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Short communication

Packed capillary liquid chromatography–electrospray mass spectrometry analysis of organophosphorus chemical warfare agents

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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 four common organophosphorus chemical warfare agents in aqueous samples. Aqueous samples containing the organophosphorus chemical warfare agents in the 0.01 to 0.1 mg/ml range were analyzed directly by packed capillary LC–ESI-MS with the chemical warfare agents and several minor related impurities being well resolved under acetonitrile–water gradient elution conditions. The ESI-MS data for isopropyl methylphosphonofluoridate (sarin or GB), *O*-ethyl *N*,*N*-dimethylphosphoramidocyanidate (tabun or GA), cyclohexyl methylphosphonofluoridate (GF) and pinacolyl methylphosphonofluoridate (soman or GD) were acquired with a sampling cone voltage 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 organophosphorus chemical warfare agents and their hydrolysis products, since they may be analyzed directly without the need for additional sample handling.

Keywords: Warfare agents; Organophosphorus compounds

1. Introduction

The Chemical Weapons Convention entered into force almost two years 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 inspection will take place at sites suspected of non-compliance. An analytical capability will be required to verify the convention, since inspectors will have the option to procure and analyze suspect samples to help establish 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 could act as an additional deterrent to non-compliance.

Gas chromatography (GC) has been used extensively for the separation and identification of chemical warfare agents [1,2], with GC–mass spectrometry

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(MS) being used most frequently for the characterization of these compounds [3–7]. Organophosphorus chemical warfare agents, scheduled under the Chemical Weapons Convention, have been studied extensively by electron impact and chemical ionization MS as the use of these complementary ionization techniques facilitates the acquisition of molecular and fragmentation ion information that may be used for unambiguous identification [8–10]. GC separation, while generally suitable for the direct analysis of organophosphorus chemical warfare agent in organic extracts, is usually not preferred for the direct analysis of aqueous samples. Aqueous samples containing organophosphorus chemical warfare agents and/or their non-volatile hydrolysis products normally require additional sample handling steps and derivatization [11–13]. Recently, water samples containing chemical warfare agents have been analyzed by GC–MS following solid-phase microextraction [14] and by microcolumn liquid chromatography (LC) with flame photometric detection [15]. Increasingly, researchers have developed LC–MS separation methods to deal with the analysis of aqueous samples containing these non-volatile hydrolysis products [16–21]. Benefits over GC analysis include reduced or no sample handling and no requirement for derivatization to increase the volatility of the hydrolysis products.

Use of thermospray MS [16–19] and more recently the atmospheric pressure ionization [e.g., electrospray (ESI), ionspray and atmospheric pressure CI] techniques [20–26] has enabled the direct mass spectrometric analysis of the hydrolysis products of organophosphorus chemical warfare agents. Both techniques may be interfaced to LC for component separation, with thermospray having been largely superseded by atmospheric pressure ionization (API) for most applications. Most recent API-MS and LC–API-MS papers have focussed on the analysis of the hydrolysis products of chemical warfare agents, with the exception of a recent presentation dealing with the direct aqueous LC–ESI-MS analysis of a degraded *O*-ethyl *N*,*N*-dimethylphosphoramidocyanidate sample [26]. ESI-MS, the most sensitive technique for these applications [21], has not been previously used for the characterization of the organophosphorus chemical warfare agents, isopropyl methylphosphonofluoridate (sarin or GB), *O*-ethyl *N*,*N*-dimethylphosphoramidocyanidate (tabun or GA), cyclohexyl methylphosphonofluoridate (GF) or pinacolyl methylphosphonofluoridate (soman or GD). This short communication focuses on the ESI-MS characterization of these four organophosphorus chemical warfare agents and the development of a LC–ESI-MS method for the direct analysis of these compounds in aqueous samples.

2. Experimental

2.1. Samples

Isopropyl methylphosphonofluoridate (sarin or GB), *O*-ethyl *N*,*N*-dimethylphosphoramidocyanidate (tabun or GA), cyclohexyl methylphosphonofluoridate (GF) and pinacolyl methylphosphonofluoridate (soman or GD) were synthesized and purified locally by the Organic Chemistry Laboratory at Defence Research Establishment Suffield. The chemical warfare agents were purified to 95% (or better) with the exception of *O*-ethyl *N*,*N*-dimethylphosphoramidocyanidate which was purified to 90%. Stock solutions containing each of the four compounds were prepared in water (pH 5 to 6) at concentrations of 0.1 mg/ml and 0.01 mg/ml.

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 25 or 50 V were utilized. Nitrogen (Very Dry, Liquid Carbonic, Scarborough, Canada) bath gas was introduced into the interface (80°C) at a flow-rate of 300 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 $4 \cdot 10^{-4}$ 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 600 to 60 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 mm×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% to 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 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 extensively for the detection and identification of organophosphorus chemical warfare agents in organic extracts [1,2], but this separation method does not generally permit direct analysis of aqueous samples containing organophosphorus chemical warfare agents and their hydrolysis products. The development of a complementary LC–MS method for these compounds would be beneficial as it would allow simultaneous identification of both organophosphorus chemical warfare agents and their hydrolysis products in a single analysis based on both LC retention time and mass spectrometric data. LC–ESI-MS methods have been recently demonstrated for a series of alkyl methylphosphonic acid standards [21] and for the analysis of degradation products related to the hydrolysis of munitions grade mustard [27]. Packed capillary LC columns with an inner diameter of 0.32 mm were selected for this study and the prior mustard hydrolysis study since the 5 μl/min flow-rates typically used during chromatographic separation with these packed capillaries 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 dependance of MS detection.

Chromatographic separation of the four organophosphorus chemical warfare agents was achieved using a 1% to 75% B gradient program over 30 min (Fig. 1). The molecular masses of four organophosphorus chemical warfare agents, as well as some minor tabun impurities, were established from the

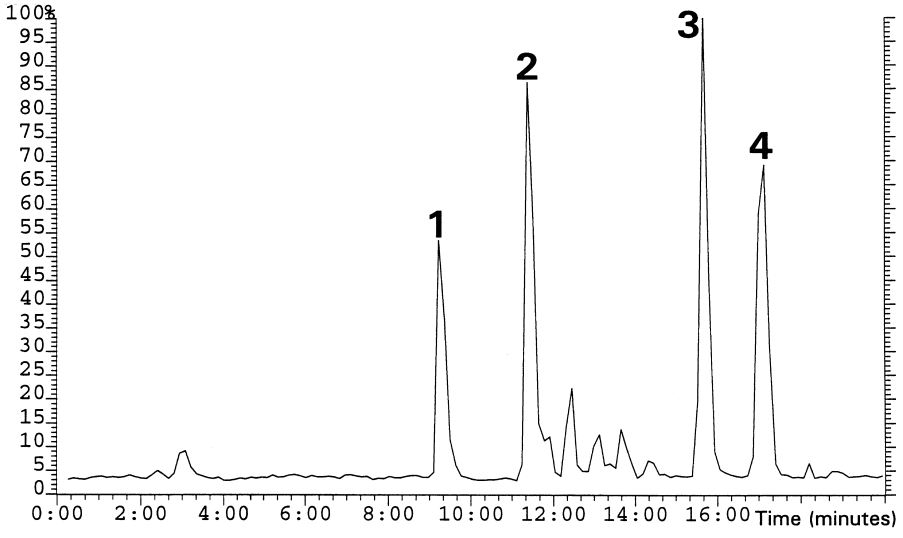

Fig. 1. Packed capillary LC–ESI-MS total ion current (600 to 60 u) chromatogram obtained for an aqueous sample containing isopropyl methylphosphonofluoridate (1), *O*-ethyl *N*,*N*-dimethylphosphoramidocyanidate (2), cyclohexyl methylphosphonofluoridate (3) and pinacolyl methylphosphonofluoridate (4) at 0.1 mg/ml.

acquired ESI-MS data. These *O*-ethyl *N*,*N*-dimethylphosphoramidocyanidate impurities, with retention times in the 12–14 min range, were the same as several that have been reported previously during GC–MS study [3].

Fig. 2 illustrates the ESI-MS data that were obtained for isopropyl methylphosphonofluoridate (molecular mass 140), *O*-ethyl *N*,*N*-dimethylphosphoramidocyanidate (molecular mass 162), cyclohexyl methylphosphonofluoridate (molecular mass 180) and pinacolyl methylphosphonofluoridate (molecular mass 182) with a sampling cone voltage of 25 V. All the spectra exhibited $(M+H)^+$ and $(M+H+ACN)^+$ ions and protonated dimers that could be used to confirm the molecular mass of each compound. Some additional minor adduct ions due to $(M+NH_4)^+$ and $(M+H_3O)^+$ were also observed [e.g., ions at m/z 158 and m/z 159 for isopropyl methylphosphonofluoridate (Fig. 2a)].

Product ions due to alkene loss from the alkoxy

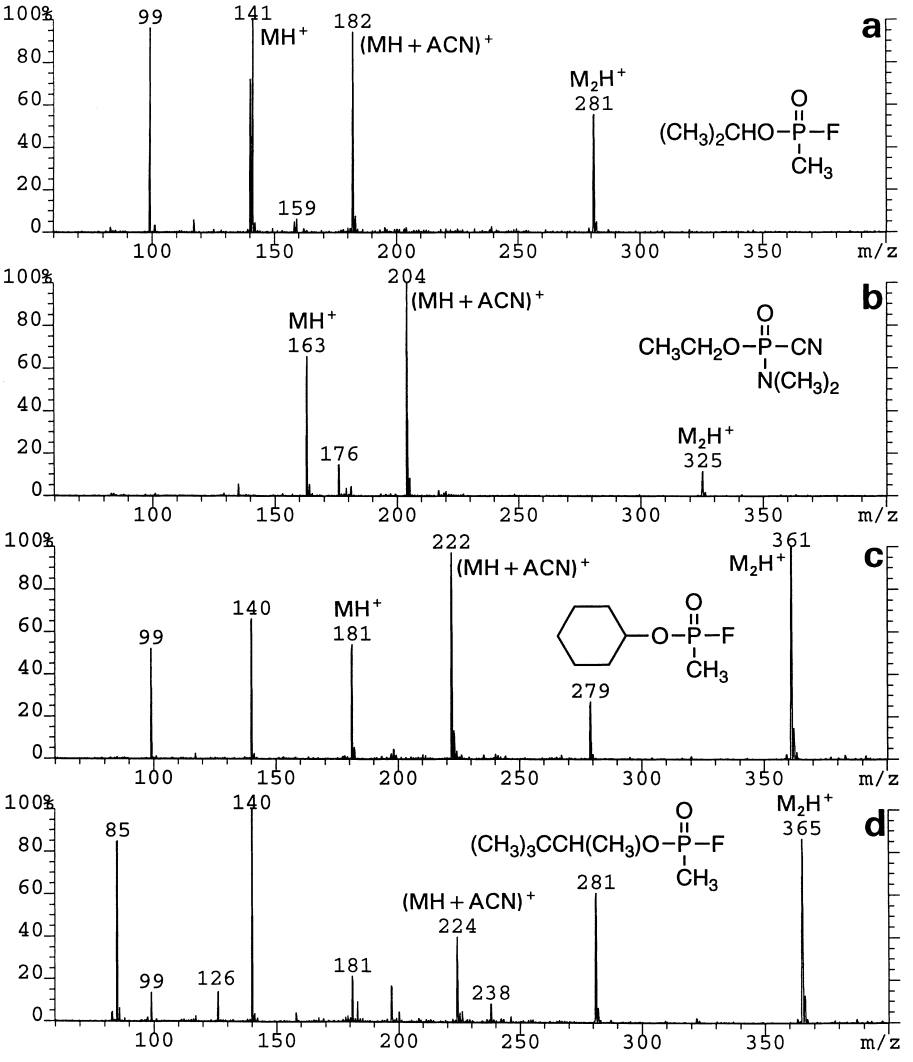

Fig. 2. Typical ESI-MS mass spectra (sampling cone voltage: 25 V) acquired for (a) isopropyl methylphosphonofluoridate, (b) *O*-ethyl *N*,*N*-dimethylphosphoramidocyanidate, (c) cyclohexyl methylphosphonofluoridate and (d) pinacolyl methylphosphonofluoridate during LC–ESI-MS analysis (0.1 mg/ml).

substituents and the acetonitrile adduct associated with these product ions were observed for all four compounds. The ESI-MS data for isopropyl methylphosphonofluoridate (Fig. 2a) contained a significant product ion at m/z 99 due to loss of C_3H_6 from the $(M+H)^+$ ion as well as its acetonitrile adduct at m/z 140. A product ion due to loss of C_2H_4 was observed for *O*-ethyl *N*,*N*-dimethylphosphoramidocyanidate (Fig. 2b) at m/z 135 along with its acetonitrile adduct at m/z 176. The ESI-MS data acquired for cyclohexyl methylphosphonofluoridate exhibited product ions at m/z 279 and m/z 99 (and its acetonitrile adduct at m/z 140) due to loss of the cyclic alkene, C_6H_{10}. Pinacolyl methylphosphonofluoridate was characterized by product ions due to loss of C_6H_{12} (and the associated acetonitrile adducts) from the protonated monomer and dimer at m/z 99 and m/z 281, respectively, and a product ion at m/z 85 due to $(C_6H_{13})^+$ and its associated acetonitrile adduct at m/z 126. The relatively low intensity of the $(M+H)^+$ ion suggests a lower proton affinity for pinacolyl methylphosphonofluoridate. Minor ions at m/z 238, m/z 197 and m/z 181 were due to $[M_2H+ACN-(C_6H_{12})_2]^+$, $[M_2H-(C_6H_{12})_2]^+$ and $[M+H+(ACN)_2-C_6H_{12}]^+$, respectively.

The formation of significant acetonitrile adducts was of initial concern. While this behavior has been observed during some pharmaceutical analyses [28] it was not significant during a prior LC–ESI-MS study of mustard hydrolysis products [27]. Adduct formation, similar to what has been observed for these compounds during ammonia CI-MS [9], was likely due to the lower proton affinity of the compounds coupled with the opportunity for multiple collisions within the ESI interface. Increasing the sampling cone voltage increased the relative abundance of the product ions due to alkene loss, but had little overall effect on the presence of acetonitrile adducts. The effect of the organic component in the mobile phase was then investigated by replacing the acetonitrile with methanol during gradient programming LC–ESI-MS analysis of a degraded sample of *O*-ethyl *N*,*N*-dimethylphosphoramidocyanidate [26]. The most notable change in the acquired mass spectra for *O*-ethyl *N*,*N*-dimethylphosphoramidocyanidate and related compounds in the sample was the presence of methanol as opposed to acetonitrile adducts. Finally the presence of these solvent adducts was confirmed on a completely different instrument, a Hewlett-Packard 1100 mass-selective detector (quadrupole), equipped with an ESI interface of their own design.

A detailed detection limit study was not undertaken as the study focussed more on the separation and characterization of the organophosphorus chemical warfare agents. A full scanning (600 to 60 u) detection limit of 5 ng, based on the acquisition of an interpretable mass spectrum, was estimated during analysis of the 0.01 mg/ml standard mixture and a relatively pure tabun standard with the same concentration [26]. This limit was similar to that obtained for thiodiglycol, the hydrolysis product of mustard [27]. Selected ion monitoring, which typically results in a 10–100-fold increase in sensitivity, was not evaluated.

4. Conclusions

This study represents the first application of packed capillary column LC–ESI-MS for the characterization of organophosphorus chemical warfare agents and demonstrates application of this method for the direct analysis of these compounds in aqueous samples. The ESI-MS data were collected with sampling cone voltages in the 25–50 V range. In general the most informative mass spectra were acquired with the lower sampling cone voltage, a setting that promoted collisionally activated dissociation, and resulted in the acquisition of mass spectra containing both molecular and product ion information.

An LC–ESI-MS method has been demonstrated for the direct analysis of nanogram quantities of organophosphorus chemical warfare agents in aqueous samples, extending the range of analytical options available to the researcher confronted with the identification of chemical warfare agents or their hydrolysis products. The developed method appears to be an attractive alternative to GC–MS for the analysis of aqueous samples since they may be analyzed directly reducing the need for additional sample handling or derivatization steps. Use of this method resulted in the ESI-MS characterization of isopropyl methylphosphonofluoridate, *O*-ethyl *N*,*N*-

dimethylphosphoramidocyanidate, cyclohexyl methylphosphonofluoridate and pinacolyl methylphosphonofluoridate. The ESI-MS data generated during LC–ESI-MS analysis would be valuable during chemical weapons destruction monitoring of countries in compliance with the Chemical Weapons Convention, for the verification of these compounds in aqueous samples collected during challenge inspections of suspect production facilities or, in support of allegations of chemical warfare agent use claims.

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