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# Trace determination of herbicides in estuarine waters by liquid chromatography–high-flow pneumatically assisted electrospray mass spectrometry

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## Abstract

High-flow pneumatically assisted electrospray (ESP) was applied to the characterization of triazine (atrazine, simazine, ametryne, cyanazine, deethylatrazine and deisopropylatrazine), phenylurea (chlortoluron, isoproturon, diuron, linuron and diflufenbuzon) and other priority herbicides (alachlor, metolachlor). In LC–ESP–MS the  $[M + Na]^+$  ion was used as the base peak in most cases, with the exception of chlorotriazines, which showed  $[M + H]^+$  as the base peak. When LC–TSP–MS was used,  $[M + H]^+$  was the base peak for many of the pesticides, with the exception of linuron and diflufenbuzon, which showed  $[M + NH_4]^+$  as the base peak. The ESP results were compared with those obtained with thermospray (TSP). LC–TSP–MS offered greater sensitivity for triazines than phenylurea herbicides, whereas the use of LC–ESP–MS offered an enhancement in sensitivity for phenylurea herbicides. As regards the fragmentation obtained using both techniques, ESP offered a considerable amount of structural information for the different chlorotriazines studied when the extraction voltage was increased from 20 to 40 V. Liquid–liquid extraction with dichloromethane was used for the trace enrichment of the different herbicides in estuarine water samples from the Elorn river (France). The presence of the different triazine metabolites, atrazine, simazine, metolachlor, isoproturon and diuron was confirmed by both LC–MS techniques.

## 1. Introduction

Herbicides such as the chlorotriazines and phenylureas, alachlor and metolachlor are compounds used in Europe [1] and the USA [2] for agricultural applications. The various usage lists include atrazine, alachlor, metolachlor, chlortoluron, isoproturon and diuron as the main pesticides used worldwide with amounts of several millions pounds of active ingredient per year. After application, the different pesticide residues constitute a source of pollution of different

environmental waters with levels varying from 1 to 800 ng/l [3–6].

Chlorotriazine pesticides together with alachlor and metolachlor are usually determined by gas chromatography–mass spectrometry (GC–MS) with electron impact (EI) and/or positive- and negative-ion chemical ionization (PCI and NCI, respectively) [3–5,7,8]. GC–MS–MS has also been applied [9,10], offering higher selectivity and additional structural information. Liquid chromatographic techniques, involving diode-array determinations, MS generally with a thermospray (TSP) or particle beam interface and MS–MS have been used for the determi-

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nation of the above-mentioned herbicides and also for phenylurea herbicides [6,11–15].

Although LC–TSP–MS has been widely used for the determination of the above-mentioned herbicides, it was the purpose of this work to use electrospray (ESP) ionization for the determination of the same herbicides. LC–ESP–MS has rarely been used in environmental analysis, as pointed out recently by Budde [16], only 5 out of the 54 presentations on LC–MS in environmental analysis at the 42nd ASMS Conference reported the use of ESP. Atmospheric pressure chemical ionization (APCI) is the most popular LC–MS technique. Recently, high-flow ESP–MS has been developed and is now commercially available. It has the advantage over conventional ESP that it does not require low flow-rates (10  $\mu$ l/min) that need either a split (when using conventional LC pumps) or syringe LC pumps, with the additional time and extra cost involved. In addition, the ideal situation of an environmental laboratory will be to perform the same analysis under conventional LC–UV or LC–diode-array conditions using LC–MS. In a previous paper [17], we evaluated the use of LC with high-flow ESP–MS for the trace determination of thermally labile organophosphorus pesticides in water samples.

In this work, we used high-flow LC–ESP–MS for the determination of a variety of herbicides in estuarine waters. The aims were (i) characterization by LC–ESP–MS of the different herbicides studied and (ii) comparison of the results obtained by LC–ESP–MS with those obtained by LC–TSP–MS for the trace determination of herbicides in estuarine waters.

## 2. Experimental

### 2.1. Chemicals

HPLC-grade water and methanol were obtained from J.T. Baker (Deventer, Netherlands) and were passed through a 0.45- $\mu$ m filter before use. The herbicides were purchased from Promochem (Wesel, Germany).

### 2.2. Liquid chromatography–mass spectrometry

#### *Electrospray*

The eluent was delivered by a gradient system from Waters Model 616 pumps controlled by a Waters Model 600S controller (Waters–Millipore, Milford, MA, USA). The LC eluent conditions varied from methanol–water (30:70) to pure methanol in 15 min, maintaining the latter for 10 min and using a flow-rate of 0.3 ml/min. The LC column was kept at the starting LC conditions for 10 min as the equilibrium time. A 300 mm  $\times$  2.1 mm I.D. column, packed with 10- $\mu$ m particles coated with a C<sub>18</sub> stationary phase (Waters–Millipore) was used.

The gradient LC system was connected to a VG Platform ESP from Fisons Instruments (Manchester, UK) equipped with a Megaflo ESP probe. The design of this ESP consists of a coaxial flow probe; a detailed description of the interface has been published elsewhere [17]. The operational parameters of ESP optimized using an eluent flow-rate of 0.3 ml/min and a source temperature of 150°C as follows were: nitrogen drying gas flow-rate, 250–300 l/h; nitrogen ESP nebulizing gas flow rates, 10 l/h; ESP voltage, 3.1 kV; HV lens voltage, 0.3 kV; extraction voltage, 20–40 V; and focus voltage and ion energy as reported previously [17].

After the LC separation, the sample is introduced into the ESP source together with a nebulizing gas, which flows directly through the probe tip, maximizing the efficiency of the nebulizing. A drying gas is added to flush out any solvent which may have entered the gas line by capillary action and as a declustering medium for solvated molecules. The instrument control and data processing utilities included the use of MassLynx application software installed in a Digital DEC PC 466 computer. The high-flow pneumatically assisted ESP using a VG Platform instrument was used at a flow-rate of 0.3 ml/min and a source temperature of 150°C.

#### *Thermospray*

When the TSP interface was used, the LC gradient elution varied from methanol–water (10:90) to pure methanol in 18 min at a flow-rate

of 0.6 ml/min. Ammonium formate (0.05 M) was used as the mobile phase additive and it was added with a T-junction at a flow-rate of 0.4 ml/min using an LC pump from Knauer (Bad-Homburg, Germany).

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 98 to 90°C at 0.3°C/min with respect to the stem. The tip and ion source temperatures were set at 206 and 250°C, respectively. The filament-on mode and conventional positive-ion TSP ionization were used in all experiments.

### 2.3. Sample preparation

The extraction procedure follows a method reported previously [3,6] involving liquid–liquid extraction (LLE) with dichloromethane (2 × 100 ml) of 2.5 l of estuarine water. After agitation the extract was evaporated to dryness and the residue was dissolved in 400 µl of methanol. Volumes of 10 and 20 µl were injected into the

LC–ESP-MS and LC–TSP-MS systems, respectively.

### 2.4. Quantification

External standard calibration was used with quantification of the extract after LLE with a standard. The system was linear in most cases using 5–8 points from 0.01–0.5 to 20–100 ng. The calibration equations for the different pesticides analysed are given in Table 1. These calibration equations show, in some cases, large non-zero intercepts on the ordinate as compared with the abscissa, e.g., linuron. It should be noted that the linearity range in ESP is very much dependent on the compound, the number of points selected for calibration (usually 5–8) and the concentration linearity range. There is always a deviation from the intercept on the ordinate because it is not linear in the low concentration range (below 0.1–0.2 ng with few exceptions) and that when more points are used for the calibration graphs, this deviation increases. Analysis of the water extracts was achieved by using selected-ion monitoring (SIM) using either the  $[M + H]^+$  or  $[M + Na]^+$  ion, depending on the compound.

Table 1  
Calibration data for herbicides in the range 0.2–40 ng (0.0016–0.32 µg/l)

Analyte	Peak No.	Calibration equation	$R^2$	L.O.D. (pg)	
				ESP	TSP
Deisopropylatrazine	1	$y = 55513x + 22130$	0.997	450	200
Deethylatrazine	2	$y = 15801x + 1714$	0.999	450	200
Cyanazine	3	$y = 29652x + 8548$	0.998	300	150
Simazine	4	$y = 13444x + 3220$	0.999	450	200
Atrazine	5	$y = 18642x + 138$	0.999	450	200
Ametryne	6	$y = 234535x + 18696$	0.999	10	150
Alachlor	7	$y = 188311x + 5655$	0.999	20	400
Metolachlor	8	$y = 270930x + 18715$	0.999	15	300
Chlortoluron	9	$y = 56747x + 18106$	0.999	35	90
Isoproturon	10	$y = 119637x + 16901$	0.999	120	200
Diuron	11	$y = 33416x + 61515$	0.999	230	250
Linuron	12	$y = 6744x + 27869$	0.993	500	500
Diflubenzuron	13	$y = 27895x + 2924$	0.998	130	150

Calibration was performed by plotting peak area ( $y$ ) versus amount injected ( $x$ ). For ametryne, alachlor, metolachlor and chlortoluron, the linearity started at 0.01 ng whereas for atrazine and linuron from 0.5 to 120 ng (0.004 to 1.0 µg/l).

### 3. Results and discussion

#### 3.1. General considerations

Although TSP has been used extensively for the determination of pesticides, nowadays the use of APCI techniques is replacing this popular interfacing system. There are different APCI interfacing systems in LC–MS based on ultrasonic nebulization [18] and pneumatically assisted [17] and heated ESP [19] for the ionization of the analytes. The system shown in this paper was described in detail in a previous paper [17] and it is based on the use of pneumatically assisted ESP-MS. It does not heat the interface or the tip, but the nebulization is assisted by nitrogen nebulizing gas at 10 l/h. There is no heat in the interface itself and only the source is kept at 150°C. The nitrogen drying gas is used at 250 l/h. Both gases are introduced at room temperature.

A flow-rate of the LC eluent of 0.3 ml/min was used. This was not changed, although we performed a few experiments (not reported here) that showed a substantial decrease in sensitivity when the flow-rate was increased up to 0.5–0.6 ml/min.

The calibration equations for the different herbicides when using LC–TSP-MS have already been given previously [20,21] and indicate that LC–TSP-MS offers good linearity for many of the herbicides studied here. However, compounds such as alachlor and metolachlor elute at the end of the chromatographic trace at 100% of organic modifier with difficulties associated with maintaining a stable TSP tip temperature, and consequently high standard deviations were observed with no linearity in the quantification values [20]. The use of LC–ESP-MS is advantageous, specially in this case, for the accurate determination of these two analytes. Basically, because of the interface design with no heat in the interface, instability of the tip temperature was avoided. The fact that no heat is applied in the interface permitted the accurate determination of the thermally labile organophosphorus pesticide trichlorfon [17].

Fig. 1 shows different chromatograms ob-

tained for the herbicide standards using both LC–TSP-MS and LC–ESP-MS.

#### 3.2. Structural information

Table 2 shows the main ions obtained for the different herbicides using LC–ESP-MS at two extraction voltages. The first consideration is that when using a higher extraction voltage, generally much more fragmentation is obtained, as shown previously for organophosphorus pesticides [17]. The second question arises from the fact that on changing the extraction voltage from 20 to 40 V, the sensitivity usually decreases from 50 to 25%. Of the different herbicides studied, chlorotriazines gave good structural information on increasing the extraction voltage. The fragmentation obtained resembles the use of MS–MS [10,12,14]. This is known and it has been reported previously that this occurs by changing the capillary–skimmer potential difference. The spectra generated by ESP in the transport region at a capillary–skimmer potential difference of 30–50 V closely resembles those obtained from the  $[M + H]^+$  ion by a triple quadrupole mass spectrometer using a 30-eV collision energy [22].

The ion at  $m/z$  71 of deisopropylatrazine, commonly found using TSP-MS–MS, corresponds to  $[\text{CH}_3\text{CH}_2\text{NHCN} + \text{H}]^+$ . Ions corresponding to ring-opening reactions such as the ion at  $m/z$  132, which are common to deisopropylatrazine and simazine, corresponds to the  $[\text{CH}_3\text{CH}_2\text{NHC}(\text{NH})\text{NCCl}]^+$  moiety. Loss of HCl from this ion generates a signal at  $m/z$  96, and loss of the  $\text{CH}_2\text{CH}_2$  group generates a signal at  $m/z$  104. Pesticides bearing an isopropyl group (such as atrazine and deethylatrazine) show an ion derived from the loss of this moiety at  $m/z$  174 and 104. Elimination of HCN in the case of cyanazine affords the  $m/z$  214 ion. In addition to the above-mentioned diagnostic ions of the chlorotriazines, there is the possibility of the formation of adduct ions with  $\text{Na}^+$  of different diagnostic ions as reported for organophosphorus pesticides. This is a typical feature in high-flow pneumatically assisted ESP-MS [17], an example of which is the signal at  $m/z$  236 for cyanazine.

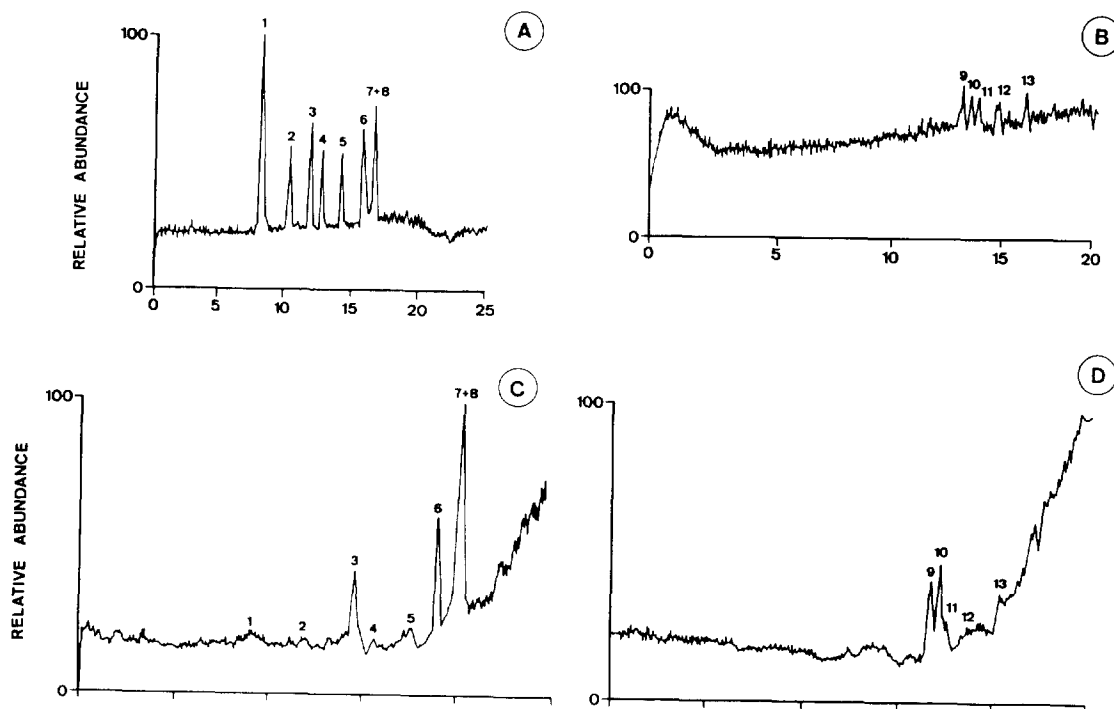


Fig. 1. (A and B) LC-TSP-MS and (C and D) LC-ESP-MS under full-scan conditions of herbicide standards injected at 200 ng (TSP) or 100 ng (ESP) for compounds corresponding to peaks 1–8 and at 100 ng (TSP) or 50 ng (ESP) for compounds corresponding to peaks 9–13. For peak identification, see Table 1. For gradient and column conditions, see Experimental.

Fig. 2 shows the increasing fragmentation observed for deethylatrazine when the extraction voltage was increased from 20 to 40 V. This feature is particularly relevant for environmental analysis, since it shows that for chlorotriazines high-flow pneumatically assisted ESP-MS can be used as a screening method for the identification of unknown chlorotriazine compounds. This is possible because the spectra obtained at an extraction voltage of 40 V resembles the fragmentation obtained under MS-MS conditions.

The remaining pesticides investigated showed virtually the same features when the extraction voltage was increased from 20 to 100 V, with  $[M+Na]^+$  as the base peak. The increased fragmentation observed for the chlorotriazines relative to the other groups of herbicides could be attributed to the facility of bond cleavage in such pesticides as compared with the other herbicides studied, which generally have an

aromatic structure that is more difficult to fragment under ESP-MS conditions.

### 3.3. Environmental analysis

Typical chromatograms of an estuarine water extract of 15‰ salinity from the Elorn river (France) obtained using LC-TSP-MS and LC-ESP-MS with SIM are shown in Fig. 3. The concentration of the different herbicides present in river water varied between 20 and 1000 ng/l. Both techniques permitted the different compounds present in the river water to be identified. The different intensities of the ions using the two techniques have been already discussed and the real sample follows expectations. In this respect, LC-TSP-MS offers higher sensitivity for chlorotriazines (peaks 1–5) whereas LC-ESP-MS offers considerably higher sensitivity than for

Table 2

Important mass spectral fragments, relative intensities of herbicides using methanol–water (50:50) as LC eluent and extraction voltages of 20 and 40 V under ESP-MS conditions

$M_r$	$m/z$	Compounds and ions: tentative identification	Relative intensity (%)	
			20 V	40 V
173		Deisopropylatrazine		
	174	$[M + H]^+$	100	100
	132	$[CH_3CH_2NHC(NH)NCCl]^+$	–	26
	104	$[132 - C_2H_4]^+$	–	14
	96	$[132 - HCl]^+$	–	40
	71	$[CH_3CH_2NHCN + H]^+$		6
187		Deethylatrazine		
	188	$[M + H]^+$	100	62
	146	$[CH_3CH_2CH_2NHC(NH)NCCl]^+$	–	100
	104	$[146 - C_3H_6]^+$	–	6
240		Cyanazine		
	263	$[M + Na]^+$	100	100
	236	$[M - HCN + Na]^+$	20	33
	214	$[M - HCN + H]^+$	–	5
201		Simazine		
	224	$[M + Na]^+$	4	35
	202	$[M + H]^+$	100	100
	166	$[M - HCl]^+$	–	10
	132	$[CH_3CH_2NHC(NH)NCCl]^+$	–	54
	104	$[132 - C_2H_4]^+$	–	8
215		Atrazine		
	238	$[M + Na]^+$	–	100
	216	$[M + H]^+$	100	26
	174	$[M - C_3H_6]^+$	–	74
227		Ametryne		
	250	$[M + Na]^+$	21	100
	228	$[M + H]^+$	100	69
	186	$[M - C_3H_6]^+$	–	36
269		Alachlor		
	292	$[M + Na]^+$	100	100
283		Metolachlor		
	306	$[M + Na]^+$	100	100
303		Chlortoluron		
	326	$[M - Na]^+$	100	100
206		Isoproturon		
	229	$[M + Na]^+$	100	100
232		Diuron		
	255	$[M + Na]^+$	100	100
248		Linuron		
	271	$[M + Na]^+$	100	100
310		Diffubenzuron		
	333	$[M + Na]^+$	100	100

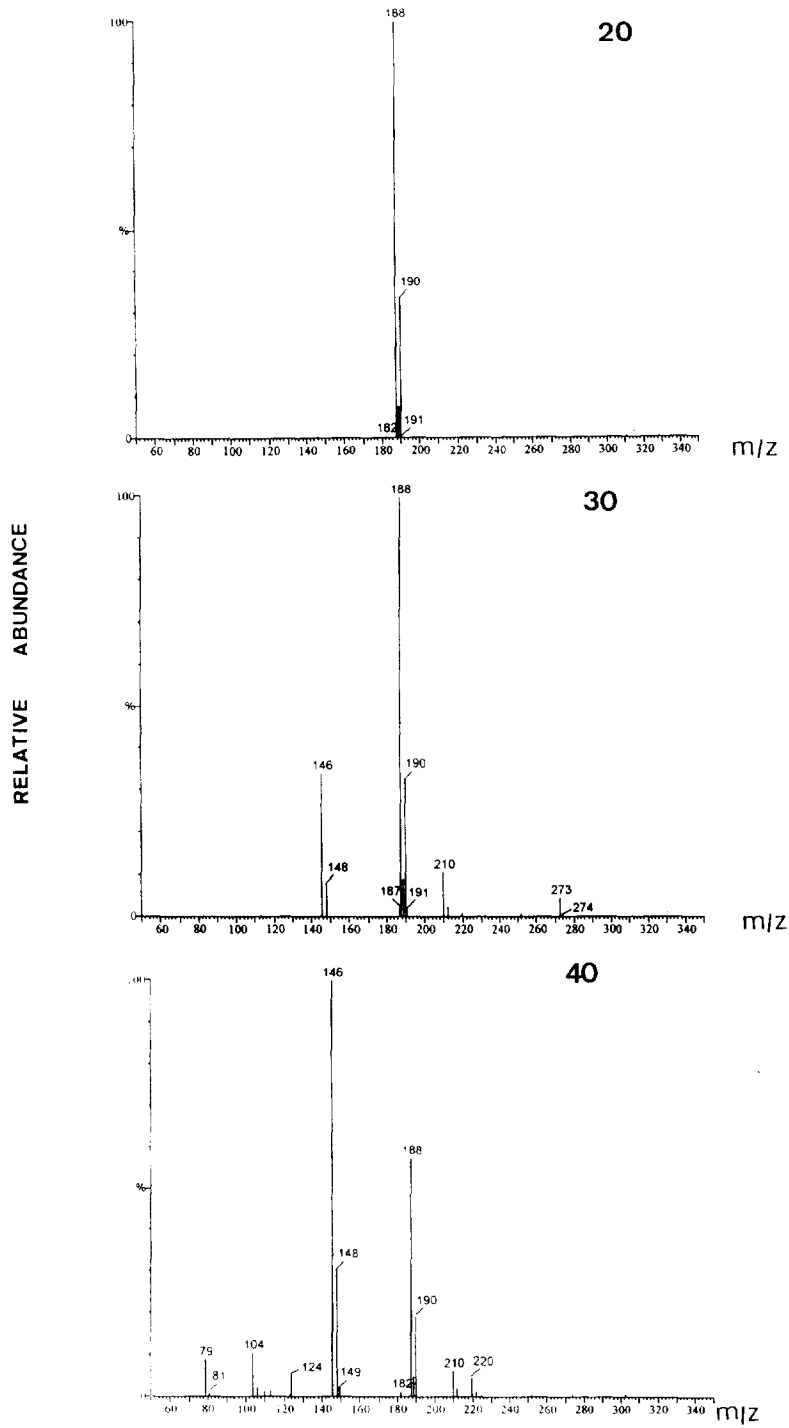


Fig. 2. LC-ESP-MS of deethylatrazine at extraction voltages of 20, 30 and 40 V. For identification of fragments, see Table 2.

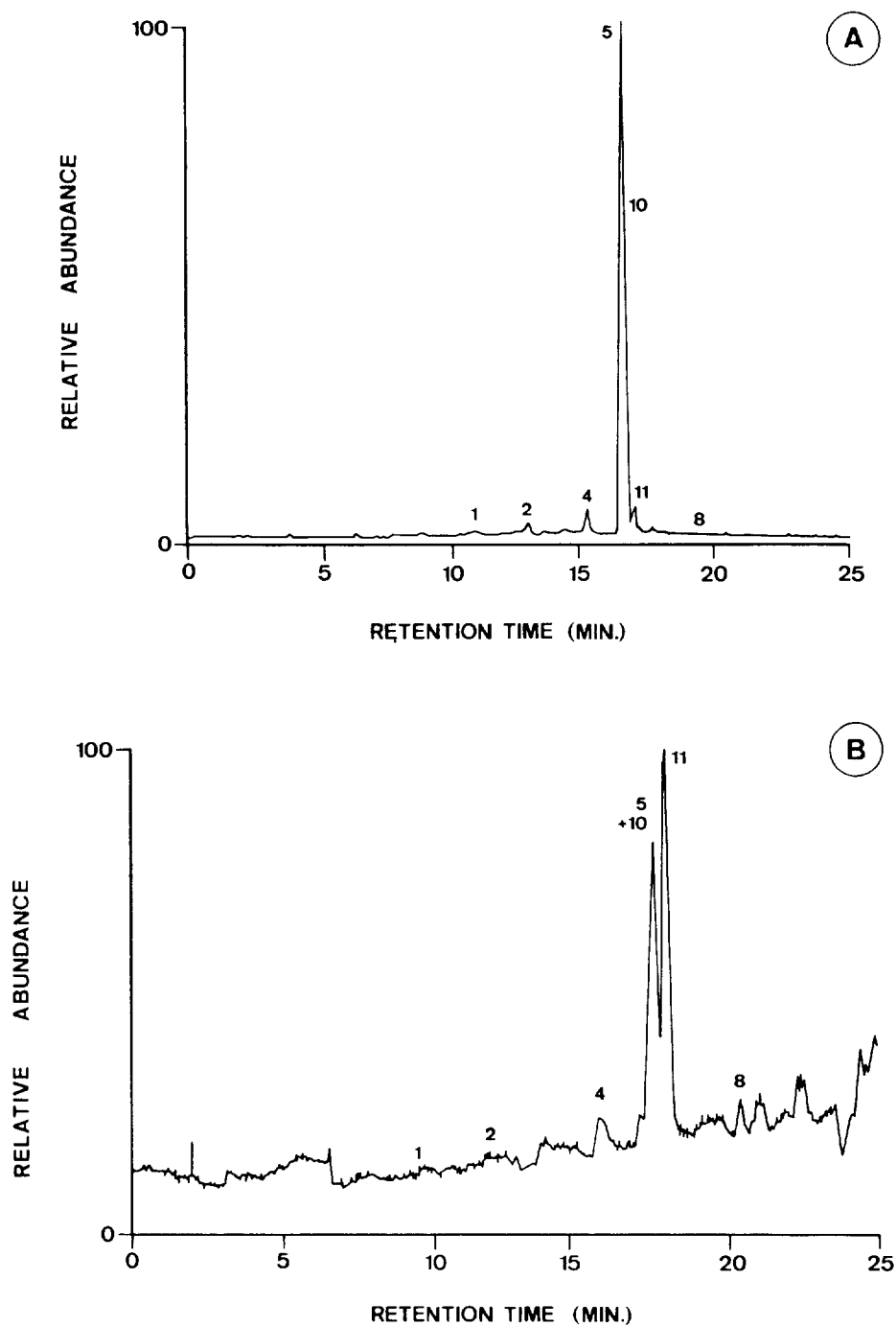


Fig. 3. (A) LC-TSP-MS and (B) LC-ESP-MS SIM traces obtained after dichloromethane LLE of 2.5 l of natural river water sample. The water sample contained (1) 20 ng/l of deisopropylatrazine, (2) 80 ng/l of deethylatrazine, (4) 400 ng/l of simazine, (5) 1000 ng/l of atrazine, (8) 200 ng/l of metolachlor, (10) 50 ng/l of isoproturon and (11) 600 ng/l of diuron. For gradient and column conditions, see Experimental and Fig. 1.



phenylureas and metolachlor (peaks 8, 10 and 11).

LC-ESP-MS can be applied to the determination of herbicides in natural estuarine water samples and has been demonstrated to be a useful technique that can easily compete with other LC-MS techniques in this field of application. From the results reported in Table 1 we can conclude that in general LC-TSP-MS give better limits of detection (L.O.D.s) for chlorotriazines (twofold). LC-ESP-MS is a much better method for alachlor, metolachlor, ametryne and chlortoluron, which show L.O.D.s in the range 10–30 pg (see Table 1). The L.O.D.s varied from 10 to 500 pg using the two techniques and were calculated by using a signal-to-noise ratio of 3–6 (the ratio between the peak intensity with SIM conditions and intensity of the noise was used). The reported L.O.D.s correspond to herbicides spiked into estuarine river waters. When using clean river water or drinking water samples, the L.O.D.s will be better, owing to less matrix interferences and/or better extraction recoveries of the pesticides from the water matrices.

#### 4. Conclusions

The application of LC-ESP-MS for the determination of different herbicides in environmental waters has been established. As regards fragmentation, the use of this technique achieves a comparable fragmentation pattern to TSP-MS-MS for chlorotriazines, whereas phenylurea, alachlor and metolachlor did not show fragmentation when the extraction voltage was increased. LC-ESP-MS offers greater fragmentation than conventional LC-TSP-MS for chlorotriazines although the sensitivity is poor. The L.O.D.s varied from 10 to 500 pg, making it a useful technique for environmental analysis. Of particular attention are the low L.O.D.s for compounds such as ametryne, alachlor, metolachlor and chlortoluron, from 10 to 30 pg, which makes the sensitivity of this technique competitive with GC-MS approaches. Another advantage for compounds not amenable to GC, such as the phenylurea herbicides, it is the much

better L.O.D. than other LC-MS techniques such as TSP or particle beam.

In view of the results obtained, we are interested in using LC-ESP-MS at high extraction voltages as a screening method for compounds that can give good structural information, e.g., chlorotriazines. This will be useful for detecting so-called "alarm" levels of chlorotriazine pesticides in different rivers, which are set at 2–5  $\mu\text{g/l}$ .

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