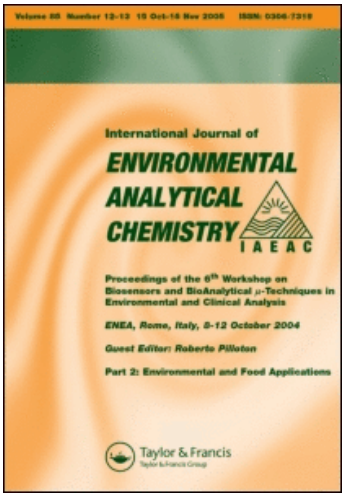

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Analysis of Atrazine in Water and Vegetables Using Immunosensors Working in Organic Media

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ANALYSIS OF ATRAZINE IN WATER AND VEGETABLES USING IMMUNOSENSORS WORKING IN ORGANIC MEDIA

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Flow immunosensors using a competitive capture format have been studied and applied to the analysis of atrazine in extracts of water and vegetables containing high percentages of organic solvents. Four organic mixtures have been assayed: M1 50% methanol–50% buffer, M2 25% isopropanol–25% methanol–50% buffer, M3 25% acetonitrile–75% buffer, and M4 10% ethyl acetate–25% methanol–65% buffer. Three polyclonal antisera and two haptens conjugated to horseradish peroxidase and alkaline phosphatase as enzyme tracers have been studied with each mixture. Although sensitivity is better in aqueous medium, good results have been obtained with the four organic mixtures tested, being best in M1, with a limit of detection of 0.15 $\mu g\,L^{-1}$ for the sensor employing peroxidase as label. Selectivity, expressed as cross-reactivity, and precision of the assays have been shown to be better in organic media than in aqueous one. More than 400 assay cycles can be run with the same immunosensor. Good recoveries have been obtained when the methanolic extracts of atrazine-spiked water and vegetables were analysed after solid phase extraction on C_{18} cartridges. These results show the potential of organic immunoassays.

Keywords: Organic solvents; Immunosensor; Pesticides; Water; Vegetables

INTRODUCTION

Immunoanalysis of organic chemicals has become common over recent years [1]. Development of haptens, antibodies and immunoassay formats for monitoring organic targets in environmental, food, clinical and agricultural areas is now a common research topic. Immunoassays are suitable methods when a large number of samples has to be screened for a low number of analytes. The application of immunochemistry in automated systems has led to the development of immunosensors, very useful for field and on-line monitoring [2].

Usually, immunochemical analyses are carried out in aqueous buffered media, since the antibody–antigen interactions involved are optimum in this environment. However, the employment of organic solvents in immunoassays is many times desirable. Solid samples such as food and soil must be extracted prior to analysis [3]. Even many

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aqueous samples, e.g., biological fluids, wastewater or aqueous extracts of solid samples, need a previous clean-up step prior to be analyzed, in order to eliminate non-specific interferences from species that influence the biological activity of the immunoreagents and enzymes employed in these assays [1]. In this sense, a study of the compatibility of organic solvents is usually carried out when developing and optimizing batch immunoassays. The most common result is that immunoassays tolerate low percentages (10–15%) of aqueous-miscible solvents, such as methanol, with loss of sensitivity in some cases [4] and gain in others [5], but larger amounts lead to non-acceptable results, although the immunochemical reaction still takes place, because tolerance of immunoreagents to these media is limited and recognition of the analyte is worse [6]. Nevertheless, there is a recent interest concerning the application of organic solvents to immunoassays, enhanced by the fact that standard methods of analysis of organic compounds employ well-established and validated sample preparation techniques, whose application in immunoanalysis would accelerate its validation. As an example, Stöcklein *et al.* [7] detected triazine and phenylurea pesticides by enzyme-linked immunosorbent assay in pure solvents, with detection limits below $0.2\,\mu g\,L^{-1}$ in hexane. The employment of immunosensors is an attractive approach for the application of immunochemistry to organic extracts. In immunosensing, all the basic operations of the protocol are carried out in a very controlled manner, so that the effect of organic solvents on immunoreagents can be controlled more accurately. Working with immunosensors, unlike batch immunoassays, the time contact between the immunoreagents and the sample matrix is usually low, especially in flow systems, thus minimizing the deleterious influence of organic solvents in assay performance. These two aspects have led to a recent interest in applying organic solvents in immunosensor studies, as it is reflected in some works [8,9] and overviews [10,11] dealing with this topic. Within this tendency, a general study about the influence of organic solvents on the properties of immunosensors working under different conditions (type of antibody, assay format, immobilization support) has been performed [12], and the main conclusions of this study have allowed to develop immunosensors applied to the determination of the algaecide Irgarol 1051 in seawater [13], 1-naphthol in river water [14], and carbaryl in fresh and processed vegetables [15].

In this article, the behaviour and performances, in terms of sensitivity and selectivity, of an immunosensor for atrazine working in organic media is studied, employing a set of three polyclonal antibodies and two competition haptens combined with two enzyme labels. The results are compared with those previously obtained by using the same reagents in aqueous buffer [16].

Atrazine has been one of the most widely herbicides used in crop protection. It has a moderate solubility in water ($70\,mg\,L^{-1}$), high persistence in the environment, as well as chemical stability for hydrolysis and decomposition. All this implies that atrazine is a polluting pesticide not only for foods but also for underground, surface and sea waters [17].

On the other hand, the high solubility of atrazine in organic solvents (e.g. $18\,g\,L^{-1}$ in methanol) makes very suitable the use of liquid–liquid extraction or solid-phase extraction (SPE), employing solvents such as methanol or ethyl acetate to isolate atrazine from aqueous matrices prior to chromatographic analysis [18–20]. It is therefore interesting to develop an immunosensor able to work in organic solvents, so as to apply the established extraction procedures to a sensitive, selective and rapid screening and quantitation methodology such as immunosensing.

The immunosensor works under a competitive capture assay format, in which the competition between the analyte, an enzyme tracer and the antibody takes place in solution, the immunocomplexes being further captured by immobilized protein A/G. This assay format shows higher sensitivity and operational lifetime than immobilized-antibody or immobilized-hapten formats. The immunosensor optimized for working in organic mixtures is applied to the analysis of atrazine in water and vegetables after SPE.

EXPERIMENTAL

Chemicals and Biochemicals

Standards of atrazine (Fig. 1) and other *s*-triazine derivatives were obtained from Dr. Ehrenstorfer (Augsburg, Germany), Ciba-Geigy (Barcelona, Spain) and Riedel de Häen (Seelze, Hannover, Germany). Stock solutions were prepared in *N,N′*-dimethylformamide and kept at −20°C until use. Alkaline phosphatase (AP), bovine serum albumin (BSA), 3-(*p*-hydroxyphenyl)-propanoic acid (HPPA) and 3,3′,5,5′-tetramethylbenzidine (TMB) were from Sigma (St. Louis, MO). Horseradish peroxidase (HRP) was purchased from Boehringer Mannheim (Mannheim, Germany). Ultralink Immobilized Protein A/G was from Pierce (Rockford, IL), and the 1,2-dioxetane luminogenic substrate (di-sodium 2-chloro-5-(4-methoxyspiro{1,2 dioxetane-3,2′-8,5′chloro)-trichloro[3.3.1.1]decane}-1-phenyl phosphate[3,7]) CDP-Star, was from Tropix (Bedford, MA). All other reagents were analytical grade. The solutions employed were vacuum-filtered through a 0.22 μm Durapore membrane filter (Millipore, Madrid, Spain).

Antisera R-10, R-11 and R-12, as freeze-dried powder, and 2b and 2d haptens (Fig. 1), were kindly given by Drs. M.P. Marco and D. Barceló. These immunoreagents have been previously characterized by ELISA [21] and employed in the development of aqueous immunosensors [16]. The conjugation of haptens to enzymes was carried out using the mixed anhydride method [22].

A 0.02 M sodium phosphate buffer pH 8.0 solution (PB) was used working with HRP label and TBS buffer (21 mM tris(hydroxymethyl)aminomethane, 138 mM NaCl, 27 mM KCl, 0.02% (w/v) NaN_3, and HCl to pH 8.0) was used for AP assays. The dissociation agent was 0.1 M glycine–HC1 buffer, pH 2.0.

In order to enhance immunoreagents stability [23], 10 mg/mL BSA was added to antibody solutions, while HRP tracers were prepared in PB containing 0.06 mM TMB.

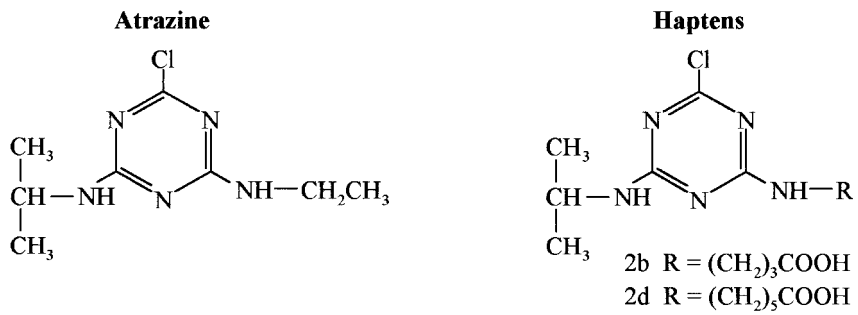

FIGURE 1 Structures of atrazine and the haptens employed in this work.

The organic media assayed were: 50% methanol-50% buffer (Ml), 25% isopropanol–25% methanol–50% buffer (M2), 25% acetonitrile–75% buffer (M3), and 10% ethyl acetate–25% methanol–65% buffer (M4). These mixtures have shown to be the best performing in a previous work [12].

System Design

The manifold employed has been described elsewhere [16]. Briefly, it consists of a 2.5 mL syringe pump directly connected to a distribution valve system with 15 ports (Kloehn Ltd., Las Vegas, NV), both components being automatically managed by a computer program (Winpump, Kloehn). The protein A/G surface is confined into a packed-bed tubular reactor, 4 mm length, 4 mm inner diameter, between a valve port and the detector. The detector for HRP activity was a Turner model 450 fluorometer (Biomolecular, Inc., Reno, NV) equipped with suitable filters at λ_{ex} 320 nm, λ_{em} 405 nm and a 15 μL Hellma flow cell. The luminescence detector for AP was a TD-20e luminometer (Turner Designs, Sunnyvale, CA) equipped with a spiral-shaped glass homemade flow cell (inner diameter 1 mm, spiral outer diameter 3 cm). Signals were registered on-line using Chrom-Card Manager software package (Fisons Instruments, Rodano, Italy). Sigmaplot 2.0 (Jandel Scientific, San Rafael, CA) was used for data treatment.

Assay Protocol

An analysis cycle includes the steps of competition, immunocomplex capture, signal display and sensor regeneration, all basic operations being carried out automatically [16]. The competition is performed by on line mixing 200 μL of antibody solution with 200 μL of hapten-enzyme conjugate solution and 800 μL of standard or sample (in buffer or the mixture under study). The mixture is incubated for 1 min while the mixing process is being carried out. 1 mL of the mixture is then injected at 0.25 mL min^{-1} through the reactor, where the immunocomplexes are captured by the protein A/G support. After washing with buffer (3 mL at 2 mL min^{-1}), the activity of the enzyme retained on the protein A/G surface is measured by injecting the substrate (on-line prepared mixture of 0.8 g L^{-1} HPPA and 0.012% (v/v) H_2O_2 solutions in PB for HRP; 0.075 mM 1,2-dioxetane solution in 0.1 M dietanolamine–HCl buffer containing 1 mM $MgCl_2$ for AP), followed by incubation (3 min) and injection of buffer (1.5 mL at 2 mL min^{-1}) so as to carry the reaction product to the detector. Finally, the sensor regeneration is accomplished by injecting 2.5 mL of dissociation buffer at 0.5 mL min^{-1} and a further washing cycle (3 mL at 2 mL min^{-1}). The total assay time is around 20 min for the HRP sensor and 19 min for the AP one, the difference being in the additional substrate mixing step performed for HRP.

Sample Treatment

Bottled drinking water was obtained from a local market. Lake and sea water samples were collected from Albufera Lake (Valencia, Spain) and Valencia and Castellón (Spain) seaports, respectively. Agricultural wastewater was collected from Acequia de Vera (Valencia, Spain). All water samples were split into portions, spiked with atrazine at different levels and filtered through a Whatman No. 1 filter paper in order to remove

suspended matter. SPE on C_{18} Sep-pack cartridges (Waters, Milford, MA) was then performed as follows: the cartridge was activated with methanol (2 mL) followed by distilled water (3 mL). Then, a 10 mL aliquot of the water sample was flushed through the cartridge, which was washed with 5 mL of distilled water and allowed to dry by flushing air. Bound species were eluted with 2 mL of methanol. All the processes were performed at 7 mL min^{-1} flow rate, except for the elution that was carried out at 5 mL min^{-1} Finally, the methanolic eluate was mixed with 2 mL of PB and the mixture directly introduced in the immunosensor. For samples to be analyzed by the AP-based sensor, the extraction procedure was carried out with acetonitrile instead of methanol, and the final extract was mixed with 6 mL of TBS.

Fresh broccoli, green bean, tomato, celery, watermelon and lettuce were commercial samples. All vegetables were blended and split into portions, spiked with atrazine at different levels and slowly stirred for 12 h. Aliquots of each sample (5 g) were extracted with 25 mL of distilled water and gentle stirring for 30 min. The mixture was vacuum-filtered through a G-3 glass filter, washing the residual solid with additional 15 mL of water that was added to the filtrate. The whole final aqueous extract was cleaned-up by SPE, employing the same cartridges and protocol as for water samples, and the final organic eluate (methanol or acetonitrile) was diluted with the corresponding buffer (PB or TBS) and analyzed by the immunosensor.

RESULTS AND DISCUSSION

System Performance

Competitive calibration was performed using atrazine standards prepared in both aqueous buffer and the studied organic mixtures, and the application of each pair antibody-tracer was optimized for sensitivity. Signals measured were normalized to the maximal signal obtained in each case in absence of analyte, and experimental points were fitted to a four-parameter logistic equation. For each mixture assayed, all the possible combinations antibody-tracer were tested, employing different concentration ranges, which depended on the signals obtained, in each case. The selection of the optimal conditions was performed on the basis on obtaining a good signal (higher than 25% of the whole scale of the detector) and maximal sensitivity. The best immunoreagent combinations and their respective concentrations (to be used in the assay protocol described in Experimental Section) are shown in Table I.

Figure 2 shows the optimal calibration curves for each medium assayed, including aqueous. The sensitivity of the competition in logistic curves is expressed as the parameter I_{50}, analyte concentration that inhibits the binding of the tracer to the antibody at 50%. The I_{50} values and the limits of detection LD, defined as the analyte concentration that generates a normalized signal of 90%, are shown in Table I. As it was expected from the preliminary works and data found in the literature, sensitivity reached in organic media is worse than that of the aqueous medium, since the I_{50} values measured are higher by a factor of 40 or more. However, the possibility of employing preconcentration of the analyte by SPE and the immunosensor working in organic media, allows to measure atrazine at a level lower than those achieved employing directly the immunosensor in aqueous medium, since a preconcentration factor of 200 or higher can be reached.

TABLE I Optimized sensitivity of the immunosensors for both aqueous and organic mixtures

	*Fluorimetric HRP detection**					*Luminometric AP detection*†*				
Medium	PB‡	M1	M2	M3	M4	TBS	M1	M2	M3	M4
Antibody (mg/L)	R-11 2	R-10 10	R-10 10	R-11 5	R-10 30	R-10 0.5	R-10 0.3	R-10 1.5	R-10 1.5	R-10 2.0
Tracer (mg/L)	2b-HRP 0.20	2d-HRP 1.00	2d-HRP 1.00	2d-HRP 0.50	2b-HRP 3.00	2d-AP 0.25	2d-AP 0.15	2d-AP 0.50	2d-AP 0.75	2d-AP 0.50
I_{50} (μg/L)¶	0.05	1.9	19.0	9.4	16.0	0.97	24.1	82.1	12.9	53.0
LD (μg/L)§	0.01	0.15	1.68	0.72	2.70	0.04	0.76	4.03	0.69	6.66

PB phosphate buffer; TBS tris buffer saline; M1 50% methanol–50% buffer; M2 25% isopropanol–25% methanol–50% buffer; M3 25% acetonitrile–75% buffer; M4 10% ethyl acetate–25% methanol–65% buffer; *Mixtures employing PB as buffer; †Mixtures employing TBS as buffer; ‡Data from [16]; ¶I_{50} analyte concentration that inhibits 50% of the binding of the tracer; §LD limit of detection, analyte concentration that inhibits 10% of the binding of the tracer.

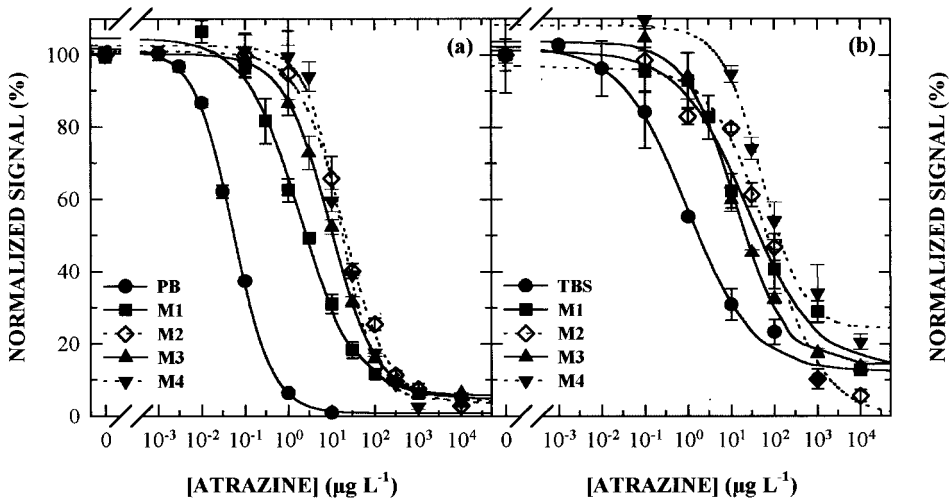

FIGURE 2 Optimized competition curves obtained in buffer and organic mixtures. (a) Employing HRP as label and fluorometric detection. (b) Employing AP as label and luminometric detection. Immunoreagents and their concentrations according to Table I.

The behaviour of immunoreagents in organic media was different from that in pure buffer. In PB [16], the combination R-11/2b-HRP is the best in sensitivity, with the I_{50} value being only half of that achieved with the other two antisera (that perform better with 2d hapten). In organic media, R-10 and R-11 antisera give similar results, being R-10 slightly better in most cases. Serum R-12 was the worse with I_{50} 2–10 fold higher than for R-10 and R-11. Also, 2d hapten works much better than 2b one, that only functions properly conjugated to HRP in M4. It is clear that the presence of solvents in the competition medium influences the binding properties of the antibodies, which are very susceptible to small conformational changes in the structures of the analyte, the competition hapten and the antibody itself. This susceptibility is also viewed when working with free and immobilized antibodies, and even when changing the immobilization support [24].

Great differences can also be observed when comparing data obtained with HRP and those achieved with AP. Immunoreagents concentration necessary for obtaining

acceptable signals is lower when employing AP and luminescence detection, which is reasonable since the 1,2-dioxetane substrates for AP show a very high yield [25], and luminometry is more sensitive than fluorometry. Also, the organic media require higher concentrations of antibody and tracer than the aqueous one, but the difference in concentrations is lower when working with AP (Table I), which means that this enzyme is less susceptible to denaturation by organic solvents. It is worth mentioning that the best organic mixture is different for HRP (50% methanol) and for AP (25% acetonitrile), which can also be explained on the basis on a difference in the influence of organic solvents on both enzymes, as well as on the substrate and the detection system used. Nevertheless, sensitivity is worse for AP immunosensor in all media. All this indicates that the label employed has strong influence in the final performance of the assay.

The reproducibility of the methods was tested by measuring an atrazine standard at concentration near the I_{50} along several days. The results showed that precision is better for fluorimetric immunosensors (RSD for normalized signals lower than 3.5% in all the mixtures, $n=8$) than for luminometric ones (RSD between 4 and 10%, $n=8$), which can be due to a higher background noise in the luminometer baseline. It should be noted that, for HRP immunosensors, reproducibility was better in organic media (RSD lower than 3% in all cases) than in aqueous one (RSD = 3.5%). This means that the presence of organic solvents enhances the repeatability of the competition process.

The long-term stability of the immunosensors was very good. Working with standards in organic media, a single protein A/G reactor could be employed for more than 400 assay cycles in the fluorometric immunosensor, and for more than 500 cycles in the luminometric one. This difference can be due to the presence of a preservative (0.02% (w/v) NaN_3) in TBS, that cannot be employed with HRP since it deactivates the enzyme, and that can avoid the bacterial growth on the support. The operational lifetime of the immunosurface is higher when working in aqueous buffer (600 assays) [16], because the organic solvents cause damage in the protein A/G and in the flow properties of the support (copolymer bis-acrylamide–azlactone). However, the reusability is very good in all cases, much higher than those achieved employing antibodies covalently bound to the support [24]. Regarding the working life of immunoreagents solutions, in previous works [16] it was stated that the addition of BSA to antibody and tracer solutions enhanced the stability of these reagents, but it was observed that the tracer solution needed to be replaced after four days, since this stabilization was little effective for enzymes. It was found in the literature that HRP could be stabilized by adding a substrate different from the one to be employed in the sensor, such as TMB [23]. Indeed, the addition of 0.06 mM of this substrate enlarged the life of HRP tracer solutions, that could be employed for more than a week without loss of absolute signal. In addition, the autonomy (ability for working without the presence of an operator) of the immunosensor is one week for the HRP-based ones and four days for those working with AP as label.

Selectivity

The selectivity of the immunosensor was studied by performing calibration curves with a series of compounds structurally related to atrazine (two atrazine metabolites and other *s*-triazine pesticides), determining their I_{50} and calculating the cross-reactivity,

TABLE II Cross-reactivity percentages obtained with the organic mixtures studied

	*Fluorimetric HRP detection**					*Luminometric AP detection*†				
Mixture	PB‡	M1	M2	M3	M4	TBS	Ml	M2	M3	M4
Atrazine	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Ametryn	11.9	0.9	< 1.1	2.1	7.2	106.0	6.7	16.0	1.1	2.3
Deethylatrazine	1.0	0.3	0.8	1.0	< 0.2	1.7	3.7	2.1	1.6	0.9
Deisopropylatrazine	< 0.1	0.4	< 0.7	< 0.4	< 0.3	1.1	2.5	3.3	1.0	2.3
Cyanazine	0.2	< 0.4	< 1.0	< 0.5	1.5	2.2	31.9	8.3	3.2	3.0
Irgarol 1051	1.0	0.2	< 1.0	< 0.5	< 0.5	62.3	48.3	3.2	2.6	1.3
Prometryn	4.5	0.3	4.4	3.3	9.5	17.2	21.2	9.6	2.2	1.8
Propazine	127.0	16.8	43.9	74.9	35.1	140.0	263.0	8.5	52.5	186.6
Simazine	11.9	1.4	0.6	0.9	0.3	0.9	0.2	3.3	0.5	1.9
Terbumeton	–	< 0.1	< 0.5	6.2	0.1	1.6	2.3	3.1	0.4	4.0
Terbuthylazine	18.8	1.1	3.9	5.2	3.6	7.6	15.1	33.5	2.5	3.0
Terbutryn	–	< 0.1	< 0.3	< 0.1	< 0.2	0.9	18.6	2.8	0.3	1.0

PB phosphate buffer; TBS tris buffer saline; M1 50% methanol–50% buffer; M2 25% isopropanol–25% methanol–50% buffer; M3 25% acetonitrile–75% buffer; M4 10% ethyl acetate–25% methanol–65% buffer. For each organic mixture, the best performing immunoreagents and concentrations, shown in Table I, are used. *Mixtures employing PB as buffer; †Mixtures employing TBS as buffer. ‡Data from [16].

expressed as the percent ratio between the I_{50} for the analyte and I_{50} for the interferent. Table II shows the cross-reactivity obtained with all the mixtures studied using the best performing immunoreagents combinations. In general, selectivity is better in organic mixtures than in buffer, which has also been observed in the literature [10,12], and the selectivity improvement is better for HRP-based immunosensors. This behaviour is probably due to conformational changes in interfering substances induced by the presence of the organic solvent in the assay medium. The improvement in selectivity allows use of the immunosensor in a particular organic mixture for the analysis of atrazine in order to avoid a potential interference from a related compound such as propazine.

Application to Water and Vegetable Samples

The immunosensors working in the best performing organic mixture (M1 for HRP-based sensor and M3 for AP-based one) were used for the analysis of atrazine in spiked water and vegetable samples, after the extraction procedures described in the Experimental Section.

The analysis of samples using the AP-based immunosensor was performed employing agricultural wastewater and tomato, treated as described above but eluted with acetonitrile so as to employ the immunosensor working in M3 mixture (the most sensitive for AP immunosensor). Results (data not shown) indicated that this immunosensor is acceptable as screening tool from a qualitative point of view, since recoveries were unacceptable in all measurements, although contaminated samples gave positive signals, and no false positives were found.

The application of the HRP-based immunosensor achieved better results, which are shown in Table III. Recoveries are good (between 80 and 125%) for all water samples, which indicates the suitability of using the immunosensor working in M1 for the analysis of water samples that show troubles if analyzed directly by an immunochemical method, such as wastewater with high contents of organic matter, or even seawater. The SPE procedure for water samples is rapid and easily automated by employing a

TABLE III Results of the analyses of atrazine-spiked samples employing the HRP-based immunosensor in M1 mixture

Sample no.	*[Atrazine] added*	*[Atrazine] found**					
		Drinking water	Agricultural wastewater	Albufera lake water	Valencia seawater	Castellón seawater	
0	0.0	< LD†	< LD	< LD	< LD	< LD	
1	0.4	0.36 ± 0.10	0.40 ± 0.02	0.46 ± 0.08	0.35 ± 0.04	0.35 ± 0.09	
2	0.8	0.70 ± 0.01	0.67 ± 0.04	0.68 ± 0.09	0.67 ± 0.07	0.80 ± 0.10	
3	1.6	1.98 ± 0.14	1.70 ± 0.30	1.90 ± 0.30	1.74 ± 0.08	1.80 ± 0.20	
4	3.2	3.30 ± 0.30	3.14 ± 0.14	3.70 ± 0.30	3.40 ± 0.40	3.30 ± 0.20	
		Broccoli	Green bean	Tomato	Celery	Watermelon	Lettuce
0	0.0	< LD	< LD	< LD	< LD	< LD	< LD
1	0.8	1.10 ± 0.20	0.50 ± 0.05	1.00 ± 0.30	0.89 ± 0.02	1.01 ± 0.17	1.20 ± 0.30
2	1.6	1.80 ± 0.40	1.92 ± 0.15	1.55 ± 0.09	1.30 ± 0.30	1.80 ± 0.30	1.60 ± 0.60
3	3.2	3.70 ± 0.80	3.45 ± 0.12	4.30 ± 0.80	2.40 ± 0.80	2.10 ± 0.50	4.30 ± 0.50
4	6.4	5.80 ± 0.80	7.50 ± 0.60	7.18 ± 0.01	7.00 ± 0.60	5.80 ± 1.40	7.20 ± 0.20

M1 50% methanol–50% buffer, [Atrazine] in μg L^{-1} (water) and μg kg^{-1} (vegetables). *Mean ± *SD* of four replicates; †LD limit of detection.

syringe pump system analogous to that used in the immunosensor. Furthermore, volumes higher than 10 mL can be processed, thus achieving a good preconcentration factor and enhancing the sensitivity of the method.

The analysis of vegetable samples by water extraction, clean-up in C_{18} cartridges and immunosensing produced acceptable recovery values (between 60 and 140%), showing that this methodology is appropriate for a rapid semi-quantitative screening of atrazine in vegetables, that can be followed by confirmation by chromatographic methods, performed on the final eluate, for samples giving results indicating an unacceptable contamination. The method is more rapid, clean and easy to perform that the application of multiresidue extraction methods, usually based on liquid–liquid extraction, rotavaporation and solvent change. The only drawback is that water extraction is only applicable to pollutants showing good solubility in water such as atrazine, and cannot be employed for very hydrophobic pesticides, for which an alternative extraction method should be tested.

CONCLUSIONS

The possibility of immunosensing determination of atrazine in four mixtures containing high percentages of organic solvents has been demonstrated. The mixture 50% methanol–50% buffer has shown to be the best performing, although the employment of other solvents such as isopropanol, acetonitrile and ethyl acetate has also led to acceptable results. The assay sensitivity achieved is lower than those obtained with immunosensors applied to aqueous buffer, but it can be improved by the analyte preconcentration accomplished in organic solvent extraction process. The assay selectivity is improved when applying the immunosensor to organic mixtures, which allows to measure the analyte in presence of habitual cross-reacting substances with lower interference. The sensor working in 50% methanol can applied to the screening and analysis of atrazine in water and vegetable samples after rapid extraction procedures.

The performances of the sensors using HRP as label and fluorimetric detection are better than those of sensors employing AP and luminometric detection. However, the use of AP and a luminometer is interesting since this detection system is simpler and cheaper, and allows to develop a smaller and more compact immunosensor system to be employed in field conditions.

Finally, the development of immunosensors able to detect the analyte in samples containing high percentages (50%) of organic solvents will allow the validation of the immunochemical technology for environmental and food analysis employing the established sample treatment procedures.

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