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IDENTIFICATION OF ORGANOPHOSPHORUS INSECTICIDES AND THEIR HYDROLYSIS PRODUCTS BY LIQUID CHROMATOGRAPHY IN COMBINATION WITH UV AND THERMOSPRAY–MASS SPECTROMETRIC DETECTION

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SUMMARY

Liquid chromatography with ultraviolet detection (LC–UV) and positive and negative ion mode (PI and NI, respectively) thermospray LC–mass spectrometry (LC–MS) were used for the analysis of the organophosphorus pesticides azinphos-methyl, diazinon and parathion-methyl and their corresponding breakdown products obtained after basic hydrolysis (pH 7–11). LC analysis was performed in the reversed-phase mode using methanol–water (80:20) or methanol–water (70:30) + 0.1 *M* ammonium acetate for LC–UV or LC–MS, respectively. By employing NI thermospray LC–MS the identification of *p*-nitrophenol, showing the $[2M-H]^-$ ion as the base peak, was feasible and confirmed the LC–UV chromatogram at 220 nm. When the PI mode was used, $[M+NH_4]^+$ and $[M+H]^+$ions were obtained as base peaks for azinphos-methyl and diazinon, respectively. The degradation rates varied from diazinon, which showed no degradation during a period of ten days, to azinphos-methyl and parathion-methyl, for which degradation occured rapidily when the pH was increased from 7 to 11.

INTRODUCTION

Organophosphorus pesticides are of interest owing to their wide use in agriculture and, in some instances, their high mammalian toxicity[1]. Their low persistency is known and hydrolysis can easily occur, either enzymatically as in organisms[2], or microbially in soils[3] or biotically and abiotically as in freshwaters and estuarine waters[4]. Further, the biodegradation rates in waters are dependent on the microbial community biomass and activity, chlorophyll, pH and total organic carbon[4].

Organophosphorus pesticides and their corresponding metabolites are currently analysed by capillary gas chromatography (GC) with thermionic[5,6] or flame photo-

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metric detection[7]. Mass spectrometric (MS) detection has been frequently employed in combination with capillary GC in the electron impact[8,9] or in the positive chemical ionization mode with methane [9–11], isobutane[12] and ammonia[10] or in the negative chemical ionization mode with methane[11,13], methane–oxygen[11] and dichloromethane[14]. However, organophosphorus pesticides which are thermally labile, such as trichlorfon[7,8], or polar such as parathion[5] and malathion[15], are difficult to analyse by GC. More problems arise when analysing organophosphorus pesticide breakdown products. In such instances even chemical derivatization is needed, *e.g.*, with *p*-nitrophenol, a metabolite of parathion obtained under basic hydrolysis, which is usually derivatized with pentafluorobenzyl bromide and subsequently analysed by GC with electron-capture detection[16].

Because of these problems, the analysis of organophosphorus pesticides and their corresponding metabolites has been carried out by liquid chromatography (LC) using either UV[17], reductive amperometric[18], selective thermionic[19] or MS detectors, employing either direct liquid introduction[20–22], moving belt[23] or thermospray interfaces[24–26]. Recently, even the applicability of supercritical fluid chromatography (SFC)–MS[27] and tandem mass spectrometry (MS–MS)[28] has been reported.

In the work described here LC–UV and LC–MS with a thermospray (TSP) interface have been applied to the analysis of the organophosphorus pesticides parathion-methyl, diazinon and azinphos-methyl and their corresponding breakdown products obtained under basic hydrolysis. Solutions of the decomposed analytes at pH 7–11 were directly injected into the LC system with UV detection at 220 nm and MS detection. The results demonstrated the utility of both positive ion (PI) and negative ion (NI) TSP LC–MS, as reported previously for a group of chlorophenols and herbicides[29]

EXPERIMENTAL

Materials

HPLC-grade water and methanol (Carlo Erba, Milan, Italy) were passed through a 0.45-μm filter from Scharlau (Barcelona, Spain) before use. Analytical-reagent grade ammonium acetate was obtained from Panreac, Montplet & Esteban (Montcada i Reixach, Barcelona, Spain), azinphos-methyl and diazinon from Polyscience (Niles, IL, U.S.A.) and parathion-methyl and *p*-nitrophenol from Dr. Su. I. Ehrenstorfer (Augsburg, F.R.G.).

Chromatographic system

Eluent delivery was provided either by two Model 510 high-pressure pumps, coupled with Model 680 automated gradient controller (Waters Chromatography Division, Milipore, Milford, MA, U.S.A.) and a Model 7125 injection valve with a 20-μl loop from Rheodyne (Cotati, CA, U.S.A.) or by a Spectra-Physics (San José, CA, U.S.A.) Model SP-8700 solvent delivery unit with an SP-8440 UV–VIS detector and an SP-4270 integrator (San José, CA, U.S.A.). Stainless-steel columns of dimensions (30 × 0.40 cm I.D. (Tracer Analítica, Barcelona, Spain) and 12 × 0.4 cm I.D. packed with 10-μm particle diameter Spherisorb ODS-2 (Merck, Darmstadt, F.R.G.) and Nucleosil C_{18} (Knauer, Bad Homburg, F.R.G.), respectively, were used.

Mass spectrometer

A Hewlett–Packard (Palo Alto, CA, U.S.A.) Model 5988A TSP LC–MS quadrupole mass spectrometer and a Hewlett-Packard Model 35741B instrument for data acquisition and processing were employed. The TSP temperatures were: stem 107°C, tip 178°C, vapour 194°C and ion source 264°C with the filament on. In all the experiments the filament-on mode of the TSP was used, in which conventional positive and negative chemical ionization can be carried out by using the vaporized mobile phase as the chemical ionization reagent gas. In essence, the vaporized mobile phase acts as a moderating gas to produce a high population of thermal-energy electrons which can be captured by the sample molecules. The TSP interface works at its highest sensitivity with at least 10% aqueous and with a sufficient concentration of ionizing additive, generally 0.1 *M* ammonium acetate. During the TSP vaporization process, interaction between the solvent and the volatile ionizing additive provides a soft ionization process, usually with little fragmentation of the primary ions[24–26].

RESULTS AND DISCUSSION

Degradation studies by LC with UV detection

The hydrolysis of the three model organophosphorus pesticides was studied by adjusting the pH of the standard aqueous solutions with ammonia and keeping the temperature at 20°C. Three different pHs, 7, 9 and 11, were considered for the hydro-

TABLE I

DEGRADATION OF PARATHION-METHYL, AZINPHOS-METHYL AND DIAZINON AT DIFFERENT pH AS A FUNCTION OF TIME

Temperature: 20°C. Results given are concentrations (mg/l) for an initial concentration of each pesticide of 100 mg/l.

Pesticide	*Time (h)*	*pH*		
		7	*9*	*11*
Parathion-methyl	2	100	98	95
	4	100	95	87
	6	100	93	77
	24	100	91	76
	48	100	86	63
	72	95	75	46
	144	88	55	27
	336	87	32	10
Azinphos-methyl	1	100	97	88
	4	100	78	47
	6	100	68	26
	48	100	55	0.1
	144	100	49	ND*
	216	100	40	ND*
Diazinon	240	No degradation observed		

* ND = Not detected.

lysis studies. This last value was used to simulate an analytical procedure for removing organophosphorus pesticides from polluted wastewater and the other two pH values are more similar to those found in natural waters[4].

The degradation of pesticides and the appearance of metabolites were followed by LC–UV. The Results obtained at different pH values and times are given in Table I. No degradation was observed for diazinon at any basic pH during ten days, whereas parathion–methyl and azinphos-methyl commenced to degrade considerably, to the extent of 14% and 45%, respectively, after 48 h at pH 9. These values agree in principle with the half-lifes reported for these pesticides[1], diazinon showing the longest half-life of the three compounds.

The corresponding LC–UV chromatograms of the three pesticides after degradation at pH 11 for 48 h are shown in Fig. 1. Parathion-methyl and azinphos-methyl showed one and three metabolites, respectively, before the peak of the compound, whereas for diazinon no metabolite was observed.

Thermospray LC–MS of organophosphorus pesticides and metabolites

By employing LC–MS the characterization of the investigated pesticides was confirmed and some metabolites could be identified. Similarly to GC–MS when using ammonia as reagent gas[10], azinphos-methyl showed an $[M+NH_4]^+$ ion as the base peak, as can be seen in Fig. 2. Degraded solutions of azinphos-methyl were also analysed. Fig. 3A shows a typical PI TSP LC–MS trace of a degraded solution of azinphos-methyl with an increase in the baseline noise at retention times from 2 to 6 min. The mass spectrum of this baseline noise showed an intense fragment at m/z = 141, which does not correspond to the reagent gas spectrum. This fragment ion can presumably be attributed to $[(CH_3O)_2PSO]^+$, corresponding to the functional group of the pesticide after a sulphur–oxygen exchange and obtained after hydrolysis.

The favoured formation of $[M+NH_4]^+$ ion as the base peak for organophosphorus pesticides in PI TSP LC–MS[23–25] indicates that such compounds exhibit proton affinities slightly lower than that for ammonia (858 kJ/mol)[30]. An exception is diazinon (see Fig. 2) with a pyrimidinyl group in its structure, yielding higher proton affinity values than ammonia and so ensuring the formation of $[M+H]^+$ion as the base peak. Other fragments corresponding to adduct ions between $[M-FG]^+$, where FG = $(C_2H_5O)_2PSO$, and the reagent gas adduct ions such as $[M-FG+NH_4]^+$, $[M-FG+(CH_3OH)NH_4]^+$ and $[M-FG+(CH_3COO)NH_4]^+$, with relative intensities of 50%, 30% and 50%, respectively, were also obtained. With diazinon no degradation peaks or fragments were observed in either LC–UV and LC–MS.

Fig. 4 shows the NI TSP LC–MS spectrum of parathion-methyl which shows an $[M-R]^-$ ion as the base peak, as previously reported for other organophosphorus pesticides[26]. The stability of this fragment may be explained by its quinone-like structure, which can easily delocalize the unpaired electron in the negative ion radical, as in NCI GC–MS[13]. Such a fragment ion, characteristic of organophosphorus pesticides, has already been observed in direct liquid introduction LC–MS but with much lower intensity (*ca.* 4%)[21]. Other fragments observed for parathion-methyl are at m/z = 183, which may correspond to $[(CH_3O)_2PS + CH_3COO - H]^-$, and at m/z = 198 and 277, from the *p*-nitrophenol structure of the parathion-methyl. The NI TSP LC–MS spectrum of *p*-nitrophenol, the main hydrolysis product of parathion-methyl, it is also shown in Fig. 4. The base peak corresponds to $[2M-H]^-$, which is

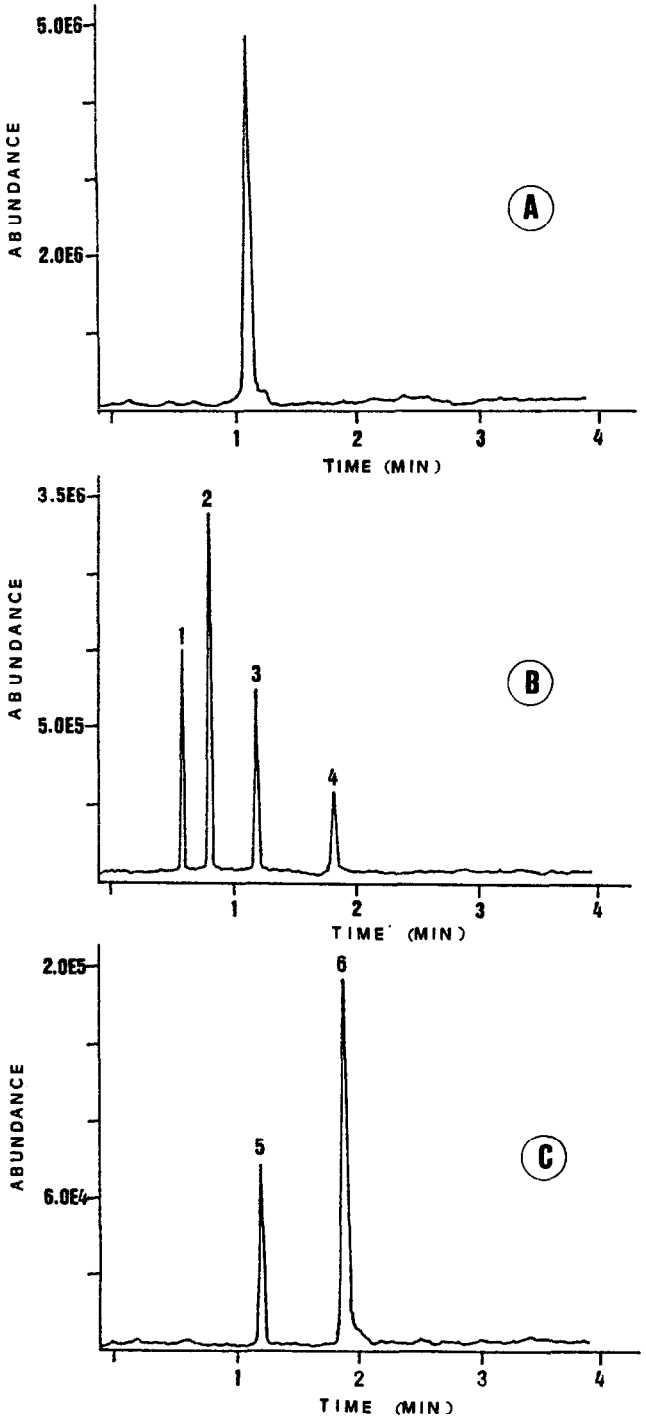

Fig. 1. LC trace with UV absorbance at 220 nm of solutions containing 100 ppm of each component after 48 h at pH 11: (A) diazinon: (B) azinphos-methyl (peak 4); and (C) *p*-nitrophenol (peak 5) and parathion-methyl (peak 6). Peaks 1, 2 and 3 are unknown breakdown products of azinphos-methyl. Column packing, 10-μm Nucleosil C_{18}; mobile phase, methanol–water (80:20) at a flow-rate of 1 ml/min; loop volume, 10 μl.

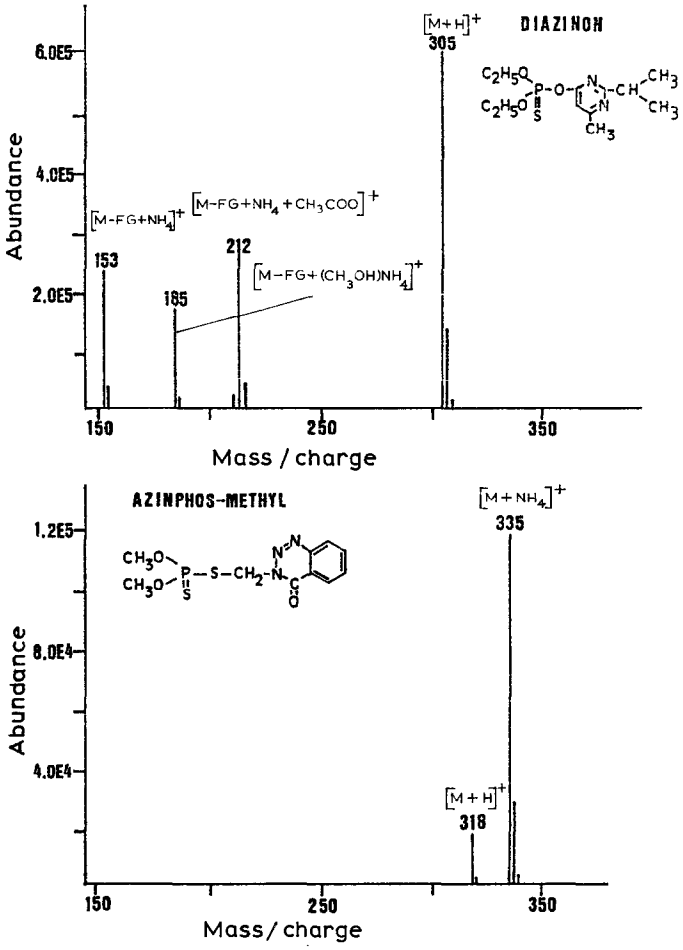

Fig. 2. Direct flow injection PI TSP MS spectra of diazinon and azinphos-methyl. Carrier stream, methanol–water (70:30) + 0.1 *M* ammonium acetate; flow-rate 1 ml/min; injection, 1 μg of each compound.

due to the fact that *p*-nitrophenol, similarly to chlorophenols[29] and nitrohydroxy polycyclic aromatic compounds[31], has a gas-phase acidity value much lower than 1464 kJ/mol, indicating stronger acidity than acetic acid, and hence acetate should deprotonate it. The high intensity of $[2M-H]^-$ has been attributed to the aromatic moiety containing electron-attracting nitro groups which stabilize the $[2M-H]^-$ ion by electron delocalization. The $[M-H]^-$ ions was not observed for *p*-nitrophenol, similarly to 2,4-dichlorophenol[29], owing to the relatively high scan values (above $m/z = 140$) required[25].

In PI TSP LC–MS no detection occurred when 200 ng of *p*-nitrophenol where injected under conditions similar to those above, whereas for parathion-methyl better sensitivities were obtained by employing PI TSP LC–MS, as generally occurs for organophosphorus pesticides[25,26]. In order to obtain the necessary information

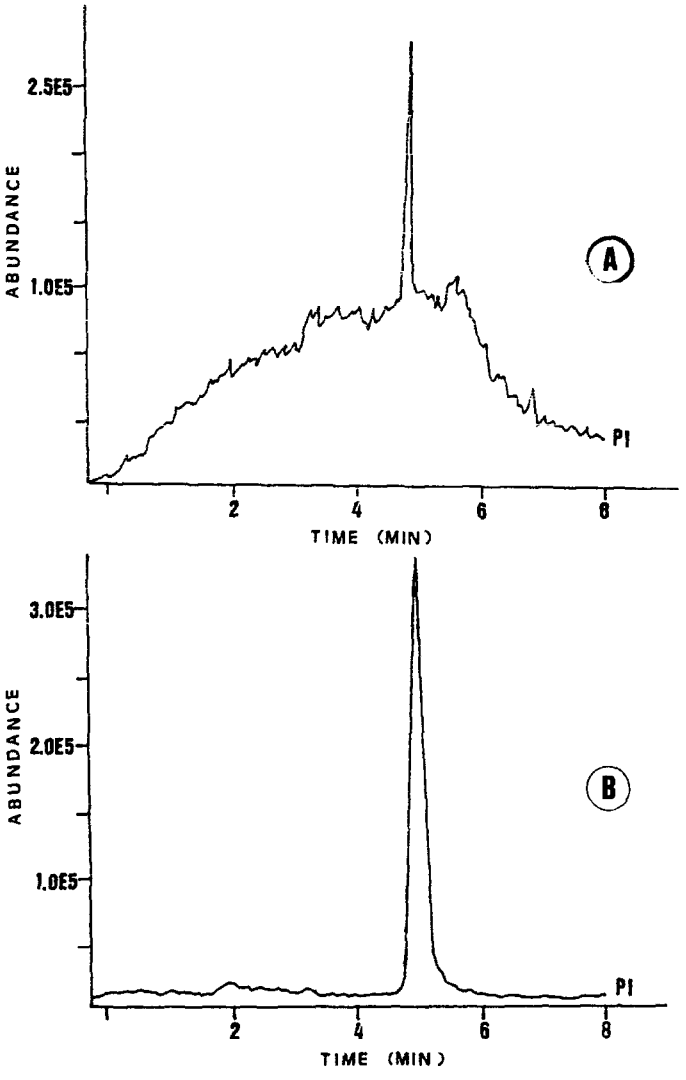

Fig. 3. Reconstructed ion chromatograms in PI TSP LC–MS of (A) 250 ppm of degraded solution of azinphos-methyl at pH 11 after 6 h and (B) 250 ppm of azinphos-methyl standard solution. Column packing, 10-μm Spherisorb ODS-2; mobile phase, methanol–water (70:30) + 0.1 *M* ammonium acetate at a flow-rate of 1 ml/min; loop volume, 20 μl.

about the formation of *p*-nitrophenol, a metabolite of parathion-methyl, NI TSP LC–MS was used as shown in Fig. 5, where chromatograms of a standard and a degraded solution of parathion-methyl are shown. The detection limit in NI TSP LC–MS for *p*-nitrophenol was at the low nanogram level (0.5 ng) whereas for parathion-methyl it was about 500 ng, using full scan conditions in both cases. The lower detection limit for *p*-nitrophenol is due to the fact that the molecular anion obtained as the base peak for *p*-nitrophenol, $[2M-H]^-$, is more stable than the corresponding base peak for parathion-methyl, $[M-R]^-$.

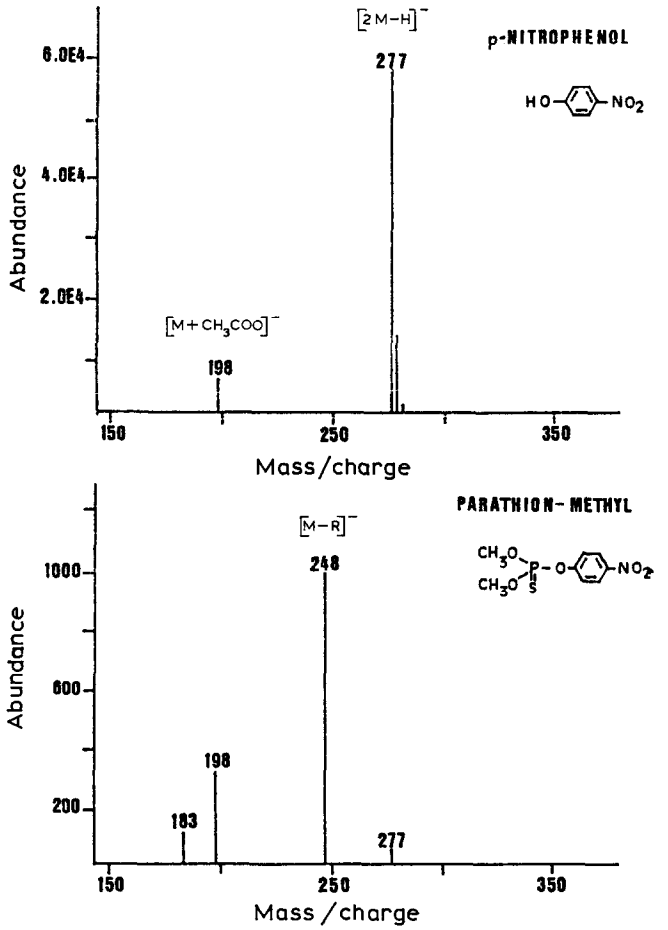

Fig. 4. Direct flow injection NI TSP MS spectra of *p*-nitrophenol and parathion-methyl. Conditions as in Fig. 2.

CONCLUSIONS

The combination of LC with UV detection at 220 nm and PI and NI TSP LC–MS allowed the unequivocal identification of azinphos-methyl, diazinon, parathion-methyl and the main breakdown product of parathion-methyl, *p*-nitrophenol, obtained after induced basic hydrolysis. Different behaviours were observed for the organophopshorus pesticides. Thus, for diazinon no degradation of solutions containing 100 ppm at pH 7–11 occurred after 10 days, whereas for azinphos-methyl and parathion-methyl 50% and 15% degradation, respectively, occurred after 4 h for solutions containing similar concentrations of pesticide and of similar pH. Possible degradation products of azinphos-methyl could be observed in the LC–UV chromatogram at 220 nm whereas in LC–MS no other peaks appeared. This fact can be attributed either to bad ionization of the degradation products or to the relatively

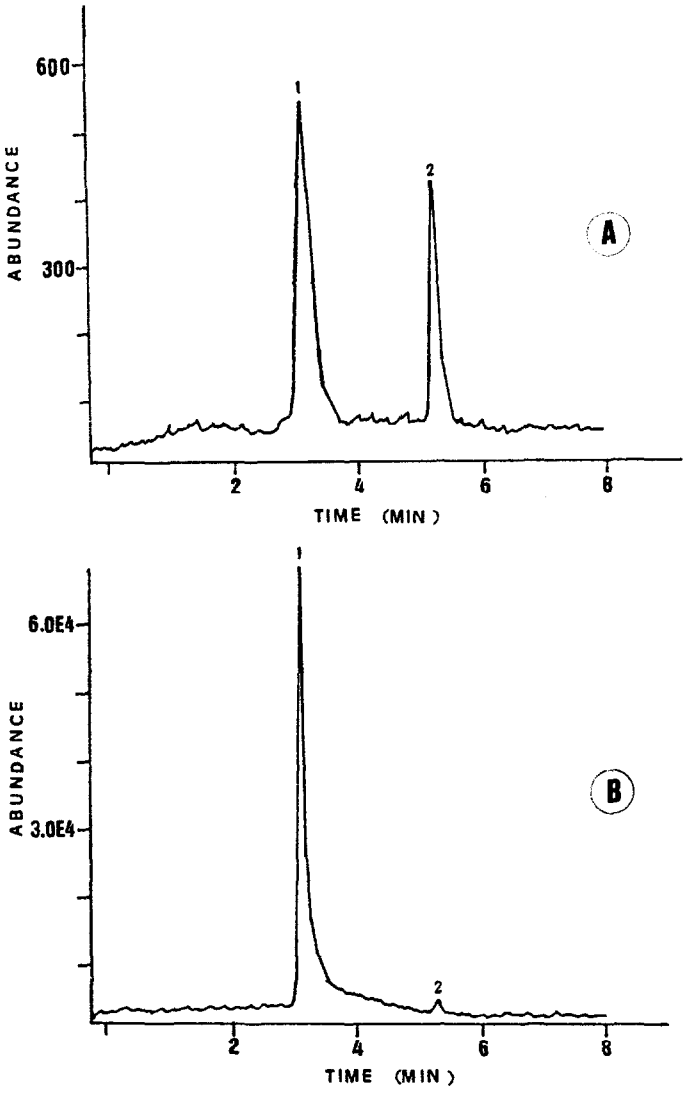

Fig. 5. Reconstructed ion chromatograms in NI TSP LC–MS of (A) 500 ppm of degraded solution of parathion-methyl (peak 2) at pH 11 after 6; peak 1 was identified as *p*-nitrophenol; (B) 2000 ppm of a standard solution containing *p*-nitrophenol (peak 1) and parathion-methyl (peak 2). Other experimental conditions as in Fig. 3.

high scan values (up to $m/z = 140$) which will not allow fragment ions to be detected below this value. The baseline noise showed a fragment ion at $m/z = 141$, probably attributable to $[(CH_3O)_2PSO]^+$.

Future developments will include the use of other organophosphorus pesticides as model compounds in order to study the formation of metabolites induced by hydrolysis by comparison with results in real environmental situations. Such model

compounds will be chosen in order to yield metabolites that can easily be measured by LC as an alternative to GC methods that require derivatization; *e.g.*, 2,4,5-trichlorophenol, the main metabolite of the organophosphorus pesticides ronnel and tetrachlorvinphos, can be analysed by LC–UV at 230 nm and NI LC–MS[28].

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