Journal of Chromatography, 474 (1989) 163–173
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 326

CHARACTERIZATION OF ORGANOPHOSPHORUS COMPOUNDS AND PHENYLUREA HERBICIDES BY POSITIVE AND NEGATIVE ION THERMOSPRAY LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY

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SUMMARY

Positive-ion (PI) mode and negative-ion (NI) mode thermospray liquid chromatography–mass spectrometry (LC–MS) were used to characterize three different groups of environmentally significant compounds: organophosphorus pesticides, phenylurea herbicides and trialkyl phosphates. Reversed-phase LC was performed with acetonitrile–water (25:75, 50:50 and 75:25) and methanol–water (50:50) + 0.1 *M* ammonium acetate as eluents. By employing PI thermospray LC–MS, the base peak was $[M+NH_4]^+$ for all the studied compounds when the NI mode was used, $[M + \text{acetate}]^-$ was usually obtained as base peak for organophosphorus pesticides and phenylurea herbicides. Other adduct ions obtained in the NI mode corresponded to $[M - R]^-$, $[M + Cl]^-$, $[M - H]^-$, and to large cluster ions with the eluent.

Detection limits at low nanogram level were achieved for all the studied compounds in the PI mode. Sensitivities in the NI mode were much poorer for organophosphorus pesticides and phenylurea herbicides, and trialkyl phosphates were not detectable at the nanogram level. An application of PI thermospray LC–MS is illustrated for the determination of vamidothion, paraoxon-methyl and azinphos-methyl in a sediment sample.

INTRODUCTION

The use of liquid chromatography–mass spectrometry (LC–MS) is becoming most popular with the increasing number of LC–MS interfaces commercially available: moving belt, direct liquid introduction (DLI), thermospray, plasmaspray, thermabeam and monodisperse aerosol generated interface. This technique plays an important role in environmental organic analysis and, compared with gas chromatography–mass spectrometry (GC–MS), offers major advantages for analyzing thermally labile and/or highly polar compounds[1–3]. The thermospray interfacing technique is probably the most widely used, and typically involves the use of reversed-phase columns and volatile buffers such as ammonium acetate[4].

The work described here will demonstrate the usefulness of filament-on thermospray LC–MS in combination with postive-ion and negative-ion modes (PI and NI, respectively) for the characterization of three different groups of pollutants:

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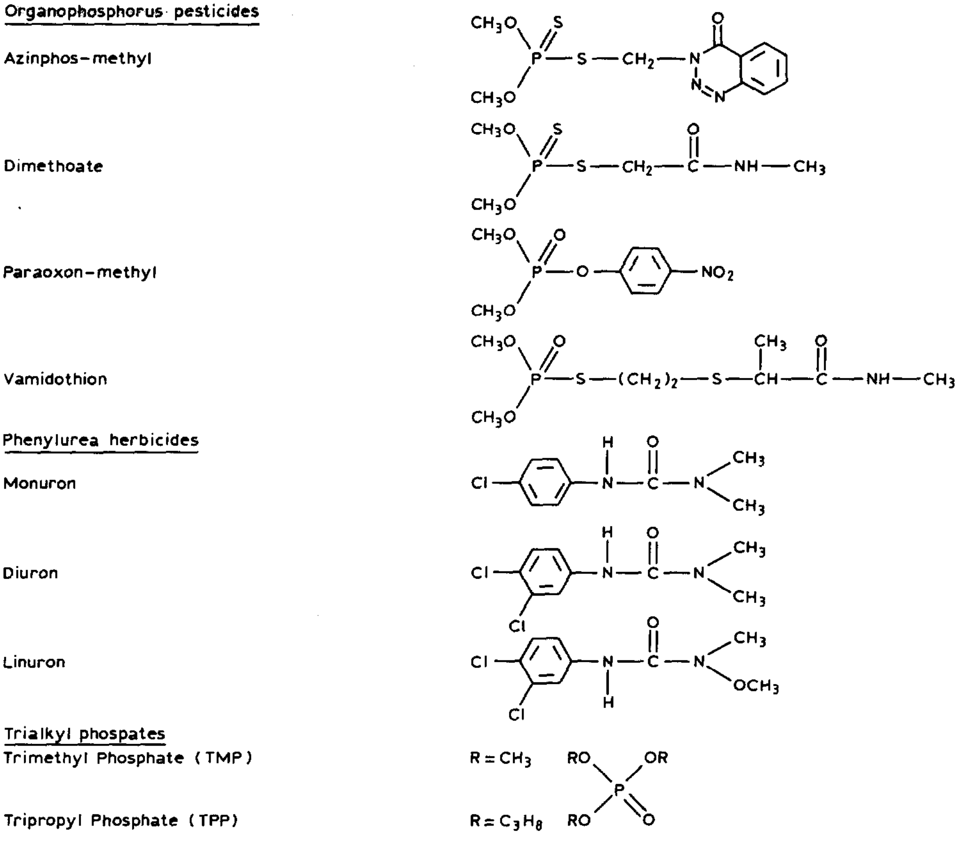

Fig. 1. Compounds analyzed.

organophosphorus pesticides, phenylurea herbicides and trialkyl phosphates (Fig. 1). Complementary information is obtained by both modes of operation. Such compounds exhibit difficulties when analysed by conventional GC because they either decompose, as do some thermally labile organophosphorus pesticides[5], or they need previous derivatization, for instance phenylurea herbicides[6].

As regards the trialkyl phosphates, although they can be analysed by GC–MS[7], there is no selective LC detector, and because of that a conventional GC thermionic detector has recently been developed[8]. The study of their fragmentation pattern is also of interest in comparison with compounds of similar structure, such as the organophosphorus pesticides, to achieve a better understanding of the thermospray LC–MS ionization behaviour.

EXPERIMENTAL

Materials

HPLC-grade water (Farmitalia Carlo Erba, Milan, Italy), methanol (Scharlau, Barcelona, Spain) and acetonitrile (Romil, Shepshed, U.K.) were passed through a 0.45-μm filter (Scharlau, Barcelona, Spain) before use. Analytical-reagent grade ammonium acetate was obtained from Panreac (Barcelona, Spain), azinphos-methyl, dimethoate, diuron, linuron, monuron and vamidothion from Polyscience (Niles, IL, U.S.A.), paraoxon-methyl from Dr. Su. I. Ehrenstorfer (Augsburg, F.R.G.) and trimethyl phosphate (TMP) and tripropyl phosphate (TPP) from Kodak (Rochester, NY, U.S.A.).

Sample preparation

Pretreatment of a sediment sample was carried out by a method commonly used for pesticide residue analysis, and described in the literature[9]. In summary, 25 g of sediment (wet weight) after spiking with 0.2 ppm of vamidothion and azinphos-methyl and 0.1 ppm of paraoxon-methyl was placed in a Soxhlet apparatus and extracted for 4 h with 150 ml of methanol–water (9:1). Afterwards, the aqueous methanol extract was concentrated in a rotary evaporator to 2 ml.

Chromatographic conditions

Eluent delivery was provided by two Model 510 high-pressure pumps coupled with a Model 680 automated gradient controller (Waters Chromatography Division, Millipore, Bedford, MA, U.S.A.) and a Model 7125 injection valve with a 20 μl loop from Rheodyne (Cotati, CA, U.S.A.). Stainless-steel columns (30 × 0.40 cm I.D.) from Tracer Analítica (Barcelona, Spain) packed with 10-μm particle diameter Spherisorb ODS-2 (Merck, Darmstadt, F.R.G.) were used. Four different LC mobile phase compositions were tested: acetonitrile–water (25:75, 50:50 and 75:25) and methanol–water (50:50) + 0.1 M ammonium acetate, at flow-rates between 1 and 1.2 ml/min.

Mass spectrometric analysis

A Hewlett-Packard (Palo Alto, CA, U.S.A.) Model 5988A thermospray LC–MS quadrupole mass spectrometer and a Hewlett-Packard Model 35741B instrument for data acquisition and processing were employed. The thermospray temperatures were: stem: 100°C, tip: 178°C, vapour: 194°C and ion source 296°C, with the filament on. In all the experiments the filament-on mode (ionization by an electron beam emitted from a heated filament) was used. In this mode of operation conventional positive and negative chemical ionization can be carried out by using the vaporized mobile phase as the chemical ionization reagent gas[4].

RESULTS AND DISCUSSION

Organophosphorus pesticides

The analysed compounds were azinphos-methyl, dimethoate, paraoxon-methyl and vamidothion (Fig. 1). Mobile phase composition was generally acetonitrile–water (50:50) + 0.1 M ammonium acetate. In the PI mode the base peak was the $[M+NH_4]^+$ ion, which is the expected behaviour for this type of compounds in LC–MS[10] or in GC–MS with ammonia as the reagent gas[11]. The favoured formation of $[M+NH_4]^+$ indicates that the proton affinity of these compounds is slightly lower than that of ammonia (858 kJ/mol)[12]. Diazinon, with a pyrimidinyl group in its structure, has been reported as an exception to this rule, and showed $[M+H]^+$ as base peak[13]. The formation of $[M+H]^+$ reached relatively high values in some cases, up 50% for vamidothion (see Fig. 2), which indicates an intermediate basicity for such compounds. Other adduct fragment ions are formed with the solvent, *e.g.*, $[M+(CH_3CN)NH_4]^+$ (Fig. 2).

Fragmentation for dimethoate was studied at higher water percentages in the eluent (Fig. 3). At 75% of water the base peak was still $[M+NH_4]^+$ but the intensity of $[M+H]^+$ increased up to 35%, as compared to a value of 10% when 50% of water was used[4]. It is worth mentioning that at this high water percentage the sensitivity was about 5 times higher than with the standard acetonitrile–water (50:50).

In the NI mode, similarly to a negative chemical ionization process, the ions detected are usually influenced by electron capture, dissociative electron capture and ion–molecule reactions in the source. These modes of negative ion formation can be due to impurities in the reagent gas, radical species in the ion source plasma and interactions with the ion source walls. Depending on the electron affinities, the bond strengths and the energy of the captured electron, dissociative electron capture reactions will take place together with electron capture reactions[14]. These three mechanisms have been observed for the different organophosphorus pesticides:

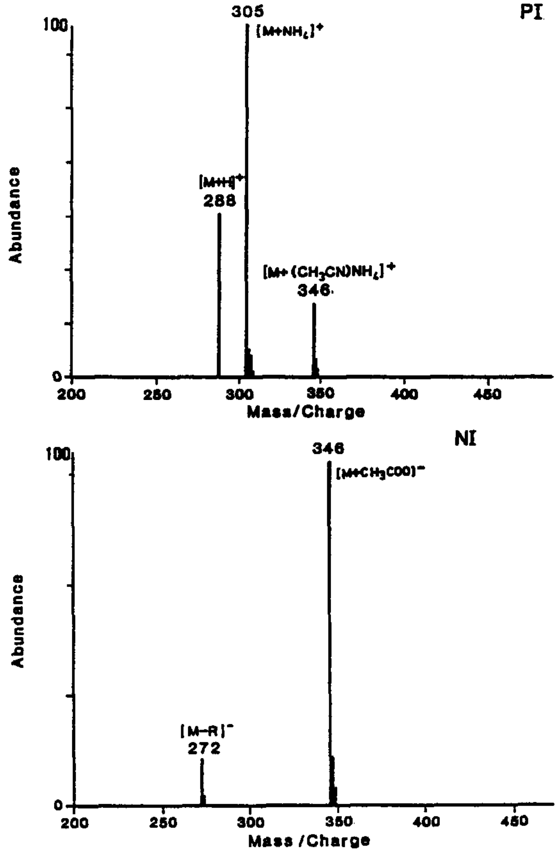

Fig. 2. Direct flow injection PI and NI thermospray MS spectra of vamidothion. Carrier stream, acetonitrile–water (50:50) + 0.1 *M* ammonium acetate; flow-rate, 1 ml/min; injection, 500 ng.

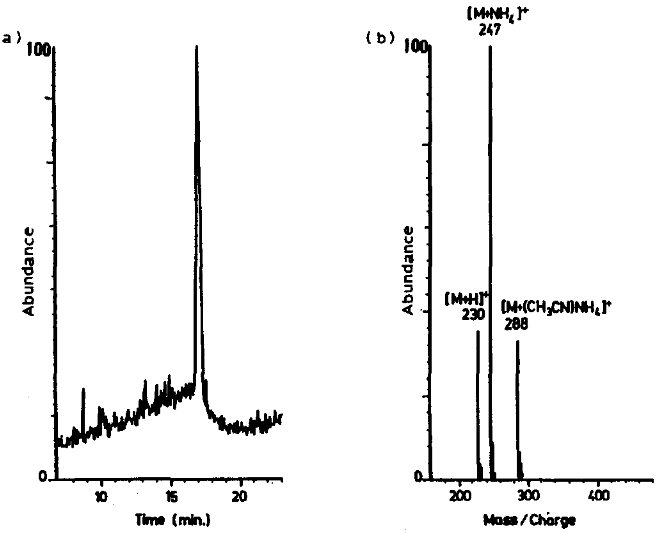

Fig. 3. (a) Reconstructed ion chromatogram and (b) mass spectrum in PI thermospray LC–MS of dimethoate. Carrier stream, acetonitrile–water (25:75) + 0.1 *M* ammonium acetate; flow-rate, 1 ml/min; injection, 500 ng.

electron capture $[M]^{-\cdot}$, dissociative electron capture $[M-R]^-$, where $R=CH_3$ or C_2H_5, and ion–molecule reactions $[M+CH_3COO]^-$ and/or $[M+Cl]^-$[4]. Formation of $[M+Cl]^-$ can be important for organophosphorus pesticides containing Cl in the molecule, such as trichlorfon[2]. The mass spectrum of vamidothion in the negative ion mode, by thermospray LC–MS, is shown in Fig. 2. The base peak corresponds to the anion attachment peak $[M + \text{acetate}]^-$, while $[M-R]^-$ is also obtained and corresponds to a typical fragment of organophosphorus pesticides as previously reported in GC–MS with negative chemical ionization[15] and in DLI LC–MS[2]. The most relevant conclusion as regards sensitivity is that the PI mode is three orders of magnitude more sensitive than the NI mode.

Phenylurea herbicides

First, it should be mentioned that in this case methanol was used in preference to acetonitrile as organic modifier because a gain of 1–2 orders of magnitude was observed when analysing compounds containing aryl halide rings[16]. Similarly to the previously mentioned compounds, the mass spectra of the studied herbicides, namely monuron and diuron, exhibit the $[M+NH_4]^+$ ion as the base peak in the PI mode (Fig. 4). Other phenylurea herbicides, *e.g.*, linuron, also exhibited the same feature[17–19], although the $[M+H]^+$ ion was the base peak for several phenylurea herbicides when volatile salt ionization (thermospray ionization) was employed[20]. Similarly to organophosphorus pesticides, the formation of $[M+NH_4]^+$ as base peak and of $[M+H]^+$, with relative intensity values close to 20%, indicates that the phenylurea herbicides containing aryl halide rings have proton affinities below that of ammonia (858 kJ/mol)[12]. When other LC–MS systems have been employed, such as DLI with a micro-LC[21], split[22] or moving belt[23], $[M+H]^+$ was always the base peak. Other fragment ions observed in Fig. 4 correspond to values higher than the molecular

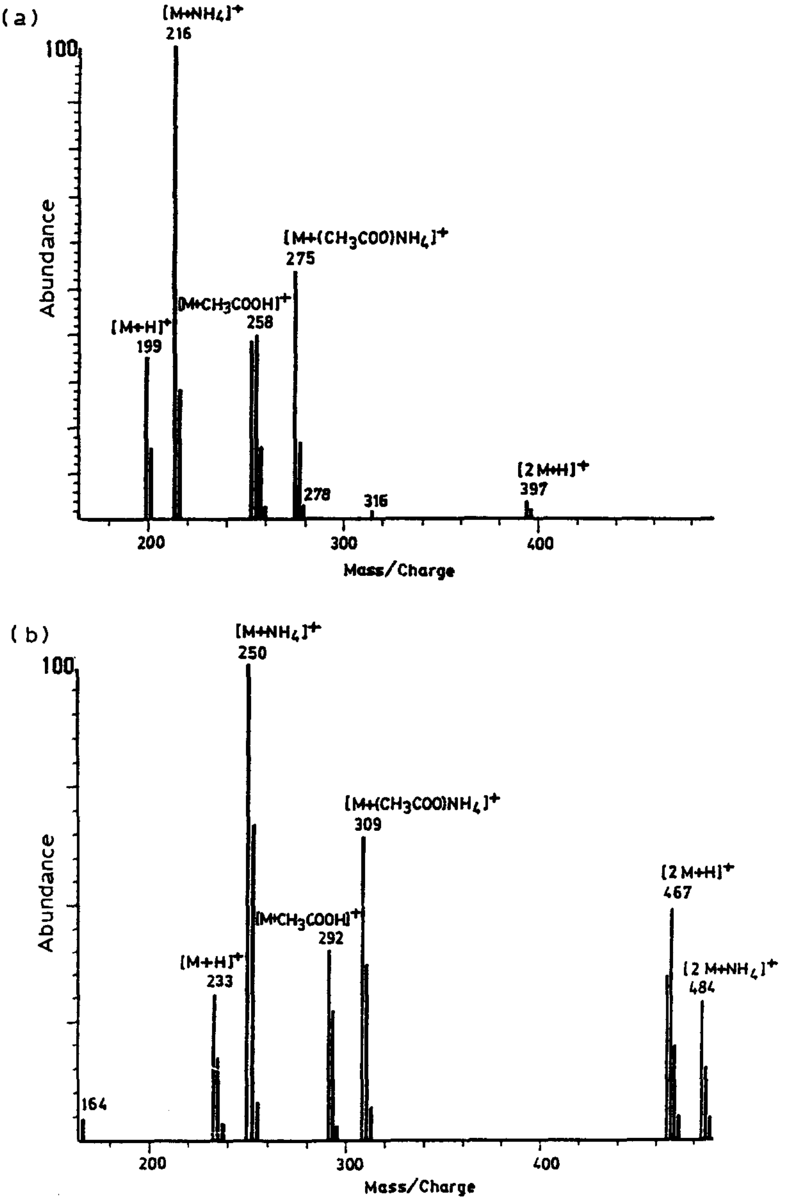

Fig. 4. Direct flow injection PI thermospray MS spectra of (a) monuron and (b) diuron. Carrier stream, methanol–water (50:50) + 0.1 *M* ammonium acetate; flow-rate, 1.2 ml/min; injection, 300 ng.

weight, and correspond to adducts with the ionizing additive, such as $[M + CH_3COOH]^+$ and $[M + (CH_3COOH)NH_4]^+$ and the dimers $[2M + H]^+$ and $[2M + NH_4]^+$.

In the NI mode, the base peak was always the anion attachment $[M + CH_3COO]^-$ ion, with a slight incidence of chloride attachment $[M + Cl]^-$ (Fig.

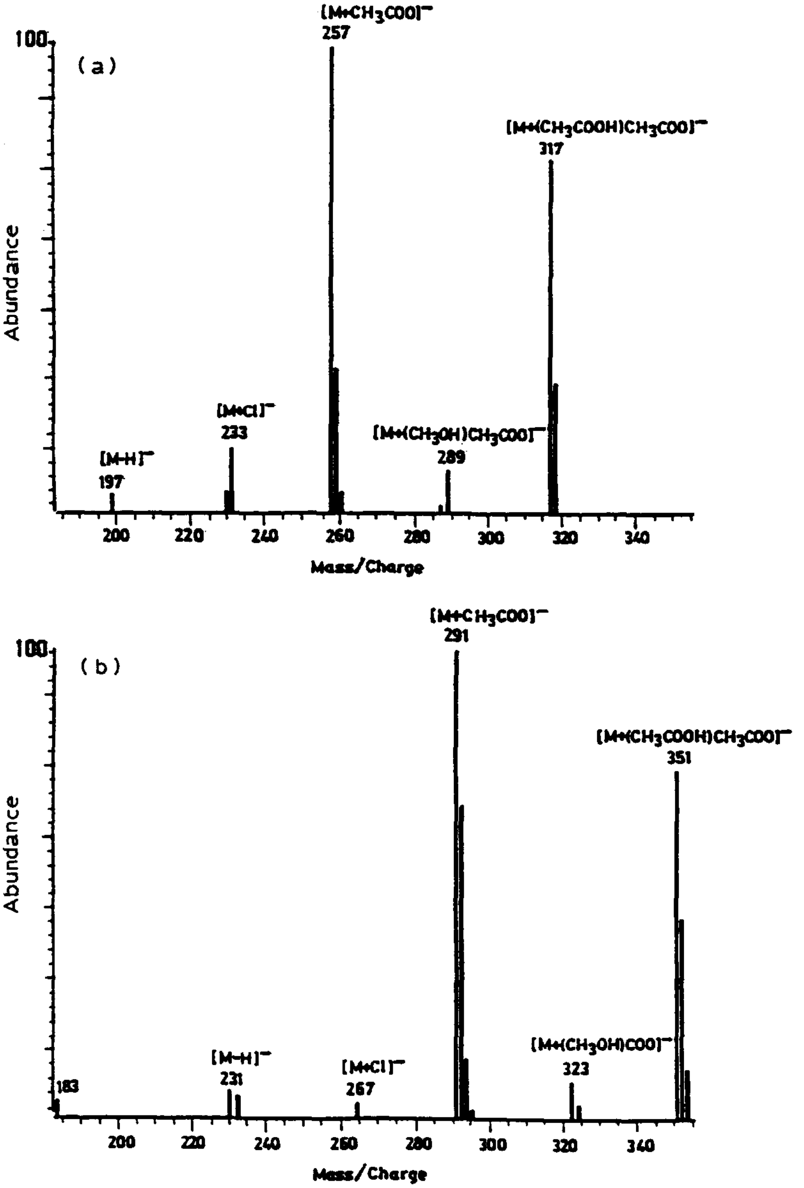

Fig. 5. Direct flow injection NI thermospray MS spectra of (a) monuron and (b) diuron under similar experimental conditions to Fig. 4.

5), as previously reported for linuron[18]. Consequently, there is no need to develop a post-column system for buffer addition in order to avoid degradation of the phenylurea herbicides[19]. To demonstrate that fact, the reconstructed ion chromatogram (RIC) in PI thermospray LC–MS for a mixture of monuron, diuron and linuron, obtained using methanol–water (50:50) + 0.1 *M* ammonium acetate, is shown in Fig.

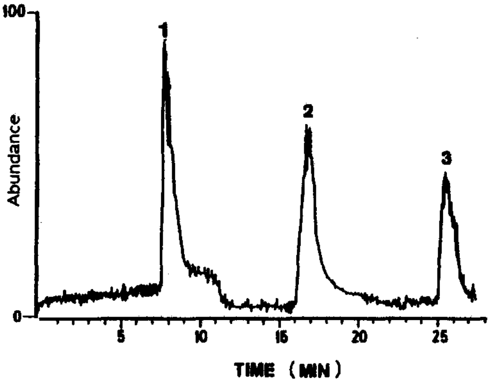

Fig. 6. Reconstructed ion chromatogram in thermospray LC–MS for a mixture of (1) monuron, (2) diuron and (3) linuron. Injected amount of each component, 300 ng. Column packing, 10 μm Spherisorb ODS-2; mobile phase, methanol–water (50:50) + 0.1 *M* ammonium acetate at a flow-rate of 1 ml/min; loop volume, 20 μl.

6. Another difference from the observations of Voyksner *et al.*[19] concerns the sensitivities in the NI mode. In our experiments, in NI thermospray LC–MS the phenylurea herbicides exhibit about 30% lower sensitivity than in the PI mode and not 4–5 orders of magnitude[19]. This large discrepancy can only be attributed to the different mode of ionization (filament-on in our experiments, *versus* filament-off ionization in their work).

Trialkyl phosphates

The PI thermospray LC–MS mass spectra of trimethyl phosphate and tripropyl phosphate obtained under conditions similar to those for organophosphorus pesticides are shown in Fig. 7. In both cases the $[M+NH_4]^+$ ion was the base peak and the $[M+H]^+$ ion was also observed. In contrast, in the literature[7] it has been reported that, when using GC–positive chemical ionization MS with ammonia as reagent gas for analyzing triethyl phosphate and tributyl phosphate, the $[M+H]^+$ ion was the base peak. This fact indicates a partial difference between ammonia chemical ionization and thermospray filament-on ionization, and conflicts with the similarities reported in the literature when comparing both ionization modes for different groups of pesticides[17]. In Fig. 7 it is also possible to observe the tendency to form adduct ions such as $[M + (CH_3CN)NH_4]^+$ and the dimer $[2M + H]^+$ ions at mass values higher than in ammonia chemical ionization.

In the NI thermospray LC–MS, the studied phosphates were not detected when 200 ng of each compound were injected. This fact could be due to their higher proton affinity than acetate ion preventing the detection by NI thermospray LC–MS[24].

Application to residue analysis

A sediment sample was spiked with three different organophosphorus pesticides: vamidothion (0.2 μg/g), azinphos-methyl (0.2 μg/g) and paraoxon-methyl (0.1 μg/g). Figure 8 shows the PI thermospray LC–MS chromatogram of the sediment after a pre-treatment procedure derived from that reported from Muir *et al.*[9]. The LC–MS

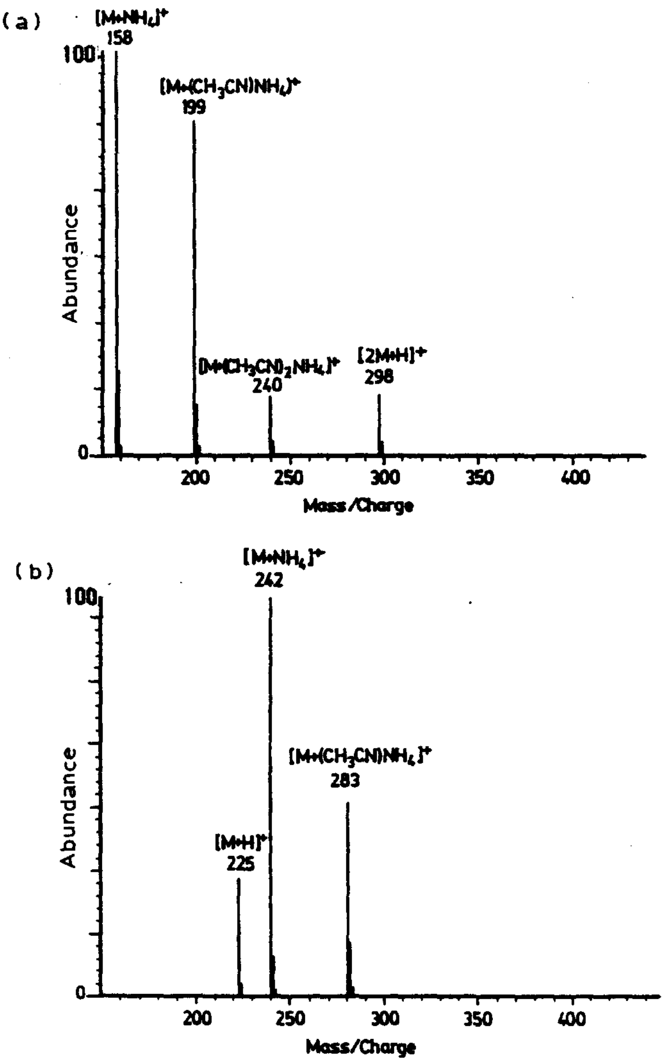

Fig. 7. Direct flow injection PI thermospray MS spectra of (a) trimethyl phosphate and (b) tripropyl phosphate under similar experimental conditions to Fig. 2.

was operated in the selected ion monitoring (SIM) mode, and three different *m/z* values per analyte were monitored, corresponding to the $[M + H]^+$, $[M + NH_4]^+$ and $[M + (CH_3CN)NH_4]^+$ ions. Vamidothion was monitored at m/z = 288, 305 and 346, azinphos-methyl at m/z = 318, 335 and 376, and paraoxon-methyl at m/z = 248, 265 and 306. As regards selectivity, only a small interference at the beginning of the chromatogram was noticed, and the sensitivity varied between 5 and 10 ng per compound (signal-to-noise ratio of 3–4). The thermospray LC–MS analyses were not performed in the NI mode of operation owing to its poor sensitivity as compared with the PI mode (about 3 orders of magnitude lower).

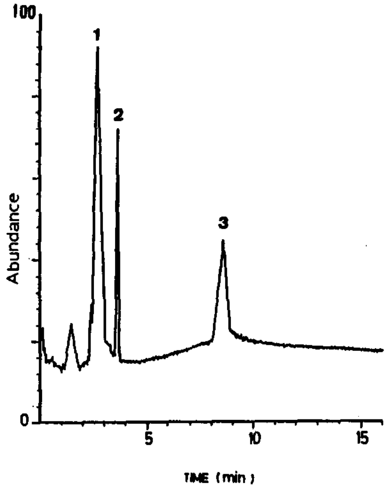

Fig. 8. Selected ion monitoring chromatogram in PI thermospray LC–MS of a sediment sample spiked with 0.2 ppm of vamidothion (peak 1), 0.1 ppm of paraoxon-methyl (peak 2) and 0.2 ppm of azinphos-methyl (peak 3). Injected amount: 5–10 ng. Column packing, 10-μm Spherisorb ODS-2; mobile phase, acetonitrile–water (75:25) + 0.1 *M* ammonium acetate at a flow-rate of 1 ml/min; loop volume, 20 μl.

CONCLUSION

The use of filament-on thermospray LC–MS in environmental analytical chemistry is a valuable technique with some points of similarity in the ionization process with other LC–MS approaches, such as DLI, and with ammonia chemical ionization GC–MS. By using two different modes of ionization in thermospray LC–MS, PI and NI modes, molecular weight information has been generally obtained with the formation of $[M+NH_4]^+$ and $[M + \text{acetate}]^-$ as base peaks, respectively. Sensitivities were always better in the PI mode than in the NI mode for the three groups of compounds studied. The lack of fragmentation is still a disadvantage in thermospray LC–MS when analyzing most environmental pollutants, for which an unequivocal identification is required.

ACKNOWLEDGEMENTS

Financial support was provided by the Consejo Superior de Investigaciones Científicas (C.S.I.C.), and Comision Asesora de Investigación Científica y Técnica (CAICYT), R. Alonso (C.S.I.C.) is thanked for technical assistance.

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