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Presence of Pharmaceuticals and Hormones in Waters from Sewage Treatment Plants

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Abstract This paper describes the presence of 33 pharmaceuticals and hormones in waters from two sewage treatment plants (STPs) situated in Catalonia, in northeastern Spain. The target compounds were one psychoactive stimulant, one antiepileptic, four analgesics and non-steroidal anti-inflammatories, one lipid regulators, two anti-ulcer agents, nine antibiotics (sulfonamides and macrolides), two beta-blockers, two metabolites, and 11 hormones (free and conjugates). The determination was performed using liquid chromatography coupled to tandem mass spectrometry after enrichment by solid-phase extraction with Oasis HLB sorbent. Most of the pharmaceuticals were found in both influent and effluent samples from the two STPs. The most frequently detected were caffeine, acetaminophen, carbamazepine, diclofenac, ibuprofen, naproxen, sulfamethoxazole, sulfapyridine, sulfathiazole, ranitidine, omeprazole, estrone 3-sulfate, and estradiol 17-glucuronide. Specifically, the highest concentrations found in influents were 19,850 ng/L (acetaminophen), 9,945 ng/L (caffeine), 4,215 ng/L (ibuprofen), 5,695 ng/L (sulfamethoxazole), and 5,140 ng/L (sulfathiazole). Most of the pharmaceuticals present in influent waters were found in effluents at lower concentrations. The highest concentrations in effluents were 970 ng/L (caffeine), 670 ng/L (sulfamethoxazole), 510 ng/L (bezafibrate), and 1,032 ng/L (diclofenac).

Keywords Hormones · Pharmaceuticals · LC-MS-MS · SPE · Wastewaters

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1 Introduction

Pharmaceutical compounds are considered emerging organic contaminants, which have recently attracted much attention from the scientific community. These compounds are not completely removed in wastewater treatment, and they might prove to be an issue in the quality of water supplies. Not only pharmaceuticals but also some of their metabolites have been detected at low levels in sewage treatment plant effluents, river water, and also drinking water (Hernando et al. 2006; Radjenovic et al. 2008; Vanderford and Snyder 2006; Zhao and Metcalfe 2008). In recent years, there have been an increasing number of studies on the occurrence of pharmaceuticals in environmental waters (Gros et al. 2006; Hernández et al. 2007). These contributions help to raise awareness of the fact that sewage treatment plants (STPs) need improved treatments. Recently, relative success has been achieved in the use of advanced technologies such as granular activated carbon, membrane technology, ozonation, and ultravi-

olet radiation in the removal of some pharmaceuticals from water (Benítez et al. 2009; Benner and Ternes 2009; Bolong et al. 2009). For example, oxidation with ozone (O_3) is usually applied in water treatment to remove micropollutants. Compounds with C=C groups, activated aromatic structures, or heteroatoms such as nitrogen or sulfur are vulnerable to ozonation, while compounds with amide structures are ozonation resistant (Nakada et al. 2007; Zwiener 2007). In contrast, with conventional treatment, ozonation represents a quantitative removal for certain compounds, such as diclofenac, carbamazepine, and sulfamethoxazole (Zwiener 2007).

Despite all the efforts made, pharmaceuticals are not completely removed in STPs and some of them enter the environment, either unaltered or in their main metabolite form. Studies conducted in various countries around the world (Farré et al. 2008b; Khalaf et al. 2009; Wu et al. 2008) showed that non-steroidal anti-inflammatory drugs (NSAIDs) are the most frequently found in wastewaters at low micrograms per liter, since in some countries most of them are available without the need for a prescription. Lacey et al. (2008) found salicylic acid (metabolite of acetylsalicylic acid) and ibuprofen to be the most abundant compounds of a total of 20 pharmaceuticals with maximum levels of 9.17 and 3.20 μg/L, respectively, in influent wastewaters in Ireland. A median as high as 360 ng/L of ibuprofen has been found in river water, in China (Peng et al. 2008). Not only do NSAIDs cause environmental concern but also antibiotics are viewed as emerging environmental contaminants because of their potential adverse effects on ecosystems and human health. Antibiotics cause ecological damage when they are released into water communities because of the potential development of antibiotic-resistant bacteria (Baquero et al. 2008; Hernández et al. 2007). Macrolides, sulfonamides, and trimethoprim are the most frequently prescribed antibiotics for use by humans, and some of them (e.g., sulfamethazine, sulfathiazole, trimethoprim, etc.) are also used in veterinary medicine (Managaki et al. 2007). Some of these antibiotics (erythromycin, roxithromycin, and tylosin) have been detected in drinking water at values lower than 5 ng/L (Ye and Weinberg 2007).

Another group of emerging contaminants which causes concern is that of hormones, which have been identified as the greatest contributors to the endocrine-disrupting compounds in waters (Auriol et al. 2006; Farré et al. 2006). Particular attention has been given to the natural hormones estradiol and estrone, as well as to the synthetic estrogen 17α-ethinylestradiol, which is for being strongly estrogenic (Viglino et al. 2008; Xu et al. 2006). All of these have actually been detected in surface and groundwaters at very low levels (Benotti et al. 2009; Vulliet et al. 2008). For example, estrone was detected at levels between 0.3 and 3.5 ng/L in these kinds of samples in France (Vulliet et al. 2008). When such contaminants reach the rivers, they enter the food chain and may become a potential risk to water consumed after the drinking treatment process. Some reports have identified this risk, and for example, sulfamethoxazole (<0.25 ng/L; Vanderford and Snyder 2006) and estriol (11.6 ng/L; Kuster et al. 2008) have been found in tap water.

Requirements of low limits of detection and the complexity of the matrix impose the use of highly sensitive and selective methods for trace determination of these compounds. Recent developments in liquid chromatography coupled to tandem mass spectrometry (LC-MS-MS) such as triple quadrupole (QqQ), quadrupole time-of-flight, or quadrupole ion trap analyzers have meant that LC-MS-MS has become the most preferred technique by which to determine pharmaceuticals and hormones in multi-residue analysis (Coetsier et al. 2007). For example, Kuster et al. (2008) determined a group of hormones in river water, using LC-QqQ, and they found maximum values of estriol of 11.60 ng/L.

Working with a hybrid triple quadrupole-linear ion trap mass spectrometer, Ding et al. (2009) achieved limits of detection (LODs) of 2–6 ng/L in a method for the determination of macrolide antibiotics. The same detector was used by Vulliet et al. (2008) to determine estrogenic compounds in the 0.3–3.5-ng/L range in surface and groundwaters, and Gros et al. (2009) determined 73 pharmaceuticals in surface and wastewaters. However, QqQ working with multiple reaction monitoring (MRM) is the most widely used technique by which to achieve low LODs in target analysis (Managaki et al. 2007).

A list of 33 target compounds (pharmaceuticals and some metabolites, hormones, and conjugates) is given whereby a classification of the substances is under investigation. Pharmaceuticals were selected so as to provide a representative selection of the pharmaceuticals most used in human and veterinarian medicine and to reflect their environmental impact. Some of the selected compounds in this study were

identified by the UK Environment Agency for priority investigation as a potential risk to the aquatic environment (Hilton and Thomas 2003). The main aim of this research was to determine and evaluate the presence of these 33 compounds in two sewage water treatment plants in Catalonia to ascertain what is going to end up in rivers. The occurrence of these contaminants was studied using seven sample sets in 2007–2008 in both STPs.

2 Materials and Methods

2.1 Chemicals

The standards were purchased from Sigma-Aldrich Chemie (Steinheim, Germany): acetaminophen, caffeine, metoprolol tartrate salt, propranolol hydrochloride, salicylic acid, carbamazepine, clofibric acid, naproxen (NPX), bezafibrate (BZF), diclofenac, ibuprofen, sulfamethoxazole, sulfadiazine, sulfamethazine, sulfapyridine, sulfathiazole, tylosin, erythromycin, roxithromycin, omeprazole, ranitidine, trimethoprim, estrone (E1), estrone 3-sulfate (E1-3S), estrone 3-glucuronide (E1-3G), 17β-estradiol (E2), estradiol 3-sulfate (E2-3S), 17β-estradiol 17-acetate (E2-17A), estradiol 17-glucuronide (E2-17G), 17α-ethinylestradiol (EE2), 17α-estradiol (α-E2), estriol (E3), and diethylstilbestrol (DSB). Individual standard solutions of 1,000 mg/L were prepared in MeOH for all the compounds, except BZF and NPX, which were prepared in MeOH/H_2O (50:50). A mixed standard solution of 10 mg/L was prepared weekly in H_2O. All solutions were stored at 4°C except the hormones, which were stored at −5°C.

Kitasamycin hydrate, the surrogate standard (100 mg/L in acetonitrile), was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Ultra-pure water was obtained with a Milli-Q water purification system (Millipore, Bedford, MA, USA), acetonitrile and methanol were HPLC grade from SDS (Peypin, France), and nitrogen was from Carburos Metálicos (Tarragona, Spain). Chlorhydric acid (HCl), sodium hydroxide (NaOH), and acetic acid from Prolabo (Bois, France) were used to adjust the pH of the sample and the mobile phase.

2.2 Site and Sample Locations

Twenty-four composite (24-h) samples from Catalonia (NE Spain), sampled from March 2007 to March 2008, were analyzed in this study. The wastewater samples were collected from the influent and effluent of two domestic STPs in two cities located on the coast, each with approximately 140,000 inhabitants. The STPs use activated sludge biological treatment, and the biological oxygen demand (BOD_5) for influent water is about 400 mg/L. The average flow rate was 30,000 m^3/day for STP1 and 16,000 m^3/day for STP2.

The study was divided into seven sample sets, but one of these seven (November 2007) was only carried out in STP2 because it was not available in STP1. All samples were collected by using pre-cleaned amber glass bottles acidified to pH 3 (HCl) and stored at −20°C until analysis.

2.3 Sample Preparation

Solid-phase extraction was used to preconcentrate the samples, and Oasis HLB cartridges (500 mg, Waters, Milford, MA, USA) were selected for all the compounds. Extraction methods were adapted from the previously optimized methods (Pedrouzo et al. 2007, 2008, 2009) and are explained briefly below (methods A, B, and C). In all cases, the sorbent was sequentially conditioned using 5 mL of MeOH and 2 mL of Milli-Q water and was then allowed to dry for 10 min in a vacuum. Sample volumes of 100 and 250 mL were extracted for the influent and effluent of the STP, respectively. Prior to the extraction procedure, samples were filtered using a 0.45-μm nylon filter (Whatman, Maidstone, UK) using a manifold (Teknokroma, Barcelona, Spain) and a pump as a vacuum source. They were passed through the cartridge at a flow rate of 10–15 mL/min. The eluate was reduced to dryness under a stream of nitrogen and redissolved with 1 mL of water with a different proportion of MeOH in each case. The residue was filtered through a 0.45-μm nylon membrane (Scharlab, Barcelona, Spain), and 50 μL of this solution was injected into the chromatographic system.

In method A, the samples were adjusted to pH 3 with HCl. The retained analytes were eluted using 6 mL of MeOH. The eluate was redissolved, after evaporation, with 1 mL of MeOH/H_2O (50:50). In method B, the samples were adjusted to pH 7 with NaOH and spiked with the surrogate (kitasamycin). The retained analytes were eluted from the cartridge with 2 mL of MeOH and 2 mL of MeOH adjusted to

basic conditions (NH_4OH 0.1%, pH 9). The eluate was reconstituted, after evaporation, with 1 mL of H_2O containing 1% MeOH. In method C, the samples were adjusted to pH 7 with NaOH. The analytes retained were eluted using 5% ACN in 5 mL of MeOH. Extracts were redissolved, after evaporation, with 1 mL of MeOH/H_2O (80:20). All the compounds included in each method are summarized in Table 1.

2.4 Chromatographic Analysis

The LC-MS-MS chromatographic analysis was conducted on an Agilent 1200 series LC and a 6400 series triple quadrupole mass spectrometer from Agilent Technologies (Waldbronn, Germany) using an electrospray interface (ESI), an automatic injector, a degasser, a quaternary pump, and a column oven. The chromatographic column was a Kromasil 100 C_{18} (25.0×0.46 cm) with a 5-μm particle size (Teknokroma, Barcelona, Spain), and the volume injected was 50 μL. A binary mobile phase with a gradient elution was used. For all the analysis, solvent A was Milli-Q water with acetic acid (pH 3), and solvent B was acetonitrile.

Nitrogen was used as the nebulizing gas in both negative and positive ionization modes. Optimization of parameters and fragment ions for the different methods (methods A, B, and C) was performed by flow injection analysis of each compound. These are compiled in Table 1.

2.4.1 Method A

The chromatographic conditions were described in Pedrouzo et al. (2007), where a quadrupole was used as detection system. In this study, we determined these compounds by LC-MS-MS and lower concentrations and confirmation data were obtained. Since both positive and negative ionizations were needed, two different gradient elution programs were used. The gradient for negative ionization mode was 55% B, which was increased to 60% in 6 min, kept constant for 3 min, increased to 80% in 12 min, to 100% in 2 min, kept constant for 3 min, and then decreased to 55% for 3 min. The gradient for the positive ionization mode was 18% B, which increased to 20% in 4 min, to 55% in 5 min, to 60% in 6 min, to 100% in 5 min, remained constant for 3 min, and finally decreased to 18% in 2 min. Temperature was kept at 30°C, the mobile phase flow rate was 1 mL/min, and the injection volume was 50 μL.

The optimization of the MS-MS parameters was performed by injecting each compound. Parameters for ESI-positive ionization were nebulizer pressure (40 psi), drying gas T^a (300° C), capillary voltage (3,000 V), and drying gas flow rate (13 L/min). For ESI in negative mode, these are nebulizer pressure (30 psi), drying gas flow rate temperature (350°C), capillary voltage (3,500 V), and drying gas flow rate (12 L/min). Values of the cone voltage and the collision energy for each MRM transition are specified in Table 1. It is noteworthy that two MRM transitions were achieved for all the compounds except for ibuprofen (Table 1). Although the identity of ibuprofen could not be confirmed because the MS-MS spectrum contained one diagnostic ion only, we decided not to exclude this compound from the study because of its high consumption; thus, concentrations are given.

2.4.2 Method B

The chromatographic separation was described in Pedrouzo et al. (2008). The gradient was 10% B, which was increased to 15% in 10 min, to 26% in 5 min, and to 60% in 4 min; then it was increased to 100% in 4 min, kept constant for 2 min, and finally returned to 10% B in 2 min. All the compounds eluted within 22 min.

We used a LC-MS-MS, as described above. Parameters for ESI-positive ionization were nebulizer pressure (40 psi), drying gas flow rate (12 L/min), drying gas temperature (350°C), and capillary voltage (4,000 V). As can be seen in Table 1, values of cone voltage were between 75 and 175 V and collision energy were between 10 and 35 V. Product ions used for monitoring were selected on the basis of the MS-MS spectra.

2.4.3 Method C

A LC-MS-MS method for simultaneous determination of hormones and their conjugates was applied as described in detail by Pedrouzo et al. (2009). A binary mobile phase with a gradient elution was used to optimize the extraction conditions. Solvent A was Milli-Q water with acetic acid (pH=2.8) and solvent B was acetonitrile. The gradient was performed as

Table 1 Classification and LC-MS-MS conditions for all the studied compounds

Group	Compound	Method	MRM transitions	Cone voltage (V)	Collision energy (V)	Ionization mode
Analgesics/ anti-inflammatories	Acetaminophen	A	152→93 152→110	100	25 15	PI
	Naproxen	A	229→140 229→185	50	30 5	NI
	Salicylic acid	A	137→93 137→65	75	15 30	NI
	Diclofenac	A	294→214 294→250	75	20 10	NI
	Ibuprofen	A	205→161	75	5	PI
Psychoactive stimulants	Caffeine	A	195→110 195→138	125	25 15	PI
Anti-epileptics	Carbamazepine	A	237→179 237→193	150	35 35	PI
Lipid regulators	Clofibric acid	A	213→85 213→127	50	5 10	NI
	Bezafibrate	A	360→154 360→274	100	30 15	NI
β-Blockers	Metoprolol	A	268→116 268→159	125	15 20	PI
	Propranolol	A	260→116 260→183	125	15 15	PI
Antibiotics	Trimethoprim	B	291→145 291→249	125	35 20	PI
Sulfonamide antibiotics	Sulfamethoxazole	B	254→108 254→156	100	20 10	PI
	Sulfapyridine	B	250→108 250→156	75	25 15	PI
	Sulfadiazine	B	251→108 251→156	75	25 10	PI
	Sulfamethazine	B	279→124 279→186	100	25 15	PI
	Sulfathiazole	B	256→108 256→156	75	20 10	PI
Macrolide antibiotics	Tylosin	B	916→174 916→772	150	35 30	PI
	Erythromycin	B	735→158 735→576	150	30 30	PI
	Roxithromycin	B	838→679 838→158	175	15 30	PI
Anti-ulcer agents	O	B	346→151 346→198	75	15 10	PI
	Ranitidine	B	315→176 315→270	100	15 10	PI
Hormones	E1	C	269→145 269→143	150	45 55	NI
	E2	C	271→145 271→183	60	30 45	NI
	EE2	C	295→145 295→159	60	45 30	NI

Table 1 (continued)

Group	Compound	Method	MRM transitions	Cone voltage (V)	Collision energy (V)	Ionization mode
	α-E2	C	271→145 271→183	60	30 45	NI
	E3	C	287→171 287→145	150	45 45	NI
	DSB	C	267→222 267→237	150	30 55	NI
Conjugate hormones	E1-3S	C	349→269 349→145	150	30 55	NI
	E1-3G	C	445→269 445→113	150	45 20	NI
	E2-3S	C	351→271 351→145	150	30 55	NI
	E2-17A	C	313→253 313→145	100	30 55	NI
	E2-17G	C	447→271 447→113	150	30 20	NI

Description of the methods A: Pedrouzo et al. (2007), B: Pedrouzo et al. (2008), C: Pedrouzo et al. (2009)

follows: 10% B, kept constant for 10 min, increased to 40% B for 5 min, to 60% for 10 min, to 100% B for 5 min, and then decreased to 10% B for 2 min. The system was re-equilibrated for 3 min between injections.

MS-MS analysis was performed in the negative ionization mode with an optimized spray potential of 3,000 V, a nebulizer of 45 psi, and a source temperature of 350°C and 12 L/min of drying gas flow rate. Nitrogen was used as the collision, nebulizing, and desolvation gas.

3 Results and Discussion

3.1 Quantification and Method Validation

Recovery values for effluent matrix are given in Table 2, and as it was expected, they were similar to the recoveries found in the previous studies. Also, comparing with the LC-MS method, the MS-MS method allowed us to calculate the recovery for acetaminophen (method A), and it was 45% (RSD <19%) in influent waters. However, because of the low recoveries of salicylic acid (metabolite of acetylsalicylic acid), bezafibrate, and E2-17A, the concentrations were not determined in influents.

Influent samples were quantified with a Milli-Q calibration curve, and recoveries for each compound were applied. Calibration curves of the entire method were used for quantification of effluent samples. Only compounds from method B required a surrogate standard to quantify the samples. The use of a surrogate (kitasamycin) was justified by the high level of ion suppression of the matrices. The linear range for effluent samples is shown in Table 2. Limits of quantification (LOQs) were calculated as the lowest point of the calibration curve, this being higher in influents (10–200 ng/L) than in effluents (3–100 ng/L) because of the different sample volume. LODs were set as the concentration at which the s/n ratio was 3 (Table 2).

3.2 Occurrence of Pharmaceuticals and Hormones in STPs

As Tables 3 and 4 show, several families of pharmaceuticals and hormones were found in the sewage waters from the two STPs. Confirmation criteria applied to the target compounds were the following: presence of two characteristic MRM transitions at the correct retention time and the correct ratio between product ions (except for ibuprofen with only one MRM transition).

Caffeine is one of the most widespread pharmaceuticals, and values between 950 and 9,945 ng/L were determined in influent from both STPs, as noted

Table 2 Validation data for all the compounds

Compound	Recoveries[a] (% R)		LODs (ng/L)		Linear range (ng/L)
	Influent	Effluent	Influent	Effluent	Effluent
Acetaminophen	45	49	5	2	5–500
Caffeine	55	80	8	3	5–1,000
Metoprolol	75	75	8	3	5–400
Propranolol	50	85	8	3	5–1,000
Carbamazepine	35	65	5	2	5–1,000
Salicylic acid	–	40	5	2	5–400
Bezafibrate	–	67	3	1	3–2,000
Naproxen	50	55	15	7	10–2,000
Clofibric acid	37	57	5	2	5–2,000
Diclofenac	38	52	8	2	5–2,000
Ibuprofen	52	89	15	7	10–2,000
Ranitidine	65	68	8	2	5–2,000
Trimethoprim	58	63	15	10	25–2,000
Sulfamethazine	62	72	15	10	25–2,000
Sulfathiazole	51	58	15	10	25–2,000
Sulfapyridine	57	62	15	10	25–1,000
Sulfadiazine	43	47	15	5	10–2,000
Tylosin	61	55	25	15	25–2,000
Erythromycin	42	44	25	15	25–2,000
Roxithromycin	52	48	10	2	5–2,000
Omeprazole	20	22	10	2	5–1,000
Sulfamethoxazole	25	29	20	10	25–2,000
E1	60	51	50	25	100–1,500
E2	53	61	100	70	100–1,500
EE2	37	52	100	70	100–1,500
α-E2	30	49	100	70	100–1,500
E3	56	59	50	25	100–1,500
DSB	58	76	50	25	100–1,500
E1-3S	57	74	15	10	30–1,500
E1-3G	66	65	50	25	100–1,500
E2-3S	57	78	15	10	30–1,500
E2-17A	–	25	100	70	100–1,500
E2-17G	23	28	50	25	100–1,500

[a] Samples spiked at 250 ng/L (effluents) and 500 ng/L (influents).
% RSD<19, n=3

– recoveries <10%

herein and in agreement with the literature (Nikolaou et al. 2007). Due to the high level found in influents, the samples were diluted to be quantified. Even higher concentrations of caffeine had been found in previous papers (Pedrouzo et al. 2007), with maximum values of 40 μg/L in influents. This concurred with Huerta-Fontela et al. (2008), who reported, also in Spain, concentrations of caffeine up to 209 and 24 μg/L in 40 studied influent samples. This level of caffeine is not only due to the amount present in pharmaceuticals but also to its presence in some products such as coffee, tea, chocolate, or sports

Table 3 Concentrations (nanograms per liter) found in STP1, RSD<15% (n=3)

Compound	March 2007		May 2007		July 2007		September 2007		January 2008		March 2008	
	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
Acetaminophen	9,792	9	11,250	–	8,652	–	12,247	30	7,665	<LOQ	19,640	<LOQ
Caffeine	9,945	19	6,540	882	4,655	580	5,154	672	8,296	34	8,750	375
Metoprolol	<LOQ	–	<LOQ	–	–	–	73	–	<LOQ	–	–	–
Propranolol	34	–	20	–	–	–	<LOQ	–	–	150	<LOQ	10
Carbamazepine	90	76	252	110	250	8	322	52	105	48	308	52
Salicylic acid	n.m	13	n.m	68	n.m	182	n.m	9	n.m	134	n.m	15
Bezafibrate	n.m	385	n.m	302	n.m	289	n.m	225	n.m	220	n.m	283
Naproxen	235	15	1,160	241	360	155	639	38	220	39	685	30
Diclofenac	145	243	374	427	133	1,032	415	303	270	146	520	368
Ibuprofen	532	30	3,615	190	2,215	<LOQ	1,175	137	172	<LOQ	3,062	81
Ranitidine	85	406	870	120	320	–	320	12	<LOQ	60	504	70
Sulfathiazole	1,870	161	965	–	1,326	205	1,340	<LOQ	3,165	–	<LOQ	–
Sulfapyridine	1,445	538	73	165	<LOQ	–	310	<LOQ	730	152	380	–
Sulfamethoxazole	3,210	670	2,490	110	1,140	<LOQ	1,195	45	2,985	420	453	44
E2-17G	376	–	<LOQ	–	615	–	415	–	316	–	<LOQ	–
E1-3S	<LOQ	35	320	<LOQ	<LOQ	<LOQ	–	255	<LOQ	–	<LOQ	–
E2-17A	n.m	<LOQ	n.m	–	n.m	176	n.m	166	n.m	–	n.m	85

n.m not measured, – <LOD

Table 4 Concentrations (nanograms per liter) found in STP2, RSD <17% (n=3)

Compound	March 2007		May 2007		July 2007		September 2007		November 2007		January 2008		March 2008	
	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
Acetaminophen	19,250	–	17,655	–	13,300	<LOQ	19,150	–	19,850	–	11,470	<LOQ	9,150	–
Caffeine	3,435	10	3,365	970	1,360	120	3,790	<LOQ	7,514	205	950	243	6,380	170
Metoprolol	15	<LOQ	40	–	30	–	<LOQ	–	<LOQ	–	–	–	<LOQ	–
Propranolol	22	–	20	–	<LOQ	<LOQ	<LOQ	15	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Carbamazepine	55	95	405	170	22	<LOQ	20	60	205	70	44	65	85	40
Salicylic acid	n.m	125	n.m	9	n.m	115	n.m	50	n.m	80	n.m	20	–	200
Bezafibrate	n.m	510	n.m	340	n.m	100	n.m	200	n.m	260	n.m	140	n.m	300
Naproxen	1,300	395	605	260	330	30	2,280	85	1,100	80	550	220	840	691
Diclofenac	315	474	275	505	160	260	410	130	350	870	<LOQ	160	265	520
Ibuprofen	1,405	325	4,215	495	1,100	<LOQ	3,580	–	1,795	15	660	120	3,140	955
Ranitidine	20	–	650	–	270	–	730	–	2,175	<LOQ	<LOQ	<LOQ	350	355
Sulfamethazine	55	–	–	–	50	<LOQ	<LOQ	<LOQ	<LOQ	–	–	<LOQ	<LOQ	–
Sulfathiazole	975	–	30	–	5,140	<LOQ	870	<LOQ	560	–	157	–	2,235	–
Sulfapyridine	–	–	165	–	<LOQ	<LOQ	360	–	2,475	<LOQ	<LOQ	–	195	<LOQ
Sulfamethoxazole	1,715	515	1,935	<LOQ	2,665	410	3,395	<LOQ	5,695	75	1,030	<LOQ	3,460	<LOQ
E2-17G	–	–	<LOQ	–	225	<LOQ	–	<LOQ	–	–	220	<LOQ	–	–
E1-3S	160	52	640	<LOQ	143	<LOQ	–	<LOQ	520	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
17α-EE2	154	–	–	–	–	–	<LOQ	–	–	–	–	–	<LOQ	–
E2-17A	n.m	–	n.m	–	n.m	135	n.m	<LOQ	n.m	–	n.m	<LOQ	n.m	–

n.m not measured, – <LOD

drinks, among others. This widespread use makes that caffeine was even detected in river waters. Three Catalan rivers showed values between 106 and 305 ng/L (Pedrouzo et al. 2007), and Conley et al. (2008) found levels between 23.2 and 38.8 ng/L in the Tennessee River with a frequency of 100% in the samples analyzed.

The widespread use of non-prescription consumption analgesics was corroborated because of the high levels of acetaminophen found in the influents under study, with a maximum concentration of 19,850 ng/L (sample diluted to be quantified). Although influent samples could not be quantified, values between 9 and 200 ng/L of salicylic acid were found in effluents. These results concurs with Sponberg and Witter (2008), who found levels between 0.4 and 8 μg/L in influents and also Lacey et al. (2008) found levels of 0.3–9.1 μg/L of this compound in influents and <0.1 μg/L in effluents.

NSAIDs were also found in waters. As can be seen in Tables 3 and 4, the highest levels correspond to ibuprofen with a maximum concentration of 4,215 ng/L in influents (STP2, May 2007). However, these high concentrations decreased to a maximum of 955 ng/L in effluent samples. An example of an effluent chromatogram is shown in Fig. 1. Another NSAID, such as naproxen, was found at levels between 220 and 1,160 ng/L in influents from STP1 and 330 and 2,280 ng/L in influents from STP2. These values for NSAIDs agree with the results reported by Farré et al. (2008b).

High levels of antibiotics were also found in both STPs. This is the case of sulfamethoxazole, which was found in both influents at values between 453 and 5,695 ng/L. This concurs with several studies (Díaz-Cruz et al. 2008; Göbel et al. 2004), which declared sulfamethoxazole to be one of the highest pharmaceutical found in environmental waters. For example, Göbel et al. (2004) found maximum concentrations of 641 ng/L in influent sewage waters. Karthikeyan and Meyer (2006) found trimethoprim and sulfamethoxazole in wastewaters with values of frequency of 70% and 80%, respectively. In our study, only one influent sample revealed concentrations of trimethoprim (505 ng/L), and data for this pharmaceutical were excluded from Tables 3 and 4. However, Vanderford and Snyder (2006) reported levels of trimethoprim of 1,140 ng/L in influent and lower than 0.50 ng/L in effluent. Antibiotics like tylosin, erythromycin, and roxithromycin belong to the family of macrolides, and they are used in both veterinary and human medicine. Our study only showed some values <LOQ and they are also not shown in Tables 3 and 4. However, these antibiotics were found in influent waters by Göbel et al. (2004) at values between 44 and 67 ng/L (erythromycin) and 22 and 30 ng/L (roxithromycin).

Pharmaceuticals showed similar behavior in both STPs, but certain compounds showed some differences. Such is the case of sulfamethazine, an antibiotic used in veterinary medicine. Possibly because of the proximity of some veterinary industries to the area, STP2 showed concentrations of 55 ng/L (influents) and <LOQ (effluents), whereas STP1 only showed values <LOQ in some influent samples (data not shown in Table 3).

Carbamazepine, an antiepileptic, was found in all the influent waters at concentrations between 20 and 405 ng/L. This compound showed low removal and sometimes similar values could be seen in effluents and influents (Tables 3 and 4).

β-Blockers are used particularly for the management of cardiac arrhythmias. In this study, two β-blockers (metoprolol and propranolol) were found at concentrations up to 73 and 34 ng/L, respectively, in influent waters, but higher values were found by Gros et al. (2008). In their study of eight β-blockers, values of metoprolol (2,408 ng/L) and propranolol (117 ng/L) in influents were reduced to 375 and 104 ng/L, respectively, in effluents. Bezafibrate, a blood lipid regulator, which had been detected in levels up to micrograms per liter in sewage water by Heberer (2002), showed maximum concentrations of 510 ng/L in effluents.

Ranitidine and omeprazole are pharmaceuticals used as anti-ulcer agents. In our study, ranitidine was present in all the influent samples with values between 20 and 2,175 ng/L. However, the presence of omeprazole was not so evident, and only values <LOQ were found in a few samples (data not shown). Other compounds with concentrations lower than LOQ were clofibric acid (metabolite of clofibrate) and some hormones (E2, E3, E2-3S, E1, E1-3G, and DSB), and consequently, these results were omitted from the Tables 3 and 4. Hormones are removed via degradation process, and although a considerable amount is adsorbed to sludge (Nieto et al. 2008), some of the compounds remain still soluble in the effluents. Although we could not determine some of these hormones, because of the LODs, 17β-estradiol was detected in influents in a range of concentration

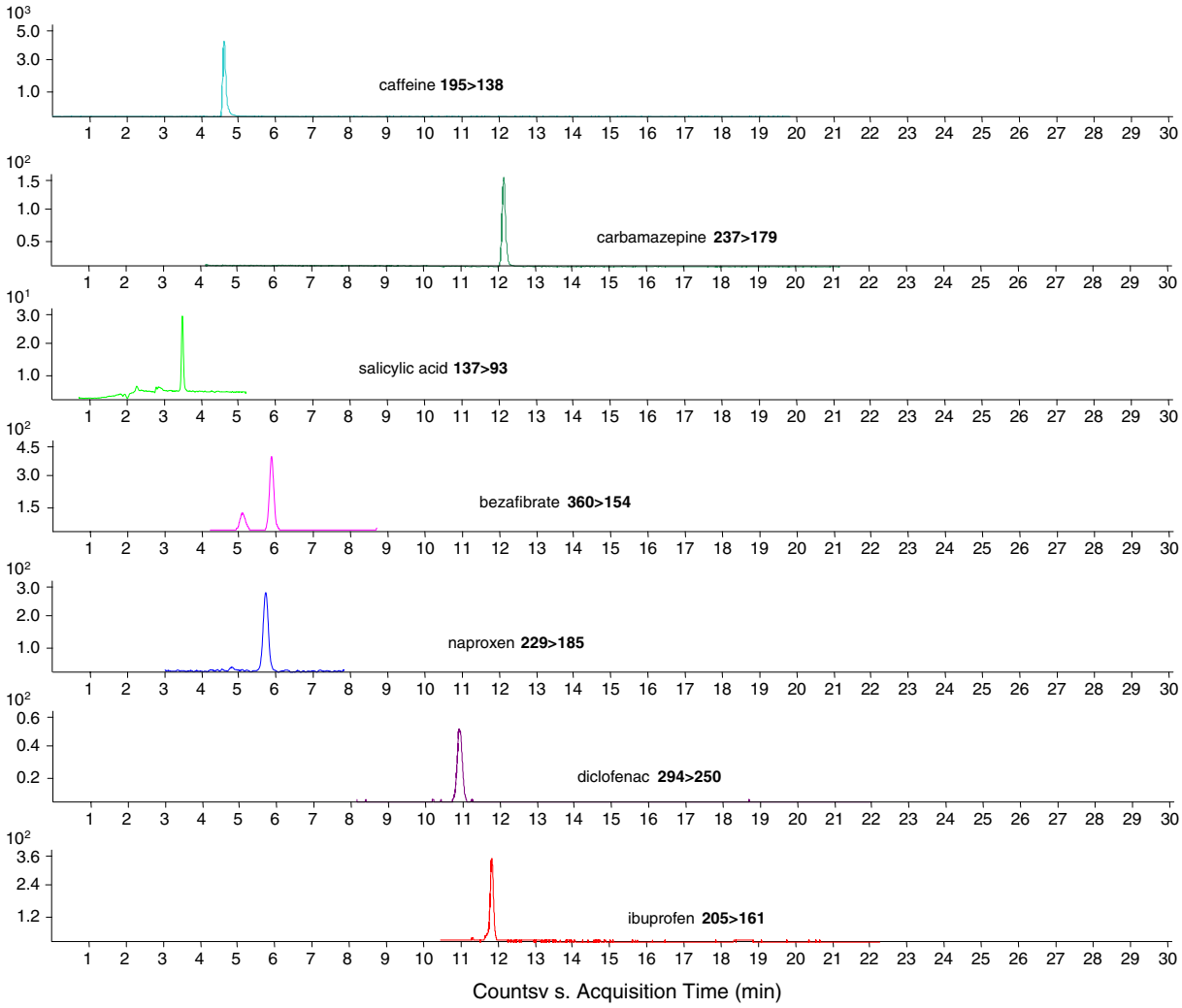

Fig. 1 MRM chromatograms of a sample from an STP2 effluent in May 2007

between 49.4 and 93.3 ng/L (Hernando et al. 2004), and Farré et al. (2006) reported concentrations of estrone between 7.9 (effluents) and 13.9 ng/L (influent) waters.

When Viglino et al. (2008) studied the occurrence of synthetic and natural hormones in influents, they found the highest values for estriol (125 ng/L) and 17α-ethinylestradiol (243 ng/L), while estrone was detected at trace levels. Lower values of the synthetic estrogen 17α-ethinylestradiol were found by Farré et al. (2006) at levels below LOD (<2 ng/L) in influent waters. In our study, only a few influent samples showed 17α-ethinylestradiol, with a maximum concentration of 154 ng/L, and it was not detected in effluent samples (Table 4).

It is known that hormones are typically excreted by mammals in the conjugate, inactive form (glucuronide or sulfate conjugates; Kvanli et al. 2008; Servos et al. 2005). Although glucuronides were not found in the earlier paper by our group (Pedrouzo et al. 2009), in this study, our LOQs were lower and both STPs showed concentrations in similar concentrations of estradiol 17-glucuronide (<LOQ, 615 ng/L) in influents. In effluents, only STP2 showed values of estradiol 17-glucuronide below LOQ, whereas in STP1 it was not detected. Other conjugates yielding positive results, as was reported in our previous paper (Pedrouzo et al. 2009), are sulfate conjugates with the highest concentration of estrone 3-sulfate (640 ng/L in influents and 52 ng/L in effluents). Some samples

showed values <LOQ of estradiol 3-sulfate in influents and effluents (data not shown in Tables 3 and 4). However, the conjugate E2-17A showed maximum values of 176 ng/L in effluents, whereas its presence was not measured in influents because of the low recovery.

3.3 Removal of Pharmaceuticals and Hormones in STPs

Although not as many samples were studied as to conclude a complete study of removals of pharmaceuticals in STPs, we could estimate the behavior of these pharmaceuticals in STPs (influents and effluents). As can be seen in Fig. 2, in general, effluent samples showed values of pharmaceuticals at lower concentration than influent samples. There are several compounds, such as diclofenac and carbamazepine which are not well removed in the STPs studied, which concurs with the literature (Gagnoc and Lajeunesse 2008; Tixier et al. 2003; Zhou et al. 2009). In our study, diclofenac was present in the effluent samples at maximum levels of 1,032 ng/L (Table 3). Even higher values were found in effluents than in influents when both results were compared. Therefore, we assumed an incomplete removal for this compound. We conclude that some sulfonamides are well removed with values between 70% and 85% for sulfamethoxazole, sulfapyridine, and sulfathiazole. The highest removals were acetaminophen (almost 100%), caffeine (>85%), and ibuprofen (>80%), in agreement with Gagnoc and Lajeunesse (2008) who reported a removal of ibuprofen higher than 95%. Also most of our samples revealed an efficient removal of ranitidine (>90%) in STP1 and slightly lower in STP2. But in some cases, higher concentrations were found in effluent than in influent, in agreement with Farré et al. (2008a).

As regards sulfamethoxazole, significant lower removals were found in the literature (24%; Ternes et al. 2007). Not only did these authors study sulfamethoxazole but also its metabolite (N^4-acetyl-sulfamethoxazole) to avoid the underestimation of removal rates. However, as it was previously mentioned, this study showed higher removals for this compound.

The present study shows the removals in STPs with conventional treatments. However, removal rates published in the literature vary greatly depending on the treatment facilities and the nature of the contaminant. For example, Gebhardt and Schroder (2007) stated that the oxidation methods using O_3/UV and H_2O_2/UV successfully led to the complete elimination of persistent and hardly eliminable pharmaceutical compounds such as carbamazepine, diclofenac, and clofibric acid. The study reported by Nakada et al. (2007) demonstrated that the efficiencies of removal during advanced treatment (filtration and ozonation) of secondary effluent gave efficient removals of sulfonamides, macrolides, and trimethoprim (>90%), the ozonation being the greatest contributor. After prechlorination and sand filtration, estrone and estrone-3-sulfate were found to have been completely removed from waters (Rodríguez-Mozaz et al. 2004).

Although there is an increasing focus on the use of advanced post-treatment units in STPs (e.g., ozone,

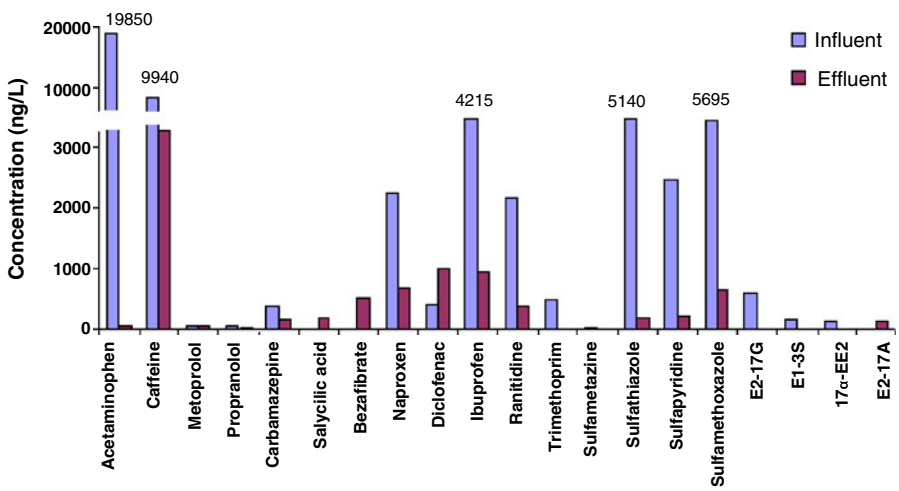

Fig. 2 Maximum concentrations found in influents and effluents from both STPs

advanced oxidation process) to enhance the removals of these contaminants, most conventional STPs do not have these high-cost treatment processes. The incomplete removal of these compounds in STPs is an evident environmental problem with adverse impact in surface waters that represents a matter of concern.

4 Conclusions

Several groups of pharmaceuticals and metabolites (caffeine, analgesics, anti-inflammatories, lipid regulators, β-blockers, anti-epileptics, antibiotics) and some hormones (free and conjugates) were the subject of the monitoring program carried out in two sewage treatment plants in Catalonia. Three different methods were successfully applied for the appropriate determination of pharmaceuticals at low levels in the samples. In influent waters, the dominant compounds that were found were acetaminophen (7,665–19,850 ng/L) and caffeine (950–9,945 ng/L). Residual amounts of these compounds were observed in effluent samples, but only caffeine showed the highest values with a maximum of 970 ng/L. The estimation of the removal in these STPs was also discussed. The lowest removals were seen for some pharmaceuticals, such as carbamazepine and diclofenac. However, acetaminophen was removed almost at 100%, ibuprofen >80%, caffeine over 85%, sulfathiazole, sulfapyridine, and sulfamethoxazole between 70% and 85%.

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