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Analysis of *O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothiolate (VX) and its degradation products by packed capillary liquid chromatography–electrospray mass spectrometry

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Abstract

Packed capillary column liquid chromatography (LC)–electrospray mass spectrometry (ESI–MS) was used for the first time to detect and identify *O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothiolate (VX) and its degradation products, including compounds containing a P-CH_3 bond, bis(diisopropylamino)thioalkanes and ureas commonly employed as VX stabilizers. The reported ESI–MS data were generally acquired with a higher sampling cone voltage, a setting that promoted collisionally activated dissociation, and resulted in the acquisition of informative mass spectra containing both molecular and product ion information. The developed method appears to be an attractive alternative to GC–MS for the analysis of aqueous samples containing the degradation products of VX, since they may be analysed directly with little risk of thermal decomposition and without the need for additional sample handling or derivatization. Application of this method to a degraded VX sample resulted in the detection of a number of novel polar and higher-molecular-mass degradation products, not previously associated with VX during GC–MS analysis.

Keywords: Warfare agents; Methylphosphonothiolate; Phosphonic acids

1. Introduction

The Chemical Weapons Convention entered into force just over a year ago, effectively banning the production, stockpiling and use of chemical weapons by all signatory nations. A strong, compliance monitoring regime involving site inspections was built into the convention to ensure a verifiable treaty. Routine inspections have or will take place at declared sites, including small scale production, storage and destruction sites, and challenge inspections will take place at sites suspected of non-compliance. An analytical capability will be required to verify compliance with the convention, since inspectors will have the option to procure and analyse suspect samples to help establish compliance or non-compliance. Ongoing development of new, specific methods [1] for the detection and identification of chemical warfare agents, their degradation products and related compounds would benefit the inspectorate, as an improved analytical capability

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could act as an additional deterent to non-compliance.

Gas chromatography (GC) has been used extensively for the separation of chemical warfare agents [1], including *O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothiolate (VX), and VX degradation products [2,3] in rat plasma [4], human serum [5] and decontamination solutions [6]. This separation technique while suitable for VX and many of its related degradation products, cannot be used for the identification of the nonvolatile acid hydrolysis products of VX without prior derivatization [7]. Increasingly, researchers have been developing liquid chromatographic (LC) [8,9] and capillary electrophoretic (CE) [10–15] separation methods to deal with the analysis of samples containing these nonvolatile hydrolysis products. Use of these separation techniques offer several benefits over GC analysis, including reduced sample handling and no requirement for derivatization.

Past experience during the analysis of chemical warfare agent samples [2,16,17], including VX, has indicated that samples may contain a significant number of other compounds related to degradation of the original agent or the synthetic procedures used. In some cases the sample may have undergone sufficient weathering such that the intact chemical warfare agent was no longer present. In these situations, the detection and identification of other chemical warfare agent related compounds would augment the detection of acid hydrolysis products, and greatly strengthen the argument for prior VX presence. Mass spectrometry (MS), and in particular GC–MS, has been used most frequently for the detection and identification of these additional compounds.

GC–MS under both electron impact (EI) and chemical ionization (CI) conditions have been used for the mass spectrometric characterization of VX and VX related compounds [2,3,18]. Under EI conditions the mass spectra acquired for many VX related compounds were remarkably similar. Acquired EI data generally did not contain a molecular ion and were dominated by a base ion at m/z 114 due to $[CH_2N(iPr)_2]^+$ and ions related to the $-SC_2H_4N(iPr)_2$ group. Molecular ion information, critical for the confirmation of these compounds, were only obtained following ammonia or methane CI–MS analysis. The acid hydrolysis products related to VX, ethyl hydrogen methylphosphonate (ethyl methylphosphonic acid) and dihydrogen methylphosphonate (methylphosphonic acid), cannot be analysed directly and required derivatization [3,7] to enhance their volatility for GC–MS analysis.

Use of thermospray mass spectrometry [19–22] and more recently the atmospheric pressure ionization (e.g., electrospray, ionspray and atmospheric pressure CI) techniques [23–28] has enabled the direct mass spectrometric analysis of the acid hydrolysis products of VX and those of other organophosphorous chemical warfare agents. Both techniques may be interfaced to LC or CE for component separation, with thermospray having been largely superceeded by atmospheric pressure ionization (API) for most applications. Most recent API–MS papers have focussed on the analysis of the acid hydrolysis products of organophosphorus chemical warfare agents, as well as the principal degradation products (e.g., thiodiglycol) resulting from hydrolysis of other chemical warfare agents scheduled under the Chemical Weapons Convention. Electrospray MS (ESI–MS), the most sensitive technique for these applications [28], has not been used for the analysis of organophosphorus chemical warfare agents or the numerous related compounds commonly found in samples. This paper focuses on the development of a LC–ESI–MS method for the separation and characterization of VX and the additional compounds commonly associated with degraded VX.

A sample of VX, known to contain numerous degradation products, as well as relatively pure samples of VX, were selected for detailed characterization by packed capillary LC–ESI–MS. ESI–MS provided ample molecular ion information and structurally important product ion information were generated by promoting collisionally activated dissociation (CAD) in the ESI interface. The ESI–MS data generated during characterization of VX and its degradation products would be valuable during chemical weapons destruction monitoring by countries in compliance with the Chemical Weapons Convention, for the verification of these compounds in samples collected during challenge inspections of suspect production facilities or, in support of allegations of chemical warfare agent use.

2. Experimental

2.1. Samples

A degraded sample of VX was obtained from a glass container that had been in storage for approximately 15 years. Preliminary analysis of the sample by GC–MS indicated that VX accounted for about 10% of the volatile organic content. This sample was dissolved in water to a concentration of 1 mg/ml prior to LC–ESI–MS analysis. A second, relatively pure sample of VX, synthesized locally, was dissolved in isopropanol at 0.05 mg/ml and analysed directly by ESI–MS. The *N,N′*-dicyclohexylurea standard was obtained from Aldrich (Milwaukee, WI, USA).

2.2. Instrumental

All electrospray mass spectra were acquired using a Micromass Autospec-Q tandem mass spectrometer (Manchester, UK) equipped with the Mark II electrospray interface. The electrospray needle was operated at 7.6 kV and ions were accelerated into the mass spectrometer at 4 kV. Sampling cone voltages of 50 or 100 V were utilized. Nitrogen (Very Dry, Liquid Carbonic, Scarborough, Canada) bath gas was introduced into the interface (80°C) at a flow-rate of 400 l/h. Nitrogen nebulizer gas was introduced at a flow-rate of 14 l/h. The electrospray interface was pumped with both a rotary and a turbomolecular pump, which enabled maintenance of a $4 \cdot 10^{-4}$ and $7 \cdot 10^{-6}$ Pa within the source and analyzer regions of the instrument, respectively. LC–ESI–MS data were acquired in the continuum mode by scanning the magnetic sector from 500 to 50 u (6 s/decade) or 600 to 100 u (7 s/decade) with a resolution of 1000 (10% valley definition). Two to three scans were typically averaged to enhance the signal-to-noise ratio.

All LC separations were performed with an Applied Biosystems Model 140B dual syringe pump (Foster City, CA, USA) equipped with a Zorbax 150×0.32 mm I.D. C_{18} SB (5 μm) packed fused-silica capillary column and a Rheodyne 8125 (Cotati, CA, USA) injector with a 5-μl sample loop. The following solvent compositions were prepared for sample introduction: 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 over 30 min. In order to minimize dead volume effects and ensure reproducible mixing, the mobile phase was delivered at 200 μl/min and split prior to the injector such that the flow through the column was 5 μl/min.

3. Results and discussion

GC–MS has been used for the detection and identification of VX and a number of related degradation products [2], but this approach does not allow for direct analysis of relatively nonvolatile compounds, including the acids formed on hydrolysis. The development and demonstration of a complementary LC–MS method would be advantageous for the analysis of, in particular, aqueous samples containing these compounds. LC–ESI–MS methods have been recently demonstrated for a series of alkyl methylphosphonic acid standards [28] and for the analysis of degradation products related to the hydrolysis of munitions grade mustard [29] and tabun [30]. Packed capillary LC columns with an internal diameter of 0.32 mm were selected for the mustard and tabun analyses since the 5 μl/min flow-rate typically used during chromatographic separation with these columns approaches the lower flow-rate limit for spraying in the ESI interface used. Optimal sensitivity resulted at this flow-rate limit due to the concentration dependence of MS detection. Chromatographic separation of many of the VX sample components was achieved using a 1–75% B gradient over 30 min. Fig. 1 illustrates a typical LC–ESI–MS total-ion-current chromatogram obtained for the degraded VX sample with a sampling cone voltage of 100 V. The molecular masses of 38 sample components were established from the acquired ESI–MS data, with two thirds of those compounds being identified, or tentatively identified, after interpretation of the ESI–MS data. Table 1 lists the compounds identified and indicates those that have been previously detected in VX samples by GC–MS.

The compounds identified in the degraded VX sample fall into two general categories, those still containing phosphorous and the P-CH_3 bond, and

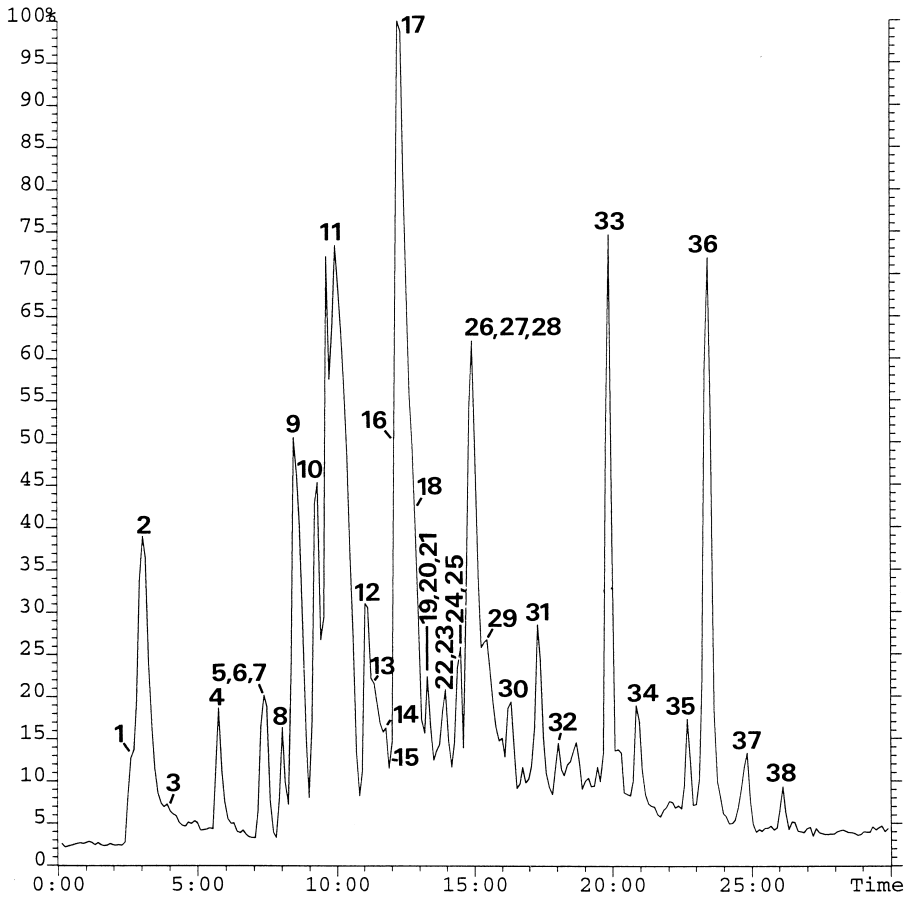


Fig. 1. Packed capillary LC–ESI–MS total-ion-current (600 to 100 u) chromatogram (sampling cone voltage: 100 V) obtained for a degraded VX sample. (Numbered peaks are identified in Table 1 and the *x*-axis is in minutes).

those that do not contain phosphorus but exhibit long chain bis(diisopropylamino)thiaalkane structures. Several ureas, compounds used to stabilize VX, were also detected. All the ESI–MS spectra acquired exhibited protonated adduct ions and in some cases dimers and trimers that could be used to establish molecular mass. Structural information was obtained from the product ions formed by increasing the sampling cone voltage in the ESI interface. Sulfur content for molecular and product ions was estimated by the observed M to M+2 ratios in Figs. 2–8. Doubly charged isotopic clusters were also observed for the longer chain bis(diisopropylamino)thiaalkanes, which were capable of stabilizing the additional charge at lower sampling cone voltages.

3.1. ESI–MS of P-CH_3 containing compounds

A number of hydroxy substituted organophosphorus degradation products, containing a P-CH_3 bond, were detected and identified in the degraded VX sample during LC–ESI–MS analysis. These compounds eluted in the first third of the chromatogram illustrated in Fig. 1, consistent with the more polar nature of compounds containing a hydroxyl group. Fig. 2 illustrates ESI–MS data obtained for two non-sulfur containing degradation products, ethyl hydrogen dimethylpyrophosphonate and ethyl hydrogen methylphosphonate, that may have formed following hydrolysis of diethyl methylpyrophosphonate and VX, respectively. The molecular mass of ethyl

Table 1
Compounds identified in degraded VX sample

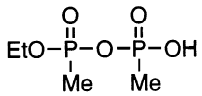
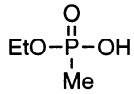
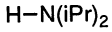
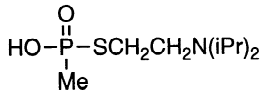
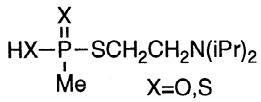
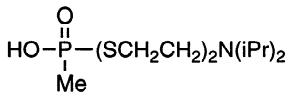
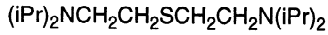
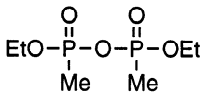
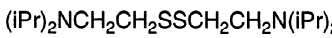
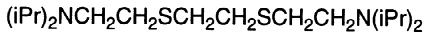
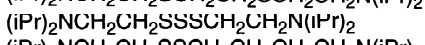
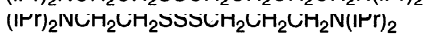
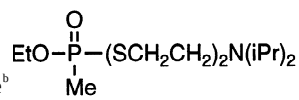
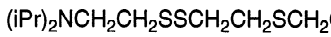
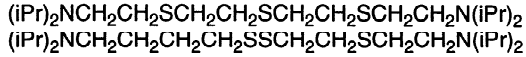
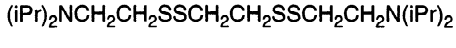
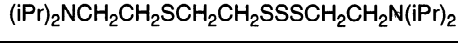
Peak no.[a]	M_r	Compound name/structure	
1	202	Ethyl hydrogen dimethylpyrophosphonate[c]	
2	124	Ethyl hydrogen methylphosphonate[b]	
3	101	Diisopropylamine[b]	
4	255	*S*-[2-(Diisopropylamino)ethyl] methylphosphonothiolate[c]	
5	255	*S*-[2-(Diisopropylamino)ethyl] methylphosphonodithioate[c]	
6	235	Unknown	
7	304	Unknown	
8	299	*S*-[5-(Diisopropylamino)-3-thiapentyl] methylphosphonothiolate[c]	
9	288	Bis[2-(diisopropylamino)ethyl] sulfide[b]	
10	230	Diethyl dimethylpyrophosphonate[b]	
11	320	Bis[2-(diisopropylamino)ethyl] disulfide[b]	
12	348	1,8-Bis(diisopropylamino)-3,6-dithiaoctane[b]	
13	352	Bis[2-(diisopropylamino)ethyl] trisulfide[c]	
14	348	1,8-Bis(diisopropylamino)-3,4-dithiaoctane[c]	
15	366	1,8-Bis(diisopropylamino)-3,4,5-trithiaoctane[c]	
16	233	Unknown	
17	327	*O*-Ethyl *S*-[5-(diisopropylamino)-3-thiapentyl] methylphosphonothiolate[b]	
18	380	1,9-Bis(diisopropylamino)-3,4,7-trithianonane[b]	
19	374	Unknown	
20	408	1,11-Bis(diisopropylamino)-3,6,9-trithiaundecane[c]	
21	408	1,11-Bis(diisopropylamino)-3,6,7-trithiaundecane[c]	
22	306	Unknown	
23	412	1,10-Bis(diisopropylamino)-3,4,7,8-tetrathiadecane[c]	
24	458	Unknown	
25	412	1,10-Bis(diisopropylamino)-3,4,5,8-tetrathiadecane[c]	

Table 1. Continued

Peak no.[a]	M_r	Compound name/structure
26	283	*S*- or *O*-Ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonodithioate[b]
27	387	*O*-Ethyl *S*-[8-9diisopropylamino)-3,6-dithiaoctyl] methylphosphonothiolate[b]
28	440	1,12-Bis(diisopropylamino)-3,6,7,10-tetrathiadodecane[b]
29	387	*O*-Ethyl *S*-[8-(diisopropylamino)-5,6-dithiaoctyl] methylphosphonothiolate[c]
30	372	Unknown
31	427	Unknown
32	343	*S*- or *O*-Ethyl *S*-[5-(diisopropylamino)-3-thiapentyl] methylphosphonodithioate[c]
33	224	*N,N′*-Dicyclohexylurea[b]
34	268	Unknown
35	291	Unknown
36	240	*N,N′*-Dicyclohexylthiourea[b]
37	307	Unknown
38	305	Unknown

[a] Refer to Fig. 1.

[b] Identification based on ESI–MS data and LC retention behaviour. Compound has been previously analysed by GC–MS (EI–CI) in VX samples at DRES.

[c] Tentative identification based on ESI–MS data and LC retention behaviour. Another isomer may be possible.

hydrogen dimethylpyrophosphonate was clearly established by the presence of $(M_2Na)^+$, $(M_2H)^+$, $(MNa)^+$ and $(MH)^+$ ions at, *m/z* 427, 405, 225, and 203, respectively (Fig. 2a). Product ions at *m/z* 216 and 175 were likely due to loss of C_2H_4 from $(MH+ACN)^+$ and $(MH)^+$ ions, indicative of ethoxy substitution, while the product ion at *m/z* 281 was likely due to $(M_2H\text{-}CH_3P(O)(OH)OEt)^+$. The molecular mass of ethyl hydrogen methylphosphonate (Fig. 2b) was established by the presence of ions at *m/z* 373, 271, 249, 166, 143, and 125 due to $(M_3H)^+$, $(M_2Na)^+$, $(M_2H)^+$, $(MH+ACN)^+$, $(MH+H_2O)^+$ and $(MH)^+$, respectively. Product ions at *m/z* 327, 138 and 115, due to $(M_3H\text{-}C_2H_6O)^+$, $(MH+ACN\text{-}C_2H_4)^+$ and $(MH+H_2O\text{-}C_2H_4)^+$, respectively, were consistent with ethoxy substitution. The data acquired for ethyl hydrogen methylphosphonate were similar to that reported by Black and Read during LC–ESI–MS analysis of a standard [28].

Figs. 3a, 3b and 4a illustrate ESI–MS data obtained for several hydrolysis products containing a $(SCH_2CH_2)_nN(iPr)_2$ (where $n=1$ or 2) substituent. M to M+2 ratios for these compounds was consistent with the sulfur content presented in the structures. Fig. 3a illustrates the ESI–MS data obtained for *S*-[2-(diisopropylamino)ethyl] methylphosphonothiolate, a compound characterized by ions at *m/z* 240, 198, 162, 139 and 128 due to

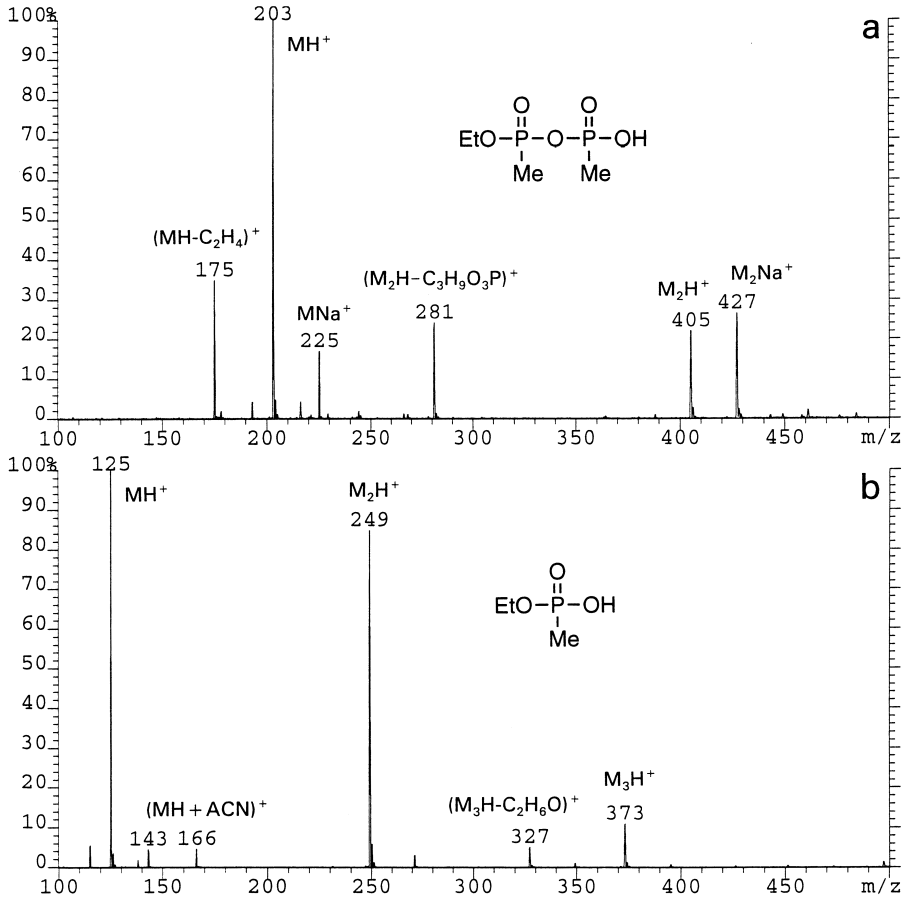


Fig. 2. ESI–MS mass spectra (sampling cone voltage: 50 V) acquired for (a) ethyl hydrogen dimethylpyrophosphonate (peak 1 in Fig. 1) and (b) ethyl hydrogen methylphosphonate (peak 2 in Fig. 1) identified during analysis of a degraded VX sample.

$(MH)^+$, $(MH\text{-}C_3H_6)^+$, $(MH\text{-}CH_3PO_2)^+$, $(MH\text{-}HN(iPr)_2)^+$ and $(CH_2CHNH(iPr)_2)^+$, respectively. Similar fragmentation was observed for *S*-[2-(diisopropylamino)ethyl] methylphosphonodithioate, the sulfur containing analogue of *S*-[2-(diisopropylamino)ethyl] methylphosphonothiolate. The exact position of the sulfur in the structure could not be determined as the fragmentation was consistent with either HS-P=O or HO-P=S (Fig. 3b). Ions at *m/z* 256, 214, 162, 155 and 128 were due to $(MH)^+$, $(MH\text{-}C_3H_6)^+$, $(MH\text{-}CH_3POS)^+$, $[MH\text{-}HN(iPr)_2]^+$ and $[CH_2CHNH(iPr)_2]^+$, respectively. The ion at *m/z* 111 may be due to a co-eluting component.

S-[5-(diisopropylamino)-3-thiapentyl] methylphosphonothiolate (Fig. 4a), a likely hydrolysis product of *O*-ethyl *S*-[5-(diisopropylamino)-3-thiapentyl] methylphosphonothiolate (Fig. 4b), exhibited a significant $(M+H)^+$ ion at *m/z* 300 and structurally significant product ions. Products ions at *m/z* 222, 199, 180, 171 and 139 due to $(MH\text{-}CH_3PO_2)^+$, $[MH\text{-}HN(iPr)_2]^+$, $(MH\text{-}CH_3PO_2\text{-}C_3H_6)^+$, $[MH\text{-}EtN(iPr)_2]^+$, $[MH\text{-}HSC_2H_4N(iPr)_2]^+$, respectively, were observed for [5-(diisopropylamino)-3-thiapentyl] methylphosphonothiolate. *O*-Ethyl *S*-[5-(diisopropylamino)-3-thiapentyl] methylphosphonothiolate exhibited a similar series of ions due to $(MH)^+$, $[MH\text{-}HN(iPr)_2]^+$, $[MH\text{-}EtN(iPr)_2]^+$ and $[MH\text{-}HSC_2H_4N(iPr)_2]^+$ at *m/z* 328, 227, 199 and 167, respectively. Four additional related organophosphorus compounds with longer chain bis(diisopropylamino)thiaalkane substituents were also observed (peak numbers 26, 27, 29 and 32). Several of

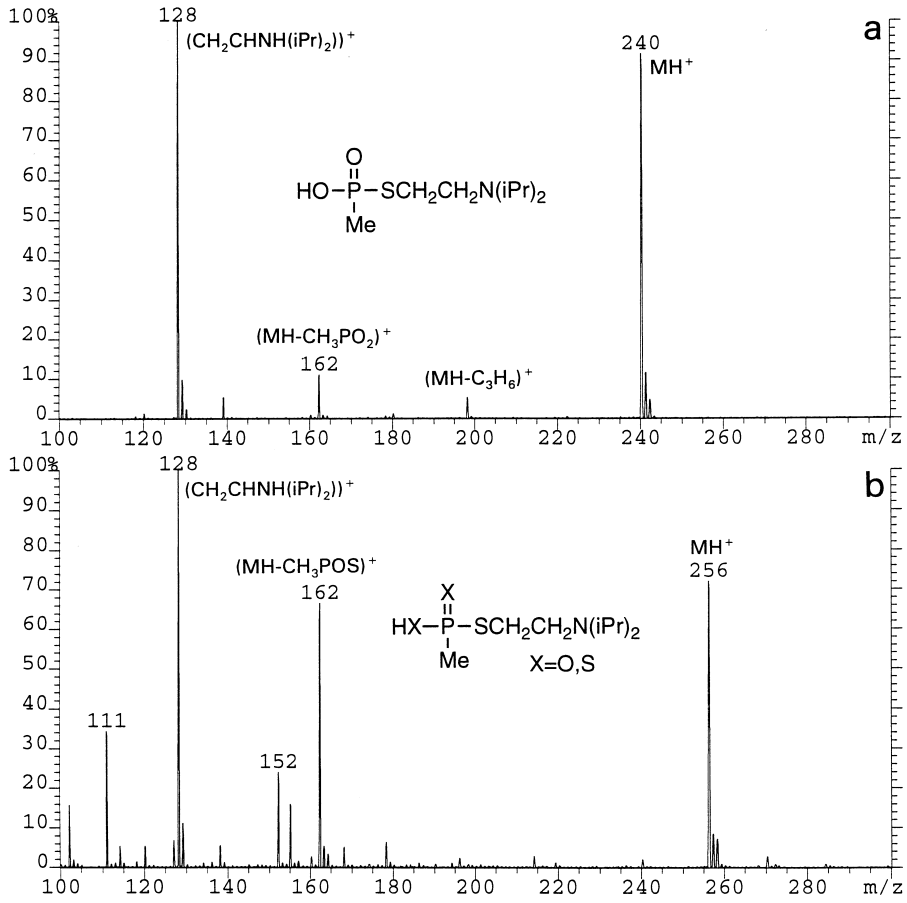


Fig. 3. ESI–MS mass spectra (sampling cone voltage: 100 V) acquired for (a) *S*-[2-(diisopropylamino)ethyl] methylphosphonothiolate (peak 4 in Fig. 1) and (b) *S*-[2-(diisopropylamino)ethyl] methylphosphonodithioate (peak 5 in Fig. 1) identified during analysis of a degraded VX sample.

these compounds co-eluted making assignment of the product ions to an individual structure difficult.

3.3. ESI–MS of bis(diisopropylamino)thiaalkanes

A number of bis(diisopropylamino)thiaalkanes were detected including, bis[2-(diisopropylamino)ethyl] sulfide and bis[2-(diisopropylamino)ethyl] disulfide, two degradation products often associated with degraded VX [2,3,20]. These two compounds were part of a series of the general form, $(iPr)_2NC_2H_4S_nC_2H_4N(iPr)_2$, where $n=1$, 2 and 3. Fig. 5 illustrates the ESI–MS data obtained with a sampling cone voltage of 100 V for these three homologues. All three compounds exhibited $(M+H)^+$ ions as well as product ion(s) due to loss of $HS_nC_2H_4N(iPr)_2$, where n represents the number of sulfur atoms contained in each of the compounds. Additional product ions due to losses of $HN(iPr)_2$ and/or $EtN(iPr)_2$ from the protonated adduct were also observed.

The ESI–MS data acquired varied with sampling cone voltage, with 100 volts generally being preferred over 50 V as both molecular ion and product ion information were usually obtained at this setting. Figs. 6a and 6b illustrate the ESI–MS data obtained for 1,8-bis(diisopropylamino)-3,6-dithiaoctane at the two sampling cone voltages. At the lower sampling

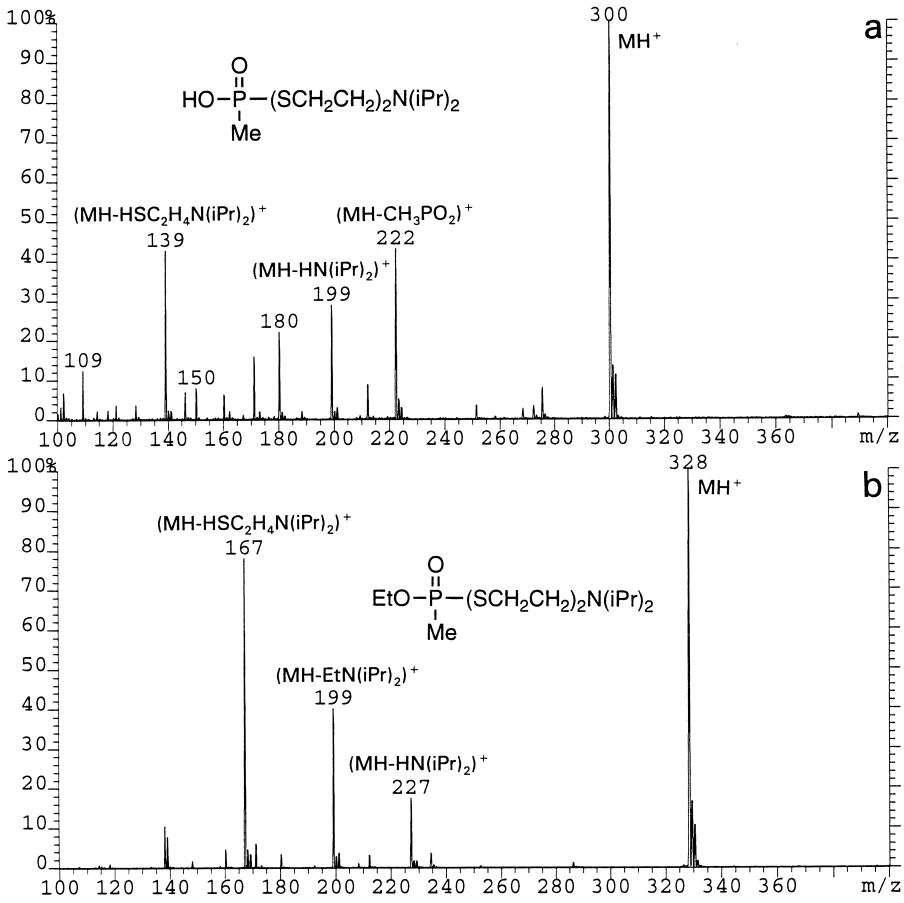


Fig. 4. ESI–MS mass spectra (sampling cone voltage: 100 V) acquired for (a) *S*-[5-(diisopropylamino)-3-thiapentyl] methylphosphonothiolate (peak 8 in Fig. 1) and (b) *O*-ethyl *S*-[5-(diisopropylamino)-3-thiapentyl] methylphosphonothiolate (peak 17 in Fig. 1) identified during analysis of a degraded VX sample.

cone voltage the only significant ions were due to the $(MH)^+$ and $(MH_2)^{2+}$ isotopic clusters, indicative of a molecular mass of 348. The doubly charged isotopic cluster was well resolved, as illustrated by the expanded insert in Fig. 6a. Product ion abundance increased with sampling cone voltage, giving rise to product ions at m/z 160 and 128 due to $[MH\text{-}EtSC_2H_4N(iPr)_2]^+$ and $[MH\text{-}HSC_2H_4SC_2H_4N(iPr)_2]^+$, respectively (Fig. 6b). Other possible isomers, were also detected and tentatively identified in the degraded VX sample. For example, Fig. 6c illustrates the ESI–MS data obtained for 1,8-bis(diisopropylamino)-3,4-dithiaoctane, an isomer of 1,8-bis(diisopropylamino)-3,6-dithiaoctane that may have formed due to the presence of an impurity containing $-C_4H_8N(iPr)_2$. The mass spectra differ with respect to product ion abundance and intensity. Product ions at m/z 188, 154 and 128 likely due to $[MH\text{-}HSC_2H_4N(iPr)_2]^+$, $[MH\text{-}HSC_2H_4N(iPr)_2\text{-}H_2S]^+$ and $[MH\text{-}HSSC_4H_8N(iPr)_2]^+$, respectively, suggested the following isomer, 1,8-bis(diisopropylamino)-3,4-dithiaoctane. A number of other bis(diisopropylamino)thiaalkanes were also detected, all of which featured an intense $(M+H)^+$ ion from which the molecular mass and sulfur content could be determined and lower mass product ions resulting from thiaalkane cleavage. At lower sampling cone

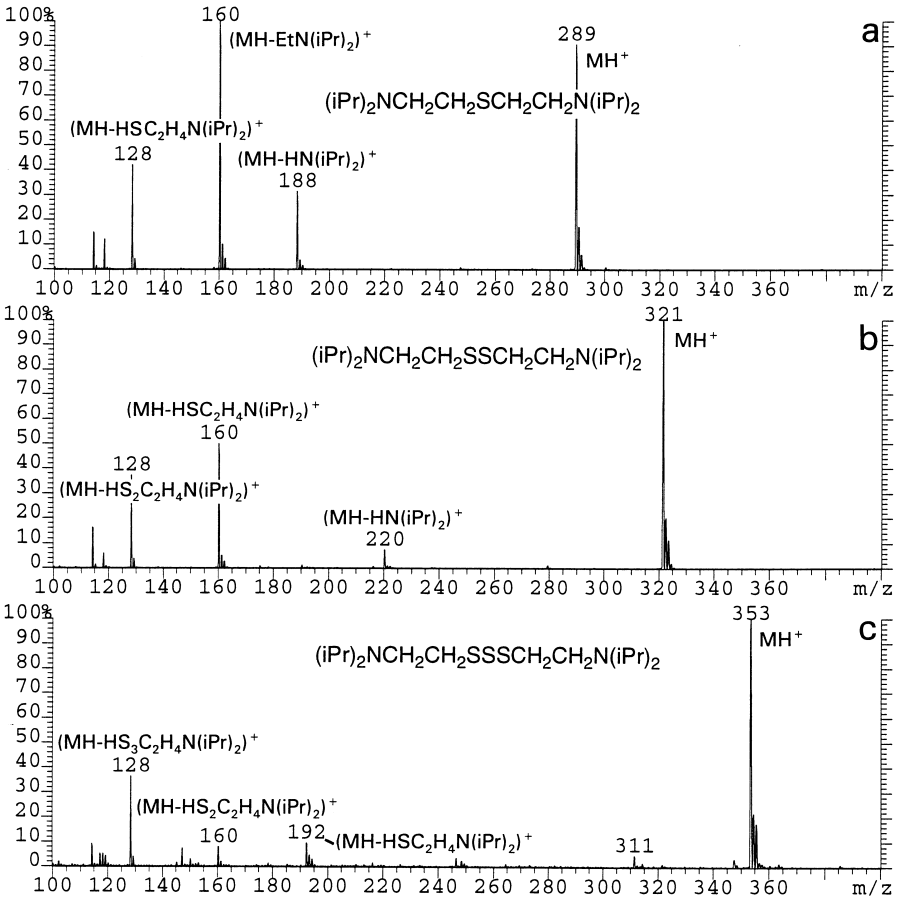


Fig. 5. ESI–MS mass spectra (sampling cone voltage: 100 V) acquired for (a) bis[2-(diisopropylamino)ethyl] sulfide (peak 9 in Fig. 1), (b) bis[2-(diisopropylamino)ethyl] disulfide (peak 11 in Fig. 1) and (c) bis[2-(diisopropylamino)ethyl] trisulfide (peak 13 in Fig. 1) identified during analysis of a degraded VX sample.

voltages $(M+2H)^{2+}$ isotopic clusters were also observed for many of the longer chain bis(diisopropylamino)thiaalkanes.[1]

3.3. ESI–MS of ureas

Both *N,N′*-dicyclohexylurea and its thio analogue, *N,N′*-dicyclohexylthiourea, have been detected in samples of VX in the past [2,3]. The ESI–MS data acquired for *N,N′*-dicyclohexyl-urea, a common VX stabilizer, agree with that acquired for a standard under identical LC–ESI–MS conditions. At a sampling cone voltage of 50 V, both ureas exhibited only adduct ions. *N,N′*-dicyclohexylurea was characterized by ions at *m/z* 225, 266 and 449 due to $(MH)^+$, $(MH+ACN)^+$ and $(M_2H)^+$, respectively, while only the $(MH)^+$ ion at *m/z* 241 was significant for *N,N′*-dicyclohexylthiourea. At a sampling cone voltage of 100 volts, product ions due to $(MH+ACN\text{-}C_6H_{10})^+$, $(MH\text{-}C_6H_{10})^+$, $(MH\text{-}C_5H_8O)^+$ and $(C_6H_{14}N)^+$ were observed at *m/z* 184, 143, 141 and 100, respectively for *N,N′*-dicyclohexylurea (Fig. 7a). Similar product ions at *m/z* 159, 141 and 100 due to $(MH\text{-}C_6H_{10})^+$, $(MH\text{-}C_5H_8S)^+$ and $(C_6H_{14}N)^+$, respectively, were

[1] $(MH)^+$ ions were obtained for all the compounds identified in this study, with data for representative compounds being presented in Figs. 2–7. The ESI–MS data obtained for the additional compounds is available from the author on request.

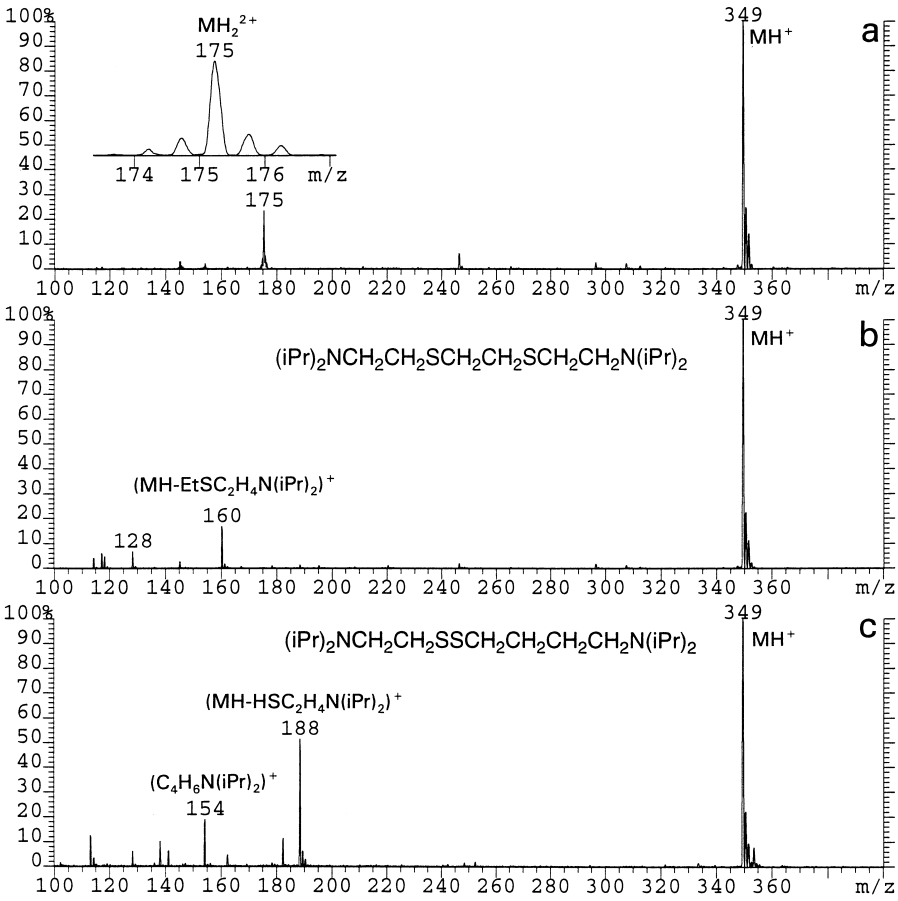


Fig. 6. ESI–MS mass spectra acquired for (a) 1,8-bis(diisopropylamino)-3,6-dithiaoctane (sampling cone voltage: 50 V), (b) 1,8-bis(diisopropylamino)-3,6-dithiaoctane (sampling cone voltage: 100 V) (peak 12 in Fig. 1) and (c) 1,8-bis(diisopropylamino)-3,4-dithiaoctane (peak 14 in Fig. 1) identified during analysis of a degraded VX sample.

detected for *N,N′*-dicyclohexylthiourea. The presence of significant acetonitrile adducts for *N,N′*-dicyclohexylurea was atypical of the degraded VX sample components, but adducts of this type were common during LC–ESI–MS of tabun samples [30].

A detailed detection limit study was not undertaken as the study focussed more on the separation and characterization of the VX degradation products. *N,N′*-dicyclohexylurea was used to estimate the full scanning (500 to 50 u) detection limit of the LC–ESI–MS method, since VX underwent some degradation during analysis. A full scanning detection limit of 5 ng, based on the acquisition of an interpretable mass spectrum was estimated. This limit was similar to that obtained for thiodiglycol, the hydrolysis product of mustard [29]. Selected-ion-monitoring, which typically results in a 10–100-fold increase in sensitivity, was not evaluated.

3.4. Relatively pure VX samples

The small amount of VX in the degraded sample hydrolysed prior or during LC–ESI–MS analysis. Had it been present at a higher concentration, as was the case for a relatively pure VX sample in water, a residual amount would have been detected in the chromatogram at 13.25 min. The hydrolysis of VX was minimized in relatively pure samples of VX by

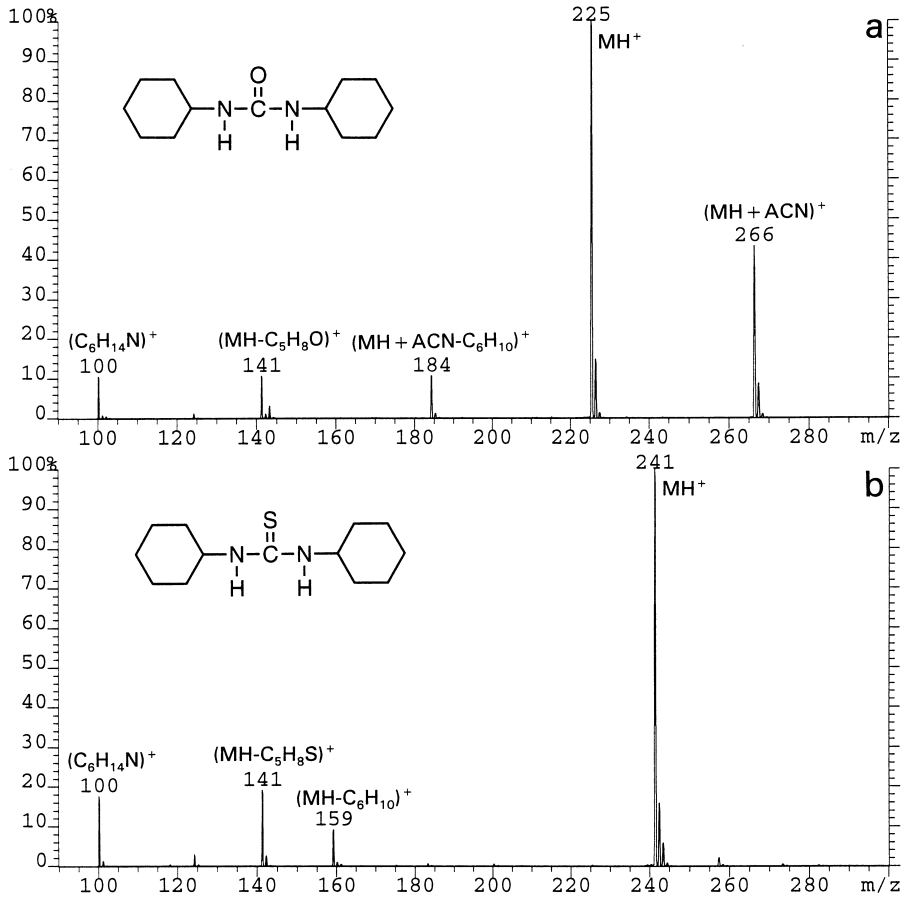


Fig. 7. ESI–MS mass spectra (sampling cone voltage: 100 V) acquired for (a) *N,N′*-dicyclohexylurea (peak 33 in Fig. 1) and (b) *N,N′*-dicyclohexylthiourea (peak 36 in Fig. 1) identified during analysis of a degraded VX sample.

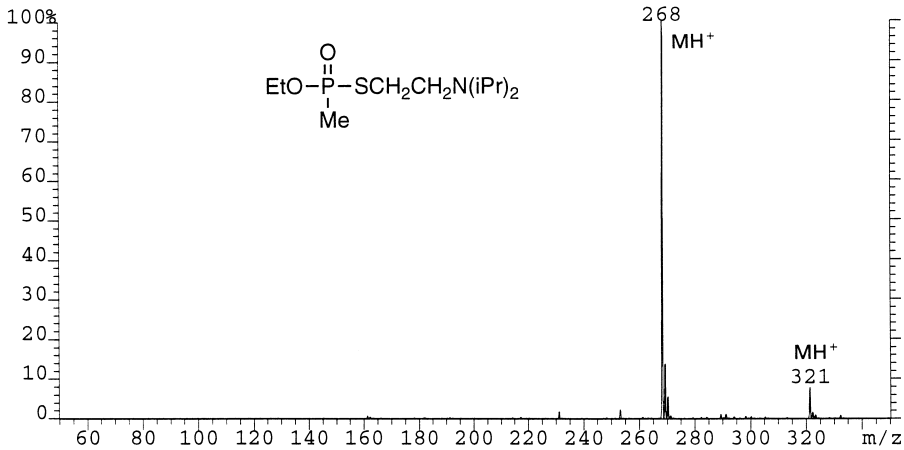


Fig. 8. ESI–MS mass spectra acquired for a relatively pure sample of VX in isopropanol with a sample cone voltage of 50 V. Both VX (MH^+ at m/z 268, structure inset) and bis[2-(diisopropylamino)ethyl] disulfide (MH^+ at m/z 321) were detected.

performing loop injections of the standard into a ACN–water (50:50) mobile phase. VX was easily detected under these conditions as the residence time of the VX in the aqueous mobile phase was in the order of two min. Fig. 8 illustrates the ESI–MS data obtained for the relatively pure standard of VX at 50 V. $(MH)^+$ ions were observed for VX and an impurity in the sample, bis[2-(diisopropylamino)ethyl] disulfide.

4. Conclusions

This study represents the first application of packed capillary column LC–ESI–MS for the characterization of VX and numerous VX related degradation products, including phosphorus compounds containing a P-CH_3 bond, long chain bis-(diisopropylamino)thiaalkanes and ureas commonly employed as VX stabilizers. The ESI–MS data were collected with sampling cone voltages of 50 and 100 V. In general the most informative mass spectra were acquired with the higher sampling cone voltage, a setting that promoted collisionally activated dissociation, and resulted in the acquistion of mass spectra containing both molecular and product ion information.

A LC–ESI–MS method has been demonstrated for the analysis of VX degradation products, extending the range of analytical options available to the researcher confronted with the identification of chemical warfare agents or their degradation products. The developed method appears to be an attractive alternative to GC–MS for the analysis of aqueous samples containing the degradation products of VX since they may be analysed directly with little risk of thermal decomposition and without the need for additional sample handling or derivatization. Use of this method resulted in the detection of a number of novel polar and higher molecular mass degradation products, not previously associated with VX during GC–MS analysis. The reported ESI–MS data could prove valuable for the verification of VX and VX related degradation products in samples collected during Chemical Weapons Convention inspections. In the absence of VX, the presence of the reported degradation products would greatly strengthen the argument for prior presence of VX in samples.

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