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Selective trace enrichment on immunosorbents for the multiresidue analysis of phenylurea and triazine pesticides

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Abstract

The use of immunosorbents for the selective solid-phase extraction of phenylureas and triazines from environmental water is presented. The choice of the haptens used for the synthesis of antibodies that are able to trap the whole group of triazines and phenylureas is discussed. A better knowledge of the nature of the binding sites has been obtained by studies of capacity for the antigen and also for different compounds in the sample either alone or in a mixture. These experiments have proved the occurrence of competition phenomena between the different compounds and have shown their influence on the calibration curves. Applications to surface waters showed the high selectivity of these sorbents. Most of the studied compounds were easily detected in highly contaminated surface water at the 0.1 $\mu g\ l^{-1}$ level with no clean-up. The simultaneous identification of different herbicides within a group in unknown samples is strongly supported by this selective multiresidue preconcentration.

Keywords: Immunosorbents; Sorbents; Water analysis; Environmental analysis; Solid-phase extraction; Trace enrichment; Pesticides; Phenylureas; Triazines; Antibodies

1. Introduction

Solid-phase extraction (SPE) is now widely accepted in environmental analysis as an alternative to the laborious and time consuming liquid–liquid extraction (LLE) [1–3]. The on-line coupling of an enrichment step using SPE with liquid chromatography (LC) has been shown to be an appropriate technique for the multiresidue analysis of pesticides in environmental water because of its suitability for simultaneously analysing thermodegradable and other pesticides over a large range of polarity without the need for a derivatization step. Fully automated devices are now commercially available and on-line methodology can be easily performed in any laboratory, using a precolumn that is placed in the sample loop position of a six-port switching valve [4,5].

The lack of selective sorbents to trap organic compounds in water is certainly the most important weakness of the SPE technique. Retention of analytes by the widely used C_{18} silica sorbents or by apolar copolymers is due to non-specific hydrophobic interactions. More selective interactions are involved with immunoaffinity sorbents. It is now possible to produce antibodies against some target

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compounds, including some small molecules such as pesticides. This is illustrated by the development of many pesticide immunoassays in recent years. However, solid-phase extractants based on immunoaffinity chromatography, so-called immunosorbents (IS), have not been developed yet for pesticide analyses as they have been for the clean-up and analyses of drugs in biosamples. One work reported the use of an antibody to selectively trap the carbendazim [6] and another one reported the use of antibodies to trap atrazine and two of its main metabolites [7].

In a previous work [8], we have presented information on the preparation and the evaluation of two immunosorbents for the selective trace solid-phase extraction of phenylurea and triazine herbicides. The potential of such ISs for multiresidue analysis was shown. An IS made with polyclonal antiisoproturon antibodies covalently bound to a silica sorbent, was able to concentrate nine phenylureas in a mixture of thirteen. Another one containing antiatrazine antibodies was able to trap six triazines in a mixture of nine. This is due to the cross-reactivity of the polyclonal antibodies that can recognize the antigen and other compounds with similar structures. The ISs were used in off-line cartridges, as simply as C_{18} silica sorbents, with percolation of the sample and then elution of the pesticides with a few ml of water and methanol (30:70, v/v). They proved to be very stable, easily regenerated and re-usable, and they could also be packed in precolumns for on-line coupling with liquid chromatography [9]. The main IS characteristic was its high selectivity so that phenylureas could be detected in contaminated surface waters at the 0.1 $\mu g\ l^{-1}$ level without any clean-up step.

For pesticide analysis, it is interesting to extract as many compounds as possible within a given group of pesticides. Several phenylureas, mainly isoproturon, chlortoluron, diuron, monuron and linuron are often detected together in some agricultural areas, as well as atrazine, simazine, terbuthylazine and their dealkylated and hydroxymetabolites for the group of triazines. This study first explains how the haptens should be selected to obtain antibodies able to trap the whole group of triazines and phenylureas. Since the affinities are different for individual compounds within the group, the breakthrough volumes of each analyte were measured and the results compared with those obtained on classical sorbents. The capacity of an IS is often measured with the antigen pesticide. However, competition for the same binding sites can decrease the capacity, when the antigen is present in a mixture containing other compounds of the group. In order to get a better knowledge of the nature of the binding sites, the behaviour of the compounds were compared alone or in a mixture. This was performed by comparing the capacity measured for compounds alone and in a mixture. The consequence on the calibration curves was also studied. Applications to the determination of phenylureas and triazines in surface waters, using either off-line cartridges or on-line preconcentration are presented.

2. Experimental

2.1. Apparatus

LC analyses were performed with a Varian LC System Workstation including a Varian Star 9010 solvent-delivery system and a Model 9065 Polychrom diode-array detector. Immunoprecolumn and analytical column switching was accomplished with two Rheodyne (Berkeley, CA, USA) valves. On-line percolation of samples was performed using a Varian 2010 (Palo Alto, CA, USA) pump.

For the off-line methodology, trace enrichment of the diluted elution fractions was performed with disposable precolumns using the Prospekt (Spark Holland, Emmen, Netherlands) which is an automated programmable sample preparation unit allowing direct elution to the LC. Conditioning of the cartridges and sampling were performed via a Solvent Delivery Unit (SDU) (Spark Holland). The procedure using the Prospekt has been previously described [8].

2.2. Stationary phases and columns

The analytical column was a 25 cm × 0.46 cm I.D. column prepacked with the Bakerbond Narrow Pore C_{18} (J.T. Baker, Deventer, Netherlands). Preconcentrations were made through experimental stainless steel precolumns (3 cm × 0.46 cm I.D.) or cartridges (3 ml) prepacked with 100–200 μm aldehyde-activated silicas particles, 30 nm pore size

(Boehringer, Mannheim, Germany) and on pre-columns prepacked with styrene–divinylbenzene copolymer, 10 mm × 2 mm I.D., 15–25 μm (Polymer Laboratories, Church Stretton, UK). Polyclonal antibodies immobilized on this adsorbent were supplied by Prof. Le Goffic (ENSCP, Paris, France).

2.3. Chemicals

HPLC-grade acetonitrile was from J.T. Baker and methanol was from Prolabo (Paris, France). LC-grade water was prepared by purifying demineralized water in a Milli-Q filtration system (Millipore, Bedford, MA, USA). Other chemicals were obtained from Prolabo, Merck or Fluka (Buchs, Switzerland). The various pesticides were supplied by Riedel-de Haën (Seelze, Germany) and C.I.L. (Ste-Foy-la-Grande, France). Stock solutions of selected solutes were prepared by weighing and dissolving them in methanol. These standard solutions were stored at 4°C and used for the preparation of dilute working standard solutions and for spiking water samples. The final standard solutions did not contain more than 0.5% of methanol.

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.

2.4. Procedure

On-line procedure

The first step of the procedure consisted of conditioning the immunosorbent (0.22 g of bonded silica) with 6 ml of PBS and then 3 ml of LC-grade water. The sample was percolated through the immunosorbent at a flow-rate of 1 ml min^{-1} and then 3 ml of LC-grade water were passed through the precolumn. By switching the valve (Fig. 1), compounds trapped on the immunosorbent were eluted on-line from the precolumn to the analytical column by an acetonitrile gradient with phosphate buffer (pH 7) at a flow-rate of 1 ml min^{-1}

Off-line procedure

0.5 g of bonded silica was introduced into an empty disposable cartridge. The first step of the procedure consisted in conditioning the immunosorbent with 10 ml of PBS and then with 5 ml of LC-grade water. The sample was percolated through the immunosorbent followed by 5 ml of LC-grade water. The compounds were eluted from the cartridge first with 4 ml of a mixture containing 70% methanol and 30% LC-grade water and then with 1 ml of LC-grade water. In order to save time and to analyze the whole extract, we did not follow the usual procedure which consisted of evaporating these 5 ml samples to 250–500 μl and injecting an aliquot. These 5 ml samples were simply diluted in 200 ml LC-grade water for their further on-line preconcentration via the Prospekt, using prepacked PLRP-S cartridges.

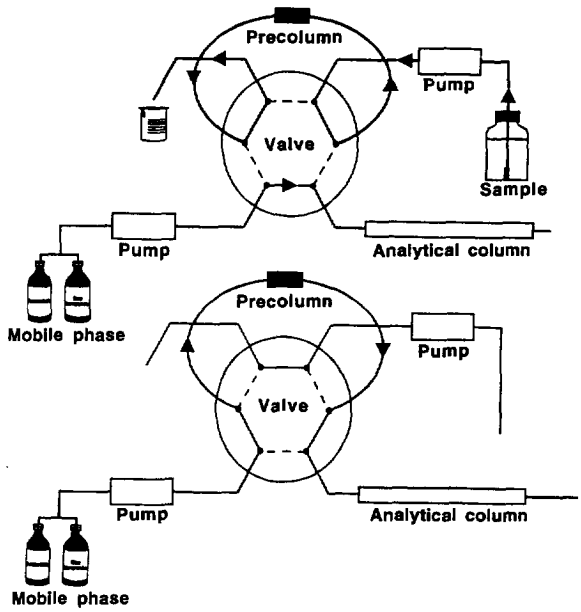

Fig. 1. Experimental set-up for on-line procedure.

The calibration curves for triazines were obtained in off-line methodology by percolating 25 ml of LC-grade water spiked in the trace level range of 0.1–3 μg/l.

When the immunosorbent was not in use, it was stored at 4°C in a solution of PBS after a washing step using 70% methanol (5 ml). The gradient used for the separation of triazines was as follow: 20% to 50% acetonitrile from 0 to 50 min, 50% to 100% from 50 to 70 min. The detection was performed at 220 nm.

3. Results and discussion

3.1. Cross-reactivity and screening potential

In previous studies, the screening potential of an antiisoproturon IS and an antiatrazine IS has been shown for some compounds with similar structure [8]. We have also noticed that a strong affinity can be obtained rapidly for the antigen pesticide, but, the affinity for compounds other than the antigen was more or less strong and was only obtained after a rather long period of immunization.

In order to make ISs capable of trapping the highest number of pesticides within a group, we predicted the most appropriate antigen(s) by having a close look at the structure of the compounds and by reading the literature about the cross-reactivity observed in the immunoassays. From the structures, it was easy to predict that antiatrazine antibodies would recognize atrazine, but would also recognise propazine and deethylatrazine (DEA), compounds that contain a chloride and an isopropyl group in their structure too, and certainly hydroxyatrazine (OHA) and prometon that contain only the isopropyl group (see Fig. 2). For the same reasons, antisimazine antibodies were expected to trap deisopropylatrazine (DIA), atrazine, cyanazine and terbuthylazine that contain a Cl- and an ethyl- group and possibly simetryne (see Fig. 2). Therefore, to cover all the triazine group including DEA and DIA, a hapten analogue of atrazine and a hapten analogue of simazine were chosen by the derivatization of the ethyl group.

For antiphenylurea antibodies, the choice of the hapten was more difficult because of the structural disparity within this chemical group (see Fig. 3). Nowadays, isoproturon is one of the most widely used herbicides. Therefore, it was important to synthesize an antibody specific to this compound. To trap the other phenylureas, antichlortoluron antibodies were chosen. Chlortoluron contains a disubstituted phenyl ring as do many phenylureas such as methoxuron, diuron, linuron, chlorbromuron and neburon. Antichlortoluron antibodies were produced to trap those phenylureas and even the monosubstituted ones.

Polyclonal antibodies were synthesized against atrazine and simazine for triazines and isoproturon and chlortoluron for phenylureas according to the procedure described in our previous study [8]. Pesticides were modified by the introduction of a carboxylic group so that they could be linked to BSA before injection into rabbits. In order to obtain a

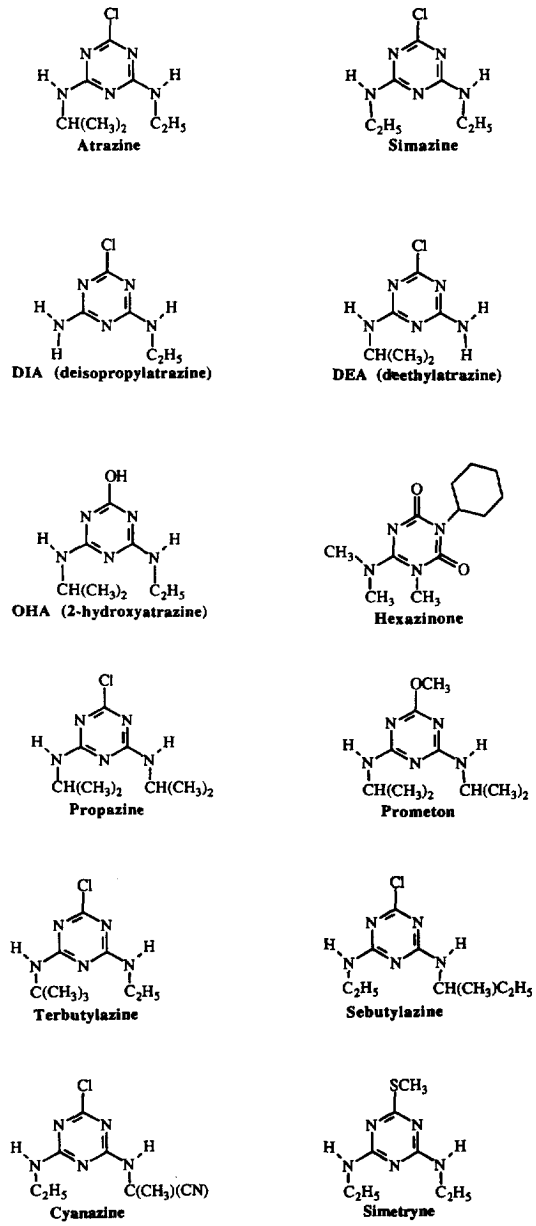

Fig. 2. Structures of triazines.

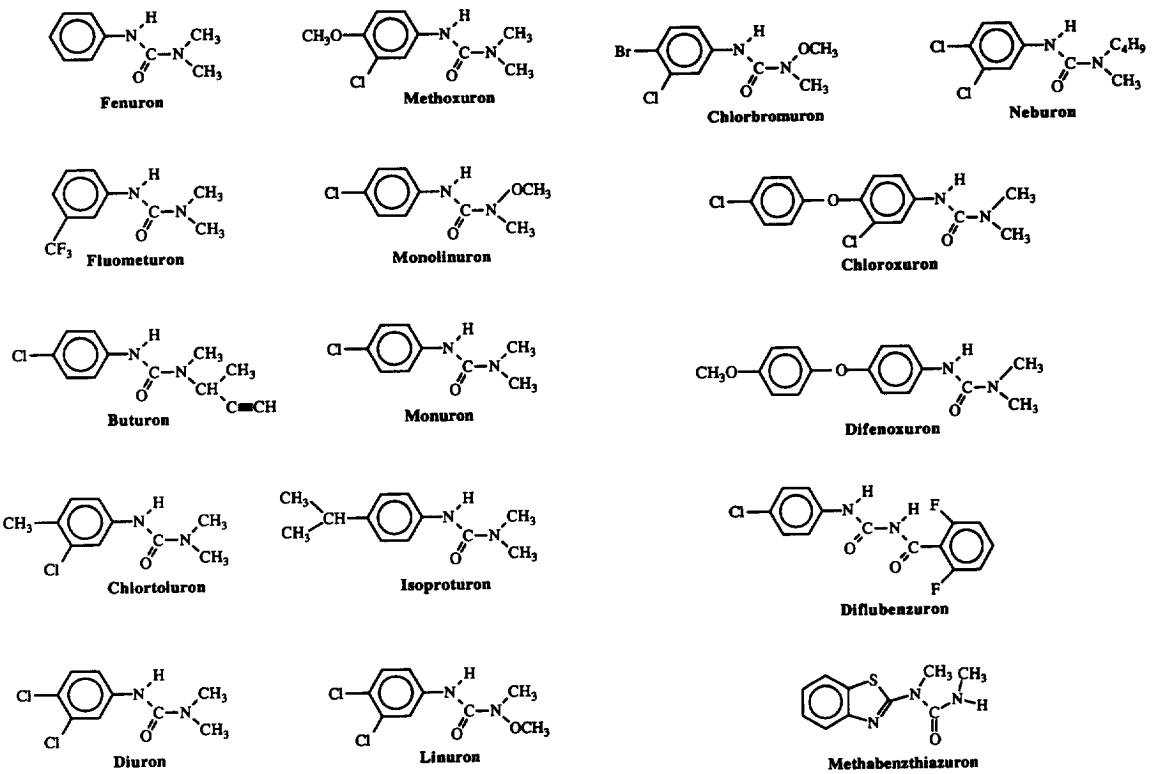

Fig. 3. Structures of phenylureas.

pressure-resistant sorbent, the antibodies were covalently bound to a silica matrix following the mixed anhydride method.

Table 1 presents the recoveries of extraction of triazines on the antiatrazine and antisimazine ISs after the percolation of 25 ml of LC-grade water spiked at 3 $\mu g\ l^{-1}$ with a mixture of triazines. High recoveries are obtained for most of the triazines on the two ISs. These results agree with those predicted from the triazines' structures and also show the high specificity of these antibodies: deethylatrazine is retained better on the antiatrazine IS than on the antisimazine IS. Deisopropylatrazine is retained by the antisimazine IS but is not trapped by the antiatrazine IS. In the same way, hydroxyatrazine and prometon are only trapped on the antiatrazine IS. The antiatrazine IS is very efficient since all the recoveries are above 60% except for DIA. Hexazinone has a different structure (see Fig. 2), but was

Table 1
Recoveries of extraction obtained after the percolation of 25 ml of LC-grade water spiked at 3 $\mu g\ l^{-1}$ with a mixture of triazines

Triazines	Recoveries (%)	
	Antiatrazine IS[a]	Antisimazine IS[a]
DIA	0	56
OHA	60	0
DEA	98	30
Hexazinone	0	0
Simazine	99	93
Cyanazine	91	74
Simetryne	63	17
Atrazine	99	88
Prometon	65	0
Sebuthylazine	88	91
Propazine	101	57
Terbuthylazine	98	85

[a]IS: 0.5 g of activated silica bonded with antiatrazine or antisimazine antibodies.

introduced in the percolated sample as a probe of the selectivity since this compound should not be extracted by the two ISs as shown here.

The recoveries for analytes in the phenylurea group are reported in Table 2. They were obtained after the percolation of 50 ml of LC-grade water spiked at 0.5 $\mu g\ l^{-1}$ with a mixture of phenylureas. According to the study of the structures of phenylureas, the antiisoproturon IS only traps the antigen, i.e., isoproturon with a high recovery. The other phenylureas are trapped, but with recoveries lower than 50%. The antichlortoluron IS is more appropriate for a screening purpose since all recoveries are higher than 75% except those for fenuron, methabenzthiazuron, monolinuron and difenoxuron, that are lower than 25%, and buturon (62%). In spite of the large structural disparity in this group, the antichlortoluron IS permits the extraction of eleven of the thirteen phenylureas studied and can even trap isoproturon with a recovery of 90%. These antiphenylurea antibodies also show high selectivity, for example, fenuron, characterized by a non-substituted phenyl ring, is not retained at all on both ISs.

If the two antibodies against triazines show a good complementarity especially, for the metabolites of atrazine (DIA, DEA and OHA), only chlortoluron antibodies have to be used for the extraction of most of the phenylureas. However, the antiisoproturon IS permits the extraction of several phenylureas and thus can be used for many applications.

3.2. Breakthrough volumes

In a solid-phase extraction process, breakthrough of analytes can occur due to an insufficient retention or to the overloading of the sorbent [1,10]. It is generally considered that in trace analysis where compounds are present at the $\mu g/l$ order, the latter effect does not occur with C_{18} silica or apolar copolymer sorbents, so that breakthrough mainly occurs because of insufficient retention. In previous studies, the capacity of the antiisoproturon IS was estimated to be 3.6 $\mu g/g$ of IS. This capacity is low, but sufficient for $\mu g/l$ analyses because the sample volumes required for analysis at this level is low, 50–200 ml, due to the high selectivity of the sorbent.

The retention of a solute on the IS depends on the affinity developed by the antibodies. Since the affinities vary for the different solutes within the group, the breakthrough volumes were measured for each compound in order to choose the sample volume that could be used without causing important losses of compounds. Several volumes of LC-grade water spiked with 25 ng of each triazine were

Table 2
Recoveries of extraction obtained after the percolation of 50 ml of LC-grade water spiked at 0.5 $\mu g\ l^{-1}$ with a mixture of phenylureas

Phenylureas	Recoveries (%)	
	Antiisoproturon IS[a]	Antichlortoluron IS[a]
Fenuron	0	0
Methoxuron	5	80
Monuron	39	78
Methabenzthiazuron	8	25
Chlortoluron	46	95
Monolinuron	ns[b]	16
Fluometuron	2	75
Isoproturon	98	90
Diuron	ns	95
Difenoxuron	24	17
Buturon	28	62
Linuron	19	85
Chlorbromuron	31	102
Diflubenzuron	39	76
Neburon	51	92

[a] IS: 0.22 g of activated silica bonded with antiisoproturon or antichlortoluron antibodies.
[b] ns: not studied.

Table 3
Average recoveries obtained with the percolation of 25, 50, 100 and 150 ml of LC-grade water spiked with 25 ng of each triazine on 0.5 g of antiatrazine and antisimazine IS

Triazines	Recoveries (%)							
	Antiatrazine IS				Antisimazine IS			
	25 ml	50 ml	100 ml	150 ml	25 ml	50 ml	100 ml	150 ml
DEA	59	57	56	29	47	42	43	15
Simazine	88	85	77	40	90	83	76	64
Cyanazine	45	40	5	0	51	44	47	44
Simetryne	60	48	20	0	60	58	59	41
Atrazine	100	100	100	85	85	84	78	71
Prometon	59	54	57	30	0	0	0	0
Sebuthylazine	85	79	75	45	60	58	59	50
Propazine	100	100	100	100	68	66	61	49
Terbuthylazine	100	100	100	84	72	68	55	50

(n=3, R.S.D. = 5–8%)

percolated through the IS containing either antiatrazine or antisimazine antibodies. The average recoveries are reported in Table 3. Breakthrough occurs for most of the compounds at 50 ml on the two ISs, except for atrazine, propazine and terbuthylazine on the antiatrazine IS; only propazine shows a recovery of 100% with a 150-ml sample. However, a sample volume of 50 ml is enough for trace level detection below the $\mu g\,l^{-1}$ level and most of the triazines are extracted with recoveries higher than 50% with this volume of sample on both ISs.

A similar experiment was carried out on the antichlortoluron IS. Breakthrough occurs at 50 ml for most of the compounds except for chlortoluron, isoproturon, diuron, chlorbromuron and neburon. Those compounds are extracted with recoveries of 100% even with a sample volume of 150 ml. However, as shown in Table 2, eleven of the fifteen phenylureas studied are extracted with good recoveries in a 50-ml sample on this IS.

If the capacity is not exceeded, breakthrough volumes are related to the retention volumes of analytes in water in elution chromatography. Therefore, these values are also related to the affinity of the antibodies for the analytes and results in Tables 1–3 show the affinity order within the group.

3.3. Capacity

The capacity is related to the number of binding or recognition sites bonded at the surface of the silica. Polyclonal antibodies are a mixture of antibodies. The measurement of the capacity is therefore more difficult as we don't know the exact nature of the recognition sites and the way they interact with analytes. If there is just one type of binding site, the interaction of other compounds will be in competition with that of the antigen, although that compound shows the strongest affinity. In other words, the capacity that is measured for the antigen can be different when the antigen is alone in the sample or when it is accompanied by other phenylureas.

The behaviour of the various ISs has been studied, using the pesticide antigen alone or in a mixture with other compounds of the group. Table 4 shows the amounts of triazines bonded on the antiatrazine and antisimazine ISs after the percolation of 50 ml of LC-grade water spiked with a large amount of each triazine (2.5 μg). This bonded amount depends on the structures of the compounds. For the antiatrazine IS, the higher bonded amount is obtained for propazine that contains two isopropyl groups and therefore can be recognized better by the antibodies than the antigen (atrazine) itself. For the antisimazine IS, the highest bonded amounts are obtained for compounds that contain an ethyl group, such as sebuthylazine, terbuthylazine and cyanazine. These results were obtained after the percolation of the mixture containing ten compounds. By percolating a large amount of atrazine alone in the sample (2.5 μg), the bonded amount is higher than that obtained with a mixture of ten compounds: 630 ng of atrazine

Table 4
Bonded amounts of each analyte on the antiatrazine and antisimazine IS after the percolation of 25 ml of LC-grade water spiked at 100 $\mu g\ l^{-1}$ with a mixture of triazines

Triazines	Bonded amounts (ng)	
	Antiatrazine IS[a]	Antisimazine IS[a]
DEA	27	38
Hexazinone	0	0
Simazine	43	81
Cyanazine	34	56
Simetryne	35	44
Atrazine	90	75
Prometon	26	0
Sebuthylazine	60	55
Propazine	150	54
Terbuthylazine	72	74

[a] IS: 0.5 g of activated silica bonded with antiatrazine or antisimazine antibodies.

could be retained rather than the 90 ng previously reported. This result indicates that the affinity of the antibodies for atrazine is not really much stronger than that for the other triazines; thus there is a competition between the different analytes to occupy the same recognition sites. This result is also consistent with the prediction from the structures of the triazines, since it can be predicted that the antibodies against atrazine will not differentiate between atrazine and propazine. Furthermore, as propazine has two symmetrical isopropyl groups, we can explain that this molecule will be better recognized, as shown by the higher bonded amount of propazine (150 ng) compared to the bonded amount of atrazine (90 ng) when these two compounds are at the same concentration in the mixture (see Table 4).

A similar result is obtained for simazine: 214 ng of simazine can be trapped on the antisimazine IS when this compound is percolated alone compared with 75 ng that can be trapped when it is in a mixture. The consequences for the calibration curves will be shown below.

A different behaviour is observed with the antiisoproturon IS. Samples of 50 ml of LC-grade water that were spiked at 10, 50 and 100 $\mu g\ l^{-1}$ with a mixture containing thirteen phenylureas were percolated on the antiisoproturon IS. The binding curves that represent the amount bonded on the IS versus the concentration in the percolated sample have been drawn for nine phenylureas of the thirteen (Fig. 4a). First, the shape of the curve for the antigen is different from those obtained for the other phenylureas. The bonded amounts of the antigen (isoproturon) increase strongly at low concentrations and then reach a plateau corresponding to the maximal amount that can be loaded on the IS. For the other phenylureas, the trapped amounts increase constantly and can even be higher than the maximal amount of antigen bonded on the IS. This is particularly well illustrated by the binding curves of diflubenzuron and neburon. At a first approximation, the comparison of the slopes observed in the linear part on these binding isotherms allows the comparison of the binding constants. The binding constant is much higher for isoproturon than for the other phenylureas. The capacity of diflubenzuron is higher than the capacity of isoproturon on the same IS. However, it has been shown previously that the breakthrough volume of isoproturon is higher (more than 500 ml) on the same antiisoproturon IS than the breakthrough volume of diflubenzuron (less than 200 ml) [8]. The breakthrough volumes are therefore more related to the binding constant than to the capacity.

These two differing behaviours obtained for the binding of phenylureas indicate the occurrence of different sites of recognition on this IS. This is confirmed by studying the behaviour of compounds on their own in the sample. The binding curves of monuron, isoproturon and diflubenzuron depicted in Fig. 4a are reported in Fig. 4b with the bonded amounts obtained for these compounds when they are alone in the percolated sample. Whether isoproturon is alone or in a mixture, the bonded amount is almost the same. On the contrary, for monuron and diflubenzuron, the bonded amount is higher when these compounds are alone in the sample than when they are in a mixture with twelve other phenylureas. It is the same phenomenon of competition as the one encountered for triazines and previously depicted. This antiisoproturon IS seems to contain antibodies that are highly specific to isoproturon and other antibodies that present different affinities for other phenylureas. When the sample contains a large amount of several phenylureas, competition takes place between these compounds for their binding to the recognition sites of the antibodies.

The capacities measured for the different herbicides on the four IS are not high but are sufficient

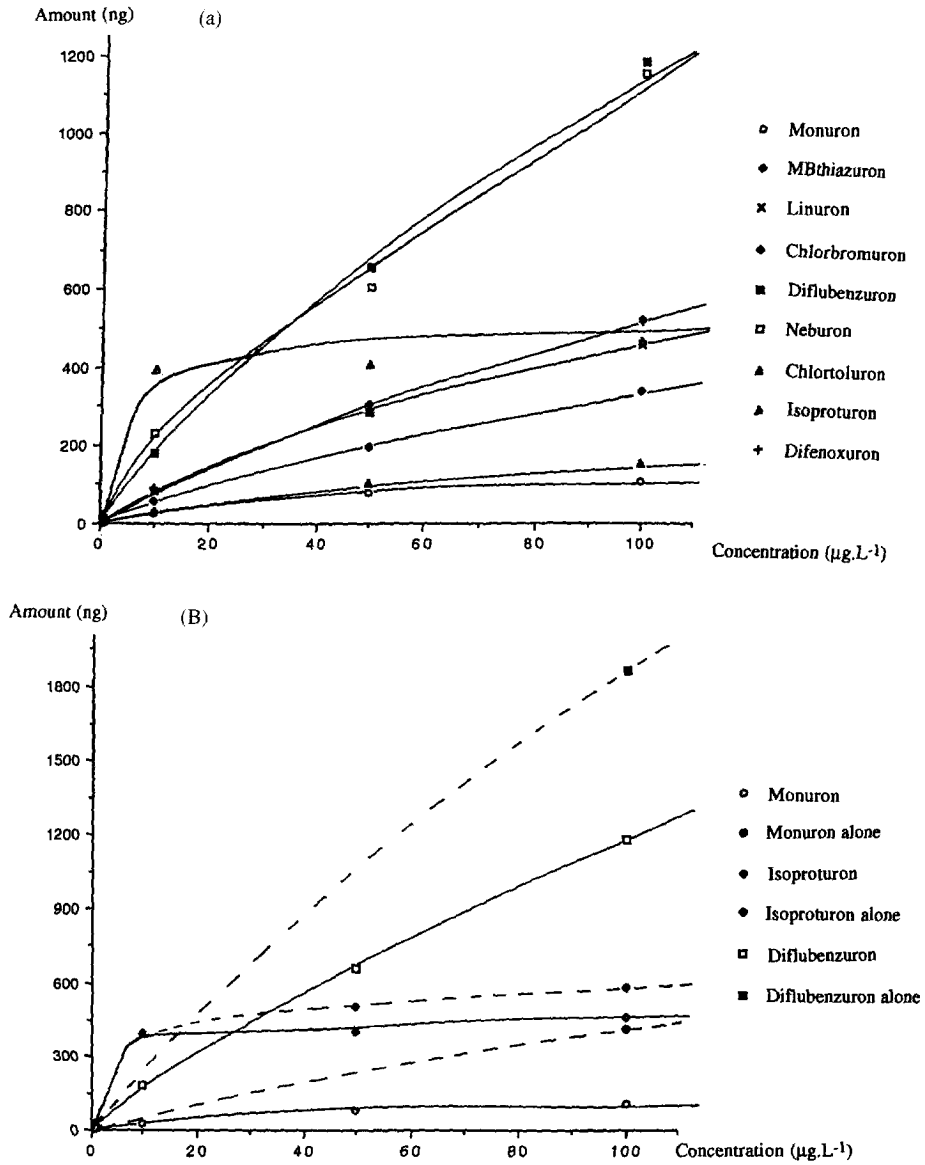

Fig. 4. Binding curves of several phenylureas obtained after the percolation of 50 ml of LC-grade water spiked at high concentration levels (10, 50 and 100 $\mu g\ l^{-1}$) with a mixture of thirteen phenylureas (a) and comparison of the binding curves obtained for several phenylureas when compounds are in a mixture or alone in the percolated samples (b).

for trace analysis at the $\mu g\ l^{-1}$ level. Moreover, if larger capacities are required, they can be increased by adding a purification step of the antibodies based on affinity chromatography with immobilized haptens. To take the low capacity into account, we recommend the establishment of calibration curves and verification of their linearity for the concentration range under study.

3.4. Calibration curves

The calibration curves for several triazines were drawn with spiked LC-grade water in the trace level range of 0.1–3 $\mu g\ l^{-1}$ ($n=7$) and with sample volumes of 25 ml. The experiments were performed with the antiatrazine and antisimazine IS (Fig. 5a and Fig. 5b, respectively). The curves are linear for the

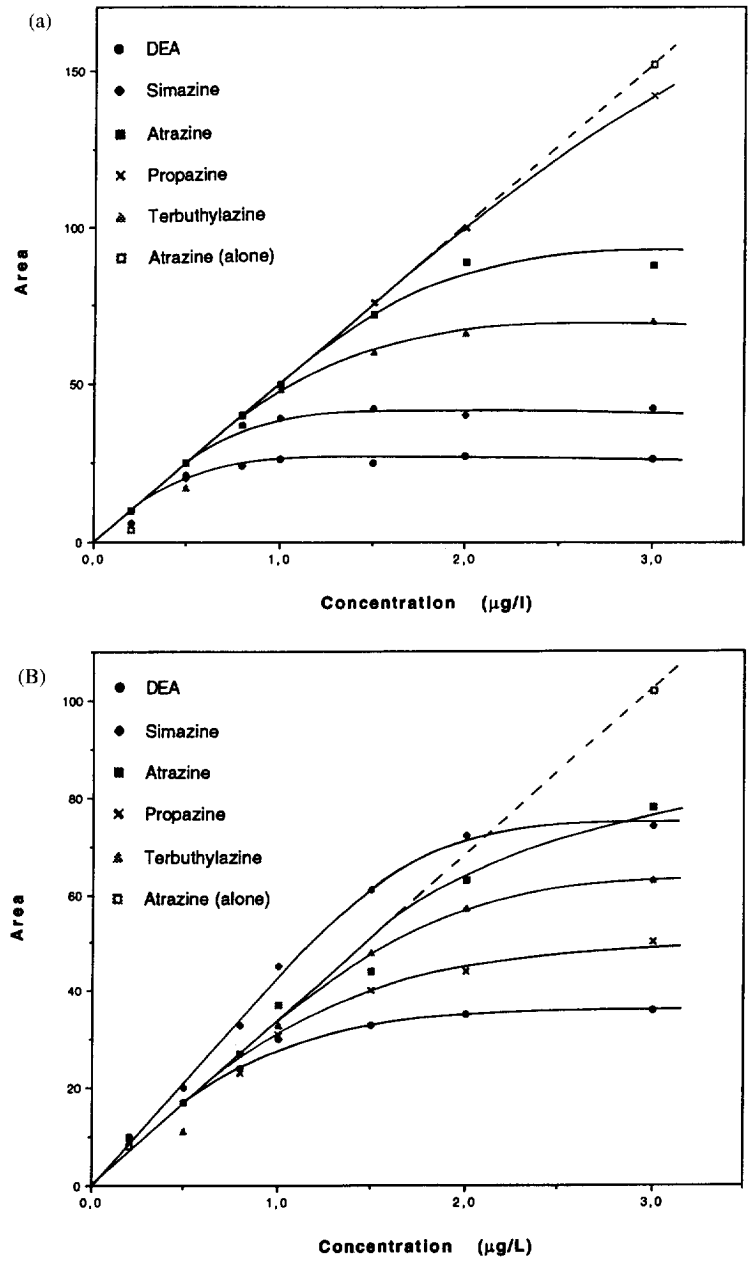

Fig. 5. Calibration curves obtained after the percolation of 25 ml of LC-grade water spiked with a mixture of triazines at concentrations from 0.1 to 3 $\mu g\ l^{-1}$ on 0.5 g of antiatrazine (a) and antisimazine (b) IS.

lowest concentration levels and reach a plateau for the highest concentration levels. The concentrations for which the curves are no longer linear depend on the affinity developed by antibodies to the different compounds. On antiatrazine antibodies, the highest concentration levels (corresponding to the limit of linearity) are obtained for propazine (2.5 $\mu g\ l^{-1}$) and atrazine (about 1.5 $\mu g\ l^{-1}$) and the lowest one is obtained for DEA (about 0.5 $\mu g\ l^{-1}$). On antisimazine antibodies, the highest concentration

levels are obtained for simazine and atrazine (between 1.5 and 2 $\mu g\ l^{-1}$). These concentration levels can be correlated with the affinities of the antibodies to every pesticide. The higher affinity with anti-atrazine antibodies was obtained for propazine because this compound possesses a chloro group and more importantly, two aminoisopropyl groups. In the same way, the higher affinity with antisimazine antibodies was obtained for simazine that is characterized by a chloro group and also two aminoethyl groups.

When atrazine is percolated on its own on the two ISs, the resulting calibration curves are linear for the entire calibration range. In this case, no competition occurs and the capacity of the IS is not reached even by percolating samples spiked at the highest concentration levels, so that the whole percolated amount of atrazine can be retained on the ISs. From a qualitative point of view, it can be noticed that, when the IS is not overloaded, the slope of the calibration curve of a compound is constant, whether the compound is alone or in a mixture (Fig. 5a and b). Decreasing the competitive binding increases the capacity for the target analyte and consequently the linear range of the calibration curve.

The aim of this work was the selective extraction of pesticides at trace levels. The range of linearity obtained here is therefore sufficient, as will be illustrated below. If higher concentration levels are required, the use of a smaller volume of sample (10 ml) will allow one to work with higher linear ranges (factor 2.5) than those shown in Fig. 5. Moreover, the increase of the capacity by adding a purification step, as previously mentioned, will allow one to extend the range of concentrations that can be studied.

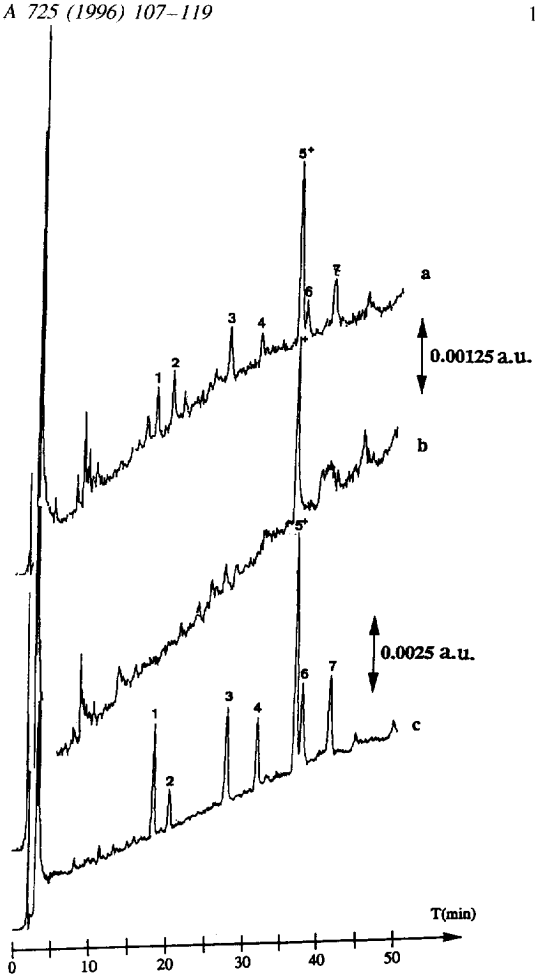

Fig. 6. Off-line preconcentration of 50 ml of River Seine water spiked at 0.1 $\mu g\ l^{-1}$ with (a) triazines; and (b) non-spiked on a cartridge containing 0.5 g of silica bonded with antiatrazine antibodies; and (c) on-line preconcentration of 100 ml of LC-grade water spiked at 0.5 $\mu g\ l^{-1}$ on PLRP-S. Analytical conditions are described in Section 2.4. Detection was measured at 220 nm. Compounds: 1=simazine, 2=cyanazine, 3=atrazine, 4=prometon, 5=sebuthylazine, 6=propazine, 7=terbuthylazine and +=unknown compounds.

3.5. Selectivity and consequences for detection limits in surface waters

The limit of detection that can be reached for a given compound with such a system depends on the recovery of extraction obtained on the IS and consequently on the affinity developed by the immobilized antibodies towards this compound. However, the selectivity of the preconcentration is so high that sample volumes can be reduced compared to results obtained with non selective sorbents. Fig. 6 shows the chromatogram corresponding to the preconcentration of 50 ml of water from the River Seine, non-spiked (Fig. 6a) and spiked with 0.1 $\mu g/l$ of triazines (Fig. 6b). The high selectivity of the immunoaffinity system allows for the easy detection of the triazines introduced into the sample at this low concentration level in this rather highly contaminated surface water (River Seine sample that was taken in Paris City). The peak identified by a cross and coeluted with sebutylazine comes from the LC-grade water used for diluting the off-line extract and for

analyzing it by an on-line system. The chromatogram corresponding to the direct on-line preconcentration using the Prospekt with on-line preconcentration through a PLRP-S cartridge of a 100-ml sample of LC-grade water spiked at 0.5 $\mu g\ l^{-1}$ proves the occurrence of this impurity in this water (Fig. 6c). So the conclusion is that the River Seine extract has been polluted by the added LC-grade water. Identification of the compounds at this low concentration level can be obtained using the retention time and the UV spectrum match given by the diode array detector. No triazines were found in the non spiked water sample as shown in Fig. 6a. Table 1 shows the complementarity of the antiatrazine and antisimazine ISs for the extraction of triazines and particularly of the atrazine metabolites. By mixing both immunosorbents, it was possible to extract most of the triazines and to identify them at this low concentration level (0.1 $\mu g\ l^{-1}$) even in highly contaminated water.

For phenylureas, the antichlortoluron IS can be used alone to extract most of them. Fig. 7 shows the chromatogram corresponding to the preconcentration of 50 ml of River Seine water spiked at 0.1 $\mu g\ l^{-1}$ with thirteen phenylureas (a) and non-spiked (b) on the antichlortoluron IS. The increase of UV absorbancy at the end of the chromatogram is due to the gradient and is explained by the rapid increase of acetonitrile content at the end of the gradient and the low sensitivity range in absorbancy units of the detector. The preconcentration of the spiked Seine river sample (Fig. 7a) shows that detection limits of 0.1 $\mu g\ l^{-1}$ can easily be reached for ten of the thirteen phenylureas selected for this application. Some traces of monuron and isoproturon were found in the raw sample (less than 0.1 $\mu g\ l^{-1}$). Their identification was made more easily due to the high selectivity of the IS, which generates a very clean base-line. For compounds having characteristic UV spectra, confirmation by mass spectrometry is no

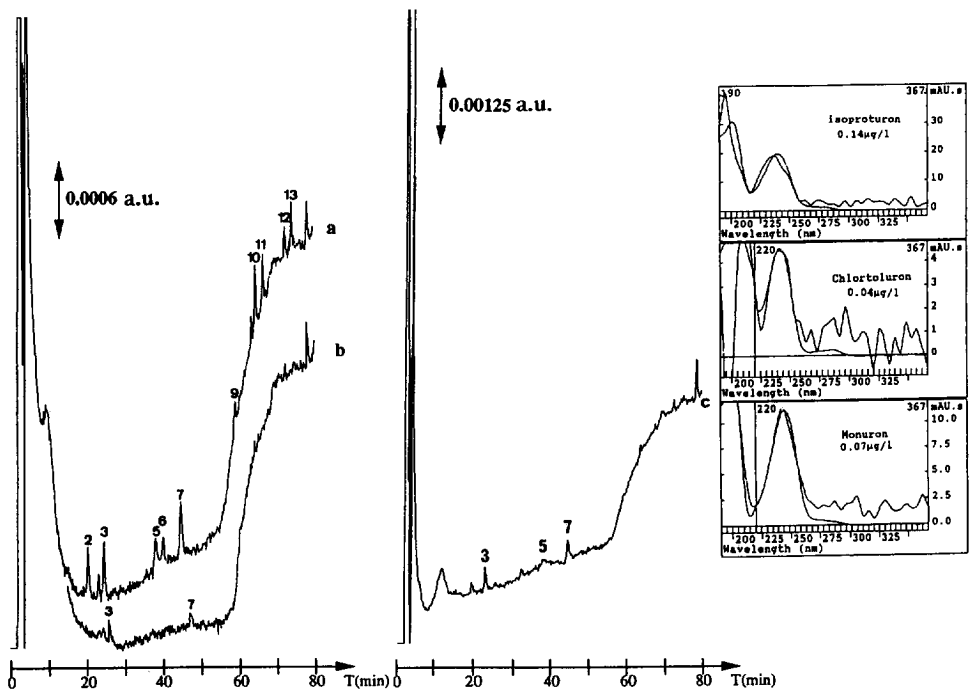

Fig. 7. On-line preconcentration of 50 ml of River Seine water spiked at 0.1 $\mu g\ l^{-1}$ with (a) phenylureas; and (b) non-spiked on a precolumn containing 0.22 g of silica bonded with antichlortoluron antibodies; and (c) identification of three compounds in a non-spiked River Seine water. Analytical conditions are described in Section 2.4. Detection was measured at 244 nm. Compounds: 1=fenuron, 2=methoxuron, 3=monuron, 4=methabenzthiazuron, 5=chlortoluron, 6=fluometuron, 7=isoproturon, 8=difenoxuron, 9=buturon, 10=linuron, 11=chlorbromuron, 12=diflubenzuron and 13=neburon.

longer required. The identity of the analyte is confirmed by the selective trapping (since only phenylureas can be trapped on the IS), the retention time and the match with the UV spectrum. As an example, Fig. 7c presents the identification of three phenylureas at very low levels in another sample of River Seine water.

4. Conclusion

We have demonstrated that it is possible to use immunosorbents for multiresidue analyses of pesticides. A close look at the structure of the compounds allowed the selection of the antigen required for the production of antibodies that are able to trap the highest number of compounds within a chemical group. Antisimazine and antiatrazine antibodies have shown their complementarity to cover all the triazines. Antichlortoluron IS was used to trap the most part of the phenylureas. Studies of capacity have proven the occurrence of different kind of recognition sites and many competition phenomena between the different compounds. The combination of these highly selective ISs with the LC analysis and diode array UV detection will allow a simple confirmation of analytes and an easy determination at very low levels in highly contaminated surface water.

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References

[1] M.W.F. Nielen, R.W. Frei and U.A.Th. Brinkman in R.W. Frei and K. Zeich (Editors), Selective Sample Handling and Detection in High-Performance Chromatography, Part A (Journal of Chromatography Library, Vol. 39A), Elsevier, Amsterdam, 1989, pp. 5–78.

[2] M.-C. Hennion and P. Scribe in D. Barcelo (Editor), Environmental Analysis; Techniques, Application and Quality Assurance, Elsevier, Amsterdam, 1993, pp 23–77.

[3] G. Font, J. Manes, J.C. Molto and Y. Pico, J. Chromatogr., 642 (1993) 135.

[4] V. Pichon and M.-C. Hennion, J. Chromatogr. A., 665 (1994) 269.

[5] J. Slobodnik, E.R. Brouwer, R.B. Geerdink, W.H. Mulder, H. Lingeman and U.A.Th. Brinkman, Anal. Chim. Acta, 268 (1992) 55.

[6] G.S. Rule, A.V. Morderhal and J. Henion, Anal. Chem., 66 (1994) 230.

[7] D.H. Thomas, M. Beck-Westernmeyer and D.S. Hage, Anal. Chem., 66 (1994) 3823.

[8] V. Pichon, L. Chen, M.-C. Hennion, R. Daniel, A. Martel, F. Le Goffic, J. Abian and D. Barcelo, Anal. Chem., 67 (1995) 2451.

[9] V. Pichon, L. Chen and M.-C. Hennion, Anal. Chim. Acta, 311 (1995) 429.

[10] R.W. Frei and K. Zeich in R.W. Frei and K. Zeich (Editors), Selective Sample Handling and Detection in High-Performance Chromatography, Part A (Journal of Chromatography Library, Vol. 39A), Elsevier, Amsterdam, 1989, p. 1.