Evaluation and Characterization of a Commercial Immunosorbent Cartridge for the Solid-Phase Extraction of Phenylureas from Aqueous Matrices

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Abstract

The behavior and main characteristics of a commercial immunosorbent (IS) cartridge for the solid-phase extraction of phenylureas are determined in this work. The measured capacity for the analyte–antigen (isoproturon) in a new cartridge is 215 ng and, after more than 100 adsorption–desorption cycles, the remaining capacity still is approximately 70 ng, demonstrating the good stability of the bonded antibody and the interesting possibility of extensive cartridge reuse. Only isoproturon and diuron are specifically retained in this sorbent. The weak nonspecific retention of other pesticides, including other phenylureas, can be avoided by increasing the sample volume during the loading step. Thus, a very selective and sensitive method for the determination of isoproturon and diuron in natural and potable waters is developed by loading a 50-mL sample adjusted to pH 7.4 in the IS cartridge, eluting with methanol–water (60:40, v/v), and analyzing the eluate by high-performance liquid chromatography with UV detection. The clean chromatograms, low detection limits (~ 0.1 µg/L), and good precision (< 5%) obtained with this rapid and simple method demonstrate that immunoaffinity extraction can be an excellent alternative for sample preparation in the environmental monitoring of particular pesticides in water matrices.

Introduction

The development of new selective sorbents for the extraction, preconcentration, and cleanup of pesticides, toxins, and other pollutants from complex samples has been the object of numerous research works in recent years. The interest has been particularly directed toward materials that allow the structure recognition of the target analytes, such as the immunosorbents (IS) (1–7) and molecularly imprinted polymers (MIP) (8–10). Thus, considerable efforts have been made to optimize all stages of the sorbent preparation process and to study and define the relevant parameters affecting the extraction/elution of the analytes (1,4–7,9). Until the present, the IS technology has reached a higher degree of maturity, as testify the reported applications of these sorbents in the solid-phase extraction (SPE) of trace pollutants from environmental samples and food extracts (11–14). Indeed, analytical methods for the determination of herbicides in water, using experimental antiphenylurea or antiatrazine IS precolumns coupled to high-performance liquid chromatography (HPLC) with various detection modes, have already been validated (15–16), and the first commercial IS cartridges for the SPE of aflatoxins, phenylureas, and triazines have appeared in the market in the last few years. Besides, some excellent reviews dealing with the online or offline SPE of low-molecular-mass analytes using IS have been published (17–19). Comparatively, the preparation and application of MIP for environmental analyses is still at a research stage, but because of their potential advantages (concerning reproducibility, sample capacity, stability, and cost), great improvements are expected in this area in the near future (20,21).

The basis of immunoaffinity extraction is the highly selective antigen–antibody interaction. Antibodies raised against a specific compound can be immobilized by covalent bonding to the surface of an adequate support (1,3,4,12) or, alternatively, they can be encapsulated into the pores of a solid matrix (5–7). These biomaterials will selectively retain the analyte–antigen present in, for example, a surface water sample, thus effecting extraction, preconcentration, and cleanup in the same step (17). However, because of the unavoidable cross-reactivity of antibodies, especially when they are raised against a small molecule with few determinant groups (as is the case for most pesticides), other structurally related compounds may also be retained by the IS. This cross-reactivity has been exploited to develop class-selective sorbents that can be used for the simultaneous SPE of several members of the same chemical family (12,14–18). It has been stated that sample preparation methods using IS also result in enhanced sensitivity of analysis for complex samples because, as the obtained extracts are in principle free of most matrix interferences, it is possible to use highly sensitive detection conditions (11,13).

Despite the interesting advantages of immunoaffinity extraction, its application in the environmental field still is restricted to

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some research laboratories, mainly because of the limited availability and high cost of immunosorbents as compared with conventional SPE phases. To overcome these drawbacks and expand the use of the technique, it would be necessary to interest the industry in the production of a greater volume and variety of IS phases, which can only be done if the potential users are convinced of the reliability of the commercial cartridges and the benefits of their use.

The aim of this work was to study and characterize a commercial antiphenylurea IS cartridge and to evaluate its application for the offline SPE of diuron, isoproturon, linuron, and fluometuron from various aqueous matrices. These four herbicides are the most commonly used phenylureas in Mexico. Important properties of the IS cartridges, such as the capacity, reusability, and cross-reactivity, were examined. Besides, a stability study of the adsorbed analytes over a long time period was performed in view of proposing the cartridges as an alternative means for the transport of water samples.

Experimental

Reagents and materials

LC-grade acetonitrile and methanol were from Prolabo (Paris, France). Type 1 reagent water was obtained from a Nanopure (Barnstead International, Dubuque, IA) deionizer. All other chemicals (sodium azide; sodium chloride; potassium chloride; phosphoric acid; sodium hydroxide; and perchloric, formic, and acetic acids) were analytical-grade reagents from various furnishers; they were used without further purification. The phenylurea herbicides (isoproturon, diuron, fluometuron, monuron, linuron, and neburon) and two other pesticides (methiocarb and parathion-methyl) were obtained from Chem Service (West Chester, PA) with certified purity of 99%. Stock solutions (1000 mg/L) of each herbicide were prepared in acetonitrile and stored at –20°C when not in use. Working standards were prepared by dissolving appropriate aliquots of the stock solutions in acetonitrile–water (40:60, v/v) or in phosphate-buffered saline solution (PBS), depending on the experiments. PBS was 0.02M (in phosphate), unless otherwise indicated. It was prepared by dilution of the adequate phosphoric acid volume in reagent water, addition of NaCl (0.137M) and KCl (0.0027M), and adjustment to pH 7.4 with a sodium hydroxide solution. For some experiments requiring other buffer concentrations, the PBS was prepared keeping the same phosphate–NaCl–KCl molar ratio.

The immunosorbent cartridges were purchased from Abkem (Ottawa, Canada). A package of ten ImmunoSep phenylurea cartridges (same lot) was used throughout this work. The cartridges are guaranteed for retention of 450 ng of isoproturon and for three uses without alteration. According to information given in the furnisher catalog, polyclonal antibodies raised against isoproturon were covalently bonded to a modified silica support to prepare the IS. The specified cross-reactivity of this sorbent for the phenylureas considered in this work, with respect to the retention of the analyte-antigen is: isoproturon 100%, diuron 43%, monuron 9%, linuron 28%, and neburon 36% (supplier information for single tested phenylurea). The measured sorbent bed in the syringe-type plastic cartridges (5-mm i.d.) was between 3–4 mm, which is equivalent to a bed volume of 0.06–0.08 mL. The cartridges were washed and stored in PBS at 4°C when not in use. For storage periods of more than one week, sodium azide (1%, w/v) was added to the buffer in order to prevent mold and bacterial growth.

Chromatography

Phenylurea recoveries in the different experiments were determined by chromatographic analysis of the eluates obtained from the IS cartridge; in some cases, the percolated sample flow through was also analyzed to confirm the results. The LC system consisted of model 305 and 306 pumps, a model 811 B dynamic mixer, a model 805 manometric module (all from Gilson, Middleton, WI), and a 7125 Rheodyne (Rohnert Park, CA) injector with a 100-µL loop. A spectrophotometric UV detector, model SPD-10 AVVP from Shimadzu (Kyoto, Japan), set at a wavelength of 254 nm (unless otherwise indicated), and a 3396-B Hewlett-Packard (Palo Alto, CA) integrator were used for the detection and quantitation of the herbicides.

The chromatographic separation was carried out on an Envirosep-pp column (125- × 3.2-mm i.d.) from Phenomenex (Torrence, CA). This column, packed with a 5-µm polymeric C_{18} reversed phase (RP), permitted us to achieve a good separation of fluometuron, isoproturon, and diuron. Other conventional (monomeric) RP phases were not capable of adequately resolving the three compounds. Depending on the experiments, isocratic elution with acetonitrile–water mixtures or gradient elution (six phenylureas together) was used. For the latter, the weak and strong mobile phases were acetonitrile–water 10:90 (v/v) and 75:25 (v/v), respectively, and the program was a linear gradient from 9% to 92% B in 20 min and then constant for 10 min. A flow

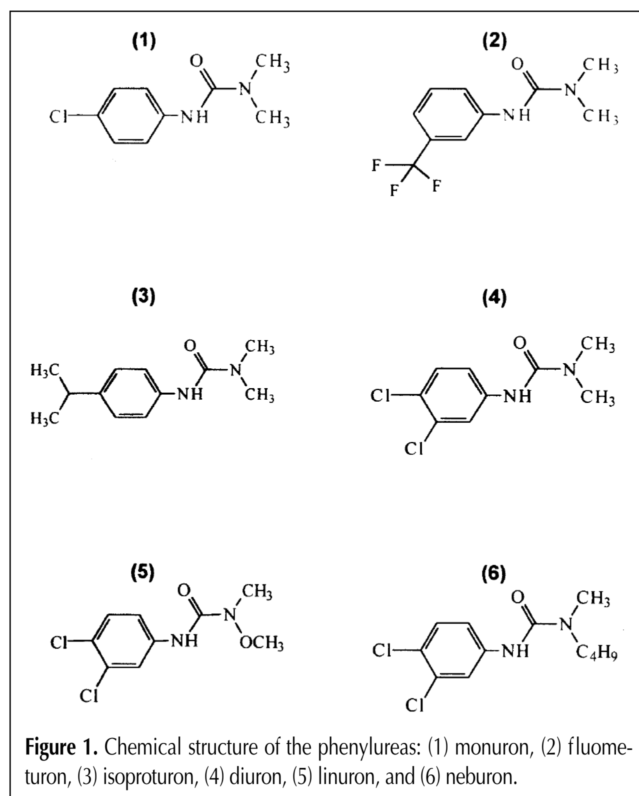

Figure 1. Chemical structure of the phenylureas: (1) monuron, (2) fluometuron, (3) isoproturon, (4) diuron, (5) linuron, and (6) neburon.

rate of 1 mL/min was used throughout.

Immunosorbent characterization

The effect of several experimental parameters on the recovery of the analyte–antigen was studied to determine the optimal operation conditions for the IS cartridges. To characterize the elution behavior, a fixed amount of isoproturon (73 ng) dissolved in PBS was first loaded in the cartridge followed by a 2-mL water rinsing; then, the solute desorption was performed with (*a*) different methanol–water mixtures, (*b*) a methanol–aqueous buffer (60:40, v/v) at different pH values of the buffer, (*c*) various eluent flow rates, and (*d*) increasing eluent volumes. For the adsorption study, the following parameters were sequentially varied: analyte concentration, sample volume, pH, and PBS concentration. Finally, the mean cartridge capacity was determined using 4 new (unused) cartridges, which were loaded with an excess of isoproturon in PBS, rinsed with 2 mL of water, and eluted with methanol–water (60:40, v/v).

The selectivity of the antibody toward different members of the same family was examined by processing samples with the six phenylureas, as single compounds or in mixture, in an IS cartridge. In the former case, 1-mL samples containing 60 ng of a phenylurea in 1 mL of PBS were studied. Besides, two different phenylurea mixtures were also tested: first, a sample containing the six phenylureas (25 ng each) in 1 mL of PBS; then, two samples containing four phenylureas (fluometuron, linuron, diuron, and neburon, 25 ng each) in 25 or 75 mL of PBS, respectively. In all these experiments, after loading the cartridge with the corresponding sample, it was rinsed with water (2 mL) and eluted with methanol–water (60:40, v/v) to determine the analyte recoveries. Figure 1 shows the structure of the studied phenylureas for reference. Additionally, the specificity of the sorbent for phenylureas was verified with a mixture of isoproturon, methiocarb, and parathion-methyl. A constant amount of the three pesticides (50 ng each) was dissolved in different PBS volumes, and these samples were percolated through a cartridge. Rinsing and elution were performed as before, and the eluates were analyzed by HPLC, setting the detector wavelength at 230 nm in this case.

Analytical method for the determination of isoproturon and diuron in water samples

The previous experiments showed that the interaction of linuron and fluometuron with the antibody was negligible. Therefore, the analytical method was only established for the determination of isoproturon and diuron at trace concentration levels in aqueous samples. The experimental parameters for the SPE of these compounds in the ImmunoSep cartridges are shown in Table I. The certification of the method (linearity, recovery, precision, accuracy, and limit of detection) was carried out on four cartridges that had been previously used only once.

Finally, a stability study for isoproturon and diuron adsorbed on the IS and kept in the cartridge for different time periods before elution and analysis was also carried out. Five cartridges were loaded with 50 ng of each analyte and rinsed according to the conditions indicated in Table I. One cartridge was immediately eluted and analyzed and the recovered amount of isoproturon and diuron was taken as the time zero reference. The other cartridges were kept in refrigeration at 4°C and subsequently analyzed, one at a time, over a total period of 103 days. For comparison, an identical parallel study was carried out with five reagent water samples spiked at 25 µg/L of each compound. These more concentrated samples were analyzed by direct injection in the chromatograph.

Table I. Experimental Conditions for the SPE of Isoproturon and Diuron from Water Samples Using the Phenylurea ImmunoSep Cartidges*

Parameter	Loading	Rinsing	Elution
Composition	Water sample with PBS 0.02M, pH7.4 (ionic strength, 0.19M)	Water	Methanol–water
Flowrate	5 mL/min	1 mL/min	1 mL/min
Volume	50 mL	2 mL	2 mL

* Before application of the sample, the cartridge was conditioned with 15 mL of PBS and 2 mL of reagent water; after elution, it was regenerated with an estra 5-mL volume of eluent and 10 mL of PBS. A recuperation time of at least 15 min was necessary between consecutive operation cycles.

Results and Discussion

Optimization of the conditions for loading and elution

Figure 2 shows the effect of the eluent composition and volume

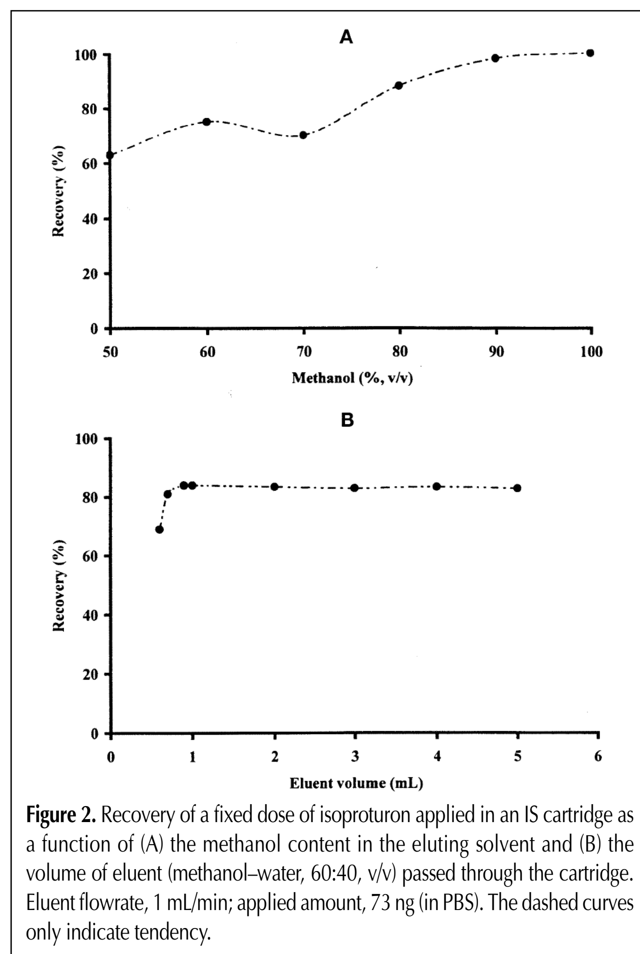

Figure 2. Recovery of a fixed dose of isoproturon applied in an IS cartridge as a function of (A) the methanol content in the eluting solvent and (B) the volume of eluent (methanol–water, 60:40, v/v) passed through the cartridge. Eluent flowrate, 1 mL/min; applied amount, 73 ng (in PBS). The dashed curves only indicate tendency.

on the recovery of isoproturon. Apparently, the interaction of the analyte-antigen with the antibody is quite strong, as it required a high methanol content in the eluent to be broken. Probably, the dissociation of the complex is caused by a modification of the tertiary structure of the protein molecule induced by the rich organic phase. However, the conformational change was not permanent, and the antibody was capable of recovering its active form after passing PBS through the cartridge and leaving the sorbent embedded in this favorable environment for at least 15 min between consecutive operation cycles (5,14). Indeed, the cartridge used for these experiments did not lose the capacity to retain isoproturon, even after the passage of pure methanol through it. Nevertheless, it was preferred to use milder conditions to preserve as much as possible the lifetime of the cartridges. As shown in Figure 2, a recovery of approximately 80% (of the 73 ng of isoproturon loaded) can be obtained with eluents containing 60% (v/v) of methanol and eluent volumes of at least 1 mL. To improve the analyte recovery, the aqueous part of the eluent was replaced by different acid buffers (phosphoric: pH 2, formic: pH 3.8, or acetic: pH 4.7), but no effect was observed with any of them. The use of acid or basic buffers, as an alternative to organic solvent–water mixtures, for the elution of compounds from immunosorbent cartridges has been reported (6,18); however, our results show that the combination of both does not lead to a synergistic effect. Finally, a test was performed varying the flow rate of the eluent from approximately 0.012 (gravity flow) to 2 mL/min. A slight improvement was observed for the slowest flow rate (recovery ~ 90%), but at the expense of very long elution times. For more practical flow rates, between 0.5–2 mL/min, this parameter did not have any influence. From these results, the conditions indicated in Table I were established for elution.

Next, the parameters affecting the adsorption of the analyte were examined. Figure 3 shows the effect of the isoproturon concentration in the sample (loaded volume 1 mL). Two series of experiments were carried out, one in a cartridge that had been previously used in 13 adsorption–desorption cycles and the other in an extensively used cartridge (114 previous cycles). For both cartridges, the retained solute amount first increases with its concentration in the applied sample and then arrives to a plateau, indicative of the sorbent saturation. Consequently, the solute recovery, which is initially high and almost constant, begins to decrease as the plateau is approached and then continually falls down. The amount of retained isoproturon corresponding to the plateau is the actual capacity of the cartridge for this solute and is highly dependent on the history or previous uses of the cartridge. Thus, the capacity for the less used cartridge was approximately 140 ng, whereas for the extensively used cartridge it only was of approximately 70 ng. This loss of capacity indicates that the antibody bonded to the support is progressively denaturized during the SPE experiments, most probably because of the methanol used in the elution step. However, it was surprising to observe that after more than 100 uses, the cartridge still was capable of retaining a nonnegligible amount of isoproturon. Obviously, the immobilization of the antibody confers a very high resistance to this biomolecule.

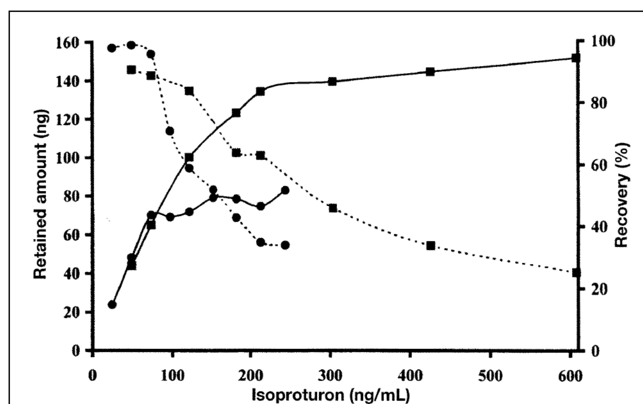

Figure 3. Retained amount (solid line) and %recovery (broken line) of isoproturon from IS cartridges with different previous uses as a function of the analyte concentration in the applied sample. (n) Cartridge previously used in 13 SPE experiments and (=) cartridge previously used in 114 SPE cycles. Sample: isoproturon in PBS, flowrate 1 mL/min, volume 1 mL; rinsing and elution of the cartridge as indicated in Table I.

Considering the previous results, the capacity was measured in 4 unused cartridges that were loaded with 500 ng of isoproturon in 1 mL of PBS. The obtained capacity for this lot of commercial cartridges was 213 ± 28 ng of isoproturon, which is significantly lower than the capacity reported by the furnisher (450 ng of the same solute). This difference can be, in part, explained by the fact that our purchased cartridges had remained stored in refrigeration for several months before this determination was carried out. It is known that the antibody activity decreases with time, even if it is immobilized in a solid support and not submitted to harsh conditions. Any way, for the application of the cartridges in trace analysis, the measured capacity is largely sufficient.

On the other hand, the comparison of the measured capacities for an unused cartridge (213 ng), a cartridge with 13 uses (140 ng), and a cartridge with more than 100 uses (70 ng) shows that the antibody activity rapidly decreases during the initial SPE experiments and then very slowly continues to diminish as the number of operation cycles increases. It would seem that, depending on their location in the support, the bonded antibody molecules are more or less protected against denaturation. Thus, the most exposed biomolecules probably lose their activity from the first contacts with the eluting solvent, whereas the antibody located in the internal pores of the solid matrix is capable of better resisting the repeated conformational changes provoked by the continuous switching between PBS and the methanol–water mixture.

To get rid of variations caused by the different cartridge capacities depending on their previous use, the experiments for determining the effect of a particular parameter were all carried out successively in the same cartridge, and the solute amount in the applied sample was always restricted to less than 100 ng.

The retention of the analyte in an IS cartridge may be affected by the flow rate of the percolated sample if the formation of the solute–antibody complex or the diffusion of the solute through the support matrix are slow. To test this variable, a cartridge was loaded with 73 ng of isoproturon in 3 mL of PBS at flow rates varying from 1 to 5 mL/min. The recovery of the analyte in this range of flow rates remained constant, indicating a rapid kinetics for both the solute diffusion and the complexation reaction. In the next test, the sample volume loaded in a cartridge (with 14 previous uses) was varied from 1 to 250 mL, keeping constant the

amount of isoproturon at 73 ng. A flow rate of 1 mL/min was used to percolate the smallest volumes, and it was increased to 4 mL/min for volumes of 50 mL or more. The results in Figure 4 show that the solute recovery first decreases as the volume of sample increases and then remains practically constant, between 70–80%, for the largest volumes. This behavior may be caused either by inhomogeneity of the antibody active sites or by nonspecific retention. Thus, the solute molecules that interact with the weakest active sites or nonspecifically (with the support or the nonactive part of the antibody), are easily eluted from the cartridge by the sample itself when the percolated volume is increased. On the contrary, the solute retained by strong specific interactions cannot be eluted during the loading step, even if very large sample volumes are processed.

An aqueous solution of phosphoric, formic, and acetic acids (0.002M each) adjusted with NaOH was used (instead of PBS) to study the effect of the sample pH on the adsorption of isoproturon. Sample volumes of 100 mL containing 18 ng of the herbicide were loaded in the cartridge. A constant recovery (~ 65%) was obtained in the studied pH range (3.5–7.4), probably because phenylureas do not have acid–base properties, and thus electrostatic interactions through ionizable groups are not implicated in the formation of the solute–antibody complex. However, this recovery was lower than in previous experiments in which PBS (0.02M) was used, indicating that the buffer concentration or the ionic strength of the sample (or both) affect the antibody performance. In their natural media, antibodies are immersed in biological fluids of neutral pH and high saline concentration (~ 0.1M), and these conditions are probably necessary to develop their full activity. In fact, additional experiments performed with samples at pH 7.4, but with different PBS concentrations, showed that a recovery close to 100% can be obtained for the same solute amount (18 ng) and sample volume (100 mL) if PBS is ≥ 0.002M. For lower buffer concentrations, the recovery abruptly decreased. Considering the relative proportions of alkaline salts and phosphate in PBS, as well as the pH, a concentration of 0.002M in phosphate is equivalent to an ionic strength of 0.019M. Comparatively, the calculated ionic strength for a solution of phosphoric, acetic, and formic acids 0.002M each at pH 7.4 is 0.007M. Therefore, it is the ionic strength and not really the buffer concentration that affects the behavior of the immunosorbent.

The conclusions of the adsorption–desorption study are reflected in the experimental conditions proposed in Table I for the immunoextraction of phenylureas from aqueous samples.

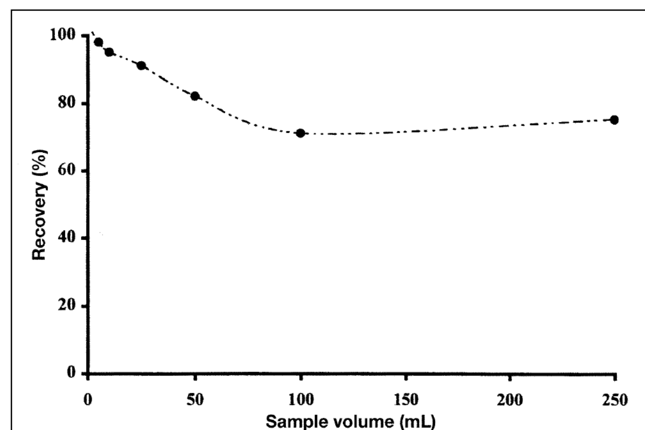

Figure 4. Effect of the applied sample volume on the recovery of isoproturon. Loaded amount, 73 ng (in PBS) and flow rate, 1 mL/min for sample volumes lower than 50 mL and 4 mL/min for the largest volumes. Other conditions are the same as in Table I.

Selectivity and specificity of the IS cartridge

Table II shows the recovery results obtained for several phenylureas that were loaded in a cartridge independently and in two different mixtures. A high recovery, similar to that of isoproturon, was obtained for diuron and neburon in the experiments in which single compounds (60 ng) were loaded in a small sample volume (1 mL of PBS). For other phenylureas, the retention decreased in the order: monuron > linuron > fluometuron. However, when a mixture of the six compounds (25 ng each) in the same PBS volume was loaded, the competition effect provoked a decrease in recovery that was proportionally different for each phenylurea. In this case, isoproturon and diuron remained as the most retained compounds, whereas the recovery of monuron, linuron, and neburon became practically identical, and fluometuron was completely lost. Furthermore, increasing the volume of percolated sample to 25 mL for a mixture of four phenylureas (without the analyte–antigen) resulted in the total loss of neburon and linuron. From the results in Table II, the following conclusions were drawn. Fluometuron probably formed a weak complex with some antibody molecules, but this weak interaction was completely disrupted in the presence of isoproturon or when the sample volume was largely increased (75 mL). Linuron and especially neburon were probably only retained by nonspecific interactions, as they were completely lost when the sample volume was increased (25 mL) and a strong competitor (i.e., diuron) was present in the same sample. Besides isoproturon, diuron was the only phenylurea that seemed to specifically interact with the immunosorbent to a significant extent. However, the high retention observed for this solute at small sample volumes also involved nonspecific interactions, as demonstrated by the loss of an important fraction of diuron at larger sample volumes.

The specificity of the immunosorbent for phenylureas was

Table II. Recovery of Phenylureas Loaded in an ImmunoSep Cartridge as Single Compounds or in Mixture (Different Sample Volumes)

	Recovery (%)			
Phenylurea	Single compund (60 ng; sample, 1 mL)	Mixture (25 ng each; sample, 1 mL)	Mixture (25 ng each; sample, 25 mL)	Mixture (25 ng each; sample, 75 mL)
Isoproturon	87	64	–*	–
Diuron	92	75	60	40
Fluometuron	10	n.d.†	13	n.d.
Linuron	35	35	n.d.	n.d.
Monuron	58	29	–	–
Neburon	90	34	n.d.	n.d.

* The compound was not added in this mixture.
† n.d., not detected in the eluate from the cartridge.

tested loading a cartridge with a mixture of isoproturon and two other pesticides from different families, methiocarb (carbamate) and parathion-methyl (organophosphorous). Samples containing a fixed amount of each compound (50 ng) in different volumes were loaded in a cartridge. Figure 5 shows the recovery obtained for the three pesticides in these experiments. For comparison, the recovery of isoproturon, loaded as a single compound in the same cartridge, is included in the figure. It is observed that the presence of the other pesticides had a minimal effect on the recovery of isoproturon. Although parathion-methyl and methiocarb were notably retained when the sample volume was very small, this retention became negligible for a sample volume of 25 mL. The latter clearly indicates that the retention of these compounds at low sample volumes was only because of nonspecific interactions.

The mentioned results show that the immunosorbent is indeed specific for phenylureas, but a volume of at least 25 mL of the sample must be processed in the cartridge to avoid or limit the nonspecific retention of other pesticides (or other compounds of medium polarity and hydrophobicity). From the six phenylureas studied in this work, only isoproturon and diuron had a significant interaction with the antibody. However, to obtain a constant recovery, it is necessary to work with relatively high sample volumes that minimize the nonspecific retention of these solutes or their interaction with the less-active sites (or both).

Determination of isoproturon and diuron in water samples

Considering the results from the previous study, the following simple procedure was established for the determination of the two phenylureas at trace concentration levels in water: (step 1) 5 mL of 0.2M PBS (pH 7.4) were added to 45 mL of the sample and the mixture was filtered through a nylon 66 membrane with a pore diameter of 0.45 µm; (step 2) a phenylurea ImmunoSep cartridge was equilibrated with 15 mL of 0.02M PBS and 2 mL of reagent water; (step 3) the filtered sample was percolated through the cartridge, and the latter was rinsed and eluted according to the conditions in Table I; (step 4) 100 µL of the eluate were analyzed by HPLC with UV detection; and (step 5) the cartridge was regenerated with an additional 5-mL volume of the eluting solvent followed by 10 mL of 0.02M PBS. After this procedure, the sorbent must be left under PBS for at least 15 min before the next use of the cartridge.

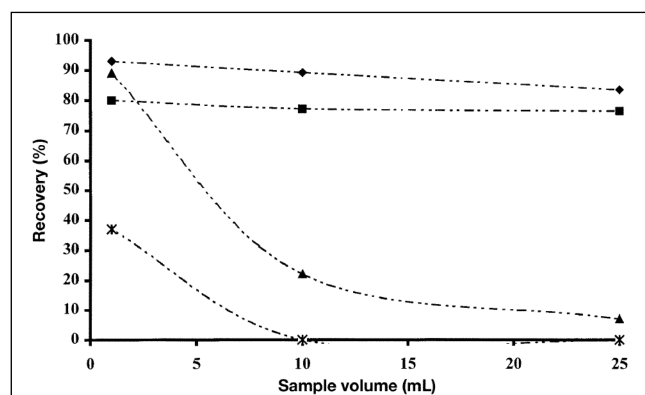

Figure 5. Recovery of isoproturon (n), methiocarb (s), and parathion-methyl (*) (50 ng of each compound) as a function of the sample volume applied in an IS cartridge. Same study with a sample containing isoproturon alone (u). Other conditions as in Table I.

Reagent water samples (4 replicates) were spiked with the two phenylureas at various concentrations and analyzed according to the method. A different cartridge was used for the SPE of each replicate. The obtained response versus concentration curves were linear in the concentration range of approximately 0–3.3 µg/L (0–150 ng of each solute in the sample). The calibration equations obtained by statistic analysis of the data in the linear range were:

$$Y = (12.48 \times 10^4)X - 0.21 \times 10^4, r^2 = 0.997 \text{ (isoproturon)} \quad \text{Eq. 1}$$

$$Y = (14.07 \times 10^4)X + 0.95 \times 10^4, r^2 = 0.998 \text{ (diuron)} \quad \text{Eq. 2}$$

where Y is the peak area and X is the phenylurea concentration in the sample (µg/L). The ordinates in both equations were statistically equal to zero. By comparing the peak areas of the analyzed samples with those of injected standards, a curve of recovered amount versus loaded amount was generated for each solute. The slope of the corresponding equation was 0.67 for isoproturon and 0.37 for diuron. This slope represents the fraction of recovered solute (mean recovery) in the linear concentration range.

The method accuracy, precision, limits of quantitation (LOQs), and limits of detection (LODs) were determined from the analysis of seven reagent water samples spiked at 0.88 µg/L (40 ng in 45 mL) of each phenylurea. The results are shown in Table IIIA. For each sample, the isoproturon and diuron concentrations (found concentration) were calculated using the obtained response and the calibration equations; these concentrations were statistically equivalent to the spiked concentrations at a confidence level of 95%. The relative standard deviation (RSD) was lower than 5%, which is excellent for sub-ppb (< 1 µg/L) concentration levels. The LOQs were defined as the concentration corresponding to 10 times the standard deviation (SD) of the results obtained in the analysis of the seven samples, and the LODs were evaluated using the relation:

Table III. Performance and Selectivity of the Analytical Method for the Determination of Isoproturon and Diuron in Water Samples Using ImmunoSep Cartridges for Sample Preparation

(A)

	Concentration (µg/L)				
Compound	Spiked	Found	RSD (%)	LOQs (µg/L)	LODs (µg/L)
Isoproturon	0.88	0.91	2.97	0.27	0.10
Diuron	0.88	0.84	4.17	0.35	0.13

(B)

	Determined concentration in different water samples (µg/L)				
Sample	Isoproturon	Diuron	Fluometuron	Methiocarb	Parqathion-methyl
River water	0.40	0.44	n.d.*	n.d.	n.d.
Ground water	0.40	0.47	n.d.	n.d.	n.d.
Tap water	0	0.47	n.d.	n.d.	n.d.

* n.d., not detected in the eluate from the SPE cartridge.

$$MDL = t_{(6, 99\%)} \times SD \qquad \text{Eq. 3}$$

where $t_{(6, 99\%)}$ is the Student t test for a confidence level of 99% and 6 degrees of freedom ($n - 1$). For the two analytes, the LOQs were lower than 0.5 ppb, and the LODs were of approximately 0.1 ppb, demonstrating the good sensitivity that can be achieved with a very simple analytical procedure when IS cartridges are used for the sample preparation step.

It must be mentioned that similar or even lower LODs have been reported for the LC–UV determination of phenylureas in water after online or offline SPE of the sample in conventional RP sorbents (C_{18} or styrene–divinylbenzene copolymers) (22–24). However, this high sensitivity has been reached by processing large sample volumes (generally 500–1000 mL) to conveniently detect the target analytes because a considerable portion of the dissolved organic matter (DOC) present in the sample is also extracted. Thus, the chromatograms obtained from the analysis of natural waters often show an initial large matrix peak that very slowly returns to the baseline, followed by other important humps. These perturbations render quite difficult the quantitation of phenylureas at low concentration levels and, therefore, the precision and accuracy of the analysis become highly dependent on the DOC content of the sample.

Considering this, the performance of the proposed method was tested with three different samples: tap water, surface water from a river in the state of Mexico, and groundwater from Tolantongo Grottes in the state of Hidalgo. The analysis of the blank samples indicated that isoproturon and diuron were not present at detectable levels in these waters. Then, an aliquot of the samples was spiked with a mixture of fluometuron, diuron, isoproturon, methiocarb, and parathion-methyl at 0.44 µg/L (20 ng in 45 mL) of each compound, and reanalyzed. The results in Table IIIB show that the good accuracy of the method at this low concentration level is independent of the water matrix. As an example, Figure 6 shows the chromatograms of the blank and spiked river water samples; for comparison, a chromatogram of an injected standard containing the five pesticides is also included. The excellent selectivity of the method, because of the specificity of the immunosorbent, is evident from the clean chromatograms that only show the perturbation provoked by the injection (100 µL) and the peaks of isoproturon and diuron in the spiked sample. Similar chromatograms were obtained with the groundwater and tap water samples, except that for the latter the isoproturon peak did not appear in the chromatogram of the spiked sample. Apparently isoproturon was completely degraded in this water. To verify this hypothesis, an aliquot of the tap water sample was spiked with the two analytes at higher concentration (40 µg/L) and successive direct injections were made in the chromatograph. The isoproturon peak became smaller at each injection, and for the third one it was no longer detectable (~ 20 min after the spike of the

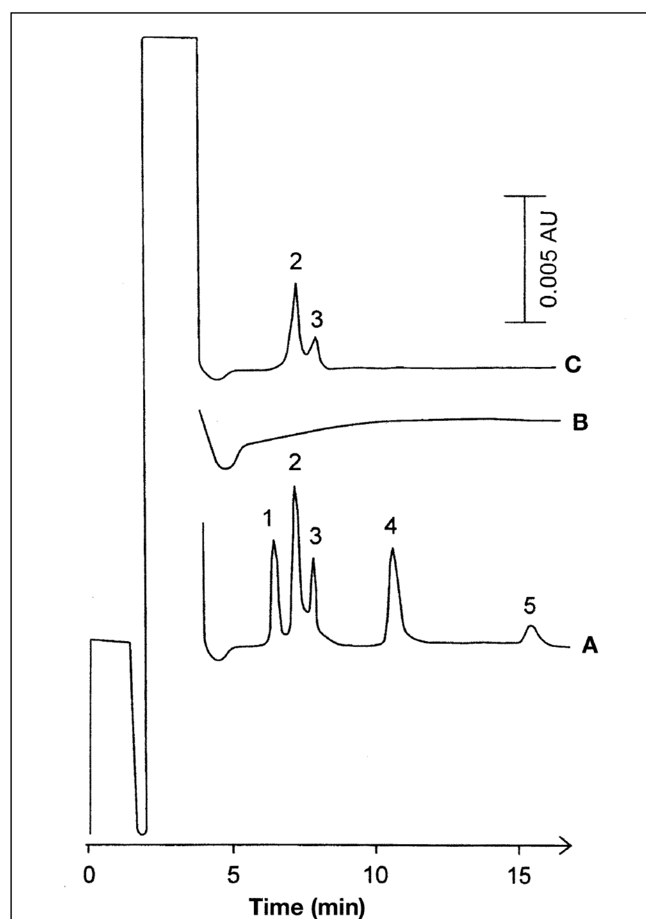

Figure 6. Chromatograms obtained from the analysis of a river water sample. (A) Injected standard, (B) blank sample, and (C) sample spiked with 5 pesticides at 0.44 µg/L of each one. UV detection at 230 nm. Solutes: (1) fluometuron, (2) isoproturon, (3) diuron, (4) methiocarb, and (5) parathion-methyl.

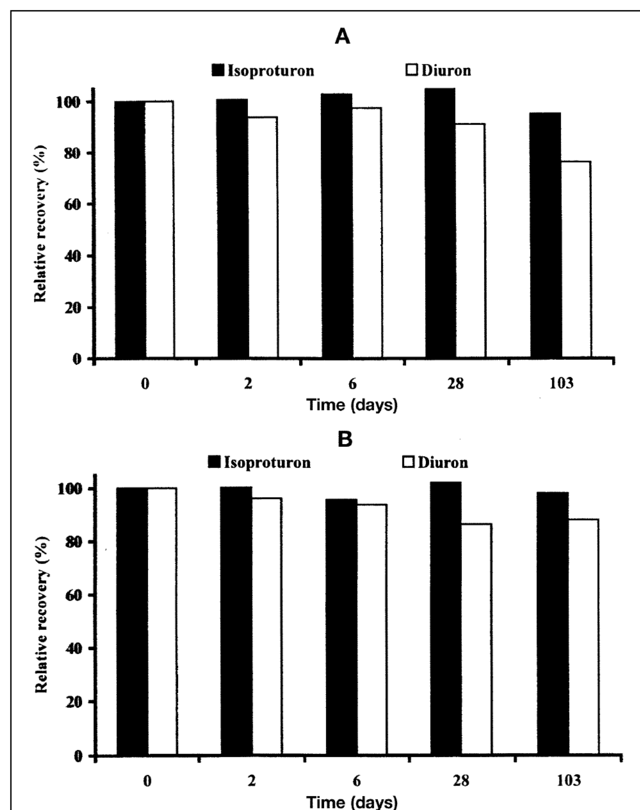

Figure 7. Relative recovery of isoproturon and diuron over a time period of 103 days (A) dissolved in reagent water samples and (B) trapped in IS cartridges. A water sample and a cartridge were immediately analyzed (time zero reference); the other liquid samples and cartridges were kept at 4°C until analysis.

sample). On the contrary, the diuron peak remained constant during this time. Therefore, it was deduced that the residual chlorine (0.5–1 mg/L, according to the Mexican norm for tap water) was capable of rapidly degrading isoproturon, but not diuron.

Finally, the stability of isoproturon and diuron in solution (reagent water) and trapped in the IS cartridge was studied over a period of 103 days. The variation of the relative analyte recovery during this period in both media is shown in Figure 7. It is seen that the compounds are quite stable either in the liquid phase or adsorbed on the immunosorbent. In fact, after 103 days isoproturon was not degraded, and only approximately 20% of diuron was lost. In this context, the interest of the cartridges is their small size, which makes them ideal as an alternative means for the transport of water samples. As shown in this work, the required operations before the SPE of the samples in the IS cartridges are minimal (PBS addition and filtering), and therefore the whole sample preparation can easily be performed onsite. In this way, many water samples could be comfortably transported in the cartridges from remote sampling sites to the laboratory for their final analysis.

Conclusion

The commercial immunosorbent cartridges evaluated in this work have proven to be an effective and reliable tool for the selective SPE of isoproturon and diuron from different aqueous matrices. These cartridges cannot be considered as "class selective" because, from the six tested phenylureas, only two were specifically recognized by the immobilized antibody. The main advantage of their use is the rapidity and simplicity of the sample preparation procedure that provides a very clean extract for direct analysis by HPLC with UV detection. Other interesting features are the possibility of reusing the same cartridge in many adsorption–desorption cycles (apparently there is no accumulation of impurities on the sorbent) and the feasibility of using these cartridges as an alternative means for the transport of water samples (the adsorbed analytes remain stable for a very long time period).

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