Journal of Hazardous Materials 164 (2009) 1509–1516

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

# Occurrence of pharmaceutically active compounds during 1-year period in wastewaters from four wastewater treatment plants in Seville (Spain)

J.L. Santos, I. Aparicio, M. Callejón, E. Alonso*

*Department of Analytical Chemistry, University of Seville, C/ Virgen de África 7, E-41011 Seville, Spain*

ARTICLE INFO

*Article history:*
Received 11 February 2008
Received in revised form 15 June 2008
Accepted 19 September 2008
Available online 26 September 2008

*Keywords:*
Pharmaceutical compound
Wastewater
Removal
Temporal evolution
Wastewater quality
WWTP operational parameters

ABSTRACT

Several pharmaceutically active compounds have been monitored during 1-year period in influent and effluent wastewater from wastewater treatment plants (WWTPs) to evaluate their temporal evolution and removal from wastewater and to know which variables have influence in their removal rates. Pharmaceutical compounds monitored were four antiinflammatory drugs (diclofenac, ibuprofen, ketoprofen and naproxen), an antiepileptic drug (carbamazepine) and a nervous stimulant (caffeine). All of the pharmaceutically active compounds monitored, except diclofenac, were detected in influent and effluent wastewater. Mean concentrations measured in influent wastewater were 6.17, 0.48, 93.6, 1.83 and 5.41 μg/L for caffeine, carbamazepine, ibuprofen, ketoprofen and naproxen, respectively. Mean concentrations measured in effluent wastewater were 2.02, 0.56, 8.20, 0.84 and 2.10 μg/L for caffeine, carbamazepine, ibuprofen, ketoprofen and naproxen, respectively. Mean removal rates of the pharmaceuticals varied from 8.1% (carbamazepine) to 87.5% (ibuprofen). The existence of relationships between the concentrations of the pharmaceutical compounds, their removal rates, the characterization parameters of influent wastewaters and the WWTP control design parameters has been studied by means of statistical analysis (correlation and principal component analysis). With both statistical analyses, high correlations were obtained between the concentration of the pharmaceutical compounds and the characterization parameters of influent wastewaters; and between the removal rates of the pharmaceutical compounds, the removal rates of the characterization parameters of influent wastewaters and the WWTP hydraulic retention times. Principal component analysis showed the existence of two main components accounting for 76% of the total variability.



## 1. Introduction

Nowadays, one of the most common relevant topics in the environmental field is water quality. During the last three decades, the organic pollutants monitored in the aquatic media have mainly been pesticides, polycyclic aromatic hydrocarbons and polychlorinated biphenyls [1]. Currently, special attention is being paid to the presence of pharmaceutical compounds in the aquatic environment because of their potential toxic effect to the aquatic media [2,3] which could even affect to human health [4]. The main source of pharmaceutical compounds to the environment is the discharge of wastewater from wastewater treatment plants (WWTPs) where the pharmaceutical compounds arrive from the sewer system [1]. Pharmaceutical compounds are continuously thrown to the sewer system through urine and faeces discharges being detected not only in wastewater [3,5–10] but also in rivers [11–15] and groundwater [16,17]. The study of the occurrence of pharmaceutical compounds in wastewater treatment works could indicate how and in which degree these compounds are infused into the environment [18-20]. Nevertheless, the concentrations of the pharmaceutical compounds in the environment, their temporal evolution and the possible synergic and antagonist effects not only depend on the discharges from WWTPs but also on the geographical area and climatologic conditions.

In a previous work [3], concentration levels of the antiinflammatory drugs diclofenac, ibuprofen, ketoprofen and naproxen, the antiepileptic drug carbamazepine and the nervous stimulant caffeine in influent and effluent wastewater from four WWTPs located in Seville city (South of Spain) during summer (July–September 2004) were reported. In this paper, the study has been completed by monitoring the concentration levels of the pharmaceutical compounds in the above mentioned WWTPs during 1-year period in order to obtain new information about their: temporal (seasonal) evolution, removal efficiency and relationship with wastewater quality and WWTP operational parameters.

* Corresponding author. Tel.: +34 9 5455 2858; fax: +34 9 5428 2777.
*E-mail address:* ealonso@us.es (E. Alonso).

0304-3894/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2008.09.073

## 2. Experimental

### 2.1. Wastewater samples

Sixty-three influent and effluent wastewater samples were collected from June 2004 to June 2005 from each of the four WWTPs located in Seville city (South of Spain). Daily-composite samples were obtained by mixing sample volumes collected every hour during 24 h by an automatic device. Sample volumes collected each hour were proportional to influent and effluent flows. Aliquots of 2.5 L of the total sample volume collected were transferred to amber glass bottles and extracted within 24 h after collection. Treatments in the four WWTPs include primary treatment (settling) and secondary treatment based on activated sludge. Some of the operational parameters of the WWTPs studied can be seen in Table 1.

### 2.2. Chemicals and reagents

HPLC-grade acetonitrile and methanol were purchased from Romil Ltd. (Barcelona, Spain). Hexane, ethyl acetate (both of HPLC grade) and potassium dihydrogen phosphate of analytical grade were obtained from Panreac (Barcelona, Spain). Ultrapure water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Caffeine (CF) was obtained from Merck (Darmstadt, Germany). Carbamazepine (CRB), diclofenac (DCL), ibuprofen (IBU), ketoprofen (KTP) and naproxen (NPX) (97–100% purity) were purchased from Sigma–Aldrich (Steinheim, Germany). 3-mL solid phase extraction cartridges, packed with 60 mg of Oasis HLB, were purchased from Waters (Milford, Massachusetts, USA). A stock solution containing 500 μg/mL of each compound was prepared in methanol and stored at 4 °C. Working solutions were prepared by dilution of the stock solution in methanol.

### 2.3. Determination of wastewater characterization parameters

Parameters measured to characterize influent and effluent wastewater in each WWTP were total suspended solid (TSS), chemical oxygen demand (COD), biological oxygen demand (BOD), pH, oil and grease content (Oil), total Kjeldahl nitrogen (TKN) and total phosphorus content (TP). Measurements were carried out according to the standard methods compiled by APHA [21].

### 2.4. Sample treatment and analysis of the pharmaceutically active compounds

Analytical procedure was based on a previously reported validated method for the determination of CF, CRB, DCL, IBU, KTP and NPX in wastewater samples from WWTPs [22]. Sample treatment was based on solid phase extraction which allowed enrichment factors of 1000 for influent samples and 2000 for effluent samples. Extracts were analyzed by HPLC using gradient elution and detection by diode array and fluorescence detectors [22].

Chromatographic analysis was performed on a LaChrom® HPLC instrument (Merck–Hitachi, Barcelona, Spain) equipped with a quaternary L-7100 pump, a L-7455 diode array (DAD) and a L-7485 fluorescence (Fl) detectors connected on line. Separations were carried out using a LiChrospher® 100 RP-18 (125 mm × 4 mm i.d., 5 μm) cartridge column (Merck, Darmstadt, Germany) protected by a LiChrospher® 100 RP-18 (4 mm × 4 mm i.d., 5 μm) guard column (Merck, Darmstadt, Germany).

### 2.5. Removal rates in the wastewater treatment plants

Daily removal rates of each pharmaceutical compound in each of the four WWTPs evaluated were calculated from the equation:

$$\%R = \frac{C_{\mathrm{inf}} - C_{\mathrm{eff}}}{C_{\mathrm{inf}}} \times 100$$

where $C_{\mathrm{inf}}$ is the concentration measured in the influent wastewater and $C_{\mathrm{eff}}$ is the concentration measured in the effluent wastewater.

### 2.6. Data analysis

Statistical techniques, correlation analysis and principal component analysis, were used to evaluate the existence of relationships between the concentrations of the pharmaceutical compounds, their removal efficiencies, the characterization parameters of influent wastewater, the removal of wastewater characterization parameters and WWTP operational parameters. Statistical analysis was carried out using Statistical 6.0 software for Windows.

## 3. Results and discussion

### 3.1. Characterization parameters

The values of wastewater characterization parameters measured during the monitoring period in each of the four WWTPs are shown in Table 2. A wide variability of the wastewater characterization parameters was observed in influent wastewater samples from the four WWTPs with R.S.D.s in the range from 0.13 to 283%, being within the common range of concentrations in urban wastewater samples from the geographical location of the WWTPs sampled. Nevertheless, after treatment, similar values were obtained with R.S.D. in the range from 0.09 to 17%. COD/BOD ratios were 1.63, 1.93, 2.02 and 2.49 in North, South, East and West WWTP, respectively, what indicate that influent wastewater in all of the evaluated WWTPs mainly contain urban wastewater. Around neutral pH was measured in influent wastewater samples what corroborates the low industrial content of wastewater discharges to the evaluated WWTP.

From characterization parameters values measured in influent and effluent wastewater samples the efficiencies of removal of TSS, COD, BOD, Oil, TKN and TP achieved in each of the WWTPs were calculated (Table 2). TSS, COD, BOD and Oil content were reduced in the range from 84 to 95% in the four WWTPs. These values are in accordance with those fixed in the Council Directive 91/271/EEC [23] where percentages of reduction of TSS, COD and BOD in urban wastewater treatment plants are regulated. TKN and TP were poorly

**Table 1**
Characteristics of influent wastewater discharges and operational parameters of the WWTP studied.

| WWTP | Equivalent inhabitants | Wastewater discharge | Volume treated ($m^3$/day) | SRT (days) | HRT (h) |
|---|---|---|---|---|---|
| North WWTP | 350,000 | Urban | 62,000 | 1.5 | 12 |
| South WWTP | 950,000 | Urban and hospital | 164,500 | 2.7 | 17 |
| East WWTP | 200,000 | Urban and industrial | 40,900 | 1.9 | 12 |
| West WWTP | 200,000 | Urban and industrial | 23,150 | 5.1 | 16 |

SRT: solid retention time; HRT: hydraulic retention time.

**Table 2**
Characterization parameters (mg/L), concentrations of the pharmaceutical compounds (μg/L), R.S.D. and mean removal efficiencies (*R*) in influent and effluent wastewater from each of the evaluated WWTPs over 1-year monitoring period.

| | Influent wastewater | | | | Effluent wastewater | | | | *R* (%) |
|---|---|---|---|---|---|---|---|---|---|
| | Minimum | Maximum | Mean | R.S.D. (%) | Minimum | Maximum | Mean | R.S.D. (%) | |
| North WWTP | | | | | | | | | |
| TSS | 82 | 280 | 150 | 43 | 6 | 27 | 17 | 6 | 88 |
| COD | 162 | 664 | 357 | 88 | 21 | 83 | 56 | 15 | 84 |
| BOD | 118 | 393 | 219 | 58 | 11 | 71 | 18 | 8 | 91 |
| pH[a] | 7.08 | 7.90 | 7.66 | 0.14 | 7.24 | 7.85 | 7.65 | 0.13 | – |
| Oil | 29 | 48 | 38 | 10 | 4 | 8 | 6 | 2 | 84 |
| TKN | 26 | 40 | 35 | 8 | 14 | 22 | 19 | 4 | 45 |
| TP | 5.9 | 10.3 | 7.4 | 2.5 | 3.8 | 5.0 | 4.2 | 0.7 | 41 |
| CF | 2.33 | 27.9 | 7.37 | 82 | 0.17 | 5.45 | 1.62 | 79 | 75 |
| CRB | <LOD[b] | 3.78 | 0.53 | 104 | <LOD[f] | 1.18 | 0.58 | 41 | 11 |
| DCL | <LOD[c] | <LOD[c] | <LOD[c] | – | <LOD[g] | <LOD[g] | <LOD[g] | – | – |
| IBU | 3.73 | 353 | 69.7 | 98 | <LOD[h] | 26.5 | 4.13 | 123 | 87 |
| KTP | <LOD[d] | 5.52 | 1.58 | 84 | <LOD[i] | 2.27 | 0.86 | 67 | 52 |
| NPX | 2.02 | 8.50 | 4.83 | 33 | 0.54 | 5.09 | 2.74 | 37 | 43 |
| South WWTP | | | | | | | | | |
| TSS | 20 | 328 | 234 | 40 | 11 | 35 | 24 | 5 | 88 |
| COD | 53 | 895 | 648 | 105 | 53 | 95 | 75 | 9 | 87 |
| BOD | 17 | 495 | 336 | 73 | 11 | 25 | 20 | 4 | 93 |
| pH[a] | 7.10 | 8.00 | 7.60 | 0.13 | 7.51 | 8.30 | 7.85 | 0.17 | – |
| Oil | 82 | 230 | 130 | 16 | 0.31 | 21 | 7 | 3 | 94 |
| TKN | 33 | 54 | 45 | 3 | 29 | 58 | 40 | 6 | 16 |
| TP | 7.00 | 11.4 | 10.2 | 1.0 | 1.4 | 10.3 | 4.6 | 1.4 | 55 |
| CF | 0.54 | 26.1 | 4.87 | 93 | 0.51 | 5.65 | 2.44 | 47 | 44 |
| CRB | <LOD[b] | 2.10 | 0.47 | 86 | 0.15 | 1.29 | 0.61 | 37 | 7 |
| DCL | <LOD[c] | <LOD[c] | <LOD[c] | – | <LOD[g] | <LOD[g] | <LOD[g] | – | – |
| IBU | <LOD[e] | 294 | 84.4 | 76 | <LOD[h] | 40.2 | 6.69 | 138 | 84 |
| KTP | <LOD[d] | 6.47 | 1.74 | 99 | <LOD[i] | 1.95 | 0.80 | 65 | 56 |
| NPX | 2.03 | 52.9 | 8.07 | 95 | 0.22 | 3.52 | 1.64 | 44 | 71 |
| East WWTP | | | | | | | | | |
| TSS | 86 | 472 | 253 | 71 | 8 | 34 | 15 | 6 | 93 |
| COD | 224 | 987 | 614 | 167 | 5 | 85 | 49 | 17 | 92 |
| BOD | 97 | 884 | 303 | 137 | 5 | 89 | 17 | 16 | 95 |
| pH[a] | 7.27 | 8.10 | 7.62 | 0.16 | 7.70 | 8.20 | 7.92 | 0.11 | – |
| Oil | 28 | 57 | 43 | 12 | 3 | 7 | 5 | 2 | 87 |
| TKN | 25 | 50 | 39 | 11 | 15 | 21 | 18 | 3 | 44 |
| TP | 6.2 | 10.5 | 7.0 | 2.3 | 2.8 | 6.7 | 3.4 | 3.1 | 48 |
| CF | 0.75 | 43.9 | 7.09 | 119 | 0.18 | 3.87 | 1.68 | 60 | 64 |
| CRB | <LOD[b] | 1.31 | 0.41 | 76 | <LOD[f] | 0.84 | 0.49 | 39 | 7 |
| DCL | <LOD[c] | <LOD[c] | <LOD[c] | – | <LOD[g] | <LOD[g] | <LOD[g] | – | – |
| IBU | <LOD[e] | 319 | 105 | 86 | <LOD[h] | 55.0 | 10.16 | 123 | 80 |
| KTP | <LOD[d] | 8.56 | 1.91 | 116 | <LOD[i] | 3.92 | 0.82 | 89 | 72 |
| NPX | 1.63 | 27.4 | 4.69 | 72 | 0.83 | 3.64 | 2.18 | 33 | 48 |
| West WWTP | | | | | | | | | |
| TSS | 108 | 630 | 214 | 77 | 6 | 22 | 11 | 3 | 94 |
| COD | 324 | 2135 | 605 | 283 | 34 | 85 | 58 | 2 | 94 |
| BOD | 144 | 497 | 244 | 66 | 7 | 18 | 14 | 12 | 89 |
| pH[a] | 7.10 | 9.40 | 7.73 | 0.27 | 7.40 | 8.00 | 7.63 | 0.09 | – |
| Oil | 41 | 57 | 49 | 11 | 0.39 | 7 | 4.5 | 3.6 | 85 |
| TKN | 31 | 52 | 42 | 7 | 29 | 40 | 34 | 3 | 9 |
| TP | 7.3 | 15.7 | 11.4 | 4.2 | 1.2 | 13.3 | 4.0 | 2.7 | 73 |
| CF | 0.22 | 22.00 | 5.34 | 85 | 0.15 | 5.12 | 2.32 | 59 | 55 |
| CRB | <LOD[b] | 2.15 | 0.49 | 77 | 0.15 | 1.55 | 0.56 | 47 | 8 |
| DCL | <LOD[c] | <LOD[c] | <LOD[c] | – | <LOD[g] | <LOD[g] | <LOD[g] | – | – |
| IBU | <LOD[e] | 603 | 115 | 94 | <LOD[h] | 48.2 | 7.62 | 126 | 87 |
| KTP | <LOD[d] | 5.70 | 2.07 | 80 | <LOD[i] | 2.03 | 0.88 | 65 | 58 |
| NPX | 1.14 | 9.10 | 4.28 | 42 | 0.29 | 4.28 | 1.67 | 50 | 60 |

[a] pH units; LOD: lower than the limit of detection of the method.
[b] 0.04 μg/L.
[c] 0.28 μg/L.
[d] 0.02 μg/L.
[e] 0.25 μg/L.
[f] 0.02 μg/L.
[g] 0.14 μg/L.
[h] 0.12 μg/L.
[i] 0.01 μg/L.

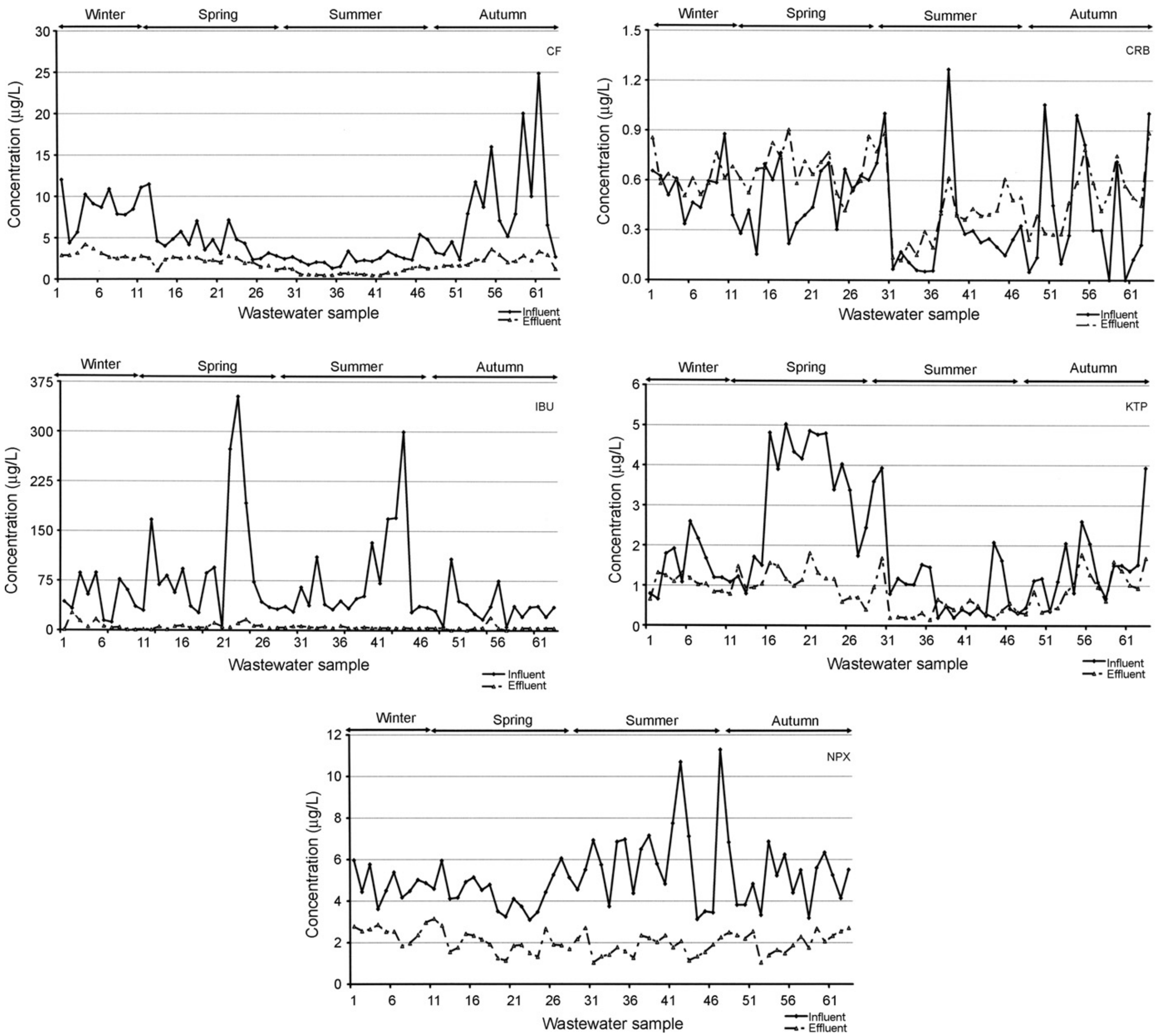


**Fig. 1.** Temporal evolution of the mean concentration of the pharmaceutical compounds in the four WWTPs during the 1-year monitoring period.

removed with removal rates in the range from 9 to 45% for TKN and 41–73% for TP.

### 3.2. *Concentrations of the pharmaceutical compounds in wastewater samples*

As can be seen in Table 2, all of the pharmaceutical compounds monitored, except DCL, were detected in the wastewater samples analyzed. CF and NPX were detected in all of the analyzed samples whereas CRB, IBU and KTP were found in the 70, 83 and 78% of the influent samples and in the 96, 65 and 88% of the effluent samples, respectively. Mean concentrations of CF, CRB, IBU, KTP and NPX were 6.17, 0.48, 93.6, 1.83 and 5.47 µg/L in influent samples and 2.02, 0.56, 8.20, 0.84 and 2.10 µg/L in effluent samples, respectively. IBU was the pharmaceutical compound present at the highest concentration level. Concentration levels of IBU in the WWTPs studied were ranged from 3.73 to 603 µg/L in influent wastewater samples and from 1.27 to 55.0 µg/L in effluent wastewater samples which are consistent with those previously reported by other authors in wastewater samples from Finland [5], Australia [24], Sweden [25], Italy [26] and Spain [27,28]. Taking into account that the four WWTPs evaluated discharge to the same river, it could be estimated discharges of IBU to the river in the range from 40.2 to 2236 g/day.

### 3.3. *Temporal evolution of the pharmaceutical compounds during the sampling period*

The temporal evolution of the concentration of the pharmaceutical compounds in influent and effluent wastewater during the monitoring period can be seen in Fig. 1. Each point shows the mean concentration of the concentrations measured in the four WWTPs. An increase of the concentration of CF in influent wastewater was observed in the coldest period of the year in Seville which corresponds with the period January–March (samples 1–13) and November–December (samples 51–63). Concentration of CF increases from a mean value around 4 µg/L during April–October to concentration values in the range from 7.9 to 24.9 µg/L during

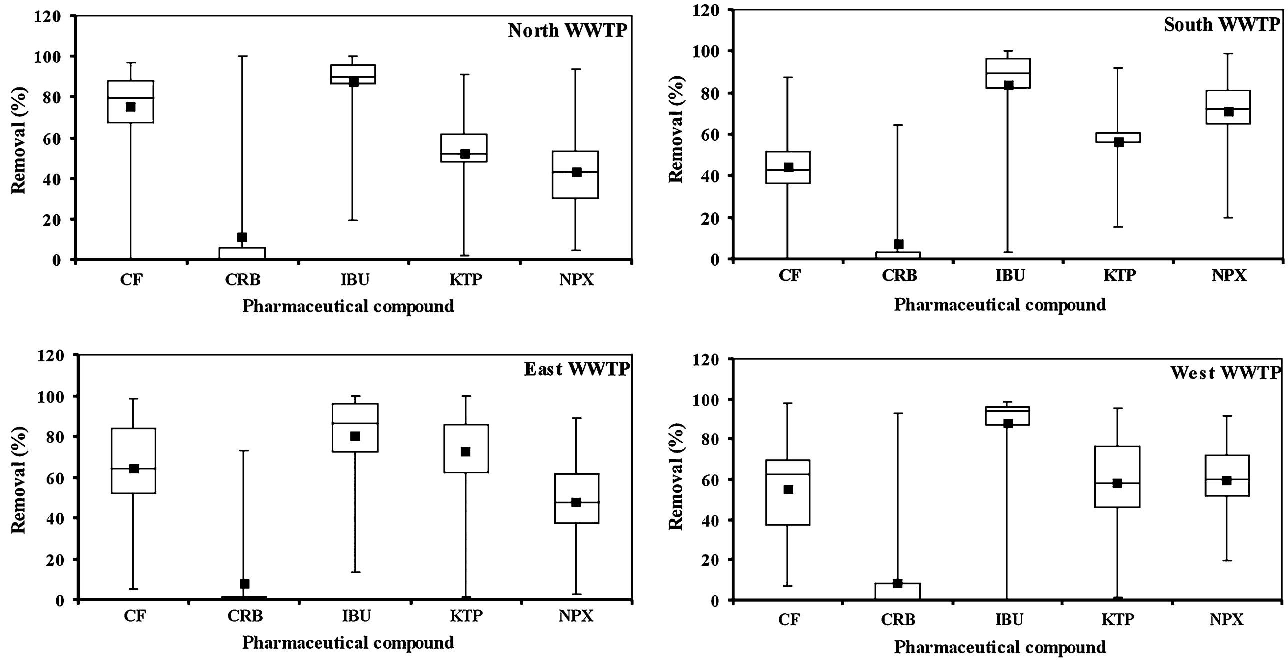


**Fig. 2.** Box-and-Whisker graphs of the removals of the pharmaceutical compounds in each of the four WWTPs evaluated ($n = 63$).

January–March and November–December. The concentration increase of CF during the coldest months can be associated to the increase of coffee consumption. Another seasonal influence was observed in the concentration of IBU that increases greatly from May–June (12.1–167 μg/L) to August–September (71.2–353 μg/L) and in the concentration of KTP that increases slightly from April (0.66–2.60 μg/L) to July (3.38–5.02 μg/L). No seasonal influence was observed in the concentration of CRB and NPX in influent wastewater neither in the concentration of any of the pharmaceutical compounds in the effluent wastewater samples.

Maximum concentration levels of caffeine, carbamazepine, diclofenac, ketoprofen and naproxen in effluent wastewater samples were lower than their predicted no effect concentration (PNEC) (182, 6.36, 13.5, 15.6, and 21.2 μg/L, respectively [3]). PNEC is the maximum concentration of a certain compound for which no adverse effect is expected to occur. Ibuprofen was the only one of the pharmaceutical compounds evaluated at concentration levels higher than its PNEC value (9.1 μg/L). Mean concentration level of ibuprofen was higher than its PNEC value in only one of the WWTPs sampled but maximum concentration levels in all of the WWTPs were above the PNEC value. However, the dilution produced after releasing effluent wastewater to receiving water is expected to reduce concentration levels of ibuprofen to concentrations with no toxicological effect to the aqueous environment.

### 3.4. Removal of the pharmaceutical compounds

A Box-and-Whisker graph of the removal rates of each pharmaceutical compound in each WWTP during the 1-year monitoring period can be seen in Fig. 2. Lines in each box show the first quartile, the median and the third quartile of the concentration values measured of each pharmaceutical compound. The whiskers or lines outside each box show the lowest and the highest concentrations measured. The point inside each box shows the mean concentration. Mean removal rates of CF, CRB, IBU, KTP and NPX were in the ranges 44–75%, 8–15%, 80–88%, 52–72% and 43–71%, respectively. These results are consistent with those previously reported by other authors [5,6,20,25,29–31]. Not only a wide variability of the removal efficiency of one pharmaceutical compound to another was observed, but also different removal efficiencies in each WWTP. For example, some compounds as CRB and IBU are removed in the same degree in the four WWTPs studied whereas others as CF, KTP and NPX are more efficiently removed in some WWTPs than in the others.

### 3.5. Statistical analysis: correlation analysis

Correlation analysis was used to evaluate the existence of relationships between the concentration of the pharmaceutical compounds (CF, CRB, IBU, KTP and NPX) or their removal rates ($R_{CF}$, $R_{CRB}$, $R_{IBU}$, $R_{KTP}$ and $R_{NPX}$) and the influent wastewater characterization parameters (TSS, BOD, COD, pH, TP, TKN and Oil content), the removal of those wastewater characterization parameters after wastewater treatment ($R_{TSS}$, $R_{BOD}$, $R_{COD}$, $R_{TP}$, $R_{TKN}$ and $R_{Oil}$) and the WWTP operational parameters (flow, SRT and HRT). Table 3 shows the correlations between the above-mentioned parameters which were used as variables in the construction of the correlation matrix. Data from the four WWTPs studied were used. Influent and effluent sampling sites from each of the WWTP were used as cases. Positive and negative correlations were written in bold.

#### 3.5.1. Relationships between the concentration and removal of pharmaceutical compounds and wastewater characterization parameters

As can be seen in Table 3, correlations are observed between the concentration of the pharmaceutical compounds and some of the influent characterization parameters (TSS, BOD, COD, TP and Oil). Positive correlation coefficients were obtained for the compounds CF, IBU, KTP and NPX while negative correlation coefficient was obtained for CRB. Positive correlations could be explained through the association of these compounds to the dissolved organic matter present in wastewater which is commonly characterized by BOD and COD values. Furthermore, BOD and COD are parameters highly correlated with TSS what, consequently, explains the high correlations between pharmaceutical compound concentrations and TSS. The only one pharmaceutical compound negatively correlated with

**Table 3**
Correlation matrix of the variables: influent wastewater characterization parameters, removals of the wastewater characterization parameters, concentrations of the pharmaceutical compounds, removals of the pharmaceutical compounds and WWTP operational parameters.

| | TSS | BOD | COD | pH | TP | TKN | Oil | $R_{TSS}$ | $R_{BOD}$ | $R_{COD}$ | $R_{TP}$ | $R_{TKN}$ | $R_{Oil}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TSS | 1.00 | 0.99 | 0.99 | −0.35 | 0.98 | 0.65 | 0.86 | 0.06 | 0.18 | 0.14 | 0.01 | −0.20 | 0.20 |
| BOD | | 1.00 | 0.97 | −0.41 | 0.93 | 0.63 | 0.91 | −0.04 | 0.11 | 0.05 | 0.11 | −0.15 | 0.17 |
| COD | | | 1.00 | −0.35 | 0.99 | 0.67 | 0.87 | 0.13 | 0.26 | 0.22 | −0.03 | −0.27 | 0.26 |
| pH | | | | 1.00 | −0.28 | 0.20 | −0.40 | −0.13 | 0.25 | 0.11 | 0.30 | −0.36 | 0.40 |
| TP | | | | | 1.00 | 0.65 | 0.78 | 0.21 | 0.28 | 0.27 | −0.12 | −0.26 | 0.25 |
| TKN | | | | | | 1.00 | 0.62 | 0.31 | 0.72 | 0.60 | −0.02 | −0.77 | 0.77 |
| Oil | | | | | | | 1.00 | −0.13 | 0.23 | 0.09 | 0.29 | −0.33 | 0.38 |
| $R_{TSS}$ | | | | | | | | 1.00 | 0.71 | 0.89 | −0.93 | −0.49 | 0.36 |
| $R_{DBO}$ | | | | | | | | | 1.00 | 0.95 | −0.39 | −0.96 | 0.92 |
| $R_{COD}$ | | | | | | | | | | 1.00 | −0.65 | −0.84 | 0.75 |
| $R_{TP}$ | | | | | | | | | | | 1.00 | 0.13 | 0.01 |
| $R_{TKN}$ | | | | | | | | | | | | 1.00 | −0.99 |
| $R_{Oil}$ | | | | | | | | | | | | | 1.00 |

| | CF | CRB | KTP | IBU | NPX | $R_{CF}$ | $R_{CRB}$ | $R_{KTP}$ | $R_{IBU}$ | $R_{NPX}$ | Flow | SRT | HRT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TSS | **0.79** | **−0.90** | **0.97** | **0.96** | **0.89** | −0.20 | −0.20 | −0.06 | −0.15 | 0.20 | 0.24 | 0.09 | 0.20 |
| BOD | **0.80** | **−0.93** | **0.92** | **0.91** | **0.94** | −0.17 | −0.17 | −0.15 | −0.20 | 0.18 | 0.30 | −0.01 | 0.15 |
| COD | **0.73** | **−0.90** | **0.96** | **0.96** | **0.87** | −0.26 | −0.26 | −0.03 | −0.17 | 0.26 | 0.25 | 0.17 | 0.27 |
| pH | −0.27 | **0.70** | −0.37 | −0.31 | −0.51 | −0.41 | −0.42 | −0.38 | −0.49 | 0.43 | 0.38 | −0.04 | 0.36 |
| TP | **0.71** | **−0.84** | **0.98** | **0.98** | **0.78** | −0.24 | −0.24 | 0.06 | −0.09 | 0.23 | 0.15 | 0.23 | 0.26 |
| TKN | 0.49 | −0.43 | 0.58 | 0.61 | 0.48 | **−0.77** | **−0.77** | −0.16 | −0.54 | **0.77** | 0.52 | 0.43 | **0.77** |
| Oil | 0.51 | **−0.89** | **0.71** | 0.69 | **0.96** | −0.39 | −0.40 | −0.37 | −0.47 | 0.40 | 0.57 | −0.05 | 0.34 |
| $R_{TSS}$ | −0.08 | −0.06 | 0.22 | 0.24 | −0.25 | −0.32 | −0.30 | **0.81** | 0.38 | 0.27 | −0.48 | **0.99** | 0.47 |
| $R_{BOD}$ | −0.17 | −0.07 | 0.16 | 0.20 | −0.01 | **−0.90** | **−0.89** | 0.16 | −0.39 | **0.87** | 0.27 | **0.82** | **0.96** |
| $R_{COD}$ | −0.14 | −0.07 | 0.20 | 0.23 | −0.11 | **−0.72** | **−0.70** | 0.46 | −0.09 | 0.68 | −0.03 | **0.95** | **0.83** |
| $R_{TP}$ | 0.01 | 0.04 | −0.20 | −0.20 | 0.31 | −0.05 | −0.08 | **−0.97** | **−0.70** | 0.10 | **0.77** | **−0.85** | −0.11 |
| $R_{TKN}$ | 0.17 | 0.06 | −0.12 | −0.15 | −0.08 | **0.98** | **0.98** | 0.11 | 0.62 | **−0.97** | −0.52 | −0.63 | **−1.00** |
| $R_{Oil}$ | −0.17 | −0.06 | 0.09 | 0.12 | 0.12 | **−1.00** | **−1.00** | −0.25 | **−0.73** | **1.00** | 0.63 | 0.52 | **0.99** |
| CF | 1.00 | −0.64 | **0.81** | **0.80** | 0.64 | 0.17 | 0.17 | 0.03 | 0.12 | −0.17 | −0.01 | −0.10 | −0.17 |
| CRB | | 1.00 | **−0.85** | **−0.82** | **−0.93** | 0.05 | 0.05 | −0.03 | 0.01 | −0.05 | −0.13 | −0.07 | −0.06 |
| KTP | | | 1.00 | **1.00** | **0.76** | −0.08 | −0.08 | 0.17 | 0.07 | 0.07 | 0.01 | 0.22 | 0.12 |
| IBU | | | | 1.00 | **0.73** | −0.12 | −0.11 | 0.17 | 0.05 | 0.10 | 0.01 | 0.24 | 0.15 |
| NPX | | | | | 1.00 | −0.13 | −0.14 | −0.33 | −0.30 | 0.15 | 0.44 | −0.20 | 0.08 |
| $R_{CF}$ | | | | | | 1.00 | **1.00** | 0.29 | **0.75** | **−1.00** | −0.67 | −0.48 | **−0.99** |
| $R_{CRB}$ | | | | | | | 1.00 | 0.31 | **0.77** | **−1.00** | −0.68 | −0.46 | **−0.98** |
| $R_{KTP}$ | | | | | | | | 1.00 | **0.85** | −0.34 | **−0.89** | **0.70** | −0.13 |
| $R_{IBU}$ | | | | | | | | | 1.00 | **−0.79** | **−0.98** | 0.22 | −0.63 |
| $R_{NPX}$ | | | | | | | | | | 1.00 | **0.70** | 0.43 | **0.98** |
| Flow | | | | | | | | | | | 1.00 | −0.32 | 0.53 |
| SRT | | | | | | | | | | | | 1.00 | 0.62 |
| HRT | | | | | | | | | | | | | 1.00 |

Correlations greater than 0.70 are significant at $p < 0.05$ (shown in bold).

characterization parameters was carbamazepine what points out the different behaviour observed for this compound in wastewater samples compared to the others evaluated.

The removal rates of the pharmaceutical compounds were highly correlated with the removal of, at least, two of the wastewater characterization parameters evaluated. Nevertheless, different behaviour was observed for each pharmaceutical compound. In some cases, there was a positive correlation between the removal of the pharmaceutical compound and the removal of the wastewater characterization parameter ($R_{CF}$, $R_{CRB}$ and $R_{TKN}$; $R_{KTP}$ and $R_{TSS}$; $R_{NPX}$ and $R_{BOD}$, $R_{Oil}$) whereas in other cases there was a negative correlation ($R_{CF}$, $R_{CRB}$ and $R_{BOD}$, $R_{COD}$; $R_{IBU}$, $R_{KTP}$ and $R_{TP}$; $R_{NPX}$ and $R_{TKN}$; $R_{CF}$, $R_{CRB}$, $R_{IBU}$ and $R_{Oil}$).

### *3.5.2. Relationships between the removal of the pharmaceutical compounds and WWTP operational parameters*

Removal rates of the pharmaceutical compounds were found to be correlated with the flow of wastewater treated by the WWTPs. This correlation was positive in the case of the removal of NPX and negative in the case of the removal of the other pharmaceutical compounds evaluated. Removal rates of IBU and KTP were highly influenced by WWTP wastewater influent flow with correlation coefficients higher than −0.88 what is consistent with correlations observed by others authors [6].

A poor correlation was obtained between the removal of the pharmaceutical compounds and SRT. Only the removal of KTP was influenced by SRT with a positive coefficient of correlation. The poor correlation observed between the removal of the pharmaceutical compounds and SRT could be explained by the small values of SRT in the studied WWTPs (1.5–5.1 days).

The removal of CF, CRB and NPX were highly correlated with HRT values with coefficients of correlation of at least 0.98. Nevertheless, whereas positive correlation coefficient was obtained for NPX, negative coefficients were obtained for CF and CRB. Negative correlation coefficients could be explained by a poor removal of the pharmaceutical compounds in the biological reactor where the compounds end up being concentrated. Nevertheless, in spite of the negative correlation coefficient between CF and HRT, the high removal of caffeine before wastewater treatment can be indicative of the existence of another way of elimination, different from degradation in the biological reactor as, for instance, elimination by retention into particulate matter.

### *3.6. Statistical analysis: principal component analysis*

A principal component analysis was carried out for all of the variables evaluated. Two components with eigenvalues >1 (Factor 1 = 11.7; Factor 2 = 8.1) accounting for 76.1% of the total variability

**Table 4**
Results of principal components analysis.

| Variable | Factor 1 | Factor 2 |
|---|---|---|
| TSS | 0.12 | **0.99** |
| BOD | 0.07 | **0.99** |
| COD | 0.19 | **0.97** |
| pH | 0.40 | −0.46 |
| TP | 0.20 | **0.93** |
| TKN | **0.73** | 0.57 |
| Oil | 0.24 | **0.88** |
| $R_{TSS}$ | 0.54 | −0.02 |
| $R_{BOD}$ | **0.98** | 0.06 |
| $R_{COD}$ | **0.87** | 0.03 |
| $R_{TP}$ | −0.20 | 0.06 |
| $R_{TKN}$ | **−0.99** | −0.08 |
| $R_{Oil}$ | **0.98** | 0.09 |
| CF | −0.22 | **0.81** |
| CRB | 0.02 | **−0.94** |
| KTP | 0.07 | **0.94** |
| IBU | 0.10 | **0.92** |
| NPX | −0.02 | **0.93** |
| $R_{CF}$ | **−0.97** | −0.09 |
| $R_{CRB}$ | **−0.96** | −0.09 |
| $R_{IBU}$ | −0.57 | −0.11 |
| $R_{KTP}$ | **−0.90** | 0.04 |
| $R_{NPX}$ | **0.95** | 0.10 |
| Flow | 0.45 | 0.22 |
| SRT | 0.68 | 0.00 |
| HRT | **0.99** | 0.08 |
| Variance (%) | 44.9 | 31.2 |

Correlations greater than 0.70 are significant at $p < 0.05$ (shown in bold).

were identified (Table 4). The first component reflects a close correlation between the content of TKN in influent wastewater, the removal of some of the wastewater characterization parameters ($R_{DBO}$, $R_{COD}$, $R_{TKN}$ and $R_{oil}$), the removal of most of the pharmaceutical compounds monitored ($R_{CF}$, $R_{CRB}$, $R_{KTP}$ and $R_{NPX}$) and the WWTP operational parameter HRT. The second component reflects a close correlation between some of the wastewater characterization parameters (TSS, BOD, TP and oil) and the concentration of all of the pharmaceutical compounds monitored (CF, CRB, KTP, IBU and NPX). Factor 1 describes an elimination pattern of pharmaceutical compounds and wastewater characterization parameters where HRT has a significant contribution. Factor 2 describes an urban contamination pattern loaded by wastewater characterization parameters and pharmaceutical compound concentrations. In Fig. 3, the correlations between the investigated variables and the two factors are represented. Plot of the variables on the plane Factor 1 versus Factor 2 allows to easily evaluate which variables have positive or negative contribution in each or both factors together with their degree of contribution. It can be seen that variables are mainly grouped into two groups, one group formed by the concentrations of the pharmaceutical compounds, except NPX, and wastewater characterization parameters, except TKN and pH, and another group formed by removal rates of NPX and wastewater characterization parameters BOD, COD and oil.

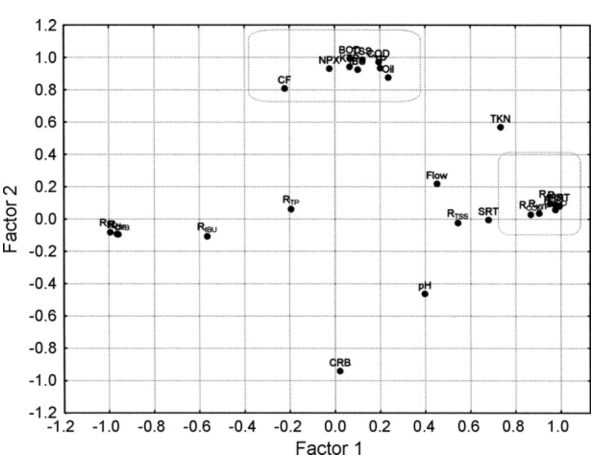


**Fig. 3.** Plot of the variables on the plane Factor 1 versus Factor 2.

## 4. Conclusions

A previously validated analytical method has been used for the determination of the pharmaceutical compounds CF, CRB, DCL, IBU, KTP and NPX in influent and effluent wastewater samples from WWTPs in Seville city during a 1-year period. All of the pharmaceutical compounds monitored, except DCL, were detected in influent and effluent wastewater samples at mean concentrations that decreased from IBU to CRB in this order: IBU > CF > NPX > KTP > CRB. A seasonal evolution of the concentration of some of the pharmaceutical compounds evaluated, CF and IBU, in influent wastewater was observed. Although wastewater treatments were similar in all of the WWTPs studied, different removal efficiencies of the pharmaceutical compounds were obtained from one WWTP to the next with mean removal rates in the range from 8.1% (CRB) to 87.5% (IBU).

By means of correlation analysis and principal component analysis, the existence of relationships between the concentration of the pharmaceutical compounds and influent wastewater characterization parameters (COD, BOD, TSS, TP and Oil) and between the removal of the pharmaceutical compounds and the removal of wastewater characterization parameters and the operational parameters flow and HRT were found to exist. Principal component analysis showed the existence of two main components accounting for 76% of the total variability.

## Acknowledgements

This research was supported by the Spanish Ministerio de Educación y Ciencia, project CGL2007-62281. The authors thank the Empresa Municipal de Abastecimiento y Saneamiento de Aguas de Sevilla (EMASESA) for supplying wastewater samples.

## References

[1] C.G. Daughton, T.A. Ternes, Pharmaceuticals and personal care products in the environment: agents of subtle change? Environ. Health Perspect. 107 (1999) 907–938.
[2] M. Crane, C. Watts, T. Boucard, Chronic aquatic environmental risks from exposure to human pharmaceuticals, Sci. Total Environ. 367 (2006) 23–41.
[3] J.L. Santos, I. Aparicio, E. Alonso, Occurrence risk assessment of pharmaceutically active compounds in wastewater treatment plants. A case study: Seville city (Spain), Environ. Int. 33 (2007) 596–601.
[4] K. Fent, A.A. Weston, D. Caminada, Ecotoxicology of human pharmaceuticals, Aquat. Toxicol. 76 (2006) 122–159.
[5] N. Lindqvist, T. Tuhkanen, L. Kronberg, Occurrence of acidic pharmaceuticals in raw and treated sewages and in receiving water, Water Res. 39 (2005) 2219–2228.
[6] A. Tauxe-Wuersch, L.F. De Alencastro, D. Grandjean, J. Tarradellas, Occurrence of several acidic drugs in sewage treatment plants in Switzerland and risk assessment, Water Res. 39 (2005) 1761–1772.
[7] L. Lishman, S.A. Smyth, K. Sarafin, S. Kleywegt, J. Toito, T. Peart, B. Lee, M. Servos, M. Beland, P. Seto, Occurrence and reductions of pharmaceuticals and personal care products and estrogens by municipal wastewater treatment plants in Ontario, Canada, Sci. Total Environ. 367 (2002) 544–558.
[8] N. Nakada, T. Tanishima, H. Shinohara, K. Kiri, H. Takada, Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment, Water Res. 40 (2006) 3297–3303.
[9] M.J. Gómez, M.J. Martínez-Bueno, S. Lacorte, A.R. Fernández-Alba, A. Agüera, Pilot survey monitoring pharmaceuticals and related compounds in a sewage treatment plant located on the Mediterranean coast, Chemosphere 66 (2007) 993–1002.
[10] M.D. Hernando, M.J. Gómez, A. Agüera, A.R. Fernández-Alba, LC–MS analysis of basic pharmaceuticals (beta-blockers and anti-ulcer agents) in wastewater and surface water, Trends Anal. Chem. 26 (2007) 581–594.

[11] S. Weigel, R. Kallenborn, H. Hünerfuss, Simultaneous solid-phase extraction of acidic, neutral and basic pharmaceuticals from aqueous samples and ambient (neutral) pH and their determination by gas chromatography–mass spectrometry, J. Chromatogr. A 1023 (2004) 183–195.
[12] L. Comoretto, S. Chiron, Comparing pharmaceutical and pesticide load into a small Mediterranean river, Sci. Total Environ. 349 (2005) 201–210.
[13] E. Zuccato, S. Castiglioni, R. Fanelli, Identification of the pharmaceuticals for human use contaminating the Italian aquatic environment, J. Hazard. Mater. 122 (2005) 205–220.
[14] M. Gross, M. Petrovic, D. Barceló, Development of a multi-residue analytical methodology based on liquid chromatography–tandem mass spectrometry (LC–MS/MS) for screening and trace level determination of pharmaceuticals in surface and wastewaters, Talanta 70 (2007) 678–690.
[15] S. Zhang, Q. Zhang, S. Darisaw, O. Ehie, G. Wang, Simultaneous quantification of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and pharmaceuticals and personal care products (PPCPs) in Mississippi river water, in New Orleans, Louisiana, USA, Chemosphere 66 (2007) 1057–1069.
[16] N. Kreuzinger, M. Clara, B. Strenn, B. Vogel, Investigation on the behaviour of selected pharmaceuticals in the groundwater after infiltration of treated wastewater, Water Sci. Technol. 50 (2004) 221–228.
[17] A.A.M. Stolker, W. Niesing, E.A. Hogendoorn, J.F.M. Versteegh, R. Fuchs, U.A. Brinkman, Liquid chromatography with triple-quadrupole or quadrupole-time of flight mass spectrometry for screening and confirmation of residues of pharmaceuticals in water, Anal. Bioanal. Chem. 378 (2004) 955–963.
[18] H.R. Andersen, M. Hansen, J. Kjølholt, F. Stuer-Lauridsen, T. Ternes, B. Halling-Sørensen, Assessment of the importance of sorption for steroid estrogens removal during activated sludge treatment, Chemosphere 61 (2005) 139–146.
[19] J.H. Al-Rifai, C.L. Gabelish, A.I. Schdfer, Occurrence of pharmaceutically active and non-steroidal estrogenic compounds in three different wastewater recycling schemes in Australia, Chemosphere 69 (2007) 803–815.
[20] T.A. Ternes, M. Bonerz, N. Herrmann, B. Teiser, H.R. Andersen, Irrigation of treated wastewater in Braunschweig, Germany: an option to remove pharmaceuticals and musk fragrances, Chemosphere 66 (2007) 894–904.
[21] APHA, Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association, Washington, DC, 1998.
[22] J.L. Santos, I. Aparicio, E. Alonso, M. Callejón, Simultaneous determination of pharmaceutically active compounds in wastewater samples by solid phase extraction and high-performance liquid chromatography with diode array and fluorescence detectors, Anal. Chim. Acta 550 (2005) 116–122.
[23] Council Directive 91/271/EEC of 21 May 1991 Concerning Urban Waste Water Treatment, The Council of the European Communities.
[24] Z. Chen, P. Pavelic, P. Dillon, R. Naidu, Determination of caffeine as a tracer of sewage effluent in natural water by on-line solid-phase extraction and liquid chromatography with diode-array detection, Water Res. 36 (2002) 4830–4838.
[25] D. Bendz, N.A. Paxéus, T.R. Ginn, F.J. Loge, Occurrence and fate of pharmaceutically active compounds in the environment, a case study: Höje River in Sweden, J. Hazard. Mater. 122 (2005) 195–204.
[26] B. Ferrari, N. Paxéus, R. Giudice, A. Pollio, J. Garric, Ecotoxicologial impact of pharmaceuticals found in treated wastewater: study of carbamazepine, clofibric acid, and diclofenac, Ecotoxicol. Environ. Saf. 55 (2003) 359–370.
[27] M. Farré, I. Ferre, A. Ginebreda, M. Figueras, L. Olivella, L. Tirapu, M. Vilanova, D. Barceló, Determination of drugs in surface water and wastewater samples by liquid chromatography-mass spectrometry: methods and preliminary results including toxicity studies with Vibrio fishery, J. Chromatogr. A 938 (2001) 187–197.
[28] M. Carballa, F. Omil, J. Lema, M. Llompart, C. García-Jares, I. Rodríguez, M.J. Gómez, T.A. Ternes, Behaviour of pharmaceuticals, cosmetic and hormones in a sewage treatment plant, Water Res. 38 (2004) 2918–2926.
[29] I.J. Buerge, T. Poiger, M.D. Müller, H.R. Buser, Caffeine, an anthropogenic marker for wastewater contamination of surface waters, Environ. Sci. Technol. 37 (2003) 691–700.
[30] M. Clara, B. Strenn, N. Kreuzinger, Carbamazepine as a possible anthropogenic marker in the aquatic environment: investigations on the behaviour of carbamazepine in wastewater treatment and during groundwater infiltration, Water Res. 38 (2004) 947–954.
[31] A. Joss, E. Keller, A.C. Alder, A. Göbel, C.S. McArdell, T. Ternes, H. Siegrist, Removal of pharmaceuticals and fragrances in biological wastewater treatment, Water Res. 39 (2005) 3139–3152.