Trace-Level Determination of Benzidine and 3,3'-Dichlorobenzidine in Aqueous Environmental Samples by Online Solid-Phase Extraction and Liquid Chromatography with Electrochemical Detection

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Abstract

A simple, rapid, and reliable online methodology for the determination of benzidine and 3,3'-dichlorobenzidine (3,3'-DCB) in natural waters is proposed. The analytes are extracted and preconcentrated from aqueous samples in a small stainless steel precolumn packed with a polymeric PLRP-S phase. The precolumn is further online-analyzed by reversed-phase gradient-elution chromatography with a highly sensitive and selective coulometric detection at *E* = 700 mV. Recoveries greater than 90% and a relative standard deviation of approximately 5% are achieved with samples spiked at low micrograms-per-liter concentration levels. The detection limits of the method in fortified reagent water samples are 100 ng/L for benzidine and 50 ng/L for 3,3'-DCB.

Introduction

Benzidine, 3,3'-dichlorobenzidine (3,3'-DCB), and other derivatives of biphenyl-4,4'-diamines have been widely used as intermediates in the manufacturing of dyes, pigments, and various pesticides. It is now generally agreed that benzidine and its salts are potent human-bladder carcinogens. Even though 3,3'-DCB has only been found to be carcinogenic in rodents (1), it has been demonstrated that it can be progressively dehalogenated in lake sediments to yield the more toxic benzidine (2). Therefore, both compounds have been included in the priority pollutant lists of most countries, and their production and use are strictly regulated (3).

Because of its polarity and the possibility of protonation in medium acidic media, benzidine has the potential to be widely transported and dispersed in the aquatic environment. Indeed, water-quality criteria have been established for benzidine and 3,3'-DCB (4). It is thus necessary to count with relatively simple, rapid, and sensitive procedures, which can be used for the regular monitoring of these compounds in natural waters.

Various methods have been published for the determination of benzidine and 3,3'-DCB at very low concentration levels in water. EPA Method 605 and other related methods (5,6) based on the liquid–liquid extraction (LLE) of the analytes and their separation and determination by reversed-phase (RP) liquid chromatography (LC) with electrochemical detection (ED) provide detection limits (LODs) in the 0.05- to 0.1-µg/L range. Their disadvantage is the very long time and extensive labor required for sample preparation. The direct injection of relatively large sample volumes (≥ 50 µL) in an RPLC–ED system has also been reported, and LODs of approximately 1 µg/L or less have been claimed (6,7). Although this procedure is very rapid and simple, it is the most susceptible to interference and column fouling when complex samples (i.e., river water or wastewater) are analyzed. Micellar electrokinetic chromatography with UV detection has also been successful in the sensitive and selective determination of benzidines after their LLE from water samples (8).

Solid-phase extraction (SPE) in the on- or offline modes is actually the technique of choice for the extraction and preconcentration of organic compounds from aqueous samples. However, there are two cases in which the optimization of conditions for the extraction with the classical RP sorbents is rather difficult. One is the extraction of fairly polar analytes, such as some phenols and anilines (9–11), which are poorly retained in the sorbents. The other is the extraction of a family or group of compounds with very different hydrophobicity (12,13). In fact, the two problems are implicated in this work because the polarity of benzidine is similar to that of phenol (14) and the difference in the hydrophobicity between benzidine and 3,3'-DCB is considerable. Riggin et al. (6) used an ODS cartridge for the offline SPE of benzidines from wastewater. Only 10 mL of sample were loaded in the cartridge, but it is evident from the chromatograms that some benzidine was lost during this process. Lacorte et al. (15) tested five polymeric SPE cartridges containing different commercial sorbents and different amounts of sorbent for the

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offline extraction of chloroanilines and benzidines from water samples. Recoveries varying from 38% to 83% for benzidine and 67% to 104% for 3,3'-DCB were obtained when 200 mL of LC water spiked at 1 µg/L of each analyte was extracted in the different cartridges. The authors indicated that the amount of sorbent was not significant to improve the recovery at this level of concentration. Recently, the use of highly selective immunosorbents has been proposed for the SPE of polar and nonpolar organic compounds from various matrices (16–18). The adsorption process in these materials is not based on hydrophobic interactions but on selective analyte–antibody interactions. Therefore, extraction, concentration, and cleanup are achieved in one step and the previously mentioned problems (for polar analytes or for mixtures of compounds with very different hydrophobicity) are not encountered. Bouzige et al. (14) used an antibenzidine immunosorbent for the online SPE of benzidine, 3,3'-DCB, and related azo-dyes from surface water and polluted effluents.

Although the immunosorbent approach is very attractive, these materials are still in development and the commercialized SPE cartridges remain quite expensive. Therefore, it was considered of interest to develop an alternative online SPE method using the classical styrene–divinylbenzene sorbents for the determination of benzidine and 3,3'-DCB in environmental waters. The objective was to propose a relatively rapid and reliable method for the accurate and precise determination of these compounds in the routine analysis of water samples and provide the conditions for the automation of this process.

Experimental

Apparatus

The LC consisted of two Gilson (Villiers-le-Bel, France) Model 305 and 306 pumps, a Gilson 805 manometric module, a Gilson 811 dynamic mixer, a 7125 Rheodyne (Berkeley, CA) injector with an in situ calibrated (12) 24-µL loop, and a coulometric detector Coulochem II from ESA (Chelmsford, MA) equipped with a Model 5011 analytical cell and a Model 5020 guard cell. Oxidation potentials of 700 and 900 mV relative to the internal reference electrode of the cells were set for the analytical and guard cells, respectively. According to data given by the manufacturer, the internal reference electrode potential at a working pH of 7 is 180 mV versus the normal hydrogen electrode. Chromatograms were recorded and integrated by a Hewlett Packard (Avondale, PA) Model 3396 Series II integrator. Quantitation was always based on peak area measurements.

The sample pretreatment section consisted of a Beckman (Berkeley, CA) 110 B isocratic pump (sample pump) with a manual three-channel selector valve adapted to the pump inlet and a 7000 Rheodyne switching valve with the SPE precolumn placed between ports 1 and 4. The valve was connected to the sample pump through port 6. For the online coupling of this section with the analytical one, the switching valve was inserted between the injector and the HPLC column using ports 2 and 3, respectively, for connection. With this setup, the sample loading and elution of the precolumn were carried out in the same direction.

Stationary phases and columns

A Haskel (Burbank, CA) Model 29426 packing system was used for the home packing of the precolumn and analytical column. The stainless steel precolumn (20- × 2-mm i.d.) from Upchurch Scientific (Oak Harbor, WA) was packed at a constant pressure of 120 bar with an acetonitrile–water (20:80, v/v) slurry of the styrene–divinylbenzene copolymer PLRP-S (100 Å, 10–15 µm) from Polymer Laboratories (Amherst, MA). The analytical column (150- × 4.6-mm i.d.) was packed at a constant pressure of 525 bar with an ethanol–acetone (50:50, v/v) slurry of Hypersil ODS (5 µm) from Thermoquest (Cheshire, U.K.).

Separations were carried out at ambient temperature using a mobile phase gradient at a flow rate of 1 mL/min. Eluents A and B were acetonitrile–water–sodium citrate buffers (1M, pH 6) in the proportions of 10:89:1 (v/v) and 70:29:1 (v/v), respectively. The gradient system used eluent B, and it was programmed in the following sequence: 0 min = 5%, 12 min = 5%, 13 min = 30%, 30 min = 100%, and then constant for 5 min. Separations of the injected standards were also carried out with the precolumn online-coupled to the analytical column because both of them participate in solute retention and peak shape.

Reagents

HPLC-grade acetonitrile and methanol were from Prolabo (Paris, France) and EM Science (Gibbstown, NJ), respectively. Reagent water was obtained from a Nanopure deionizer (Barnstead Thermolyne, Dubuque, IA). Ammonium acetate (obtained from Productos Quimicos Monterrey, Monterrey, Mexico) and sodium hydroxide and citric acid (from Baker, Phillipsburgh, NJ) were analytical-grade reagents. Benzidine and 3,3'-DCB were purchased from Chem Service (West Chester, PA) with a certified purity of 99%. Benzidine is a polar weak base with pK_a values of 3.57 and 4.66 at 30°C (19) and a log K_{ow} (octanol–water partition coefficient) of 1.34 (2). 3,3'-DCB is still a weaker base with reported pK_a values of 1.6 and 3.2, but it is considerably more hydrophobic than benzidine, as shown by its log K_{ow} of 3.5 (2). Figure 1 shows the structures of these compounds. Stock solutions (1000 mg/L) of each benzidine were prepared by weighing and dissolving the corresponding compound in

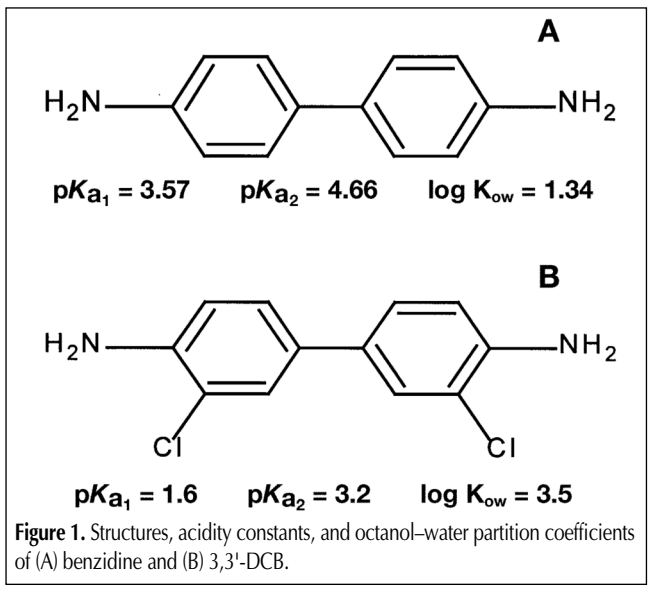

Figure 1. Structures, acidity constants, and octanol–water partition coefficients of (A) benzidine and (B) 3,3'-DCB.

methanol or acetonitrile. Working mixed standards of different concentrations in acetonitrile–water (50:50, v/v) were prepared from the stock solutions. These standards were used to spike water samples and also for direct loop injection to calculate solute recoveries. All of the benzidine solutions were stored in amber glass bottles at 4°C. Taking into consideration the high toxicity of benzidines, the stock solutions and the mixed standards were always prepared and handled in a hood using eye shields and a toxic gas respirator.

Procedures

Sample preparation

Water samples (200 mL) were collected in amber glass bottles with Teflon-lined caps. When required, the samples were directly spiked with the benzidines in the sampling bottle. The fortified reagent water samples that were used to develop the method were also placed in similar bottles. A nylon 66 membrane (0.2-µm pore size) previously soaked in methanol for 1 to 2 h and rinsed with fresh methanol and reagent water was used to filter the sample. Then, the sampling bottle was rinsed with two 5-mL aliquots of methanol and the rinsing solvent was passed through the same filter and collected in the same flask as the sample. Finally, a 2-mL aliquot of a 1M ammonium acetate solution was directly added to the flask, and the mixture was gently stirred and sonicated for 3 min. The flask was used as a reservoir of the sample pump for the SPE.

The sample prepared in this way had a pH near 7 and contained approximately 4.7% (v/v) of methanol in a total volume of 212 mL. The preparation procedure could accept small variations in the initial sample volume (± 5 mL) without any modification. However, the exact sample volume must first be determined in order to calculate the analyte concentrations.

Preconcentration and analysis

The following 6-step procedure was finally adopted for the online extraction, preconcentration, and analysis of the sample: (step 1) the PLRP-S precolumn was conditioned with 10 mL of a methanol–water–1M ammonium acetate solution (5:94:1, v/v); (step 2) the precolumn was loaded with 50 mL of the prepared sample, and then simultaneously the conditioning of the analytical column with the initial mobile phase (95% eluent A, 5% eluent B) was begun; (step 3) the precolumn was flushed with 0.2 mL of reagent water and the conditioning of the HPLC-column was simultaneously continued; (step 4) the 7000 Rheodyne valve was switched, the gradient run, and the sample analyzed; (step 5) the precolumn and analytical column was conditioned with 15 mL of the initial mobile phase; and (step 6) a standard was injected for quantitation.

Table I. Timetable for the Online Preconcentration and Analysis of Benzidines in Water Samples

Time (min)	Step	Operation	Solution	Flow rate (mL/min) P_1	Flow rate (mL/min) P_2	Valve*
0.0		Purge P_2	S_1†	0.0	3.0	Inject
2.0	1	Condition precolumn	S_1	0.0	2.0	Load
7.0		Purge P_2	Sample	0.0	3.0	Inject
9.0	2	Load sample	Sample	0.6	2.0	Load
			Condition column		S_2‡	
31.0		Stop P_1	Sample	0.0	2.0	Load
34.0		Purge P_2	LC-water	0.0	3.0	Inject
36.0	3	Flush precolumn	LC-water	1.0	0.2	Load
		Condition column	S_2			
37.0	4	Analyze sample	Gradient	1.0	0.0	Inject
72.0	5	Condition column + precolumn	S_2	1.0	0.0	Inject
87.0	6	Analyze standard	Gradient	1.0	0.0	Inject
122.0		Begin new cycle				

* Switching valve with precolumn.
† S_1, methanol–water–1M ammonium acetate (5:94:1, v/v).
‡ S_2, eluent A–eluent B (95:5, v/v).

The timetable corresponding to this procedure is presented in Table I. The position of the switching valve, the flow rates in the sample pump (P_2) and the LC pump (P_1), and the different solvents or solutions used at each time interval are also described in Table I. Before steps 1, 2, and 3 of the general procedure were performed, a fast purging of the sample pump was included to fill the pumphead and lines with the corresponding solution. At the end of step 6, a new cycle may begin for the analysis of the next sample. The time required for the online operations in this method (including the chromatographic runs for the sample and the standard) is 122 min; however, only 37 min is needed for the extraction and preconcentration of the sample. In sequential analysis, the offline operations for one sample (filtration, sampling bottle rinsing, and pH adjustment) may be carried out while the previous sample is being analyzed, and the injection of a standard may be programmed after two or three sample analyses and not necessarily after each one. In this way, the analysis time per sample could still be substantially reduced.

In this work, the pumps and valves were manually controlled in order to optimize all the experimental conditions. However, the online operations can be fully automated for routine analysis using an appropriate controlling system programmed according to Table I.

The proposed method includes a regeneration of the precolumn and column by the passage of 5 mL of the strong mobile phase at the end of the gradient-elution program. The efficiency of this regeneration is demonstrated by the fact that the same column and precolumn were used for all the experiments reported in this work and no degradation of their performance was observed. Nevertheless, it is advisable to change the precolumn frits after the analysis of approximately 10 samples because they become gradually clogged, especially when working with surface water samples. It is probable that some colloidal material arrives and passes through the nylon membranes during sample filtration, thus provoking this problem.

Results and Discussion

Sample preparation

Preliminary experiments showed that considerable losses of 3,3'-DCB occurred during sample filtration, probably because this compound is sufficiently hydrophobic enough to remain adsorbed on the walls of the sampling bottle, the surface of the fil-

tering membrane, or both. In previous studies (12,13) we have eliminated this problem by rinsing all materials that were in contact with the aqueous sample with a small volume of an organic solvent, which was then added to the filtered sample. This artifice also prevented subsequent adsorption of the compounds of interest on the walls of the final flask or on the filters and tubing of the sample pump. However, the addition of organic solvents to aqueous samples has the inconvenience of reducing the volume of sample that can be loaded in the SPE precolumn, thus decreasing the sensitivity of the method. Therefore, it is important to carefully optimize the volume of rinsing solvent according to the hydrophobicity of the studied compounds. For the benzidines, it was found that 10 mL of methanol was the minimum volume required to adequately rinse the sampling bottle and the nylon membrane in order to obtain good recoveries of 3,3'-DCB. Methanol was preferred over acetonitrile because it has been observed that its elution strength in polymeric RPs is considerably lower. However, when the nylon membrane is in contact with pure methanol (or acetonitrile), it releases organic substances that can interfere with the determination of the target compounds. In order to avoid this contamination, the membrane must be previously soaked in the organic solvent and rinsed with reagent water. Finally, by adjusting the pH of the sample to approximately 7, the molecular form of benzidine predominates and its retention in the SPE precolumn is maximal.

Extraction and preconcentration

Initially, SPE experiments were assayed in precolumns packed with a C18 phase or the polymeric CHP-3C phase (Mitsubishi, Tokyo, Japan); they were both unsuccessful. Benzidine was not sufficiently retained in the C18 phase. However, both benzidines were well-retained in the CHP-3C phase, but when the precolumn was online-eluted with mobile phases containing acetonitrile, the pressure in the system dramatically increased. It is probable that the crosslinking of this copolymer was too low and a strong swelling occurred in the presence of acetonitrile; interestingly, this phenomenon was not observed with mobile phases containing methanol. However, the elution and transfer of 3,3'-DCB from the CHP-3C precolumn to the analytical C18 column with methanolic eluents seemed to be unfavorable, and very large and asymmetric peaks were obtained. Finally, a precolumn packed with the polymeric PLRP-S phase was assayed with good results. Up to 50 mL of the prepared sample could be loaded in the precolumn without a breakthrough of the most polar solute. Besides, the PLRP-S copolymer did not swell in the presence of acetonitrile, and mobile phases containing this organic modifier could be used for the online elution of the precolumn. In these conditions, relatively narrow and symmetrical peaks were obtained for the two analytes.

Polymeric phases have the advantage of being strongly retentive, which makes them ideal for the SPE of polar and medium-polar compounds. The counterpart is their lack of selectivity. For solutes with acid–base properties, it is sometimes possible to perform a cleanup of the extract obtained in a polymeric precolumn by the selective transfer of the ionized analytes to a second precolumn packed with an ion exchanger (11–13). A small volume of an aqueous solution having an appropriate pH (containing if necessary a little amount of an organic modifier) is used to ionize and desorb the solutes from the first precolumn and transfer them to the second one in which they remain retained. This procedure was assayed for the benzidines considering the possibility of protonation of the amino groups. However, because of the high hydrophobicity and very low basicity of 3,3'-DCB, it was not possible to desorb it from the PLRP-S phase, even with acid solutions at pH 0 containing up to 15% of methanol. Besides, in these conditions benzidine was no longer retained in the second precolumn that was packed with a cation exchanger.

Chromatography

Considering the good redox properties of most aromatic amines, electrochemistry was chosen as the detection mode to compensate the lack of selectivity during the sample pretreatment process. The analytical cell of the Coulochem detector used in this work contained two sequential electrode chambers that could be used to increase the selectivity of detection by imposing different potentials to their working electrodes. In the first chamber, the working electrode of high surface area (coulometric) can eliminate some electroactive impurities present in the sample without electrolyzing the compounds of interest. Then, in the second working electrode of low surface area (amperometric) that was set at higher potential, the analytes can be selectively oxidized.

Figure 2 shows the benzidine peak heights as a function of the imposed potential in the two working electrodes. Each compound was separately studied using isocratic conditions, a flow rate of 1 mL/min, and a mobile phase composition giving a retention time of approximately 7 min. From Figure 2 it is observed that

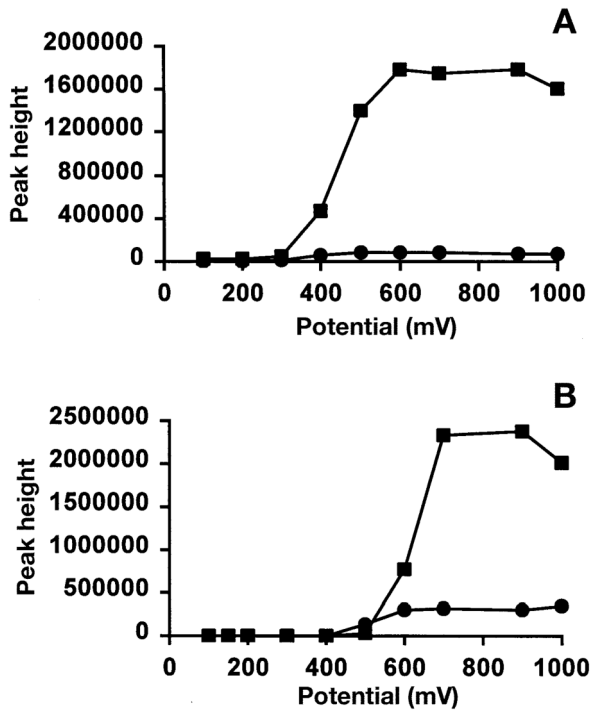

Figure 2. Variation of the response of (A) benzidine and (B) 3,3'-DCB as a function of the imposed potential in the coulometric (■) and amperometric electrode (●) of the Coulochem detector. Injected amount was 216 ng, and the mobile phase was acetonitrile–water–1M citrate buffer at a ratio of 28:71:1 (v/v) for benzidine and 50:49:1 (v/v) for 3,3'-DCB.

benzidine begins to be oxidized from a potential of 300 mV and reaches a plateau at approximately 600 mV, and 3,3'-DCB begins at 500 mV and reaches a plateau at 700 mV. The comparison of the curves clearly shows the great difference in the response obtained at the two working electrodes. From this study it was decided that the first electrode (coulometric) would be used for solute detection because of its higher sensitivity and also because the oxidation of benzidine begins at very low potentials, preventing a substantial gain in selectivity. Moreover, by setting a potential of 700 mV to this electrode, the detection would already be quite selective and have optimal sensitivity.

Benzidine and 3,3'-DCB can be easily separated in C18 columns, but their great difference in hydrophobicity and the chosen detection mode somewhat complicate the problem. It is well-known that a matrix peak generally appears at the beginning of the chromatogram when some samples (such as surface waters) are preconcentrated in a nonselective mode (i.e., RP-SPE or LLE) prior to analysis. In order to avoid interference in the quantitation of the analytes, it is advisable to use a mobile phase that elutes the first compound of interest with a retention volume of at least 10 mL. However, in the case of benzidines it is impossible to sufficiently retain benzidine and elute 3,3'-DCB with the same mobile phase; therefore, gradient elution was required. When electrochemical detection is used, the baseline control during gradient elution is very difficult because of the strong changes in the conductivity of the mobile phase. Therefore, several experiments were carried out using different types and concentrations of buffers. Only with a 0.01M citrate buffer of pH 6 in the weak and strong mobile phases was it possible to obtain a good signal for the analytes and an acceptable baseline. The gradient program described in the Experimental section (with an abrupt increase of solvent B between minutes 12 and 13) was designed to obtain a good retention for benzidine, a reasonable elution time for 3,3'-DCB, and as less as possible of a perturbation from the steep baseline change on the analyte peaks.

Table II. Analysis of Reagent Water Samples* Spiked at 1.5 µg/L of Each Benzidine by Online SPE and Gradient-Elution RP-HPLC–ED

Compound	Recovery (%)	RSD (%)
Benzidine	93	4.8
3,3'-DCB	95	5.2

* n =10.

Table III. Analysis of Reagent Water Samples Spiked with the Benzidines in the Range of 0.05 to 40 µg/L by Online SPE and Gradient-Elution RP-HPLC–ED*

Compound	Intercept† (µg)	Slope†	LOQ (µg/L)	LOD (µg/L)
Benzidine	0.002 ± 0.042	0.93 ± 0.04	0.5	0.1
3,3'-DCB	–0.009 ± 0.044	1.04 ± 0.05	0.5	0.05

* Recovered amount versus added amount.
† Intervals for the intercepts and slopes calculated for a confidence level of 5% (n = 6, correlation coefficient = 0.999).

Table IV. Analysis of Water Samples Spiked at 5 µg/L of Each Benzidine by Online SPE and Gradient-Elution RP-HPLC–ED*

Compounds	Recovery (%)		
	Tap water	Groundwater	River water
Benzidine	14	91	96
3,3'-DCB	83	97	94

* No benzidines were found in the blank samples.

Performance of the method and application to environmental water samples

Table II shows the recovery and relative standard deviation (RSD) determined from the analysis of ten identical reagent water samples spiked at 1.5 µg/L of each benzidine. Recoveries were calculated by comparing the peak areas of the samples with those obtained from the direct loop injection of a standard. These results demonstrated that benzidine and 3,3'-DCB can be quantitated with good accuracy and precision at concentration levels in the order of the parts-per-billion level (1 µg/L) in water.

The linearity, LODs, and limits of quantitation (LOQs) were determined from the analysis of reagent water samples spiked at a progressively decreasing concentration of the benzidines. The upper concentration was 40 µg/L for each analyte and the lowest

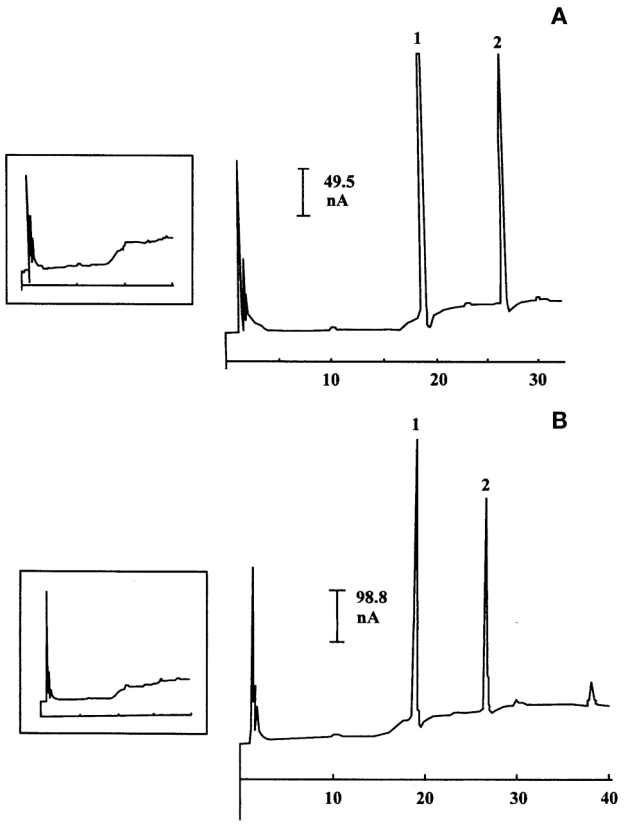

Figure 3. Chromatograms obtained from the analysis of (A) groundwater and (B) river water. The left graph represents the blank samples, and the right graph is the same samples fortified at 5 µg/L of (1) benzidine and (2) 3,3'-DCB.

was 0.05 µg/L. Recoveries were calculated and analyzed as relations of the recovered amount versus the added amount. In the concentration range of 0.5 to 40 µg/L the method was linear with correlation coefficients of 0.999 for the two benzidines. Table III shows the corresponding intercepts and slopes. It is observed that the former are statistically equal to zero and the latter (representing the fraction of solute recovered) are higher than 0.90, thus confirming the good accuracy of the method in this concentration range. The LOQ was arbitrarily defined as the lowest concentration that gave a recovery in the interval of 100% ± 15%; in fact, this limit corresponds to the lowest point of the linear range. In these samples, the LODs of the method for a signal-to-noise ratio of 3 were measured at approximately 0.1 µg/L for benzidine and 0.05 µg/L for 3,3'-DCB.

For the application of the method, three different water samples were analyzed: tap water, groundwater from a well used for irrigation in the state of Puebla in Mexico, and surface water from Rio Atoyac (also in the state of Puebla). No benzidines were detected in the blank samples, but in order to test the method in these waters the same samples were fortified at 5 µg/L benzidine and 3,3'-DCB and then reanalyzed. The analyte recoveries determined in the three samples are reported in Table IV, and the chromatograms obtained from the blank and fortified groundwater and river water samples are shown in Figure 3.

It was observed that the chromatograms of the blank samples were very clean and only some relatively small matrix peaks eluted at low retention times. In the spiked samples the two analyte peaks were very well-defined and completely free of interference. It was thus confirmed that the imposed potential of 700 mV allowed for a very selective detection of benzidines. Besides, the recoveries in groundwater and surface water samples were practically identical to those obtained with the reagent water samples. This indicates that the organic matter content in these samples did not provoke the breakthrough of the analytes from the SPE precolumn. Therefore, the proposed method was well-adapted for the monitoring of these compounds in environmental waters.

On the contrary, the recoveries were surprisingly low in the tap water sample, especially for benzidine. Probably, the residual chlorine present in the sample provoked an oxidation of these amines. Although this parameter was not measured, the official norm in Mexico for residual chlorine in tap water is 0.5–1 mg/L. In a previous work (13), a similar rapid degradation of chlorophenols in tap water samples was observed. The effect of chlorine on the stability of benzidines was also studied by Riggin et al. (6). They found that after seven days in an aqueous solution containing 2 ppm of NaOCl, benzidine and 3,3'-DCB were completely degraded.

Conclusion

Benzidine and 3,3'-DCB can be selectively and accurately determined at trace concentration levels in environmental waters using SPE online-coupled to LC with coulometric detection. The method proposed in this work provides LODs similar to those obtained with the longer and more complicated LLE-based procedures. In this method, the offline operations are minimal and very simple and all the conditions for the online SPE–LC–ED section have been carefully optimized, thus the recoveries for the two compounds and the precision of recovery are very good. Indeed, the method is perfectly suited for the routine screening of a series of samples because of its simplicity, reliability, and the possibility of automation. We consider the reliability to be caused in great part by the robustness of the polymeric precolumn, which can be regenerated and used many times without any change in its retention properties. Besides, the precolumn effectively protects the HPLC column, thus extending its lifetime. Therefore, an additional advantage for routine analysis is the considerable reduction of costs in comparison to other reported methods.

Acknowledgments

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