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Identification of degradation products of some chemical warfare agents by capillary electrophoresis-ionspray mass spectrometry

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ABSTRACT

Capillary zone electrophoresis-ionspray mass spectrometry (CZE-IS-MS) in the negative-ion mode was applied in the identification of five organophosphonic acids, which are the primary hydrolysis products of nerve agents. The spectra exhibit a very abundant $(M-H)^-$ ion with minimal fragmentation. Fragment ions were produced by raising the nozzle – skimmer voltage difference in the first vacuum stage between the atmospheric pressure ion source and the mass analyzer. CZE-IS-MS provides extremely good separation efficiency and very high sensitivity for the phosphonic acids. Sensitivity in the range 10–30 pg was achieved with standard solutions using selected ion monitoring. Linear regression analysis data showed correlation coefficients to be between 0.994 and 0.999, indicating good quantitative linearity of the method.

INTRODUCTION

The reliable analysis of certain alkyl-substituted organophosphorus acids, which are the primary hydrolysis products of nerve agents, has become very important in the last few years owing to the likelihood of an international agreement that will forbid the development, production and stockpiling of chemical warfare (CW) agents and weapons. This agreement will also require the destruction of all existing chemical weapons. In the verification process of this agreement, these compounds must often be unambiguously identified from various matrices.

Alkyl-substituted organophosphorus acids are the primary hydrolysis products of physiologically active organophosphorus compounds such as nerve agents and pesticides. Many of these compounds contain a methyl group directly bound to a phosphorus atom. This leads to alkylmethylphosphonic acids and ultimately to methylphosphonic acid on hydrolysis [1,2]. The hydrolysis is rapid in alkaline matrices or matrices containing water. The degradation products are polar, have low volatility and are easily isolated from various matrices by extraction with water. Therefore, these compounds are difficult to determine directly using gas chromatography (GC) and chemical derivatization is required before it can

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be employed. Derivatizations have been carried out by methylation with diazomethane [3,4], by the formation of trimethylsilyl ethers [5] or *tert.*-butyldimethylsilyl ethers [6] and by methylation with dimethylphenylammonium hydroxide or benzylation with 1-benzyl-3-(4-chlorophenyl) triazene [7]. To achieve sensitive detection and unambiguous identification at the same time, mass spectrometric (MS) detection is required, however.

These acids can be determined directly in aqueous solution by reversed-phase liquid chromatography. Sensitive detection with ultraviolet (UV) or fluorescence detection requires chemical derivatization, however. This has been carried out by precolumn derivatization into compounds with UV [8] or fluorescence properties [9]. Another approach is to combine micro-LC with a phosphorus-sensitive GC detector [10,11]. MS detection is, however, the only technique to provide sufficient information for unambiguous identification. Wils and Hulst [12] successfully determined several organophosphorus acids with LC-thermospray. Using two different eluent compositions they were able to separate and determine thirteen different organophosphorus acids.

The recently introduced electrospray (ES) [13,14] and ionspray (IS) [15] (nebulizing gas-assisted electrospray) techniques are displacing TSP in analyses for ionic and polar compounds. ES- and IS-MS provide high sensitivity, good stability and an extremely mild ionization process, operation is easy and the contamination inside the vacuum system of a mass spectrometer is minimal. The possibility of determining molecular masses up to 100 000 via multiple charging with high accuracy (0.01%) by ES- and IS-MS has opened up new avenues in the analysis of large biomolecules [16]. HPLC and CZE can be easily connected with a mass spectrometer via ES or IS, whereas the connection of CZE with TSP is not possible. CZE-IS-MS combines superior separation efficiency and extremely good sensitivity for ionic compounds. The method has been applied to proteins, peptides, nucleotides, medicines, etc. [17–20]. This paper describes the identification of some phosphonic acids, which are degradation products of nerve agents, by CZE-IS-MS in aqueous solution.

EXPERIMENTAL

The degradation products (Table I) methylphosphonic acid (MPA) and methylphosphonic acid 1-methylethyl ester (IPMPA) were obtained from the Research Centre of Finnish Defence Forces (Lakiala, Finland) and methylphosphonic

TABLE I

THE METHYLPHOSPHONIC ACIDS STUDIED [$R_1R_2P(O)CH_3$]

Compound	CAS Registry Number	Functional groups		M_r
		R_1	R_2	
Methylphosphonic acid (MPA)	993-13-15	OH	OH	96
Methylphosphonic acid ethyl ester (EMPA)	1832-53-7	OH	OCH_2CH_3	124
Methylphosphonic acid 1-methylethyl ester (IPMPA)	1832-54-8	OH	$OCH(CH_3)_2$	138
Methylphosphonic acid 1,2,2-trimethylpropyl ester (PMPA)	616-52-4	OH	$OCHCH_3C(CH_3)_3$	180
Methylphosphonothionic acid O-ethyl ester (EMTPA)	18005-40-8	SH	OCH_2CH_3	140

acid ethyl ester (EMPA), methylphosphonic acid 1,2,2-trimethylpropyl ester (PMPA) and methylphosphonothionic acid O-ethyl ester (EMTPA) were obtained from the Defence Technology and Procurement Agency, NC Laboratory (Spiez, Switzerland). All the compounds were dissolved in water purified using a Milli-Q system (Millipore). Five standard solutions containing 5, 15, 35, 60 and 112 ng/μl of the degradation products were prepared for linearity studies.

Injections were made by a Prince (Lauerlabs, Netherlands) microprocessor-controlled injector using a pressurized injection (6 s, 50 mbar). The injection volume for the pressurized injection can be calculated according to Poiseuille's law for fluid flow through a circular tube:

$$V_{inj} = \mathrm{Ap}\, t\pi d^4 / 128\eta L \qquad \text{(I)}$$

where Ap = pressure difference across capillary, t = duration of the pressure difference, d = capillary inner diameter, η = bulk fluid viscosity, L = capillary length.

According to eqn. 1, the injection volume was 5.7 nl in our experiments. The injection volume was confirmed by measuring the flow-rate at a pressure of 50 mbar. The voltage of 30 kV was provided by a Glassman power supply controlled by the Prince injector. The fused-silica capillary (uncoated, 0.8 m x 50 μm I.D.) was obtained from Polymicro Technologies. A buffer solution consisting of 20 mM ammonium acetate in Milli-Q-purified water was prepared, the pH being adjusted to 9 with ammonia solution.

The fused-silica capillary was connected to a coaxial ionspray interface, described in detail earlier [21]. A voltage of 3 kV was applied to a stainless-steel tube (15 cm x 0.40 mm I.D.). The nebulizing gas (99.9% nitrogen) pressure was 3 bar. The make-up flow (5 μl/min) was provided by a micro gradient system (Brownlee Labs.). The make-up solution was methanol (Merck, gradient grade).

The mass spectrometer was Nermag R 3010 equipped with a laboratory-made atmospheric pressure ionization (API) source described in detail earlier [22]. Nitrogen (99.9%) was used as a curtain gas. The spectra were recorded in the negative-ion mode with nozzle – skimmer voltage differences (AV) of 55 and 155 V. The mass spectrometer was scanned from 70 to 400 u with a scan rate of 1.5 s per scan.

RESULTS AND DISCUSSION

The spectra of the degradation products were recorded in the negative ion mode with nozzle – skimmer voltage differences (AV) of 55 (Fig. 1)

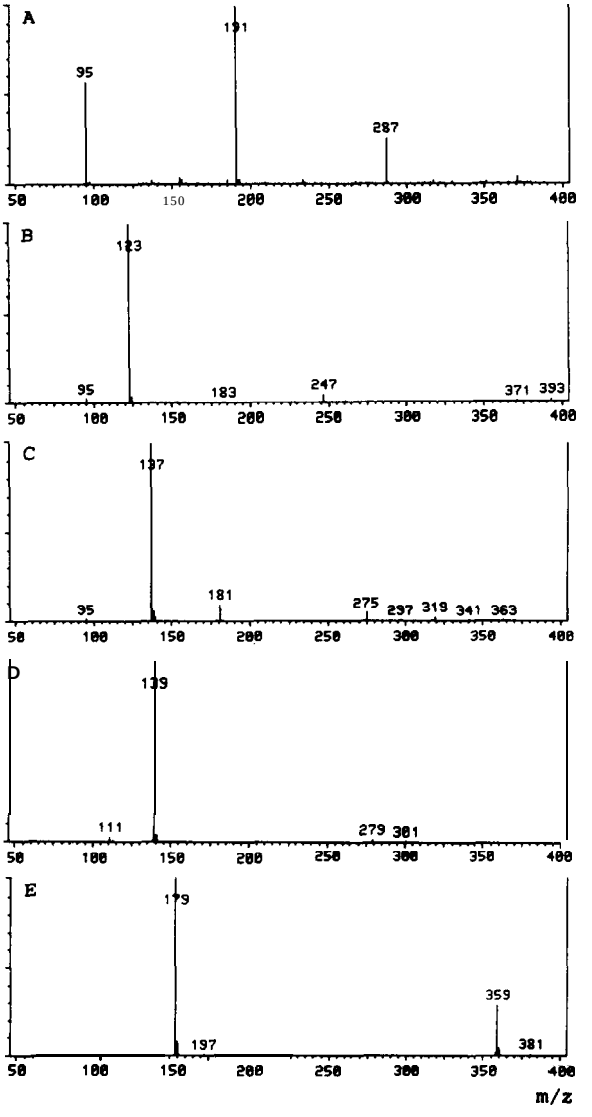

Fig. 1. Negative ionspray mass spectra of (A) MPA, (B) EMPA, (C) IPMPA, (D) EMTPA and (E) PMPA obtained using a nozzle – skimmer voltage difference of 55 V.

and 155 V (Fig. 2). The samples were introduced by a continuous pressure (50 mbar) feed via the CE capillary. All the spectra show a very abundant (M-H)- ion, dimers, trimers, some adduct ions and little fragmentation. The spectra recorded with AV= 155 V show more abundant fragment ions than those recorded with AV= 55 V. This is because the kinetic energy of the ions is increased when the nozzle voltage is raised,

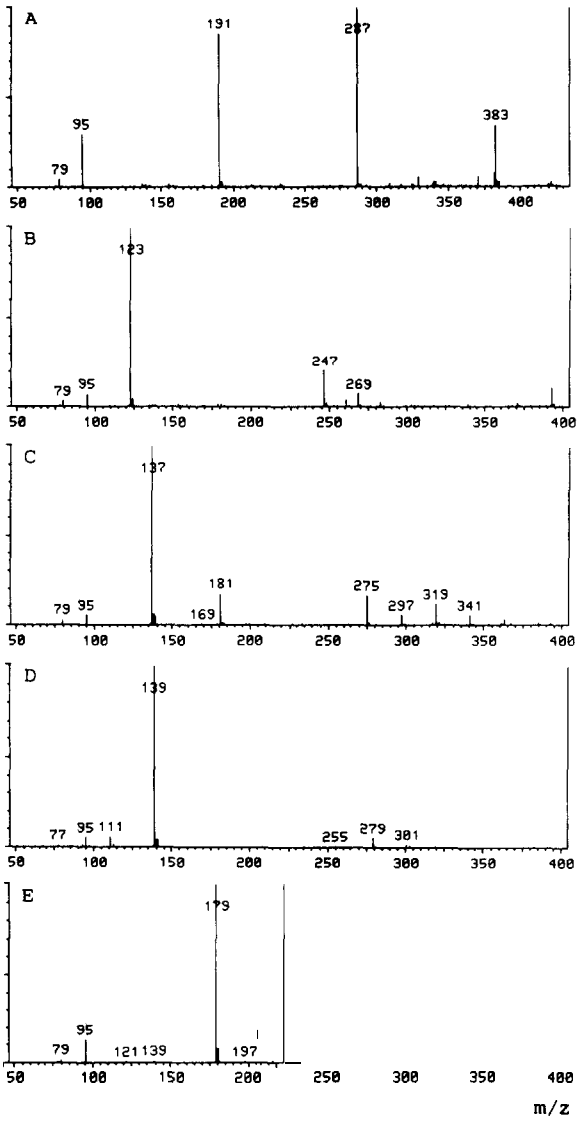

Fig. 2. Negative ionspray mass spectra of (A) MPA, (B) EMPA, (C) IPMPA, (D) EMTPA and (E) PMPA obtained using a nozzle – skimmer voltage difference of 155 V.

which leads to more energetic collisions of the ions with nitrogen molecules in the nozzle–skimmer region, and fragmentation becomes favoured. However, the abundances of the fragment ions are relatively low also with AV= 155 V, indicating relatively high stability of $(M-H)^-$ ions.

The spectra of EMPA, IPMPA and PMPA exhibit common fragment ions at *m/z* 95 and 79, which are formed by the loss of alkyl and alkoxy groups, respectively. The respective ions in the spectrum of EMTPA appear at m/z 111 and 95. The mass shift of 16 u is because EMTPA contains a thiol group instead of a hydroxy group. The ion at *m/z* 95 is very useful in the monitoring of phosphonic acids by CZE-IS-MS, although its structure in the spectrum of EMTPA is different than that in the other spectra.

The degradation products show a high tendency to form dimers and trimers in the negative-ion mode. The spectrum of MPA also shows a tetramer at m/z 383. The relatively high concentration (112 ng/μl) of the phosphonates may explain the formation of dimers and trimers, as the formation of these type of ions is favoured by high concentrations. The relative abundances of dimers and trimers compared with the $(M-H)^-$ ion increased when the nozzle – skimmer voltage difference was raised. This seems to indicate that the stabilities of the dimers and trimers are higher than those of the $(M-H)^-$ ion. However, the increased abundance is probably due to better focusing of larger ions at higher nozzle – skimmer voltage differences.

The selection of the solvent and additives in CZE-IS-MS is important and may have a significant effect on the sensitivity and separation efficiency. A make-up liquid is necessary in CZE-IS-MS, as the flow-rates in CZE are too low for efficient droplet formation. Methanol was chosen as the make-up liquid. The low surface tension and good conductivity of methanol promote the formation of gas-phase ions. Ammonium acetate was chosen as the buffer, as only volatile buffers can be used without contamination problems in the API source.

The selected ion electropherogram of the degradation products (Fig. 3) shows that good

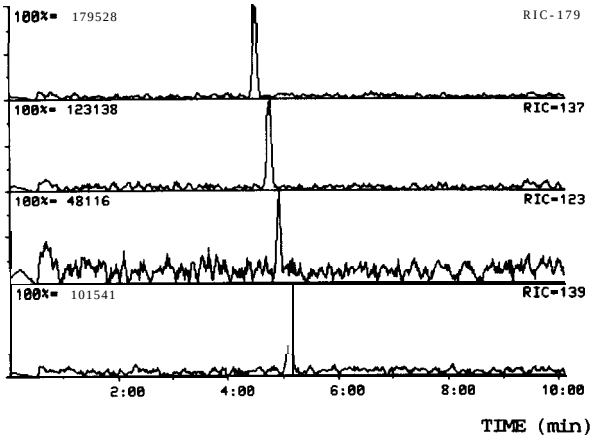

Fig. 3. Selected ion electropherograms of (from top to bottom) PMPA (m/z 179), IPMPA (m/z 137), EMPA (m/z 123) and EMTPA (m/z 139). Amount injected, 30 pg (5 ng/μl).

separation was achieved within 5 min. The elution order PMPA, IPMPA, EMPA and EMTPA shows that the electrophoretic mobility of the phosphonic acid increases with the number of carbon atoms in the alkyl chain. Further, the migration time for IPMPA, containing a hydroxy group, is significantly shorter than that for EMTPA, containing a thiol group. This suggests that the presence of a thiol group in the phosphonic acids decreases the electrophoretic mobility compared with the phoshonic acids containing a hydroxy group.

The peaks were 10 s wide in our experiments (Fig. 3), which is wider than typical in CZE. The linear nebulizing gas velocity in the ionspray is relatively high, leading to a decreased pressure at the tip of the stainless-steel capillary. The aspiration by the pneumatic nebulizer will suck the buffer solution into the capillary, causing an additional laminar flow, which leads to peak broadening. This problem could be avoided by positioning the fused-silica capillary inside the stainless-steel capillary. To avoid a dead volume, the inner diameter of the tip of the stainless-steel capillary should be the same as that of the fused-silica capillary. The preparation of this type of tip will be examined in the near future. Anyway, the peak width of 10 s is still very good and provides a high separation power. Further, too narrow peaks may be a problem in mass spectrometry, as normally in the full-scan mode the scan time is about 1 s per decade.

Fig. 3 demonstrates the sensitivity of CZE–IS-MS using selected negative-ion monitoring for the phosphonic acids. The concentrations of the compounds injected were 5 ng/μl. The injection volume was 5.7 nl. The minimum detectable amounts (signal-to-noise ratio = 3) were about 10, 20, 20 and 30 pg for PMPA, IPMPA, EMTPA and EMPA, respectively. These results suggest that the sensitivity depends on the number of carbons in the alkyl chain: the higher the number, the better is the sensitivity. This might be because the solvation energy decreases when the number of carbons in the alkyl chain increases. It has been reported that the emission of gas-phase ions from the charged droplets is promoted by low solvatation energies [22].

The recorded detection limits are one to two orders of magnitude better than those reported for TSP-MS [12], indicating very high sensitivity of IS-MS. However, the sample concentration in CZE-IS-MS must be about one order of magnitude higher than that in LC-TSP-MS, owing to the small injection volumes used in CZE. To achieve a sensitivity level needed for real environmental analyses, the sample preconcentration must be considered. The use of XAD-4 adsorbent [23] and an ion-exchange [24] resin in the preconcentration of organophosphorus acids has been reported. Sep-Pak C_{18} cartridges pretreated with a tetra-n-butylammonium salt have been used in the preconcentration of PMPA [12]. Alternatively, specific injection methods, e.g., isotachophoresis [25], can be used in CZE to increase the injection volume. The use of micro-LC-IS-MS with flow-rates of 5-100 μl/min and injection volumes of a few microlitres is also possible. However, the separation efficiency is better with CZE than with micro-LC.

The linear regression analysis data for the phosphonic acids obtained by CZE-IS-MS are presented in Table II. The correlation coefficients recorded for the phosphonic acids were between 0.994 and 0.999, indicating good quantitative linearity of the method between concentrations of 5 and 100 ng/μl.

TABLE II

LINEAR REGRESSION ANALYSIS DATA AND DETECTION LIMITS IN CZE-IS-MS

No. of data points = 5; concentration range = 5-112 ng/μl.

Compound	Correlation coefficient	Slope	Intercept	Detection limit (pg)
EMPA	0.9957	10.44	64.0	30
IPMPA	0.9984	29.35	107.4	20
EMTPA	0.9964	25.37	55.0	20
PMPA	0.9945	40.12	126.0	10

CONCLUSIONS

CZE-IS-MS has been shown to be a very powerful technique for the determination of the degradation products of chemical warfare agents directly in water samples. The method provides very rapid separation and reliable identification of the degradation products without time-consuming derivatization. The detection limits were 10–30 pg, indicating very high sensitivity of IS-MS. However, to achieve the sensitivity level needed for real environmental analyses, a sample preconcentration must be considered, as only a few nanolitres can be injected in CZE-IS-MS. The method showed good quantitative linearity between concentrations of 5 and 100 ng/μl. CZE-IS-MS is well suited for use in the verification process of the proposed agreement to ban the development, production and stockpiling of chemical warfare agents and weapons.

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