Analysis of 65 pharmaceuticals and personal care products in 5 wastewater treatment plants in Portugal using a simplified analytical methodology

R. Salgado, J. P. Noronha, A. Oehmen, G. Carvalho and M. A. M. Reis

ABSTRACT

Pharmaceuticals and personal care products (PPCPs) are becoming increasingly recognised as important micropollutants to be monitored in wastewater treatment plants (WWTPs), since WWTP effluents represent an important point source to natural aquatic systems. In this study, the abundance of 65 PPCPs was analysed in 5 Portuguese WWTPs during the spring and autumn. Due to the fact that analytical approaches normally used to quantify the abundance of these compounds are labour intensive and require various specific procedures, this study proposes a set of simplified analytical methods for the quantification of pharmaceutically active compounds (PhACs) and polycyclic musks in liquid and sludge samples. The analytical methods were validated using influent wastewater matrices, showing comparable limits of detection and quantification as literature values for most PPCPs, with the exception of the estrogenic compounds. The PhAC concentrations detected in the WWTP survey were in the range of 0.050–100 $\mu g\,L^{-1}$ in the influent and up to 50 $\mu g\,L^{-1}$ in the effluent, where the non-steroidal anti-inflammatory drugs (NSAIDs) were the most abundant and frequently detected group. Some musks were detected up to 11.5 $\mu g\,L^{-1}$ in the influent and 0.9 $\mu g\,L^{-1}$ in the effluent, and adsorbed in the sludge up to 22.6 $\mu g\,g^{-1}$.

Key words | musks, pharmaceutical active compounds (PhAC), solid phase extraction (SPE), solid phase microextraction (SPME), wastewater treatment

R. Salgado
J. P. Noronha
A. Oehmen
G. Carvalho (corresponding author)
M. A. M. Reis
REQUIMTE/CQFB, Chemistry Department,
Faculdade de Ciências Tecnologia,
Universidade Nova de Lisboa,
2829-516 Caparica,
Portugal
E-mail: *gildacarvalho@dq.fct.unl.pt*

G. Carvalho
IBET/ITQB, Apt.12,
2781-901 Oeiras,
Portugal

R. Salgado
Computing and Systems Department,
IPS/Setúbal, EST Setúbal,
Campus IPS, Estefanilha,
2910-761 Setúbal,
Portugal

INTRODUCTION

Pharmaceuticals and personal care products (PPCPs) are commonly occurring micropollutants with a potentially significant environmental impact. The impact in the environment and public health arises not only from wastewater effluents discharged in aquatic media (Bartelt-Hunt *et al.* 2009), but also from sludge application in agriculture, since they can desorb and contaminate the groundwater (Carrara *et al.* 2008). Therefore, it is important to monitor these compounds to know their concentration in the liquid and solid phases after treatment in wastewater treatment plants (WWTP). While many studies have been carried out in different countries and geographical locations (Comeau *et al.* 2008; Okuda *et al.* 2008; Santos *et al.* 2009), the occurrence of PPCPs in wastewater and environmental samples is highly dependent on the local diseases, treatment habits and market profiles, thus, the pollution profile and can vary significantly between different countries (Zuccato *et al.* 2006). This was the motivation for the present study, since little information on the occurrence of PPCPs in WWTPs in Portugal is available.

The most common PPCPs are the pharmaceutical active compounds (PhAC) and the polycyclic musk fragrances. PhACs include the antidepressives, anticonvulsants, non-steroidal anti-inflammatory drugs (NSAID), steroidal anti-inflammatory drugs (SAID), drugs for asthma and allergic diseases, antihypertensives, beta-blockers, lipid regulators, antibiotics, and estrogens.

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Due to the high diversity of compounds displaying a wide variance of chemical structures, many previous studies have elected to perform a combination of analytical methods targeting specific families of compounds (Sacher *et al.* 2001; Ternes 2001). While this strategy can be advantageous for the analysis of each target group, the time-consuming and labour-intensive nature of the analytical procedures makes numerous methodologies undesirable when the goal is to make an overall assessment of PPCPs present in environmental samples and WWTPs. This work proposes a simplified methodology adapted from previously published analytical methods that can be applied to wastewater and sludge samples for the detection of 65 PhACs and musks. All PhACs were analysed through LC-DAD-MS(ESI +) with the same set of conditions after solid-phase extraction (SPE) using two different materials for either neutral or acidic compounds. The musks were analysed by GC-MS after solid-phase microextraction (SPME). Sludge samples were pre-treated with an ultrasonication step prior to PhAC and musk analysis.

The methodology proposed in this study was applied to the influent, effluent and sludge samples from 5 Portuguese WWTPs. The validity of the simplified analytical methodology was assessed using the influent of the different plants. The PhAC compounds covered in this survey were selected based on the top-ranking sales figures for 2003 and 2007 provided by INFARMED (Portuguese Authority for Medication and Health Products), which is a similar approach as adopted by e.g. Erickson (2002) and Zuccato *et al.* (2006).

METHODS

Chemicals and reagents

HPLC-grade acetonitrile, methanol, *n*-hexane and formic acid were purchased from Panreac (Portugal), all pharmaceutical active compound standards from Sigma-Aldrich (Steinheim, Germany) and the musks from LGC-Promochem (Spain). Stock solutions (1 mg mL^{-1}) of each pharmaceutical and musk were prepared in methanol or hexane, respectively, and stored at 4°C.

Sampling collection and properties of the WWTPs

The characteristics of the 5 WWTPs assessed in this study are presented in Table 1. All plants contained screening and primary clarification prior to the secondary treatment process, as well as secondary clarifiers. Grab samples were collected at the influent (prior to primary treatment), the final effluent and the sludge (in the recycle from the

Table 1 | Characteristics of the WWTPs

	Setúbal	Cussena	Valdeão	Quinta da Bomba	Fernão Ferro
Average influent flow (m^3d^{-1})	11,195	773	1,634	15,536	3,579
Wastewater	Domestic + hospital + industrial (8%)	Domestic + industrial (20%)	Domestic + hospital	Domestic	Domestic
Process	Activated sludge†	Activated sludge	Activated sludge	Trickling filter	Trickling filter
Volume biological reactor (m^3)	765* 7,000** 2,083***	696*	1,352*	9,120	628
Hydraulic retention time (h)	33.6 h	11 h	20 h	14 h	7.6 h
Sludge age (d)	15	9	–	–	–
Sludge waste flow (m^3d^{-1})	354	40	No wastage	28	12

†Process includes tertiary treatment by UV-radiation.
*Aerobic; **Anaerobic; ***Anoxic.

secondary clarifier). 5 L samples were collected in plastic (PET) bottles and preserved at 4°C during transportation. The samples were filtered by 0.45 μm glass fiber membranes (GF 6, Whatman, England) and stored at −20°C.

Extraction and clean-up

Acidic and neutral pharmaceuticals by SPE

SPE was used for the extraction and clean-up of the liquid wastewater samples. OASIS HLB cartridges (60 mg, 30 μm, Waters, Eschborn, Germany) were used for the acidic PhACs and RP-C_{18ec} cartridges (500 mg, 50 μm, Waters, Milfort, U.S.) for the neutral PhACs. Each cartridge was previously conditioned with 1 mL methanol followed by 1 mL of Milli-Q water, then dried in a N_2-stream. For the acidic PhACs, 200 mL of filtered wastewater and 10 μL of meclofenamic acid (as internal standard) were passed through the OASIS HLB cartridges at pH 2–3. For the neutral PhACs, 500 mL of filtered wastewater and 50 μL of meclofenamic acid were passed through the RP-C_{18ec} cartridges at pH 7–7.5. Samples were passed through the SPE cartridges at a flow rate of 20 mL min^{-1} and vacuum pressure of −5 psi, and the cartridges were eluted four times with 1 mL of methanol. The methanol extracts were evaporated to 1 mL by a N_2-stream. Then, 50 μL of extract was injected into the LC/MS.

Sludge samples–ultrasonic solvent extraction prior to SPE

The secondary sludge collected in the WWTPs was centrifuged for 5 min at 10,000 rpm. 2 g of the centrifuged sludge was mixed with 4 mL methanol in an ultrasonic bath for 5 min. The slurry was then centrifuged for 1 min at 10,000 rpm. The supernatant was collected in a separate vial and 2 mL of methanol were again added to the sludge. Centrifugation and supernatant collection were then repeated. To ensure the extraction was complete, 2 mL of acetone were then added to the sludge and the same procedure (i.e. ultrasonic bath, centrifugation, supernatant collection) was repeated. Then, the 4 extracts (2 × 2 mL of methanol and 2 × 2 mL of acetone) were combined and evaporated to a volume of ca. 1 mL. The concentrated extract was diluted in 150 mL of Milli-Q water prior to SPE.

Polycyclic musk fragrances by SPME

The extraction of musks from the wastewater and sludge samples was carried out by solid phase micro extraction (SPME) with 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) fibres (Supelco, Spain). The fibres were pre-conditioned prior to use for 30 min at 250°C. 2 g of wastewater or sludge was added to 0.5 g NaCl and 10 μL of mirex as internal standard. The PDMS/DVB fibre was exposed to the sample headspace in a sealed vial with a Teflon lid for 15 min at 90°C. The fibre was thermally desorbed and analysed by GC-MS.

Analytical procedures

Detection of acidic and neutral PhACs by LC-MS

Reverse-phase chromatography (LiChroCART 250-4 Purospher Star RP18 column, Merck) was performed with a diode array detector (DAD) from 200 to 400 nm with a 0.6 mL min^{-1} flow, using a degassed mobile phase with 0.1% water/formic acid (A) and acetonitrile (B). The following binary gradient was used: start with 2 min, 15% B at 0.6 mL min^{-1}; 20 min, 100% B at 0.6 mL min^{-1}; 25 min, 100% B at 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}. HPLC-DAD-MS(ESI +) was carried out in a HPLC system (Waters) coupled with a pump and controller (Waters 600), an in-line degasser (X-Act-4 channels, Jour Research), an autosampler (Waters 717 plus), a photodiode array detector (DAD, Waters 996) and a quadrupole VG Platform spectrometer (Micromass, UK) equipped with an electrospray ionisation (ESI) source operating in positive mode. A split ratio of 1:10 was used between the HPLC column and the mass spectrometer. The capillary temperature was 100–120°C, the scanning cone voltage was 35–100 V and the capillary voltage was 3.5 kV. Nitrogen was used as drying and nebulising gas at 300 mL min^{-1} and 10 mL min^{-1}, respectively. The mass/charge spectrum range used was 100–450 Da with a MassLynx™ software data acquisition system. All samples were analysed in triplicate.

Detection of polycyclic musk fragrances

Analysis was performed using a Hewlett-Packard 5890 GC fitted with a QMD1000 Carlo Erba mass spectrometric

detector. 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, increased to 250°C at 10°C min^{-1}, then raised to 310°C at 20°C min^{-1}, and held for 13 min. The MS spectra were obtained with 70 eV, mass range m/z 50–500 and using MassLab™ software (Micromass). The injector temperature during SPME splitless analysis was 250°C for PDMS/DVB.

Method validation

Determination of recoveries

Samples were spiked with analytes dissolved in a stock solution (each at 1 mg mL^{-1} methanol). The influent wastewater samples were spiked with 100 μg L^{-1} of analyte and internal standard (i.e. meclofenamic acid). After spiked, the samples were stirred for homogenisation and to enable a sufficient contact of analytes and standards with the matrix. Relative recoveries were determined relative to a MilliQ water standard solution, also spiked with 100 μg L^{-1} of analyte and internal standard. Recoveries of the pharmaceuticals in the individual clean-up steps were determined by SPE in wastewater influent matrices and in MilliQ water, and analysed by LC-DAD-MS as described above. The relative recoveries were calculated from the analyte areas in the influent matrix, subtracting the analyte area quantified in the original unspiked matrix, divided by the area of the MilliQ standard sample.

Calibration curves, limits of detection (LOD) and quantification (LOQ)

Standards were prepared from the stock solutions diluted in methanol. A six-point calibration curve was used for each compound, ranging from 5–200 ng L^{-1}. The regression coefficient of the resulting calibration curves was >0.95 for all compounds. Ten blank samples were analysed by LC-MS (with methanol) and GC-MS (with *n*-hexane) to determine the lowest signal/noise ratio of each analyte. Limits of detection (LOD) for the analytes were calculated by the formula $3 \times SD/m$, where SD is the standard deviation of the lowest signal/noise ratio of the analyte and m is the slope of the calibration curve. Limits of quantification (LOQ) were calculated as $10 \times SD/m$.

RESULTS AND DISCUSSION

Validation of the analytical approach

The analytical methodology selected for the PhAC survey carried out in this work was based on SPE followed by LC-DAD-MS(ESI +). Although two different SPE materials were used to best enrich either acidic or neutral pharmaceuticals, the analysis of both extracts was done using the same LC-MS conditions (see methods), in order to detect the highest number of compounds with the lowest analytical effort. The selection of positive mode for the MS electrospray ionisation was based on preliminary tests using standards of the target compounds. For the majority of the compounds it was found that, when compared to ESI −, the ESI + resulted in precursor molecular ions ($[M + H]^+$ or $[M - H]^-$, respectively for ESI + or ESI −) with higher relative peak intensity, thus ESI + was selected.

The LOD, LOQ and recoveries obtained with this approach are presented in Table 2 for the compounds detected in this study. The results showed that the analytical procedure for PhAC enables the detection of a substantial number of pharmaceuticals with LOD and LOQ comparable to those reported in literature using a combination of analytical methods designed for specific groups of compounds. The estrogens are the main exception, where tandem MS should be used in order to detect these compounds to a concentration that is relevant to assess their potential environmental impact (Ternes 2001). However, some other compounds, namely carbamazepine, showed lower LOD and LOQ with SPE (RP-C_{18}) followed by LC-DAD-MS(ESI +) when compared to results obtained with SPE (RP-C_{18}) and GC-MS (Sacher *et al.* 2001). The LOQ for carbamazepine in this study was similar to that found by Ternes (2001) through LC-MS/MS. In Sacher *et al.* (2001), three separate analytical methods were used for antibiotics, whereas in this study, antibiotics were

Table 2 | Limits of detection and quantification, and recovery of the PhACs found in this study. The results are compared with literature studies

Compound	LOD ng L^{-1}	LOQ ng L^{-1}	Recovery** %	LOD ng L^{-1}	LOQ ng L^{-1}	Recovery %	LOQ ng L^{-1}	Recovery %
Neutral PhACs								
Atenolol	3	10	>94 ± 5	2.4*	8.2*	67*	50†	98†
Caffeine	27	91	>82 ± 1					
Carbamazepine	2	7	>75 ± 1	9.6*	32*	74*	10†	92†
Chlorazepate	17	57	–					
Dimethylaminophenazone	29	95	>83 ± 3	4.3*	14*	66*	100†	93†
Domperidone	3	9	–					
Etofenamate	20	67	–					
Fentiazac	–	–	–					
Fluoxetine	17	57	>60 ± 6					
Fluticasone	25	85	>87 ± 1					
Hydroxazine	18	60	73 ± 1					
Indapamide	6	18	>86 ± 1					
Nimesulide	14	46	>82 ± 6					
Paroxetine	27	89	>86 ± 12					
Piroxicam	–	–	–					
Ramipril	9	31	–					
Salbutamol	11	36	>94 ± 1	2.6*	9.1*	66*	50†	61†
Tramadol	20	67	>86 ± 2					
Acidic PhACs								
Captopril	5	15	66 ± 10					
Clofibric acid	15	50	>98 ± 1	5.3*	18*	103*	50†	82†
Diclofenac	7	24	>79 ± 5	8.7*	29*	70*	50†	89†
Enalapril	8	28	>88 ± 3					
Flurbiprofen	18	58	>65 ± 1					
Furosemide	19	63	–					
Ibuprofen	14	46	>89 ± 9	3.5*	12*	110*	50†	81†
Indomethacin	7	23	>96 ± 5	5.4*	18*	114*	50†	90†
Ketoprofen	21	69	>86 ± 2	4.8*	16*	104*	50†	94†
Naproxen	18	59	102 ± 6	3.8*	13*	105*	50†	91†
Meclofenamic acid	4	14	>74 ± 4				50†	89†
Antibiotics								
Amoxicillin	13	43	>83 ± 4	4.6*	15*	36*		
Ampicillin	3	11	>67 ± 1					
Estrogens								
17-α-ethynylestradiol	21	69	–				1†	76†
Estrone	18	60	104 ± 12				1†	82†
β-estradiol	4	12	–				1†	76†
Musks								
Cashmeran	1	4	83 ± 3	21‡				
Celestolide	2	6	85 ± 4	16‡				

Table 2 | (*continued*)

Compound	LOD ng L^{-1}	LOQ ng L^{-1}	Recovery** %	LOD ng L^{-1}	LOQ ng L^{-1}	Recovery %	LOQ ng L^{-1}	Recovery %
Galaxolide	1	4	94 ± 2	11‡			20§	82§
Phantolide	1	4	97 ± 2	17‡				
Tonalide	1	2	82 ± 3	8.4‡			20§	78§
Traseolide	2	6	85 ± 4	13‡				
Mirex	1	2	89 ± 3					

*Sacher *et al.* (2001); recoveries obtained with surface water.
†Ternes (2001); recoveries obtained with WWTP effluent.
‡Smyth *et al.* (2008).
§Ternes *et al.* (2005); recoveries obtained with groundwater.
**Signifies that the recoveries were obtained with WWTP influent. The value presented is the minimum value obtained from the 5 WWTPs.

analysed together with the other acidic compounds. While Sacher *et al.* (2001) found lower LOD and LOQ for amoxicillin, their recovery was lower than in this study (36% vs. 83%). The remaining compounds that were not detected in this study had LOD values ranging from 1–25 ng L^{-1} and LOQ values ranging from 3–83 ng L^{-1}.

PhAC recoveries for the analytical process (SPE followed by LC-DAD-MS) were obtained with samples of influent wastewater for the compounds that were detected in that plant. These tests aimed at assessing the effect of the wastewater matrix on the analytical method as well as interferences due to the manipulation associated with the extraction and analytical process. The precision and reproducibility of the method indicated a relative standard deviation varying from 1 to 12%. Despite some variation of the PhAC recoveries with the matrix (results not shown), the results were above 70% except for four compounds (Table 2). Fluoxetine showed the lowest recovery (60 ± 6%) with wastewater from Valdeão, suggesting that a different SPE material or GC after derivatisation should have been used for better detection of this compound. Nevertheless, the recoveries of fluoxetine for other WWTPs were >79 ± 1%. With this methodology, a very wide range of compounds with different structures were covered, using a reduced number of analytical processes.

Measurement of PPCPs in wastewater influents

In this study, two sampling campaigns were performed for the 5 WWTPs analysed, in the spring and in autumn (Table 3). The results showed that 33 out of 59 pharmaceuticals were detected in the influents to the plants, at varying frequencies of occurrence. The concentrations detected were in the range of approximately 50 ng L^{-1} to 100 μg L^{-1}. The most dominant class of compounds present in the WWTPs were the non-steroidal anti-inflammatory drugs (NSAIDs), which appeared at significantly higher concentration levels than the other PhAC groups (Figure 1).

The total quantity of the major PhAC families present in the influent of most plants surveyed in this study (with the exception of Quinta da Bomba, see Figure 1) was found to be between 30 and 70 μg L^{-1}, and went up to 120 μg L^{-1} in one sample. In previous surveys covering a wide range of PhACs in other countries, the total PhAC concentration reported was approximately 23 μg L^{-1} in a Japanese study (Okuda *et al.* 2008), 143 μg L^{-1} in a Spanish study (Rosal *et al.* 2009) and 320 μg L^{-1} in the U.K. (Kasprzyk-Hordern *et al.* 2009).

The most commonly detected and abundant NSAIDs found in the WWTP analysed were ibuprofen, ketoprofen, flurbiprofen, diclofenac and indomethacin. Caffeine was present in all plants and generally detected in high concentrations. Santos *et al.* (2009) also found ibuprofen, ketoprofen, and caffeine in the μg L^{-1} range of concentrations in WWTP influents of Seville, Spain, which is consistent with the present study. In addition to the NSAIDs, the antihypertensive (enalapril, captopril and furosemide) and lipid regulator groups were also occasionally found at μg L^{-1} levels, whereby clofibric acid represented the sole lipid regulator detected. No readily observable pattern was found between the frequency of detection and quantity of PhACs measured in influent samples collected in the spring or autumn from the different plants (Figure 1).

Three of the musks analysed (galaxolide, tonalide and cashmeran) were found in each plant (Table 3), while

Table 3 | Pharmaceuticals and musks detected at the influent, effluent and secondary sludge of 5 different WWTPs in Portugal during the spring (23 May to 7 July) and autumn (2 to 25 October)

Compound	Infl. Occur-rence	Infl. Min ng/L ± SD	Infl. Max ng/L ± SD	Effl. Occur-rence	Effl. Min ng/L ± SD	Effl. Max ng/L ± SD	Sec. Sludge Occur-rence	Sec. Sludge Min ng/g ± SD	Sec. Sludge Max ng/g ± SD
Neutral PhACs									
Atenolol	**5/10**	65 ± 5	4,757 ± 25	**4/9**	119 ± 25	1,297 ± 14	**0/9**		
Caffeine	**10/10**	258 ± 13	36,160 ± 36	**4/9**	437 ± 20	4,392 ± 22	**3/9**	1,788 ± 12	8,423 ± 29
Carbamazepine	**2/10**	664 ± 49	994 ± 11	**1/9**	238 ± 17	238 ± 17	**0/9**		
Clorazepate	**1/10**	6,227 ± 16	6,227 ± 16	**0/9**			**1/9**	181 ± 9	181 ± 9
Dimethylamino-phenazone	**4/10**	158 ± 8	3,664 ± 19	**6/9**	252 ± 65	4,278 ± 12	**3/9**	158 ± 8	1,361 ± 17
Domperidone	**1/10**	163 ± 67	163 ± 67	**0/9**			**0/9**		
Etofenamate	**7/10**	58 ± 12	7,333 ± 26	**2/9**	229 ± 6	1,620 ± 33	**2/9**	24,785 ± 121	134,431 ± 438
Fentiazac	**1/10**	5,297 ± 19	5,297 ± 19	**0/9**			**0/9**		
Fluoxetine	**5/10**	85 ± 1	1,704 ± 15	**0/9**			**1/9**	77 ± 10	77 ± 10
Fluticasone	**3/5**	196 ± 1	1,298 ± 82	**3/9**	33 ± 0.3	2,848 ± 14	**2/9**	1,473 ± 17	2,330 ± 42
Hydroxazine	**1/10**	9,344 ± 80	9,344 ± 80	**0/9**			**1/9**	43,339 ± 86	43,339 ± 86
Indapamide	**3/10**	177 ± 19	1,236 ± 23	**2/9**	90 ± 2	329 ± 13	**3/9**	47 ± 3	1,362 ± 31
Nimesulide	**1/10**	6,911 ± 13	6,911 ± 13	**0/9**			**0/9**		
Paroxetine	**3/10**	182 ± 19	1,312 ± 15	**3/9**	224 ± 15	3,367 ± 30	**0/9**		
Piroxicam	**2/10**	2,575 ± 49	9,298 ± 34	**0/9**			**0/9**		
Ramipril	**1/10**	5,445 ± 49	5,445 ± 49	**0/9**			**1/9**	488 ± 62	488 ± 62
Salbutamol	**3/10**	104 ± 21	2,158 ± 13	**1/9**	572 ± 27	572 ± 27	**2/9**	12 ± 0.6	104 ± 21
Tramadol	**2/5**	158 ± 2	1,344 ± 17	**2/9**	51 ± 3	134 ± 4	**0/9**		
Acidic PhACs									
Captopril	**5/10**	32 ± 2	13,335 ± 26	**1/9**	1,376 ± 56	1,376 ± 56	**3/9**	875 ± 16	5,516 ± 49
Clofibric acid	**9/10**	40 ± 1	6,785 ± 21	**8/9**	198 ± 3	7,286 ± 19	**4/9**	117 ± 7	15,655 ± 18
Diclofenac	**7/10**	207 ± 44	6,674 ± 24	**2/9**	26 ± 2	1,612 ± 18	**3/9**	2,259 ± 5	17,785 ± 48
Enalapril	**4/10**	51 ± 4	10,238 ± 32	**3/9**	624 ± 21	19,888 ± 22	**1/9**	61 ± 13	61 ± 13
Flurbiprofen	**5/10**	918 ± 2	9,631 ± 69	**3/9**	684 ± 2	3,011 ± 56	**2/9**	1,018 ± 84	3,544 ± 18
Furosemide	**2/10**	3,618 ± 29	15,244 ± 48	**0/9**			**1/9**	3,602 ± 30	3,602 ± 30
Ibuprofen	**8/10**	550 ± 33	106,490 ± 42	**8/9**	518 ± 33	43,653 ± 54	**3/9**	550 ± 33	3,398 ± 16
Indomethacin	**5/10**	240 ± 17	8,899 ± 17	**2/9**	1,470 ± 53	2,393 ± 5	**2/9**	20 ± 3	88 ± 3
Ketoprofen	**10/10**	260 ± 41	14,275 ± 98	**5/9**	20 ± 4	160 ± 13	**5/9**	47 ± 10	2,1989 ± 52
Naproxen	**2/10**	283 ± 2	3,894 ± 28	**0/9**			**0/9**		
Antibiotics									
Amoxicillin	**4/10**	232 ± 5	5,698 ± 99	**2/9**	1097 ± 35	4,801 ± 45	**2/9**	112 ± 5	166 ± 28

Table 3 | (continued)

Compound	Infl. Occurrence	Infl. Min ng/L ± SD	Infl. Max ng/L ± SD	Effl. Occurrence	Effl. Min ng/L ± SD	Effl. Max ng/L ± SD	Sec. Sludge Occurrence	Sec. Sludge Min ng/g ± SD	Sec. Sludge Max ng/g ± SD
Ampicillin	**3/10**	306 ± 2	4,120 ± 19	**1/9**	410 ± 5	410 ± 5	**0/9**		
Estrogens									
17-α-ethynylestradiol	**2/10**	103 ± 12	106 ± 1	**0/9**			**1/9**	221 ± 8	221 ± 8
Estrone	**2/10**	189 ± 27	2,484 ± 15	**1/9**	25 ± 2	25 ± 2	**2/9**	8 ± 0.5	181 ± 18
B-estradiol	**1/10**	344 ± 10	344 ± 10	**0/9**			**0/9**		
Musks									
Galaxolide	**10/10**	55 ± 17	11,463 ± 20	**10/10**	4 ± 1	889 ± 61	**6/6**	119 ± 63	22,649 ± 154
Tonalide	**10/10**	1 ± 0.1	2,933 ± 51	**10/10**	1 ± 0.1	225 ± 20	**6/6**	11 ± 3	5,968 ± 47
Cashmeran	**10/10**	50 ± 2	7,206 ± 125	**10/10**	4 ± 0.4	640 ± 11	**5/6**	35 ± 13	1,865 ± 108
Celestolide	**5/10**	5 ± 1	338 ± 2	**0/10**			**0/6**		

celestolide also appeared punctually, and traseolide and phantolide were not detected. In this case, the results show that in the spring the concentrations are, in general, higher than in the autumn (Figure 2). The concentration of the musks ranged from 117 ng L^{-1} to 5.0 μg L^{-1}, going up to 21.6 μg L^{-1} in one sample. In literature studies, galaxolide and tonalide are generally the most frequently detected musks, as found in this study. Most previous reports indicate that the total musk concentrations are within a similar range for domestic/industrial wastewater. For example, in a study of 11 polycyclic musks in 6 WWTPs, Smyth *et al.* (2008) found them in a concentration range of 2–40 μg L^{-1} in the influent to the plants, while other literature studies report similar values or lower (Bester 2004; Rosal *et al.* 2009).

3.3 Measurement of PPCPs in wastewater effluents and adsorption to the secondary sludge

As expected, the effluent concentrations of most PhACs were generally much lower than the influent concentrations, suggesting at least some degree of transformation via biological degradation, UV degradation or adsorption to the sludge (Table 3). Also, the diversity of compounds detected was substantially lower, as well as the frequency in their occurrence. It is noteworthy that clofibric acid and dimethylaminophenazone were present at a similar frequency and abundance in the influent and effluent of most plants (Table 3), suggesting that these compounds were difficult to degrade by the WWTPs. Clofibric acid has previously been found to have a very high persistence in the environment (Winkler *et al.* 2001), which is consistent with the results of this study.

The range of total PhAC concentrations found in the effluent of the different plants varied between 2–50 μg L^{-1}. When compared with surveys of WWTP effluents in other countries, a similar range of total PhAC concentrations were reported. The total PhAC concentrations observed in the effluent in some other locations is as follows: in Japan, 2–10 μg L^{-1}; in Atlantic Canada, 2 μg L^{-1}; in Spain, 26 μg L^{-1}; and Italy, 3 μg L^{-1} (Zuccato *et al.* 2006; Comeau *et al.* 2008; Okuda *et al.* 2008; Rosal *et al.* 2009). In the UK, an activated sludge effluent contained 43 μg L^{-1} of PhACs, while a trickling filter plant contained 93 μg L^{-1}, where the influent concentration to each plant was similar (Kasprzyk-Hordern *et al.* 2009). In this study, no apparent differences were

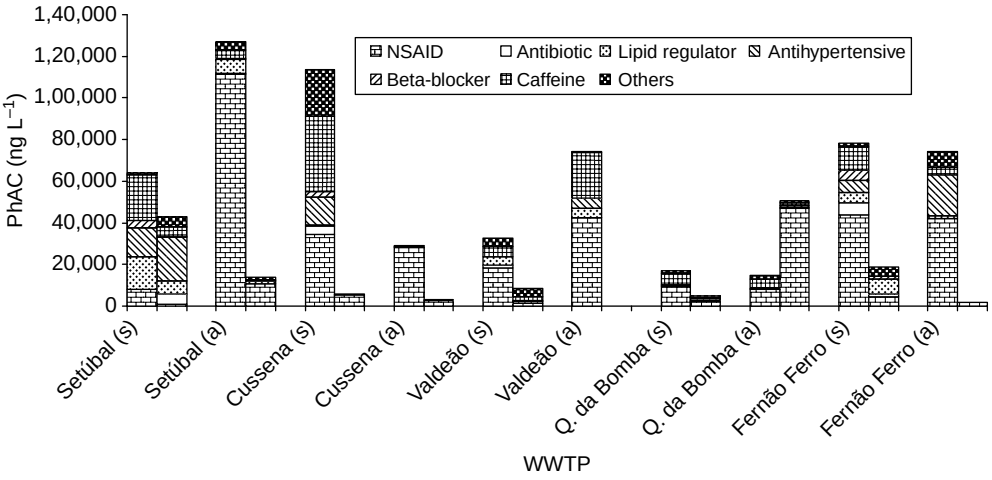

Figure 1 | Occurrence of the most important PhAC families observed in the spring (s) and autumn (a) campaigns in the 5 studied WWTPs in the influent ('left' column of each pair) and effluent ('right' column of each pair).

observed in the range of total effluent concentrations of the activated sludge plants (3–43 $\mu g\,L^{-1}$), versus the trickling filters (2–50 $\mu g\,L^{-1}$). In addition, similarities were observed between the main families of compounds detected in literature surveys (i.e. NSAIDs, antibiotics, β-blockers, lipid regulators, analgesics and caffeine), though each geographical region generally displayed differences in the particular compounds that were most abundant.

In relation to the musks, galaxolide, tonalide and cashmeran were detected in the effluent of each plant, but at lower concentrations than in the influent (Table 3). Celestolide was not detected in the effluent of any of the WWTPs. The range of musk concentrations in the effluent were between 9 $ng\,L^{-1}$ and 1.4 $\mu g\,L^{-1}$, from which 5 $ng\,L^{-1}$ to 1.1 $\mu g\,L^{-1}$ were either galaxolide or tonalide. Previous studies reporting galaxolide and tonalide concentration in WWTP effluents found on average 1.3 $\mu g\,L^{-1}$ (Rosal *et al.* 2009) or 4.8 $\mu g\,L^{-1}$ (Smyth *et al.* 2008), which is a comparable range to that found in Portuguese plants.

The high frequency of occurrence of musks in the sludge suggests that adsorption was one of the important removal mechanisms, which agrees well with previous studies (Bester 2004; Ternes *et al.* 2005). The total galaxolide and tonalide found in the sludge ranges from 0.130–28.6 $\mu g\,g^{-1}$, while Bester (2004) found 4.5 $\mu g\,g^{-1}$ and Ternes *et al.* (2005) observed 2.3–8.5 $\mu g\,g^{-1}$. The high adsorption levels detected for the musks are due to their high hydrophobicity. Some pharmaceuticals were also found in the secondary sludge at high levels, however, they were less persistent since they were not detected as frequently in all of the plants

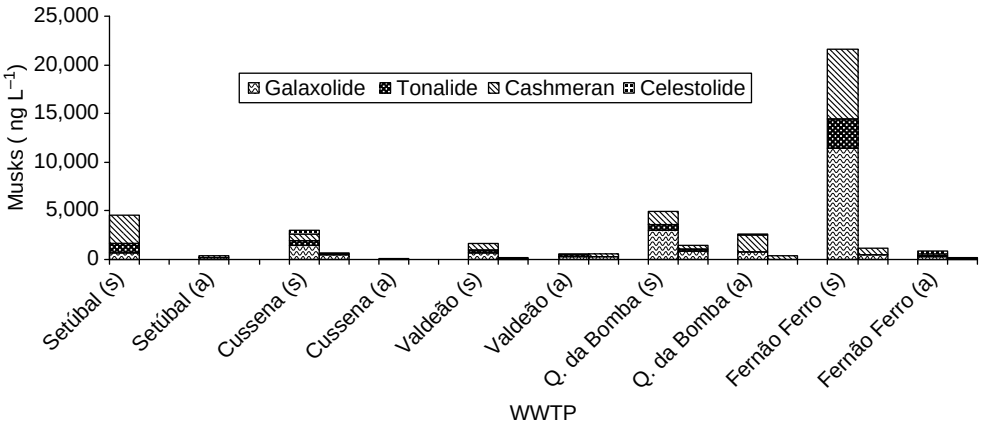

Figure 2 | Occurrence of the musks observed in the spring (s) and autumn (a) campaigns in the 5 studied WWTPs in the influent ('left' column) and effluent ('right' column).

tested. The must abundant PhACs in the sludge were diclofenac, ketoprofen, etofenamate, clofibric acid and hydroxazine, which were present at levels above 10 $\mu g\,g^{-1}$.

CONCLUSIONS

The results of this study show that the adapted analytical methodology employed in this work was effective for monitoring pharmaceutical and personal care products in wastewater treatment plants. This method reduced the analytical effort necessary to cover a wide range of compounds with different natures, and still achieved good LOD and LOQ levels (with the exception of the estrogens), with high recoveries in influent wastewater. The total PhAC and musk concentrations found in this work were in a similar range as previously reported studies. The most abundant PhACs were the NSAIDs (particularly ibuprofen), while the antihypertensives (particularly enalapril), caffeine, and clofibric acid were also present in relatively high concentrations in the influent and effluent. Clofibric acid represented one of the few compounds present at a similar range of concentrations in the influent and effluent of the plants, suggesting that little biodegradation and adsorption of this compound took place.

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