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Application of on-line solid-phase extraction followed by liquid chromatography–thermospray mass spectrometry to the determination of pesticides in environmental waters

S. Chiron[a], S. Dupas[b], P. Scribe[b], D. Barceló*[,a]

[a]Department of Environmental Chemistry, CID-CSIC, c/Jordi Girona 18–26, 08034 Barcelona, Spain

[b]Laboratory of Marine and Physical Chemistry, Université Pierre et Marie Curie, 4 Place Jussieu, 75252 Paris Cedex 05, France

Abstract

A multi-residue method for the trace-level determination of 34 pesticides and various transformation products was developed by using on-line solid-phase extraction and either ten 4.6-mm Empore extraction discs containing C_{18} or a conventional precolumn, packed with PRP-1 copolymer, followed by liquid chromatography–thermospray mass spectrometry with time-scheduled selected-ion monitoring. Two main ions (usually $[M + H]^+$ and $[M + NH_4]^+$ or $[M + CH_3CN]^+$) were used for each pesticide in the positive-ion operational mode, while $[M - H]^-$ and $[M + HCOO]^-$ ions were used in the negative-ion mode. Losses of CH_3NCO were also monitored for many of the carbamate pesticides. The proposed method requires 100 ml of sample for a limit of detection of 0.01–0.4 μg/l, depending on the particular compound and the operational mode. Calibration graphs were constructed by preconcentrating 100 ml of an estuarine water sample, spiked with the pesticide mixture at various concentration levels, varying from 0.025 to 1.2 μg/l. Good linearity was observed for sixteen of the analytes studied, the relative standard deviation ($n = 5$) being 4–13%. Some examples of the trace-level determination of various pesticides in European river and ground water samples are given.

1. Introduction

The on-line combination of LC and MS is the most powerful tool available for confirmation of the presence of pesticides in water matrices with no false-positive determinations. Of the different LC–MS systems, the thermospray (TSP) method has been widely used for water analysis, usually following off-line liquid–liquid extraction (LLE) and/or solid-phase extraction (SPE) [1]. One of the assets of LC–MS systems is their suitability for determining a wide variety of pesticides, which makes them appealing for multi-residue analysis. In this respect, LC–TSP-MS has permitted the determination of 23 and 19 pesticides in water [1] and fruits and vegetables [2], respectively. LC–particle beam (PB)–MS has also been evaluated for the characterization of pesticides in ground waters included in the National Pesticide Survey (NPS), where it proved to be sensitive enough for only 43 of the 126 polar pesticides on the NPS list [3]. Recent studies on the performance of TSP and PB in the determination of pesticides have shown TSP to excel

* Corresponding author.

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over PB with acidic herbicides {*e.g.*, (2,4-dichlorophenoxy)acetic acid (2,4-D) and (4-chloro-2-methylphenoxy)acetic acid (MCPA) [4]} and carbamates (*e.g.*, carbaryl and carbofuran [5]); the limit of detection is 10–50 times lower with TSP than that with PB, the former method also featuring a wider linear dynamic range.

In previous work, our group has characterized a wide variety of pesticides including chlorinated phenoxy acids [6], carbamates and triazines [7] and various pesticide transformation products (TPs) [8] by LC–TSP-MS. Other workers [9] have also accomplished the preconcentration of phenylurea herbicides from water samples by using on-line SPE–LC–TSP-MS and positive-ion (PI) mode detection. However, results were only obtained in the PI mode, quantification aspects were lacking and no examples of on-line preconcentration of a broad range of pesticides (only a few TPs were addressed) were reported, with also few applications to real environmental samples. In two previous papers, we reported on the optimization of two on-line systems based on Empore SPE discs and a PRP-1 precolumn for the preconcentration of 30 pesticides and various transformation products included on the NPS list of the US Environmental Protection Agency (EPA) and the European Community [10,11] and for the characterization of phenylurea and triazine herbicides [12]. As a rule, quantification was performed by UV spectrophotometry at 220 nm or by using a postcolumn reaction with fluorescence derivatization (for carbamate pesticides).

Based on previous studies, the purpose of this work was to apply on-line SPE–LC–MS by using two different precolumn sorbents (Empore C_{18} discs and PRP-1) and two detection modes (PI and NI) for the determination of pesticides bearing various chemical groups and selected TPs at concentrations of 0.01–0.5 μg/l in real environmental water samples.

2. Experimental

2.1. Chemicals

HPLC-grade water, acetonitrile (gradient-grade LiChrosolv) and methanol (Merck Darmstadt, Germany) were passed through a 0.45-μm filter before use. Ammonium formate and formic acid were also purchased from Merck. Aldicarb sulphoxide, butocarboxim sulphoxide, aldicarb sulphone, oxamyl, methomyl, deisopropylatrazine, 3-hydroxy-7-phenolcarbofuran, deethylatrazine, 3-hydroxycarbofuran, methiocarb sulphoxide, methiocarb sulphone, methiocarb, 3-ketocarbofuran, 3-ketocarbofuranphenol, 1-naphthol, carbofuran, butocarboxim, aldicarb, bentazone, symazine, baygon, carbaryl, chlortoluron, MCPA, atrazine, isoproturon, propanil, molinate, alachlor, metolachlor, diuron, propazine, terbuthylazine, linuron, propoxur, neburon and metoxuron were purchased 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 Chromatography Division, Millipore, Bedford, MA, USA) and a Model 7125 injection valve furnished with a 20-μl loop (Rheodyne, Cotati, CA, USA). The general scheme of the system used for carrying out the on-line preconcentration of pesticides from water samples was similar to that described elsewhere [10–12]. However, in this work we used thermospray mass spectrometry instead of conventional diode-array and/or fluorescence detection. The general scheme of the method used is shown in Fig. 1.

After the membrane discs has been placed in the disc holder, this holder was fitted in a MUST column-switching device (Spark Holland, Emmen, Netherlands) and connected to an SSI Model 300 LC pump (Scientific Systems, State College, PA, USA) which delivered the water samples containing the pesticides. The discs were first 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 and TPs at concentrations of 0.2 μg/l were preconcentrated on ten membrane extraction discs of 4.6 mm diameter at a flow-rate of 5 ml/min. Following the preconcentration step, the MUST valve was switched and the com-

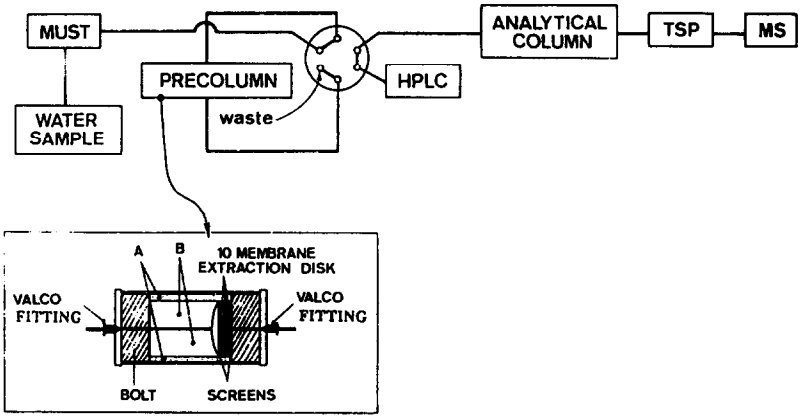

Fig. 1. General scheme of the system used for the preconcentration and determination of pesticides in water samples. A = Cylinder to adjust diameter; B = cylinder to adjust length of holder.

ponents were desorbed and separated in an analytical column. Instead of the discs, a conventional precolumn was also used and the system operated under conditions similar to those with the discs.

Two multi-residue analyses were carried out. The first involved a 250 × 4.6 mm I.D. analytical column (Interchim, Paris, France) packed with ODS-2 silica of 5-μm particle size. Preconcentration was carried out through a precolumn of 20 × 3 mm I.D. packed with PRP-1 copolymer (Brownlee Columns, Applied Biosystems, San Jose, CA, USA) of 10-μm particle size at a flow-rate of 4 ml/min. Gradient elution was accomplished in 35 min from an eluent containing 80% of solvent A (water + 0.05 *M* ammonium formate) and 20% of solvent B (acetonitrile) to 80% A–20% B at a flow-rate of 1 ml/min. The second analysis used a precolumn consisting of ten Empore C_{18} extraction discs, 4.6 mm in diameter, and preconcentrating the water at a flow-rate of 3 ml/min. The analytical column was a LiChroCART cartridge column (250 × 4.6 mm I.D.) packed with 4-μm Supersphere 60 RP-8 from Merck. Gradient elution was performed from an eluent containing 5% of solvent A [acetonitrile–methanol–water (40:40:20) + 0.0075 *M* ammonium formate)] and 95% of solvent B [acetonitrile–water (10:90) + 0.05 *M* ammonium formate–formic acid buffer (pH 3)] to 20% A–80% B (15 min), from 20% A–80% B to 30% A–70% B (20 min) and from 30% A–70% B to 55% A–45% B (20 min). The isocratic mode was used for 10 min, followed by gradient elution from 55% A–45% B to 100% of A (10 min), then the isocratic mode for 5 min and back to the initial conditions in 5 min. The post-run time was 10 min (flow-rate 0.85 ml/min).

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 used varied from 90 to 80°C (stem) and from 190 to 180°C (tip) at the beginning and end of the gradient. The ion source temperature was set at 240°C. The filament-on mode was used in all experiments, with conventional positive- and negative-ion chemical ionization. Chromatograms were recorded under time-scheduled selected-ion monitoring (SIM) conditions as shown in Table 1.

2.4. Quantitative analysis

The linearity and reproducibility of the response of the on-line system used was examined. Calibration graphs were constructed by injecting estuarine water samples spiked at five different concentration levels encompassing the range of interest; they were linear over the range 0.025–1.2 μg/l (see Table 2). Sixteen compounds

Table 1
Time-scheduled SIM conditions, with preconcentration through a PRP-1 precolumn and through Empore C_{18} discs

Preconcentration	Time (min)	Compounds (m/z monitored) Positive-ion mode	
PRP-1	From 0 to 12	Deisopropylatrazine (174, 215) Deethylatrazine (188, 229)	
	From 12 to 18	Simazine (202, 243) Metoxuron (229, 270)	
	From 18 to 23	Chlortoluron (213) Isoproturon (207, 248) Atrazine (216, 257)	
	From 23 to 27	Propazine (230, 271) Terbuthylazine (230, 271)	
	From 27 to 35	Neburon (275, 316)	
		Compounds (m/z monitored)	
		Positive-ion mode	Negative-ion mode
Empore	From 0 to 12	Butocarboxim sulphoxide (207, 224) Aldicarb sulphoxide (207, 224)	
	From 12 to 15.5	Aldicarb sulphone (223, 240) Oxamyl (220, 237)	(267)
	From 15.5 to 23	Methomyl (163, 180) Deisopropylatrazine (174, 215) 3-Hydroxycarbofuranphenol	(225)
	From 23 to 35	3-Hydroxycarbofuran (238, 255) Methiocarb sulphoxide (185, 226) Deethylatrazine (188, 229) Methiocarb sulphone (218, 259)	(183, 229) (199, 245)
	From 35 to 50	3-Ketocarbofuranphenol Butocarboxim (191, 208) Aldicarb (191, 208) 3-Ketocarbofuran	(178, 223) (178, 235)
	From 50 to 59.5	Simazine (202, 243) Propoxur (210, 227) Carbofuran (222, 239) Bentazone	(239)
	From 59.5 to 72	Carbaryl (219, 260) Chlortoluron (213, 254) MCPA 1-Naphthol Atrazine (216, 257) Isoproturon (207, 248)	(199, 245) (143, 189)
	From 72 to 77	Propanil Methiocarb (226, 243) Molinate (188, 213)	(217, 262)
	From 77 to 80	Alachlor (238, 270, 287) Metolachlor (284)	

Table 2
Calibration data for selected pesticides (spiked at 0.025, 0.1, 0.4, 0.8 and 1.2 μg/l) after preconcentration of 100 ml of estuarine water

Analyte[a]	Calibration equation	R^2	R.S.D. (%)[b]	LOD (μg/l)
Aldicarb sulphone	$y = 69.2x + 2.56$	0.962	7	0.1
Methomyl	$y = 1.43x + 1.26$	0.975	10	0.05
Deisopropylatrazine	$y = 15.9x + 1.43$	0.915	9	0.02
3-Hydroxycarbofuran	$y = 8.31x + 1.67$	0.995	7	0.1
Deethylatrazine	$y = 14.1x + 1.18$	0.990	8	0.02
Butocarboxim	$y = 9.21x + 1.20$	0.977	10	0.05
Aldicarb	$y = 13.5x + 1.76$	0.922	11	0.02
Simazine	$y = 18.7x + 1.45$	0.995	8	0.01
Propuxor	$y = 24.8x + 1.62$	0.931	13	0.02
Carbofuran	$y = 152.4x + 7.51$	0.992	12	0.02
Carbaryl	$y = 192.6x + 3.12$	0.931	11	0.02
Chlortoluron	$y = 122.5x + 9.69$	0.922	13	0.01
Atrazine	$y = 23.1x + 1.32$	0.977	6	0.02
Isoproturon	$y = 27.2x + 1.58$	0.961	4	0.01
Methiocarb	$y = 143.6x - 5.68$	0.938	11	0.05
Molinate	$y = 51.8x + 1.86$	0.918	6	0.01

Calibration was performed by plotting peak area (y) *versus* amount injected on to the on-line precolumn system (x, μg/l) using positive-ion mode time-scheduled SIM.

[a] Aldicarb sulphoxide, butocarboxim sulphoxide and oxamyl could not be properly determined as their LODs were *ca.* 0.4 μg/l. Alachlor and metholachlor were not measured owing to difficult quantification (large variation in the tip temperature of the TSP interface) when working at 100% of organic modifier in the LC eluent.

[b] Relative standard deviation ($n = 5$) at 0.4 μg/l.

showed a good linearity range in the positive-ion mode and with time-scheduled SIM. Aldicarb sulphoxide, butocarboxim sulphoxide and oxamyl could not be measured owing to a poor limit of detection (LOD), close to the concentration range studied. Other compounds such as 3-hydroxycarbofuran and 3-hydroxy-7-phenolcarbofuran could not be measured owing to the lack of detection. Methiocarb sulphone and 1-naphthol showed instability in water during the preconcentration step. Alachlor and metolachlor could not be properly measured owing to their elution at 100% of organic modifier, making it difficult to keep the tip temperature of the thermospray interface stable and consequently high standard deviations were observed with no linearity in the quantification values. Other compounds showed good sensitivity only in the NI mode, such as 3-hydroxycarbofuranphenol, 3-ketocarbofuranphenol, 3-ketocarbofuran, bentazone, MCPA and propanil.

The dynamic range provided by TSP was consistent with reported values whether the PI (carbamate pesticides) [5] or the NI mode (chlorinated phenoxy acid herbicides) [4] was used. However, none of these previous studies used an on-line preconcentration system as described here.

The LODs in Table 2 were calculated by using a signal-to-noise ratio of 3–4 and assuming that 1 cm was the minimum peak height that could be measured with reasonable confidence. This corresponds, in the Hewlett-Packard TSP mass spectrum, to *ca.* 500 arbitrary units (the noise level is *ca.* 150 units). Table 2 gives the values for the compounds measured using the PI mode. The LODs using the NI mode varied from 0.02 to 0.4 μg/l depending on the compound studied,

the worst values being obtained for the TPs of carbofuran and methiocarb.

3. Results and discussion

3.1. Mass spectral information

As ion formation and fragmentation patterns in thermospray LC–MS may be strongly influenced by various operational parameters (*e.g.*, the nature and composition of the mobile phase, the source and vaporization temperature), each group of pesticides should be studied in detail prior to tackling trace-level determinations in surface waters. The major ions and their relative abundances obtained by TSP-MS in the PI and NI modes for a selection of eight analytes are given in Table 3. Because complete separation of all the pesticides studied entailed the use of gradient elution (see Fig. 2), the major ions for each compound were obtained at three different eluent compositions, depending on the retention time in the liquid chromatogram.

Each compound gave two main ions corresponding to $[M + H]^+$ and $[M + NH_4]^+$ in the PI mode. Ammonium formate was chosen as an eluent additive instead of ammonium acetate because it enhanced the chromatographic resolution at pH 3 and also adduct formation as a result of its slightly higher gas-phase acidity [6,13]. However, the sensitivity was similar in both instances and $[M + NH_4]^+$ appeared to be the base peak for the whole group of carbamate pesticides except carbofuran, which had $[M + H]^+$ as its base peak. This behaviour meets the expectations for carbamates [5,7,14,15] except for carbofuran, the ions of which did not match previous findings published by our group showing $[M + H]^+$ as the base peak [7]. This difference in the relative abundances of the ions may be ascribed to the presence of formic acid in the mobile phase; hence, depending on the compounds and experimental conditions used, TSP is a solution-dependent ionization technique, being affected by the pH of the mobile phase. Chlorotriazine and phenylurea pesticides usually generate $[M + H]^+$ as their base peak, thus meeting the expectations for compounds that exhibit a higher proton affinity that does ammonia [9,16–18]. Fragmentation was only observed in a few isolated instances. Methiocarb sulphoxide and methiocarb sulphone showed a loss corresponding to CH_3NCO, and the herbicide alachlor exhibited a fragment at m/z 238 resulting from the loss of the CH_3OH moiety [1].

The NI mode was also assessed as it generally involves more fragmentation processes, so it provides complementary structural information to that offered by the PI mode; however, few compounds provide adequate sensitivity. Formate adducts (propanil, MCPA, aldicarb sulphone, 3-hydroxy-7-phenolcarbofuran) and/or deprotonated molecular ions (bentazone, 1-naphthol) were observed as base peaks. Electron capture was observed to occur for 3-ketocarbofuranphenol and 3-ketocarbofuran, leading to $[M]^{-\cdot}$ as the base peak; on the other hand, the N-methylcarbamates methiocarb sulphoxide, methiocarb sulphone and 3-ketocarbofuran showed fragmentation, with the loss of a CH_3NCO moiety. The relative abundances of the ions from the four metabolites of carbofuran were low owing to thermal decomposition in the probe. When electrospray (EPS)-MS experiments were performed they exhibited $[M + Na]^+$ and $[2M + Na]^+$ as their base peak and main adduct ion, respectively [19]. Monitoring them by ESP may be easier, but our current ESP system is not yet operational with the proposed multi-residue method.

3.2. Water analysis

Efficient monitoring of surface water samples requires LODs close to 0.1 μg/l, which is the maximum allowable concentration established by the European Community in its Directive on the Quality of Water Intended for Human Consumption [20]. LC–TSP-MS provides LODs of 10 μg/l (0.2 ng if a 20-μl loop is used) by SIM, so obtaining a three orders of magnitude lower LOD entails the use of a preconcentration step. The advantages of SPE over conventional LLE are well documented [21]; also, the use of SPE coupled on-line to LC with UV and diode-array

Table 3
Main ions and their relative abundances (%) for eight compounds using TSP-MS in the PI and NI modes and filament-on conditions

Peak No.	M_r	Compound and ions	Eluent	PI mode	NI mode
7	180	3-Hydroxycarbofuranphenol	1	n.d.[a]	
	225	$[M + HCOO]^-$			100
8	237	3-Hydroxycarbofuran	1		n.d.
	238	$[M + H]^+$		12	
	255	$[M + NH_4]^+$		100	
9	241	Methiocarb sulphoxide	1		
	185	$[M + H - CH_3NCO]^+$		100	
	226	$[M - CH_3NCO + H + CH_3CN]^+$		55	
	183	$[M - H - CH_3NCO]^-$			20
	229	$[M - CH_3NCO + HCOO]^-$			100
11	257	Methiocarb sulphone	1		
	218	$[M - CH_3NCO + NH_4]^+$		100	
	259	$[M - CH_3NCO + NH_4 + CH_3CN]^+$		20	
	199	$[M - H - CH_3NCO]^-$			45
	245	$[M - CH_3NCO + HCOO]^-$			100
12	178	3-Ketocarbofuranphenol	2	n.d.	
	178	$[M]^{-\cdot}$			100
	233	$[M + HCOO]^-$			5
15	235	3-Ketocarbofuran	2	n.d.	
	178	$[M - CH_3NCO]^{-\cdot}$			60
	235	$[M]^{-\cdot}$			100
18	221	Carbofuran	2		n.d.
	222	$[M + H]^+$		100	
	239	$[M + NH_4]^+$		40	
29	269	Alachlor	3		n.d.
	238	$[M + H - CH_3OH]^+$		50	
	270	$[M + H]^+$		100	
	287	$[M + NH_4]^+$		50	

The relative abundances indicated were obtained on injecting 100 ng of each individual compound (after preconcentrating 100 ml of a solution of 1 μg/l of each analyte) and using the following eluent compositions: (1) water–acetonitrile–methanol (80:10:10) with ammonium formate–formic acid buffer (pH 3); (2) water–acetonitrile–methanol (50:25:25) with ammonium formate–formic acid buffer (pH 3); (3) water–acetonitrile–methanol (20:40:40) with ammonium formate–formic acid buffer (pH 3).
[a] Not detected.

detection has been reported [22–24]. Recently, Empore C_{18} discs were used with similar detection systems to accomplish the highly efficient trace enrichment of both polar and non-polar analytes from surface water samples [10,11,25]. In addition to C_{18} SPE, PRP-1 copolymer has been used as a sorbent for the preconcentration of relatively polar pesticides [12].

Both SPE sorbents, Empore C_{18} discs and PRP-1, were employed in a multi-residue approach to the determination of 34 pesticides and various transformation products in water samples. In order to compare both sorbents, the average recoveries for four compounds are given in the Table 4; they range from 20% for compounds with low breakthrough volumes (*e.g.*, deisopropylatrazine) to 92% for compounds with high breakthrough volumes (*e.g.*, atrazine).

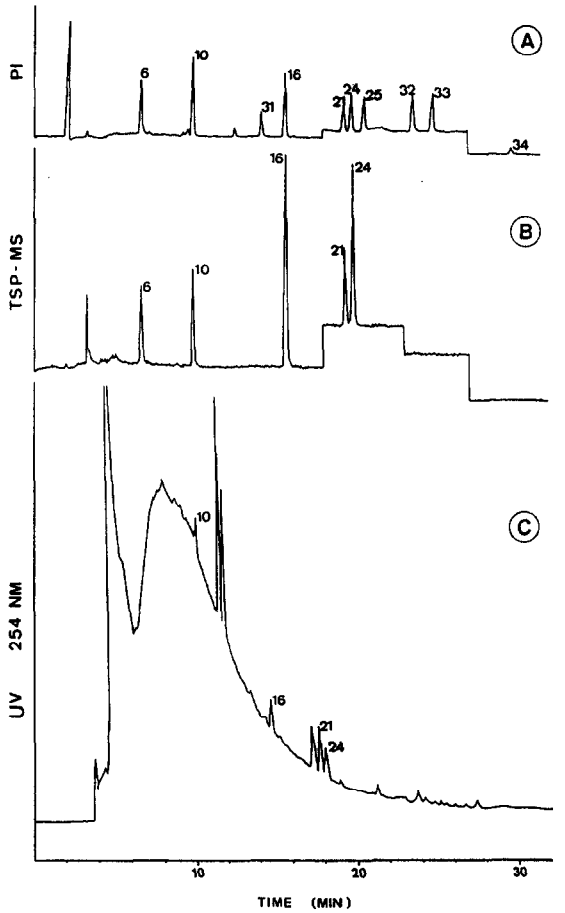

Fig. 2. (A) On-line SPE using PRP-1 sorbent followed by LC–TSP-MS of a river water sample spiked with 0.2 μg/l each of (6) deisopropylatrazine, (10) deethylatrazine, (31) metoxuron, (16) simazine (21) chlortoluron, (24) atrazine, (25) isoproturon, (32) propazine, (33) terbuthylazine and (34) neburon obtained with a preconcentration volume of 50 ml in the PI mode under SIM conditions (for details, see Table 1). The analytical LC column (250 × 4.6 mm I.D.) was packed with ODS-2 silica. Gradient elution programme: from 80% of A (water–0.05 *M* ammonium formate) and 20% of B (acetonitrile) to 80% of A–20% of B in 35 min and back to the initial conditions 10 min post-run. Flow-rate: 1 ml/min. (B) On-line SPE using PRP-1 sorbent followed by LC–TSP-MS of 50 ml of non-spiked River Dropt water obtained in the PI mode under SIM conditions (for details, see Table 1). The PI mode confirmed (6) deisopropylatrazine, (10) deethylatrazine, (16) simazine, (21) chlortoluron and (24) atrazine. Other experimental conditions as in (A). (C) On-line SPE using PRP-1 sorbent with LC–DAD at 254 nm of 200 ml of the same River Dropt water as in (B). Other experimental conditions as in (A) and (B) except that eluent B contained potassium acetate–0.1 *M* acetic acid (pH 4.7) instead of 0.05 *M* ammonium formate.

These two on-line methods usually require the use of 50–100 or 150–200 ml of water as the preconcentration volume when MS or UV detection, respectively, is employed [10,11,24]. Using a lower preconcentration water volume an additional advantage is gained as the analysis time is decreased (*ca.*, 30 min) and recoveries are improved. Typical results for the analysis of 50 ml of spiked (0.2 μg/l) water from the River Dropt (France) and 100 ml of spiked (0.3 μg/l) water from the River Ebro (Spain) are shown in Figs. 2A and 3A, respectively. PRP-1 (Fig. 2A) and Empore C_{18} discs (Fig. 3A) were used as on-line solid-phase sorbents, followed by LC–TSP-MS in the PI mode under time-scheduled SIM conditions. By using this detection mode, the sensitivity was increased by one order of magnitude relative to the traditional SIM detection mode. The two m/z values selected for each compound are given in table 1. Only the base peak was selected for monitoring at trace-level concentrations when the relative abundance of the second ion was very low (5–10%). The time-scheduled SIM monitoring is shown in Table 1. Fig. 3B shows an on-line LC–TSP-MS trace obtained in the NI mode under time-scheduled SIM conditions by preconcentrating 150 ml of spiked (0.3 μg/l) water from the River Ebro. Despite the well known lower sensitivity of the NI relative to the PI mode for most of the pesticides studied [2,7], the LODs for pesticides possessing an electrophilic moiety (acidic and phenolic compounds) were lower in the NI than the PI mode, consistent with previous results [4,6]. Two of the pesticide TPs studied, methiocarb sulphone and 1-naphthol, could not be determined by this method owing to instability in water during the preconcentration step and to the latter not being trapped on the C_{18} sorbent at an acidic pH (pH 3) [11]. Monitoring 3-hydroxycarbofuran and 3-hydroxy-7-phenolcarbofuran was impossible because of the lack of sensitivity of MS detection, which was ascribed to the thermal decomposition of the analytes in the TSP probe during analysis.

Fig. 2B and C illustrate the analysis of a non-spiked real river water sample using on-line SPE and PRP-1 copolymer followed by MS

Table 4
Breakthrough volumes (V_B) and average recovery of pesticides using on-line SPE with ten Empore C_{18} discs or a PRP-1 precolumn

Compounds	Empore C_{18} discs			PRP-1 precolumn		
	V_B (ml)[a]	Recovery (%)[b]		V_B (ml)[a]	Recovery (%)[b]	
		A	B		A	B
Deisopropylatrazine	8	17	60	14	22	65
Deethylatrazine	70	55	90	64	51	90
Simazine	>150	87	95	>160	90	94
Atrazine	>150	92	97	>320	92	100

[a] Breakthrough volumes were calculated as described in refs. 10 and 12.
[b] Average recoveries were calculated by preconcentrating (A) 150 or (B) 50 ml of water at 2 ml/min with a spiking level of 0.3 μg/l.

detection in the PI mode under SIM conditions and or diode-array detection (DAD) at 254 nm, respectively. In the LC–DAD spectrum, the matrix peak appears at the beginning of the chromatogram (20–30 min) and can vary according to the water type and gradient elution performed. Low recoveries were obtained for the first few compounds eluted (deethylatrazine, deisopropylatrazine) when 150–200 ml of water were used, and are attributed to fulvic and humic substances present in the river water. In Fig. 2B it can be observed that the use of MS detection under SIM conditions removed all kinds of interfering peaks and permitted the determination of deisopropylatrazine in real water samples with a volume of only 50 ml, giving a better recovery than using 150 ml of preconcentrated water (see Table 4).

Although all the dirty water samples were injected directly into the source of the MS apparatus, no decrease in sensitivity was observed during our analyses.

The LODs obtained with the method are given in Table 2. We noted an increase in the sensitivity for all the compounds studied with respect to LC–DAD, ranging from a factor of 200 for aldicarb sulphoxide to a factor of 10 for atrazine when comparing the results at the same preconcentration volume (150 ml). The performances of the two types of preconcentration procedures were similar for the common compounds studied, as illustrated in Table 4; however the recoveries were better when using smaller volumes as a result of the lower breakthrough volumes of the chlorotriazine transformation products. It should be noted that the Empore C_{18} discs were replaced after five analyses in order to avoid band broadening; the PRP-1 precolumn can be used much longer (*ca.* twenty analyses).

3.3. Confirmation of environmental levels

Our groups are currently working on the trace-level determination of pesticides in water samples from three different areas. The River Dropt area (south-western France) is mainly used for cereals and triazine and phenylurea herbicides are applied extensively. The Ebro delta (Tarragona, Spain) is a typical rice cultivation area, where propanil, molinate and bentazone are used [26]. The third region is Almeria (southern Spain), where carbamate insecticides are used for green and fruit crops. Fig. 2B shows a chromatogram obtained in the PI mode under SIM conditions following preconcentration of 50 ml of water from the River Dropt which allows the unequivocal determination of deisopropylatrazine, deethylatrazine, simazine, atrazine and chlortoluron at 0.2, 0.2, 0.3, 0.3 and 0.25 μg/l, respectively. Fig. 4A shows an LC–TSP-MS trace obtained in the PI mode under SIM con-

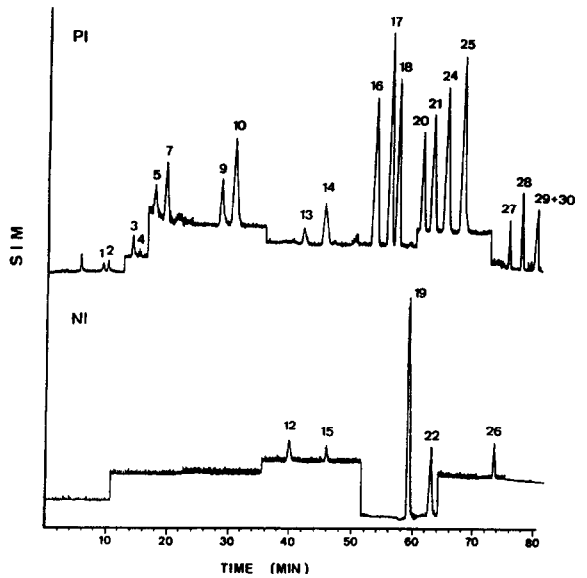

Fig. 3. On-line SPE with C_{18} Empore discs followed by LC–TSP-MS of a River Ebro water sample spiked with 0.3 μg/l each of (1) butocarboxim sulphoxide, (2) aldicarb sulphoxide, (3) aldicarb sulphone, (4) oxamyl, (5) methoxyl, (6) 3-hydroxy-7-phenolcarbofuran, (7) deisopropylatrazine, (8) 3-hydroxycarbofuran, (9) methiocarb sulphoxide, (10) deethylatrazine, (11) methiocarb sulphone, (12) 3-ketocarbofuran, (13) butocarboxim, (14) aldicarb, (15) 3-ketocarbofuran, (16) simazine, (17) baygon, (18) carbofuran, (19) bentazone, (20) carbaryl, (21) chlortoluron, (22) MCPA, (23) 1-naphthol, (24) atrazine, (25) isoproturon, (26) propanil, (27) methiocarb, (28) molinate and (29 + 30) alachlor + metolachlor, obtained after preconcentrating 100 and 150 ml of sample in the PI and NI modes, respectively, and under SIM conditions (for time-scheduled SIM conditions, see Table 1). Precolumn packed with ten Empore C_{18} extraction discs and LiChroCART cartridge column (25 cm × 4.6 mm I.D.) packed with 4-μm Supersphere 60 RP-8. Gradient elution programme: from 5% of A [acetonitrile–methanol–water + 0.075 *M* amonium formate (40:40:20)] and 95% of B [acetonitrile–0.05 *M* ammonium formate–formic acid buffer (pH 3) (10:90)] to 20% A–80% B in 15 min, from 20% A–80% B to 30% A–70% B in 20 min, from 30% A–70%–B to 55% A–45% B in 20 min, isocratic for 10 min, from 55% A–45% B to 100% A in 10 min, isocratic for 10 min and back to initial conditions 10 min post-run. Flow-rate: 0.85 ml/min.

ditions following preconcentration of 100 ml of water from Almeria where simazine, carbofuran and methiocarb were detected at 0.03, 0.02 and 0.05 μg/l, respectively. Fig. 4B shows an LC–TSP-MS trace of Ebro river water obtained

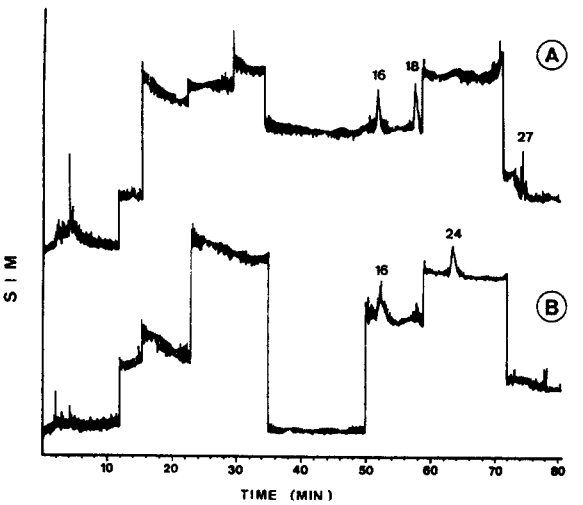

Fig. 4. (A) On-line SPE using Empore C_{18} extraction discs followed by LC–TSP-MS in the PI mode under time-scheduled SIM conditions (see Table 1) of 100 ml of Almeria ground-water. Compounds present were (16) simazine, (18) carbofuran and (27) methiocarb at concentrations of 0.03, 0.02 and 0.05 μg/l, respectively. For other experimental conditions, see Experimental. (B) On-line SPE using Empore C_{18} extraction discs followed by LC–TSP-MS in the PI mode under time-scheduled SIM conditions (see Table 1) of 100 ml of River Ebro water samples. Compounds detected at 0.01 μg/l were (16) simazine and (24) atrazine.

under the same conditions. The presence of simazine and atrazine at 0.01 μg/l was confirmed. The herbicide concentrations in the last sample are ten times lower than those usually expected [26] because the samples were collected during the winter, when herbicides are used sparingly.

4. Conclusions

The proposed on-line SPE–LC–TSP-MS method uses preconcentration of only 50–100-ml volumes for the determination of pesticides in water samples at concentrations of 0.02–0.4 μg/l. It is worth emphasizing that early-eluting compounds (*e.g.*, deisopropylatrazine and deethylatrazine), which usually cannot be determined by DAD with the same on-line system owing to low breakthrough volumes and/or interferences from humic substances in the water matrix, were

unequivocally identified by on-line SPE LC–TSP-MS using time-scheduled SIM. A good linearity range was obtained for sixteen compounds on preconcentrating 100 ml of estuarine water samples spiked at 0.025–1.2 μg/l. When determination was performed at the 0.4 μg/l level, the relative standard deviation for the different pesticides varied from 4 to 13%, which is acceptable for the on-line SPE–LC–TSP-MS technique used. A better detection limit was also obtained with on-line SPE–LC–TSP-MS than with DAD as only 50–100 ml of water were required compared with 150–200 ml in order to obtain a similar LOD.

PRP-1 and Empore C_{18} discs provide similar results for the breakthrough volumes of various triazine herbicides. The proposed method permits the unequivocal determination of deisopropylatrazine and deethylatrazine at concentrations as low as 0.15 and 0.2 μg/l, respectively, in river water samples, which was impossible with our current monitoring system based on on-line coupled SPE–LC–DAD. This is particularly important for deisopropylatrazine, which is a ubiquotus water pollutant and frequently escapes detection owing to interferences and/or extraction problems. Removal of fulvic and humic interferents is the greatest advantage of this technique over DAD.

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