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On-line and off-line sample preparation of acidic herbicides and bentazone transformation products in estuarine waters

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Abstract

The isolation and trace-enrichment of the acidic herbicides benazolin, bentazone, (2,4-dichlorophenoxy)acetic acid (2,4-D), (4-chloro-2-methylphenoxy)acetic acid (MCPA), 2-(4-chloro-2-methylphenoxy)propionic acid (MCP) and the transformation products (TPs) of bentazone 6- and 8-hydroxy in estuarine waters is described. The pesticides were spiked at 0.3 $\mu\text{g/l}$ and 1–3 $\mu\text{g/l}$ (for the TPs) in estuarine waters and were preconcentrated using solid-phase extraction (SPE) methods using C_{18} and styrene divinylbenzene (SDB) Empore extraction disks of 47 mm and 4.6 mm diameter in the off-line and on-line modes, respectively, followed by liquid chromatography–diode array (LC–DAD), fluorescence (FD) and/or thermospray mass spectrometric (TSP–MS) detection.

In the off-line mode 5 liters of water were preconcentrated and recoveries of 40–76% were obtained except for the TPs that were not recovered at all. Blanks were investigated within a pH range of 0.7–2, thus recommending the use of SDB over C_{18} Empore disks. When on-line SPE was used only 150 ml of river water were needed, with a limit of detection (LOD) varying between 5–100 ng/l and higher recoveries than in the off-line mode, followed by LC–DAD in both cases.

When on-line SPE with Empore disks was used for the determination of the acidic herbicides in real estuarine waters followed by LC–FD, only bentazone showed similar detection levels as in DAD whereas MCPA and MCP exhibited quenching interferences from the water matrix.

Confirmation of the acidic herbicides in estuarine waters was accomplished by on-line SPE–LC–TSP–MS using selected-ion monitoring (SIM) and negative-ion mode thus showing similar LOD to those achieved with on-line SPE–LC–DAD.

Illustrative examples of the trace level determination of the acidic herbicides bentazone and MCPA in real estuarine water samples of the Ebro delta (Tarragona, Spain) are shown.

1. Introduction

The acidic herbicides are of interest within the European Community (EC) countries. In a recent report [1] it was shown that the acidic herbicides benazolin, bentazone, (2,4-dichlorophenoxy)acetic acid (2,4-D), (4-chloro-2-methylphenoxy)acetic acid (MCPA), 2-(4-chloro-2-methylphenoxy)propionic acid (MCP) and

TCA are being used in Europe in amounts over 500 tons per year. Since most of them are considered transient and probable leachers through the soil and into the ground-water, they may pose problems for the ground-water resources. In addition to the parent pesticides, in the same report [1] it was recommended to monitor their toxic transformation products (TPs). Due to their current use they have been identified in environmental water samples. Bentazone, for instance, is being applied as rice

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herbicide and it has been determined together with 8-hydroxybentazone in estuarine water samples from the Ebro delta (Tarragona, Spain) when using our current monitoring programmes, either based on liquid–liquid extraction (LLE) [2] or on-line solid-phase extraction [3] (SPE) followed by liquid chromatography with diode array (LC–DAD) and mass spectrometric (TSP–MS) detection.

The official and most current methods for the determination of acidic herbicides in water samples are based on the adjustment of the waters to basic pH followed by LLE with diethyl ether or dichloromethane, their conversion to methyl esters by diazomethane or as pentafluorobenzyl derivatives and gas chromatography–electron capture detection (GC–ECD) [4]. Recently, the use of single or double Empore disks at pH from 1–7 was described as a previous step replacing the tedious LLE and using the same determination protocol as the official methods of analysis [5].

To avoid the derivatisation step, the use of either LLE at acidic pH < 2 and LC with DAD and FD has been proposed [2,6]. Another alternative is to replace LLE by SPE techniques with Carbo-pack cartridges, that permit high flow-rates and high water volumes of 100 ml/min and 2 l, respectively. In this way a LOD of 0.1 µg/l can be obtained when using LC–UV detection [7].

On-line SPE coupled to LC has been recently reported for the determination of various acidic herbicides [3,8–10]. Such SPE systems use either PLRP-S, C₁₈ with large volume injection or anion exchange. One of the difficulties encountered with the analysis of acidic herbicides in real river water samples was the interference of humic substances that could not be totally removed even when working at different pH values [3,8].

One of the key parameters in environmental analysis is the confirmation of the trace organics tentatively identified by the UV spectra of the LC–DAD. In this respect, MS is the ideal technique that avoids false positive determinations. Thermospray LC–MS in the negative-ion mode of operation has been recommended for the identification of acidic herbicides [11–14]. In

addition, the use of chloroacetonitrile as eluent additive in TSP–MS has proved to be useful for obtaining additional structural information via chloride attachment to chlorinated phenoxy acids [11,13].

The aim of this work was to develop and compare various LC methods for the determination of acidic herbicides and bentazone TPs in estuarine waters at levels of 0.1 µg/l. The approach has consisted in the use of (i) an off-line method with Empore extraction disks of C₁₈ and SDB by preconcentrating 5 liters of water at acidic pH of 0.7–2; (ii) an on-line SPE method using Empore disks followed by LC–DAD, LC–FD and LC–TSP–MS which involved the preconcentration of 150 ml and (iii) the systems developed for the determination of the different herbicides in real estuarine waters of the Ebro delta (Tarragona, Spain).

2. Experimental

2.1. Chemicals

HPLC grade water and acetonitrile gradient grade LiChrosolv from Merck (Darmstadt, Germany) were passed through a 0.45 µm filter before use. Ammonium formate, formic acid, sulphuric acid and trifluoroacetic acid were also purchased from Merck. All of these chemicals were of analytical reagent grade. Tripropylamine was obtained from Fluka (Buchs, Switzerland). Tripropylammonium formate was prepared by mixing amounts of tripropylamine (98%) and formic acid. Benazolin, 8-hydroxybentazone, 6-hydroxybentazone, bentazone, 2,4-D, MCPA and MCPP were purchased from Promochem (Wesel, Germany). Stock standard solutions (1000 µg/ml) of the herbicides were prepared in acetonitrile. The stock solutions were diluted and mixed in HPLC grade water containing formic acid (pH = 3) before LC analysis.

2.2. Chromatographic conditions

The eluent was delivered by two Knauer 64 high-pressure pumps (Bad Homburg, Germany)

coupled to a 1000S Applied Biosystems diode array detector (Foster City, CA, USA) or a Model LC 240 Fluorescence detector from Perkin-Elmer (Beaconsfield, UK). Two LiChrocart cartridge columns 60 RP-8 and 60 RP-8 select B (25 cm × 4.6 mm I.D.) packed with 4- μ m Supersphere from Merck were used. Gradient elution was performed from an eluent containing 10% of A [acetonitrile–water (90:10)] and 90% of B [water–formic acid pH = 2.9] to 35% of A–65% of B in 30 min; from 35% of A–65% of B up to 45% of A–55% of B in 20 min. From 45% of A–55% of B up to 100% of A in 2 min and back to the initial conditions in 3 min. Post-run time was 10 min at a flow-rate of 0.9 ml/min.

Quantification of the compounds was done by external calibration and peak area measurements. The calibration graphs were constructed for compounds showing recoveries higher than 70%. The concentration range studied ran from 0.02 to 2 μ g/l using preconcentration volumes of 100–150 ml and UV detection at 200 nm.

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 temperature parameters were programmed from 100°C to 90°C and from 180°C to 170°C for the stem and tip, respectively. Source temperature was set at 220°C when using ammonium formate as mobile phase additive and at 250°C when using tripropylammonium formate as mobile phase additive. In all experiments the filament-on mode was used in which conventional negative-ion chemical ionization can be carried out. The chromatograms were recorded under time scheduled selected-ion monitoring (SIM) conditions as shown in Table 1.

2.4. Sample pretreatment

Estuarine water samples from Ebro Delta (Tarragona, Spain) of 150 ml were filtered

Table 1
Time scheduled selected ion monitoring (SIM) conditions under TSP-MS with NI detection

Time in min	Ions monitored
0 to 35	242, 288, 255, 239, 283
38 to 45	239, 199, 245, 219, 265
45 to 50	213, 259

through a 0.45 μ m membrane filter (Millipore, Bedford, MA, USA) and were then acidified at pH = 1–1.5 in case of C₁₈ disks and at pH = 1 in case of SDB disks with sulphuric acid. Samples were spiked with the different pesticides giving final concentrations in the range of 0.3–3 μ g/l. In the off-line method a Baker Empore Extraction System filtration apparatus, which was a gift from J.T. Baker (J.T. Baker, Deventer, Netherlands) was used. The general extraction procedure using 4–5 liters of water samples was described elsewhere [15]. In the on-line method a MUST column switching device from Spark Holland (Emmen, Netherlands) was used. Sample preconcentration was carried out with an SSI Model 300 LC pump from Scientific Systems Inc., (State College, PA, USA). The precolumn consisted of a stainless steel membrane disks holder constructed in the workshop of the Free University of Amsterdam (Netherlands) where 10 × 4.6 mm I.D. C₁₈ or SDB Empore extraction disks were placed, as described elsewhere [3]. Extraction disks manufactured under the trade mark Empore were a gift of J.T. Baker (Deventer, Netherlands).

3. Results and discussion

3.1. Analytical LC separation

It has been shown that the use of a C₁₈ bonded silica column with an acidic mobile phase, e.g., perchloric acid at pH = 2, allows a good separation of the acidic herbicides [10]. When the acidic mixture contains the TPs, then the separation between closely related compounds becomes critical. This is the case of 2,4-D and

MCPA or 6-hydroxybentazone and 8-hydroxybentazone. To achieve a complete separation of all these compounds a high-resolution end-capped LC column of 25 cm length and 4- μm particle size becomes necessary exhibiting a better resolution for the two TPs of bentazone with a reduction in the peak tailing.

The influence of the pH on the separation was also studied. Due to the ionization suppression, the separation of the acidic herbicides increased with a pH decrease with an optimal separation at $\text{pH} = 2.82$. This pH value was selected as a compromise that prevented a decrease of the column life. When a polymeric column Zorbax SB phenyl of 250×4.6 mm I.D. 5- μm particle size was used, a lack of separation efficiency was noticed even though its use was possible over a pH range 1–13. Two acid modifiers were investigated: trifluoroacetic acid and formic acid. The former allowed a good background line at wavelengths lower than 220 nm and the full separation of 6-hydroxybentazone and the 8-hydroxybentazone, but 2,4-D and MCPA were not resolved (figure not shown). The latter one, used at the same pH but with formic acid, permitted a really good resolution between 2,4-D and MCPA but 6-hydroxybentazone and benazolin coeluted.

When performing on-line precolumn technology, a good separation is necessary because otherwise the extra band broadening caused by the coupling may lead to an overlapping of peaks when analyzing real samples [10]. Therefore, we have selected formic acid as modifier for further analysis, even though benazolin and 6-hydroxybentazone coelute. This coelution was not so important for us, as compared to 2,4-D and MCPA (these two compounds are also much more in use in the area of study). With respect to the UV detection a wavelength of 200 nm was used as the result of a compromise between selectivity and sensitivity.

In addition, it was difficult to find a commercially available 6-hydroxybentazone standard of high purity. In ethylacetate solvent, that was commercially available, three peaks with UV detection appeared. These peaks did not come from a degradation process on the analytical

column but from impurities present in the analytical standard.

3.2. Preconcentration studies with LC-DAD

Off-line SPE

The aim of the off-line preconcentration procedure was to compare two kinds of Empore disk materials: C_{18} and SDB. Previous studies have shown that, in general, PRP-1 or PLRP-S were more suitable than C_{18} silica sorbent for the extraction of relative polar compounds from water [8,10].

It is known that in reversed-phase chromatography the trapping capacity of an ionisable analyte depends on its ionisation state. The retention of a compound is higher in its neutral than in its ionic form. Since the pK_a values of phenoxy acids is around 2.5 [10] a pH value lower than the pK_a is needed to achieve good recovery values.

In our experiments a $\text{pH} = 1$ in the water sample was established in order to prevent recovery losses. This creates a problem since C_{18} bonded silica material is no more stable at $\text{pH} = 1$, especially when preconcentrating large water volumes like 5 liters, which require 150 min of preconcentration time leading to decomposition of the sorbent and high blank interferences (figure not shown). So, when using C_{18} disks the pH was kept at 1.5 to avoid these decomposition problems. In contrast, when using SDB disks even at pH 0.7 the blanks are quite clear. The main advantage of SDB Empore disks was the possibility of using them over the pH range 0.7–13 without any decomposition of the sorbent material in the off-line method. Table 2 shows the recoveries and LODs when using such water volumes. The recoveries varied from 40–76%, bentazone being the only compound that shows recovery values over 70% in both sorbents. In general, there is a surplus of the breakthrough volumes for most of the compounds with no recovery at all for TCA and the two TPs of bentazone. The somewhat higher recoveries values for SDB as compared to C_{18} can be explained by the structural molecular properties

Table 2

Average % recovery (Av) and relative standard deviation (R.S.D.) of pesticides in estuarine waters using off-line SPE with SDB or C₁₈ Empore extraction disks of 47 mm diameter

Compound	UV (nm) absorption	SDB ^a		C ₁₈ ^b		LOD g/l
		Av	R.S.D.	Av	R.S.D.	
Benazolin	220	45	12	39	15	1
Bentazone	220	76	7	72	6	0.05
MCPA	200	65	9	53	11	0.2
2,4-D	200	59	11	44	9	0.2
MCPP	200	70	8	58	12	0.1

Water volume preconcentrated: 5 l at a flow-rate of 30 ml/min. Spiking level: 0.25 µg/l. Analysis: LC-DAD. 6-hydroxy-bentazone, 8-hydroxybentazone and TCA were not recovered.

^a Water pH = 0.7.

^b Water pH = 1.5.

involving π - π interactions between polymeric matrix and organic compounds with free electrons.

On-line SPE

When using on-line SPE only 150 ml of water were needed for the preconcentration studies. The contact time between the mobile phase and the sorbent is shorter and we did not see any alteration of the C₁₈ sorbent at pH = 1. Table 3 gives the recoveries and the coefficient of variation of each compound spiked at 0.3 µg/l in a estuarine water after preconcentrating a volume of 150 ml followed by LC-DAD.

As noticed in Table 3, the on-line SPE method using SDB Empore disks allowed only a slight improvement of the recovery values as compared to C₁₈ Empore disks due to some decrease in the interferences of the early eluting peaks. Fig. 1A and 1B show typical chromatograms obtained with the on-line method after preconcentrating 150 ml of Ebro river surface water spiked at 0.3 µg/l using C₁₈ and SDB disks, respectively. The use of a wavelength of 200 nm permitted the detection of most of the compounds. In the case of bentazone a better quantitation is obtained by the use of $\lambda = 215$ nm (Fig. 1C). Benazolin and 8-hydroxybentazone exhibited low recoveries

Table 3

Average % (Av) recovery and relative standard deviation (R.S.D.) of pesticides in surface water using on-line SPE with 10 SDB or 10 C₁₈ Empore extraction disks of 4.6 mm diameter

Compound	UV (nm) absorption	SDB		C ₁₈		LOD µg/l
		Av	R.S.D.	Av	R.S.D.	
Bentazone	220	95	5	98	3	0.01
Benazolin	220	65	10	58	8	0.3
MCPA	200	80	5	78	6	0.05
2,4-D	200	82	6	74	8	0.05
MCPP	200	85	3	81	7	0.05
8-Hydroxybentazone	220	33	20	30	22	0.7

Surface water (pH = 1) volume preconcentrated: 150 ml at a flow-rate of 3 ml/min ($n = 6$ for each pesticide). Spiking level: TCA: 500 µg/l; 6-hydroxybentazone: 1.5 µg/l; 8-hydroxybentazone: 3 µg/l; others: 0.3 µg/l. Analysis: LC-DAD. 6-Hydroxybentazone and TCA could not be recovered.

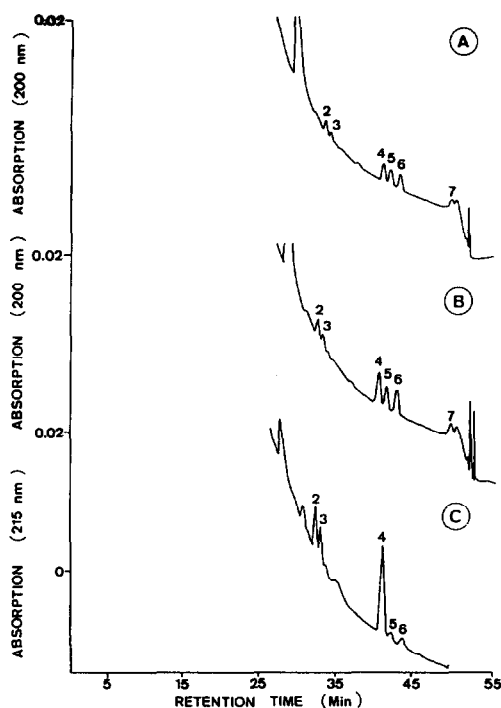


Fig. 1. LC-Diode array chromatograms at 200 nm obtained after on-line preconcentration of 150 ml of Ebro river water sample spiked at $0.3 \mu\text{g/l}$: (A) on 10 C_{18} Empore extraction disks; (B) on 10 SDB Empore extraction disks; (C) same analysis as (B) but recorded at 215 nm. Peaks: (1) benazolin, (3) 8-hydroxybentazone, (4) bentazone, (5) 2,4-D, (6) MCPA, (7) MCPP. LC conditions: see Experimental.

due to matrix interferences present in the estuarine waters.

Similar explanations as discussed in the off-line method can be given for the somewhat higher recoveries obtained for SDB as regards to C_{18} Empore disks (see Table 3). 6-Hydroxybentazone and TCA were not recovered at all due to matrix interferences and poor detection, respectively. The present study expands the application of the use and comparison of SDB and C_{18} Empore disks. In previous studies from our group [3] we observed that C_{18} was superior to SDB Empore disks for a variety of carbamate and triazine pesticides.

Because of evident difficulties (due to degradation) encountered in previous studies when analysing carbamates in river water samples immediately after sampling [3], it was decided to

perform an analyte stability study during 20 days in water. All the 8 compounds were spiked into river water, stored at 4°C in the dark and kept at $\text{pH} = 4.8$ with ammonium formate-formic acid buffer that avoids biological degradation. All the 8 compounds remained stable.

3.3. Preconcentration studies with LC-FD

Schüssler [6] used fluorescence detection after LLE of MCPA, MCPP and bentazone from water sample. Fig. 2A shows a typical LC-FD of the pesticide standard mixture. 2,4-D, which has a closely related structure, does not fluoresce naturally. It is known that with halogen substituents on the ring fluorescence can be reduced or even eliminated [16]. 6-Hydroxybentazone

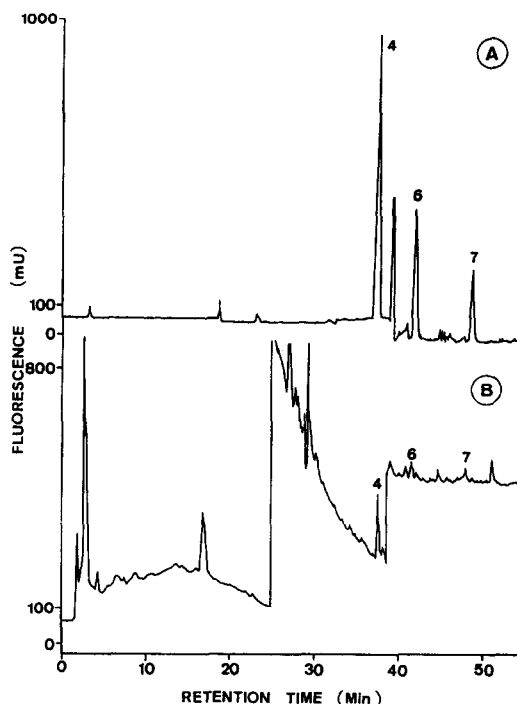


Fig. 2. (A) LC-FD chromatogram of the pesticide containing 10 mg/l (injection $20 \mu\text{l}$) each of (4) Bentazone (Ex: 340 nm , Em: 430 nm), (6) MCPA (Ex: 280 nm , Em: 310 nm), (7) MCPP (Ex: 280 nm , Em: 310 nm). (B) LC-FD chromatogram obtained after on-line preconcentration on Empore extraction disks of SDB 150 ml of Ebro river water sample spiked at $0.3 \mu\text{g/l}$. Other experimental conditions as in Fig. 1.

and 8-hydroxybentazone exhibited moderate fluorescence in a mobile phase water–acetonitrile (50:50, v/v) with neutral media [17]. The fluorescent intensity is strongly influenced by the pH and the type of the mobile phase. When using an acidic mobile phase, the chromatographic separation of 6-hydroxybentazone and 8-hydroxybentazone showed very low fluorescence even when injecting high amounts of the compounds, the LOD being 200 ng and 250 ng, respectively. At this LOD it is impossible to use the FD for trace level monitoring in water.

For bentazone, MCPA and MCPP the LOD were 0.5 ng, 1 ng and 1.5 ng, respectively. It was necessary to preconcentrate a volume of 100–150 ml in the on-line SPE method to reach the LOD of 0.1 $\mu\text{g/l}$, in a similar way to the use of LC–DAD. Average recoveries and LOD using SDB and C_{18} Empore extraction disks are indicated in Table 4. Fig. 2B shows a LC–FD chromatogram obtained after preconcentrating 150 ml of estuarine water. We can see that the recoveries for MCPA and MCPP are low. This is attributed to humic substances, considered to be polydisperse hydrophobic polymers of average molecular mass 1000–10 000, that contain structures as methoxy aromatics, phenols and aryl ketones [3]. Due to their aromatic structure, humic substances including humic acids and fulvic acids could be directly responsible for the quenching phenomenon explaining the low recovery values and the high coefficient of variation obtained in the case of MCPA and MCPP. Besides, the interference level depends strongly on the excitation and emission wavelength val-

ues. Actually, only bentazone was monitored in estuarine waters using LC–FD. This was demonstrated by performing the recovery studies using HPLC water instead of estuarine water with recoveries in the range of 85–95% for the three acidic herbicides, demonstrating the negative effect of the natural humic materials on the recoveries. Bentazone, with a less acidic structure as compared to the chlorinated phenoxy acids, showed good recoveries using estuarine waters.

3.4. Thermospray LC–MS

In order to confirm the compounds of interest in the HPLC chromatogram and avoid false positives, an on-line SPE–LC–TSP–MS method has been developed in our laboratory for a variety of pesticides [18]. In this paper we have optimized such a method for the acidic herbicides using NI mode detection, as recommended [11–14]. The major ions, relative abundances and LOD obtained under SPE–LC–TSP–NI MS are shown in Table 5.

In general, two mechanisms of ion formation were observed: proton abstraction and anion attachment. When ammonium formate–formic acid buffer (pH = 2.9) was used as additive different base peaks were obtained depending on the compound: for phenoxy acids and benazolin $[\text{M} + \text{HCOO}]^-$ ion, following expectations [11,12], whereas the $[\text{M} - \text{H}]^-$ ion for bentazone and 8-hydroxybentazone [19] and the $[\text{M} - \text{H} - \text{OH} + \text{HCOO}]^-$ ion for 6-hydroxybentazone. The use of tripropylammonium for-

Table 4

Average % recovery (Av) and relative standard deviation (R.S.D.) of pesticides in estuarine water (pH = 1) using on-line SPE with 10 SDB or 10 C_{18} Empore disks of 4.6 mm

Compound	Wavelength (nm)		SDB		C_{18}		LOD ($\mu\text{g/l}$)
	Ex	Em	Av	R.S.D.	Av	R.S.D.	
Bentazone	340	430	85	8	86	7	0.01
MCPA	280	310	22	30	18	24	1
MCPP	280	310	31	19	25	15	1

Water volume: 150 ml, spiking level 0.3 $\mu\text{g/l}$. Detection by fluorescence. (When HPLC water was used instead of estuarine water recoveries were of 85–95% for all three compounds at the same spiking level.)

Table 5

Main ions, relative abundance and LOD (when preconcentrating 100 ml of estuarine water) for acidic herbicides under on-line SPE–LC–TSP–MS under NI mode of operation

M_w	Compound and ions (m/z)	Carrier stream				LOD $\mu\text{g/l}$
		1	2	3	4	
240	Bentazone 239 $[\text{M} - \text{H}]^-$	100	100	100	100	0.02
200	MCPA 199 $[\text{M} - \text{H}]^-$ 245 $[\text{M} + \text{HCOO}]^-$ 235 $[\text{M} + \text{Cl}]^-$	12 100	18 100 18	17 100	16 100	0.1
214	MCPP 213 $[\text{M} - \text{H}]^-$ 259 $[\text{M} + \text{HCOO}]^-$ 249 $[\text{M} + \text{Cl}]^-$	10 100	10 100 10	23 100	16 100	0.1
221	2,4-D 219 $[\text{M} - \text{H}]^-$ 265 $[\text{M} + \text{HCOO}]^-$ 256 $[\text{M} + \text{Cl}]^-$	12 100	8 100 8	30 100	100 7	0.1
243	Benazolin 242 $[\text{M} - \text{H}]^-$ 288 $[\text{M} + \text{HCOO}]^-$	25 100	30 100	30 100	30 100	2
256	8-Hydroxybentazone 255 $[\text{M} - \text{H}]^-$	100	100	100	100	2
256	6-Hydroxybentazone 239 $[\text{M} - \text{OH}]^-$ 255 $[\text{M} - \text{H}]^-$ 283 $[\text{M} - \text{H} - \text{OH} + \text{HCOO}]^-$	23 23 100	20 20 100	5 15 100	10 10 100	4

Carrier stream: (1) water–acetonitrile (65:35, v/v) with a buffer of 50 mM ammonium formate–formic acid, pH = 2.9; (2) water–acetonitrile + 2% chloroacetonitrile (65:35, v/v) with a buffer of 50 mM ammonium formate–formic acid, pH = 2.9; (3) water–acetonitrile (65:35, v/v) with a buffer of 50 mM tripropylammonium formate–formic acid, pH = 2.9; (4) water–acetonitrile (65:35, v/v) with a buffer of 50 mM tripropylammonium formate buffer, pH = 7.2.

mate (pH = 2.9) was also investigated. Since tripropylamine has a higher basicity than ammonia [20] an increased deprotonation was expected. This hypothesis was confirmed in a basic mobile phase (pH = 7.2) and was important in the case of 2,4-D, that showed $[\text{M} - \text{H}]^-$ ion as base peak instead of $[\text{M} + \text{HCOO}]^-$ (see Table 5). When formic acid was added in greater amounts to lower the pH (pH = 2.9) the $[\text{M} +$

$\text{HCOO}]^-$ ion remained as base peak. This shows once more that TSP is a solution-dependent ionization technique being affected by the pH of the mobile phase [21]. With this mobile phase a two-fold, three-fold and one order of magnitude increase in signal intensity was found for MCPA, benazolin and bentazone, respectively. In addition, it showed slightly better sensitivity for the rest of the compounds and therefore it was

selected for performing the on-line SPE–LC–MS analysis.

The addition of 2% of chloroacetonitrile to the mobile phase was also investigated in order to investigate the additional structural information obtained, as reported [13]. Although no change was observed in the base peak the abundance of the $[M + \text{HCOO}]^-$ adduct ion decreased as compared with the absence of chloroacetonitrile. Chloride attachment leads to $[M + \text{Cl}]^-$ ion formation with relative abundances of at best 18%. Although the addition of chloroacetonitrile to the LC eluent allowed complementary molecular mass information to be obtained, unfortunately a two-fold decrease in the signal intensity was noticed. Consequently, it was not used for performing further studies with the on-line system since the major analytical requirements of the present work is to achieve a low detection limit.

The chromatograms were recorded under time-scheduled selected-ion monitoring (SIM) conditions, each compound being identified by two main ions. The time scheduled SIM is reported in the experimental section. Fig. 3 shows a typical on-line SPE–LC–TPS–MS separation in the NI mode of the pesticides mixture obtained after preconcentrating 100 ml of Ebro river water spiked at 0.6 $\mu\text{g/l}$. The monitoring of benazolin and the two bentazone TPs was impossible because of the lack of sensitivity under TSP–MS detection, and requires further investigation by other techniques, *e.g.*, electrospray. The optimization of the stem temperature showed that no thermal decomposition of the analytes occurred in the interface. The LOD obtained with the proposed method are indicated in Table 5 being somewhat higher than with LC–DAD.

In NI mode TCA gives one main ion corresponding to $[2M - 2\text{Cl} + \text{HCOO}]^-$ using water + formic acid pH = 2.82–methanol (65:35, v/v) as mobile phase when 1 μg was injected. In a mobile phase of acetonitrile–water, the relative abundance of this ion is even lower. Due to the difficulties in its response and low molecular mass, 162, this compound can not be determined

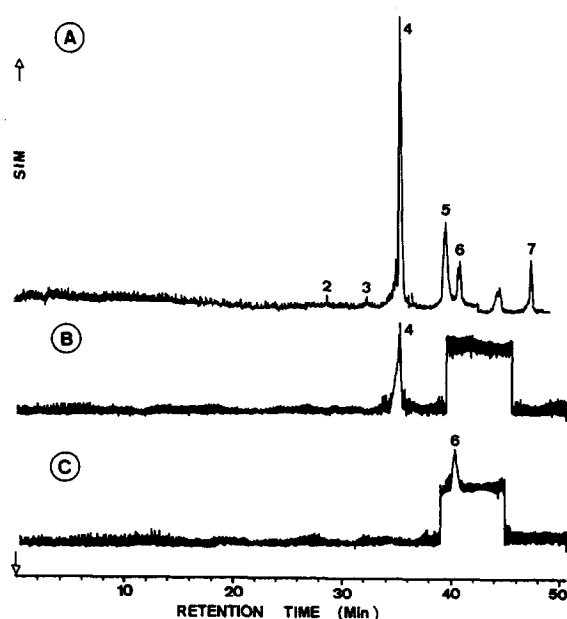


Fig. 3. (A) On-Line SPE using 10 SDB Empore extraction disks followed by LC–TSP–MS of 100 ml of Ebro river water sample spiked at 0.6 $\mu\text{g/l}$ in NI mode under SIM conditions (for details, see Experimental). Gradient elution as in Fig. 1 but the mobile phase contained as additive tripropylammonium–formic acid buffer pH = 2.9. (B) and (C) TSP–MS detection under NI and SIM detection after preconcentrating 100 ml of the same non-spiked estuarine Ebro delta water that permitted confirmation of the presence of: (B) (4) bentazone (0.12 $\mu\text{g/l}$) (stem, tip and source temperatures: 95°C, 180°C and 250°C, respectively); (C) (6) MCPA (0.18 $\mu\text{g/l}$) (stem, tip and source temperatures: 70°C, 160°C and 200°C, respectively).

at the low $\mu\text{g/l}$ level required in the present method.

3.5. Confirmation of environmental levels

The current developed methods were applied to the determination of acidic herbicides in the Ebro delta estuarine waters. A monitoring programme that measures the contamination level from acidic herbicides is continuously performed. The Ebro river delta is a typical rice cultivation area where bentazone and MCPA are applied in large amounts during spring and summer [2]. An example of the on-Line SPE

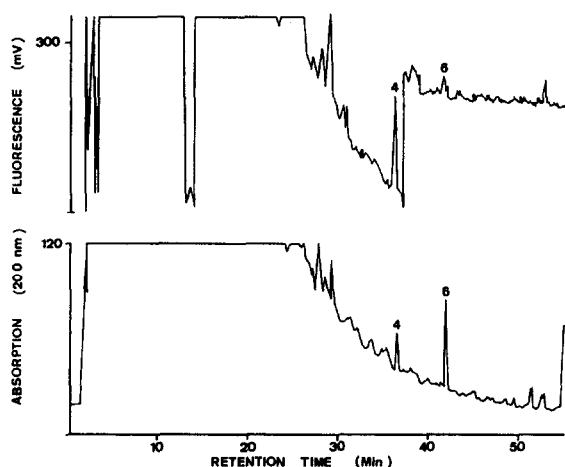


Fig. 4. LC chromatogram obtained after on-line preconcentration on SDB Empore extraction disks of a non-spiked estuarine Ebro Delta water sample using: (A) FD, (pre-concentration volume 150 ml) (B) DAD at 200 nm, (pre-concentration volume 150 ml) that permitted confirmation of: (4) bentazone at 0.12 $\mu\text{g/l}$ and (6) MCPA at 0.18 $\mu\text{g/l}$.

with LC-DAD and LC-FD traces are shown in Fig. 4A and 4B. Bentazone (peak no. 4) was clearly identified in both detectors, whereas the presence of MCPA was somewhat suspect and FD, due to the quenching effects mentioned before, could not be used for an unequivocal identification. The use of on-line SPE-LC-TSP-MS of the same sample is shown in the chromatograms of Fig. 3B and 3C. Due to the low level of MCPA, and lower signal under TSP-MS as regards to bentazone, it was necessary to perform the analysis of the sample twice. In the first case we monitored the $[\text{M} - \text{H}]^-$ ion of bentazone at m/z 239 (Fig. 3B) whereas in the second case we monitored the $[\text{M} - \text{H}]^-$ and $[\text{M} + \text{HCOO}]^-$ ions of MCPA, at m/z values of 199 and 245, respectively. In the latter case the experimental conditions were set up in order to increase the MCPA signal intensity (stem, tip, and source temperatures: 70°C, 160°C and 200°C, respectively). Under these conditions, the signal intensity of bentazone decreased by a factor of 10. In this way the unequivocal determination of bentazone and MCPA at 0.12

$\mu\text{g/l}$ and 0.18 $\mu\text{g/l}$ levels, respectively, was feasible in estuarine Ebro river waters samples collected during June of 1993.

4. Conclusions

An off-line SPE system based on the use of C_{18} or SDB Empore disks was evaluated for the determination of various acidic herbicides spiked at 0.25 $\mu\text{g/l}$ level using 5 liters of estuarine waters. The method permitted only the determination of bentazone and MCPA at 0.1 $\mu\text{g/l}$ level when using SDB Empore disks, whereas the recoveries for C_{18} Empore disks were below 60%, with the exception of bentazone. The off-line method demonstrated that with a pH close to 1 the use of SDB disks is superior to C_{18} disks since better blanks and recoveries are achieved.

The on-line SPE method using both types of material and only 150 ml of estuarine water spiked at levels of 0.3–1.5 $\mu\text{g/l}$ permitted recoveries greater than 80% for all the pesticides, with the exception of benazolin and the two TPs of bentazone. This was attributed to interferences in the LC-DAD traces caused by the humic substances present in the real estuarine waters. On-line SPE-LC with FD permitted a complementary detection to LC-DAD for bentazone in real estuarine water samples, whereas for MCPA and MCPA quenching effects were noticed. This behaviour presents a novelty in the analysis of real estuarine waters containing bentazone and it is a useful confirmation method.

The use of on-line SPE-LC-TSP-MS with NI and time-scheduled SIM permitted confirmation of most of the studied pesticides at LOD below or equal to 0.1 $\mu\text{g/l}$. Exceptions were benazolin, 6-hydroxybentazone and 8-hydroxybentazone with LOD of 2–4 $\mu\text{g/l}$.

It is our plan to investigate in the near future other adsorbent materials, e.g., carbon type, together with other MS detection systems, e.g., electrospray, to improve the LOD of certain compounds, such as benazolin, TCA and bentazone TPs.

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