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Selective on-line immunoextraction coupled to liquid chromatography for the trace determination of benzidine, congeners and related azo dyes in surface water and industrial effluents

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Abstract

A new extraction immunosorbent involving antigen–antibody interactions was coupled on-line to liquid chromatography for the selective extraction in aqueous samples of benzidine and congeners, widely used as intermediate compounds in the manufacturing of dyes and pigments. Due to the cross-reactivity of the antibodies for analytes with chemical structures closely related to that of the analyte used for immunization, the immunoextraction sorbent was shown to be able to extract aminoazobenzene and related azo dyes with good recoveries. The on-line coupling was optimized for the trace determination of benzidine, dichlorobenzidine, aminoazobenzene and some azo dyes with detection limits in the range 0.1 to 1 μg/l . The high selectivity of the immunoextraction was shown by comparing the analysis of an industrial textile effluent obtained using precolumns packed either with a non-selective polymeric sorbent or with the anti-benzidine immunosorbent. In such complex samples, extraction and clean-up are achieved in the same step.

Keywords: Extraction methods; Water analysis; Environmental analysis; Immunosorbents; Benzidines; Azo dyes

1. Introduction

The carcinogenic nature of benzidine, 3,3′-dichlorobenzidine and its congeners was recognized several years ago [1]. Although their industrial use has been reduced, they are still produced in large amounts as intermediate compounds in the manufacturing of dyes and pigments. They are included in all priority pollutants lists worldwide. The US Environmental Protection Agency (EPA) established water quality criteria for 3,3′-dichlorobenzidine and for benzidine [2]. In Europe, they are listed among the ‘very toxic substances for the environment’ and required to be controlled in industrial effluents. Textile industry is of a major concern, but one has to add industries dealing with painting pigments, printing inks and food coloring production. Although 3,3′-dichlorobenzidine is less toxic than benzidine, recent studies have shown that the fate of this analyte and its congeners in natural sediments is a transformation to benzidine [3].

The analysis of benzidine and related dyes is difficult and has received little attention up to now. Benzidine is a polar analyte as indicated by its octanol–water constant (K_{ow}) equal in log unit to 1.34 [3]. As a comparison benzidine is less polar than aniline (log $K_{ow}=0.9$), but more polar than monochloroaniline (log K_{ow} between 1.8 and 1.9) or phenol (log $K_{ow}=1.5$). Therefore, its extraction

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using classical solid-phase extraction (SPE) sorbent in off-line and on-line procedures is not so straightforward [4,5]. Sulfonated azo dyes have been determined in ground water, waste water and industrial effluents using SPE followed by liquid chromatography and capillary electrophoresis with UV or mass spectrometry detection [6–8].

Another difficulty for the trace-level determination of these compounds in industrial effluents comes from the complexity of the matrix and from the fact that many related dyes are ionic and water-soluble. An extraction step is necessary before the chromatographic determination. This has been performed using polymeric SPE sorbents for sulfonated azo dyes [6–8]. However, these extraction materials are non-selective and many interferents are co-extracted from complex matrices when for instance textile effluents are handled. In surface water, most of the polar analytes cannot be determined due to their co-elution with humic substances in the matrix peak obtained at the beginning of the chromatogram. An additional clean-up step using silica or Florisil can solve the problem by removing a great part of the matrix interferents, but it is always laborious and time-consuming.

There is a great interest in providing selective extraction procedures which eliminate the co-extraction of matrix interferences and can be simply coupled on-line with LC. These highly selective extractions have been recently obtained using new types of sorbents involving analyte–antibody interactions. Antibodies produced against a target compound are immobilized on a silica based support to form a so-called immunosorbent that is used just like a classical SPE sorbent. Antibodies principally react specifically with molecules following a mechanism of structural recognition. The target molecule being small, the antibodies are able to recognize not only the antigen that was used to initiate the immune response, but also compounds from the same structural family. In contrast to previous development of immunosorbent for single analytes and metabolites [9–12], our group took advantage of this cross-reactivity of the antibodies to develop class-selective immunosorbents for triazine and phenylurea pesticides, including their degradation products, and polycyclic aromatic hydrocarbons (PAHs) in various matrices such as water, foodstuffs, soils and sediments. In aqueous samples, extraction, concentration and clean-up are achieved in one step, whereas for solid samples immunoextraction and clean-up are applied to simple extracts [13–22]. The use of immunosorbents has been validated using certified reference materials [23–25].

Antibodies against benzidine have been produced and chemically-bonded to a silica-based sorbent resulting into an anti-benzidine immunosorbent [26]. The objectives of this study are to evaluate the performance of the anti-benzidine immunosorbent for the selective immunoextraction of benzidine, congeners and related dyes from surface water and heavy polluted effluents using on-line procedures. Like many aniline derivatives, benzidine is polar and rather volatile, so that there is an interest in eliminating any evaporation step which occurs in off-line extraction procedures. This necessitates a study of the required characteristics of the immunosorbent and especially (i) the ability to extract benzidine and other structurally related analytes, (ii) the capacity and the breakthrough volumes, (iii) the possibility of the method for quantification at the μg/l level, (iv) the selectivity of the immunosorbent, as compared to classical sorbents.

2. Experimental

2.1. Chemicals

HPLC-grade acetonitrile and methanol were obtained from Mallinckrodt Baker (Deventer, The Netherlands). LC-quality water was prepared by purifying demineralized water in a Milli-Q filtration system (Millipore, Bedford, MA, USA). Benzidine and other chemicals were purchased from Prolabo, Merck, SDS and Fluka. Stock solutions of selected solutes were prepared by weighing and dissolving them in methanol or acetonitrile and stored at 4°C. The phosphate-buffered solution (PBS) consists of a 0.01 *M* sodium phosphate buffer containing 0.15 *M* NaCl (pH 7.4) and 0.2% azide.

2.2. Apparatus

LC analyses were performed with a Varian LC system workstation including a Varian Star 9010

solvent-delivery system, a Model 9065 polychrom diode-array detector and a Varian 9075 fluorescence detector. Immunoprecolumn and analytical column switching was accomplished with two Rheodyne (Berkeley, CA, USA) valves. Conditioning of the immunoprecolumn and percolation of samples were performed using one Milton-Roy pump. The on-line system of preconcentration was totally constituted by stainless steel tubing.

2.3. Stationary phases, columns and precolumns, materials

The analytical column was a 150×4.6 mm I.D. column prepacked with octadecylsilica Bakerbond ENV from Mallinckrodt Baker. Preconcentrations were made through experimental stainless steel precolumns (30×4.6 mm I.D.) prepacked with 220 mg of the immunosorbent based on anti-benzidine antibodies bonded onto glutardialdehyde-activated silica particles of 50-nm pore size (Mallinckrodt Baker). Polyclonal anti-benzidine antibodies and the immunosorbents were supplied by N. Fischer-Durand and Professor F. Le Goffic (ENSCP, Laboratory of Bioorganic and Biotechnologies, Paris, France). These antibodies were produced by the injection of an animal with an immunizing agent obtained by coupling the 4-benzidinylbutyric acid to a carrier protein, i.e. bovine serum albumin, and by following an immunization procedure similar to that previously described [13]. The serum was collected 5 to 7 months after first immunization and 34 mg of the purified IgG fraction were bonded to 1 g of silica. Immobilization on the activated silica sorbent was achieved as previously reported [13]. The properties of the anti-triazine immunosorbent used in this study was described in Ref. [18]. Other preconcentrations on non-selective sorbents were carried out on precolumns prepacked with styrene–divinylbenzene copolymer PRP-1, 20×2 mm I.D., 5–10 μm (Hamilton, Reno, NV, USA).

2.4. LC conditions

The gradient used for the separation of benzidine, congeners and aminoazobenzene was as follows: a mixture of acetonitrile and 0.005 *M* phosphate buffer at pH 7 (25:75, v/v) from 0 to 5 min, 25% to 47% acetonitrile from 5 to 25 min and 80% acetonitrile at 40 min, the flow-rate used was 1 ml/min.

2.5. Immunoextraction procedure

In the on-line system, a precolumn packed with the sorbent used for the extraction step takes place at the loop position of a six-port switching valve. The first step of the procedure is achieved in the load position and consists in conditioning the sorbent. For the immunopreconcentration, the immunosorbent is conditioned with 5 ml of PBS and then 6 ml of LC-grade water. For the preconcentration on PRP-1, the sorbent is conditioned with 6 ml of acetonitrile, 6 ml of methanol and 6 ml of water. The sample is percolated through the precolumn at a flow-rate of 2 ml/min. By switching the valve, compounds trapped on the sorbent are eluted on-line from the precolumn to the analytical column by an acetonitrile–water gradient used for the analytical separation at a flow-rate of 1 ml/min. The valve is switched in the load position at 3 min after the transfer of all the analytes. When the immunosorbent is not in use, it is stored at 4°C in a solution of PBS (containing 0.2% of azide) after a washing step using 70% of methanol (10 ml).

Surface water samples were taken in Paris and solid PBS was added to the samples after filtration and before percolation through the immunosorbent. The industrial effluent was from a textile industry from Porto (Portugal). After filtration it was diluted in a phosphate buffer, pH 8, before percolation through the immunosorbent.

3. Results and discussion

In order to obtain antibodies by animal immunization, a hapten had first to be synthesized. It was obtained by modifying the benzidine molecule using one of the two amino groups while the other one was protected. The hapten carrier conjugate was obtained by linking 4-benzidinylbutyric acid to bovine serum albumin and was injected in rabbits [26]. Therefore, although the highest affinity should be obtained for the hapten 4-benzidinylbutyric acid, the evaluation of the immunosorbent was made using benzidine and related analytes.

3.1. Capacity measurement

In a SPE process, an incomplete recovery can be explained by analyte breakthrough which can be the result either of an insufficient retention or an overloading of the sorbent capacity. With commonly used C_{18} silicas, the capacity is high and when pollutants are present at the μg/l level, breakthrough mainly occurs because of insufficient retention. Previous studies have demonstrated that the capacity was an important parameter in SPE using immunosorbents, because when the capacity is overloaded, the recoveries are no longer constant and the calibration range no longer linear [14,15,18,22]. Although these studies have also shown that the capacity was usually sufficient for the analyte–antigen, this condition was not straightforward for the related analytes. Quantitative analysis can be made only if recoveries are constant and if the calibration curves do not depend on the competition process. Therefore, having a high capacity was shown to be very important because the linear part of the calibration curves of cross-reacting analytes increases with capacity [18,22].

The capacity was evaluated by percolating 25 ml of samples with increasing concentrations of benzidine. Results are reported in Fig. 1 and one can see that the curve has two linear portions. The first part (amount injected <0.6 μg) correspond to a constant recovery around 80–90% and the second part with a recovery around 15%. In the absence of non-specific interactions, the curve has a Langmuir type shape, with a linear portion and then a plateau. We obtained such curves for the capacity of the anti-triazine and anti-phenylurea immunosorbents [18,22]. The

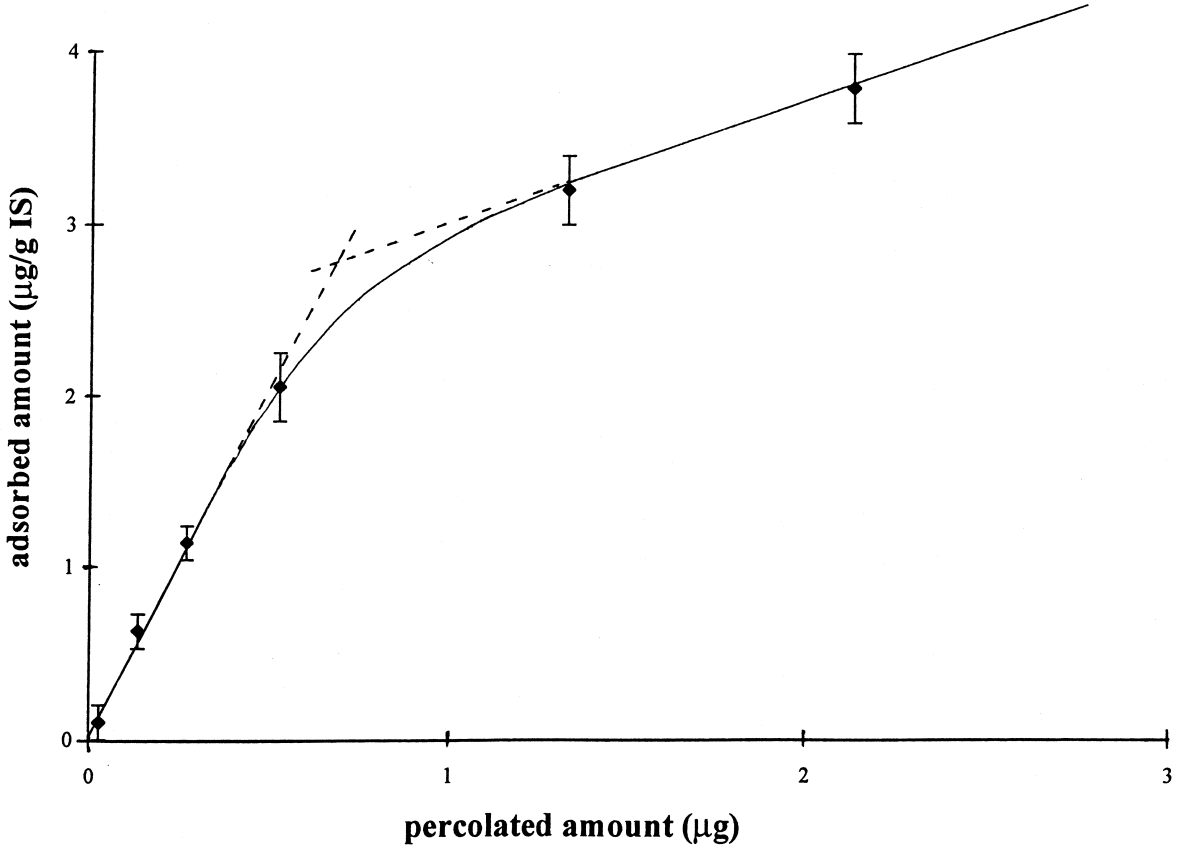

Fig. 1. Capacity measured by the corresponding amount in micrograms of benzidine adsorbed per gram of immunosorbent according to the percolated amount in micrograms, using the on-line procedure with a constant sample volume of 25 ml containing increasing concentrations of benzidine.

capacity corresponds to the concentration for which the curve is no more linear. Here, the fact that the curves does not reach a plateau, but a linear portion with a lower slope indicates that non-specific interaction occur between antibodies and benzidine. Binding between antibodies and analytes occurs if the spatial complementary between molecules is good. It involves several types of interactions such as Van der Waals, electronic, hydrogen and hydrophobic interactions. But, in addition to these specific interactions, non-specific interactions may also occur between the large protein of the antibodies and benzidine. In case of hydrophobic PAHs, the non-specific interactions were attributed to their high hydrophobicity. Since benzidine is far from being an hydrophobic molecule, non-specific interactions may involve interactions with the two free amino groups of benzidine. Interactions with the silica matrix may also occur, although the pH of the sample has been adjusted to pH 8 in order to have benzidine under its molecular form ($pK_{a,1}$ and $pK_{a,2}$ of 1.6 and 4.3, respectively), so that ionic interactions between ionized silanol groups are reduced. In order to verify the occurrence of non-selective interactions, an anti-triazine immunosorbent was used (containing 47 mg IgG fraction per gram of silica) instead of the anti-benzidine immunosorbent (containing 34 mg IgG fraction per gram of silica). The extraction recoveries were measured under the same experimental conditions and a constant recovery of 16% was obtained. Therefore, the second part of the capacity curve in Fig. 1 corresponds to non-specific interactions. The capacity was estimated between 2 and 3 μg/g of immunosorbent. As comparison, the anti-triazine immunosorbent has a capacity of 2.5±0.2 μg/g of immunosorbent. This capacity was high enough to allow the immunosorbent to extract seven triazines together with a linear range between 0 and 3 μg/l when handling on-line 25 ml of sample spiked with the seven analytes.

3.2. Potential for the extraction of chlorobenzidine, aniline derivatives and azo dyes

Previous studies have demonstrated that antibodies developed for the recognition of a small molecule were also able to recognize structurally related molecules with different affinities, i.e. the so-called cross-reactivity of antibodies. Therefore, in addition to benzidine and dichlorobenzidine, one can also expect the immunosorbent to co-extract some aniline derivatives, aminoazobenzene and related dyes as a consequence of the cross-reactivity. The structure of benzidine, the immunoconjugate carrier and of some compounds under study are reported in Fig. 2.

Several analytes were on-line analyzed using a sample volume of 10 or 25 ml. Results are reported in Table 1. Benzidine, 3,3′-dichlorobenzidine are well retained by the immunosorbent with recoveries above 70%, showing thus a good affinity for these two analytes. A good affinity was also obtained for 4-aminoazobenzene (known also as C.I. Solvent Yellow 1, intermediate in manufacturing of Acid Yellow). Two other analytes related to this dye were analyzed and one can see that azobenzene and *p*-dimethylazobenzene (also known as C.I. Solvent Yellow 2) are well extracted with recoveries above 50% from 10 ml of samples. A ionic disulfonated azo dye, Acid Blue 113, was also studied and was extracted with a lower recovery, but since this analyte is ionic, it is more difficult to extract with an on-line system using precolumns packed with C_{18} silicas or apolar copolymers so this is an interesting result. Other ionic dyes are under study. The cross-reactivity of the anti-benzidine antibodies towards some aniline derivatives was also studied, especially those having a substituent in the *para* position. Recoveries are low for the analytes 4-methyl and 4-chloro, but are higher for 4-nitroaniline. Obviously, there is a recognition for analytes with groups containing one N atom in the *para* position. This can explain why azobenzene and related azo dyes are retained by the immunosorbent. Dichloro- and trichloroanilines are also extracted, but owing to increasing hydrophobicity of the molecules due to the chloro groups, some hydrophobic interactions may be more important. Aniline could not be studied with the on-line system because the analytical mobile phase does not contained a sufficient amount of organic solvent to transfer this compound to the analytical column. It was then studied in an off-line procedure: the result was that aniline is not extracted by the anti-benzidine immunosorbent, showing the importance of the electronic distribution in the molecule for the recognition by the antibodies.

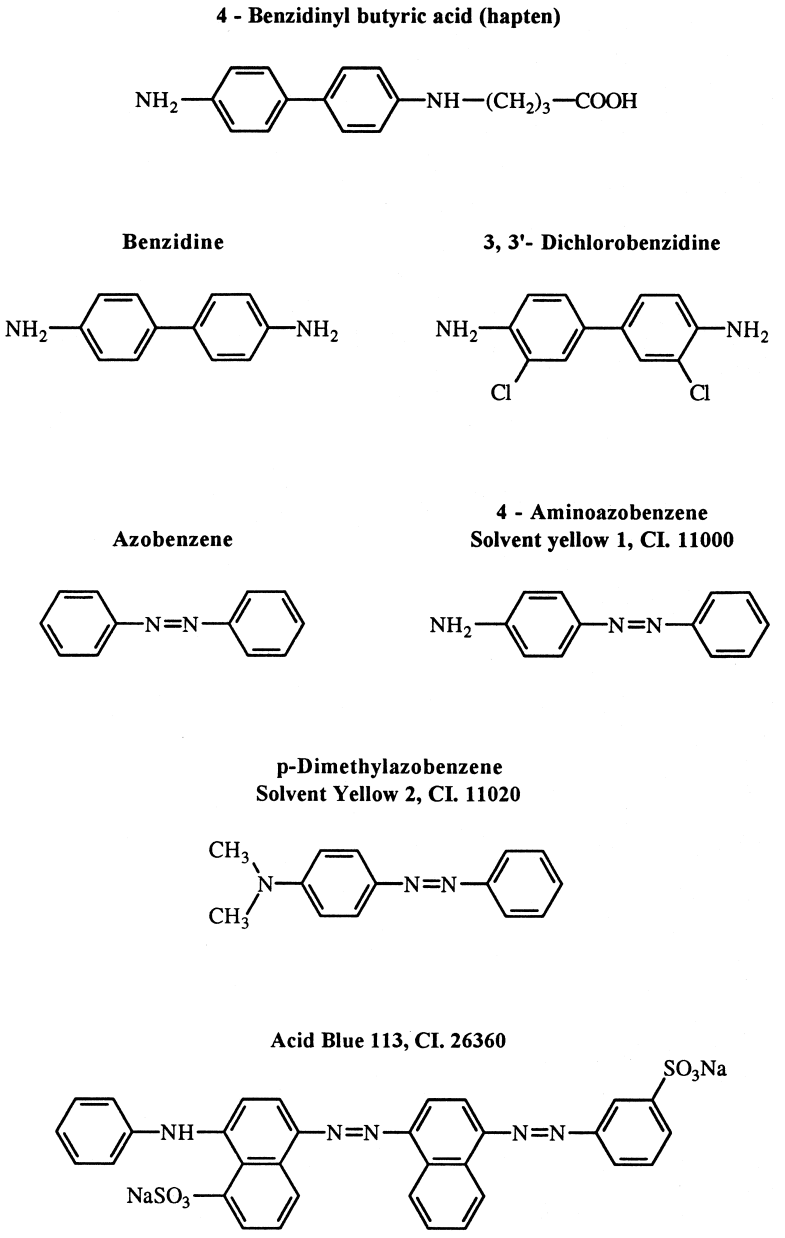

Fig. 2. Chemical structure of the hapten (4-benzidinylbutyric acid) and of some selected compounds studied.

3.3. Effect of the sample volume

A complementary means for the evaluation of the class properties of the immunosorbent is to increase the sample volume and to measure the breakthrough volumes of the various analytes. For each analytes, the recovery is constant as long as breakthrough does not occur. Table 2 reports the results of the recovery measurement for some analytes when increasing the sample volume up to 150 ml while keeping the

Table 1
Characteristics of the analytes (retention time, acidity constants, extraction recoveries from 10-ml samples spiked with 5 μg/l and 25-ml samples spiked with 2.5 μg/l)

Analyte	Retention time (min)	pK_{a1}, pK_{a2}	% Recovery (R.S.D., %)[a]	
			(a) 10 ml	(b) 25 ml
Benzidine	6.9	1.6; 3.2	86 (8)	80 (7)
3,3′-Dichlorobenzidine	21.8	3.3; 4.3	73 (6)	75 (4)
Azo dyes				
4-Aminoazobenzene	21.1	Nd	79 (4)	Nd
Azobenzene	28.1	No p*K*a	55 (7)	Nd
p-Dimethylazobenzene	31.5		48 (6)	Nd
Acid blue 113A	14.7	Ionic	38 (6)	Nd
Aniline derivatives				
4-Methoxyaniline	4.1	4.6		Nd
4-Nitroaniline	6.8	4.1	20 (3)	Nd
4-Methylaniline	7.4	5.1	Nd	<5
3-Chloro-4-methoxyaniline	7.5	4.4	Nd	<5
4-Chloroaniline	11.2	4.15	16 (4)	Nd
3-Chloroaniline	11.7	3.45	16 (5)	Nd
2,3-Dichloroaniline	19.1	–	48 (4)	Nd
2,4-Dichloroaniline	19.8	2.05	41 (6)	13 (4)
2,6-Dichloroaniline	19.8	–	47 (4)	Nd
2,5-Dichloroaniline	20.2	1.57	52 (5)	Nd
2,4,6-Trichloroaniline	24.5		72 (6)	Nd

[a] Mean recoveries of three replicates and R.S.D.; Nd: not determined.

amount of analytes in the sample constant at 50 ng, in order not to overload the capacity of the pre-column. For benzidine, 3,3′-dichlorobenzidine and 4-aminoazobenzene, the affinity for the antibodies is high, since the recoveries decrease only after the handling of a volume of 100 ml. For the three other aniline derivatives, the decrease in recoveries occurs rapidly, showing thus a lower affinity. These results confirms that the recovery of 72% obtained for the preconcentration of 10 ml of trichloroaniline is mainly due to hydrophobic interactions due to the three chloro groups.

3.4. Calibration curves

A sample volume of 50 ml was selected and the calibration curves were constructed for benzidine, 3,3′-dichlorobenzidine and 4-aminoazobenzene. The

Table 2
Effect of the sample volume on the recoveries

Analytes	% Recovery (R.S.D., %)[a]					
	10 ml	20 ml	25 ml	50 ml	100 ml	150 ml
Benzidine	86 (8)	80 (8)	80 (7)	73 (5)	69 (5)	52 (7)
3,3′-Dichlorobenzidine	73 (6)	73 (6)	75 (4)	72 (6)	60 (6)	59 (4)
4-Aminoazobenzene	79 (4)	79 (4)	83 (5)	83 (6)	69 (5)	63 (4)
2,4-Dichloroaniline	41 (6)	14 (4)	13 (4)	8 (3)	6 (3)	<3
4-Nitroaniline	20 (3)	18 (4)	Nd	11 (4)	<3	<3
2,4,6-Trichloroaniline	72 (6)	38 (6)	Nd	26 (4)	<3	<3

[a] Mean values of three replicates and corresponding R.S.D.

calibration curves were linear in the range 0.1–5 μg/l. With this sample volume, detection limits were measured around 0.05–0.1 μg/l (signal-to-noise ratio of 5) depending on the relative detection properties of the three analytes.

3.5. Application to surface water samples: selectivity and optimization of the on-line procedure

Fig. 3 represents the chromatogram corresponding to the analysis of 25 ml of the river Seine water, taken in Paris, spiked with 1 μg/l of each herbicide simazine, atrazine and the dealkylated degradation products de-ethyl atrazine and deisopropylatrazine, as well as 10 μg/l of benzidine. The chromatogram in Fig. 3A is obtained by on-line preconcentration using a precolumn packed with the apolar copolymer PRP-1. All the analytes are recovered, as well as other interfering analytes. The chromatogram represented in Fig. 3B corresponds to the on-line pre-

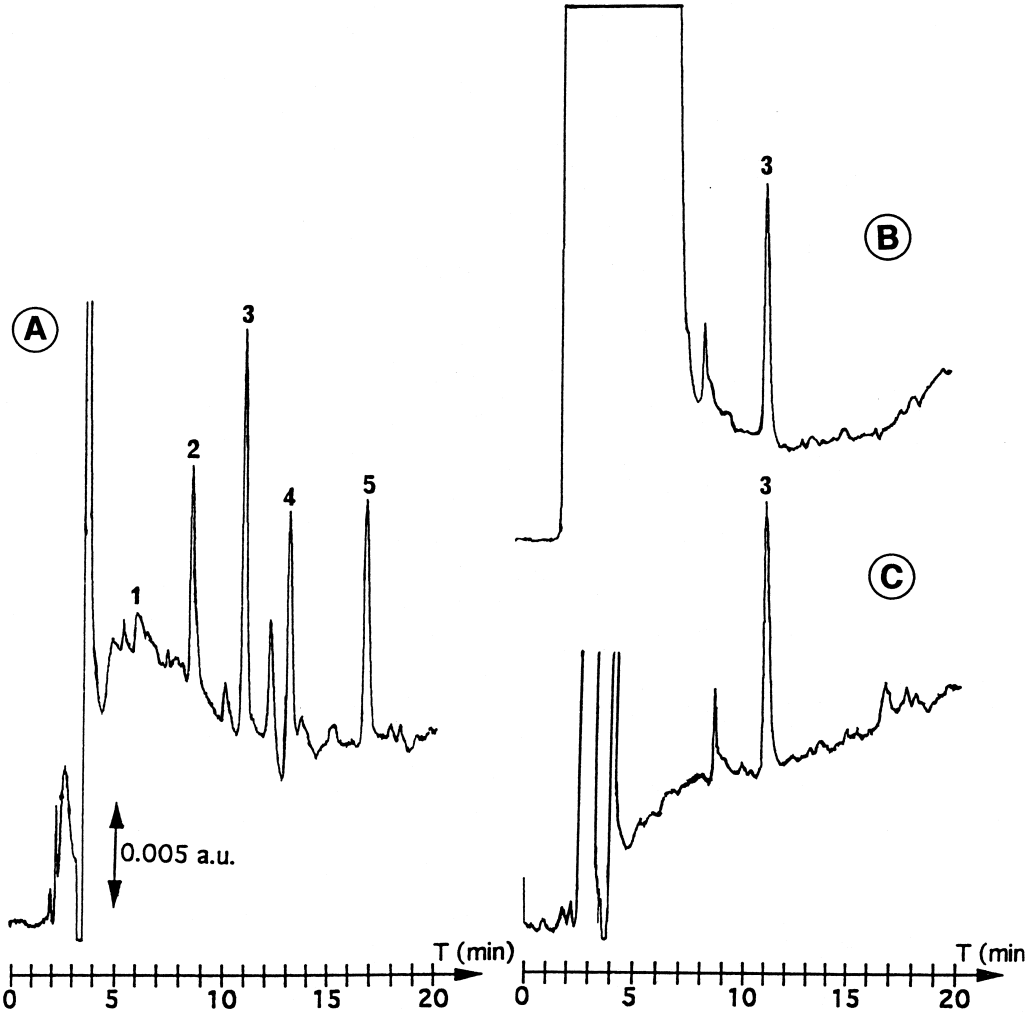

Fig. 3. On-line analysis of 25 ml of the river Seine water spiked with benzidine (10 μg/l) and a mixture of triazine (each at 1 μg/l) using the on-line system with a precolumn packed with a PRP-1 precolumn (A) and with the anti-benzidine immunosorbent (B and C). (B) Is a non-modified Seine sample and (C) is a Seine sample containing PBS. Analytical column: octadecylsilica Bakerbond, 250 mm×4.6 mm; flow-rate: 1 ml/min. Acetonitrile gradient with 0.005 *M* phosphate buffer, pH 7, from 20 to 60% in 20 min. UV detection at 220 nm. Analytes: (1) deisopropylatrazine, (2) deethylatrazine, (3) benzidine, (4) simazine and (5) atrazine.

concentration using the precolumn packed with the anti-benzidine immunosorbent. The herbicides are not extracted, thus showing the selectivity of the preconcentration, but there is a large peak during the first 7 min, which did not show up with drinking or LC-grade water. Since this peak does not occur when preconcentration is made using PRP-1, it was attributed to ionic substances which interact with antibodies or silica matrix. Therefore, the on-line procedure was modified by adding the phosphate buffer containing sodium chloride (PBS) – the same as that used for the regeneration of the immunosorbent – to the samples before percolation through the immunosorbent. The effect is shown in Fig. 3C and the large peak has disappeared. The effect of the PBS is explained by the removal of ionic interaction with the protein. The on-line procedure was therefore modified and PBS was added to all samples.

3.6. Application to textile effluents

3.6.1. Selectivity

The textile effluent was very dirty, as shown by its dark black color. A volume of 2.5 ml was diluted with 47.5 ml of PBS before analysis and then spiked with 1 μg/l of each three analytes: benzidine, 3,3′-dichlorobenzidine and 4-aminoazobenzene. The analysis was carried out using the on-line set-up with a precolumn prepacked with PRP-1 or with the anti-benzidine immunosorbent. Results are reported in Fig. 4A and B, respectively. The selectivity provided by the immunosorbent is very obvious for such industrial waste, although diluted. The number of polar interferents are very numerous with PRP-1 and benzidine cannot be detected and identified by its UV spectrum whereas, the chromatogram is much clearer in Fig. 4B, and benzidine can be easily detected and identified. However, other unknown peaks show up in this chromatogram and correspond to analytes trapped by the antibodies.

3.6.2. Recoveries and detection limits in effluent

Matrix effect can affect the recoveries which were measured by spiking the industrial effluent. Fig. 5 represents the chromatogram of 1 ml of the textile effluent diluted to 20 ml using PBS and spiked with

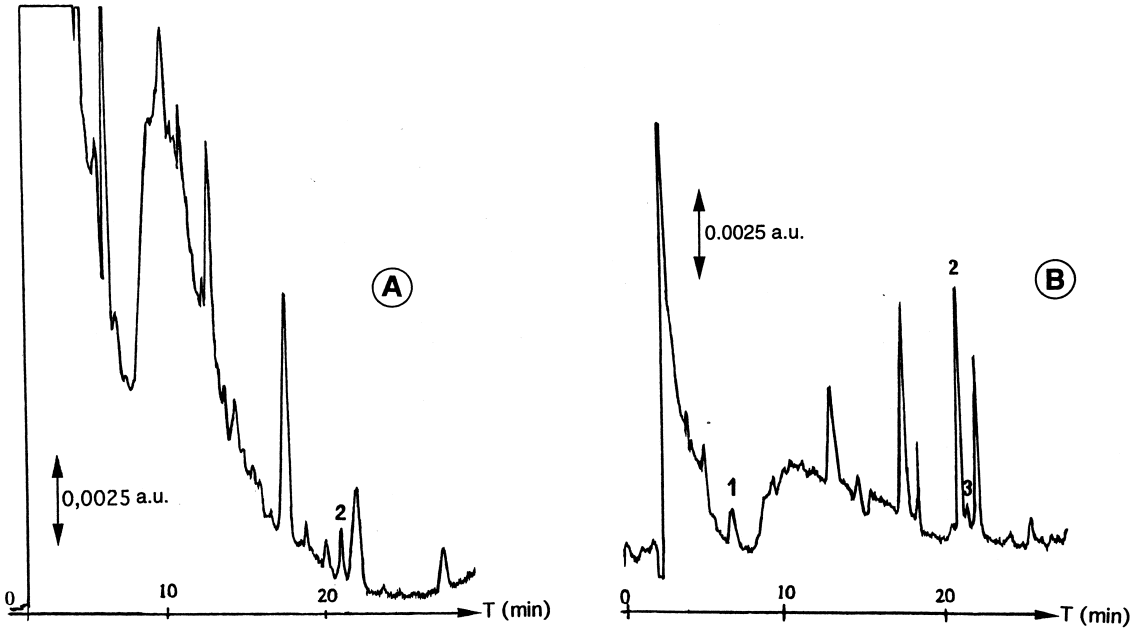

Fig. 4. On-line analysis of 2.5 ml of textile effluent diluted with 47.5 ml of PBS using a precolumn packed with (A) PRP-1 and (B) the anti-benzidine immunosorbent. The 50 ml sample was spiked with 1 μg/l of each analyte: (1) benzidine, (2) 3,3′-dichlorobenzidine, (3) 4-aminoazobenzene. Analytical column: octadecyl Bakerbond ENV, 150 mm×4.6 mm; flow-rate: 1 ml/min. Acetonitrile gradient with 0.005 *M* phosphate buffer at pH 7, 25% acetonitrile from 0 to 5 min, 47% at 25 min and 80% at 40 min. UV detection at 287 nm.

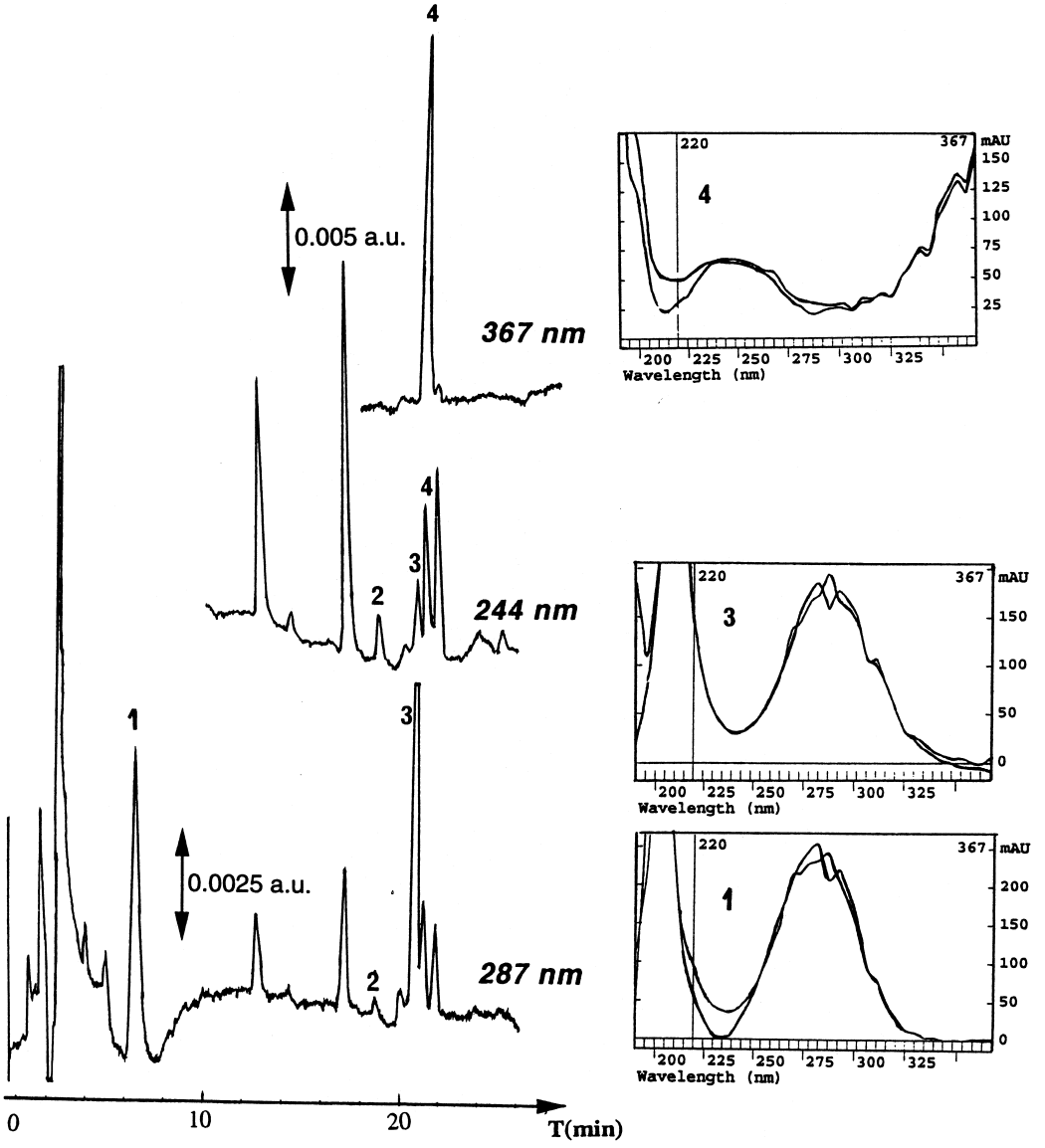

Fig. 5. On-line analysis of 1 ml of textile effluent diluted with 19 ml of PBS using a precolumn packed with the anti-benzidine immunosorbent and spiked with 5 μg/l of each analyte: (1) benzidine, (2) 2,4-dichloroaniline, (3) 3,3′-dichlorobenzidine, (4) 4-aminoazobenzene. Same conditions as Fig. 4. UV detection represented at 244, 287 and 367 nm and corresponding UV spectra in the inserts.

four analytes at a 5 μg/l concentration. Benzidine and 3,3′-dichlorobenzidine are better detected at 287 nm, which corresponds to their UV maximum, 2,4-dichloroaniline at 244 nm whereas 4-aminoazobenzene is better detected at 367 nm, which is the maximum wavelength provided by our diode array detector. Since these are colored dyes, it would be more appropriate to use a UV–visible diode array detection (DAD) system. However, 367 nm is close to the maximum which was measured at exactly 386 nm. The recoveries measured for the four analytes are 55% for benzidine, 14% for 2,4-dichloroaniline, 71% for 3,3′-dichlorobenzidine and 56% for 4-aminoazobenzene. These recoveries have to be

compared to those obtained in Table 2 for a sample volume of 20 ml. There is a good agreement between recoveries measured in pure spiked water or in waste spiked samples for 2,4-dichloroaniline and 3,3′-dichlorobenzidine, whereas the recoveries are slightly lower for benzidine and 4-aminoazobenzene. With these dirty samples, the addition of known amounts to the unknown sample for more reliable quantitative analysis can be recommended. Fig. 5 shows also that detection of the analytes in the diluted waste are

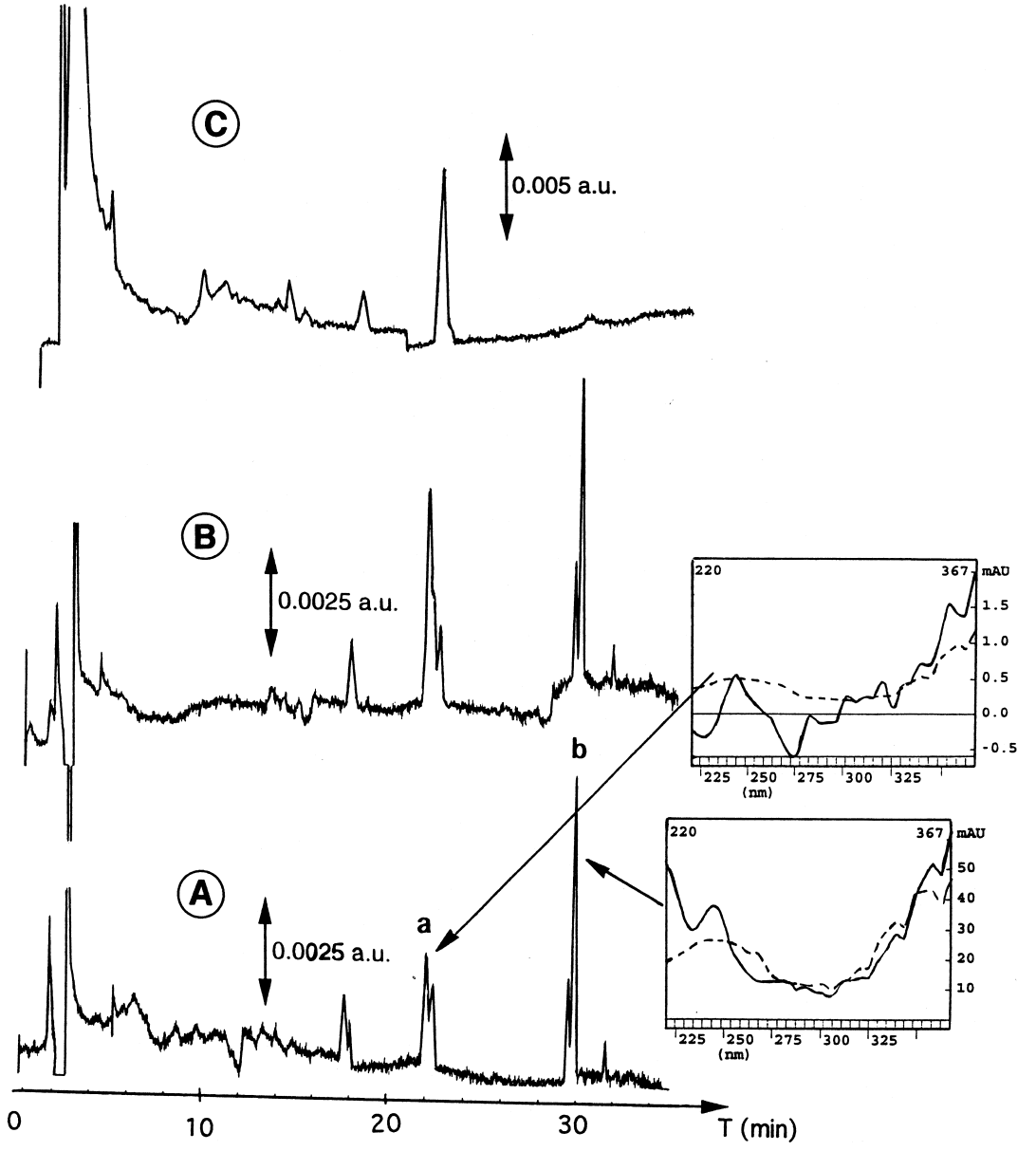

Fig. 6. On-line analysis of 2.5 ml of textile effluent diluted with 47.5 ml of PBS using a precolumn packed with (A and B) the anti-benzidine immunosorbent and (C) the polymer PRP-1. (A) A non-spiked sample (B and C) same sample spiked with 1 μg/l of 4-aminoazobenzene. Conditions as in Fig. 4. UV detection represented at 367 nm. In the two inserts, correspondence of the peak UV spectrum to that of 4-aminoazobenzene (dotted line).

easily achieved at the 5 μg/l concentration in the 20 ml and that detection limits are much lower. However, the analysis of the undiluted waste was not performed, because we wanted to re-use the immunosorbent. If one considers the spiking level in the 1 ml-aliquot, then it is 100 μg/l . Therefore, it is difficult to provide a quantification limit in waste, because it depends on whether it can be analyzed without dilution or not. If it can be analyzed without dilution, then quantification limits are in the range 0.1–0.5 μg/l for benzidine, aminobenzene and related azo dyes.

3.6.3. Application to unknown non-spiked wastes

A 50-ml volume of another diluted textile effluent (2.5 ml to 50 ml of PBS) was analyzed by the on-line immunosorbent LC–UV-DAD system and the resulting chromatogram is shown in Fig. 6A. The UV spectra were drawn for each peak and two peaks, (A) and (B), showed a similar UV spectrum to aminoazobenzene. The first peak has a retention time very close to that of 4-aminoazobenzene. The sample was spiked with 4-aminoazobenzene and the corresponding chromatogram is represented in Fig. 6B, showing that the peak a is not 4-aminoazobenzene. The textile effluent was also preconcentrated using the PRP-1 precolumn. On the non-spiked sample (not represented in Fig. 6), peaks A and B are not extracted, whereas the chromatogram in Fig. 6C, corresponding to the sample analyzed in Fig. 6B shows only 4-aminoazobenzene. No interferents are detected because of the wavelength of 367 nm. These results indicate that (A) and (B) may correspond to ionic dyes, not extracted with PRP-1 but by the immunosorbent. Table 1 has indicated a recovery of 38±6% for the ionic sulfonated azo dye Acid Blue 113A with a retention time in the chromatogram around 14 min. Several ionic dyes have some naphthalene groups which can be more strongly retained than Acid Blue 113. Identification using LC–MS is being studied and we can also point out the help provided by the immunoextraction which provides clean extracts for further identification using MS.

4. Conclusion

We have reported preliminary results on the evaluation of a new immunoextraction sorbent for the rapid monitoring of benzidine and congeners in surface water and industrial effluents. In addition to its high selectivity in such complex matrices, it has the ability to recognize compounds containing the azobenzene structure. Since the azo dyes represent a class of ionized analytes which are difficult to extract and identify, it is worthwhile to investigate the environmental impact of these analytes. Moreover, since the coupling of the immunoextraction to LC–MS detection is easy, one can expect in the near future a more efficient monitoring of textile effluents in the environment.

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