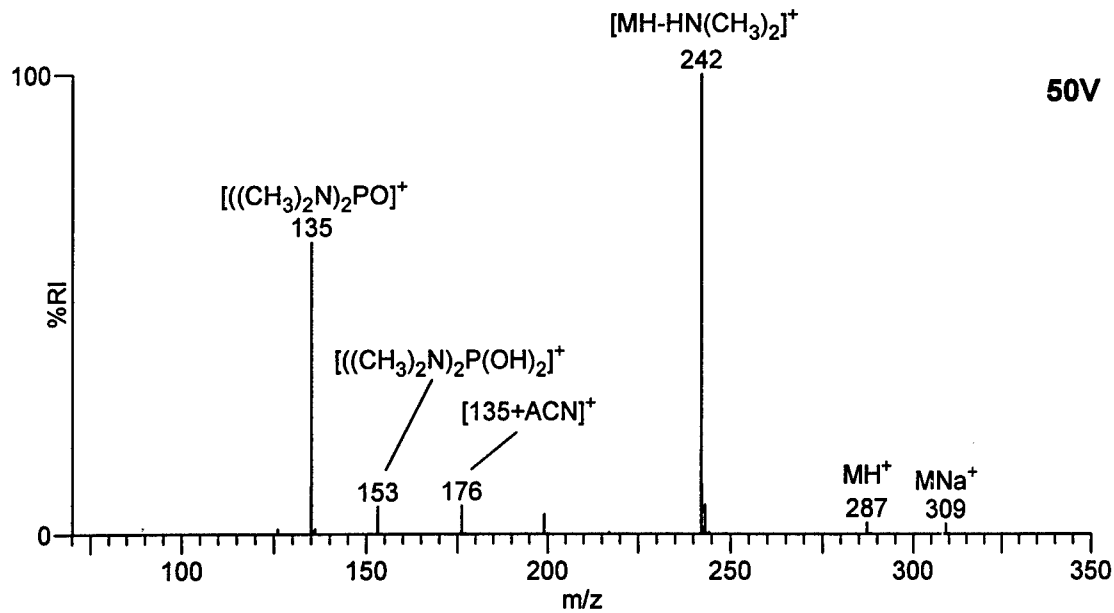


Figure 5. *ESI-MS data acquired for bis(tetramethylphosphorodiamidic) anhydride during LC-ESI-MS analysis with sampling cone voltages of a) 20 volts and b) 50 volts.*

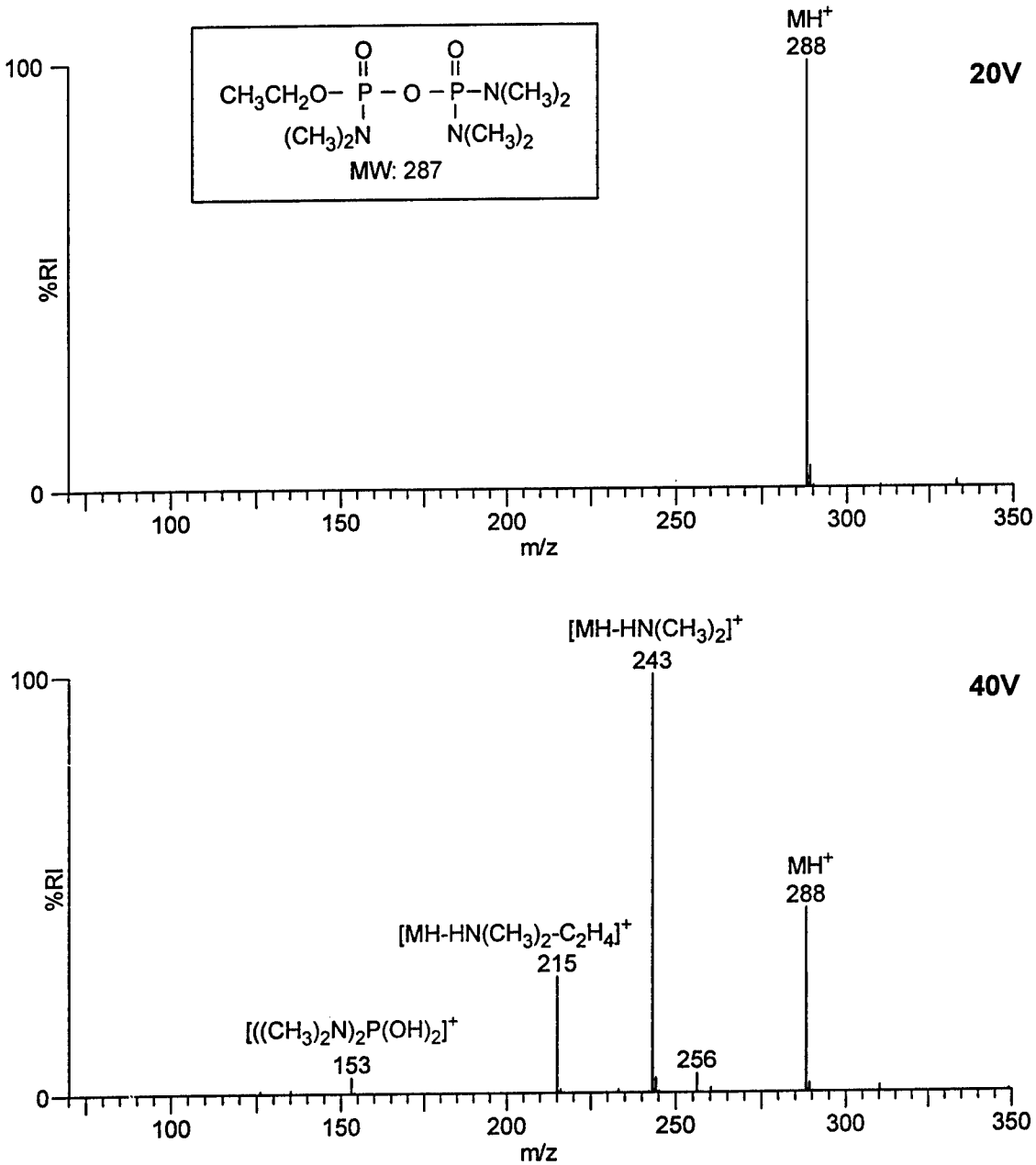


Figure 6. *ESI-MS data acquired for ethyl dimethylphosphoramidic tetramethylphosphorodiamidic anhydride during LC-ESI-MS analysis with sampling cone voltages of a) 20 volts and b) 40 volts.*

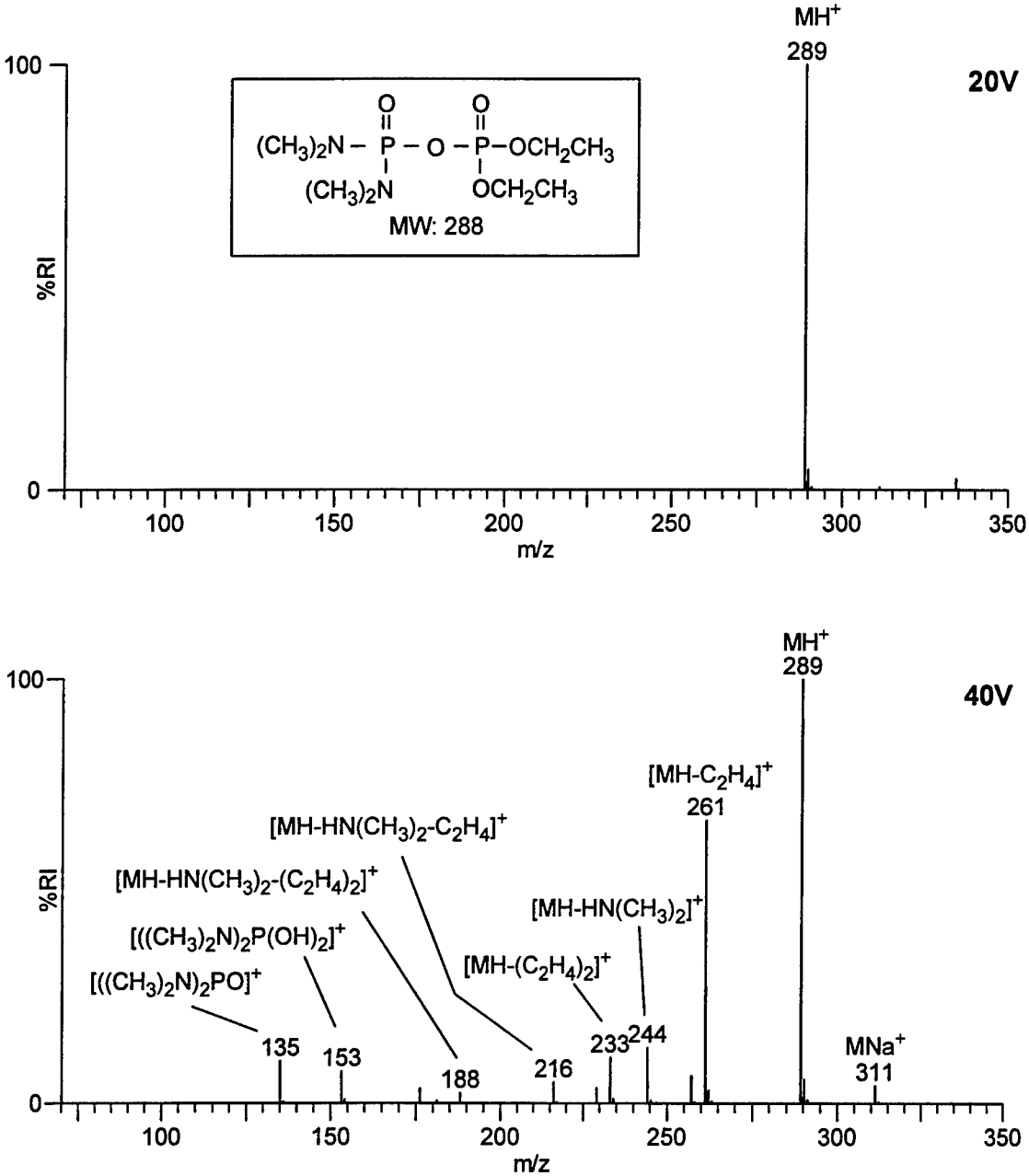


Figure 7. *ESI-MS data acquired for diethyl phosphoric tetramethylphosphorodiamidic anhydride during LC-ESI-MS analysis with sampling cone voltages of a) 20 volts and b) 40 volts.*

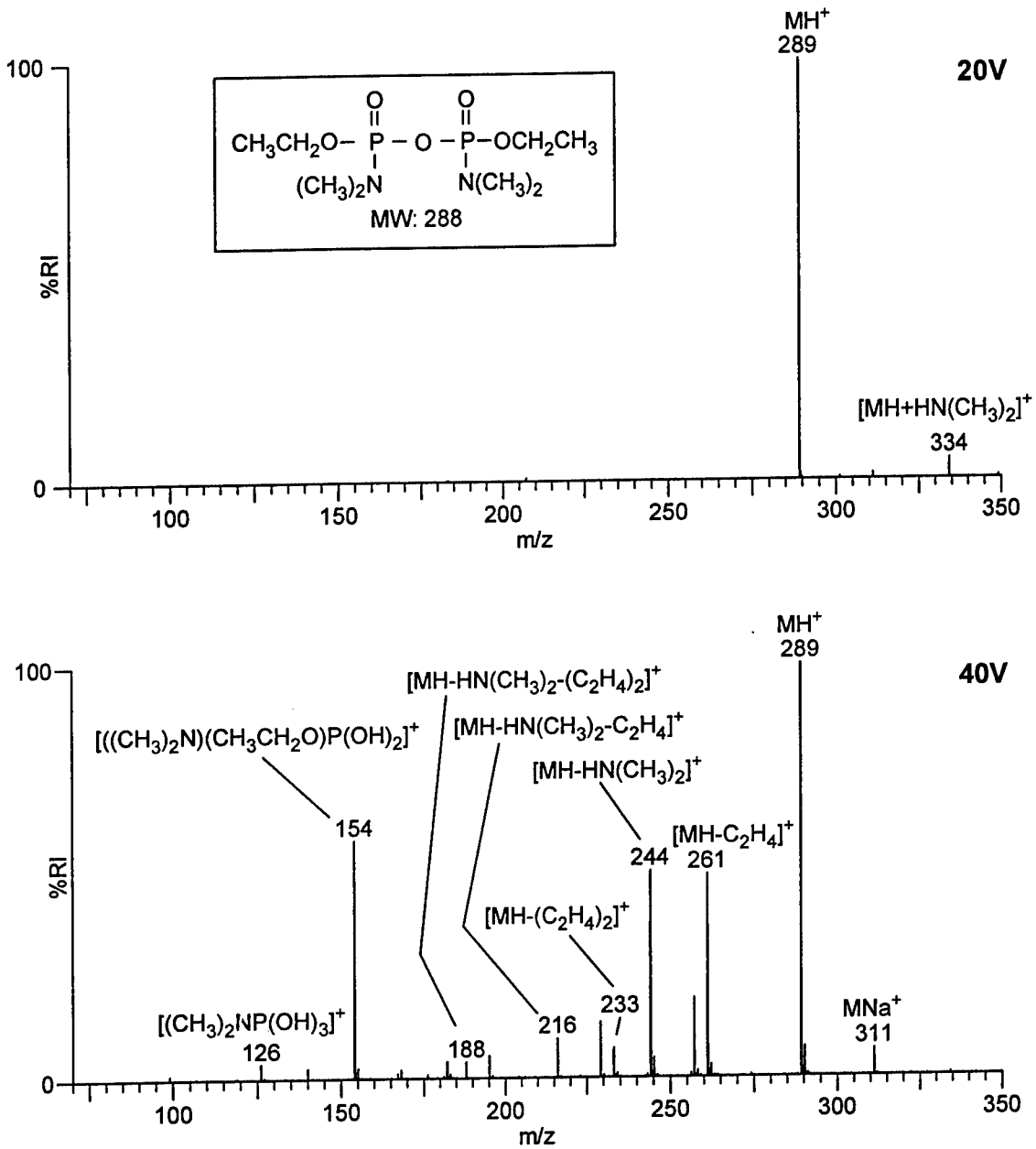


Figure 8. *ESI-MS data acquired for bis(ethyl dimethylphosphoramidic) anhydride during LC-ESI-MS analysis with sampling cone voltages of a) 20 volts and b) 40 volts.*

Sensitivity

The time-of-flight mass spectrometer instrument used in this investigation offers a significant improvement in sensitivity over the older DRES Autospec-Q instrument for the acquisition of complete electrospray mass spectra. The sensitivity of the LC-ESI-MS method has been estimated with triethyl phosphate, a compound that does not readily hydrolyse in water. A complete interpretable mass spectrum (sampling cone voltage: 20 volts) was obtained for 50 pg of TEP during LC-ESI-MS analysis. The S/N ratio for the m/z 183, $(M+H)^+$, reconstructed ion current chromatogram was approximately 25:1 (24).

The relative sensitivity of LC-ESI-MS to GC-MS were estimated in this study since the exact contribution of each sample component to the mixture used for comparison remains unknown. Relative sensitivities were compared during the acquisition of full mass spectra for the sample components in the 6000 to 1 diluted synthetic tabun samples. LC-ESI-MS (5 μL injection volume) sample sensitivity for the acquisition of a full mass spectrum was comparable to GC-MS (1 μL injection volume) for the compounds detected by both techniques. Interpretable full mass spectra and similar S/N ratios in the total-ion-current were observed for the trace sample components (e.g., peak number 11 in Table 1 and Figure 1), estimated to be present in the low nanogram or subnanogram range (based on typical ESI-MS responses), using both methods.

Conclusions

Packed capillary LC-ESI-MS and capillary column GC-MS each offer the analyst advantages for analysis of samples containing chemical warfare agents, their hydrolysis products and related compounds. Under well defined circumstances one technique may be more suitable for analysis purposes, while the analysis of samples with unknown contamination would best be tackled using both analytical techniques.

Twelve sample components were identified during packed capillary LC-ESI-MS analysis, while only ten were detected by capillary column GC-MS. The two compounds not detected by GC-MS contained hydroxyl substitution and would only be detected by GC-MS following derivatization and a second analysis. Both low volatility compounds, ethyl phosphoric tetramethylphosphorodiamidic anhydride and octmethyltetramidotriphosphoric acid, were detected along with the ten more volatile phosphate and pyrophosphates during a single analysis by LC-ESI-MS analysis. Derivatization was not required, a definite advantage for LC-ESI-MS over GC-MS for the reporting of mixtures containing chemical warfare agents, related compounds and lower volatility, hydrolysis products.

Total analysis times, including equilibration times between analyses, were similar, typically requiring about 40 to 45 minutes between analyses. Peak widths for capillary column GC-MS separations were typically an order of magnitude better than packed capillary LC-ESI-MS, offering the potential to resolve more sample components during a given analysis.

The relative sensitivity of packed capillary LC-ESI-MS to capillary column GC-MS was estimated since the contribution of each sample component to the mixture used for comparison remains unknown. LC-ESI-MS (5 μL injection volume) sample sensitivity for the acquisition of a full mass spectrum was comparable to GC-MS (1 μL injection volume) for the compounds detected by both techniques provided the analyst was not sample limited. Similar S/N ratios in the total-ion-current were observed for trace sample components, estimated to be present in the low nanogram or subnanogram range.

EI-MS data acquired for the ten tabun related compounds observed during GC-MS analysis were consistent with EI data contained in the NIST database and/or the DRDC Suffield EI Database. High resolution ESI-MS data were acquired at two different sampling cone voltages, with the lower (20 volts) voltage mass spectra being dominated by protonated molecular ions and the higher (30 to 50 volts) voltage mass spectra containing significant product ions. The ESI-MS mass spectra for previously uncharacterized tabun related compounds were included in the recently created DRDC Suffield ESI-MS Database.

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Packed capillary LC-ESI-MS and capillary column GC-MS were compared for the analysis of a synthetic tabun sample as each method has advantages for the analysis of samples containing chemical warfare agents, their hydrolysis products and related compounds. Twelve sample components were identified during LC-ESI-MS analysis of the sample, while only ten were detected by GC-MS. The two less volatile compounds not detected by GC-MS contained hydroxyl substitution and would only be detected by GC-MS following derivatization and a second GC-MS analysis. The total analysis times, including equilibration times between analyses, were similar, typically requiring about 40 to 45 minutes. Peak widths for capillary column GC-MS separations were typically an order of magnitude better than packed capillary LC-ESI-MS, offering the potential to resolve more sample components during a given analysis. The relative sensitivity of the methods was estimated since the exact contribution of each sample component to the mixture used for comparison remains unknown. Interpretable full mass spectra and similar S/N ratios in the total-ion-current were observed for the trace sample components, estimated to be present in the low nanogram or subnanogram range, using both methods.

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