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Packed capillary liquid chromatography–electrospray mass spectrometry of snow contaminated with sarin

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Abstract

Packed capillary liquid chromatography–electrospray mass spectrometry (LC–ESI–MS) was used for the analysis of a snow sample that was accidentally contaminated with an organophosphorus chemical warfare agent during the destruction of a chemical munition. Sarin, its hydrolysis products and a number of related compounds were identified on the basis of acquired LC–ESI–MS data. Full mass spectra were acquired for 14 compounds, with all exhibiting MH^+ , $[MH+ACN]^+$ ions and/or protonated dimers that could be used to confirm molecular mass. Sampling cone voltages from 20 to 70 V were utilized with the higher sampling voltages enhancing formation of structurally important product ions in the ESI interface. All data were acquired with a time-of-flight mass spectrometer with a resolution of 5000 (50% valley definition), a resolution that aided in the assignment of elemental composition of the observed ions. The application of LC–ESI–MS to snow analysis appears to be an attractive alternative to the GC–MS methods, since both chemical warfare agents and their hydrolysis products may be analysed directly, eliminating the need for additional sample handling and derivatization steps. Crown copyright © 2002 Published by Elsevier Science B.V. All rights reserved.

Keywords: Snow; Warfare agents; Environmental analysis; Sarin; Organophosphorus compounds

1. Introduction

Canada, one of more than 140 countries to ratify the Chemical Weapons Convention, complies with this treaty by maintaining only small stocks of chemical warfare agents for defensive research and development. Old chemical shells are periodically discovered worldwide on old chemical training ranges, storage sites or other locations and need to be destroyed in a safe manner. The disposal of old chemical shells in Canada usually involves perfor-

ation of the shell, resulting in a small sampling hole that can be used to access the contents for chemical warfare agent identification purposes. Recently a 1950s 105-mm chemical shell found in an old chemical training area was split apart by the perforating charge and contaminated the surrounding snow. Chemical Agent Monitor (hand-held military detection device) readings in the nerve agent mode above the snow indicated the presence of a nerve agent and a sample of the snow was collected for laboratory analysis to confirm the identity of the chemical warfare agent(s). This type of retrospective analysis, typical of those required in support of the Chemical Weapons Convention, requires the use of sensitive analytical methods that provide unambigu-

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ous proof of the presence of chemical warfare agents [1].

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 for the characterization of these compounds [1,2]. Increasingly 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 [5–17]. These ionization modes interface to liquid chromatography (LC) and capillary electrophoresis (CE) for component separation, with LC–MS [9–11,13,15–17] and CE–MS [5,12] methods being reported for the identification of low volatility chemical warfare agent hydrolysis products. Recently, several LC–ESI–MS papers were published on the identification of organophosphorus chemical warfare agents, their hydrolysis products and related compounds in aqueous samples [13,14] or aqueous extracts of contaminated soil samples [17] during a single analysis. LC–ESI–MS was an attractive alternative to GC–MS for these aqueous analyses, as both the organophosphorus chemical warfare agents and their hydrolysis products could be analysed directly without the need for additional sample handling and derivatization. A similar advantage should be possible during the analysis of contaminated snow samples.

Few reports deal with the analysis of chemical warfare agents and their hydrolysis products from snow samples. The Norwegian Defence Research Establishment conducted a number of experiments in the 1980s involving the analysis of chemical warfare agents, including sarin, soman, tabun and VX, in contaminated snow samples. Snow samples were melted, extracted with chloroform and analysed by GC–MS for the presence of chemical warfare agent(s). A second extraction and analysis by GC–MS was required to determine the presence of the chemical warfare agent hydrolysis products following derivatization with diazomethane.

This paper focuses on the development and application of packed capillary LC–ESI–MS for the identification of organophosphorus chemical warfare agents and/or their hydrolysis products observed

during analysis of a contaminated snow sample. Sarin (isopropyl methylphosphonofluoridate) and its initial hydrolysis product, isopropyl methylphosphonic acid, were identified as the principal sample components in the melted snow sample using this approach. A number of other previously uncharacterized organophosphorus compounds were also observed during LC–ESI–MS analysis, the identities of which were determined by interpreting high-resolution ESI–MS data collected at several different sampling cone voltages.

2. Experimental

A sample of the snow from near a split 105-mm chemical shell was collected for laboratory analysis to confirm the identity of the chemical warfare agent(s). The snow sample was allowed to melt and an aliquot was diluted 1:10 with distilled water prior to LC–ESI–MS analysis. The remaining snow sample was refrozen at -20°C . Sarin and isopropyl methylphosphonic acid, used for instrumental setup and reference purposes, were provided by the Canadian National Single Small Scale Facility at Defence Research Establishment Suffield.

ESI–MS data were acquired using a Micromass LCT time-of-flight (TOF) 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 70-V 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 600 to 70 u (1 s) in the continuum mode with a resolution of 5000 (50% valley definition).

LC separations were performed using an Applied Biosystems Model 140B dual syringe pump equipped with a Micro-Tech 150×0.32 mm I.D. C_{18} (Zorbax, 5 μm) packed fused-silica capillary column and a Rheodyne 8125 injector with a 5- μl sample loop. The following solvent compositions were prepared for LC separation: solvent A (0.1% trifluoroacetic acid in water) and solvent B [0.1% trifluoroacetic acid in acetonitrile (ACN)–water, 95:5]. Chromatographic separations were performed using a 1–75% B gradient program over 30 min. In order to

minimize dead volume effects and ensure reproducible mixing, the mobile phase was delivered at 200 $\mu\text{l}/\text{min}$ and split prior to the injector such that the flow through the column was 16 $\mu\text{l}/\text{min}$.

3. Results and discussion

Unexploded chemical shells, from exercises held more than 40 years ago, are periodically discovered on the Suffield Military Training Area. Disposal of chemical shells frequently involves perforation of the shell, resulting in a small sampling hole that can be used to access the contents for identification purposes. Recently an old chemical shell was accidentally split apart by the perforating charge and the contents contaminated the surrounding snow. Chemical Agent Monitor readings in the nerve agent mode above the snow indicated the presence of a nerve agent. A sample of the snow was collected for laboratory analysis to confirm the identity of the chemical warfare agent(s).

Fig. 1 illustrates the total-ion-current chromatogram acquired for the melted snow sample diluted 1:10 with distilled water. Sarin and its initial hydrolysis product, isopropyl methylphosphonic acid were identified as major components in the snow sample, based on the acquired chromatographic and ESI-MS data [14,17]. An additional 12 sample components, many of which have not been previously characterized by LC-ESI-MS, were also detected. ESI-MS data were acquired with a resolution of 5000

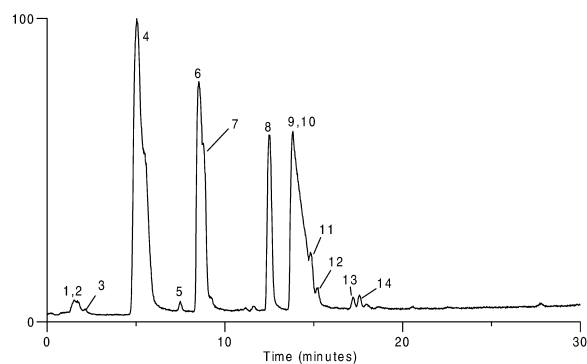


Fig. 1. LC-ESI-MS total-ion-current (600–85 u) chromatogram obtained for the snow sample with a sampling cone voltage of 20 V. Numbered peaks are identified in Table 1.

(50% valley definition), with all compounds exhibiting MH^+ , $[\text{MH}+\text{ACN}]^+$, $[\text{MH}+\text{HN}(\text{CH}_3)_2]^+$ ions and/or protonated dimers that were used to confirm molecular mass. Sampling cone voltages from 20 to 70 V were utilized with the higher sampling cone voltages enhancing formation of structurally important product ions in the ESI interface. Interpretation of all the acquired high resolution ESI-MS data resulted in the identification of 14 compounds listed in Table 1. ESI-MS characterization and identification of these additional sample components will prove valuable during future analyses, since their presence could help verify the prior presence of sarin in collected samples, indicate the synthetic procedure used for munition production or suggest the origin of the munition.

Methylphosphonofluoridic acid, methylphosphonic acid and dimethylphosphinic acid, eluted at or near the dead volume of the LC column and were identified on the basis of MH^+ data acquired using a sampling cone voltage of 20 V. MH^+ ions were observed at m/z 97.0099, m/z 99.0038 and m/z 95.0247 for the above three compounds, respectively (Table 1). Errors associated with the observed measurements were usually within 0.005 u of theoretical values, consistent with the errors typically observed during TOF-MS operation at a resolution of 5000 (50% valley definition). Phosphoric acid (theoretical mass of MH^+ ion is 98.9847 u), an organophosphorus compound with the same nominal molecular mass as methylphosphonofluoridic acid, was not considered as a possible sample component due to the large mass measurement error (0.019 u) that would be associated with this assignment. Product ions, which have been observed during prior negative ion ESI-MS-MS analysis of phosphoric acid and methylphosphonofluoridic acid [6], were not significant in this investigation.

Fig. 2 illustrates typical ESI-MS data acquired for (a) isopropyl methylphosphonic acid, (b) sarin, (c) diisopropyl methylphosphonate and (d) triisopropyl phosphate during analysis of the snow sample. All four compounds, characterized during prior LC-ESI-MS analyses of sarin samples in our laboratory, exhibited MH^+ ions and product ions due to alkene (e.g., C_3H_6) loss from the corresponding alkoxy substituent (e.g., $-\text{OC}_3\text{H}_7$). Identification was strengthened by comparing the observed masses of

Table 1
Compounds identified in the snow sample by high-resolution LC–ESI-MS (sampling cone voltage: 20 V)

Peak no. ^a	Compound name	Ion	Observed mass (u) (mean ± SD; n = 5)	Theoretical mass (u)	Average error (u)
1	Methylphosphonic acid	MH ⁺	97.0099 ± 0.0044	97.0055	0.0044
2	Methylphosphonofluoridic acid	MH ⁺	99.0038 ± 0.0007	99.0011	0.0027
3	Dimethylphosphinic acid ^b	MH ⁺	95.0247 ± 0.0014	95.0262	0.0015
4	Isopropyl methylphosphonic acid	MH ⁺	139.0455 ± 0.0019	139.0524	0.0069
		[MH–C ₃ H ₆] ⁺	97.0071 ± 0.0015	97.0055	0.0016
5	Isopropyl dimethylphosphinate ^b	MH ⁺	137.0711 ± 0.0005	137.0731	0.0020
		[MH–C ₃ H ₆] ⁺	95.0311 ± 0.0010	95.0262	0.0049
6	Sarin (isopropyl methylphosphonofluoridate)	MH ⁺	141.0444 ± 0.0021	141.0481	0.0037
		[MH–C ₃ H ₆] ⁺	99.0036 ± 0.0014	99.0011	0.0025
7	Isopropyl methyl methylphosphonate ^b	MH ⁺	153.0665 ± 0.0028	153.0681	0.0016
		[MH–C ₃ H ₆] ⁺	111.0267 ± 0.0020	111.0211	0.0056
8	Diisopropyl methylphosphonate	MH ⁺	181.1004 ± 0.0028	181.0994	0.0010
		[MH–C ₃ H ₆] ⁺	139.0547 ± 0.0019	139.0524	0.0023
9	Pentyl dibutylamine ^b	MH ⁺	200.2376 ± 0.0033	200.2378	0.0002
10	Tributylamine	MH ⁺	186.2221 ± 0.0026	186.2222	0.0001
11	Diisopropyl chloromethylphosphonate ^b	MH ⁺	215.0642 ± 0.0016	215.0604	0.0038
		[MH–C ₃ H ₆] ⁺	173.0191 ± 0.0013	173.0134	0.0057
12	Diisopropyl phosphorofluoridate ^b	MH ⁺	185.0747 ± 0.0003	185.0743	0.0004
		[MH–C ₃ H ₆] ⁺	143.0285 ± 0.0010	143.0273	0.0012
13	Triisopropyl phosphate	MH ⁺	225.1246 ± 0.0027	225.1256	0.0010
		[MH–C ₃ H ₆] ⁺	183.0814 ± 0.0005	183.0786	0.0028
14	Diisopropyl dichloromethylphosphonate ^b	MH ⁺	249.0216 ± 0.0006	249.0214	0.0002
		[MH–C ₃ H ₆] ⁺	206.9768 ± 0.0038	206.9745	0.0023

^a Refer to Fig. 1.

^b Identification based on interpretation of acquired mass spectrometric data.

the MH⁺ and [MH–C₃H₆]⁺ ions for each compound with theoretical masses (Table 1). Dimers (and a trimer for sarin) were more significant in the acquired mass spectra due to the relatively high concentration of isopropyl methylphosphonic acid, sarin and diisopropyl methylphosphonate in the snow sample. Higher mass adduct ions, due to the presence of acetonitrile and dimethylamine (possibly present in the sarin munition as a stabilizer or an impurity in the stabilizer), were also observed 41 and 45 u higher in mass than the protonated adducts (or product ions). Accurate mass measurement was used to confirm the presence of these adducts.

Additional structural information was acquired by promoting product ion formation in the ESI interface. A lower sampling cone voltage of 20 V resulted in the formation of only one significant product ion, due to loss of C₃H₆ from the MH⁺ ion, for both diisopropyl methylphosphonate and triisopropyl phosphate (Fig. 2d). Increasing the sampling cone

voltage to 30 V greatly increased product ion formation for both compounds. Product ions at *m/z* 139 and *m/z* 97 (and their acetonitrile adducts at *m/z* 180 and *m/z* 138), due to sequential loss of C₃H₆ from the MH⁺ ion, were observed for diisopropyl methylphosphonate (Fig. 2c). Similar results were also obtained at this sampling cone voltage for triisopropyl phosphate with product ions (and their acetonitrile adducts) being observed at *m/z* 183, *m/z* 141 and *m/z* 99.

Two novel chlorinated compounds, exhibiting isotopic clusters consistent with the presence of one and two chlorines, respectively, were identified as diisopropyl chloromethylphosphonate and diisopropyl dichloromethylphosphonate based on the interpretation of acquired ESI-MS data (Fig. 3). Chlorination was thought to be associated with the methyl substituent (possibly during the chlorination step in the sarin synthetic process), since both compounds exhibited sequential loss of C₃H₆ from

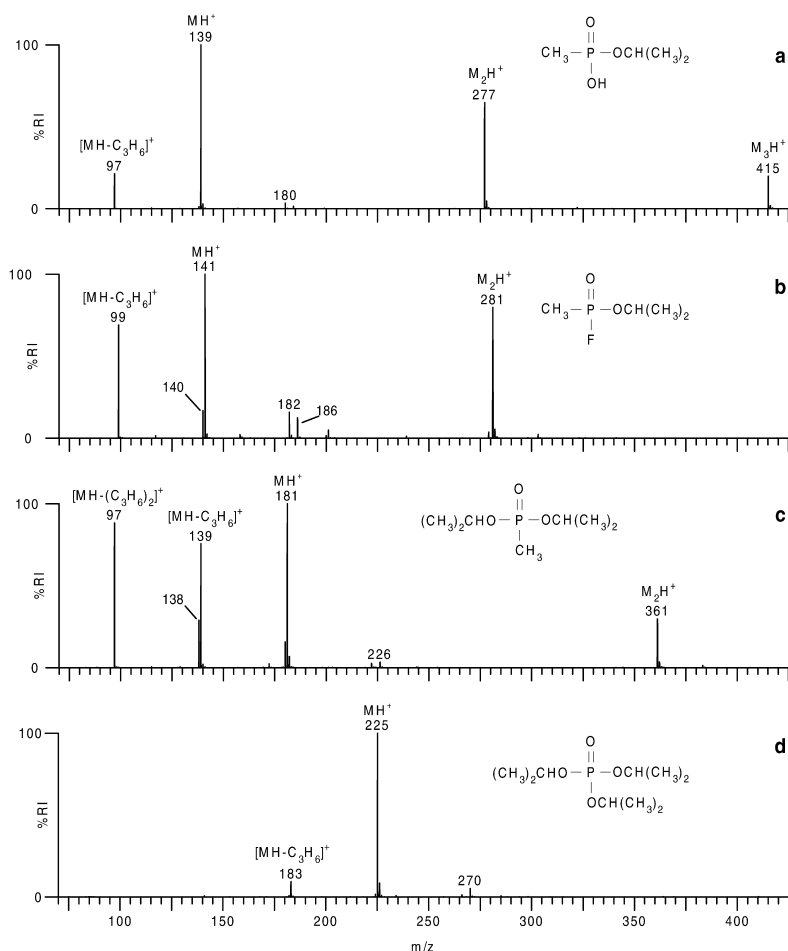


Fig. 2. ESI-MS data acquired for (a) isopropyl methylphosphonic acid (sampling cone voltage: 20 V), (b) sarin (sampling cone voltage: 20 V), (c) diisopropyl methylphosphonate (sampling cone voltage: 30 V) and (d) triisopropyl phosphate (sampling cone voltage: 20 V) during LC-ESI-MS of the melted snow sample diluted 1:10 with distilled water. RI, relative intensity.

the MH^+ ion, consistent with the presence of two isopropoxy substituents. Product ions were observed at m/z 173 and m/z 131 for diisopropyl chloromethylphosphonate and at m/z 207 and m/z 165 for diisopropyl dichloromethylphosphonate with a sampling cone voltage of 20 V. An increase in the relative intensity of the product ions was observed with a sampling cone voltage of 30 V, but the acquired data were complicated by a corresponding increase in acetonitrile adduct formation. The identification of both these compounds was strengthened by the high resolution data acquired for the MH^+ and $[MH-C_3H_6]^+$ ions for both compounds (Table 1), with the observed mass of the $[MH-C_3H_6]^+$ ion

for diisopropyl chloromethylphosphonate being mass deficient, as predicted theoretically.

Fig. 4 illustrates the ESI-MS data acquired for three additional organophosphorus compounds identified following interpretation of the acquired ESI-MS data. Molecular masses for all three compounds were established by the presence of MH^+ , $[MH+ACN]^+$, $[MH+HN(CH_3)_2]^+$ ions and/or protonated dimers. Product ions due to loss of C_3H_6 were observed at m/z 95 and m/z 111 for isopropyl dimethylphosphinate (Fig. 4a) and isopropyl methylphosphonate (Fig. 4b), respectively, indicating the presence of isopropoxy substitution for both compounds. Sequential losses of C_3H_6 from the

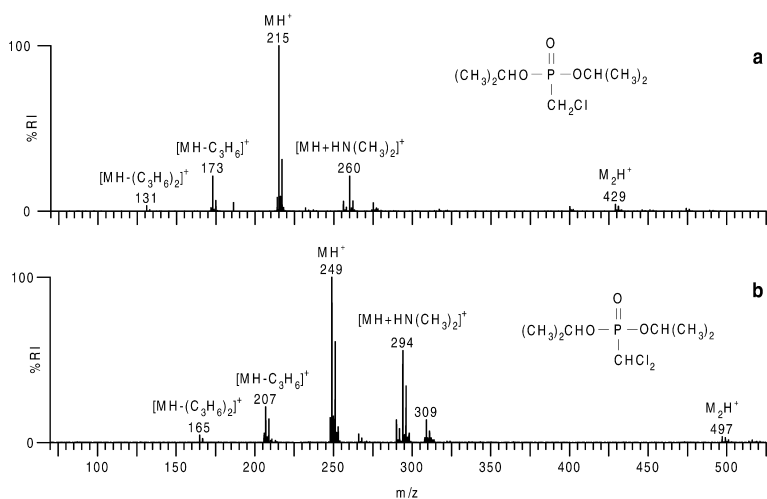


Fig. 3. ESI-MS data acquired for (a) diisopropyl chloromethylphosphonate (sampling cone voltage: 20 V) and (b) diisopropyl dichloromethylphosphonate (sampling cone voltage: 20 V) during LC-ESI-MS of the melted snow sample diluted 1:10 with distilled water.

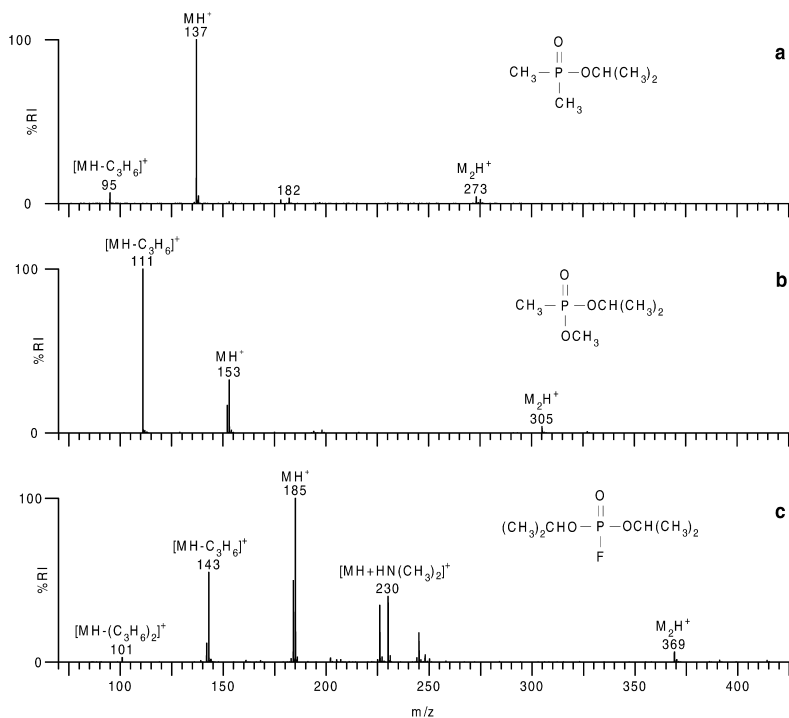


Fig. 4. ESI-MS data acquired for (a) isopropyl dimethylphosphinate (sampling cone voltage: 30 V), (b) isopropyl methyl methylphosphonate (sampling cone voltage: 20 V) and (c) diisopropyl phosphorofluoridate (sampling cone voltage: 20 V) during LC-ESI-MS of the melted snow sample diluted 1:10 with distilled water.

MH^+ ion were observed at m/z 143 and m/z 101 for diisopropyl phosphorofluoridate (Fig. 4c), suggesting the presence of a compound with two isopropoxy substituents. Finally, the assignment of the structures illustrated in Fig. 4 were consistent with the observed masses for the MH^+ and $[MH-C_3H_6]^+$ ions for all three organophosphorus compounds listed in Table 1.

A broad chromatographic component was observed just before 15 min in the LC–ESI–MS chromatogram (Fig. 1). Two components, containing an odd number of nitrogen atoms, were detected with molecular masses of 199 and 185 based on the observed MH^+ ions (sampling cone voltages ranging from 20 to 70 V). Acetonitrile adducts, generally observed for sarin, soman, tabun and related organophosphorus compounds [14,17] were not observed, suggesting the presence of two more basic sample components. The two compounds did not produce significant product ions until the sampling cone voltage was raised to 50 V, with the most structurally informative mass spectra being acquired with a sampling cone voltage of 70 V. This setting was much higher than the 30 V setting that produced significant product ions for sarin and related organophosphorus compounds. Product ions resulting from the loss of C_4H_8 suggested the two more basic compounds contained butyl substitution. Tributylamine, a stabilizer used in sarin munitions, and

a possible impurity, pentyl dibutylamine, were suspected based on this evidence. Comparison of the observed masses for the MH^+ and $[MH-C_4H_8]^+$ ions for both amines with theoretical values was consistent with this identification (Table 1), suggesting the presence of these two stabilizers in the sample (Fig. 5).

4. Conclusions

Packed capillary LC–ESI–MS was demonstrated for the analysis of organophosphorus chemical warfare agents and their hydrolysis products in a contaminated snow sample. Sarin, its hydrolysis products and a number of previously uncharacterized related compounds were identified on the basis of acquired LC–ESI–MS data. Full mass spectra were acquired for 14 compounds detected during LC–ESI–MS analysis, with all compounds exhibiting MH^+ , $[MH+ACN]^+$ ions and/or protonated dimers that could be used to confirm molecular mass. Sampling cone voltages from 20 to 70 V were utilized with the higher sampling voltages enhancing formation of structurally important product ions in the ESI interface. All data were acquired with a time-of-flight mass spectrometer with a resolution of 5000 (50% valley definition), a resolution that aided in the assignment of elemental composition.

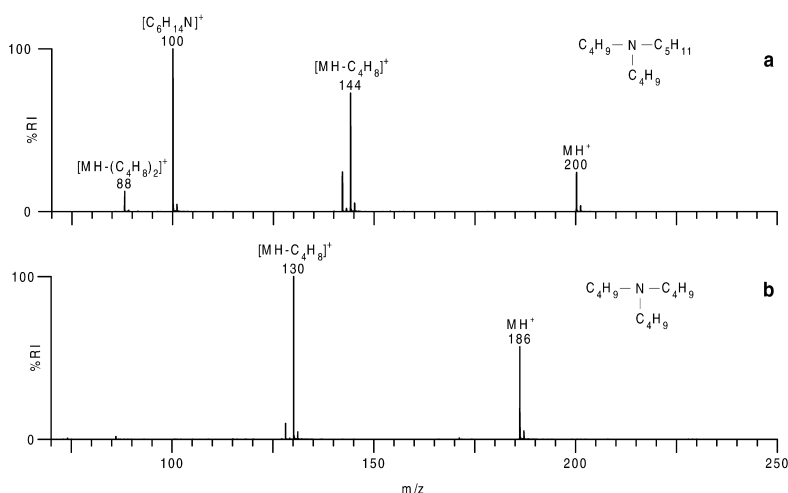


Fig. 5. ESI–MS data acquired for (a) pentyl dibutylamine (sampling cone voltage: 70 V) and (b) tributylamine (sampling cone voltage: 70 V) during LC–ESI–MS of the melted snow sample diluted 1:10 with distilled water.

The application of LC–ESI–MS to snow analysis appears to be an attractive alternative to the GC–MS methods. Melted snow samples containing both chemical warfare agents and their hydrolysis products may be analysed directly during a single analysis, eliminating the need for the additional sample handling and derivatization steps associated with GC–MS analyses. Application of the acquired ESI–MS data in support of the Chemical Weapons Convention is anticipated, since the presence of these compounds in collected samples could help verify the prior presence of sarin, indicate the synthetic procedure used for munition production or suggest the origin of the munition.

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