Environment International 36 (2010) 15–26

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes

Meritxell Gros [a,c], Mira Petrović [a,b,*], Antoni Ginebreda [a], Damià Barceló [a,c]

[a] Department of Environmental Chemistry, IDAEA-CSIC, c/Jordi Girona 18–26, 08034 Barcelona, Spain
[b] Institució Catalana de Recerca i Estudis Avançats (ICREA), Passeig Lluis Companys 23, 80010 Barcelona, Spain
[c] Institut Catalàde Recerca de l'Aigua (ICRA), c/ Emili Grahit 101, 17003 Girona, Spain

ARTICLE INFO

Article history:
Received 26 June 2009
Accepted 4 September 2009
Available online 12 October 2009

Keywords:
Multiresidue analysis
Pharmaceutical removal
Pharmaceuticals
Surface/wastewater analysis

ABSTRACT

In a long term study, which covered 4 sampling periods over three years, a total number of 84 samples, specifically 28 influent, effluent, from seven WWTP located in the main cities along the Ebro river Basin (North East of Spain), as well as receiving river waters, were analyzed to assess the occurrence of 73 pharmaceuticals covering several medicinal classes. Results indicated that pharmaceuticals are widespread pollutants in the aquatic environmental. Linking the calculation of removal rates with half-lives, assuming that compound degradation followed pseudo-first order kinetics, suggested that conventional wastewater treatments applied at the seven WWTP were unable to completely remove most of the pharmaceuticals under study. The evaluation of compound degradability, in terms of half-lives, is an important task to discuss integrated solutions for mitigation of pollutants entry into the water cycle. High half-lives observed for the majority of pharmaceuticals in WWTP suggest that, in order to enhance compound degradation, higher hydraulic retention times should be required. The wide spectrum of substances detected in receiving river waters indicates that WWTP outlets are major contributors of pharmaceuticals in the aquatic environment. However, municipal wastewater treatment represents an obligatory and final treatment step prior to their release into the aquatic media, since load of pharmaceuticals in outlets were considerably reduced after treatment.
Finally, hazard posed by pharmaceuticals in both surface and effluent wastewaters was assessed toward different aquatic organisms, (algae, daphnids and fish). The overall relative order of susceptibility was estimated to be algae > daphnia > fish. Results indicate that no significant risks could be associated to the presence of pharmaceuticals in those matrices, indicating that reduction of compound concentration after wastewater treatment as well as dilution factor once pharmaceuticals are discharged in receiving river water efficiently mitigate possible environmental hazards.

1. Introduction

In the European Union (EU) around 3000 different PhACs are used in human medicine belonging to different medicinal classes. Thus, their main route into the aquatic environment is ingestion following excretion and disposal via wastewater. After administration, pharmaceuticals can be excreted, primarily via urine and faeces, either as an unchanged parent compound or in the form of metabolites or as conjugates of glucuronic and sulphuric acid. Besides these WWTP discharges into the environment, that are usually a consequence of their incomplete removal (Petrovic et al., 2005), other environmental exposure pathways of PhACs are manufacturing and hospital effluents, land applications (e.g., biosolids and water reuse), concentrated animal feeding operations (CAFOs), and direct disposal/introduction to environment (Daughton and Ternes, 1999).

Several studies reported on the limited degradability of pharmaceuticals under conventional treatments applied in the WWTPs (Radjenovic, et al., 2007; Carballa et al., 2005), suggesting that their upgrade and implementation of advanced treatment technologies are required to achieve high-quality treated effluents (Radjenovic et al., 2009).

While most of northern European WWTPs include tertiary wastewater treatments, in Spain only primary and secondary treatments are performed, where the second one is based on conventional activated sludge, and tertiary treatments are seldom applied. Consequently, there is a need to assess the limitations of current wastewater treatment processes, as well as to evaluate which operational parameters would play a key role regarding pharmaceutical removal.

Selection of target analytes, which will be included in the analytical methods applied in monitoring programs, should be based on the sales and practices of each country (according to national sales figures and health system), compound pharmacokinetics (the percentage of excretion as non-metabolized substance), occurrence in the aquatic media (data taken from similar studies) as well as on data provided by environmental risk assessment approaches, which link the calculation

* Corresponding author. Department of Environmental Chemistry, IDAEA-CSIC, c/Jordi Girona 18–26, 08034 Barcelona, Spain. Tel.: +34934006172; fax: +34932045904.
E-mail address: mpeqam@cid.csic.es (M. Petrović).

0160-4120/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.envint.2009.09.002

Table 1
Target compounds and their frequency of detection in all matrices analyzed.

Therapeutic group	Compounds	CAS number	% Freq. detection WWI	% Freq. detection WWE	% Freq. detection RW
Analgesics and anti-inflammatories	Ketoprofen (1)	22071-15-4	93	82	n.d.
	Naproxen (2)	22204-53-1	96	96	93
	Ibuprofen (3)	15687-27-1	100	89	96
	Indomethacine (4)	53-86-1	96	89	75
	Diclofenac (5)	15307-86-5	96	86	93
	Mefenamic acid (6)	61-68-7	50	75	20
	Acetaminophen (7)	103-90-2	100	93	89
	Salicylic acid (8)	69-72-7	100	89	100
	Propyphenazone (9)	479-92-5	100	93	100
	Phenylbutazone (10)	50-33-9	n.d.	n.d.	n.d.
	Phenazone (11)	60-80-0	n.d.	80	57
	Codeine (12)	76-57-3	100	100	86
Lipid regulators and cholesterol lowering statin drugs	Clofibric acid (13)	882-09-7	54	79	54
	Bezafibrate (14)	41859-67-0	100	89	86
	Fenofibrate (15)	49562-28-9	39	14	54
	Gemfibrozil (16)	25812-30-0	39	29	100
	Mevastatin (17)	73573-88-3	n.d.	n.d.	n.d.
	Pravastatin (18)	81093-37-0	75	64	46
	Atorvastatin (19)	134523-00-5	100	89	46
Psychiatric drugs	Paroxetine (20)	61869-08-7	n.d.	39	n.d.
	Fluoxetine (21)	54910-89-3	21	100	25
	Diazepam (22)	439-14-5	50	64	39
	Lorazepam (23)	846-49-1	67	68	57
	Carbamazepine (24)	298-46-4	100	100	100
Histamine H_2 receptor antagonists	Loratadine (25)	79794-75-5	25	61	54
	Famotidine (26)	76824-35-6	61	57	n.d.
	Ranitidine (27)	66357-35-5	100	100	75
	Cimetidine (28)	51481-61-9	86	79	11
Tetracycline antibiotics	Tetracycline (29)	60-54-8	36	57	n.d.
	Doxycycline (30)	564-25-0	n.d.	n.d.	n.d.
	Oxytetracycline (31)	79-57-2	14	11	n.d.
	Chlortetracycline (32)	57-62-5	n.d.	n.d.	n.d.
Macrolide antibiotics	Erythromycin (33)	114-07-8	68	93	64
	Azithromycin (34)	83905-01-5	11	100	32
	Roxithromycin (35)	80214-83-1	39	64	25
	Clarithromycin (36)	81103-11-9	100	100	100
	Josamycin (37)	16846-24-5	n.d.	18	14
	Tylosin A (38)	1401-69-0	11	11	n.d.
	Spiramycin (39)	-	32	100	54
	Tilmicosin (40)	10850-54-0	n.d.	n.d.	n.d.
Sulfonamide antibiotics	Sulfamethoxazole (41)	723-46-6	100	100	100
	Sulfadiazine (42)	68-35-9	57	57	46
	Sulfamethazine (43)	57-68-1	57	57	71
Fluoroquinolone antibiotics	Ofloxacin (44)	82419-36-1	79	79	89
	Ciprofloxacin (45)	85721-33-1	86	75	11
	Enrofloxacin (46)	93106-60-6	36	54	11
	Norfloxacin (47)	70458-96-7	39	36	32
	Danofloxacin (48)	112398-08-0	n.d.	n.d.	n.d.
	Enoxacin (49)	74011-58-8	n.d.	n.d.	n.d.
Other antibiotics	Trimethoprim (50)	738-70-5	96	96	86
	Chloramphenicol (51)	56-75-7	14	25	n.d.
	Metronidazole (52)	443-48-1	96	93	32
	Nifuroxazide (53)	965-52-6	n.d.	n.d.	n.d.
B-blockers	Atenolol (54)	29122-68-7	100	93	89
	Sotalol (55)	3930-20-9	79	100	50
	Metoprolol (56)	37350-58-6	89	89	50
	Propranolol (57)	525-66-6	93	100	79
	Timolol (58)	26839-75-8	57	93	21
	Betaxolol (59)	63659-18-7	n.d.	n.d.	n.d.
	Carazolol (60)	57775-29-8	n.d.	n.d.	n.d.
	Pindolol (61)	13523-86-9	n.d.	n.d.	n.d.
	Nadolol (62)	42200-33-9	89	71	50
B-agonists	Salbutamol (63)	18559-94-9	89	86	14
	Clenbuterol (64)	37148-27-9	n.d.	n.d.	n.d.
Barbiturates	Butalbital (65)	77-26-9	n.d.	n.d.	n.d.
	Pentobarbital (66)	76-74-4	n.d.	n.d.	n.d.
	Phenobarbital (67)	50-06-6	n.d.	n.d.	n.d.
Antihypertensives	Enalapril (68)	75847-73-3	100	32	n.d.
	Lisinopril (70)	83915-83-7	n.d.	n.d.	n.d.
Diuretic	Hydrochlorothiazide (69)	58-93-5	100	100	68
	Furosemide (71)	54-31-9	100	100	71
Anti-diabetic	Glibenclamide (72)	10238-21-8	96	100	43
To treat cancer	Tamoxifen (73)	10540-29-1	n.d.	n.d.	n.d.

WWI: Wastewater influent; WWE: Wastewater effluent; and RW: river water.

of predicted environmental concentrations (PEC) with toxicity data in order to evaluate which compounds are more liable to pose an environmental risk for aquatic organisms (Bound and Voulvoulis, 2006; Castiglioni et al., 2004; Cooper et al., 2008).

Directives set by the US Food and Drug Administration (FDA) stipulates that an environmental risk assessment (ERA) should be part of the approval procedure of new medical substances (Cooper et al., 2008). However, few of these substances have been subjected to a complete ERA, because, in most cases, predicted environmental concentrations lie below the proposed cut-off values, fixed by these directives, making further ecotoxicological studies unnecessary. The current US and European regulatory guidance requires new pharmaceuticals to undergo standard acute toxicity tests (to algae, Daphnia magna and fish) if the predicted or measured environmental concentration (PEC or MEC) of the active ingredient is >1 μg/L for the US legislation or 10 ng/L, according to the European threshold safety value, set by the European Medicines Agency (EMEA). For compounds whose PEC exceed these values, as a second tier in the ERA procedure, predicted no-effect concentrations (PNEC) are extrapolated by dividing $E(L)_{50}$ values, (which are obtained from standard toxicity tests), by an assessment factor of up to 1000 in the EU (Cooper et al., 2008). If the quotient between the PEC or MEC and PNEC is lower than 1 (MEC or PEC/PNEC < 1), no further assessment is necessary (Cooper et al., 2008).

Over recent years, Spain has raised its position in the world and the European pharmaceutical market. It was the eighth largest world market in 2005, whereas the following year, it took up the fifth position in Europe's top pharmaceutical markets (www.farmaindustria.es; IMS Health). Such high consumption may lead to the conclusion that the problematic associated with aquatic contamination by pharmaceuticals may be an important issue that needs to be assessed and, since data regarding contamination of Spanish aquatic systems is still sparse, it is necessary to set up surveys at national or basin scale.

In the light of these concerns, the aim of the present study was to identify the loads of pharmaceuticals discharged into the aquatic environment through municipal wastewater effluents in the region of the Ebro river basin (North East of Spain). Therefore, the occurrence of 73 pharmaceuticals of major human consumption, which are listed in Table 1, was determined in both influent and effluent wastewaters from seven WWTP located in the main cities along the basin, as well as in their subsequent receiving river waters (see Fig. 1). Both removal rates and half-lives were evaluated for each compound, in all WWTP, in order to overview their biodegradability, as a consequence of the effectiveness of treatments currently applied in Spanish WWTP.

Finally, established hazard indexes were calculated in order to assess the risk towards different aquatic organisms (algae, daphnids and fish). Such indexes were obtained through the ratio between MECs in both effluent and river waters and PNECs, which were derived from acute toxicity data (EC_{50}) from the literature. Such quotients could be used as an indicator of the possible ecotoxicological risks posed by the concentrations of pharmaceuticals detected in the aquatic environment in the area under investigation.

2. Materials and methods

2.1. Pharmaceutical standards

All standards used were of high purity grade (>90%). Compounds with number 1–5 and 16 (see Table 1) were kindly supplied by Jescuder (Rubí, Spain). Compounds with number 4, 6–8, 10, 11, 13, 14, 15, 17, 18, 21, 24–27, 29, 30, 31–52, 54, 55–64, 68–73 were purchased from Sigma-Aldrich (Steinheim, Germany). Compounds with number 21, 27, 29, 30, 31, 32, 57 and 64 were provided as hydrochloride, 33 as hydrate, 18 as sodium salt, 56 as tartrate, 70 as dehydrate, 73 as citrate, 68 as maleate and 40 as a mixture of isomers. Standards with number 19 (as calcium salt), 9 and 20 (as hydrochloride) were from LGC Promochem (London, UK), while 12, 22, 23, 65, 66 and 67 were from Cerilliant (Texas, USA).

Isotopically labelled compounds, used as internal standards, were ^{13}C-phenacetin, fluoxetine-d_5 and flumequine from Sigma-Aldrich (Steinham, Germany), sulfathiazole-d_4 from Toronto Research Chemicals, diazepam-d_5 and phenobarbital-d_5 from Cerilliant (Texas, USA), atenolol-d_7, carbamazepine-d_{10}, ibuprofen-d_3 from CDN isotopes (Quebec, Canada) and mecoprop-d_3 from Dr. Ehrenstorfer (Augsburg, Germany).

Both individual stock standard and isotopically labelled internal standard solutions were prepared on a weight basis in methanol, except fluoroquinolones which were dissolved in water:methanol mixture (1:1) containing 0.2% v/v hydrochloric acid (Golet et al., 2002). After preparation, standards were stored at −20 °C. Fresh stock solutions of antibiotics were prepared monthly due to their limited stability while stock solutions for the rest of substances was renewed every three months. On the other hand, compounds with number (see Table 1) 12, 71, 65, 66 and 67, were obtained as solutions in acetonitrile, while 22 and 23 were dissolved in methanol, at a concentration of 1 mg/mL.

A mixture of all pharmaceuticals was prepared by appropriate dilution of individual stock solutions in methanol–water (25:75, v/v). Working standard solutions, also prepared in methanol–water (25:75,

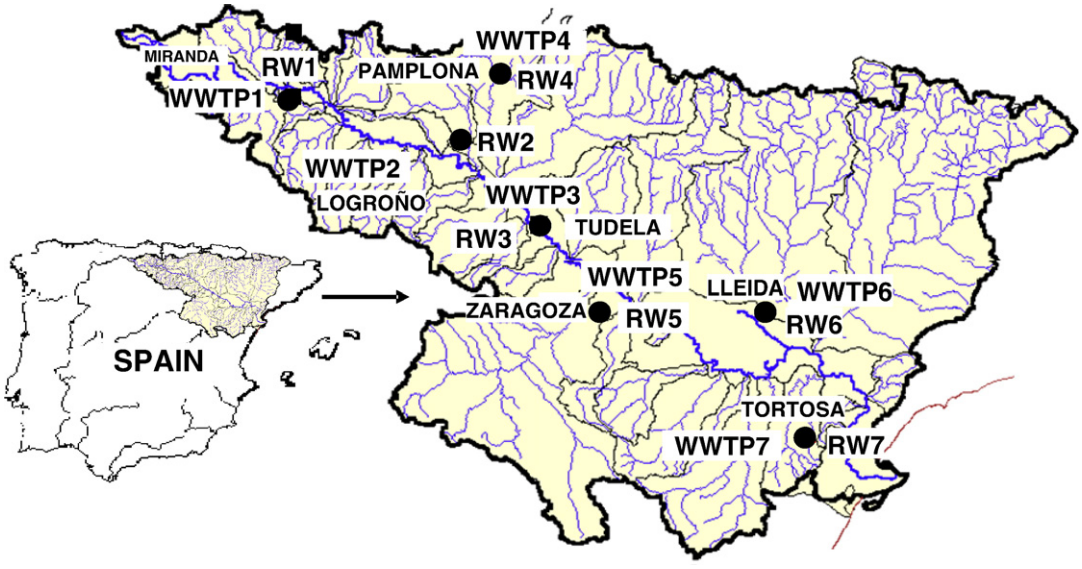

Fig. 1. Map of the sampling sites, indicating all wastewater treatment plants (WWTP) and river waters (RW) located downstream each plant.

v/v) mixture, were renewed before each analytical run. A separate mixture of isotopically labelled internal standards, used for internal standard calibration, was prepared in methanol and further dilutions also in methanol–water (25:75, v/v) mixture.

2.2. Sampling site, sample collection and pre-treatment

The Ebro river basin (northeast of Spain), (see Fig. 1), drains an area of approximately 85,000 km^2, ending in the Mediterranean Sea and forming a delta of more than 30,000 ha. The most relevant economic activity in the region is basically agriculture (vineyards, cereals, fruit, corn, horticulture and rice production), but there are also some highly industrialized regions, mainly located in the northern–central part, close to the cities of Zaragoza, Vitoria, Pamplona, Logroño, Monzón and Lleida. Around 2,800,000 inhabitants live in the area. Water quality and management along the basin is controlled by the Confederación Hidrográfica del Ebro (CHE). This organization performs regularly monitoring programs to survey and control the state of the basin. Among them, there is a monitoring network addressed to the control of regulated pollutants (priority and dangerous priority contaminants) under the provisions of Water Framework Directive (Directive 2000/60/EC, Decision 2455/2001/EC and Directive 2008/105/EC) and Directive 2006/11/CE (follow-up of the recently repealed Directive 76/464/EEC). To the author's knowledge, only one previous study reported the occurrence of 29 pharmaceuticals, belonging to different medicinal classes, along the Ebro river basin (Gros et al., 2007). In the light of the results obtained, a broader survey, including the analysis of a more extended list of 73 pharmaceuticals, was carried out covering four sampling periods, including June and November 2006, October 2007 and July 2008. As previously done (Gros et al., 2007) in waste and river waters downstream seven WWTP were monitored (see Fig. 1). Table 2 summarizes the characteristics of the WWTP studied as well as the river waters where their effluents are discharged. The majority of the plants have a primary and secondary treatment operating with conventional activated sludge, except one whose biological treatment is with biologic filters, but the main differences between them lie in their hydraulic retention times. Both time-averaged influent and effluent samples were collected to calculate removal rates of target compounds during treatment processes. Influents and effluents were 24-h composite samples whereas river waters were grab samples. Water samples were collected in 500 mL amber PET bottles, previously rinsed with ultrapure water. Once collected, samples were kept at 4 °C until arrival in the laboratory. Wastewaters were analyzed the day after while river waters were processed within a period no longer than one week.

Wastewaters were vacuum filtered through 1-μm glass fiber filters, followed by 0.45-μm, nylon membrane filters (Teknokroma, Barcelona, Spain). Otherwise, river waters were only filtered with 0.7-and 0-45-μm filters because of their lower amount of suspended particulate matter.

2.3. Analytical method

A multiresidue analytical method was previously developed to measure the 73 pharmaceuticals selected in both surface and wastewaters, as described elsewhere (Gros et al., 2009). Briefly, after filtration, an appropriate volume of aqueous solution of 5% Na_2EDTA were added to 500 mL of river water, 200 mL of effluent and 100 mL of influent wastewaters, respectively, to achieve a final Na_2EDTA concentration of 0.1% in the samples. The measured volumes were afterwards preconcentrated onto a lipophilic–hidrophilic balanced Oasis HLB (60 mg and 3 mL) cartridge, using a Baker vacuum system (J.T. Baker, Deventer, The Netherlands) at a flow rate of 5 mL/min. After sample preconcentration, cartridges were rinsed with 5 mL of HPLC grade water and were dried under vacuum for 15–20 min, to remove excess of water. Elution of target compounds was performed with 2×4 mL pure methanol. Extracts were evaporated to dryness under a gentle nitrogen stream and reconstituted with 1 mL of methanol–water (25:75, v/v). Finally, 10 μL of a 1 ng/μL standard mixture containing the internal standards were added in the extract for internal standard calibration.

Instrumental analysis was performed by liquid chromatography, using an Agilent HP 1100 HPLC (Palo Alto, CA, USA) system, equipped with an autosampler and connected in series with a 4000 QTRAP hybrid triple quadrupole-linear ion trap mass spectrometer operating with a Turbo Ion Spray source (Applied Biosystems-Sciex, Foster City, CA, USA). Chromatographic separation was achieved with a Purospher Star RP-18 endcapped column (125 mm×2.0 mm, particle size 5 μm) preceded by a C_{18} guard column (4×4, 5 μm), both supplied by Merck (Darmstadt, Germany). For the analysis in NI mode, eluent A was a mixture of acetonitrile–methanol (1:1, v/v) and eluent B was HPLC grade water at a flow rate of 0.2 mL/min, whereas the analysis in PI mode was performed using acetonitrile as eluent A and HPLC grade water with 0.1% formic acid as eluent B.

Quantification of target compounds was performed by SRM, monitoring two transitions between the precursor ion and the most abundant fragment ions for each compound. Further identification of target compounds in complex environmental waters elution gradients and method performance is described in detail elsewhere (Gros et al., 2009).

3. Results and discussion

3.1. Wastewater monitoring

It is well documented that WWTPs are major contributors of pharmaceuticals in the aquatic environment, since important loads are discharged into river waters through effluent wastewaters. This statement is supported by the information given in Table 3, which shows total loads of target pharmaceuticals in treated wastewaters that are afterwards discharged in receiving river waters. For each WWTP and sampling period, loads were calculated by multiplying total concentrations (addition of

Table 3
Total loads (indicated as g/day/1000 inhabitants) of target pharmaceuticals in each WWTP effluent, which are afterwards discharged into receiving river waters.

WWTP	Range loads	Average loads
WWTP1	[0.62–0.89]	0.76
WWTP2	[0.21–1.01]	0.72
WWTP3	[0.35–2.06]	1.18
WWTP4	[0.43–1.48]	0.89
WWTP5	[1.21–3.33]	2.52
WWTP6	[1.59–5.75]	2.73
WWTP7	[0.57–2.00]	1.08

In the table, the range of loads detected every sampling period and average values are indicated.

Table 2
Characteristics of the wastewater treatment plants (WWTP) monitored in the receiving waters where their effluents are discharged.

WWTP	Population equivalent	Flow (m^3/day)	Receiving river water	Type of wastewater treated	Hydraulic retention time (h)	Primary treatment	Secondary treatment
WWTP1	52700	10090–11395	Vallas	Urban	32	–	Activated sludge
WWTP2	466560	6000	Iregua	Urban and industrial	8	Primary settling	Activated sludge
WWTP3	65000–110000	16820–24680	Ebro	Urban	18	Primary settling	Biologic filters
WWTP4	721829–755205	95990–126749	Arga	Urban and industrial	9.5	Primary settling	Activated sludge
WWTP5	800000	169810–194600	Ebro	Urban	10	Primary settling	Activated sludge
WWTP6	162784–222049	45373–61705	Segre	Urban	6–10	Primary settling	Activated sludge
WWTP7	50000	5227–10064	Ebro	Urban	33	–	Activated sludge

individual concentrations) with the flow rates and then normalized by the population equivalent of each plant.

On the other hand, boxplots indicating levels found in both influent and effluent wastewaters, for some of the most representative pharmaceutical classes, and the ones detected at higher concentrations, are shown in Fig. 2(a) and (b). Concentration ranges for the missing groups are included in the supporting information (as Fig. 1 in SI). These graphics were built from 28 measures for both influent and effluent samples, corresponding to the addition of individual concentrations of each compound,

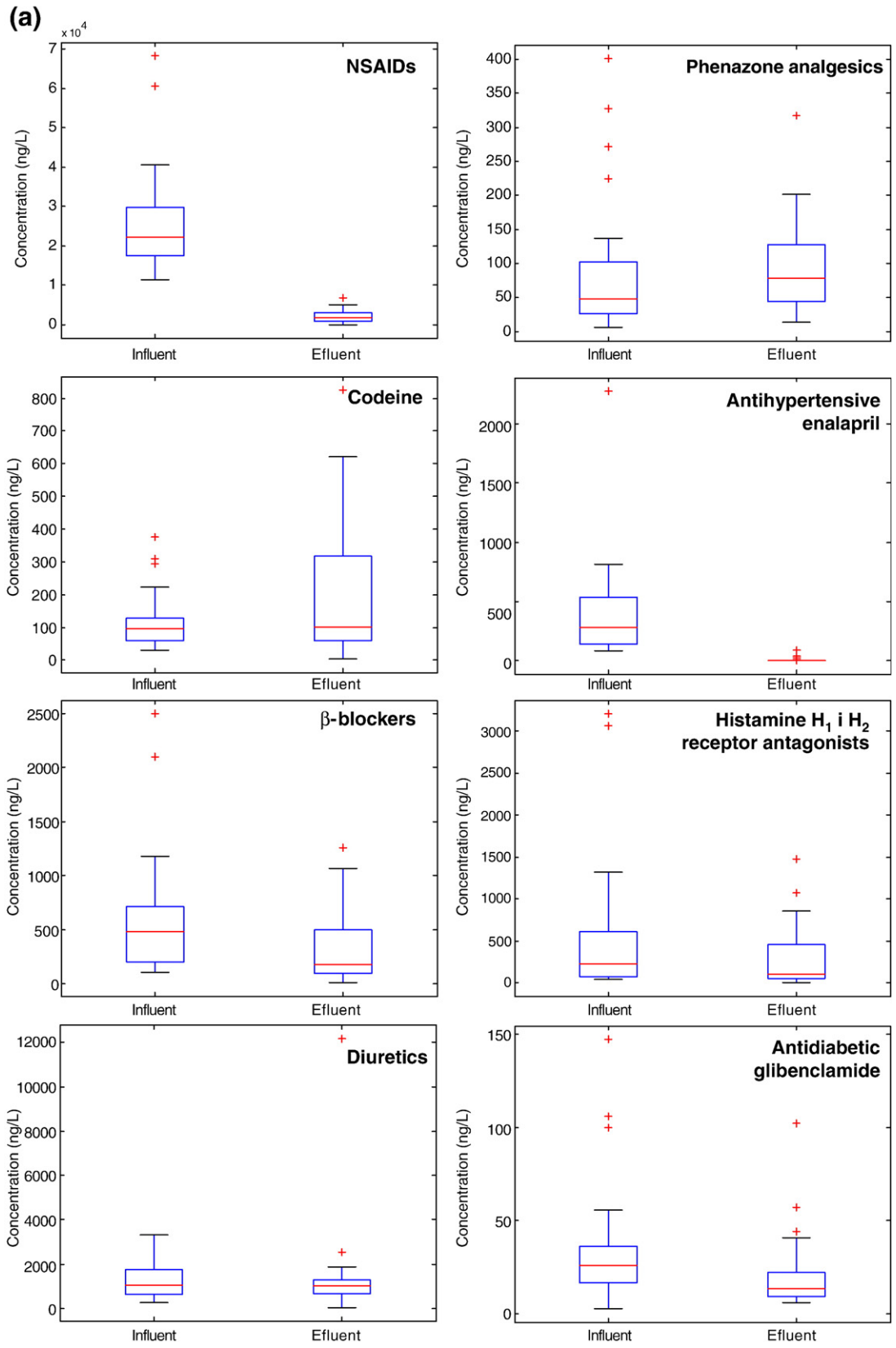

Fig. 2. Box plot indicating concentration ranges and median values of some of the most representative therapeutic groups included in the study, in both influent and effluent wastewaters. Each box plot includes 28 measures, which correspond to the sum of individual compound levels, of each therapeutic group, in all WWTP along all sampling campaigns.

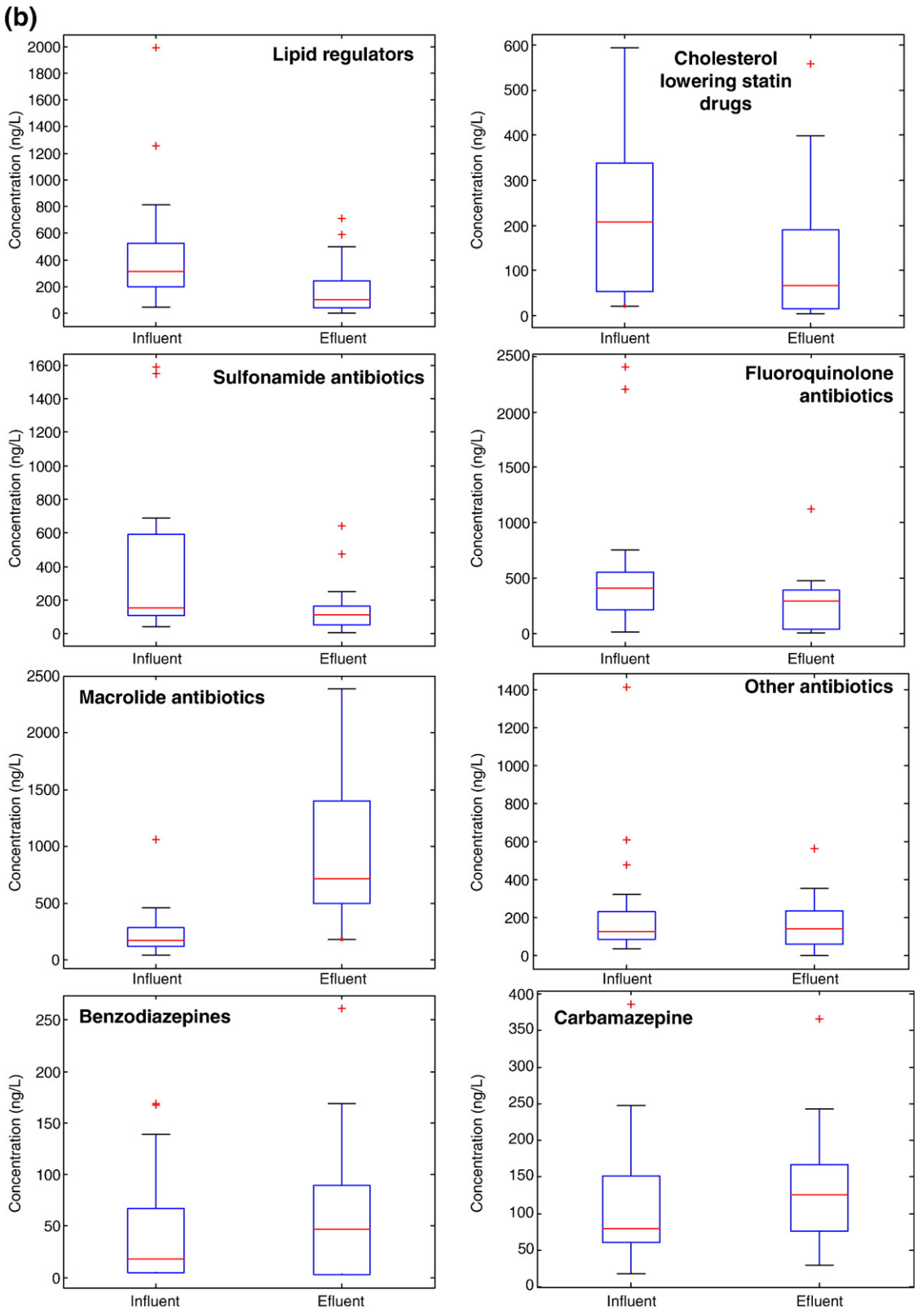

Fig. 2 (*continued*).

belonging to a determined therapeutic group, in all WWTP and including all sampling periods. For each variable, the box has lines at the lower quartile (25%), median (50%), and upper quartile (75%) values. The whiskers are the lines extending from each end of the box to show the extent of the data up to 1.5 times the interquartile range (IQR). Outlier values are marked with + symbols.

Furthermore, in order to have more detailed information about the ubiquity of single pharmaceuticals in the aquatic environment, the frequency of detection of each compound, taking into consideration all sampling campaigns, is indicated in Table 1. According to the boxplots, highest levels are observed for non-steroidal anti-inflammatory drugs (NSAIDs) and analgesics (5 μg/L in effluent and from 18 to 41 μg/L in influents). However, phenazone type analgesics and opiate analgesics (with the single contribution of codeine), presented lower total average concentrations, with general higher values around 200 ng/L in both influent and effluent. Compounds having major significance for the NSAIDs, in terms of both individual concentration (taking into

consideration data from all the WWTP) and frequency of detection, are acetaminophen, ibuprofen (with individual concentrations from 1 to 26 μg/L in influents) followed by naproxen, ketoprofen, salicylic acid and diclofenac. Lower but still significant individual levels were found for ketoprofen, naproxen, diclofenac, salicylic acid and indomethacine in influent wastewaters, with values ranging from 25–900 ng/L up to 1–7 μg/L. Conversely, concentrations in the outlets decreased considerably for all substances, from 10 ng/L up to 1 μg/L for ibuprofen, ketoprofen, naproxen, diclofenac and codeine and from around 10 to 100–200 ng/L and for salicylic acid.

Other groups showing considerably high total average concentrations were the antihypertensive enalapril, β-blockers, histamine H_2 receptor antagonists and the diuretics furosemide and hydrochlorothiazide (see boxplots). Compounds having a major role in the total average concentrations were hydrochlorothiazide for diuretics, atenolol for β-blockers and ranitidine for histamine H_2 receptor antagonists. While all of them presented similar individual concentrations in both matrices (from 50 to 1–3 μg/L), nadolol, sotalol, metoprolol, propranolol, timolol, famotidine, cimetidine and loratadine were found, generally, at levels one order of magnitude lower (from 10 to 100 ng/L and in some situations, even up to 100–200 ng/L).

Following these medicinal classes, other significant and ubiquitous groups were lipid regulators, cholesterol lowering statin drugs and antibiotics. Bezafibrate, being the most significant compound for the lipid regulators, was found in inlets at individual concentrations from 40 to 2 μg/L in inlets. However, its presence in the outlets decreased to values around half of the inlets. Even though the statin drugs pravastatin and atorvastatin were detected at similar individual concentrations than lipid regulators in effluents, lower concentrations were found in inlets (from 10 to 400 ng/L). Concerning to antibiotics, sulfamethoxazole, ofloxacin, ciprofloxacin, clarithromycin, azithromycin, spiramycin, metronidazole and trimethoprim were the compounds with major significance. Their individual level concentrations were in the same range as the ones detected for bezafibrate. Concerning psychiatric drugs, except carbamazepine, these compounds are found at much lower values (see boxplot), especially for serotonin reuptake inhibitors, with levels ranging from 2 to 20 ng/L. Finally, B-agonists and the anti-diabetic glibenclamide were found at higher levels than psychiatric drugs, but lower than the remaining groups (see boxplots).

3.2. Overall removal of pharmaceuticals during wastewater treatment

Modern WWTP can effectively accomplish carbon and nitrogen removal, as well as microbial pollution control. However, these installations receive also a large number of different trace organic polluting compounds, among them pharmaceuticals, for which conventional treatment technologies have not been specifically designed (Suárez et al., 2008). The term "removal" will be here used to refer to the conversion of a micropollutant to compounds other than parent compound. Pharmaceuticals may occur in WWTP effluents because they do not have or have low tendency to adsorb onto activated sludge or because their microbial degradation was not fast enough to be completed within the hydraulic retention time of the plants.

The range of removal rates (%RE) for the most representative compounds of each therapeutic group, in the whole set of WWTPs under investigation, is given in Table 4, altogether with their average %RE. It should be highlighted that, in the table, only pharmaceuticals showing positive removal rates were considered. Therefore, serotonin reuptake inhibitors, benzodiazepines, carbamazepine and macrolide antibiotics, (as described below), are not included. Additionally, removal rates for each therapeutic group were also evaluated, in each WWTP. Reported overall removal rates varied strongly between individual pharmaceuticals and therefore, it is difficult to establish a general trend for each one of the therapeutic groups, but in most of the cases, results indicate that elimination of most of the substances is incomplete. In a general extent, and linking the %RE of each therapeutic group with the results obtained in Table 4, three different behaviours were observed.

Table 4
Range of removal efficiencies (%RE), average (±%RSD) for some of the most representative pharmaceuticals of each therapeutic group in the whole set of WWTPs under investigation.

Compounds	Range of %RE	Average %RE (±RSD)
Sulfadiazine	[43–98]	69 (±32)
Sulfamethoxazole	[30–92]	74 (±22)
Norfloxacin	[30–98]	57 (±54)
Ofloxacin	[20–99]	40 (±64)
Ciprofloxacin	[37–99]	66 (±35)
Tetracycline	[40–89]	71 (±33)
Enalapril	[83–99]	96 (±11)
Salbutamol	[20–99]	60 (±44)
Famotidine	[30–99]	50 (±59)
Ranitidine	[50–98]	66 (±39)
Cimetidine	[30–99]	50 (±64)
Glibenclamide	[22–75]	46 (±39)
Nadolol	[25–99]	60 (±51)
Atenolol	[20–97]	59 (±50)
Bezafibrate	[23–99]	69 (±39)
Gemfibrozil	[30–99]	67 (±48)
Atorvastatin	[40–80]	58 (±44)
Propyphenazone	[30–87]	44 (±68)
Ketoprofen	[40–100]	69 (±40)
Naproxen	[60–100]	86 (±13)
Ibuprofen	[65–100]	91 (±13)
Diclofenac	[30–100]	58 (±53)
Acetaminophen	[96–100]	99 (±1)
Salicylic acid	[82–99]	96 (±8)
Furosemide	[20–96]	50 (±59)

(a) an increase in concentration along the passage through the WWTPs
Macrolide antibiotics, the anti-epileptic carbamazepine, benzodiazepines and serotonin reuptake inhibitors showed either poor or no elimination in all WWTP investigated, generally presenting higher concentrations in effluent wastewaters. These results are in good agreement with those reported in the literature. While Göbel and coworkers (Göbel et al., 2007) observed higher concentrations of several antibiotics (some sulphonamides, macrolides and trimethoprim) in effluent samples, the anti-epileptic carbamazepine followed the same behaviour in a study carried out by (Vieno et al., 2007), where the increase of carbamazepine concentration in effluent wastewaters was demonstrated to occur due to conversion of carbamazepine glucuronides and other conjugated metabolites to the parent compound by enzymatic processes taking place in the treatment plant. They confirmed this assumption by monitoring three mass transitions reported for carbamazepine-*N*-glucuronide in the LC-MS/MS system, finding intense peaks for the glucuronide in influent samples, which were afterwards hardly noticeable in effluent wastewaters (Vieno et al., 2007). In our case, since conjugates were not included in the analysis, no firm conclusion can be made about their biotransformation. However, this could be a plausible explanation for higher concentrations of these substances in the outlets.

(b) No significant to medium removal
Lipid regulators, fluoroquinolone, tetracycline antibiotics (when detected), cholesterol lowering statin drugs, histamine H_1 and H_2 receptor antagonists, β-blockers, β-agonists and the anti-diabetic glibenclamide were partially degraded, presenting average removal efficiencies between 40 and 60–70%. However, in some isolated situations (monitoring campaigns), they were not eliminated at all. For instance, the β-blockers metoprolol and propranolol were poorly (20%), (and in some cases not eliminated) removed in most of the WWTPs. Concerning the diuretics (furosemide and hydrochlorothiazide), their removal range is highly variable (see Table 4), with average elimination rates of 50% for furosemide and 32 % for hydrochlorothiazide. On the other hand, although sulphonamide antibiotics presented quite high average removal rates (around 70%), in some situations these values were lower (see Table 4). Regarding trimethoprim and metronidazole they were only quite efficiently removed in the plants with higher hydraulic retention times, with values ranging from 65 to 80% for both compounds. Finally, phenazone type analgesics and codeine (opiate analgesic) showed poor, or in some cases, no elimination, but propyphenazone presented average removal around 40%.

(c) High removal efficiency
Only NSAIDs and the antihypertensive enalapril would be fitted in this group. Whereas the former reported values ranging from 81 to 98%, enalapril was almost fully eliminated in all plants (%RE from 97 to 99%). The only exception was diclofenac, whose removal rates varied from no elimination up to 100%. These results are in good agreement with those reported by other authors (Carballa et al., 2008), who determined that the use of coagulants (ferric and aluminium salts) enhanced the removal of diclofenac up to 50–90% (Carballa et al., 2008).
Although it is not fully elucidated which factors could explain these deviations, since in many cases there are not enough operational data reported, it has been observed that, besides compound physico-chemical properties, other factors, regarding operational parameters of the plants, influence in a great extent removal during biological treatment (Suárez et al., 2008). These factors are: (i) temperature of operation (higher removal efficiencies have been observed in summer periods in comparison with colder seasons), (ii) different kinetic behaviours (degradation rates) of compounds, (iii) redox conditions and (iv) sludge retention time (SRT) and hydraulic retention time (HRT).
Linking removal rates with compounds half-lives ($t_{1/2}$) (compound degradation) and HRT of each WWTP, existing limitations of current treatments, regarding pharmaceutical removal, were demonstrated. Calculation of $t_{1/2}$ would provide more comprehensive information about compound persistence and would be also useful as an indicator of compound degradation rate and would give an idea about the required permanence time of the compounds in the biological reactor to ensure an efficient removal of the compound. In this way, half-lives were obtained from their relation with rate loss constants (k) through equation *(i)*, assuming that compound concentration decrease over time followed pseudo-first order kinetics. From the

kinetic point of view, this is a reasonable assumption since the concentration of pharmaceuticals is much lower than those of biological sludge.

$$t_{1/2} = \ln 2 / k \quad (1)$$

Rate loss constants (k) were calculated for each compound in each WWTP according to the formula:

$$\ln(C_{eff} / C_{in}) = -kt \quad (2)$$

where C_{eff} is the concentration of a particular compound detected in effluent wastewaters (which is assumed to be the final concentration after a certain time t, attributed to the hydraulic retention time of each plant), C_{in} correspond to influent concentrations (which are assumed to be the initial concentration) and t corresponds to the hydraulic retention time of each plant. In order to simplify the calculation and to obtain qualitative $t_{1/2}$, mean influent and effluent levels were used.

Half-lives and %RE for some of the most representative compounds detected in wastewaters in (a) a plant operating at high hydraulic retention time (HRT) and (b) in a WWTP working at low HRT are indicated in Fig. 3. According to the results reported, a minimum HRT is needed to accomplish the complete or high removal of pharmaceuticals. While in plants operating at lower HRT, compounds can not even accomplish the degradation of half of their initial concentration, which is translated into lower removal efficiencies, a totally different behaviour is observed in plant working at higher HRT. Therefore, low $t_{1/2}$ values (fast degradation) for non-steroidal anti-inflammatory drugs NSAIDs, the antihypertensive enalapril and lipid regulators (bezafibrate) suggest that total or high removal can be achieved within the HRT in all plants. However, higher $t_{1/2}$ for most of other groups (antibiotics, atenolol, salbutamol, famotidine, ranitidine, pravastatin, furosemide, glibenclamide, hydrochlorothiazide and propyphenazone) indicates that low to a medium percentage can be degraded at the operating HRT. More information is included in the supporting information (see Fig. 2 in SI) regarding the role of HRT in pharmaceutical removal. Taking into consideration some representative compounds in each WWTP three situations were observed: (i) compounds with high removal and degradation rate (low $t_{1/2}$) like all NSAIDs, except diclofenac and the antihypertensive enalapril and (ii) compounds with poor or no elimination and degradation (high $t_{1/2}$), like carbamazepine, HRT does not influence in compound removal and (iii) compounds with medium removal and degradation rate, where HRT seems to pay a role, since elimination rates were higher when increasing HRT. Therefore, in a great extent, it could be said that compounds that are biodegradable (high k_{iol} or $t_{1/2}$) and have low k_d values (low sludge–water distribution coefficient, which means that they show low tendency to absorb in sewage sludge) are more influenced by HRT, whereas substances that

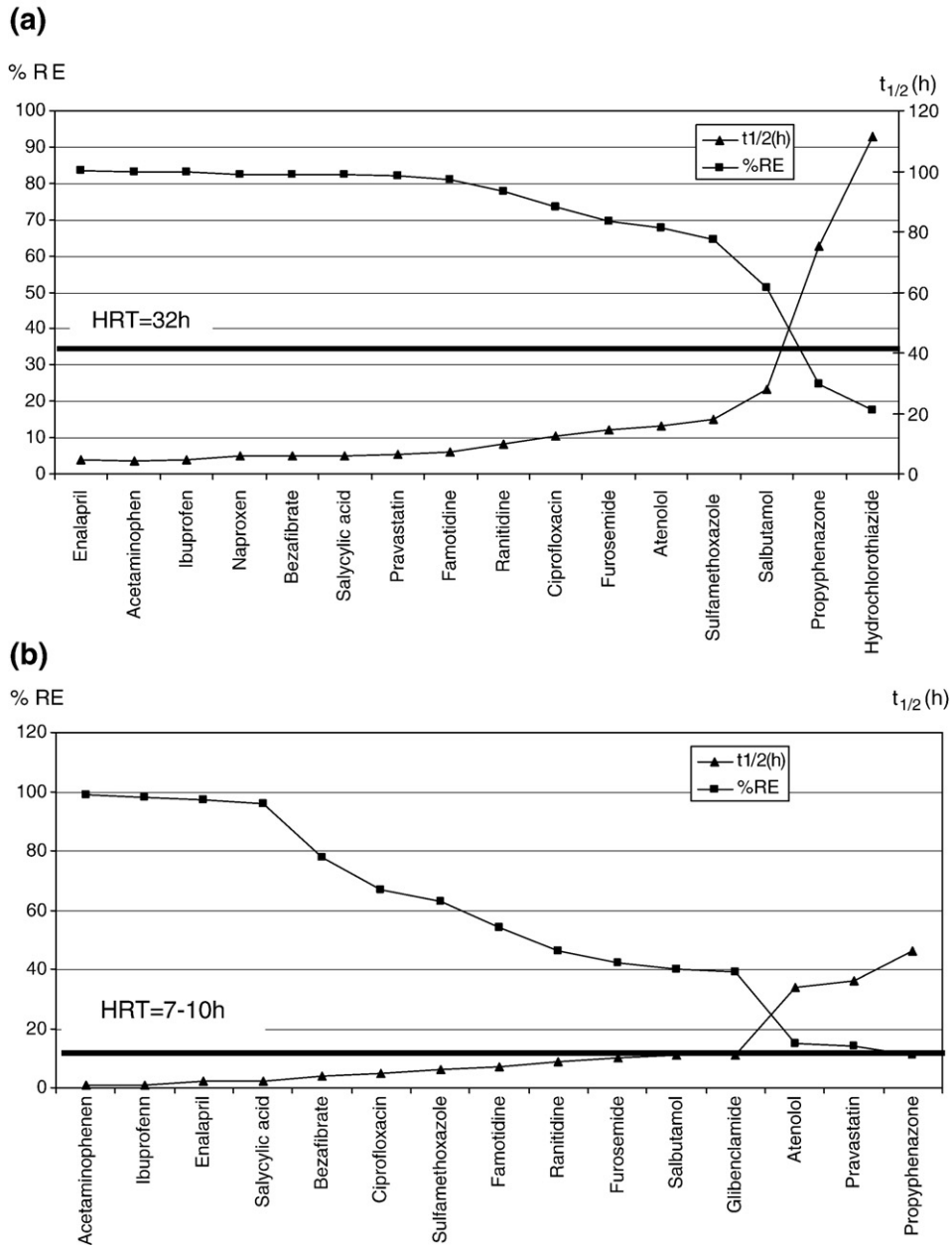

Fig. 3. Half-lives ($t_{1/2}$), expressed as hours (h) and removal efficiencies of some representative compounds in (a) a WWTP operating under high hydraulic retention time (WWTP1) and (b) WWTP operating with low hydraulic retention time (WWTP6).

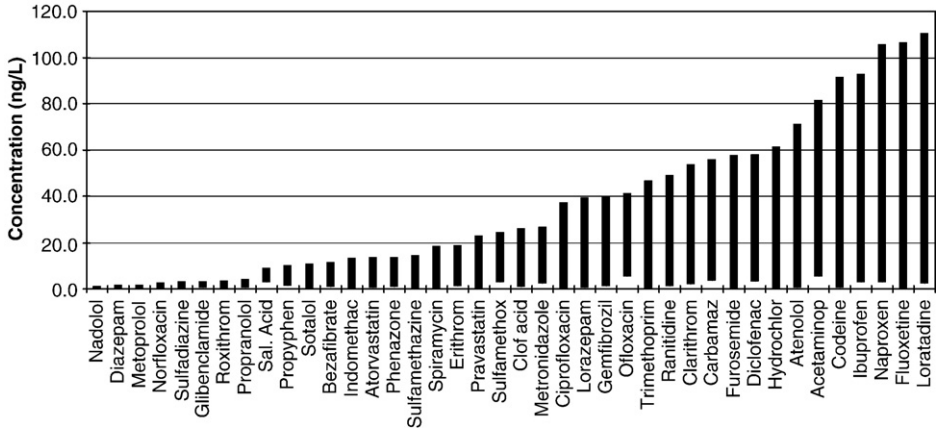

Fig. 4. Range of concentrations, expressed in ng/L, detected for the most representative pharmaceuticals in river waters.

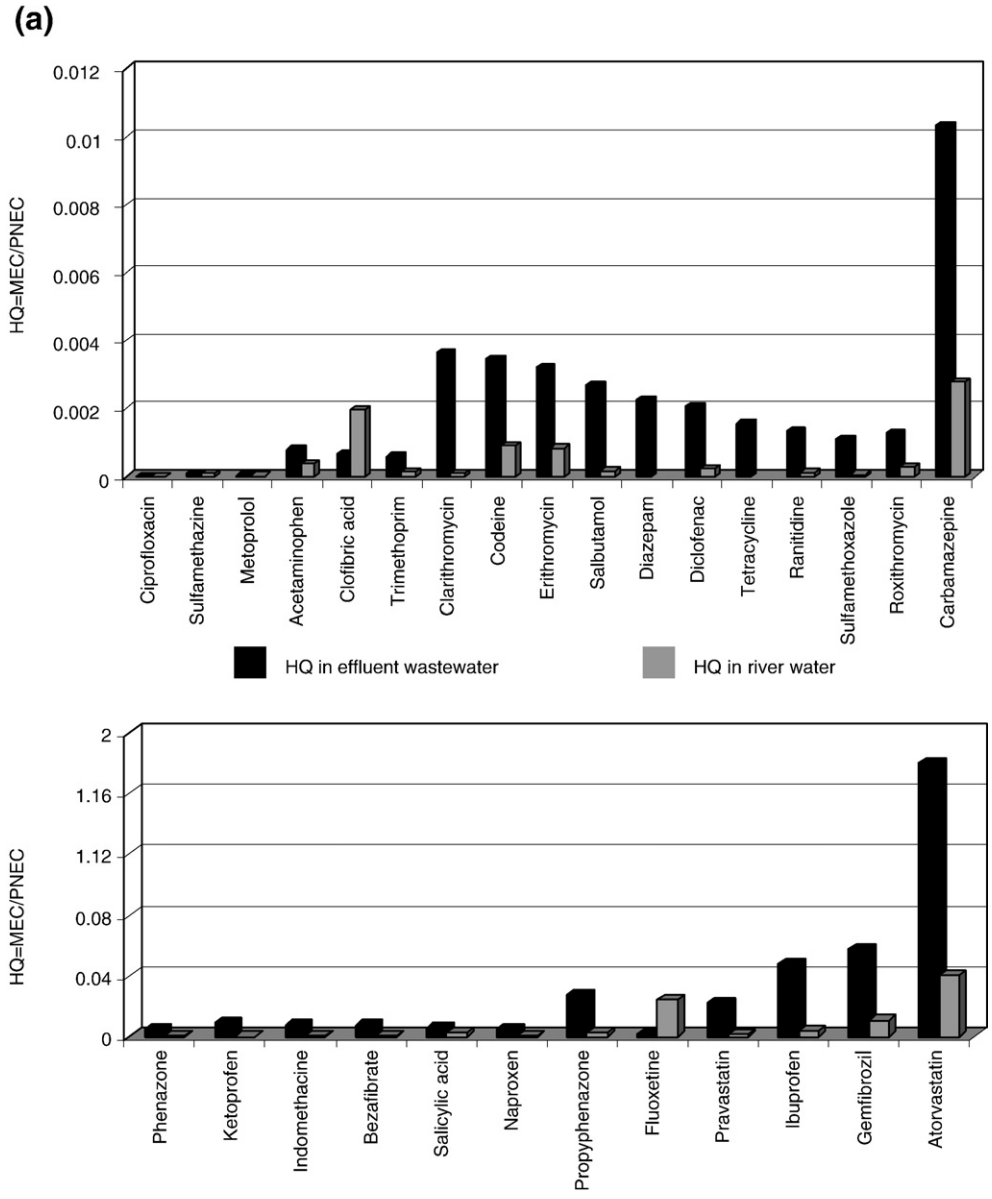

Fig. 5. Evaluation of hazards (hazard quotients, posed by pharmaceuticals detected in environmental waters towards (a) fish, (b) daphnids and (c) algae.

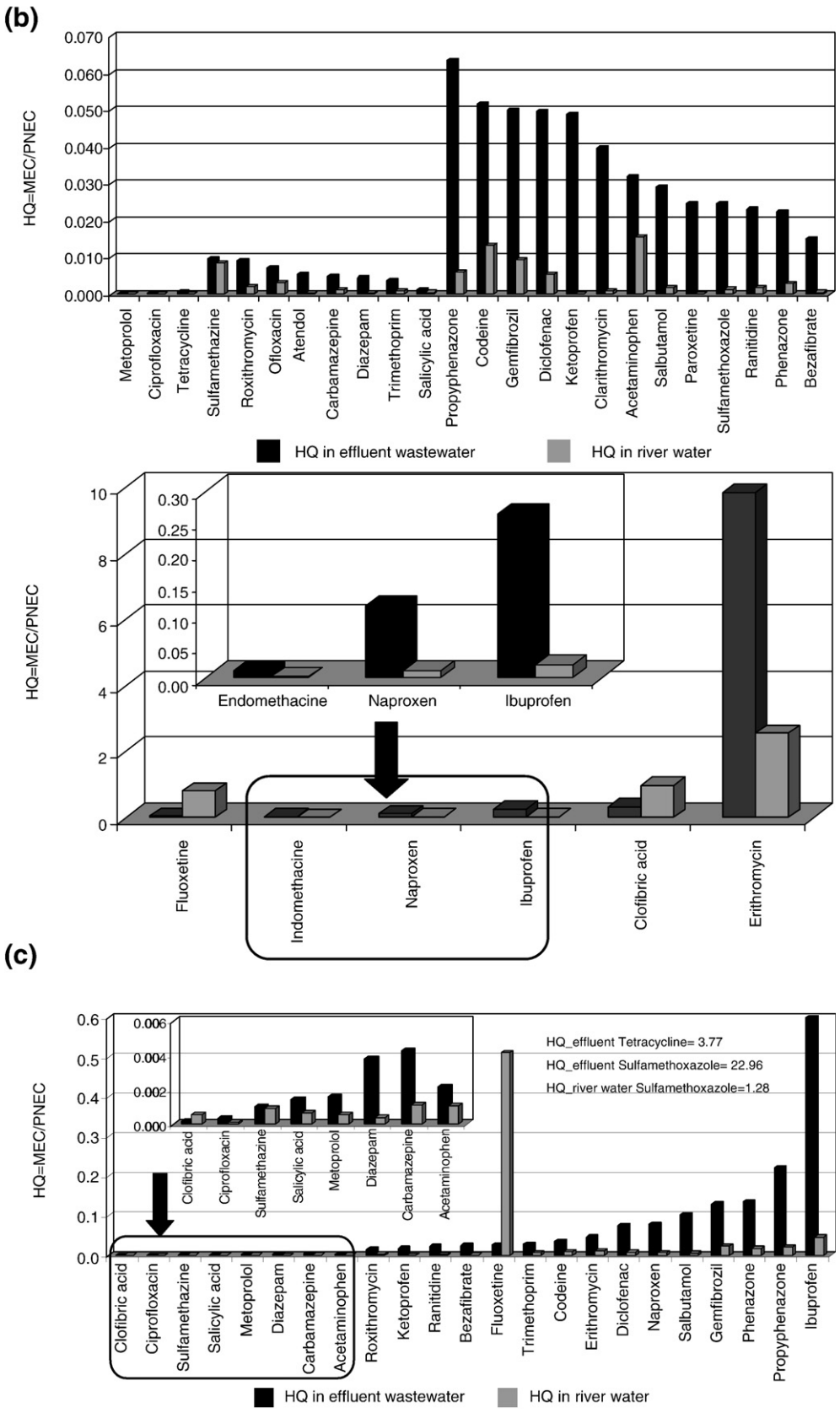

Fig. 5 (*continued*).

have high k_d and low k_{biol} are more influenced by SRT. However, there are substances, like ibuprofen and other analgesics and anti-inflammatories, which show high k_{biol} and k_d, that are very well removed independently of SRT and HRT.

From the results presented in this study, it can be concluded that HRT is a key parameter regarding pharmaceutical elimination. Nevertheless, as indicated before, there are other parameters influencing pollutants removal. Since data

about SRT was only available for two plants and all of them operated under similar reactor configurations, only the influence of HRT could be here discussed.

3.3. Entry of pharmaceuticals into the water cycle: occurrence in river waters

In Fig. 4, the range of concentrations of some of the most representative pharmaceuticals detected in river waters is represented. As indicated in the figure, pharmaceuticals more frequently detected in river waters coincide, in a great extent, with those that are more ubiquitous in effluent wastewaters. Therefore, compounds showing average and low removal rates are the ones more frequently found in receiving river waters. However, even though analgesics and anti-inflammatory drugs are highly removed after wastewater treatment (see previous Section), they are also ubiquitous and are present at considerable concentrations in river waters. This could be due to the fact that, although they are efficiently eliminated, concentrations in the inlets are so high, that levels that remain in the effluents are still significant. Nevertheless, the antihypertensive enalapril, which is also removed over 90% in all WWTP investigated, was never detected in river waters. This could be attributed to the dilution factor or that some attenuation due to abiotic processes, such as photo degradation, is taking place (Pérez et al., 2007).

Even though a wide spectrum of substances is detected, pharmaceuticals are considerably diluted when they enter river waters. Typical levels range from 10 to 100 ng/L while in effluent wastewater they are, generally, one order of magnitude higher, in the high ng/L range, even reaching, sometimes, low μg/L levels. This fact states that the dilution of pharmaceuticals when they enter river waters may reduce environmental risks posed by these compounds to aquatic organisms.

In order to confirm these assumptions, dilution factors were estimated for the sites where river flows were available. WWTP3, WWTP5 and WWTP7 discharge their effluents to the Ebro river, whilst WWTP1, WWTP2, WWTP4 and WWTP6 go to tributaries. Results indicated that dilution factor in the Ebro river is controlled (averaging 30 and 40). Conversely, when receiving river flows are lower, as for RW4 (river Arga in Pamplona), where wastewater effluents are discharged into a 9 m^3/s (year average) river, a totally different profile is observed, since compound concentration is only decreased to a factor of 5.

3.4. Ecotoxicological implications

Although it is very difficult to estimate if adverse effects to non target organisms will occur at environmental levels, the hazard quotient could be a useful measure that can be employed to characterize potential ecological risk of a stressor, in this case a pollutant (Kim et al., 2007). In most risk assessment approaches, based on EMEA guidelines, this quotient is calculated as the ratio between Predicted Environmental Concentrations (PEC) and Predicted No-Effect Concentrations (PNEC) (Grung et al., 2008; Huschek et al., 2004). However, other authors used Measured Environmental Concentrations (MEC) instead of PEC to evaluate risks posed by pharmaceuticals in a specific site (Santos et al., 2007). If this ratio is higher or equal to one, it suggests that this particular substance could cause potential adverse ecological effects.

In this context, risks towards algae, daphnids and fish, were evaluated in both river and effluent wastewaters, according to the water quality criteria fixed by the Water Framework Directive (Sanderson et al., 2003), which precludes the convenience of assessment using taxa of three different trophic levels of the ecosystem.

Fig. 5 summarizes hazard quotients (HQ) calculated as stated above. PNEC values were estimated for (a) fish, (b) daphnids and (c) algae from data literature on acute toxicity. Since data regarding chronic toxicity was lacking for many pharmaceuticals studied, acute toxicity values were used to calculate the PNEC for each substance. Specifically, dividing EC_{50} values by an arbitrary uncertainty factor, in this case, typically 1000, PNEC were derived (Sanderson et al., 2003). In fact, the lack of chronic toxicity data is a major hindrance to the effective risk assessment of pharmaceuticals, as they are most likely to induce chronic rather than acute toxic effects. However, the use of EC_{50} values, to predict PNEC, is widely used to estimate if levels detected would induce any adverse effect to aquatic organisms. Moreover, EC_{50} data for all substances was used in order to follow the same criteria for all pharmaceuticals when calculating PNEC values.

On the other hand, measured environmental concentrations (MEC) correspond to maximum levels detected for each compound in order to assess risks in the most extreme situations (with higher concentrations). Concentrations used to calculate HQ as well as EC_{50} values used in this study are given as Table 1 in the supporting information. It should be highlighted that when more than one EC_{50} value was available, only lower values were taken into consideration (Grung et al., 2008). EC_{50} values used are also indicated in the supporting information as Table 2.

According to the results shown in Fig. 5, the overall relative order of susceptibility was estimated to be algae > daphnia > fish in river water and effluent wastewaters. However, in river waters few substances were more sensitive to daphnia rather than algae. Results indicate that no risks could be associated to the presence of pharmaceuticals in surface waters. HQs higher than one in these matrices were associated to erythromycin, clofibric acid and fluoxetine for daphnia and sulfamethoxazole for algae. As expected, HQs in effluent wastewater were higher than those found in river water. Regarding wastewaters, only atorvastatin to fish, erythromycin to daphnia and sulfamethoxazole and tetracycline to algae posed an ecotoxicological hazard. Some substances presented values close to one, indicating that the margin of safety in these types of waters is narrow.

On this context, it could be concluded that dilution of wastewaters once pharmaceuticals are discharged in receiving river water efficiently mitigate possible environmental hazards.

This evaluation, however, is only focused on the toxicity that individual compounds may cause to aquatic organisms, but in the aquatic environment, pharmaceuticals are present as mixtures of a great variety of therapeutic classes, which should be taken into account when evaluating ecotoxicological effects (Pomati et al., 2008). Some studies, like those performed by Cleuvers (Cleuvers, 2004; Cleuvers, 2003) revealed that a mixture of pharmaceuticals induced toxicity at concentrations at which a single compound showed either no or only little effect.

Acknowledgments

This work has been supported by the EU project AQUATERRA (GOCE 505428) and by the Spanish Ministry of Science and Education Project CEMAGUA (CGL2007-64551). M Gros acknowledges her grant from the MSyE under the EVITA project (CTM2004-06265-C03-01). Merck is acknowledged for the gift of LC columns and Waters Corporation for the SPE cartridges. Staff from the WWTP are also acknowledged for their kindness and cooperation during the sampling.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:.10.1016/j.envint.2009.09.002.

References

Bound JP, Voulvoulis N. Predicted and measured concentrations for selected pharmaceuticals in UK rivers: implications for risk assessment. Water Res 2006;40(15):2885–92.
Carballa M, Omil F, Lema JM, Llompart M, Garcia C, Rodriguez I, et al. Behaviour of pharmaceuticals and personal care products in a sewage treatment plant of northwest Spain. Water Sci Technol 2005;52(8):29–35.
Carballa M, Omil F, Lema JM. Comparison of predicted and measured concentrations of selected pharmaceuticals, fragrances and hormones in Spanish sewage. Chemosphere 2008;72(8):1118–23.
Castiglioni S, Fanelli R, Calamari D, Bagnati R, Zuccato E. Methodological approaches for studying pharmaceuticals in the environment by comparing predicted and measured concentrations in River Po, Italy. Regul Toxicol Pharmacol 2004;39(1):25–32.
Cleuvers M. Aquatic ecotoxicity of pharmaceuticals including the assessment of combination effects. Toxicol Lett 2003;142(3):185–94.
Cleuvers M. Mixture toxicity of the anti-inflammatory drugs diclofenac, ibuprofen, naproxen and acetylsalicylic acid. Ecotoxicol Environ Saf 2004;59(3):309–15.
Cooper ER, Siewicki TC, Phillips K. Preliminary risk assessment database and risk ranking of pharmaceuticals in the environment. Sci Total Environ 2008;398(1–3):26–33.
Daughton CG, Ternes TA. Pharmaceuticals and personal care products in the environment: agents of subtle change? Environ Health Perspect 1999;107(SUPPL 6):907–38.
Göbel A, McArdell CS, Joss A, Siegrist H, Giger W. Fate of sulfonamides, macrolides, and trimethoprim in different wastewater treatment technologies. Sci Total Environ 2007;372(2–3):361–71.
Golet EM, Alder AC, Hartmann A, Ternes TA, Giger W. Trace determination of fluoroquinolone antibacterial agents in urban wastewater by solid-phase extraction and liquid chromatography with fluorescence detection. Anal Chem 2002;73(15):3632–8.
Gros M, Petrovic M, Barcelo D. Wastewater treatment plants as a pathway for aquatic contamination by pharmaceuticals in the Ebro river basin (Northeast of Spain). Environ Toxicol Chem 2007;26(8):1553–62.
Gros M, Petrovic M, Barcelo D. Tracing pharmaceutical residues of different therapeutic classes in environmental waters by using liquid chromatography/quadrupole-linear ion trap mass spectrometry and automated library searching. Anal Chem 2009;81(3):898–912.
Grung M, Kallqvist T, Sakshaug S, Skurtveit S, Thomas KV. Environmental assessment of Norwegian priority pharmaceuticals based on the EMEA guideline. Ecotoxicol Environ Saf 2008;71(2):328–40.
Huschek G, Hansen PD, Maurer HH, Krengel D, Kayser A. Environmental risk assessment of medicinal products for human use according to European Commission recommendations. Environ Toxicol 2004;19(3):226–40.
Kim Y, Choi K, Jung J, Park S, Kim PG, Park J. Aquatic toxicity of acetaminophen, carbamazepine, cimetidine, diltiazem and six major sulfonamides, and their potential ecological risks in Korea. Environ Int 2007;33(3):370–5.
Pérez S, Eichhorn P, Barceló D. Structural characterization of photodegradation products of enalapril and its metabolite enalaprilat obtained under simulated environmental conditions by hybrid quadrupole-linear-ion trap MS and quadrupole-time of flight MS. Anal Chem 2007;79:8293–300.
Petrovic M, Hernando MD, Díaz-Cruz MS, Barceló D. Liquid chromatography-tandem mass spectrometry for the analysis of pharmaceutical residues in environmental samples: a review. J Chromatogr 2005;1067(1–2):1-14.
Pomati F, Orlandi C, Clerici M, Luciani F, Zuccato E. Effects and interactions in an environmentally relevant mixture of pharmaceuticals. Toxicol Sci 2008;102(1):129–37.
Radjenovic J, Petrovic M, Barcelo D. Analysis of pharmaceuticals in wastewater and removal using a membrane bioreactor. Anal Bioanal Chem 2007;387(4):1365–77.
Radjenovic J, Petrovic M, Barcelo D. Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. Water Res 2009;43(3):831–41.

Sanderson H, Johnson DJ, Wilson CJ, Brain RA, Solomon KR. Probabilistic hazard assessment of environmentally occurring pharmaceuticals toxicity to fish, daphnids and algae by ECOSAR screening. Toxicol Lett 2003;144(3):383–95.

Santos JL, Aparicio I, Alonso E. Occurrence and risk assessment of pharmaceutically active compounds in wastewater treatment plants. A case study: Seville city (Spain). Environ Int 2007;33(4):596–601.

Suárez S, Carballa M, Omil F, Lema JM. How are pharmaceuticals and personal care products (PPCPs) removed from urban wastewaters? Rev Environ Sci Biotechnol 2008;7(2):125–38.

Vieno N, Tuhkanen T, Kronberg L. Elimination of pharmaceuticals in sewage treatment plants in Finland. Water Res 2007;41(5):1001–12.