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# Determination of chlorobenzidines in industrial effluent by solid-phase extraction and liquid chromatography with electrochemical and mass spectrometric detection

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## Abstract

The analysis of chloroanilines in industrial effluents is severely complicated by the total organic content (TOC) (10–100 mg/l) and high particle content, which reflects the presence of many compounds other than the analytes of interest. Therefore, both the extraction procedure and the application of a selective and sensitive detection technique are important. Five different polymeric solid-phase extraction cartridges were tested to extract chloroanilines and benzidines from Milli-Q and industrial effluent waters. The analyses were performed by liquid chromatography with electrochemical detection and with atmospheric pressure chemical ionization mass spectrometry in positive ionization mode. These techniques were used to detect several mono- and dichloroanilines in industrial effluents, which had a TOC between 1 and 70 mg/l. © 1999 Published by Elsevier Science B.V. All rights reserved.

**Keywords:** Industrial effluents; Solid-phase extraction; Chloroanilines; Benzidines

## 1. Introduction

In recent years, the occurrence of chloroanilines and benzidines in environmental waters has started to be of concern because of their widespread use as intermediates in the production of azo dyes and azo pigments. Moreover, chloroanilines have been found in environmental waters as a consequence of degradation of acetamide [1] and urea herbicides [2]. At the same time, monochloroanilines can be biotransformed to its degradation products chloroacetanilides [3]. Benzidines are also used as cross-linking agents, e.g., in polyurethane plastics [4,5].

The determination of these compounds in environmental waters is necessary to develop strategies for pollution prevention and minimization [6]. Therefore, the analysis of industrial effluents is necessary in order to assess the quality of these waters which will be, eventually, discharged into the environment. This objective is supported by the European Union [7,8].

Several extraction and detection methods [9–16] are proposed in the literature to analyze chloroanilines and benzidines in waters, but few refer to the analysis in industrial effluents. This type of water poses difficulties of analysis because it often has a high total organic content (TOC), contains particles or is viscous. For such reason, it is important to use a

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selective extraction procedure and a sensitive detection technique since the levels of chloroanilines and benzidines can vary from few ppb to ppm. Among the different extraction techniques available, liquid–liquid extraction (LLE) is still used [17] but is being replaced by solid-phase extraction (SPE) either in on-line mode [14,18] or off-line [19,20]. As regards to on-line SPE, previous works have indicated poor usability of this technique for analyzing industrial effluents [21] since interferences are pre-concentrated along with the analytes of interest.

The specific objectives of this work were: (i) to develop and test a SPE procedure to recover mono-chloroanilines, dichloroanilines, trichloroanilines and benzidines in Milli-Q water and in industrial effluent water and (ii) to analyze industrial effluents and determine the occurrence of these compounds. Liquid chromatography with electrochemical detection (LC–ED) was found to be a sensitive and selective technique to determine chloroanilines and benzidines at ppb levels. In order to compare and confirm the results obtained by LC–ED, LC with atmospheric pressure chemical ionization mass spectrometry (APCI-MS) was optimized to determine these compounds.

## 2. Experimental

### 2.1. Chemicals and reagents

Pure standards (98–99%) 4-chloroaniline, 3-chloroaniline, 2-chloroaniline, 2-chloro-4-methylaniline, 3,4-dichloroaniline, 2,3-dichloroaniline, 2,4-dichloroaniline, 2,5-dichloroaniline, 2,6-dichloroaniline and 3,5-dichloroaniline were purchased from Sigma–Aldrich (France). 2,3,4-Trichloroaniline and 2,4,6-

trichloroaniline were purchased from Dr. Ehrenstorfer (Ehrenstorfer Labs., Augsburg, Germany). Benzidine and 3,3'-dichlorobenzidine were from Supelco (Bellefonte, PA, USA). Acetonitrile and dichloromethane were from SDS (Peypin, France) and pure (Milli-Q) water was generated with a Maxima Ultra Pure Water (Elga, France). Solid-phase cartridges used are detailed in Table 1.

### 2.2. Sample collection

Four liters of each sample were collected in Pyrex borosilicate glass bottles. Samples were immediately stored at 5°C and were analyzed within 24 h after arrival. In this study, four samples were collected from different sites. The first one, which contained no chloroanilines nor benzidines, was collected from the reject waters of an industry and was used to test the recoveries of chloroanilines and benzidines in industrial effluents. Samples 2, 3 and 4 were rejects from processed water from different types of industries. The TOC was analyzed after reception and was of 20, 1, 36 and 70 mg/l, respectively for samples 1, 2, 3 and 4.

### 2.3. Sample preparation

The standard solution containing the chloroanilines was prepared in acetonitrile at a working concentration of 10 to 50 ng/μl. Water samples were spiked with the mixture of chloroanilines to a final concentration of 1 ng/ml. Milli-Q and tap water was not filtered previous to SPE. However, industrial effluent samples were filtered through a 0.45-μm nylon membrane filter (Whatman, UK). Samples were extracted at a neutral pH.

Table 1

Types of cartridge used in this study, the trade mark and the chemical and physical properties

Type	Trade	Sorbent	Particle size (μm)	Pore size (Å)	Sorbent (mg)	Tube (ml)
EnviCrom-P	Supelco	SDB	80–160	20–300	500	6
Porapak	Waters	DB+VP	125–150	200	500	6
Oasis HLB, 30 mg	Waters	PDB+VP	31.4	82	30	1
Oasis HLB, 60 mg	Waters	PDB+VP	31.4	82	60	3
Oasis HLB, 200 mg	Waters	PDB+VP	31.4	82	200	6

SDB=Styrene–divinylbenzene, DB=divinylbenzene, VP=vinylpyrrolidone, PDB=[poly(divinylbenzene-co-N-vinylpyrrolidone)], HLB=hydrophilic–lipophilic-balance

## 2.4. Extraction procedure

### 2.4.1. Solid-phase extraction

Extraction was carried out using the manual manifold Visiprep from Supelco. Two different extraction procedures were chosen on the basis of the type of cartridge. For cartridges of 200 or 500 mg (Oasis 200, Envicrom-P and Porapak), cartridges were rinsed with 10 ml of dichloromethane and were freed from dichloromethane under vacuum. Afterwards, the cartridges were conditioned with 10 ml of acetonitrile (by letting it soak the cartridge during 1 min) and 10 ml of water, dropwise. Avoiding the cartridge to dry, 200 ml of sample were applied at a flow-rate of 10 ml/min. After the preconcentration step, cartridges were rinsed with 2 ml of water at a flow-rate of 20 ml/min, to remove all the interferences and impurities from the matrix. Afterwards, the cartridges were dried, under vacuum, during 30 min. Elution for these type of cartridges was performed with 5 ml of acetonitrile, followed by a second elution with 5 ml of acetonitrile–dichloromethane (1:1); no residues were found after a third elution.

For Oasis cartridges of 30 mg and 60 mg, the procedure was slightly different. Cartridges were soaked with one cartridge volume of dichloromethane during 1 min and afterwards dichloromethane was freed under vacuum. Conditioning was performed with one cartridge volume of acetonitrile (by letting it soak the cartridge during 1 min), followed by one cartridge volume of water. Two hundred ml of water was applied immediately after at a flow-rate of 10 ml/min. Cartridges were rinsed by applying one cartridge volume of water at a flow-rate of 20 ml/min. After the preconcentration, the cartridges were dried under vacuum during 15 min. Elution was carried out with one cartridge volume of acetonitrile followed by one cartridge volume of acetonitrile–dichloromethane (1:1). In all cases, evaporation of the solvent was performed under a stream of nitrogen. The final sample volumes (0.5–1 ml) were corrected by weighing.

### 2.4.2. Liquid–liquid extraction

LLE was used only to analyze industrial effluents and to compare the results obtained with SPE. For such purposes, 1 l of unfiltered water sample was

extracted with 100 ml of dichloromethane at neutral pH. Three successive extractions were carried out. For samples with a high TOC, formation of emulsions often occurred and it was necessary to centrifuge the extract. In such cases, the centrifuged sediment was added to the successive extraction in order to optimize the recovery. The dichloromethane extract was rotaevaporated just to dryness so as not to lose any of the compound. This extract was transferred to small vials and dichloromethane was substituted by acetonitrile by subsequent evaporations under a stream of nitrogen. Similarly as before, the final sample volumes were corrected by weighing.

### 2.5. Breakthrough volumes

The breakthrough volumes, or the maximum amount of water that can be percolated through a cartridge without experiencing any loss of recovery, were tested for all the studied compounds. Unfiltered tap water samples were spiked at a level of 1 ng/ml. Fifty, 100, 250 and 500 ml were percolated through the Oasis 60 mg cartridges using the method described above.

### 2.6. Liquid chromatography–electrochemical detection

A Hewlett-Packard isocratic pump HP 1050 was connected to a Hewlett-Packard 1049 programmable electrochemical detector (Hewlett-Packard, Waldbron, Germany). A column oven Model CTO-10A from Shimadzu (Tokyo, Japan) was set at 40°C. Data acquisition was performed with a HP B.02.06 Chemstation. An Alltech on-line degassing system (USA) was used to degas the mobile phase. The chromatographic separation was performed with an Interchim Nucleosil C<sub>18</sub> stainless steel column (150×4.6 mm I.D.) with a particle size of 5 µm (Interchim, France). For mono- and dichloroanilines, the LC isocratic gradient consisted of a 20 mM lithium perchlorate (Fluka, Buchs, Switzerland) solution–acetonitrile (70:30, v/v) at a working pH of 6. For benzidines and trichloroanilines, the same tampon was used but mixed with acetonitrile in the proportions (50:50, v/v). The chromatographic flow-rate was set at 0.7 ml/min. The optimal potential used in the electrochemical detection was 1 V. The

electrochemical detector was set in the amperometric mode and worked with a glassy carbon electrode. An Ag/AgCl reference electrode was used. Since most of the samples were from industrial effluents, the working electrode had to be polished once a week. A proper electrode had to give a current below 50 nA.

### 2.7. Liquid chromatography–atmospheric pressure chemical ionization mass spectrometry

A VG Platform from Micromass (Manchester, UK) equipped with an APCI source interface was used. The system was connected on-line with a gradient system from Waters 616 pumps coupled to a Model Waters 600S controller (Waters, Milford, MA, USA). The same analytical column as described above was used. Elution was carried out with acetonitrile and water acidified with 0.5% of acetic acid with the following gradient: from 10% of acetonitrile and 90% of water to 50% of acetonitrile and 50% of water in 12 min, to 70% of acetonitrile and 30% of water in 10 min and to 100% acetonitrile in 8 min, at a flow-rate of 1 ml/min. Initial conditions were reached in 5 min.

In this study, the cone and corona voltages were set at values of 30 V and at 3 kV, respectively. The HV lens voltage was set at 0.18 kV whereas the focus voltage varied between 8 and 13 V. The ion source was set at 180°C and the probe temperature was of 400°C. The nebulizing gas flow-rate was set at 10 l/h and the drying gas at 250–300 l/h. Standard calibration curves and samples were recorded under time-scheduled selected ion monitoring (SIM) using acquisition windows for each group of chloroanilines [ $m/z$  128, 92 and 93 (5–13 min);  $m/z$  142 and 106 (13 to 14.7 min) and  $m/z$  162, 164, 128 and 127 (14.7 to 20 min)].

In order to characterize the chloroanilines and benzidines and select the most abundant ion for SIM studies, flow-injection analysis (FIA) LC–APCI-MS was performed with acetonitrile–water (50:50, v/v) containing 0.5% acetic acid. The flow-rate was set at 1 ml/min and the amount injected was 200 ng. Acquisition was done in full scan in positive mode of ionization.

### 2.8. Quantification

For both LC–ED and LC–APCI-MS, quantifica-

tion was performed by comparison with an external standard. Calibration graphs were constructed for all the studied by injecting a standard mixture of the studied compounds over a concentration range of 20–40 to 500–700 pg/ $\mu$ l. Ten  $\mu$ l of a standard solution were injected by LC–ED. For LC–APCI-MS, 20  $\mu$ l were injected.

## 3. Results and discussion

### 3.1. Solid-phase extraction

Styrene–divinylbenzene sorbent material is used for a universal trapping of organic pollutants, which often range in polarities and physical properties [22,23]. It overcomes the limitations of C<sub>18</sub> silica-based sorbent material since it is stable over a broad pH range and it has a better retention capacity than C<sub>18</sub>. For such reasons, it was used to extract chloroanilines and benzidines from waters. Table 2 indicates the mean recovery ( $n=3$ ) of 14 chloroanilines and benzidines in Milli-Q water using different types of cartridges. All types of sorbent gave recoveries around 100% for most of the studied compounds. This indicates that the two-step elution procedure, which contained dichloromethane in a 25% was sufficient to elute all the retained compounds. This is specially relevant for Oasis 30 mg cartridge, for which 2 ml of solvent was enough to elute all the trapped compounds. All the analysis were performed in triplicate, and since the Milli-Q water does not represent a problem of interferences, the repeatability of the method was from 2 to 8% in all cases (values not indicated in Table 2). Both Porapak and Envicrom-P were used in the format of 500 mg. This quantity ensured acceptable recoveries and the percolation of 200 ml of water did not produce breakthrough of the analytes. Benzidine was the least recovered compound with these cartridges, which can be attributed to the peak tailing under isocratic conditions which made an error in quantification. The low recovery of benzidine with the Envicrom-P cartridges were attributed to an early breakthrough of this analyte. In this case, however, the repeatability was still very good (3% R.S.D., with  $n=3$ ), which indicates the validity of the extraction method. Other polymeric cartridges tested were the Oasis, which have the property of retaining all basic, neutral and

Table 2

Recoveries obtained after SPE–LC–ED of chloroanilines and benzidines in Milli-Q water (%  $R$ ) and in industrial effluents (%  $R_{\text{ef}}$ ) spiked at a level of 1 ng/ml ( $n=3$ )

Compound	Porapak % $R$	Oasis 30 % $R$	Oasis 60		Oasis 200 % $R$	Envicrom-P	
			% $R$	% $R_{\text{ef}}$		% $R$	% $R_{\text{ef}}$
4-Chloroaniline	114	82	102	43	85	80	66
3-Chloroaniline	114	102	96	120	110	91	121
2-Chloroaniline	124	76	86	85	105	111	119
2-Chloro-4-methylaniline	114	104	88	86	105	96	78
3,4-Dichloroaniline	122	106	90	101	94	97	96
2,3-Dichloroaniline	110	93	93	120	92	102	101
2,4+2,5-Dichloroaniline	115	86	113	79	78	105	90
2,6-Dichloroaniline	106	108	95	98	114	98	76
3,5-Dichloroaniline	110	69	91	120	125	102	87
Benzidine	66	67	83	91	76	38	47
3,3'-Dichlorobenzidine	104	93	87	73	81	67	66
2,3,4-Trichloroaniline	71	46	71	42	78	88	48
2,4,6-Trichloroaniline	108	92	98	73	86	99	72

Experimental conditions and coefficients of variation detailed in Sections 2.4, 2.6 and 3.3.

acidic compounds, regardless of their polarity [24]. Contrarily to other cartridges, Oasis cartridges can become dry during the sample manipulation due to its hydrophilic–lipophilic balanced copolymer which maintains the wettability of the sorbent. This aspect becomes important in situations where extraction has to be performed with the manual SPE manifold. Oasis cartridges, available in 30, 60 and 200 mg of sorbent, have been basically used for pharmaceutical applications where a small amount of sample is applied; therefore, it was considered necessary to study the performance of all of them. Oasis 30 mg gave good recovery values by percolating 200 ml of Milli-Q water spiked at 1 ng/ml level (see Table 2), indicating no breakthrough despite the small amount of sorbent. The lower recovery values observed for 2,3-dichloroaniline and 2,3,4-trichloroaniline were attributed to poor quantification due to peak broadening. Similar recoveries were obtained with Oasis 60 and 200 mg cartridges, with slightly better recoveries for 3,5-dichloroaniline and 2,3,4-trichloroaniline. On one hand, this confirms that no breakthrough of the studied compounds occurred with Oasis 30 mg, and on the other, it indicates that the extraction efficiency of both cartridges is excellent, since it is possible to load them with 3  $\mu\text{g}$  of product (in the present experiment) without experimenting losses of the analytes. This indicates that the amount of sorbent of Oasis cartridges is not significant to improve the

recovery of chloroanilines and benzidines at this level of concentration (1 ng/ml). Oasis 60 mg and Envicrom-P cartridges were selected to study the effect of the water matrix in the recovery values. To achieve this purpose, a blank industrial effluent water sample was spiked at a level of 1 ng/ml and the same extraction procedure was followed as for the former experiments. Table 2 reports the recovery results obtained for both Oasis 60 mg and Envicrom-P cartridges after preconcentrating industrial effluent water. In general, lower recoveries were obtained compared to Milli-Q water and higher R.S.D.s (3–16%). This was attributed to matrix interferences or problems of quantification due to partial coelution with matrix components, which was specially relevant for compounds eluting at the beginning of the chromatogram. Fig. 1 compares the LC–ED chromatograms obtained after preconcentrating 200 ml of Milli-Q and industrial effluent water with Envicrom-P cartridges. Fig. 2 shows the LC–ED separation method to detect benzidines and trichloroanilines after preconcentrating industrial effluent water through Envicrom-P and Oasis 60 mg cartridges. Benzidine and 3,3'-dichlorobenzidine were better recovered with Oasis 60 mg, due to a better baseline as compared to Envicrom-P (see Fig. 2). The lower recovery of 2,3,4-trichloroaniline in both cartridges was attributed to the low peak signal at this concentration.

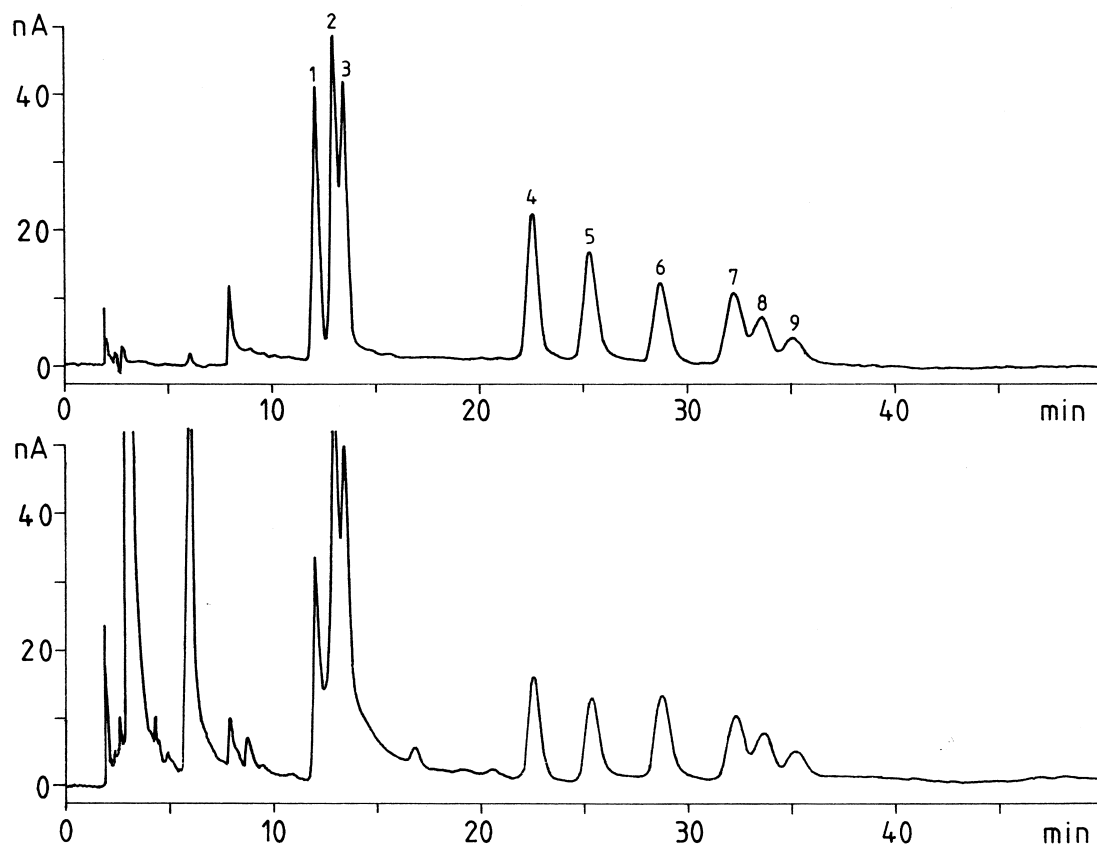


Fig. 1. LC-ED traces which correspond to (A) Milli-Q water spiked with chloroanilines at 1 ng/ml and (B) industrial effluent water spiked with chloroanilines at 1 ng/ml. Cartridges used were Envicrom-P and the separation method was to detect mono- and dichloroanilines (see Section 2.6). Peaks: 1=4-Chloroaniline, 2=3-chloroaniline, 3=2-chloroaniline, 4=2-chloro-4-methylaniline, 5=3,4-dichloroaniline, 6=2,3-dichloroaniline, 7=2,4+2,5-dichloroaniline, 8=2,6-dichloroaniline and 9=3,5-dichloroaniline.

### 3.2. Breakthrough values

The breakthrough of chloroanilines and benzidines was studied by percolating increasing volumes (50, 100, 250 and 500 ml) of a tap water sample spiked at a concentration of 1 ng/ml. If breakthrough occurred, lower recovery values would be expected at increasing the water volume percolated. No breakthrough was observed for most of the compounds studied. However, a 50–30% decrease in recovery was observed for 2,4- and 3,5-dichloroaniline, respectively, when percolating 500 ml of water. The advantage of percolating a water volume close to the breakthrough volume is that the limit of detection (LOD) can be lowered. It is expected, however, that lower breakthrough values will be encountered when

testing industrial effluent water matrix, since the efficacy of the SPE is reduced due to the obstruction of the active sites of the sorbent, which is reflected in increasing the breakthrough volumes [19]. Moreover, the higher the water volume preconcentrated, the higher will be the effect of the water matrix and more interferences will appear in the chromatogram. Therefore, the water volume preconcentrated is always a compromise between LOD and matrix effect.

### 3.3. Detection and quantification by LC-ED

Two LC separation methods were used to determine monochloroanilines and dichloroanilines on one hand and benzidines and trichloroanilines on the

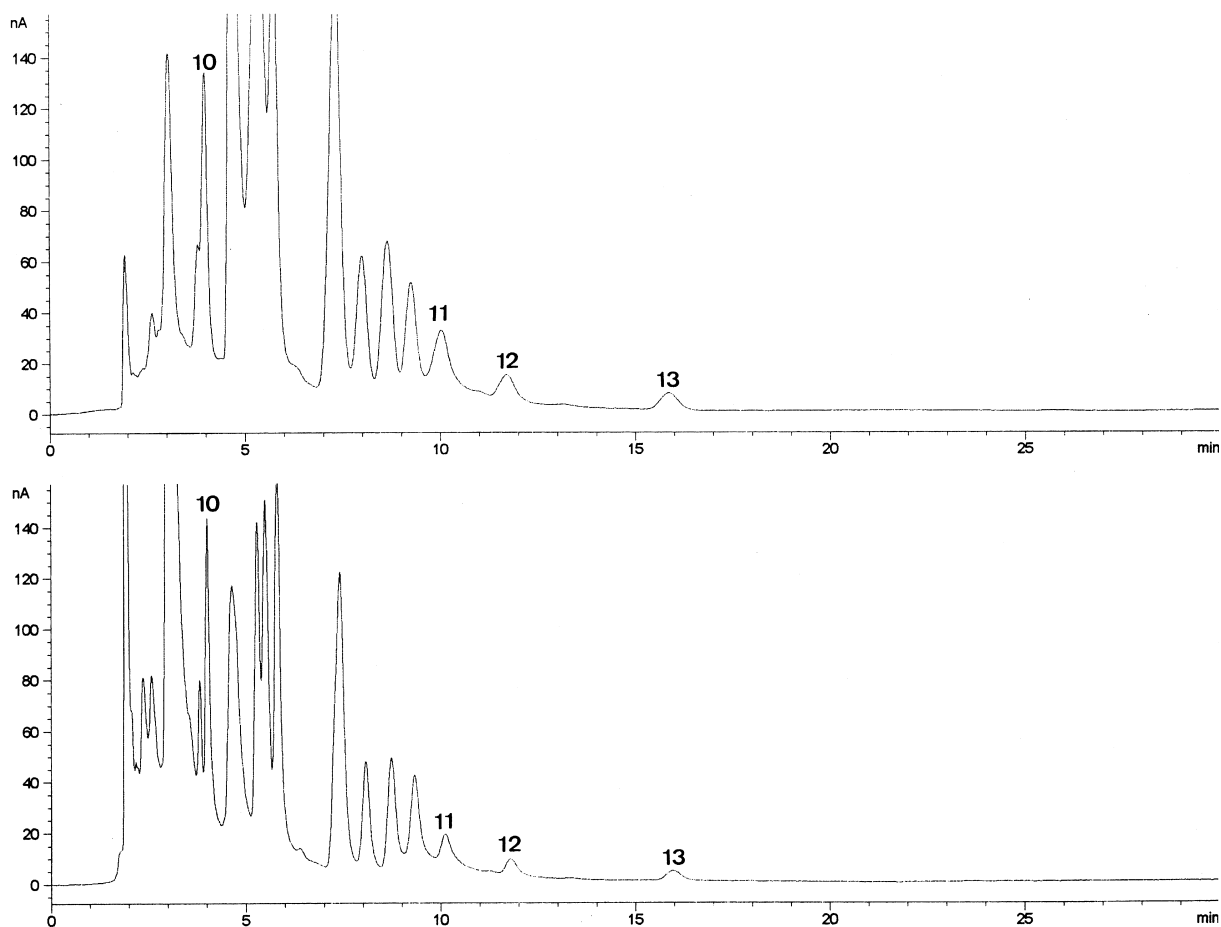


Fig. 2. LC-ED traces which compares the effect of the water matrix upon the preconcentration of (A) industrial effluent water spiked with chloroanilines and benzidines at 1 ng/ml and extracted with Envicrom-P and (B) industrial effluent water spiked with chloroanilines and benzidines at 1 ng/ml using Oasis 60 mg cartridges. The separation method was to detect benzidines and trichloroanilines (see Section 2.6). Peaks: 10=Benzidine, 11=3,3'-dichlorobenzidine, 12=2,3,4-trichloroaniline and 13=2,4,6-trichloroaniline.

other. Application of two gradients was performed to avoid excessive long runs or band broadening when working under isocratic conditions.

The system was linear over the concentration range studied (20 to 500 pg/ $\mu$ l for mono and dichloroanilines and from 20 to 1000 pg/ $\mu$ l for the trichloroanilines and benzidines). The only exception was for 2,4- and 2,5-dichloroaniline, which coeluted and quantification was not always evident specially at the low concentrations since at the same time they coeluted partially with 2,6-dichloroaniline. Therefore, they show a correlation coefficient of 0.978. For the rest of the compounds, the correlation coefficient was higher than 0.99. The LODs were

calculated from the chromatogram obtained at the lower concentration (20 to 40 pg/ $\mu$ l) taking a *S/N* of 3. For monochloroanilines, benzidines and trichloroanilines, LODs were below 10 pg/ $\mu$ l, which is in accordance with other authors for LC-ED [1]. The LODs for dichloroanilines were slightly higher (from 20 to 40 pg/ $\mu$ l) due to the band broadening of these compounds when working with isocratic conditions with a high percentage of water.

### 3.4. LC-APCI-MS

#### 3.4.1. Mass spectral information

In the present study the LC-APCI-MS instrument

was optimized in order to get maximum sensitivity due to the low concentration of chloroanilines and benzidines in the extracts. Previous studies on organophosphorus compounds indicated that at 30 V high sensitivity was obtained as compared to higher voltages [25] and provided sufficient structural information. In the present study, the cone voltage was

set at 30 V, which produced quasimolecular ions of most of the compounds as a base peak and several fragment ions which corresponded to chlorine losses. Table 3 reports the main ions formed. Except for trichloroanilines, the base peak corresponded to the protonated molecule, and presented the chlorine isotopic pattern of each group of chloroaniline,

Table 3  
Structural information obtained by FIA LC–APCI-MS in positive ionization mode

Compound	$M_r$	$m/z$ (% Relative abundance)	Identification
3-Chloroaniline	127	93 (42)	$[M+H-Cl]^+$
		111 (19)	$[M+H-NH_3]^+$
		128 (100)	$[M+H]^+$
2-Chloroaniline	127	92 (88)	$[M+H-HCl]^+$
		93 (13)	$[M+H-Cl]^+$
		128 (100)	$[M+H]^+$
4-Chloroaniline	127	93 (56)	$[M+H-Cl]^+$
		111 (14)	$[M+H-NH_3]^+$
		128 (100)	$[M+H]^+$
2-Chloro-4-methylaniline	141	106 (92)	$[M+H-HCl]^+$
		107 (19)	$[M+H-Cl]^+$
		142 (100)	$[M+H]^+$
3,4-Dichloroaniline	161	127 (55)	$[M+H-Cl]^+$
		145 (10)	$[M+H-NH_3]^+$
		162 (100)	$[M+H]^+$
2,4-Dichloroaniline	161	126 (38)	$[M+H-HCl]^+$
		162 (100)	$[M+H]^+$
2,5-Dichloroaniline	161	126 (52)	$[M+H-HCl]^+$
		162 (100)	$[M+H]^+$
2,3-Dichloroaniline	161	90 (18)	$[M+H-2HCl]^+$
		126 (69)	$[M+H-HCl]^+$
		162 (100)	$[M+H]^+$
2,6-Dichloroaniline	161	126 (60)	$[M+H-HCl]^+$
		162 (100)	$[M+H]^+$
3,5-Dichloroaniline	161	127 (61)	$[M+H-Cl]^+$
		145 (8)	$[M+H-NH_3]^+$
		162 (100)	$[M+H]^+$
Benzidine	184	168 (25)	$[M+H-NH_3]^+$
		185 (100)	$[M+H]^+$
3,3'-Dichlorobenzidine		n.a.	n.a.
2,3,4-Trichloroaniline	195	99 (100)	Not identified
		196 (26)	$[M+H]^+$
2,4,6-Trichloroaniline	195	99 (100)	Not identified
		175 (38)	Not identified
		255 (14)	$[M+CH_3COOH]^+$

The molecular mass ( $M_r$ ), fragment ions ( $m/z$  and % relative abundance) and tentative identification of each compound is indicated. n.a.=Not analyzed.



which was used for identification and confirmation purposes. In the case of monochloroanilines, a slightly different fragmentation pattern was observed and it was attributed to the position of the chlorine atom. 3-Chloroaniline and 4-chloroaniline followed exactly the same fragmentation pattern with identical relative abundances, which included an ion at  $m/z$  111 which corresponded to  $[M+H-NH_3]^+$  and a fragment ion at  $m/z$  93, corresponding to  $[M+H-Cl]^+$ . However, 2-chloroaniline which have a chlorine atom adjacent to the amino group, produced  $m/z$  92  $[M+H-HCl]^+$ . Since  $m/z$  92 was present as the second most abundant ion, with a relative abundance of 88, it was used to distinguish between the two other monochloroanilines. 2-Chloro-4-methylaniline produced a quasimolecular ion at  $m/z$  142  $[M+H]^+$  and losses of the chlorine, rendering  $m/z$  106 as the second most abundant ion, corresponding to  $[M+H-HCl]^+$ , similar to 2-chloroaniline. Dichloroanilines showed also the 3 chlorine structure at  $m/z$  162, 164 and 166, always conserving the isotopic relative abundances and followed the same fragmentation pattern corresponding to a chlorine loss, which produced both  $[M+H-HCl]^+$  or  $[M+H-Cl]^+$ , depending on whether they had a chlorine atom adjacent to the amino group or not. The ion formation of 3,4-dichloroaniline and 3,5-dichloroaniline was consistent with the ion formation of 3-chloroaniline and 4-chloroaniline (chlorine atom not adjacent to the amino group), and gave  $[M+H]^+$ ,  $[M+H-Cl]^+$  and  $[M+H-NH_3]^+$  ions.

Benzidine gave the same fragmentation pattern as reported by Castillo et al. [19], with the formation of  $[M+H]^+$  as the base peak. Trichloroanilines were hardly detected by injecting 200 ng of analytes. They underwent a different fragmentation pattern as the rest of the analytes studied, and did not form the  $[M+H]^+$  as base peak but a fragment ion at  $m/z$  99, not positively identified. The low sensitivity obtained for this compounds was due to the electronegativity of these compounds which permitted its detection at concentration levels of ng/ $\mu$ l.

#### 3.4.2. Detection and quantification

Even though LC-ED is a sensitive detection technique, LC-APCI-MS provides structural information which can be used for identification purposes and can be operated in a gradient mode, and there-

fore, in 20 min it is possible to analyze all the chloroanilines. Moreover, when working in SIM mode sensitivity and selectivity are enhanced, so that water matrix interferences, which are significant when analyzing industrial effluents with ED or diode array detection (DAD), are avoided. However, the main problem of analyzing a mixture of mono- and dichloroanilines by LC-APCI-MS is that for each degree of chlorination, they follow a similar fragmentation pattern. Therefore, identification had to be performed together with retention time. With the gradient used, 2,4- and 2,5-dichloroaniline coeluted although it was seen that 2,4-dichloroaniline was the first to elute. Since ion distinction was impossible, they were quantified in conjunction, similar to what was done with LC-ED.

Quantification of the samples was performed by external standard calibration. A calibration curve was constructed by standard injection of mono- and dichloroanilines over a concentration range of 30 to 700 pg/ $\mu$ l in SIM. The system was linear at these interval of concentration, with a correlation coefficient of 0.99 for most of the compounds. The exceptions were some dichloroanilines, and it was attributed to a low signal at the lowest concentration, which was close to the LOD. The LODs with LC-APCI-MS were similar to those obtained under LC-ED conditions.

#### 3.5. Analysis of industrial effluents

Three industrial effluent samples (samples 2, 3 and 4) were extracted by SPE and by LLE, and analyzed by LC-ED. In the case of the SPE extracts, the results were confirmed by means of LC-APCI-MS. No prior information was obtained from any of the samples analyzed. Therefore, specific information on the type of sample, whether the sample had undergone a depuration treatment or sample source was not available to us. The TOC gave an idea of the carbon charge of the sample and how it should be processed. Therefore, samples were filtered and the raw water was injected directly to the LC-ED to roughly determine the concentration of chloroanilines in the water. Direct water analysis had often many interferences, and so, extraction of the water sample was necessary to provide a mean of clean-up step, even if the sample had to be diluted afterwards.

From this point, it was possible to select the water volume that should be preconcentrated and the type of cartridge to be used. The initial purpose to test several cartridges was not to select the best one, but to study the capabilities of each and use their advantages for each type of application. For such reason, depending on the type of sample, a different extraction cartridge was used. Sample 2 was a clean sample with a TOC of 1 mg/l. It was therefore important to achieve a high preconcentration factor in order to decrease the detection limits. Using Oasis 30 mg cartridges, it was possible to percolate 200 ml of sample and elute it in a final volume of 2 ml. With a 100-fold preconcentration, with no need of evaporating the extract, it was possible to detect 3,4-dichloroaniline at an extract concentration of 37 ng/ $\mu$ l by LC-ED, equivalent to 475 ng/l in water. The same extract was analyzed by LC-APCI-MS to confirm the results and the concentration encountered was slightly lower, of 317 ng/l (see Table 4). This lower concentration might be more close to reality, since with this technique there is not a problem of matrix interferences. Fig. 3A shows the standard containing the mono and dichloroanilines at 20 pg/ $\mu$ l. Fig. 3B shows the LC-ED traces of sample 2. The same water sample was extracted by LLE and 3,4-dichloroaniline was detected at a lower con-

centration, which can be attributed to losses during manipulation or due to poor extraction efficiency.

Sample 3 represented the opposite case. With a TOC of 30 mg/l, chloroanilines were detected by direct water analysis. Therefore, 50 ml of sample were preconcentrated onto Oasis 60 mg and eluted to a final volume of 5 ml. This extract was diluted 50 times before injection by LC-ED and LC-APCI-MS. Fig. 3C shows the traces obtained with LC-ED. Fig. 4 shows the LC-APCI-MS traces of a standard solution at 20 pg/ $\mu$ l and the sample extract. Table 4 lists the compounds detected and their concentrations. For SPE extracts, since the concentrations were higher in this sample than in the previous one, less deviation in concentrations was obtained between the LC-ED and LC-APCI-MS detection techniques. However, for LLE extracts, lower concentrations were measured in all cases due to the complication and success of extracting chloroanilines in such a charged sample using this technique. Moreover, when analyzing the SPE extract by LC-APCI-MS, 2-chloroaniline, 2-chloro-4-methylaniline and 2,6-dichloroaniline were also detected. This indicates that this technique is superior to LC-ED in means of sensitivity. However, LC-ED should be always applied on first instance to discard positive and negative samples.

Table 4  
Levels of chloroanilines in industrial effluents

Compounds identified	SPE		LLE
	LC-ED	LC-APCI-MS	LC-ED
<i>Sample 2</i>			
3,4-Dichloroaniline	475 ng/l	317 ng/l	216 ng/l
<i>Sample 3</i>			
4-Chloroaniline	1.05 mg/l	1.11 mg/l	0.65 mg/l
3-Chloroaniline	2.25 mg/l	1.75 mg/l	1.50 mg/l
2-Chloroaniline	n.d.	0.21 mg/l	n.d.
2-Chloro-4-methylaniline	n.d.	0.20 mg/l	n.d.
3,4-Dichloroaniline	1.98 mg/l	2.10 mg/l	1.34 mg/l
2,3-Dichloroaniline	6.25 mg/l	5.87 mg/l	3.7 mg/l
2,6-Dichloroaniline	n.d.	0.62 mg/l	n.d.
<i>Sample 4</i>			
2,5-Dichloroaniline	47.8 $\mu$ g/l	39.5 $\mu$ g/l	106 $\mu$ g/l

SPE or LLE was performed, and analyses were carried out by LC-ED and LC-APCI-MS.

n.d.=Not detected.

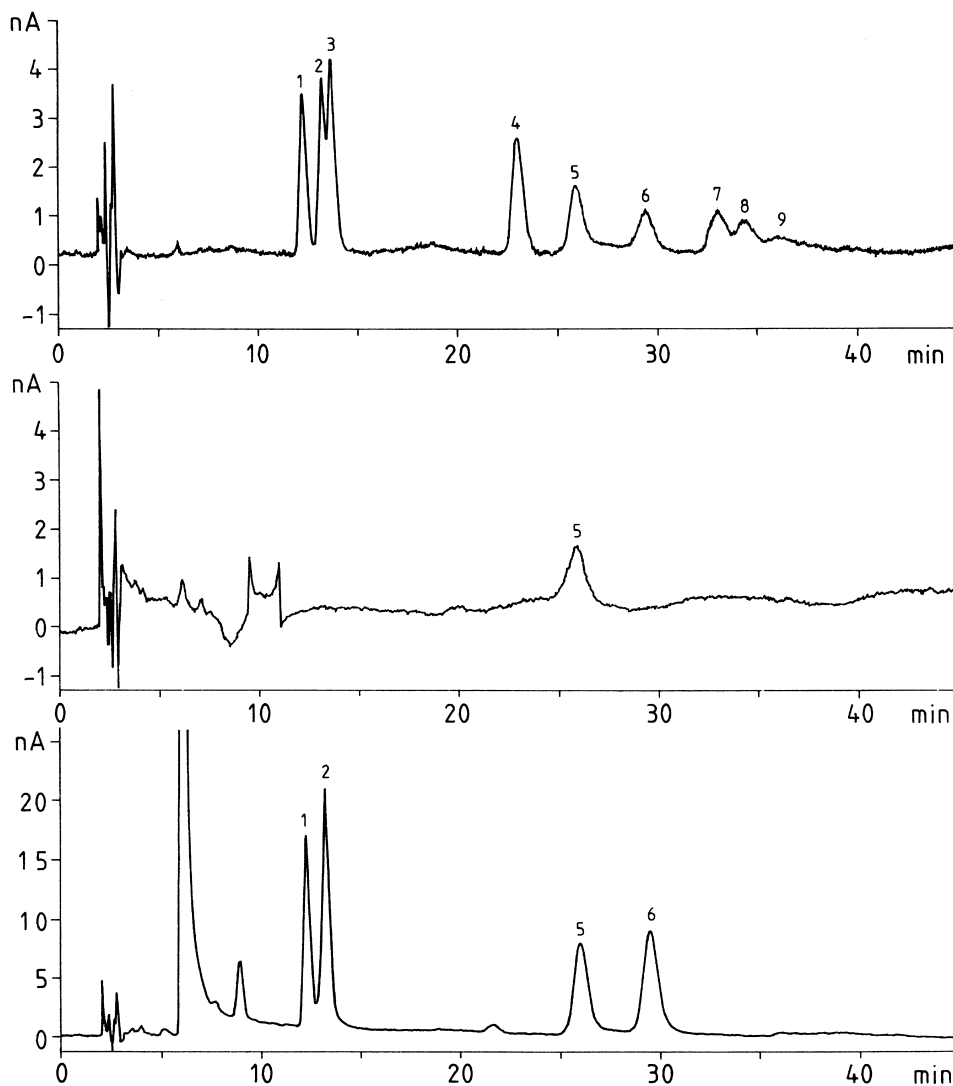


Fig. 3. LC-ED chromatograms that correspond to (A) a standard mixture at 20 pg/ $\mu$ l and analyzed to detect mono- and dichloroanilines, (B) sample 2, in which 3,4-dichloroaniline is detected and (C) sample 3 in which 4-chloroaniline, 3-chloroaniline, 3,4-dichloroaniline and 2,3-dichloroaniline were detected. Peaks as in Fig. 1.

A third case is represented by sample 4. In this case, the TOC was of 70 mg/l and with a high particle content. SPE was performed by percolating 100 ml of filtered water through Oasis 60 mg cartridges. 2,5-dichloroaniline was detected at a concentration of 47.8  $\mu$ g/l and confirmed by LC-APCI-MS. The distinction between 2,5-dichloroaniline and 2,4-dichloroaniline was done by the

slight retention time difference. In this case, the LLE extract, analyzed by LC-ED gave a higher result, which was attributed to a matrix interference.

#### 4. Conclusions

A comparative study of the performance of five

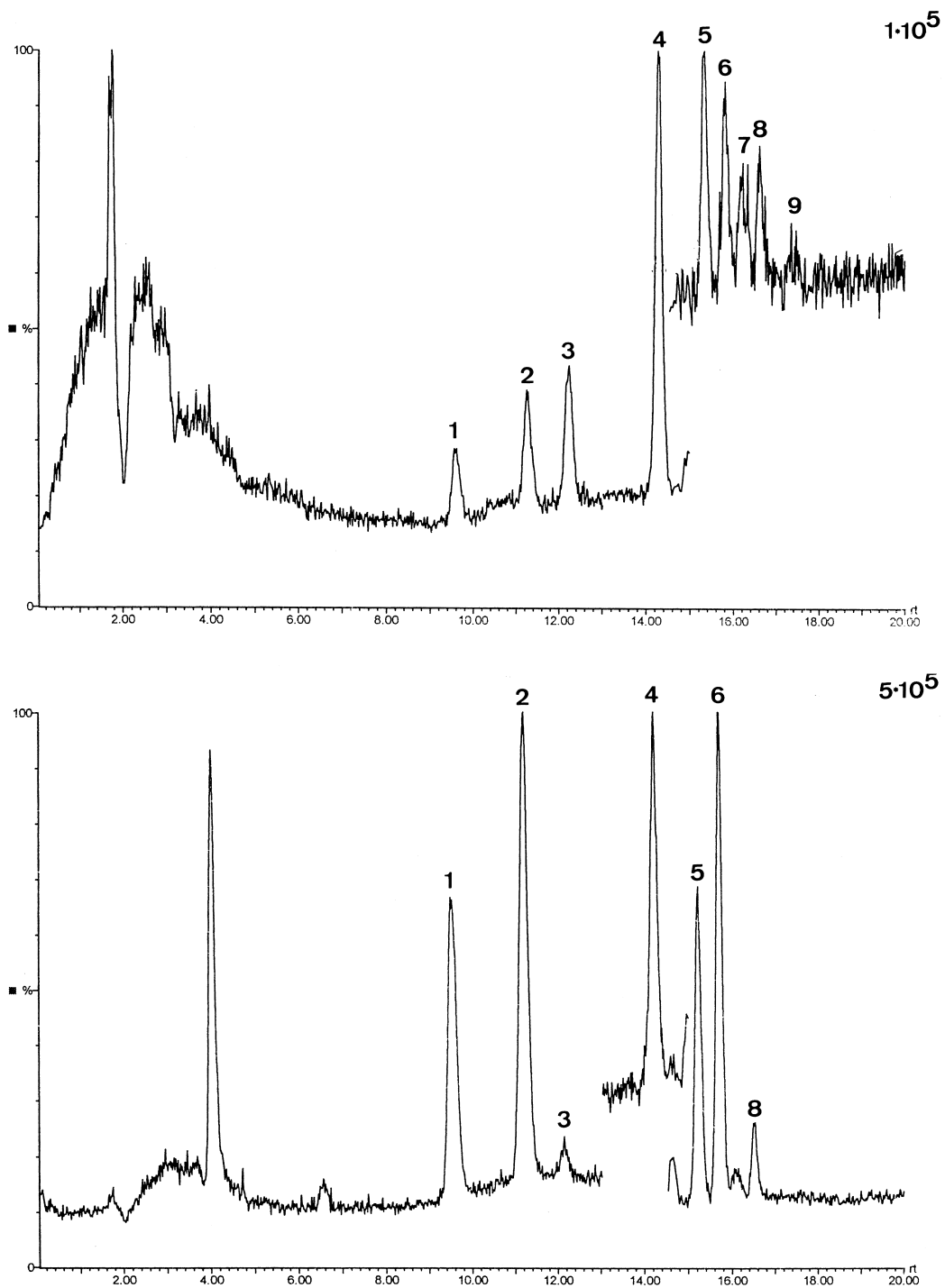


Fig. 4. LC-APCI-MS chromatogram in positive ionization mode and SIM of (A) a standard containing mono- and dichloroanilines and (B) sample 3. Peaks as in Fig. 1.

different polymeric SPE cartridges for the extraction of chloroanilines and benzidines in water was carried out. LC–ED and LC–APCI-MS provided good performance at the pg/ $\mu$ l level. No differences in recoveries were obtained using different SPE cartridges. Higher R.S.D.s were found in analyzing spiked industrial effluent water, due to matrix interferences, which made quantification difficult. Of the overall cartridges tested, Oasis 30 and 60 mg were preferred due to the fact that although the quantity of sorbent is much smaller, they rendered good recoveries in both Milli-Q and in industrial effluent water. Using these cartridges, the total amount of solvent dispensed and the drying time of the cartridges was reduced. Moreover, the final eluate was collected in a small volume of solvent (2 ml for Oasis 30 mg and 5 ml for Oasis 60 mg), which provided a preconcentration factor of at least 100, which was enough to achieve detectability of chloroanilines and benzidines in industrial effluent water samples.

FIA LC–APCI-MS in positive ionization mode was carried out to characterize chloroanilines and benzidines. At an extraction voltage of 30 V, good sensitivity and structural information was achieved.  $[M+H]^+$  was formed as base peak and fragmentation was caused by losses of chlorine or  $NH_3$ , which rendered either  $[M+H-HCl]^+$ ,  $[M+H-Cl]^+$  and  $[M+H-NH_3]^+$ . The most abundant ion of each compound was selected for SIM analysis. LODs were at the pg/ $\mu$ l level, except for trichloroanilines and benzidines, that were at the ng/ $\mu$ l level. For these compounds, a higher preconcentration of the sample is needed in order to achieve their detectability.

The developed extraction and detection techniques were used to determine the levels of chloroanilines in industrial effluents. LC–ED permitted a selective detection, at ppb/ppm levels. All the positive samples were confirmed by LC–APCI-MS in SIM mode. Such technique was considered more powerful, since in some cases it detected chloroanilines which were not encountered by LC–ED. The SPE procedure was intercompared with conventional LLE. In two samples, the lower levels of chloroanilines determined with LLE were attributed to poor extraction efficiency. Extraction of sample 4, which contained 70

mg/l of TOC, higher levels were encountered due to interferences coextracted with the compounds of interest.

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