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Assessing the diurnal variability of pharmaceutical and personal care products in a full-scale activated sludge plant

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ABSTRACT

An intensive sampling campaign has been carried out in a municipal wastewater treatment plant (WWTP) to assess the dynamics of the influent pharmaceutical active compounds (PhAC) and musks. The mass loadings of these compounds in wastewater influents displayed contrasting diurnal variations depending on the compound. The musks and some groups of PhACs tended to follow a similar diurnal trend as compared to macropollutants, while the majority of PhACs followed either the opposite trend or no repeatable trend. The total musk loading to the WWTP was 0.74 ± 0.25 g d^{-1}, whereas the total PhAC mass loading was 84.7 ± 63.8 g d^{-1}. Unlike the PhACs, the musks displayed a high repeatability from one sampling day to the next. The range of PhAC loadings in the influent to WWTPs can vary several orders of magnitude from one day or week to the next, representing a challenge in obtaining data for steady-state modelling purposes.

1. Introduction

Pharmaceuticals and personal care products (PPCP) are xenobiotics that can be detected in wastewater treatment plants (WWTP) at the influent, effluent and in waste sludges (Ternes et al., 1999, 2005; Kolpin et al., 2002; Richardson and Ternes, 2005; Zuccato et al., 2005; Jones et al., 2007; Miège et al., 2009; Santos et al., 2009; Sim et al., 2010). Many studies have been previously performed concerning the occurrence and fate of PPCPs in WWTPs from various countries. However, only few studies have attempted to assess the variability that can be expected in the diurnal PPCP loadings in the influent to WWTPs (Joss, et al., 2005; Gobel et al., 2005; Weissbrodt et al., 2009; Plósz et al., 2010). Wastewater treatment plants are known to receive discharges that vary widely according to the time of day. Diurnal variations (in particular peak loads) are important to consider for successful WWTP design and operation, not only with respect to flow, but also the pollutant loading rates in order to achieve sufficient effluent quality. Further, peak pollutant loads can have toxic or inhibitory impacts on the WWTP sludge. The inhibition of heterotrophs and nitrifiers by pharmaceutical active compounds has previously been shown (Dokianakis et al., 2004; Carucci et al., 2006; Wang et al., 2008) and can reduce treatment efficiency.

The typical dry-weather diurnal variations of organic matter, ammonia, phosphate and wastewater flow consist of a relatively large morning peak, smaller variations throughout the day and low levels overnight (Tchobanoglaus and Burtan, 1995; Almeida et al., 1999). It is still unknown if a similar-type pattern exists with respect to PPCP concentrations, and if so, how repeatable this pattern is from one day to the next. Such information is important in order to incorporate PPCP compounds into WWTP models.

Thus far, the studies that have addressed the diurnal variability of PPCPs in the influent of WWTPs have generally observed lower overnight concentrations, consistent with the wastewater flow variation (Joss et al., 2005; Gobel et al., 2005; Weissbrodt et al., 2009; Plósz et al., 2010). In most of these studies, samples were collected after primary clarification, dampening the effect of diurnal flow and PPCP loading variations. However, since the monitoring plan of WWTPs generally requires that pollutant characterisation is done at the sewer entrance of the plant (and treated effluent), it is desirable to understand quantitatively how raw influent loadings fluctuate diurnally, and indeed, how repeatable is this assessment from one day to the next in a WWTP.

In this study, 79 PPCPs, including 73 pharmaceutical active compounds (PhACs) of different families and 6 musk fragrances, have been monitored for characterising their diurnal variation in

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Table 1
Range of concentration, mean concentration, and frequency of detection of the 20 most frequently detected PhACs and musks analysed. Limits of detection and quantification, as well as the relative recovery are also shown.

Compound	Human effect	LOD (ng L^{-1})	LOQ (ng L^{-1})	Relative recovery	Week 1						Week 2					
					Monday			Tuesday			Monday			Tuesday		
		($n = 10$)	($n = 10$)	WW (%) ($n = 3$)	Range (ng L^{-1})	Mean (ng L^{-1})	Freq of detection	Range (ng L^{-1})	Mean (ng L^{-1})	Freq of detection	Range (ng L^{-1})	Mean (ng L^{-1})	Freq of detection	Range (ng L^{-1})	Mean (ng L^{-1})	Freq of detection
Diclofenac	NSAID	7	24	65 ± 6	n.d.–26,598	10,898	11/12	11,299–64,479	38,674	12/12	1257–16,963	4534	12/12	n.d.–11,742	5456	11/12
Etofenamate***	NSAID	20	67	89 ± 4	n.d.	n.d.	0/12	n.d.–40,168	7444	8/12	n.d.–3164	1541	11/12	n.d.–2979	1507	10/12
Ibuprofen*	NSAID	14	46	70 ± 2	n.d.–52,201	9102	7/12	235–13,905	4476	12/12	358–1795	1059	12/12	n.d.–1298	562	9/12
Ketoprofen*	NSAID	21	69	102 ± 13	1106–29,496	28,269	12/12	47–104,114	9255	12/12	n.d.–211	71	8/12	n.d.–250	85	8/12
Clorazepate***	Anxiolitic	17	57	92 ± 2	n.d.	n.d.	0/12	n.d.–3332	416	5/12	n.d.–427	249	11/12	n.d.–463	316	11/12
Hydroxyzine***	Antihistamine	18	60	73 ± 5	n.d.	n.d.	0/12	n.d.	n.d.	0/12	162–1168	470	12/12	n.d.–570	216	8/12
Indapamide***	Antihypertensive	6	18	86 ± 1	n.d.	n.d.	0/12	n.d.	n.d.	0/12	n.d.–15,386	2668	8/12	n.d.–6737	3476	11/12
Enalapril***	Antihypertensive	8	28	88 ± 3	n.d.	n.d.	0/12	n.d.	n.d.	0/12	n.d.–4162	696	8/12	n.d.–6244	1532	10/12
Captopril***	Antihypertensive	5	15	66 ± 10	n.d.–978	82	1/12	n.d.–509	96	3/12	n.d.–4231	784	9/12	n.d.–2267	1015	11/12
Atenolol***	β-blocker	3	10	115 ± 6	n.d.–4341	362	1/12	n.d.–427	36	1/12	141–944	476	12/12	77–1474	815	12/12
Clofibric acid**	Lipid mod. agent	15	50	96 ± 12	n.d.–41,428	8461	6/12	137–1602	602	12/12	116–722	276	12/12	n.d.–1723	442	7/12
Estrone***	Estrogen	18	60	104 ± 12	n.d.	n.d.	0/12	n.d.	n.d.	0/12	n.d.–177	82	12/12	n.d.–73	28	7/12
Ampicillin***	Antibiotic	3	11	68 ± 3	n.d.	n.d.	0/12	n.d.–126	11	1/12	n.d.–252	157	10/12	n.d.–240	100	7/12
Paroxetine***	Antidepressant	27	89	86 ± 12	n.d.	n.d.	0/12	n.d.–39,732	9676	11/12	n.d.–1927	251	10/12	n.d.	n.d.	0/12
Fluoxetine***	Antidepressant	17	57	41 ± 3	n.d.	n.d.	0/12	n.d.–3465	359	4/12	n.d.–971	219	6/12	n.d.–3090	946	10/12
Galaxolide	Musk	1	1	94 ± 2	119–1698	478	12/12	152–526	396	12/12	50–2780	465	11/11	53–617	204	11/11
Tonalide	Musk	1	1	82 ± 3	57–270	124	12/12	85–421	178	12/12	23–816	140	11/11	18–190	72	11/11
Cashmeran	Musk	1	1	83 ± 3	97–1275	599	12/12	127–2477	1362	12/12	93–4040	1447	11/11	66–2151	698	11/11
Celestolide	Musk	2	2	85 ± 4	6–428	216	12/12	288–1442	470	12/12	30–885	149	11/11	19–308	101	11/11
Traseolide	Musk	2	2	85 ± 4	35–165	87	12/12	125–676	207	12/12	7–309	53	11/11	10–79	37	11/11

n.d. – not detected.
Differences in frequency of detection (occurrences per samples analysed) between sampling days are: *statistically significant ($p < 0.05$), **very significant ($p < 0.01$), ***highly significant ($p < 0.001$).

the raw influent prior to primary sedimentation. This analysis was performed along 2 days per week over 2 consecutive weeks at a municipal WWTP in Portugal in order to investigate the repeatability of the profiles. The goals of this study were to assess the diurnal variations of PPCPs in the influent, as well as the reproducibility of the mass loading of PPCPs detected during the different days and weeks analysed. To the best of our knowledge, this is the first study to investigate the diurnal variations of this broad range of PPCPs in the raw influent of municipal wastewater.

2. Materials and methods

2.1. Influent WWTP sampling and sample preservation

The influent samples were collected at the WWTP of Fernão Ferro (Seixal, Portugal), which has a design capacity for 32,700 population equivalents and treats 2790 $m^3\,d^{-1}$ of domestic municipal wastewater. Two consecutive 48 h periods over two successive weeks have been monitored during dry-weather conditions. Samples of the influent (1 L) were collected on Monday from 10am until Wednesday 10am (a sample was collected every 2 h) using a refrigerated auto-sampler. The samples were transported to the laboratory in a refrigerated isothermal container and immediately extracted and stored at −20 °C until the analysis was performed. The samples were prepared for analysis of two different classes of pharmaceutical compounds (acidic and neutral) and of polycyclic musk fragrances according to the procedures described below.

2.2. Chemicals and reagents

HPLC-grade acetonitrile, methanol, and formic acid were purchased from Panreac (Portugal). Ultrapure water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). All of the pharmaceutical active compound (PhAC) standards were purchased from Sigma–Aldrich (Steinheim, Germany), while the musks were purchased from LGC-Promochem (Spain). Stock solutions (1 $mg\,mL^{-1}$) of each PhAC or musk were prepared and diluted in methanol or *n*-hexane, respectively. All samples were analysed in triplicate.

2.3. Extraction and analysis

2.3.1. Acidic and neutral pharmaceutical compounds

Solid-phase extraction (SPE) was used for clean-up and concentration of the samples, as detailed in Salgado et al. (2010). All wastewater samples were filtered through glass fibre filters (GF 6, <1 μm pore diameter, Whatman, England). 350 mL of filtered wastewater were spiked with an internal standard (meclofenamic acid) to a final concentration of 100 $\mu g\,L^{-1}$. SPE was carried out on the filtered and spiked wastewater samples with Waters Oasis HLB cartridges (60 mg, 30 μm, Waters, Eschborn, Germany) for the acidic pharmaceutical compounds, while Waters RP-C18 cartridges (500 mg, 50 μm, Waters, Milford, U.S.) were used for the neutral compounds. The pH of the samples was adjusted to 2 for the acidic compounds and 7 for the neutral compounds. The wastewater samples were passed through the cartridges at a flow rate of approximately 20 $mL\,min^{-1}$. The solid-phase material was then dried through a continuous nitrogen stream for 1 h and then the analytes were eluted four times with 1 mL methanol (total 4 mL). The extracts were evaporated to 1 mL by a gentle nitrogen stream.

The acidic and neutral PhAC extracts were analysed through high performance liquid chromatography coupled to mass spectrometry (HPLC-MS). For HPLC, a reverse-phase column was employed (LiChroCART 250-4 Purospher Star RP18 endcapped, 5 μm column, Merck) using a degassed mobile phase of water/formic acid 0.1% (A) and acetonitrile (B). The following binary gradient was used: 2.01 min, 15% B at 0.6 $mL\,min^{-1}$; 20 min, 100% B at 0.6 $mL\,min^{-1}$; 25 min, 100% B 1.0 $mL\,min^{-1}$; 27 min, 15% B at 1.0 $mL\,min^{-1}$ and 35 min, 15% B at 0.6 $mL\,min^{-1}$. The HPLC system (Waters) was coupled with a pump and controller (Waters 600), an in-line degasser (X-Act-4 channels, Jour Research), an auto-sampler (Waters 717 plus), a photodiode array detector (DAD, Waters 996, used at 200–400 nm) and a quadrupole VG Platform (Micromass, UK Ltd) spectrometer equipped with an electrospray ionisation (ESI) source operating in positive mode. An accurate splitter (split ratio of 1:10) was used between the HPLC column and the mass spectrometer. Capillary temperature was kept between 100 °C and 120 °C, using a scanning cone voltage from 35 to 100 V and capillary voltage of 3.5 kV. Nitrogen was used as drying and nebulising gas at 300 $mL\,min^{-1}$ and 10 $mL\,min^{-1}$, respectively. Spectra mass/charge range used was 100–450 Da with a MassLinx™ software data acquisition system.

2.3.2. Polycyclic musk fragrances

The extraction of the musks was carried out by headspace solid-phase microextraction (SPME) using fibres coated with 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB, Supelco, Spain). Two grams of wastewater sample were combined with 0.5 g NaCl and spiked with Mirex (internal standard) to a concentration of 100 $\mu g\,L^{-1}$ in 8 mL glass vials with magnetic stirring. The PDMS/DVB fibre was exposed to the sample headspace in the sealed glass vial for 15 min at 90 °C. The fibre was inserted into the injection port of the GC-MS during 3 min, where the volatile compounds were desorbed.

Analysis was performed using a Hewlett-Packard 5890 gas chromatographer fitted with a QMD1000 Carlo Erba mass spectrometric detector (GC-MS). The injection port was operated in splitless mode. A DB-5MS fused-silica capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness, Agilent-J&W Scientific, Spain) was used, with helium as carrier gas at a flow rate of 1.5 $mL\,min^{-1}$. The injection port temperature was 250 °C. The ion source and the transference line were kept at 200 and 310 °C respectively. The oven temperature was maintained at 60 °C for 3 min, raised to 250 °C at 10 °C min^{-1}, and then to 310 °C at 20 °C min^{-1}, where it was held for 13 min. The MS spectra were obtained with electron energy 70 eV, mass range m/z 50–500 and using MassLab™ software (Micromass).

2.4. Determination of recovery, LOD and LOQ

Recoveries, limits of detection (LOD) and limits of quantification (LOQ) were determined as detailed in Salgado et al. (2010). In brief, for determination of the recovery percentages, samples were spiked with standards of each of the PPCPs studied (100 $\mu g\,L^{-1}$ in methanol) and an internal standard (meclofenamic acid, also at 100 $\mu g\,L^{-1}$). After homogenisation for 30 min, the extraction and analysis was performed as detailed above. Relative recoveries were determined relative to Milli-Q water.

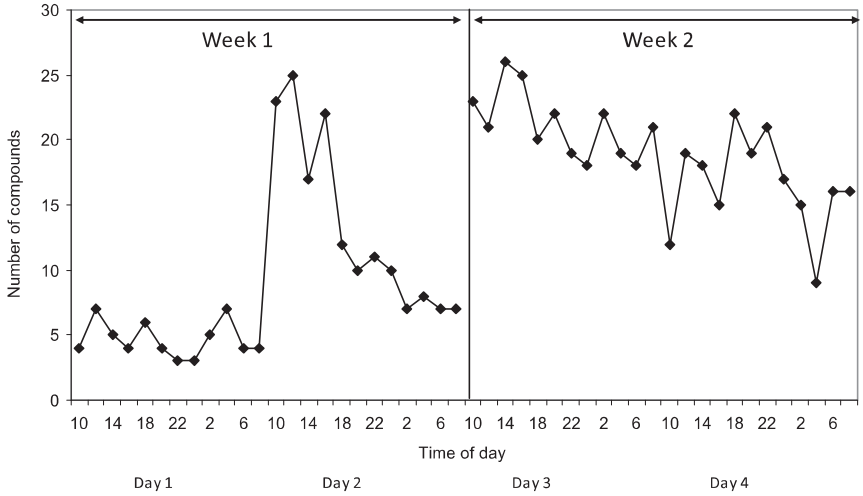

Fig. 1. Number of pharmaceutical compounds detected throughout the sampling campaign of 4 days: 2 consecutive Mondays and Tuesdays (mean and standard deviation: 1st week: Monday 5 ± 1; Tuesday 13 ± 7; 2nd week: Monday 21 ± 3; Tuesday 17 ± 4).

Table 2
Range of concentration, mean concentration, and frequency of detection of infrequently detected PhACs and musks. Limits of detection and quantification are indicated where available.

Compound	Human effect	LOD ($ng\,L^{-1}$)	LOQ ($ng\,L^{-1}$)	Monday			Tuesday			Monday			Tuesday		
				Range ($ng\,L^{-1}$)	Mean ($ng\,L^{-1}$)	Freq of detection	Range ($ng\,L^{-1}$)	Mean ($ng\,L^{-1}$)	Freq of Detection	Range ($ng\,L^{-1}$)	Mean ($ng\,L^{-1}$)	Freq of detection	Range ($ng\,L^{-1}$)	Mean ($ng\,L^{-1}$)	Freq of detection
Allopurinol	Gout treatment	22	73	n.d.–8234	1498	3/12	n.d.	n.d.	0/12	n.d.	n.d.	0/12	n.d.	n.d.	0/12
Dimethyl Phenazone	Analgesic; antiinflamatory	29	95	n.d.	n.d.	0/12	n.d.–221	18	1/12	n.d.	n.d.	0/12	n.d.	n.d.	0/12
Paracetamol	Analgesic	3	9	n.d.	n.d.	0/12	n.d.–342	29	1/12	n.d.–91	8	1/12	n.d.–266	22	1/12
Codeine	Analgesic	–	–	n.d.	n.d.	0/12	n.d.–351.2	55	3/12	n.d.	n.d.	0/12	n.d.	n.d.	0/12
Acethylsalicilic acid	Analgesic	–	–	n.d.	n.d.	0/12	n.d.	n.d.	0/12	n.d.–172	28	2/12	n.d.–404	88	3/12
Caffeine	CSN stimulant	27	91	n.d.–11,751	1047	2/12	n.d.–2380	287	2/12	n.d.–4684	1205	10/12	n.d.–9175	1389	3/12
Omeprazole	Proton pump inhibitor	–	–	n.d.	n.d.	0/12	n.d.–148	21	3/12	n.d.	n.d.	0/12	n.d.	n.d.	0/12
Domperidone	Antidopaminergic	3	9	n.d.–9145	1108	2/12	n.d.–77,349	19,150	12/12	n.d.	n.d.	0/12	n.d.	n.d.	0/12
Propranolol	β-blocker	4	15	n.d.	n.d.	0/12	n.d.–23,446	2840	4/12	n.d.	n.d.	0/12	n.d.	n.d.	0/12
Ramipril	Congestive heart failure	9	31	n.d.	n.d.	0/12	n.d.	n.d.	0/12	n.d.–2265	227	3/12	n.d.	n.d.	0/12
Betamethasone	Corticosteroid (SAID)	6	20	n.d.–61	5	1/12	n.d.–64	10	2/12	n.d.–343	145	7/12	n.d.	n.d.	0/12
Carbamazepine	Antiepileptic	2	7	n.d.	n.d.	0/12	n.d.–1950	461	4/12	n.d.	n.d.	0/12	n.d.	n.d.	0/12
Nimesulide	NSAID	14	46	n.d.	n.d.	0/12	n.d.–671	70	3/12	n.d.	n.d.	0/12	n.d.	n.d.	0/12
Naproxen	NSAID	18	59	n.d.	n.d.	0/12	n.d.–244	37	4/12	n.d.	n.d.	0/12	n.d.–78	16	3/12
Flurbiprofen	NSAID	18	58	n.d.–15,480	4564	5/12	n.d.–1777	299	4/12	n.d.–18,608	3645	5/12	n.d.–5385	752	6/12
Indomethacin	NSAID	7	23	n.d.	n.d.	0/12	n.d.–1639	254	5/12	n.d.–686	99	6/12	n.d.	n.d.	0/12
Fentiazac	NSAID	–	–	n.d.	n.d.	0/12	n.d.–317	–	1/12	n.d.–208	25	2/12	n.d.	n.d.	0/12
β-estradiol	Estrogen	4	12	n.d.	n.d.	0/12	n.d.–937	97	4/12	n.d.	n.d.	0/12	n.d.–750	95	4/12
17-α-ethynylestradiol	Estrogen	21	69	n.d.	n.d.	0/12	n.d.–76	7	2/12	n.d.–80	39	10/12	n.d.	n.d.	0/12
Escitalopram	Antidepressant	14	47	n.d.–32,228	3506	2/12	n.d.	n.d.	0/12	n.d.	n.d.	0/12	n.d.–1104	381	8/12
Salbutamol	B2-adrenergic receptor antagonist	11	36	n.d.	n.d.	0/12	n.d.	n.d.	0/12	n.d.	n.d.	0/12	n.d.–1301	317	4/12
Budesonide	Corticosteroid (asthma)	21	69	n.d.	n.d.	0/12	n.d.–12,302	1051	2/12	n.d.–191	16	1/12	n.d.–51	4	1/12
Fluticasone	Glucocorticosteroid (asthma)	25	85	n.d.	n.d.	0/12	n.d.–7809	658	2/12	n.d.–569	67	4/12	n.d.–96	23	5/12
Tramadol	Opiod centrally action	20	67	n.d.	n.d.	0/12	n.d.	n.d.	0/12	n.d.	n.d.	0/12	n.d.–1130	94	1/12
Furosemide	Loop diuretic	19	63	n.d.	n.d.	0/12	n.d.	n.d.	0/12	n.d.–801	116	2/12	n.d.	n.d.	0/12
Alprazolam	Ansiolitic, tranquilizer	–	–	n.d.	n.d.	0/12	n.d.–4705	548	6/12	n.d.–89	12	2/12	n.d.–153	66	9/12
Oxazepam	Ansiolitic	–	–	n.d.	n.d.	0/12	n.d.	n.d.	0/12	n.d.–36	–	1/12	n.d.–155	22	2/12
Bromazepam	Ansiolitic, tranquilizer	–	–	n.d.	n.d.	0/12	n.d.	n.d.	0/12	n.d.–36	5	2/12	n.d.	n.d.	0/12
Amoxicillin	Antibiotic	13	43	n.d.	n.d.	0/12	n.d.	n.d.	0/12	n.d.–569	252	9/12	n.d.	n.d.	0/12
Azithromycin	Antibiotic	3	11	n.d.	n.d.	0/12	n.d.	n.d.	0/12	n.d.	n.d.	0/12	n.d.–210	97	8/12
Ciprofloxacin	Antibiotic	1	3	n.d.	n.d.	0/12	n.d.–22,074	1840	1/12	n.d.	n.d.	0/12	n.d.–15,397	3985	4/12
Telmitarsen	Angiotensive; hypertension	–	–	n.d.	n.d.	0/12	n.d.–20	3	2/12	n.d.–187	18	2/12	n.d.	n.d.	0/12
Tiaprofencic acid	NSAID	–	–	n.d.	n.d.	0/12	n.d.–1175	131	3/12	n.d.–914	303	9/12	n.d.	n.d.	0/12
Lovastatin	Antideslipidemic	–	–	n.d.	n.d.	0/12	n.d.	n.d.	0/12	n.d.–251	145	10/12	n.d.	n.d.	0/12
Clofibrate ethyl	Lipid modifying agent	–	–	n.d.–15,568	1398	3/12	n.d.–917	111	6/12	n.d.–56	20	6/12	n.d.–44	11	4/12
Salicilic acid	Analgesic	–	–	n.d.	n.d.	0/12	n.d.	n.d.	0/12	n.d.–2780	561	5/12	n.d.	n.d.	0/12
Penincillin G	Antibiotic	14	47	n.d.	n.d.	0/12	n.d.	n.d.	0/12	n.d.–127	11	1/12	n.d.	n.d.	0/12

n.d. – not detected.

Table 3
PhACs and musks that were never detected. Limits of detection and quantification are indicated where available.

Compound	Human effect	LOD (ng L^{-1})	LOQ (ng L^{-1})	Compound	Human effect	LOD (ng L^{-1})	LOQ (ng L^{-1})
Sertraline	Antidepressant	–	–	Reserpine	Antiadrenergic agent	8	28
Digoxin	Cardiac glycoside	25	83	Warfarin	Anticoagulant	–	–
Diazepam	Ansiolitic, tranquilizer	–	–	Progestin/Progesterone	Steroid contraceptive hormone	2	6
Diltiazem	Hypertensive, calcium blocker	6	18	Extasy	Psychoactive drug	18	61
Glibenclamide	Diabetes type II treatment	6	20	Tetrahydrocannabinol	Psychoactive drug	17	58
Latanoprost	Ocular hypertension (glaucoma)	–	–	Triprolidine	Antihistaminic	–	–
Lorazepam	Ansiolitic	–	–	Zolpiden	Insomnia treatment	–	–
Nifedipine	Calcium blocker	19	62	Mexazolam	Ansiolitic; tranquilizer	–	–
Phenazone	NSAID	7	22	Valerian	Ansiolitic; tranquilizer	–	–
Piroxicam	NSAID	1	4	Mirtazepine	Antidepressant	–	–
Ranitidine	Histamine H2 receptor antagonist	–	–	Phantolide	Musk	1	1

The LOD for each of the PPCPs were calculated by the formula 3 SD/m, where SD is the standard deviation of the lowest signal/noise ratio of the analyte and m is the slope of the calibration curve. The LOQ were calculated as 10 SD/m. Ten blanks were analysed by LC/MS (with methanol) and GC-MS (with n-hexane) to determine the lowest signal/noise ratio of each analyte.

2.5. Statistical analysis

Contingency tables were used to determine if the results obtained in this study with respect to the frequency of detection of the PhACs was statistically significant from one day to the next. The entries of the contingency tables for each compound consisted of the number of detection and non-detection events in each of the four sampling days (12 sampling events per day). The corresponding χ^2-test for each compound was performed and the results are presented in Table 1. Further detail regarding this analysis is provided in the supplementary material.

3. Results and discussion

3.1. Most frequently detected compounds

The number of PhACs detected in each sample over the four days (2 consecutive Mondays and Tuesdays) of the campaign is plotted in Fig. 1. It can be observed that a small, relatively constant number of compounds was found throughout the first day (5 ± 1), which increased to 23 compounds at 10am of Day 2. After 4pm, the number of compounds detected decreased steadily to 10–12 until midnight, where afterwards it decreased further overnight (7–8 compounds). In the second week, a high number of compounds was detected throughout Day 3 (21 ± 3) and Day 4 (17 ± 4). The difference in the number of compounds observed between the 1st week and the 2nd week illustrates the high variability of PhACs, and the difficulty in obtaining a repeatable assessment of the compounds being discharged to the WWTP.

While different compounds were detected at different times of the day, some compounds were detected more frequently than others. The most frequently detected compounds are shown in Table 1, while those infrequently detected are listed in Table 2 and those never detected are listed in Table 3. From Table 1, it can be observed that diclofenac, ibuprofen, ketoprofen and clofibric acid were frequently detected at the WWTP influent during each sampling day. Etofenamate, clorazepate, hydroxyzine, indapamide, enalapril, captopril, atenolol, ampicillin, fluoxetine and estrone were all commonly detected in the second week of sampling, but were either not detected or were seldomly detected during the first week. Paroxetine was commonly detected on the first Tuesday (Day 2) and second Monday (Day 3) of the campaign, but not detected on the other two sampling days. Interestingly, 5 of the 6 musks studied (galaxolide, tonalide, cashmeran, celestolide and traseolide) were present in every sample analysed during the campaign. It should also be noted that the differences observed in the frequency of detection from one day to another were statistically significant for 14 of the 15 PhACs analysed (Table 1). This reflects the large differences in the PhAC composition of the wastewater observed between each sampling day.

3.2. Diurnal variations of the PhACs and musks in the WWTP

The diurnal variations of the total concentration of PhACs and musks detected in the filtered influent are shown in Fig. 2a and b, respectively. In general, the total concentration of musks tended to follow a trend where higher concentrations were observed during the day and low concentrations were observed at night (with the exception of 2 outliers, the 4am sample on Day 3 and the 24 h

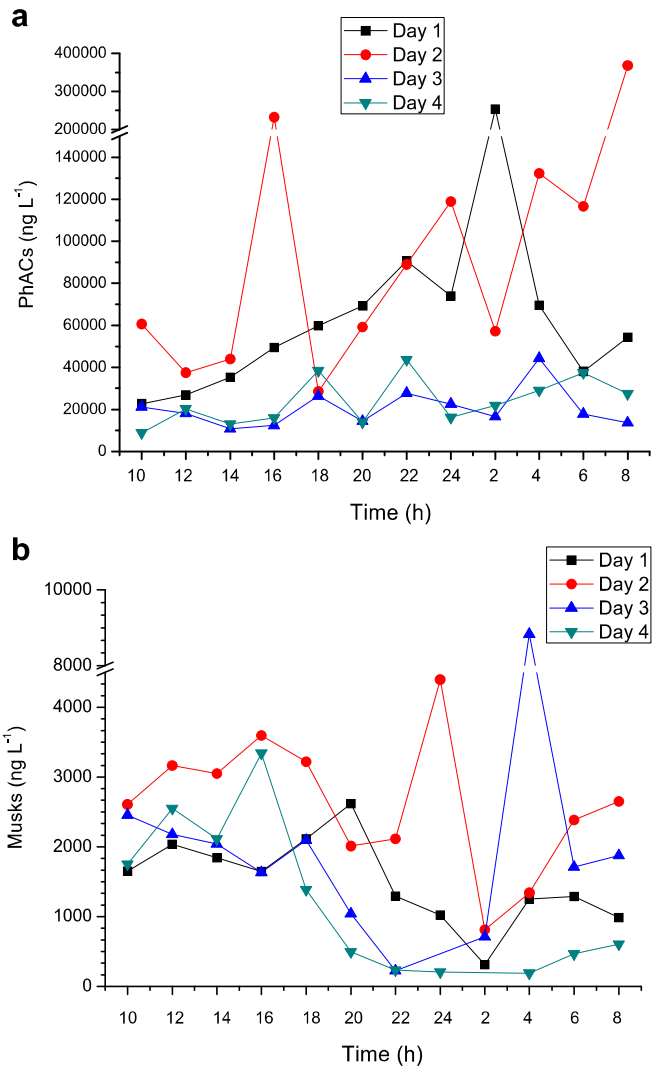

Fig. 2. Diurnal variations of the total concentration of PhACs (a) and the total concentration of musks (b) in the influent to the WWTP during each day of the campaign.

sample on Day 2). Further, this trend was repeatable between the 4 sampling days, and the total concentration of musks measured was in a similar range in each case. However, the PhAC profile observed throughout each day was less repeatable as compared to the musks. On Days 1 and 2 (Monday and Tuesday from the first week of sampling), there appeared to be a higher PhAC concentration in the evening or night as compared to during the day. On Days 3 and 4 (Monday and Tuesday from the second week of sampling), there was a lower total concentration of PhAC compounds throughout the day with much smaller fluctuations. The reason for this difference is unclear, since the sampling strategy and weather conditions were consistent during each sampling day.

Fig. 3 shows the relative contribution of the main PhAC (a) and musk (b) compounds towards the total concentration detected. It can be observed that diclofenac and ketoprofen were the most abundant of the analysed compounds, and usually responsible for the peak concentrations that were occasionally observed. Throughout the four sampling days analysed, diclofenac comprised an average of 40 ± 24% of the total PhAC concentration and was regularly present in relatively high levels. This correlates well with sales data from the official Portuguese database (INFARMED, 2005), where diclofenac showed the highest sales when compared to the other compounds detected in this study. Ketoprofen was present in high abundance more sporadically than diclofenac, comprising 36 ± 24% of the PhAC concentration on Day 1, but only 1 ± 1% on the other 3 days, excluding the 8am sample on Day 2 (36%). Overall, NSAIDs (such as diclofenac and ketoprofen) were the family of PhACs detected in highest abundance (55 ± 21%), which is consistent with findings from some literature studies (Comeau et al., 2008; Lin and Tsai, 2009; Miège et al., 2009). Cashmeran was the dominant musk detected, forming on average 52 ± 18% of the total musk concentration, while galaxolide was the second most abundant musk (22 ± 10%), with both compounds consistently present in relatively high abundance.

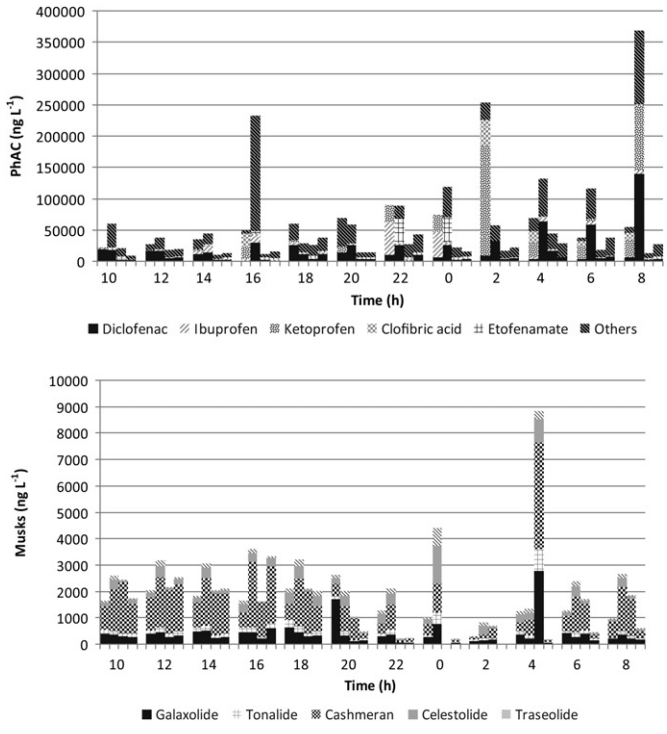

Fig. 3. Mean concentrations of the most frequently detected PhACs and musks throughout each sampling day (Days 1, 2, 3 and 4 are plotted for each 2 h-sample).

While a small number of PhACs generally constituted the bulk of the total PhAC concentration, occasionally some compounds that were only rarely detected appeared at high levels. One example of this happened at 16 h on Day 2, where omeprazole was primarily responsible for the PhAC peak load observed at this time (67% of the total PhAC concentration).

Table 4 shows the diurnal variations in the influent throughout the periods of 8–16 h (day), 16–24 h (evening) and 0–8 h (night). It can be observed that the total PhACs did not display a particular trend, while the musks were more abundant during the day. Also, the overnight mass loadings were generally quite low for the musks, with the exception of a peak load at 4am on Day 3. This pattern is similar to the wastewater flow. The WWTP influent flow rate (Table 4) shows that the wastewater flow was almost constant during the day, however, a notable decrease in flow was observed at night. Nevertheless, the total PhAC mass flow does not follow this pattern.

Fig. 4 shows the relative contribution of the 15 most frequently detected PhACs throughout the sampling days. Eight of these 15 compounds average less than 25% of their total mass flow during the night period, including ibuprofen, etofenamate, clorazepate, atenolol, captopril, ampicillin, estrone and hydroxyzine. Etofenamate presented the lowest average mass flow of the PhACs at night (9 ± 8%), which could be related to the fact that this compound is administered as a gel, lotion or cream. This is similar to the musk compounds, and correspondingly, etofenamate appears to follow the same diurnal pattern as most musks. Four compounds (ketoprofen, indapamide, paroxetine, enalapril) averaged >50% of the total mass flow during the night period, thus displaying the opposite diurnal profile. The other three compounds (diclofenac, clofibric acid and fluoxetine) did not display any repeatable diurnal trend. The differences observed between the diurnal patterns of these PhACs likely reflect the varying frequencies of administration by consumers. Indeed, PhACs are administrated for specific medical reasons and the demand for these compounds is highly variable with time, as opposed to musks, which are more often used as part of routine hygiene habits.

3.3. Mean and peak concentration of the PhACs and musks

The variability of the mean concentration of the 20 most commonly detected PhACs and musks for the 4 days of the campaign is shown in Fig. 5. It can be observed that the 5 musks presented a relatively small variability in the mean concentration among the different days of the campaign as compared to the PhACs. The total musk loading to the WWTP was 0.74 ± 0.25 g d^{-1}, showing a 34% relative standard deviation. By comparison, the total PhAC mass loading was 84.7 ± 63.8 g d^{-1}, a relative standard

Table 4
Diurnal variations of total pharmaceuticals and musks during the periods of 8–16 h, 16–24 h and 0–8 h for each day of the campaign.

	Time	% of total mass flow		
		8–16 h	16–24 h	0–8 h
PhACs	Day 1	19	40	41
	Day 2	33	25	42
	Day 3	29	41	30
	Day 4	23	44	33
	Average	**26 ± 6**	**38 ± 8**	**36 ± 6**
Musks	Day 1	43	41	16
	Day 2	43	39	17
	Day 3	38	20	42
	Day 4	74	17	9
	Average	**50 ± 16**	**29 ± 13**	**21 ± 14**
Flow	m^3 h^{-1}	147 ± 11	143 ± 18	103 ± 27

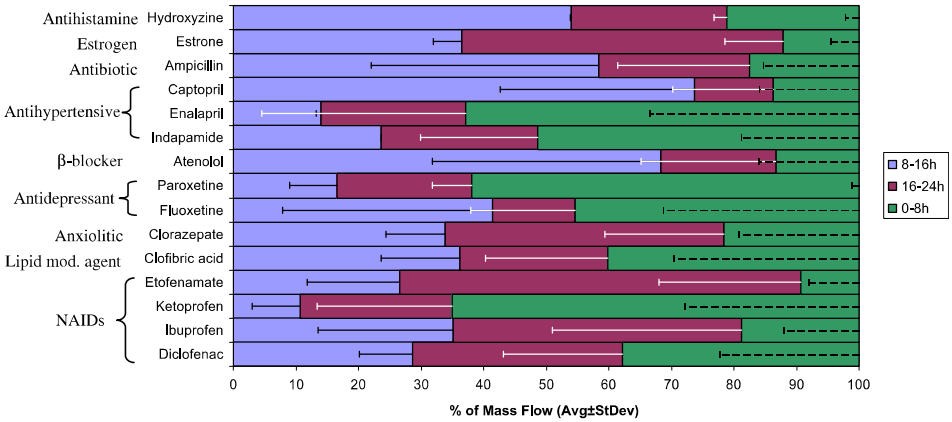

Fig. 4. Average percent of PhAC mass flow that were detected during the periods of 8–16 h, 16–24 h and 0–8 h, for different families of the most frequently detected PhACs. Error bars indicate the standard deviation found for the 4 sampling days.

deviation of 75%. Of the 5 PhACs that were detected each day of the campaign (diclofenac, ibuprofen, ketoprofen, clofibric acid and atenolol) the mean concentration varied between 1 and 3 orders of magnitude. Nine of the remaining 10 PhACs shown in Fig. 5 were detected in both days of the second week, showing generally consistent mean concentrations for each compound on these days. Eight of these nine compounds were not detected during Days 1 and 2, thus, the loading of PhACs changed significantly for this WWTP from one week to the next. This implies that frequent sampling campaigns will be required over time to obtain a representative description of the PhAC loading to the WWTP for e.g. modelling purposes.

In contrast, relatively little variability was observed for the musk compounds detected in this study. The differences in the mean concentration from each day were always far less than one order of magnitude apart. The occurrence of musks was in the same concentration range or lower than the PhACs (Fig. 5), showing that the higher repeatability observed with these compounds was not due to higher abundance. Rather, the fact that these compounds were ubiquitously present in the influent is likely reflective of their more regular and widespread usage than PhACs. This implies that obtaining a representative description of the musk compounds entering the WWTP is a far simpler task as compared to the PhACs.

The ratio of the maximum concentration detected to the mean concentration (max/mean) provides an indication of the occurrence of peak loads as well as their relative magnitude. Fig. 6 shows the ratio of max/mean for the top 20 most frequently detected PhACs and musks. In general, the max/mean values are more variable for the PhACs as compared to the musks. For the musks, the max/mean was between 2 and 4 for three of the four sampling days, while a value above 6 was observed for most musks on Day 3, corresponding to the peak load at 4am of that day. For the PhACs, the max/mean ratio of the samples from the first week generally varied more than the second week, which was more consistent. The max/mean of ketoprofen approached the theoretical maximum on Day 2 (11.2) despite the fact that this compound was detected in every sample analysed on this day. This shows that the ketoprofen maximum peak load on Day 2 (8am) far outweighed the concentration observed throughout the rest of the day; indeed, the concentration of this sample was greater than 2 orders of magnitude higher than the average ketoprofen concentration of the remaining samples. Paroxetine and clorazepate also displayed high variability in the max/mean ratio, where the highest value observed was approximately 8. On Day 1, captopril was only detected once, while atenolol was only detected once during Days 1 and 2, thus, at these times the max/mean ratios were the theoretical maximum for these two compounds.

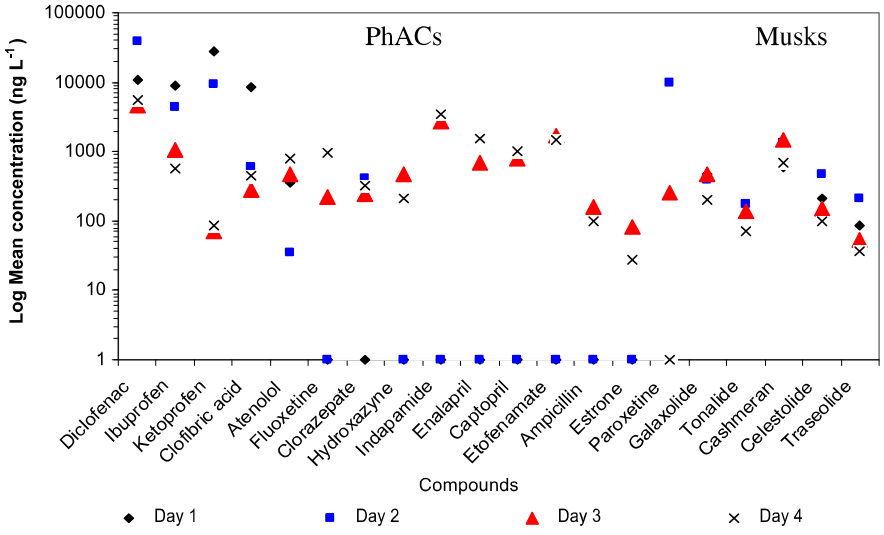

Fig. 5. Mean concentrations of the most frequently detected PhACs and musks during each sampling day of the campaign.

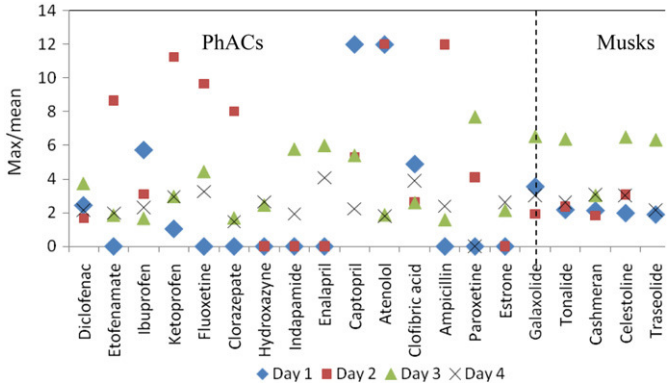

Fig. 6. Max/mean concentrations of the most frequently detected pharmaceuticals and musks for the 4 days of the campaign. Since 12 samples were analysed per day, the highest possible max/mean is 12, while the lowest possible ratio is 1, unless the compound was not detected on that day, in which case the max/mean value is represented as zero.

The max/mean generally ranges between 2 and 3 for BOD, suspended solids (SS), nitrogen and phosphorus in the influent to WWTPs (Tchobanoglaus and Burtan, 1995). While shock loads of these macropollutants are also known to occur occasionally, there is often a more repeatable pattern as compared to micropollutants such as PPCPs. As seen from Figs. 3 and 6, the influx of most PhACs do not follow a repeatable pattern and peak loads of different compounds appear to occur sporadically. However, the results of this study suggest that musk compounds do, in general, follow the typical pattern exhibited by macropollutants (i.e. higher loadings throughout the day, lower at night) and are quite repeatable over the 4 sampling days, although occasionally higher peak loads can also be expected, such as those observed for most musks during Day 3 (max/mean ~ 6).

3.4. Implications of the results and comparison with literature

Upon comparing the results from this study with those who have studied diurnal variations of PPCPs in literature, it can be observed that most studies have generally found that the micropollutants studied followed a similar pattern as compared to macropollutants; i.e. a clear decrease in the loading of these compounds was observed at night (Joss et al., 2005; Gobel et al., 2005; Plósz et al., 2010). This study has investigated a higher number of compounds, many differing from those analysed in previous works. Thus, we have focussed this comparison with literature on either the same compounds, or similar compounds (i.e. from the same therapeutic family).

For example, Joss et al. (2005) studied the diurnal pattern of two musks also found in this study, galaxolide and tonalide. Our results indicate that these compounds, as well as the other musks detected, were generally less abundant at night (Figs. 2 and 3), which agrees well with the results of Joss et al. (2005). Similarly, Plósz et al. (2010) found that estrone was also present in lower abundance at night, which was in accordance with our results (Fig. 4). Previous studies investigating antibiotic compounds (Joss et al., 2005; Gobel et al., 2005; Plósz et al., 2010) also observed a similar diurnal pattern. Although the specific antibiotic compounds detected in those studies were not widely detected at the Fernão Ferro WWTP, the one antibiotic that was frequently detected (ampicillin) was also present in low abundance at night (Fig. 4). Thus, the results of this study are in accordance with literature findings. However, many other compounds were detected in this study that displayed differing diurnal patterns (e.g. ketoprofen, paroxetine, enalapril and indapamide) where the mass loadings were higher at night. This highlights the fact that patterns observed for some micropollutant groups cannot be readily extrapolated to other types of micropollutants. It is clear that different types of PPCPs display dissimilar diurnal variations, likely due to their varying administration patterns.

Furthermore, the results from this study show that even for the most commonly detected PhACs, variations in the mean concentration greater than 1 order of magnitude were found from one day to the next. The diurnal trend observed for PhACs was also variable between the first and second weeks and the occurrence of peak loads varied widely and was highly unpredictable. Obtaining a representative description of PhAC loading to WWTPs for modelling purposes is clearly a challenging task. Indeed, a "steady-state" did not exist for PhACs, unlike the musks. It is possible that obtaining a representative description of PhACs is not practically feasible due to the typically intermittent consumption of many different compounds, where some substances are consumed and excreted only on certain days by a small number of point sources (i.e. consumers). The high cost associated with analysing these compounds further inhibits the practical feasibility of performing multiple sampling campaigns. These issues are important to be resolved in order to model the fate of these compounds in WWTPs, since wastewater influent characterisation is important for model calibration and application, and these models usually describe typical "steady-state" conditions of the WWTP. Nevertheless, it should be noted that a much higher repeatability was observed among the musks analysed, suggesting that less intensive monitoring is needed for acquiring the necessary data to model these compounds in WWTPs.

4. Conclusions

The dynamics of PPCPs in a WWTP was evaluated through an intensive sampling campaign covering a large number of pharmaceuticals and musks. It was found that the PhAC concentrations in the influent were subject to a wider variability than the musks, which were more repeatable. The typical diurnal pattern for macropollutants (i.e. higher loading during the day as compared to the night) was observed for the musks and some PhACs, while other frequently detected PhACs (e.g. ketoprofen) displayed the opposite trend or no trend. In general, the mean PhAC loadings varied between 1 and 3 orders of magnitude from one sampling day or week to the next, whereas the mean musk loadings were far less than one order of magnitude apart. This information is relevant to the design of sampling campaigns for modelling purposes.

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Appendix. Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.envpol.2011.07.004.

References

Almeida, M.C., Butler, D., Friedler, E., 1999. At-source domestic wastewater quality. Urban Water Journal 1, 49–55.

Carucci, A., Cappai, G., Piredda, M., 2006. Biodegradability and toxicity of pharmaceuticals in biological wastewater treatment plants. Journal Environmental Science Health: Part A 41, 1831–1842.

Comeau, F., Surette, C., Brun, G.L., Losier, R., 2008. The occurrence of acidic drugs and caffeine in sewage effluents and receiving waters from three coastal watersheds in Atlantic Canada. Science of the Total Environment 396, 122–146.
Dokianakis, S.N., Kornaros, M.E., Lyberatos, G., 2004. On the effect of pharmaceuticals on bacterial nitrite oxidation. Water Science and Technology 50, 341–346.
Gobel, A., Thomsen, A., Mcardell, C.S., Joss, A., Giger, A., 2005. Occurrence and sorption behaviour of sulfonamides, macrolides, and trimethroprim in activated sludge treatment. Environmental Science and Technology 39, 3981–3989.
INFARMED, 2005. Medicine statistics of 2003. Direcção de Economia do Medicamento e Produtos de Saúde, Lisboa, Portugal, pp. 73–80.
Jones, O.A.H., Voulvoulis, N., Lester, J.N., 2007. The occurrence and removal of selected pharmaceutical compounds in a sewage treatment works utilizing activated sludge treatment. Environmental Pollution 145, 738–744.
Joss, A., Keller, E., Alder, A.C., Gobel, A., McArdell, C.S., Ternes, T., Siegrist, H., 2005. Removal, of pharmaceutical and fragrances in biological wastewater treatment. Water Research 39, 3139–3152.
Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.D., Buxton, H.T., 2002. Pharmaceuticals, hormones and other organic wastewater contaminants in U.S. Streams, 1999–2000: a national reconnaissance. Environmental Science and Technology 36, 1202–1211.
Lin, A.Y., Tsai, Y., 2009. Occurrence of pharmaceutical in Taiwan's surface waters: impact of waste streams from hospitals and pharmaceutical production facilities. Science of the Total Environment 407, 3793–3802.
Miège, C., Choubert, J.M., Ribeiro, L., Eusébe, M., Coquery, M., 2009. Fate of pharmaceuticals and personal care products in wastewater treatment plants – conception of a database and first results. Environmental Pollution 157, 1721–1726.
Plósz, B.G., Leknes, H., Liltved, H., Thomas, K.V., 2010. Diurnal variations in the occurrence and the fate of hormones and antibiotics in activated sludge wastewater treatment in Oslo, Norway. Science of the Total Environment 408, 1915–1924.
Richardson, S.D., Ternes, T.A., 2005. Water analysis: emerging contaminants and current issues. Analytical Chemistry 77, 3807–3828.
Salgado, R., Noronha, J.P., Oehmen, A., Carvalho, G., Reis, M.A.M., 2010. Analysis of 65 pharmaceuticals and personal care products in 5 wastewater treatment plants in Portugal using a simplified analytical methodology. Water Science and Technology 62 (12), 2862–2871.
Santos, J.L., Aparicio, I., Callejón, M., Alonso, E., 2009. Occurrence of pharmaceutically active compounds during 1-year period in wastewaters from four wastewater treatment plants in Seville (Spain). Journal of Hazardous Materials 164, 1509–1516.
Sim, W., Lee, J., Oh, J., 2010. Occurrence and fate of pharmaceuticals in wastewater treatment plants and rivers in Korea. Environmental Pollution 158, 1938–1947.
Tchobanoglaus, G., Burtan, F.L., 1995. Wastewater Engineering – Treatment, Disposal, Reuse, third ed. McGraw Hill., New York, pp. 153–165.
Ternes, T.A., Stumpf, M., Mueller, J., Haberer, K., Wilken, R.D., Servos, M., 1999. Behavior and occurrence of estrogens in municipal sewage treatment plants – I. Investigations in Germany, Canada and Brazil. Science of the Total Environment 225, 81–90.
Ternes, T.A., Bonerz, M., Herrmann, N., Loffler, D., Keller, E., Lacida, B.B., Alder, A.C., 2005. Determination of pharmaceutical, iodinated contrast media and musk fragrances in sludge by LC/tandem MS and GC/MS. Journal of Chromatography A 1067, 213–223.
Wang, S., Holzem, R.M., Gunsch, C.K., 2008. Effects of pharmaceutically active compounds on a mixed microbial community originating from a municipal wastewater treatment plant. Environmental Science and Technology 42, 1091–1095.
Weissbrodt, D., Kovalova, L., Ort, C., Pazhepurackel, V., Moser, R., Hollender, J., Mcardell, C.S., 2009. Mass flows of X-ray contrast media and cytostatics in hospital wastewater. Environmental Science and Technology 43, 4810–4817.
Zuccato, E., Castiglioni, S., Fanelli, R., 2005. Identification of the pharmaceuticals for human use contaminating the Italian aquatic environment. Journal of Hazardous Materials 122, 205–209.