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Monitoring of estrogens, pesticides and bisphenol A in natural waters and drinking water treatment plants by solid-phase extraction–liquid chromatography–mass spectrometry[☆]

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Abstract

A multi-residue analytical method has been developed for the determination of various classes of selected endocrine disruptors. This method allows the simultaneous extraction and quantification of different estrogens (estradiol, estrone, estriol, estradiol-17-glucuronide, estradiol diacetate, estrone-3-sulfate, ethynyl estradiol and diethylstilbestrol), pesticides (atrazine, simazine, desethylatrazine, isoproturon and diuron), and bisphenol A in natural waters. In the method developed, 500 ml of water are preconcentrated on LiChrolut RP-18 cartridges. Further analysis is carried out by liquid chromatography–mass spectrometry (LC–MS) using atmospheric pressure chemical ionisation (APCI) in the positive ion mode for determination of pesticides and electrospray in the negative ionisation mode for determination of estrogens and bisphenol A. Recoveries for most compounds were between 90 and 119%, except for bisphenol A (81%) and diethylstilbestrol (70%), with relative standard deviations below 20%. Limits of detection ranged between 2 and 15 ng/l. The method was used to study the occurrence of the selected pollutants in surface and groundwater used for abstraction of drinking water in a waterworks and to evaluate the removal efficiency of the different water treatments applied. Water samples from the river, the aquifer, and after each treatment stage (sand filtration, ozonation, activated carbon filtration and post-chlorination) were taken monthly from February to August of 2002. The presence in river water of atrazine, simazine, diuron and bisphenol A were relatively frequent at concentrations usually below 0.1 µg/l. Lower levels, below 0.02 µg/l, were usual for isoproturon. Estrone-3-sulfate and estrone were detected occasionally in the river. Most of the compounds were completely removed during the water treatment, especially after activated carbon filtration.
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1. Introduction

During the last decades, the ever-increasing number of organic compounds being detected in surface waters has risen concern about the contamination of water resources. One main reason for this is the use of surface waters as recipients for wastewater. Most effluents from cities and industrial plants, containing a large variety of pollutants, even after their treatment, end up in rivers, streams or lakes. As the removal of contaminants by the water treatments applied is frequently not complete, natural waters often contain many dissolved chemicals, which can affect ecosystems and impact drinking water supplies [1]. Since it is sometimes necessary to produce drinking water from polluted surface waters [2], the quality of the raw water is extremely important. Independently of the origin of the surface water, drinking water companies have to reduce the concentration of any contaminant as much as possible to supply hazardous-free drinking water to the consumers. Assessment of the behaviour of contaminants during drinking water production and prediction of their removal [3] are thus necessary in most water production companies, especially when surface water is treated. Because 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, a group of selected analytes was monitored throughout the different purification stages at a waterworks and the removal efficiency of each stage was assessed. Target analytes were selected on the basis of their presence in natural waters and their environmental significance and include compounds representative of different sources of contamination: pesticides (agricultural), estrogens (domestic) and plasticisers (industrial).

Pesticide contamination of surface waters and ground waters from agricultural use has been well documented around the world. Most pesticides enter the environment as diffuse contamination, following normal spraying in agricultural fields and further surface run-off. However, there is an unknown amount of pesticides that are used for non-agricultural purposes: on lawns [4], as algaecides in paints and coatings [5], or as root protection agents in flat roof sealing [6]. This situation has led the European Union to set regulatory directives for some pesticides. European regulations on drinking water quality set a maximum concentration of 0.1 µg/l for individual pesticides and some of their degradation products, and 0.5 µg/l for total pesticides present in a sample (European Union Drinking Water Directive, 98/83/EC). Nevertheless, the concentration of certain herbicides, such as atrazine, metolachlor, isoproturon and mecoprop, frequently exceed these levels [7,8]. The pesticides analysed in this work belong to two different chemical groups: triazines (atrazine, simazine and the desethylatrazine) and phenylureas (isoproturon and diuron). These compounds are largely used in Europe for both pre- and post-emergence control in a wide variety of crops. They represent nearly 40% of the herbicides used in European countries [9] and are frequently detected in surface and groundwater, especially in the southern rivers of Europe [10]. Except for desethylatrazine (main degradation product of atrazine), all the pesticides selected in this work are included in the list of 33 priority substances in the field of water policy established by the Water Framework Directive (WFD, 2000/60/EC). Some studies, however, recommend the addition of several triazines, including desethylatrazine, to a list of 38 priority pesticides [11].

On the other hand, the environmental presence of compounds with estrogenic properties has become a major subject of worldwide concern. It has been hypothesized that the statistically derived decrease in sperm counts over the last decades and the increasing incidence of testicular cancer and other disorders regarding male infertility may be caused by the intake of estrogens via food or drinking water [12]. As these chemicals have been shown to provoke endocrine disruption on certain fish at sub-ng/l levels [13,14], their determination requires high-sensitivity analytical methods. Contamination of soil and groundwater by estrogens can take place through application of digested sludge from municipal STP on agricultural fields. However, the principal pathway is through wastewaters, which after incomplete removal in the municipal STP are released into the receiving waters [15]. The estrogens selected in this work include natural estrogens (estradiol, estrone, estriol), conjugates (estradiol-17-glucuronide, estradiol diacetate, estrone-3-sulfate) and synthetic estrogens (ethynyl estradiol and dietylstilbestrol).

Bisphenol A (a chemical intermediate in the synthesis of polycarbonate and epoxy resins, unsaturated polyester-styrene resins and flame-retardants) has also been included in the present monitoring study due to its ubiquitous nature and its endocrine disrupting potential [1]. Likewise bisphenol A and estrogens, pesticides have been reported to exhibit endocrine-disrupting properties in a range of laboratory and field studies [16,17].

A single method for the analysis of such different classes of selected analytes would reduce the costs and time necessary for their routine analysis in many companies and institutions concerned with the water quality. To date, various multi-residue analytical methods for the environmental determination of pollutants have been described in the literature [8,18–25]. However, to our knowledge, the present work represents the first description of a multi-residue analytical method for the simultaneous determination of estrogens, pesticides and bisphenol A

2. Experimental

2.1. Chemicals

HPLC-grade water and acetonitrile were purchased from Merck (Darmstadt, Germany) and were used to prepare the mobile phase. HPLC-grade methanol was also purchased from Merck (Darmstadt, Germany) and was used to prepare standard solutions. The pesticide standards atrazine, simazine and desethylatrazine were from Novartis (Basel, Switzerland), diuron from Riedel-de Haën (Seelze, Germany) and isoproturon from Ehrenstorfer GmbH (Augsburg, Germany). Estrogens were purchased from Sigma–Aldrich (Steinheim, Germany). $[^2H_5]$ Atrazine (atrazine-d5) was supplied by Cambridge Isotopes (Cambridge, UK) and $[^2H_4]$ equilin (equilin-d4) was kindly provided by Dr. Lee Ferguson (Marine Sciences Research Centre, State University of New York, USA). For all analytes, stocks solutions were initially prepared at 1000 mg/l by dissolving 25 mg of each compound in 25 ml of methanol. Standard mixtures of the compounds were prepared in methanol at different concentrations to be used for calibrations and for the preparation of fortified samples.

2.2. Description of the site

2.2.1. Llobregat River

The Llobregat River comes from the north west of Catalonia (Spain) and flows into the Mediterranean Sea, close

to Barcelona. This is a densely inhabited area and thus urban wastewaters represent a significant input in the river. The Llobregat River has been also polluted by the effluents from different industries located along the basin. A significant number of wastewater treatment plants (WWTPs) have been thus set up along the river during the last decade for improvement of the water quality. The river can also receive surface run-off from agricultural areas in the last section of its basin, and especially from the rivers Cardener and Anoia, tributaries of Llobregat river. Due to the diverse sources of pollution, a broad spectrum of organic chemicals including hydrocarbons, pesticides, surfactants, plasticiser etc, can be found at high levels in the river water. The presence of salt mines along the river gives the Llobregat River its characteristic salinity.

The quality of the river water, on the other hand, can change in correlation to river flow. Medium pluviometry in the basin is 650 mm/year and the medium flow of the river is 20 m^3/s with variations that go up to 4000 m^3/s. Medium temperature is 16 °C, although it has occasionally reached 33 °C as a maximum and 0 °C as a minimum.

2.2.2. Waterworks

Near the mouth of the river, in Sant Joan Despi (a town located 15 km south of Barcelona), there is a waterworks, which is operated by the company "Aguas de Barcelona" (Barcelona's waters) (AGBAR). In this plant, the water of the Llobregat River (at a flow of about 5.5 m^3/s) is processed through several treatment steps, including prechlorination and pre-dioxichlorination (to minimize subproducts formation), sand filtration, ozonation, granular activated carbon (GAC) filtration and post chlorination, to produce drinking water. The waterworks supplies approximately 1/3 of the drinking water consumed in Barcelona (with a population of 3.2 million people). Groundwater from the Llobregat aquifer, mixed with river water filtered by sand, is frequently used to produce drinking water, when the water treatment plant is in downtime or the quality of the river water is low [26]. The blending with groundwater leads also to a decrease in the levels of the trihalomethanes (THMs) formed during prechlorination step.

2.3. Sampling and sample preparation

Samples of the raw river water, the aquifer, and the water obtained after each treatment step in the waterworks were collected monthly from February to August of 2002. Samples were collected in pre-cleaned amber glass bottles and transported to the laboratory under cool conditions. Upon arrival to the laboratory, the samples were filtered though 0.45 μm HVLP filters (Millipore Bedford, MA, USA) to eliminate particulate matter and other suspended solid matter, and then stored at 4 °C in the dark. Further extraction of the samples was carried out within 24 h of collection to keep microbial degradation to a minimum.

2.4. Instrumental conditions

2.4.1. Solid-phase extraction (SPE)

Preconcentration of the samples was performed using an automated solid-phase extraction (SPE) 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). Pre-filtered water samples (500 ml) were preconcentrated on LiChrolut RP-18 cartridges (500 mg, 3 ml) from Merck. 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 completely dried using a Baker LSE 12G apparatus (J.T. Baker, Deventer, The 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) with a 5 min delay between them. The extracts were then evaporated to dryness under a stream of N$_2$ and reconstituted to a final volume of 300 μl with methanol containing a fixed concentration (0.1 μg/l) of the internal standards d$_5$-atrazine (used for the quantification of pesticides) and d$_4$-equilin (used for the quantification of estrogens and bisphenol A).

2.4.2. Chromatographic and analysis conditions; LC–MS

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. 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 and Bisphenol A. The MS conditions were as follows: nebuliser pressure, 55 psi; capillary voltage, 3500 V; drying gas flow, 5 and 13 l/min, for APCI and ESI, respectively); drying gas temperature, 300 and 350 °C, for APCI and ESI, respectively; fragmentor, 90 and 110 V, for APCI and ESI, respectively; and vaporiser temperature 350 °C (for APCI). Chromatograms were recorded in both the scan mode (*m/z* 100–500, for identification purposes) and in the selected ion monitoring (SIM) mode (for quantification). Table 1 lists the base peaks selected for determination of the target analytes. For equilin-d$_4$ and atrazine-d$_5$, ions at *m/z* val-

Table 1
Main ions selected for detection in the SIM mode of the target compounds, mean recovery percentages, standard deviations (S.D.), limits of detections (LOD), and linear correlation coefficients (R^2)

Compound	m/z of main ions	Recovery (%)	S.D. (%), $n = 6$	LOD (ng/l)	R^2
Desethylatrazine	188 $[M + H]^+$	94	11	1.61	0.992
Simazine	202 $[M + H]^+$	96	11	2.55	0.995
Atrazine	216 $[M + H]^+$	94	15	2.79	0.996
Isoproturon	207 $[M + H]^+$	99	12	4.89	0.999
Diuron	233 $[M + H]^+$	99	14	10.95	0.999
Bisphenol A	227 $[M - H]^-$	81	8	6.30	0.999
Estradiol-17-glucuronide	447 $[M - H]^-$	100	6	2.24	0.999
Estrone-3-sulfate	349 $[M - H]^-$	119	4	0.53	0.999
Estriol	287 $[M - H]^-$	94	8	5.04	0.992
Estradiol	271 $[M - H]^-$	98	10	2.50	0.993
Ethynyl Estradiol	295 $[M - H]^-$	91	13	3.22	0.985
Estrone	269 $[M - H]^-$	100	5	2.50	0.997
Diethylstilbestrol	267 $[M - H]^-$	70	23	1.64	0.995
Estradiol diacetate	313 $[M - H]^-$	98	8	14.79	0.993

ues 271 $[M - H]^-$ and 221 $[M + H]^+$, respectively, were recorded.

2.5. Identification and quantification

Quantification of the analytes was performed by the internal standard method, based on peak areas, using atrazine-d$_5$ as internal standard for determination of the pesticides and equilin-d$_4$ as internal standard for determination of the estrogens and bisphenol A. Four- to six-point calibration curves were constructed from the analysis in the SIM mode of groundwater samples spiked with the standard mixture of the analytes at concentrations ranging between 0.005 and 1 μg/l.

Analyte identification was performed by comparing both, the retention time, and the MS spectrum of the sample peaks, with those of the standard solutions. Moreover, for confirmation of the results obtained in the monitoring study, the sample extracts (along with standards and blanks) were analysed by LC-tandem MS following the method developed by Diaz-Cruz et al. [27].

2.6. Quality parameters

For estimation of the accuracy and the repeatability of the method, six replicates of groundwater (500 ml) spiked with the analytes at a concentration of 0.1 μg/l for pesticides, 0.2 μg/l for bisphenol A, and 0.05 μg/l for estrogens, were processed through the whole analytical procedure. The method accuracy was calculated from the areas obtained in the analysis of the spiked samples as a percentage of those obtained in the analysis of a standard solution with an equivalent concentration. The within-day method repeatability was expressed as the relative standard deviation of the corresponding peak areas obtained in the same approach. Limits of detection (LODs) were defined as

the concentration of a compound giving a signal-to-noise ratio of 3.

3. Results

3.1. Quality parameters of the method

The performance of the method was evaluated through estimation of its recovery, repeatability, linearity, and sensitivity (see Table 1). Mean recoveries ($n = 6$) were between 91 and 100%, except for estrone-3-sulfate (119%), diethylstilbestrol (70%) and bisphenol A (81%). Repeatability of the method was considered satisfactory, with standard deviations varying from 5 to 15%, except for diethylstilbestrol (23%). The linearity was also good for all compounds with correlation coefficients always higher than 0.99 over the studied concentration range (0.005–1 μg/l). Method detection limits varied between 2 and 15 ng/l. These LODs are in general low enough as to allow the detection of the compounds investigated at the levels at which they present toxic or endocrine disrupting effects.

3.2. Monitoring study

3.2.1. General river quality parameters

Due to the large seasonal variations of rainfall conditions in Mediterranean areas, surface waters exhibit changing chemistry or composition. Most compounds concentrations decrease with increasing flow after dilution with rainwater. On the other hand, mean temperatures during spring and summer (22 and 26 °C) may activate organic compounds degradation, producing therefore a concentration decrease. In Table 2, river quality parameters during the monitoring study, including flow and temperature, are shown. Seasonal variation in the flux of water was not observed the days

Table 2
Physico-chemical parameters of the Llobregat River on the days of sampling

	River flow (m^3/s)	pH	Conductivity (μS/cm)	TOC (mg, C/l)	Temperature (°C)	Turbidity (NTU)
February	2.2	7.72	1644	Not analysed	10.2	12
April	4.7	8.24	1775	8	18.5	33
May	5.0	8.52	1718	6.2	22.6	48
June	2.2	7.91	2080	5.2	26.0	49
July	3.7	8.14	1636	5.6	26.5	26
August	5.0	8.01	1779	5.2	22.3	230

where samples were taken. However, a high increase of turbidity was observed in August probably due to latest storms. Medium value of pH was 8.09 with little variation between months. Medium value for conductivity (1772 μS/cm) was higher than typical surface waters conductivity, due mainly to the salt mines exploitation in headwaters.

3.2.2. Pesticides

3.2.2.1. Occurrence in river water.
Table 3 summarizes the concentrations found for pesticides in the river water and along the purification stages at the waterworks during the monitoring program. The pesticides studied were found

in the river at levels varying from the detection limits up to different maximum values depending on the month. The concentrations of herbicides in surface water are affected by the combination of different factors: the proximity of crop fields, the time elapsed between the pesticides application and the first subsequent rainfall event, and the degradation rate of the herbicides, among others [10,28,29]. In the present study, atrazine and simazine were the herbicides most frequently found in the Llobregat River. The concentration profile for simazine and atrazine along the monitoring period showed a maximum of concentration in April (2.218 μg/l) and June (0.463 μg/l), respectively. Similar, major inputs of triazines in surface waters in the spring, just

Table 3
Levels of pesticides (μg/l) along the purification process at the waterworks during the monitoring study

Month	Sample	Desethylatrazine	Simazine	Atrazine	Isoproturon	Diuron
February	River	bld	0.008	0.007	0.503	
	Sand	bld	0.009	0.007		
	Ozone	bld	0.012	0.007		
	Carbon		0.006	0.008		
	Drinking water		0.006	bld		
April	River	0.002	2.218	0.020	0.021	0.064
	Sand	bld	2.313	0.022	0.023	0.070
	Ozone	0.003	1.259	0.017	0.015	bld
	Carbon		0.060	0.028	bld	
	Drinking water		0.032	0.018		
May	River	0.002	0.157	0.005	0.016	0.076
	Sand	0.002	0.047	0.008	0.014	0.055
	Ozone	0.002	0.067	0.004		
	Carbon	bld	0.026	0.002		
	Drinking water		0.015	0.001		
June	River	0.002	0.093	0.463	0.025	0.070
	Sand	0.002	0.089	0.008		0.050
	Ozone	0.002	0.077	0.007		0.011
	Carbon	bld	0.005	bld		
	Drinking water		0.005	bld		
July	River	bld	0.076	0.005	0.005	0.239
	Sand	bld	0.097	0.009		0.256
	Ozone	bld	0.049	0.003		bld
	Carbon		0.008			
	Drinking water		0.013	bld		
August	River	0.004	0.207	0.012		
	Sand		0.082	0.005		0.118
	Ozone	bld		0.006		0.068
	Carbon	bld	0.011			
	Drinking water		0.011			

bld: below limit of detection.

after their application in crops, and declining values during the rest of the year have also been reported by other authors [30,31]. Desethylatrazine exhibited relatively uniform low concentrations, close to the limit of detection (0.002 μg/l), through the whole monitoring period. Its presence can be attributed to microbial degradation of the triazine herbicides in the soil samples and subsequent transport through the river in the dissolved phase [32]. In general, the levels of triazines found in the Llobregat River were of the same order as those reported by other authors in southern European rivers [32–34]. In northern European countries, triazines are found in surface waters at lower levels, from 0.01 to 0.1 μg/l [19,35]. Regarding phenylureas, the highest concentration of isoproturon in the Llobregat River was observed in February (0.503 μg/l) being the levels detected in the other months much lower (between 0.005 and 0.025 μg/l). Neal et al. [36] reported also high concentrations of isoproturon during the winter and early spring period, which reflects the normal practice of autumn applications followed by leaching during the winter. However, the application of isoproturon at the end of February (in winter cereals) has also been reported [28]. In the case of diuron, the maximum concentration was found in July (0.239), which is in accordance with other authors' findings [32]. The importance of episodic events in the occurrence of these herbicides classes in river water is an aspect to consider that has been also highlighted by several authors [37,38]. These events may relate to individual, intensive rainstorms covering a wide area or the occurrence of several smaller-scale events in different tributaries giving rise to pulses of herbicides within the main river as a result of the combination of surface and sub-surface through-flow [10]. In the southern countries of Europe, farms are small compared with those of northern Europe. Such a distribution considerably expands the variety of pesticides and/or application methods and periods that need to be considered, because each farmer will take his own decision [29]. This phenomenon may thus serve to explain, for instance, the isolated high value of simazine found in the river water in April.

3.2.2.2. Occurrence in groundwater. Pollution of ground waters by pesticides is associated with the leaching of the chemicals and its metabolites through the soil column [29]. In the present study, atrazine and simazine were the pesticides most frequently encountered in the groundwater samples analysed (see Table 4). The occurrence of these two compounds in the Llobregat aquifer has already been asserted by Quintana et al. [31]. As pointed out before, groundwater from the Llobregat aquifer is sometimes mixed with the river water (after sand filtration) in the process to produce drinking water. This was the case in February, when the groundwater contribution amounted 30% of the total raw water. As shown in Tables 3 and 4, the concentration of simazine in the aquifer was in this period higher (0.022 μg/l) than that in the river water (0.008 μg/l). This fact explains the otherwise surprising high level of this compound in the

Table 4
Summary of the analytes concentrations (μg/l) detected in groundwater during February–August 2002

	February	April	May	June	July	August
Isoproturon						
Diuron		0.004				
Desethylatrazine	0.003	0.003		0.002	0.002	0.002
Atrazine	0.009	0.014		0.007		0.008
Simazine	0.022	0.073	0.030	0.153		0.144
Bisphenol A	0.007	bld		0.005		

bld: below limit of detection.

water collected after the ozonization step (0.012 μg/l), once that the mixture of both river water and groundwater had taken place.

3.2.2.3. Levels throughout the waterworks treatment process. Granular activated carbon (GAC) is used in many waterworks plants for the removal of organic compounds. To improve its efficiency, the introduction of a previous ozonation step is recommended. Ozone is used to increase the biodegradability of dissolved organic carbon because it promotes the breaking of complex compounds into simpler molecules [39]. Ozonation not only converts refractory organic matter into biodegradable matter but also particulate organic matter into dissolved matter [40]. In the Sant Joan Despí waterworks, the ozone–carbon activated process follows the conventional treatment prechlorination and sand filtration steps. The greatest removal of pesticides, as the data in Table 3 show, is thus achieved through ozonation and GAC filtration. The combination of these two terciary treatments leads to the complete elimination of the two phenylureas and desethylatrazine, and renders concentrations of atrazine and simazine in the final drinking water well below the concentration limit of 0.1 μg/l established in the EU drinking water directive (98/83/EC). The main inconvenient of the activated carbon process is the material saturation, phenomenon that needs to be controlled regularly by the waterworks. This is generally accomplished by classical analytical methods, and in this respect, the present procedure may represent a useful tool since it allows the monitoring of various representative compounds along the process.

3.2.3. Estrogens
Based on human daily excretion and other physicochemical parameters such as dilution factor, sorption to solid matter, etc., and the observations made by other authors, estrogens are expected to be present in aqueous environmental samples at the ng/l level [41]. Of the various estrogens investigated in this monitoring study, only estrone and its conjugate estrone-3-sulfate were detected, and solely in river water samples (see Table 5). Levels found for estrone varied between the limit of detection (2.50 ng/l, in July and August) and 21.7 ng/l (in February). Estrone-3-sulfate, on the other hand, appeared at a relatively constant level (around 6 ng/l), although it was not

Table 5
Levels of estrogens (µg/l) detected in river water during February–August 2002

	February	April	May	June	July	August
Estrone	0.022	0.008	0.004	0.004	bld	bld
Estrone-3-sulfate	0.007	bld	0.004	0.006		0.007

bld: below limit of detection.

detected in July. These levels are in agreement with those reported by other authors. To cite a few examples, estrone has been shown to be present in, for instance, some German rivers [15] at concentrations between 0.7 and 1.6 ng/l, and in the Thames river at concentrations ranging between 0.2 and 17 ng/l [42]. Natural steroids are excreted mainly in the urine, usually as glucuronide or sulfate conjugates, and a small amount in the faeces, as "free" estrogens [43]. Accordingly, conjugated estrogens (such estrone-3-sulfate) would be expected to be present in the aquatic environment at higher concentrations than "free" estrogens (such as estrone). However, in many monitoring studies, such as the present one, this ratio is often inverted. An explanation for this is the fact that the less active conjugated forms can be deconjugated during wastewater treatment and in the environment thus generating the parent compound. Other potential sources of estrone are partial biochemical oxidation of estradiol and cleavage of glucuronide conjugates of both estradiol and estrone, mechanisms that would also explained the absence of these other compounds. The total absence of estrogens in the water samples collected after the various treatment steps in the waterworks proves the satisfactory removal efficiency of the procedure applied. However, according with the studies conducted by other authors, this is not always the case: some estrogens have been detected, for instance, in groundwater and drinking water in Germany [44,45]. The groundwater samples investigated in this study were also found to be free of estrogens. Because of their physico-chemical properties, estrogenic compounds tend to adsorb to the aquatic sediments, thus minimizing leaching through the subsoil into the aquifer [46].

3.2.4. Bisphenol A

Bisphenol A was detected in all the surface water samples analysed (see Table 6). The highest concentration was found in February (0.295 µg/l). These levels are in agree-

Table 6
Levels of bisphenol A (µg/l) detected along the purification process at the waterworks during February–August 2002

	February	April	May	June	July	August
River	0.295	0.113	0.162	0.065	0.086	0.232
Sand	0.017	0.046	0.006	0.014	0.013	
Ozone	0.016	bld	0.006	0.012		
Carbon	0.007		0.006	bld		
Drinking water	0.005					

bld: below limit of detection.

ment with those reported in the literature for other rivers [47,48]. Within the waterworks, the concentration of bisphenol A decreased progressively as the water treatment process progressed. In this respect, it was remarkable the reduction of bisphenol A concentration achieved through the sand filtration step. In the final drinking water, this compound was only present in one of the samples investigated (February) but at a level below the quantification limit. Quite low levels were also found in two of the groundwater samples analysed (see Table 4): 0.007 and 0.006 µg/l in February and June, respectively.

4. Conclusions

A multiresidue analytical method based on SPE–HPLC–MS has been developed for the simultaneous determination of some representative estrogens, pesticides, and bisphenol A in environmental waters. This method has been used to monitor the occurrence of the target analytes in the river water and the ground water serving as source for abstraction of drinking water in a Spanish waterworks, as well as to study the removal efficiency of the various water treatments applied in the plant. With the purpose of investigating possible trends in the seasonal distribution of the selected pollutants, the collection of the various types of water samples was performed monthly for a six-month period.

Results indicate that there is a relationship between the observed river water pollution by pesticides and the agricultural practices of the area, as regards to seasonal application. By contrast, the content of the various pollutants detected in the groundwater (bisphenol A, and all the target pesticides except isoproturon) did not change significantly during the period of study. These findings indicate that pollution of the groundwater is rather persistent and that the degradation rate in this medium is very slow.

The results obtained for the various treated waters analysed also confirm the suitability of the water treatment process applied in the waterworks investigated: all the monitored compounds are either completely eliminated or, as in the case of simazine and triazine, removed to a level below the maximum legislated value of 0.1 µg/l in the final drinking water.

References

[1] G.R. Boyd, H. Reemtsma, D.A. Grimm, S. Mitra, Sci. Total Environ. 311 (2003) 135.

[2] T. Heberer, D. Feldmann, K. Reddersen, H.-J. Altmann, T. Zimmermann, Acta Hydrochim. Hydrobiol. 30 (2002) 24.

[3] T.P. Knepper, F. Sacher, F.T. Lange, H.J. Brauch, F. Karrenbrock, O. Roerden, K. Lindner, Waste Manag. 19 (1999) 77.

[4] W.H. Ahrens, Herbicide Handbook, Champaign, IL, 1994.

[5] K. Martinez, I. Ferrer, D. Barcelo, J. Chromatogr. A 879 (2000) 27.

[6] T.D. Bucheli, F.C. Gruebler, S.R. Mueller, R.P. Schwarzenbach, Anal. Chem. 69 (1997) 1569.

[7] A.C. Gerecke, M. Scharer, H.P. Singer, S.R. Muller, R.P. Schwarzenbach, M. Sagesser, U. Ochsenbein, G. Popow, Chemosphere 48 (2002) 307.

[8] G.R. van der Hoff, P. van Zoonen, J. Chromatogr. A 843 (1999) 301.

[9] R. Faasen, Eur. Water Pollut. Control 5 (1995) 34.

[10] W.A. House, D. Leach, J.L.A. Long, P. Cranwell, C. Smith, L. Bharwaj, A. Meharg, G. Ryland, D.O. Orr, J. Wright, Sci. Total Environ. 194–195 (1997) 357.

[11] L. Patty, A. Bruchet, E. Jaskulke, F. Acobas, F. Van Hoof, F. Sacher, I. Bobeldijk, F. Ventura, M.H. Marecos do Mote, TSM Hydrologie-Hydraulique 9 (1998) 24.

[12] R.M. Sharpe, N.E. Skakkebaek, Lancet 341 (1993) 1392.

[13] C.E. Purdom, P.A. Hardiman, V.J. Bye, N.C. Eno, C.R. Tyler, J.P. Sumpter, Chem. Ecol. 8 (1994) 275.

[14] P.-D. Hansen, H. Dizer, B. Hock, A. Marx, J. Sherry, M. McMaster, C. Blaise, Trends Anal. Chem. 17 (1998) 448.

[15] T.A. Ternes, M. Stumpf, J. Mueller, K. Haberer, R.-D. Wilken, M. Servos, Sci. Total Environ. 225 (1999) 81.

[16] T. Colborn, F.S. Vom Saal, A.M. Soto, Environ. Health Perspect. 101 (1993) 378.

[17] Endocrine-Disrupting Substances in the Environment: What Should Be Done? UK Environment Agency: Consultative Report, January 1998.

[18] H. Sabik, R. Jeannot, B. Rondeau, J. Chromatogr. A 885 (2000) 217.

[19] S. Ollers, H.P. Singer, P. Fassler, S.R. Muller, J. Chromatogr. A 911 (2001) 225.

[20] D. Loffler, T.A. Ternes, J. Chromatogr. A 1021 (2003) 133.

[21] N. Masque, R.M. Marce, F. Borrull, J. Chromatogr. A 793 (1998) 257.

[22] A. Di Corcia, M. Nazzari, R. Rao, R. Samperi, E. Sebastiani, J. Chromatogr. A 878 (2000) 87.

[23] K.M. Moore, S.R. Jones, C. James, Water Res. 29 (1995) 1225.

[24] D. Volmer, K. Levsen, G. Wunsch, J. Chromatogr. A 660 (1994) 231.

[25] M. Petrovic, A. Diaz, F. Ventura, D. Barcelo, Anal. Chem. 73 (2001) 5886.

[26] I. Marti, F. Ventura, J. Chromatogr. A 786 (1997) 135.

[27] M.S. Diaz-Cruz, M.J. Lopez de Alda, R. López, D. Barcelo, J. Mass Spectrom. 38 (2003) 91.

[28] M. Garmouma, M.J. Teil, M. Blanchard, M. Chevreuil, Sci. Total Environ. 224 (1998) 93.

[29] C. Ramos, G. Carbonell, J.M. Garcia Baudin, J.V. Tarazona, Sci. Total Environ. 247 (2000) 269.

[30] R. Carabias-Martinez, E. Rodriguez-Gonzalo, M.E. Fernandez-Laespada, L. Calvo-Seronero, F.J. Sanchez-San Roman, Water Res. 37 (2003) 928.

[31] J. Quintana, I. Marti, F. Ventura, J. Chromatogr. A 938 (2001) 3.

[32] D. de Almeida Azevedo, S. Lacorte, T. Vinhas, P. Viana, D. Barcelo, J. Chromatogr. A 879 (2000) 13.

[33] T.A. Albanis, D.G. Hela, T.M. Sakellarides, I.K. Konstantinou, J. Chromatogr. A 823 (1998) 59.

[34] C. Aguilar, I. Ferrer, F. Borrull, R.M. Marce, D. Barcelo, Anal. Chim. Acta 386 (1999) 237.

[35] B. Van der Bruggen, C. Vandecasteele, Environ. Pollut. 122 (2003) 435.

[36] C. Neal, H.P. Jarvie, R.J. Williams, L.C.V. Pinder, G.D. Collett, M. Neal, L. Bhardwaj, Sci. Total Environ. 251–252 (2000) 423.

[37] P.J. Squillace, E.M. Thurman, Environ. Sci. Technol. 26 (1992) 538.

[38] J.A. Moody, D.A. Goolsby, Environ. Sci. Technol. 27 (1993) 2120.

[39] F. Ribas, J. Frias, J.M. Huguet, F. Lucena, Water Res. 31 (1997) 639.

[40] W. Nishijima, M. Okada, Water Sci. Technol. 37 (1998) 117.

[41] C. Baronti, R. Curini, G. D'ascenzo, A. Di Corcia, R. Gentili, R. Samperi, Environ. Sci. Technol. 34 (2000) 5059.

[42] X.-Y. Xiao, D.V. McCalley, J. McEvoy, J. Chromatogr. A 923 (2001) 195.

[43] C. Desbrow, E.J. Routledge, G.C. Brighty, J.P. Sumpter, M. Waldock, Environ. Sci. Technol. 32 (1998) 1549.

[44] P. Adler, T. Steger-Hartmann, W. Kalbfus, Acta Hydrochim. Hydrobiol. 29 (2001) 227.

[45] R.D. Rurainski, H.J. Theiss, W. Zimmermann, GWF Wasser/Abwasser 118 (1977) 288.

[46] M.J. Lopez de Alda, A. Gil, E. Paz, D. Barcelo, Analyst 127 (2002) 3.

[47] H. Fromme, T. Kuchler, T. Otto, K. Pilz, J. Muller, A. Wenzel, Water Res. 36 (2002) 1429.

[48] B. Stachel, U. Ehrhorn, O.-P. Heemken, P. Lepom, H. Reincke, G. Sawal, N. Theobald, Environ. Pollut. 124 (2003) 497.