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Determination of organophosphorus pesticides and their transformation products in river waters by automated on-line solid-phase extraction followed by thermospray liquid chromatography–mass spectrometry

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Abstract

The trace-level determination of ten priority organophosphorus (OP) pesticides (e.g. chlorpyrifos-methyl, diazinon, disulfoton, fenthion, fenamiphos) and various transformation products (TPs; e.g. disulfoton sulfoxide, fenthion sulfoxide etc.) using automated on-line solid-phase extraction (SPE) with C_{18} precolumns followed by LC–MS and thermospray interface with time-scheduled selected-ion monitoring (SIM) was developed.

Two main ions (usually $[M + H]^+$ and $[M + NH_4]^+$ or $[M + CH_3CN]^+$) were used for each pesticide in the positive ion (PI) detection mode, while $[M - H]^-$ and $[M + HCOO]^-$ ions were used in the negative ion (NI) mode. The proposed method requires 100 ml of sample for a limit of detection (LOD) of 0.01–0.1 μg/l. Calibration graphs were constructed by preconcentrating 100 ml of water spiked with the pesticide mixture at various concentrations (from 0.025 to 2 μg/l). Good linearity was observed for most of the analytes studied.

The experimental setup described in this paper was applied to study the kinetics of degradation of ten organophosphorus pesticides in spiked river water samples. The different samples were first analyzed by an automated on-line precolumn exchange system (OSP-2) followed by LC with diode array detection. To confirm the identity of the organophosphorus pesticides detected, the samples were then analyzed by automated on-line SPE–LC–MS. The method permitted unequivocal identification of many of the TPs formed during the experiments, e.g. the oxo-derivatives of chlorpyrifos-methyl, temephos and pyridafenthion, fenamiphos sulfoxide. Many of these TPs are here reported for the first time since previously used MS-based techniques were not sensitive enough.

1. Introduction

The determination of organophosphorus (OP) pesticides is of concern because of their extensive use as insecticides in different types of cultivation, e.g. rice [1], and their use in the elimination of pests [2]. Residue levels of OP pesticides such as pyridafenthion and fenitrothion have been reported in environmental matrices [3,4]. These compounds can persist in the environment for several days, and biotic and abiotic degradation can occur [5], with the subsequent formation of various transformation products (TPs). Such TPs may be more toxic than the parent compounds [6], and therefore,

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pesticide degradation and their stability in the environment are becoming study subjects of increasing interest. Proof that OP pesticides are of concern is their inclusion in different water monitoring programs such as the National Pesticide Survey (NPS) and the Commission of the European Communities (CEC). In this respect, the CEC 76/464/EEC council directive list of pesticides to be monitored in the aquatic environment includes different OP pesticides, e.g., parathion, methyl-azinphos, fenitrothion, demeton, fenthion, and malathion.

On-line solid-phase extraction (SPE) techniques coupled to liquid chromatography and thermospray mass spectrometry (LC–TSP-MS) are currently applied for the determination of different pesticide groups, including organophosphorus [7], triazines [8,9], and chlorinated phenoxyacids [9,10], in different environmental water matrices. Up to now, no studies dealing with the coupling of on-line SPE followed by LC–TSP-MS for the trace determination of OP pesticides in water samples have been reported.

In our Department, we are also particularly interested in following the degradation of OP pesticides in river waters in order to measure the degradation kinetics and half-lives of pesticides under real environmental conditions [1,4]. In this respect, on-line SPE–LC–TSP-MS is a useful technique for the confirmation of unknown pesticide TPs formed during the degradation experiments.

The objectives of the present paper are: (i) the development of an on-line SPE–LC–TSP-MS method for the direct determination of trace levels of OP pesticides in water samples and the establishment of analytical parameters such as linearity and limit of detection of the analytical method, and (ii) the application of the on-line method to the quantitation of OP pesticides in river waters submitted to natural degradation with special emphasis on the degradation products formed, such as sulfoxide and oxo-metabolites. The information obtained by the developed on-line system will be of interest to environmental water analysis.

2. Experimental

2.1. Chemicals

HPLC grade water, acetonitrile, and methanol (Baker, Deventer, Netherlands) were filtered through a 0.45-μm filter before use. Ammonium formate was purchased from Fluka (Buchs, Switzerland). Chlorpyrifos-methyl, diazinon, disulfoton, fenamiphos, fenthion, isofenphos, malathion, metidathion, pyridafenthion, and temephos were obtained from Promochem (Wesel, Germany).

2.2. Chromatographic conditions

The eluent was delivered by two Model 510 high-pressure pumps coupled to a Model 680 automated gradient controller (Waters, Milford, MA, USA). The automated SPE device (OSP-2, Merck, Darmstadt, Germany) was connected on-line with the gradient pumps. The OSP-2 consists of a cartridge magazine containing the precolumns, which are held in a ring. Two electrically operated six-port switching valves are arranged in such a way that preconcentration is carried out in the preparation clamp, and afterwards the ring rotates so that the precolumn is transferred to the elution position, and the analytes are desorbed (Fig. 1). A LiChroGraph Model L-6200A intelligent pump (Merck–Hitachi) was used to deliver the solvents to condition the precolumns and the water that contained the pesticides. The precolumns were conditioned by flushing 10 ml of methanol and then 10 ml of HPLC grade water at 1 ml/min. Water volumes of 100 ml spiked with pesticides were preconcentrated on disposable precolumns (Merck, Darmstadt, Germany) prepacked with 10 μm LiChrospher Si100 RP-18. Prior to SPE, river water samples were filtered through 0.45-μm filters (Millipore) to remove suspended matter.

A Supershper cartridge column (250 × 4 mm I.D.) packed with 4 μm Superspher C_8 (Merck) was used. The LC mobile phase was acetonitrile–water with 0.05 *M* ammonium formate with

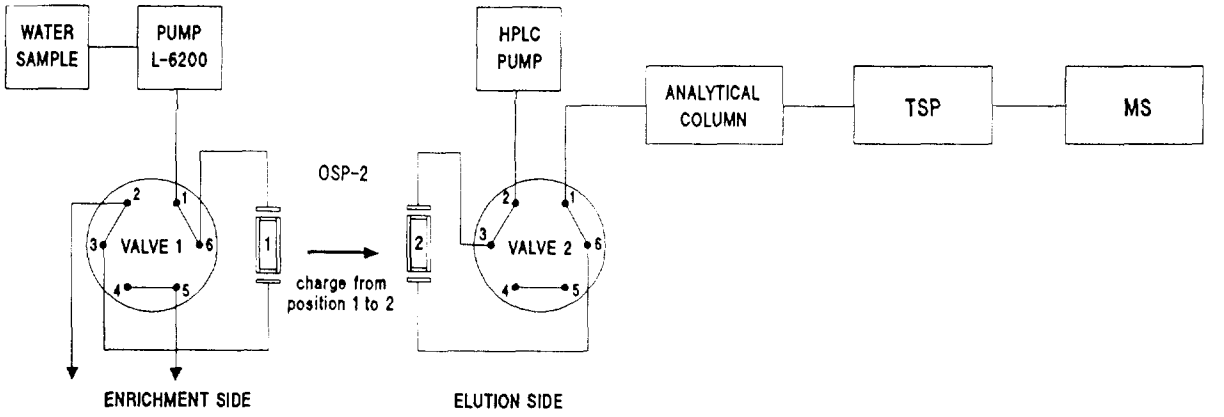

Fig. 1. General diagram of the system used for on-line preconcentration and determination of pesticides in water samples.

the following gradient: from 15:85 (v/v) to 30:70 (v/v) in 15 min, and from there to 90:10 in 15 min, and subsequently kept isocratic for 10 min. Return to the initial conditions was accomplished in 5 min.

Flow-injection (FI) TSP-MS experiments without the use of the column were performed with acetonitrile–water (50:50, v/v) with 0.05 *M* ammonium formate. In all cases, the flow-rate was set at 1 ml/min, and the amount injected was 200 ng.

2.3. Mass spectrometric analysis

A Hewlett-Packard (Palo Alto, CA, USA) 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 varied from 98 to 95°C (stem), and the tip was maintained at 198°C at the beginning and end of the gradient. The ion source was set at 250°C. The filament-on ionization mode was used in all experiments, with positive (PI) and negative (NI) ion chemical ionization.

2.4. Calibration graphs

The linearity and reproducibility of the on-line SPE system were examined. Calibration graphs were constructed for all parent pesticides by percolating 100 ml of HPLC grade water spiked at six different concentrations (from 0.025 to 2 μg/l) through C_{18} precolumns. Analytical conditions were as described above. Quantitation was performed with an external standard calibration method, using time-scheduled selected-ion monitoring (SIM); the ions selected for quantitation are italicized in Table 1.

2.5. Water analysis

Ebre river water samples were collected, filtered through 0.45-μm filters (Millipore) to remove suspended particles, and spiked with each individual pesticide (listed in Table 2) at a level of 50 μg/l, lower than the amount applied for treatment purposes (200 μg/l). These waters were placed in a transparent glass bottle on a terrace roof in Barcelona on January 19th. After four weeks they were collected and analyzed by on-line SPE–LC–TSP-MS. Chromatograms were recorded under full scan mode from m/z 150 to 550 in the PI mode. The water sample which contained chlorpyrifos-methyl was also analyzed in the NI mode.

3. Results and discussion

3.1. Mass-spectral information

Even though there is ample literature concerning the determination of OP pesticides by

Table 1
Mass fragments and relative abundances (%) observed in flow-inject thermospray LC–MS in PI and NI modes and filament-on conditions

Compound	M_r	Ions (m/z) (% abundance) PI mode	Ions (m/z) (% abundance) NI mode
Chlorpyrifos-methyl	322	*323*(58) $[M + H]^+$	
		340(100) $[M - NH_4]^+$	186(100) $[M - HCl]^-$
Diazinon	304	*305*(100) $[M + H]^+$	169(100) $[(C_2H_5O)_2POS]^-$
			303(4) $[M - H]^-$
Disulfoton	274	153(75) $[CH_3CH_2O)_2PS]^+$	185(100) $[(C_2H_5O)_2PS_2]^-$
		275(100) $[M + H]^+$	
		292(26) $[M + NH_4]^+$	
Disulfoton sulfone	306	307(100) $[M + H]^+$	185(100) $[(C_2H_5O)_2PS_2]^-$
		324(44) $[M + NH_4]^+$	
Disulfoton sulfoxide	290	159(73)	185(100) $[(C_2H_5O)_2PS_2]^-$
		291(100) $[M + H]^+$	
		308(15) $[M + NH_4]$	
Fenamiphos	303	*304*(100) $[M + H]^+$	153(15) $[CH_3S - C_6H_3 - CH_3O]$
		321(17) $[M + NH_4]^+$	302(100) $[M - H]^-$
Fenamiphos sulfone	335		185(65) $[M - 150]^{-a}$
		336(30) $[M + H]^+$	320(98) $[M - CH_3]^-$
		353(100)$[M + NH_4]^+$	334(100) $[M - H]^-$
		377(11) $[M + H + CH_3CN]^+$	380(42) $[M + HCOO]^-$
Fenamiphos sulfoxide	319		169(100) $[M - 150]^{-a}$
		320(93) $[M + H]^+$	185(82) $[169 + HCOOH - CH_2O]^-$
		337(100) $[M + NH_4]^+$	215(29) $[169 + HCOOH]^-$
		361(17) $[M + H + CH_3CN]^+$	318(85) $[M - H]^-$
Fenthion	278	183(53)	153(28) $[M - (CH_3O)_2PS]^-$
		279(25) $[M + H]^+$	279(48) $[M + H]^-$
		296(100) $[M + NH_4]^+$	340(100) $[M + HCOOH + HCOO - CO]^-$
Fenthion oxon	262	171(13) $[C_8H_9OS + NH_4]^+$	153(100) $[M - (CH_3O)_2PO]^-$
		263(24) $[M + H]^+$	194(40)
		280(100) $[M + NH_4]^+$	
		304(8) $[M + H + CH_3CN]^+$	
Isofenphos	345	287(100) $[M - NHCH(CH_3)_2]^+$	344(100) $[M - H]^-$
		346(89) $[M + H]^+$	
Malathion	330	331(12) $[M + H]^+$	157(100) $[(CH_3O)_2PS_2]^-$
		348(100) $[M + NH_4]^+$	329(33) $[M - H]^-$
Malaoxon	314	315(54) $[M + H]^+$	172(100) $[(CO_2CH_2CH_3)_2 - C_2H_2]^-$
		332(100) $[M + NH_4]^+$	230(48) $[172 + CH_3COO + H]^-$
			313(18) $[M - H]^-$
Metidathion	302	303(18) $[M + H]^+$	157(100) $[(CH_3O)_2PS_2]^-$
		320(100) $[M + NH_4]^+$	287(6) $[M - CH_3]^-$
Pyridafenthion	340	*341*(100) $[M + H]^+$	169(100) $[(C_2H_5O)_2POS]^-$
			203(10) $[M - (C_2H_5O)_2PO]^-$
			340(11) $[M]^-$
Temephos	466	466(53) $[M]^+$	341(35) $[M - (CH_3O)_2PS]^-$
		484(100) $[M + NH_4]^+$	451(100) $[M - CH_3]^-$
Temephos sulfoxide	482	348(44) $[M - (OCH_3)_2P + CH_3CN]^+$	341(6) $[M - (CH_3O)_2POS]^-$
		482(45) $[M]^+$	373(22) $[M - (CH_3O)_2PO]^-$
		483(100) $[M + H]^+$	451(100) $[M - CH_3O]^-$

Eluent: acetonitrile–water (50:50, v/v) with ammonium formate 0.05 *M*. Amount injected = 200 ng.
[a] $150 = PO(OC_2H_5) + NHCH(CH_3)_2$.

Table 2
Calibration graphs constructed by preconcentrating 100 ml of river water sample, spiked with the pesticide mixture at six different concentrations (from 0.025 to 2 μg/l)

Compound	Calibration equation	R^2	Linear range (μg/l)	LOD (μg/l)
Chlorpyrifos-m	$Y = 571066X + 45888$	0.9955	0.100–2	0.088
Diazinon	$Y = 58415859X + 2785451$	0.9833	0.025–2	0.004
Disulfoton	$Y = 11124131X + 1431491$	0.8666	0.025–2	0.002
Fenamiphos	$Y = 15544948X + 827158$	0.9803	0.025–2	0.012
Isofenphos	$Y = 2608255X + 22174$	0.9987	0.025–2	0.011
Malathion	$Y = 3688033X + 201632$	0.9831	0.025–2	0.029
Metidathion	$Y = 2555832X + 645204$	0.9860	0.025–2	0.016
Pyridafenthion	$Y = 12968626X + 1132752$	0.9947	0.025–2	0.011
Temephos	$Y = 47078X + 32472$	0.9920	0.050–2	0.038

Chromatograms were recorded under time-scheduled selected-ion monitoring (SIM).

TSP-MS [10–18], few consider the need for characterization of TPs for further studies on the biodegradation of pesticides [14,15]. For this reason, the parental pesticides selected in this study were characterized by TSP-MS together with those TPs that were commercially available. Table 1 shows the main fragment ions and relative abundances of the parent pesticides studied and all their TPs in both PI and NI mode of operation. The spectral information obtained from the different TPs was mandatory for the further identification and characterization of such compounds in spiked river water submitted to degradation.

(a) Positive ions. The favored formation of $[M + NH_4]^+$ as the base peak for OP indicates that the proton affinity for these compounds is slightly lower that of ammonia (858 kJ/mol), as reported previously [15]. There are three exceptions among the different OPs studied that certainly show $[M + H]^+$ as base peak, i.e. fenamiphos, diazinon, and pyridafenthion. This has been explained in previous articles by our group [11,14,17]. However, the formation of $[M + H]^+$ ion as the second most abundant ion (relative abundance varying from 12% to 93%) indicates that these compounds exhibit intermediate basicity, with an equal or lower proton affinity than ammonia, favoring both the formation of $[M + NH_4]^+$ and $[M + H]^+$ ion. The relative abundances of both ions can be slightly different when compared to those of the parent compounds (temephos or fenamiphos) and the corresponding TPs (sulfoxide and sulfone), as shown in Table 1. In the case of fenamiphos sulfoxide and sulfone, these TPs have a decreased proton affinity, and then $[M + NH_4]^+$ ion is formed. In the case of temephos, its sulfoxide presents a $[M + H]^+$ ion.

(b) Negative ions. Table 1 presents the NI ions of the pesticides studied using filament-on ionization. The pesticides which produced the strongest signal and fragmentation were the electronegative compounds. In this sense, the NI mode of ionization is advantageous for the determination and identification of pesticides and their TPs from environmental matrices, since it yields more structural information and is usually more selective.

The characterization of OPs by TSP-MS in the NI mode yielded specific fragment ions, as reported previously [12,16], which correspond to molecular ions or adducts with formate as the base peaks. However, under the conditions used in this experiment, strong fragmentation occurred for diazinon, disulfoton and its TPs, fenamiphos sulfoxide, fenthion oxon, malathion, malaoxon, metidathion, pyridafenthion, and temephos and its sulfoxide. In the case of diazinon and disulfoton, the base peak corresponds to m/z 169 $[(C_2H_5O)_2POS]^-$ and m/z 185 $[(C_2H_5O)_2PS_2]^-$, respectively. This be-

havior has already been observed for some OP pesticides [18]. For fenamiphos, whereas the parent compound and its sulfone exhibited $[M-H]^-$ as their base peak, the sulfoxide presents an unidentified ion at m/z 169. However, adducts with fragments have been observed for this compound at m/z $[M+16]^-$, which can be attributed to $[169+HCOOH-CH_2O]^-$ [18].

Fenthion exhibited an ion at m/z 279, which corresponds to a $[M-H]^-$ ion similar to that observed by Vreeken et al. [18] for other pesticides. The base peak for fenthion corresponded to m/z 340, which can be tentatively identified as $[M+61]^-$, corresponding to $[M+HCOOH+HCOO^- - CO]^-$. The loss of CO from the molecule has also been reported by Vreeken et al. [18] for phenyl urea pesticides. Fenthion oxon presents two fragments at m/z 153 and 194.

3.2. Calibration graphs

The calibration data of these pesticides obtained by SIM are presented in Table 2. The linear relationship between the area of each peak versus concentration was evaluated by calculating the correlation coefficient. All compounds showed an R^2 around 0.99 calculated from the limit of detection (LOD), which implies that these compounds exhibit good linearity within this range, and therefore quantitation can be performed at levels of a few μg/l using on-line SPE–LC–TSP-MS. The exception was fenthion, which could not be determined due to the fact that it is easily degraded in aqueous solution. Its instability in water solution has already been reported [19], and its exclusion from the National Pesticide Survey (USA) list proves that it is unstable in well water within a period of 14 days. The linear range of chlorpyrifos-methyl was from 0.1 to 2 μg/l. This compound was monitored at m/z 323, corresponding to $[M+H]^+$, which has an abundance of 58%. A better signal could be obtained at m/z 340, but it was discarded due to a coelution problem with pyridafenthion.

The SIM LODs of the studied pesticides are presented in Table 2. They were calculated by selecting the lowest concentration of the spiked water sample that gave a signal-to-noise ratio of 3, and measuring the height of the peaks.

The results obtained were far below the limits imposed by the EEC (0.1 μg/l), indicating the suitability of this method for screening OPs in field studies.

Calibration plots were not constructed for the TPs, since these compounds were qualitatively analyzed in spiked river water submitted to degradation. However, under the analytical conditions used in this work, it can be expected that when percolating 100 ml of water, losses of these compounds due to breakthrough might be expected [20], and thus, the recovery will not reach 100% but the LOD will be augmented. Regardless, the breakthrough value of the TPs is a parameter that has to be taken into account when performing degradation studies.

3.3. Environmental application

This technique was used to unequivocally identify the parental pesticides and TPs formed by degradation under semi-natural conditions (Ebre river water spiked at a low concentration level and exposed outdoors). These waters were analyzed four weeks after spiking by both on-line SPE–LC–diode-array detection (DAD) and on-line SPE–LC–TSP-MS in order to detect as many TPs as possible. Table 3 shows the main compounds identified by TSP-MS in the PI mode and summarizes the major ions and their relative abundances. Since not all the TP standards were characterized by flow-injection analysis, the scan mode was used in order to detect as many as possible.

The determination of TPs of OP pesticides is of importance since the oxo-derivatives and sulfoxides, which are more toxic than the parent compounds, can be formed under natural conditions and therefore may cause toxic effects to natural flora and fauna [19]. In this respect, the EEC Directive on the Quality of Water Intended for Human Consumption has already stressed the need for analyzing pesticides and TPs at levels under 0.1 μg/l. Moreover, the NSP has published a list in which pesticides and their TPs detected in ground water were automatically

Table 3
List of the OPs oxo or sulfoxide TPs studied, their molecular mass (M_r), retention time (min), and major ions obtained by TSP-MS in the PI mode of Ebre river water samples spiked at a level of 50 μg/l and analyzed four weeks after spiking

Compound	M_r	LC–TSP-MS	
		t_R	Main ions
Chlorpyrifos-m	322	33.9	323[M + H]$^+$
oxon	306	28.5	324[M + NH_4]$^+$
Diazinon	304	35.8	305[M + H]$^+$
oxon	288	29.1	289[M + H]$^+$
Disulfoton	274	30.1	275[M + H]$^+$
sulfoxide	290	24.6	291[M + H]$^+$
			308[M + NH_4]$^+$
Fenamiphos	303	28.7	304[M + H]$^+$
sulfoxide	319	20.9	320[M + H]$^+$
			337[M + NH_4]$^+$
Fenthion	278	34.1	279[M + H]$^+$
sulfoxide	294	24.9	295[M + H]$^+$
			312[M + NH_4]$^+$
			336[M + CH_3CN]$^+$
Isofenphos	345	30.3	287[M − $NHCH(CH_3)_2$]$^+$
			346[M + H]$^+$
oxon	329	25.3	330[M + H]$^+$
Malathion	330	31.7	331[M + H]$^+$
			348[M + NH_4]$^+$
Methidathion	302	31.0	303[M + H]$^+$
			320[M + NH_4]$^+$
Pyridafenthion	340	29.3	341[M + H]$^+$
oxon	324	23.5	325[M + H]$^+$
Temephos	466	31.3	484[M + NH_4]$^+$
			525[M + $(CH_3CN)NH_4$]$^+$
oxon	450	31.6	468[M + NH_4]$^+$

Analytical conditions as described in the Experimental section.

included. For this reason, there is a demand for an analytical method which accomplishes such needs.

It can be observed from Table 3 that some oxons were detected four weeks after the water samples were spiked (chlorpyrifos-methyl, diazinon, isofenphos, pyridafenthion, and temephos). The toxicity of the oxo-derivatives has been demonstrated by Miyamoto et al. [21]. In all cases, the oxygen analogues could be identified since they followed the same adduct formation as the parent pesticide (except for chlorpyriphos-methyl) and eluted before their parent compound. Temephos oxon, however, eluted after temephos, possibly due to the stronger effect of the P = S group towards the C_8 of the stationary phase compared to the P = O group.

The presence of these compounds in aqueous solution for at least four weeks means that, according to the NPS-EPA, they are stable for at least 14 days, and thus their inclusion in the NPS-US-EPA list of toxic contaminants should be contemplated. Since the NPS-US-EPA stability study was conducted without the use of LC–MS, the TPs were probably also formed but were not detected. In this respect, LC–MS is a very useful technique for the identification of OP pesticide TPs.

The production of sulfoxide derivatives was noted for disulfoton, fenamiphos, and fenthion. In the case of disulfoton sulfoxide, the ions were located at m/z 291 $[M + H]^+$ and m/z 308 $[M + NH_4]^+$, as indicated by flow-injection information. This compound has been included in the NPS-EPA list.

Fenamiphos sulfoxide was the main TP formed, which is in accordance with Ref. [22], which reports the formation of this compound by photodegradation. In neither case was the sulfone found in the water sample, simply because an other oxidation step is implied for its formation in the environment. Fenamiphos presented also an unidentified peak with a molecular mass of 288 (not shown in Table 3), which was tentatively identified as a loss of CH_3 from the original molecule. Chukwudebe et al. [23] already pointed out that after UV irradiation, dealkylation with loss of CH_3 could occur in phosphorotioate pesticides. To our knowledge, such a TP has not been reported previously as being formed in spiked river water submitted to degradation. The other ions formed were 153(67) (unknown fragment), 289(29) $[M + H]^+$, 306(100) $[M + NH_4]^+$, and 317(88) (unknown adduct).

Fenthion is a compound which can degrade easily in aqueous solution but could still be identified at m/z 279 $[M + H]^+$ four weeks after spiking. Its sulfoxide could also be identified (see Table 3). Fenthion is actually a controversial compound since it has been removed from the

NPS-EPA list, as it is considered unstable in well-water solution over a period of 14 days. However, the conditions used in this experiment permitted the detection of traces of fenthion.

In this list, fenthion sulfoxide has not yet been considered as a toxic substance discharged into the environment. Two more peak were identified as possible TPs of fenthion. Since not for all TPs standards are available, its previous characterization by flame ionization (FI) was not possible, and the identification of TPs with this technique is complex due to the presence of fragments and rearrangements which are hard to interpret. The first unknown TP eluted at 27.5 min and produced an ion at m/z 247 as the main peak, which could correspond to the molecular ion X, m/z 264(45) $[X + NH_4]^+$ and 296(37) $[X + (CH_3OH)NH_4]^+$. More sophisticated techniques are needed to identify this TP, such as MS–MS, as reported for chlorotriazines [24]. The second unidentified peak eluted at 34.02 min and presented m/z 153 as its base peak, 171(52) and 277(28). The ion at m/z 153 could correspond to the loss of $(CH_3O)_2PS$ from the parent pesticide, yielding $[C_8H_9OS]^+$. The ion at m/z 171 is identified as $[M + NH_4]^+$, following the same adduct formation as the parent pesticide.

Similarly, waters containing isofenphos hold the parent pesticide, the oxygen derivative, and an unidentified TP at m/z 287(100), at 28.16 min. Since no fragmentation occurred, its molecular structure is uncertain, but as it was similar to isofenphos, it could tentatively be identified with the loss of $(CH_3)_2CHNH$ from the parent compound.

Fig. 2 shows the LC–DAD of a water sample spiked with chlorpyrifos-methyl and analyzed after four weeks, with two peaks that correspond to chlorpyrifos-methyl and to its oxon. Confirmation was performed by means of MS detection. Water samples spiked with chlorpyrifos-methyl were analyzed in both the PI and NI mode, to gain more structural information. Compounds such as chlorpyrifos-methyl showed good sensitivity [25], and it was found that loss of chlorine was involved. The total ion chromatogram (TIC) in the PI and NI ionization mode and the ion traces at m/z 323, 324, and 198 are presented, which correspond to the parent compound and the two TPs formed, chlorpyrifos-methyl oxon (m/z 324) and 3,5,6-trichloro-2-pyridinol (m/z 198). The latter exhibited loss of chlorine atoms and proton abstraction, similar to the parent compound. One report [26] also describes the formation of 3,5,6-trichloro-2-pyridinol by hydrolysis. The presence of this compound agrees with other studies on photolysis in water using the suntest apparatus [22] and in different solid surfaces [26]. The toxicity of 3,5,6-trichloro-2-pyridinol is higher than that of chlorpyrifos, with EC_{50} values of 18.6 and 46.3 μg/ml, respectively, as calculated with the Microtox system [27]. Also, it has been reported that this compound is toxic to soil microorganisms, resulting in lower mineralization and therefore enhanced persistence of chlorpyrifos in soil.

Other samples analyzed with the NI mode of ionization were diazinon, fenamiphos, fenthion, isofenphos, pyridafenthion, and temephos. The use of TSP-NI permitted further confirmation of the results with another detection technique. Of the pesticides mentioned, temephos, fenthion, and fenamiphos gave no signal. As stated by Barceló et al. [25], sensitivity for OP pesticides of the parathion group was higher in the PI than in the NI mode; hence, these compounds could not be detected under NI mode and scanning. The only oxygen derivative found was that of pyridafenthion, with m/z at 324 $[M]^{-\cdot}$. The poor formation of oxons in the NI mode of ionization was expected since for oxygen analogues the oxo group enhances the proton affinity compared with the thio group, and therefore, the sensitivity is one order of magnitude higher with PI [25].

Waters spiked with diazinon presented two peaks at m/z 169, at 21.45 and 27.1 min. The second one corresponds to diazinon itself. The former has an unidentified m/z 235 as base peak and an ion at m/z 169(34). Not enough information is given to identify this TP.

Pyridafenthion was detected at m/z 340 $[M]^{-\cdot}$. Isofenphos was detected under NI mode, together with a TP at 16.1 min, tentatively identified as $[(CH_3)_2CHNH(CH_3CH_2O)POS]^-$ at

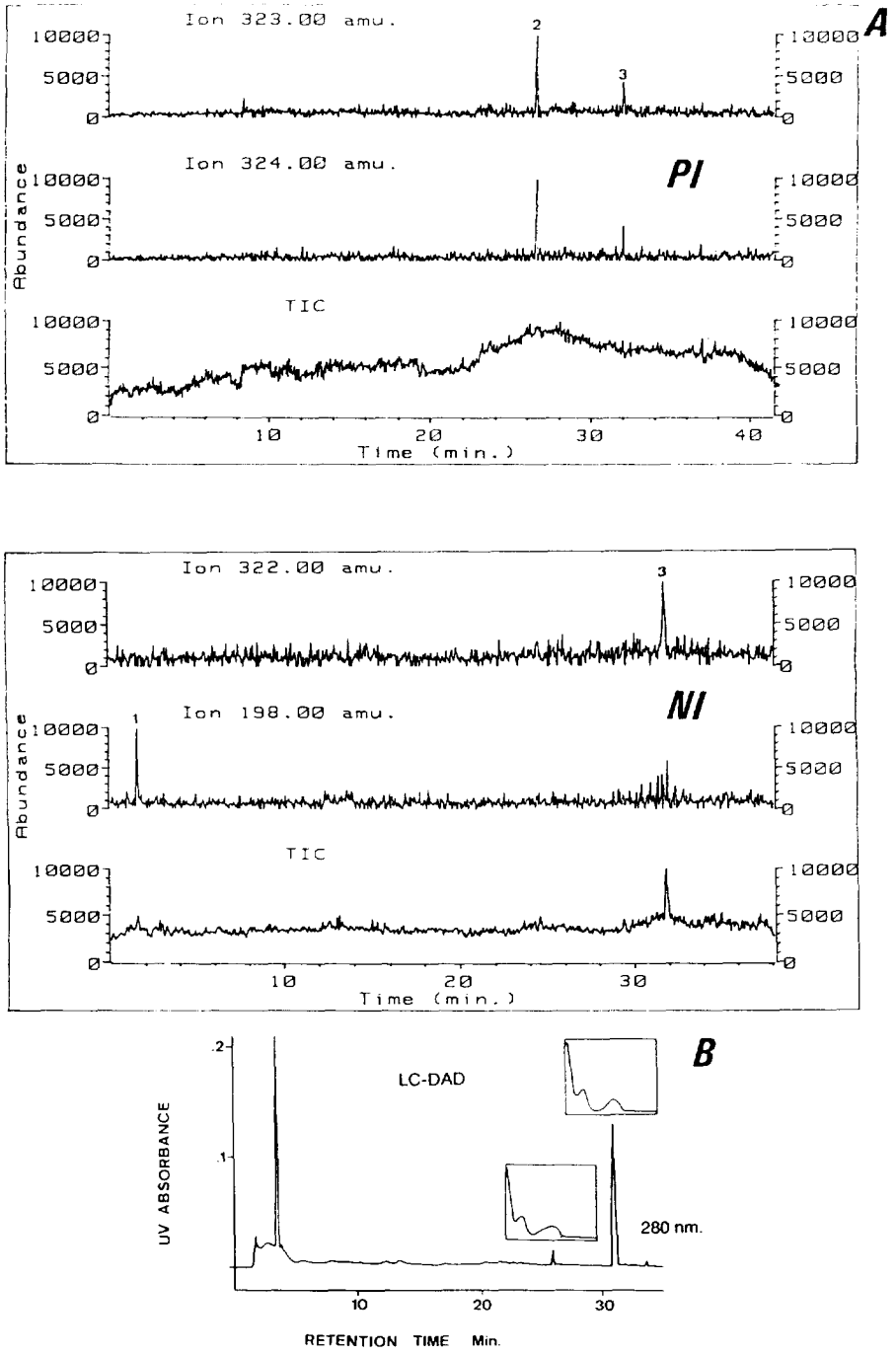

Fig. 2. (A) Reconstructed ion chromatogram obtained with on-line SPE–LC–TSP-MS with PI and NI mode of ionization of an Ebre river water sample spiked with chlorpyrifos-methyl at 50 μg/l; the water sample was analyzed four weeks after spiking. Peak identification: 1 = 3,5,6-trichloro-2-pyridinol, 2 = chlorpyrifos-methyl oxon, 3 = chlorpyrifos-methyl. (B) Same water sample analyzed by on-line SPE–LC–DAD at 280 nm. In both cases, the same analytical conditions were used (see Experimental section).

m/z 182(100). Adducts with formate were present at 228(50) [182 + HCOOH] . This compound could be formed by hydrolysis.

4. Conclusions

The application of automated on-line SPE (OSP-2) followed by LC–TSP-MS was used for the determination of OPs and their TPs at trace levels in river water samples subjected to natural degradation. It has been shown that the application of highly automated techniques permits the detection of polar TPs determined for the first time in natural waters, e.g. 3,5,6-trichloro-2-pyridinol. The use of such automated techniques combined with MS is useful in environmental degradation studies of pollutants. Few results have been published up to now on the stability of OPs in water. This subject is still a matter of controversy, and conclusions cannot be drawn. In this sense, although a few OPs were rapidly degraded in water, TPs were formed and were stable during a period of more than 3–4 weeks, which led us to pay attention to a more stable contamination in water due to TPs. More research is needed in order to verify the formation of TPs of pollutants in natural waters and to check their stability over time.

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