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# Fast and simultaneous monitoring of organic pollutants in a drinking water treatment plant by a multi-analyte biosensor followed by LC–MS validation

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**Abstract**

This work describes the application of an optical biosensor (RIver ANALyser, RIANA) to the simultaneous analysis of three relevant environmental organic pollutants, namely, the pesticides atrazine and isoproturon and the estrogen estrone, in real water samples. This biosensor is based on an indirect inhibition immunoassay which takes place at a chemically modified optical transducer chip. The spatially resolved modification of the transducer surface allows the simultaneous determination of selected target analytes by means of "total internal reflection fluorescence" (TIRF).

The performance of the immunosensor method developed was evaluated against a well accepted traditional method based on solid-phase extraction followed by liquid chromatography–mass spectrometry (LC–MS). The chromatographic method was superior in terms of linearity, sensitivity and accuracy, and the biosensor method in terms of repeatability, speed, cost and automation. The application of both methods in parallel to determine the occurrence and removal of atrazine, isoproturon and estrone throughout the treatment process (sand filtration, ozonation, activated carbon filtration and chlorination) in a waterworks showed an overestimation of results in the case of the biosensor, which was partially attributed to matrix and cross-reactivity effects, in spite of the addition of ovalbumin to the sample to minimize matrix interferences. Based on the comparative performance of both techniques, the biosensor emerges as a suitable tool for fast, simple and automated screening of water pollutants without sample pretreatment. To the author's knowledge, this is the first description of the application of the biosensor RIANA in the multi-analyte configuration to the regular monitoring of pollutants in a waterworks.


*Keywords:* Immunosensor; TIRF; Multi-analyte; Pesticides; Estrogens; Water; Waterworks

## 1. Introduction

The increasing number of pollutants in the environment calls for extensive monitoring programs. The requirements, both in terms of time and costs, of most traditional analytical methods (e.g. chromatographic methods) often constitute an important impediment for their application in these programs. Biosensors appear in this context as a suitable alternative or as a complementary analytical tool that can be applied for screening of samples providing fast and specific data of contaminated sites. They offer several advantages over current analytical methods, such as the possibility of portability and the ability of measuring pollutants in complex matrices directly or after minimal sample preparation, which may be particular useful for field applications. Moreover, the late development of sensors capable to determine several analytes simultaneously, offers an extra advantage for the implementation of such techniques in environmental monitoring. These multi-analyte devices allow a further reduction in time, sample volume and reagents required. Multichannel performance [1], planar array biosensors [2–4], ordered array capillary [5] and spatially resolved surface modifications [6] are examples of sensor designs developed in recent years, that enable the nearly real-time parallel monitoring of multiple species by means of separate immunoreactions. This work describes the application of the RIver ANAlyzer (RIANA), an optical multi-analyte immunosensor, to the simultaneous determination of various analytes in natural and treated waters in a waterworks treatment plant.

The behaviour and removal efficiency of target contaminants during drinking water production needs to be assessed in most

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water production companies, especially when surface water is treated. The quality of the raw water is, in this case, extremely important. Since it is impossible to monitor every substance that may be present in the surface water, it is necessary to define major targets of interest for water resources protection by focusing on substances that might be able to enter the drinking water supply. In this work, three selected analytes, namely, atrazine, isoproturon and estrone, were monitored throughout the different purification stages at a waterworks and the removal efficiency of each stage was assessed. Both atrazine and the phenylurea isoproturon belong to the group of pesticides included in the list of 33 priority hazardous substances or groups of substances of major concern in European waters to be monitored under the Water Framework Directive (WFD, 2000/60/EC). Estrone is a natural estrogen of growing environmental significance due to its endocrine disruption potency, whose presence in water, along with that of some other estrogens is likely to be regulated in the future.

To date, most of the literature available on biosensors reports on their use in either distilled water or buffer solutions [7]. In the last years, however, more examples of biosensors applied to real samples have appeared [8]. The application of biosensors to real samples is the late necessary step in the development of new devices. Biosensors results must also be validated by comparison with those obtained with standard protocols in order to get the acceptance of end users. This work describes the application of an optical immunosensor to the simultaneous analysis of three relevant environmental organic pollutants (atrazine, isoproturon and estrone) in real water samples and evaluates its performance in comparison with a well accepted traditional chromatographic method. This work follows a series of other articles published by the authors on the development and validation of the optical immunosensor RIANA [9–12] and represents the first description of the application of the biosensor to the monitoring of selected pollutants in the multi-analyte configuration in a waterworks.

## 2. Experimental

### 2.1. Chemicals

Potassium hydroxide, potassium dihydrogen phosphate, sodium chloride, hydrochloric acid, sodium dodecyl sulphate (SDS) and HPLC-grade water, acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Fluorescent Cy5.5-dye and bifunctional reactive *N*-hydroxysuccinimide (NHS) ester were obtained from Amersham Life Science (Braunschweig, Germany). Di-isopropylcabodiimide (DIC) and Ovalbumin (OVA) were purchased from Sigma (Deisenhofen, Germany). Pesticide standards atrazine and isoproturon were obtained from Riedel-de-Haen (Seelze, FRG). Deuterated $d_5$-atrazine was purchased from Cambridge Isotopes (Cambridge, UK) Deuterated $d_4$-equilin was kindly provided by Dr. Lee Ferguson (Marine Sciences Research Centre, State University of New York, USA).

Lyophilised anti-estrone, anti-isoproturon and anti-atrazine antibodies and the analyte derivatives used in this study were kindly supplied by Dr. Ram Abuknesha (King's College London, UK).

Phosphate buffered saline (PBS) was prepared in bidistilled water at a concentration of 150 mM sodium chloride and 10 mM mono-potassium phosphate, and adjusted with a potassium hydroxide solution to pH 7.4. A stock standard solution of the target analytes was prepared in methanol at 1000 mg/l. Two sets of working standard solutions were then prepared at various concentrations by appropriate dilution of the stock solution both with methanol and PBS for LC–MS and RIANA determination, respectively. Final aqueous standard solutions did not contain more than 0.1% of methanol.

### 2.2. Sampling and sample preparation

In the Sant Joan Despí waterworks, the water of the Llobregat River is processed through several treatment steps, including prechlorination, rapid sand filtration, ozonation, granular activated carbon (GAC) filtration and post-chlorination, to produce drinking water for the Barcelona's metropolitan area. The waterworks is located in the south of Barcelona, close to the mouth of the river Llobregat, which, at this point, presents an important load of organic matter due to the various domestic and industrial discharges that take place upstream. Due to the diverse sources of pollution, a broad spectrum of organic chemicals including hydrocarbons, pesticides, surfactants, plasticizers, etc. can be found at high levels in the river water [8,13–16].

Groundwater from the Llobregat aquifer is also frequently used to produce drinking water, mixed with the river water that has already undergone prechlorination and sand filtration. Blending of the river water with groundwater leads to a better water quality and also to a decrease in the levels of the trihalomethanes (THM) formed during the prechlorination step.

Samples of the raw river water, and the water obtained after each treatment step in the waterworks were collected in precleaned amber glass bottles and transported to the laboratory at 4 °C. Water was filtered through 0.45 μm HVLP filters (Millipore Bedford, MA, USA) to eliminate particulate matter and other suspended solid matter, and subsequently stored at 4 °C in the dark. Samples were processed within 24 h of collection to avoid microbial degradation.

### 2.3. Biosensor analysis

#### 2.3.1. Preparation of the transducer

*2.3.1.1. Spatially resolved surface modification.* One key step in the development of biosensors is the immobilization of the biological component at the transducer surface. The immobilization procures both the stabilization of the biomaterial and the proximity between the biomaterial and the transducer. In the present immunosensor, aminodextran derivatives of the target analytes were covalently bound on three separated areas on the transducer glass slide following the procedure described by Barzen et al. [17]. Briefly, a glass interference layer of 58 mm × 10 mm × 1.5 mm with 45° bevel on short side was cleaned by immersion in a freshly prepared hot mixture of concentrated $H_2SO_4/H_2O_2$ 2:1 for 30 min,

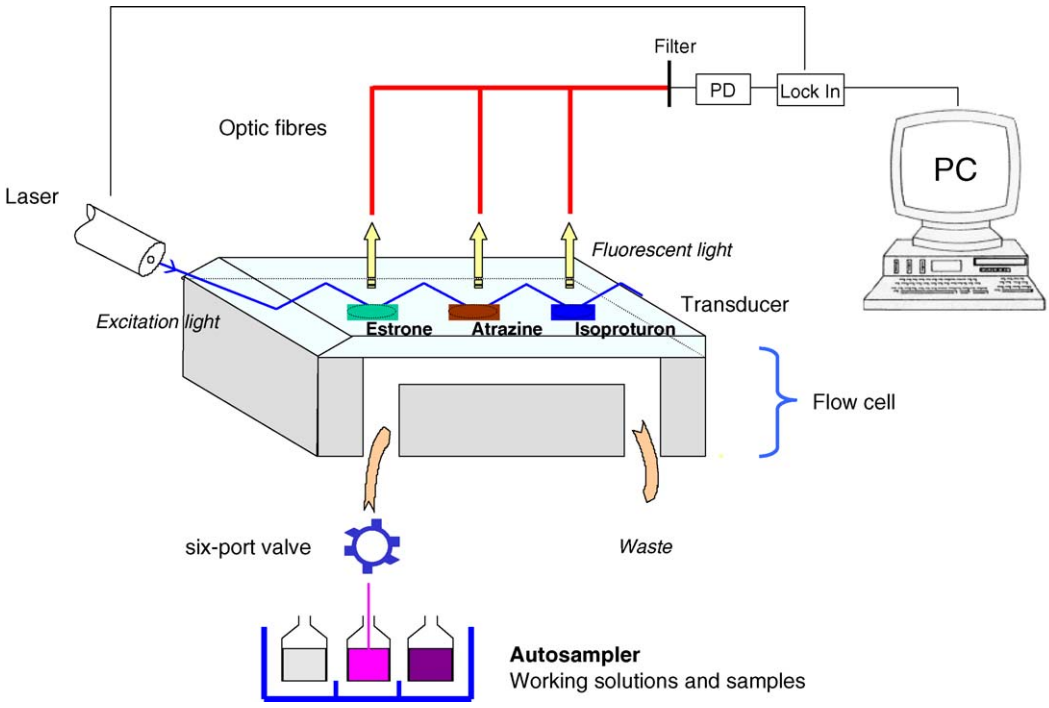


Fig. 1. Scheme of the immunosensor RIANA system configured for multi-analyte determination.

rinsed with water afterwards and dried at room temperature. Silanisation of the surface was achieved by treatment with 3-glycidoxypropyltrimethoxysilanxysilane (GOPTs) for not more than 1 h. Thereafter, it was rinsed with acetone and dried in a nitrogen stream. Conjugates of the analyte derivatives with aminodextran (aminodextran-atrazine, -estrone and -isoproturon derivatives) were then placed in separated areas of the activated transducer surface allowing the reaction to take place. Such multi-analyte transducer with predefined detection spots permits the performance of simultaneous multi-analyte measurements.

### 2.3.2. Biosensor set-up

A scheme of the RIANA immunosensor is presented in Fig. 1. The flow-injection system (FIA) equipped with a six-port valve and a 1 ml syringe pump delivers the buffer solution, the sample or the regeneration solutions to the flow-cell where the multi-analyte transducer is mounted. Light from a collimated and modulated He–Ne laser source (633 nm, 7 mW) is directly coupled and guided through the length of the transducer by total internal reflection (TIRF). An evanescent field is produced at each reflection spot and penetrates a few hundreds of nanometres into the external medium, provoking the excitation of the fluorescently labelled antibodies, bound in each specific area. The fluorescence light is subsequently collected and conduced separately through optic fibres, filtered and detected by photodiodes using lock-in detection. As a result, three independent measurements are obtained simultaneously. To deliver the samples to the FIA system, an autosampler AS90/91 from Perkin-Elmer is used. Fluid handling and data acquisition is fully automated and computer controlled.

### 2.3.3. Performance of measurements

Previous to the analysis, lyophilised anti-estrone, anti-isoproturon and anti-atrazine antibodies (labelled with the fluorescent dye Cy5.5) are reconstituted with phosphate buffer in a single solution containing the mixture of the antibodies. Thereafter, to perform the binding measurements, 0.9 ml of the standard solutions, as well as the unknown samples, are mixed in 1 ml Eppendorf tubes with ovalbumin (200 μg/ml) and with the antibodies (labelled with Cy5.5) at a final concentration of 0.35, 0.33 and 0.05 μg/ml for anti-estrone, anti-isoproturon and anti-atrazine, respectively. After incubation for at least 15 min, to reach the binding equilibrium between analytes molecules and antibodies, the solution is delivered into the flow-cell by the flow-injection system. Only unbound antibodies are then able to bind to the corresponding analyte derivatives covalently bound on the surface. During this process, the laser is switched off to avoid photobleaching of the dyes. After automatic washing with buffer, the laser is turned on, and the fluorescence signal is recorded. The difference in the signals obtained before and after the binding is then measured to obtain an estimation of the number of molecules bound to the transducer layer. Increasing analyte concentrations lead to a decrease in the fluorescence signal because the number of free antibodies able to bind to the surface is diminished. After each measurement, rinsing with SDS solution (0.5 g/100 ml, pH 1.9) achieves regeneration of the transducer, which allows the performance of a new run. The total analysis time for a single measuring cycle is 15 min.

Calibration curves are built by measuring standard solutions of the analytes at different concentrations. All fluorescent signals are normalised with the signal corresponding to a blank (maximum fluorescence). Average values (measurements made

in triplicate) obtained for the calibration standards are fitted to a logistic function to plot the corresponding calibration curve. Real water samples containing unknown concentrations of the analytes are processed in the same way as standard solutions, once that the ionic strength and the pH of the samples have been adjusted to those of the standards by adding appropriated amounts of a phosphate buffer solution. A common analytical sequence consists of nine "blank" (analyte-free) measurements, nine calibration standards (minimum of five) from 0 to 100 μg/l, and the samples to analyse. After incubation and mix, samples are randomly placed in the autosampler and analysed automatically.

### 2.4. Chromatographic analysis

As previously indicated, the performance of the biosensor was evaluated against an already validated, more traditional method based on solid-phase extraction (SPE) followed by LC–MS [8]. This method includes as target analytes, in addition to those considered in the present study, the hormones estradiol-17-glucuronide, estrone-3-sulfate, estriol, estradiol, ethynyl estradiol, diethylstilbestrol and estradiol diacetate, the pesticides desethylatrazine, simazine and diuron, and bisphenol A.

#### 2.4.1. Sample preconcentration

Preconcentration of the samples was carried out using an automated solid-phase extraction sample processor ASPEC XL (Automated Sample Preparation with Extraction Columns), fitted with a 817 switching valve and an external 306 LC pump for selection and dispensing of samples, respectively, all from Gilson (Villiers-le-Bel, France). Water samples (500 ml), previously filtered through 0.45 μm glass fibre filters, were preconcentrated on LiChrolut RP-18 cartridges (500 mg, 3 ml) from Merck (Darmstadt, Germany). The cartridges were first conditioned with 5 ml of methanol and 5 ml of water, at a flow rate of 4 ml/min. After loading of the sample at a rate of 5 ml/min, the cartridges were dried using a Baker LSE 12G apparatus (J.T. Baker, Deventer, Netherlands) connected to a vacuum system set at −15 psi. Elution was performed by passing a total volume of 8 ml of methanol, which was dispensed in two steps (2 × 4 ml) with a 5 min delay between them. The extracts were evaporated to dryness under a stream of $N_2$ and reconstituted to a final volume of 300 μl with methanol containing a fixed concentration of 0.1 μg/ml of the internal standards $d_5$-atrazine and $d_4$-equilin, used for the quantification of pesticides and estrogens, respectively.

#### 2.4.2. LC–MS analysis

The HPLC system consisted of an HP 1100 autosampler with the volume injection set to 20 μl and a HP 1090A LC pump, both from Hewlett Packard (Palo Alto, CA, USA). Chromatographic separation was achieved in a reversed-phase C18 analytical column (LiChrospher 100 RP-18, 250 mm × 4 mm, 5 μm particle diameter) preceded by a guard column (4 mm × 4 mm, 5 μm) of the same packing material from Merck (Darmstadt, Germany). Gradient elution was performed as follows: from 10% acetonitrile and 90% HPLC water to 100% acetonitrile in 30 min and back to the initial conditions in 10 min. Flow rate was set at 1 ml/min.

Liquid chromatography-atmospheric pressure chemical ionisation-mass spectrometry (LC-APCI-MS) in the positive ion mode of operation was used for the determination of pesticides and liquid chromatography-electrospray-mass spectrometry (LC-ESI-MS) in the negative ionisation mode was used for the determination of estrogens, both in the selected ion monitoring (SIM) mode. MS experimental conditions are described in detail in [8]. For quantitation, six-point calibration curves were constructed. Standard solutions of the analytes in methanol where prepared at concentrations ranging from 10 to 2000 ng/ml (equivalent to 5–1000 ng/l in water, considering the concentration factor of the method, which is 2000).

## 3. Results and discussion

### 3.1. Analytical performance of the multi-analyte biosensor

#### 3.1.1. Matrix effect

The interactions between antibodies, analytes and analyte derivatives can be affected by the pH, the ionic strength or matrix substances. Dissolved organic carbon, for example, can interact weakly with the antibodies inducing an overestimation of the immunoassay response [17]. Previous studies [9,10,12] indicate that the present biosensor can be successfully applied to the analysis of most natural water samples, while its applications to more complex matrices, like wastewaters, seem to be importantly restricted. Nevertheless, interferences can be removed by applying appropriate clean-up procedures or by employing simple pretreatment methods. In this assay, the background protein ovalbumin was added to real samples as an immolation protein to saturate any non-specific binding site [6,18,19]. Thus, the probability of the antibody to adhere to passive particles or non-specific binding sites is minimised.

#### 3.1.2. Cross-reactivity

The antibodies can react with compounds structurally similar to the analytes also present in the sample and lead to the determination of wrong concentrations of the target substances (cross-reactivity). The selectivity of the antibodies used in this work towards the target analytes in the presence of potential cross-reactants has been previously investigated [10,12]. According with these studies, cross-reactivity values of diuron, linuron and chlortoluron in the determination of isoproturon were 92.8, 53.4 and 46.3%, respectively, whereas, deisopropylatrazine, simazine and desethylatrazine exhibited, respectively, 18.7, 19 and 42.8% of cross-reactivity in the determination of atrazine.

On the other hand, the simultaneous presence of the three analytes and their corresponding antibodies on the same assay has been shown not to interfere in the analysis (except for an insignificant, non-specific binding of the isoproturon antibodies in the atrazine and estrone spots) [9]. Fig. 2 shows, as an example, the calibration curves obtained for atrazine when using an individual solution of anti-atrazine antibodies and when using a mixture of the three antibodies. As shown in the figure, data resulting from both approaches did not differ significantly, being

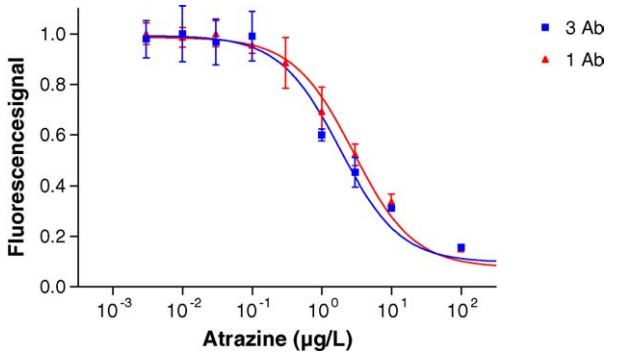


Fig. 2. Standard calibration curves of atrazine after incubation with anti-atrazine antibodies and with a mixture of anti-estrone, anti-atrazine and anti-isoproturon antibodies.

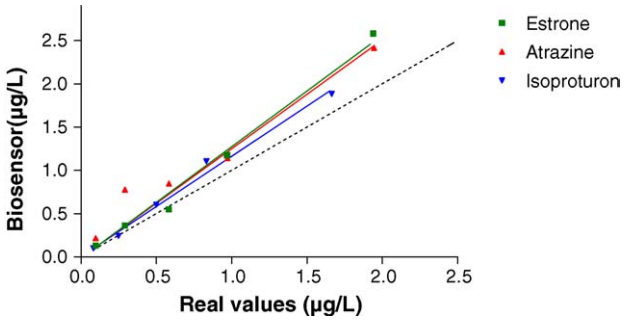


Fig. 3. Correlation between real and experimental values obtained with the immunosensor in the analysis of spiked river water. Regression equations were: (a) estrone $y = 1.346x - 0.084$, $r^2 = 0.990$; (b) atrazine $y = 1.100x + 0.223$, $r^2 = 0.965$; (c) isoproturon $y = 1.153x + 0.020$, $r^2 = 0.989$.

the inhibition concentrations at 50% of absorbance ($IC_{50}$ values) in the same range.

### 3.1.3. Performance parameters

The biosensor performance was evaluated through estimation of the linearity, repeatability, accuracy and sensitivity of the method. Table 1 summarises the analytical parameters obtained for the analysis of the three target compounds with the immunosensor. For comparison purposes, these parameters are presented in parallel with those obtained by the SPE-LC–MS analysis.

Both methods were shown to be linear, with correlation coefficients over the studied concentration ranges higher than 0.99 for all compounds except for atrazine when measured with the biosensor ($r^2 = 0.979$).

As regards sensitivity, limits of detection achieved with the biosensor (calculated at 90% of maximal absorbance) were somewhat higher than those obtained by SPE-LC–MS (experimentally estimated from the analysis of spiked groundwater samples as the minimum concentration of an analyte giving a signal-to-noise ratio of 3), but still close to the levels of concern: 0.1 ug/l in the case of isoproturon and atrazine (as set in the EU drinking water directive 2000/60/EC as maximum admissible concentration for individual pesticides), and around 0.01–0.1 ug/l in the case of estrone (lowest concentration at which this compound is estimated to exert estrogenic effects in fish) [20,21].

In terms of accuracy, the SPE-LC–MS method used for validation of the biosensor system was found to be very satisfactory, with recovery percentages, calculated from the replicate ($n = 6$) analysis of groundwater spiked with estrone at 0.05 μg/l and the pesticides atrazine and isoproturon at 0.1 μg/l, between 94 and 100%. In the case of the biosensor, a slight overestimation of results can be expected from the recovery percentages obtained: between 117 and 175%. The accuracy of the immunosensor method was assessed by comparing the results obtained from the analysis of river water samples spiked with varying concentrations of the target analytes (from 0.1 to 2 μg/l) and the known spiking levels. The recovery percentages listed in Table 1 correspond to the average recovery of those calculated at each spiking level. As it can be seen in Fig. 3, good agreement was, in general, observed between the experimental and the real values for all target compounds (correlation coefficients ranging between 0.989 and 0.990), although, as it is common in other immunoanalytical assays [22,23], the calculated concentrations were slightly higher than theoretical ones.

The repeatability of the biosensor, calculated as the relative standard deviation (R.S.D.) of the triplicate analysis of a blank, was quite satisfactory, and slightly better than that of the SPE-LC–MS method.

Main advantages of the biosensor method developed are time saving, automation and high throughput analysis. The samples, once mixed with the antibodies solution, can be analysed completely automated and unattended. On the other hand, being the overall analysis time per sample 15 min, up to 25 samples, together with nine standards and nine blanks (all in triplicate) can be analysed per day. From the economical point of view, the biosensor presents also some advantages. Thus, typical expenses of SPE methods, such as the nitrogen consumed in the concentration of the extracts or the eluting solvents, are avoided. In

Table 1
Analytical quality parameters corresponding to the biosensor and LC–MS analysis of the target analytes: linear correlation coefficients, working range, limit of detection (LOD); accuracy (expressed as recovery percentage) and relative standard deviation (R.S.D.; $n = 6$ for SPE-LC–MS and $n = 3$ for immunosensor measurements)

| Analytical parameters | Estrone | | Atrazine | | Isoproturon | |
|---|---|---|---|---|---|---|
| | Biosensor | LC–MS | Biosensor | LC–MS | Biosensor | LC–MS |
| $r^2$ | 0.992 | 0.997 | 0.979 | 0.996 | 0.998 | 0.999 |
| Working range (μg/l) | 0.170–10.7 | 0.005–1 | 0.350–1.47 | 0.005–1 | 0.110–2.83 | 0.005–1 |
| LOD (μg/l) | 0.080 | 0.002 | 0.160 | 0.003 | 0.050 | 0.005 |
| Accuracy (%) | 122 | 100 | 175 | 94 | 117 | 99 |
| R.S.D. (%) | 7 | 5 | 1 | 15 | 3 | 12 |

Table 2
Levels of pesticides and estrogens (in μg/l) detected at the various water treatment stages in the Sant Joan Despí waterworks, as determined by the biosensor (individual compounds concentrations) and the SPE-LC–MS method (sum of concentrations within a chemical class)

| Samples | Estrogens | | | | Triazines | | | | | Phenyl-ureas | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | RIANA | LC–MS | | | RIANA | LC–MS | | | | RIANA | LC–MS | | |
| | | E1 | E1-sulf | Total estrogens | | DEA | Simazine | Atrazine | Total triazines | | Isoproturon | Diuron | Total phenylureas |
| May | | | | | | | | | | | | | |
| River water | 0.579 | 0.004 | 0.004 | 0.008 | 0.392 | 0.002 | 0.157 | 0.005 | 0.164 | 0.620 | 0.016 | 0.076 | 0.092 |
| Sand filtration | 0.509 | 0.005 | | 0.005 | 0.348 | 0.002 | 0.047 | 0.008 | 0.057 | 0.515 | 0.014 | 0.055 | 0.069 |
| Ozonation | 0.124 | | | | bld | 0.002 | 0.067 | 0.004 | 0.073 | | | | |
| GAC filtration | nd | | | | | bld | 0.026 | 0.002 | 0.028 | | | | |
| Drinking water | 0.132 | | | | bld | | 0.015 | 0.001 | 0.016 | 0.158 | | | |
| June | | | | | | | | | | | | | |
| River water | 0.124 | 0.0060 | 0.004 | 0.010 | 0.395 | 0.002 | 0.093 | 0.463 | 0.558 | 4.116 | 0.025 | 0.070 | 0.095 |
| Sand filtration | 0.112 | | | | 0.454 | 0.002 | 0.089 | 0.008 | 0.099 | 0.730 | | 0.050 | 0.050 |
| Ozonation | 0.145 | | | | 0.980 | 0.002 | 0.077 | 0.007 | 0.086 | 0.771 | | 0.011 | 0.011 |
| GAC filtration | | | | | bld | bld | 0.005 | bld | 0.005 | | | | |
| Drinking water | 0.667 | | | | 0.739 | | 0.005 | bld | 0.005 | 2.408 | | | |
| July | | | | | | | | | | | | | |
| River water | 0.265 | bdl | | | 0.171 | bld | 0.076 | 0.005 | 0.081 | 0.214 | 0.005 | 0.239 | 0.244 |
| Sand filtration | 0.307 | | | | 0.481 | bld | 0.097 | 0.009 | 0.106 | 0.117 | | 0.256 | 0.256 |
| Ozonation | 0.599 | | | | 0.577 | bld | 0.049 | 0.003 | 0.052 | 0.228 | | bld | |
| GAC filtration | | | | | | | 0.008 | | 0.008 | | | | |
| Drinking water | 0.101 | | | | | | 0.013 | bld | 0.013 | | | | |
| August | | | | | | | | | | | | | |
| River water | 0.175 | 0.0070 | bld | 0.007 | 1.347 | 0.004 | 0.207 | 0.012 | 0.223 | 0.705 | | | |
| Sand filtration | 0.408 | | | | 1.873 | | 0.082 | 0.005 | 0.087 | 0.428 | 0.118 | | 0.118 |
| Ozonation | 0.178 | | | | 0.673 | | | 0.006 | 0.006 | 0.492 | 0.068 | | 0.068 |
| GAC filtration | | | | | | | 0.011 | | 0.011 | | | | |
| Drinking water | 0.081 | | | | bld | | 0.011 | | 0.011 | 0.237 | | | |

Abbreviations: E1, estrone; E1-sulf, estrone-3-sulfate; DEA, desethylatrazine; GAC, granular activated carbon (GAC); bdl: below detection limit; blank fields mean not detected.

addition, the RIANA system requires very low maintenance and the operation is quite simple.

Also, regeneration of the sensing layer, which is accomplished through washing of the transducer with the SDS solution to completely remove the antibodies from the surface after each measurement, allows for its continuous, long use. The transducer employed in this work exhibited a long operational time allowing the performance of at least 300 measurements properly.

### 3.2. Application to real samples

As previously mentioned, in the development of new devices, the demonstration of their proper performance in the analysis of real samples and their subsequent validation through the comparison of results with those obtained by other accepted protocols, is the late necessary step to get the acceptance of end users.

In this work, the performance of the biosensor was evaluated through its application to the analysis of the target analytes in various different types of water collected at the Sant Joan Despí waterworks and validated against the described SPE-LC–MS method applied in parallel to the analysis of the same samples. Both methods were thus used to simultaneously monitor the presence of the target compounds and to check the removal efficiency of the various water treatments applied along the purification process. The results of this monitoring, which was performed in four consecutive months in year 2002, are shown in Table 2.

In all cases, the results obtained with the biosensor were higher than expected from the recovery percentages calculated for the immunosensor (121–145%) and higher also than those obtained with the chromatographic method. The overestimation of results, which is common to many immunoanalytical techniques, is normally attributed to matrix effects, cross-reactivity effects, or both. The existence of matrix effects cannot be ruled out in this case, provided that the plant is located in the last stretch of the river, close to its mouth, where the river has already received a large input of numerous domestic and industrial discharges. Thus, high amounts of unknown compounds can be present in the samples and may bind to the antibodies, in addition to the target analytes, leading to an overestimation of the real analyte concentrations. The observed overestimation of results, however, does not seem to follow a common pattern, in the sense that the more complex samples (raw river water and the waters collected after the first treatment steps) do not always present the largest overestimation of results, as it could be expected from their complexity. In this respect, it is, for instance, remarkable the high values, interpreted as false positives, found with the RIANA for all the compounds tested,

in comparison with the LC–MS method, in the drinking water samples obtained after post-chlorination. This might be, in part, due to the formation of halogenated derivatives or halogenated degradation products of the target compounds [24] with, eventually, binding capacities to the corresponding antibodies. Another explanation could be the effect of the hypochlorite on the antibody and/or on the fluorescent compound (bleaching) that results in lower signal values. Moreover, as previously pointed out, the determination of atrazine suffers from a high percentage of cross-reactivity in the presence of desethylatrazine (40%) and, to a less extent, of simazine (18%), whereas other triazines, not considered in the cross-reactivity study and not detected either by the LC–MS method, such as, for instance, metribuzine, which has large application as herbicide in the Llobregat river, may also be present in the samples and contribute to the elevated concentrations calculated with the biosensor for atrazine. The same explanation can be inferred in the case of isoproturon, whose determination by the biosensor may also be affected by the presence of other phenylureas, such as diuron (cross-reactivity 92.8%, also determined by the LC–MS method), or linuron and chlorotoluron (not considered in the LC–MS method and with 53.4 and 46.3% of cross-reactivity, respectively). Both diuron and linuron are among the herbicides most used in agriculture in the Llobregat area. Likewise, cross-reactivity effects might also be taking place in the case of estrone, provided that structurally similar compounds, such as estrone-3-sulfate, as determined by the LC–MS method, or other glucuronide or sulfate conjugates, not investigated here, may also be present in the samples.

The presence of cross-reactivity effects cannot always be assessed as a drawback. In fact, this feature is quite often exploited in immunoassays to determine class-related compounds all together. Thus, even though this biosensor technique cannot compete with standard chromatographic methods in terms of accuracy, it may represent a very useful tool for the rapid screening not only of the targeted compounds but also of unknown, structurally related compounds.

As regards the fate of the selected analytes in the waterworks, the results obtained by both techniques indicate that the compounds investigated are, in general, gradually eliminated through the various water treatments applied. In this respect, it is remarkable the removal attained through the activated carbon filtration step, observed both by the biosensor and the LC–MS method.

Finally, it may be worth mentioning that, based on the multianalyte detection capabilities of the present biosensor, a new, more advanced immunosensor device, called AWACSS, has been recently developed [25]. This new biosensor, which shares the most notable advantages of the RIANA in terms of speed, low-cost, automation, etc., allows the determination of up to 32 compounds simultaneously, the selection of which depends on the particular interests of the end users, the nature of the water pollution or the legislation requirements, provided that the corresponding antibodies are available. The recently adopted Decision No. 2455/2001/EC [26], which amends Directive 2000/60/EC [27], has established a list of 33 priority substances in the field of water policy, the third part of which are pesticides. These compounds cannot be determined in a single run by either LC or GC, whereas with an approach similar to that presented here and the newly developed biosensor, the analysis of these compounds could be performed in one unique run and in a very short period of time (one measurement cycle, including the regeneration step takes 15 min).

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## References

[1] C.E.H. Berger, T.A.M. Beumer, R.P.H. Kooyman, J. Greve, Anal. Chem. 70 (1998) 703.
[2] C.A. Rowe-Taitt, J.P. Golden, M.J. Feldstein, J.J. Cras, K.E. Hoffman, F.S. Ligler, Biosens. Bioelectron. 14 (2000) 785.
[3] C.A. Rowe-Taitt, J.W. Hazzard, K.E. Hoffman, J.J. Cras, J.P. Golden, F.S. Ligler, Biosens. Bioelectron. 15 (2000) 579.
[4] R.M. Wadkins, J.P. Golden, L.M. Pritsiolas, F.S. Ligler, Biosens. Bioelectron. 13 (1998) 407.
[5] C. Mastichiadis, S.E. Kakabakos, I. Christofidis, M.A. Koupparis, C. Willetts, K. Misiakos, Anal. Chem. 74 (2002) 6064.
[6] C. Barzen, A. Brecht, G. Gauglitz, Biosens. Bioelectron. 17 (2002) 289.
[7] S. Rodriguez-Mozaz, M.-P. Marco, M. Lopez de Alda, D. Barceló, Pure Appl. Chem. 76 (2004) 723.
[8] S. Rodriguez-Mozaz, M. Lopez de Alda, D. Barcelo, J. Chromatogr. A 1045 (2004) 85.
[9] S. Rodriguez-Mozaz, S. Reder, M. Lopez de Alda, G. Gauglitz, D. Barceló, Biosens. Bioelectron. 19 (2004) 633.
[10] E. Mallat, C. Barzen, A. Klotz, A. Brecht, G. Gauglitz, D. Barcelo, Environ. Sci. Technol. 33 (1999) 965.
[11] E. Mallat, C. Barzen, R. Abuknesha, G. Gauglitz, D. Barcelo, Anal. Chim. Acta 427 (2001) 165.
[12] E. Mallat, C. Barzen, R. Abuknesha, G. Gauglitz, D. Barcelo, Anal. Chim. Acta 426 (2001) 209.
[13] J. Rivera, J. Caixach, F. Ventura, I. Espadaler, Chemosphere 14 (1985) 395.
[14] P. Eichhorn, T.P. Knepper, F. Ventura, A. Diaz, Water Res. 36 (2002) 2179.
[15] M. Petrovic, A. Diaz, F. Ventura, D. Barcelo, Environ. Sci. Technol. 37 (2003) 4442.
[16] J. Quintana, I. Marti, F. Ventura, J. Chromatogr. A 938 (2001) 3.
[17] M.-C. Hennion, D. Barcelo, Anal. Chim. Acta 362 (1998) 3.
[18] M. Nakata, A. Fukushima, H. Ohkawa, Pest. Manage. Sci. 57 (2001) 269.
[19] J. Tschmelak, G. Proll, G. Gauglitz, Anal. Bioanal. Chem. 379 (2004) 1004.
[20] E.J. Routledge, D. Sheahan, C. Desbrow, G.C. Brighty, M. Waldock, J.P. Sumpter, Environ. Sci. Technol. 32 (1998) 1559.

[21] M. Petrovic, E. Eljarrat, M. Lopez de Alda, D. Barcelo, Anal. Bioanal. Chem. 378 (2004) 549.
[22] J. Gascon, G. Durand, D. Barcelo, Environ. Sci. Technol. 29 (1995) 1551.
[23] I. Toscano, J. Gascon, M.-P. Marco, J.C. Rocha, D. Barcelo, Analysis 26 (1998) 130.
[24] K. Moriyama, H. Matsufuji, M. Chino, M. Takeda, Chemosphere 55 (2004) 839.
[25] J. Tschmelak, G. Proll, J. Riedt, J. Kaiser, P. Kraemmer, L. Barzaga, J.S. Wilkinson, J.P. Hole, R. Nudd, M. Jackson, R. Abuknesha, D. Barcelo, S. Rodriguez-Mozaz, M.J. Lopez de Alda, F. Sacher, J. Stien, J. Slobodnik, P. Oswald, H. Kozmenko, E. Korenkova, L. Tothova, Z. Krascsenits, G. Gauglitz, Biosens. Bioelectron. 20 (2005) 1499.
[26] Council of the European Communities, Off J. Eur. Commun., 2001.
[27] Council of the European Communities, Off J. Eur. Commun., 2000.