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Pilot survey monitoring pharmaceuticals and related compounds in a sewage treatment plant located on the Mediterranean coast

M.J. Gómez [a], M.J. Martínez Bueno [a], S. Lacorte [b], A.R. Fernández-Alba [a], A. Agüera [a,*]

[a] *Pesticide Residue Research Group, University of Almería, 04120 Almería, Spain*
[b] *Department of Environmental Chemistry, IIQAB-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain*

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Abstract

A one-year monitoring study was performed to evaluate the occurrence, persistence and fate of a group of 14 organic compounds in a sewage treatment plant (STP) located in the south of Spain. These results are part of a more extensive study, financed by the Spanish Ministry of Research with the aim to evaluate the traceability of new pollutants on the Mediterranean coast and to determine the removal efficiency of sewage treatment plants (STP) for these pollutants. The compounds which have been analyzed include pharmaceuticals of various therapeutic categories (ibuprofen, acetaminophen, dipyrone, diclofenac, carbamazepine and codeine), pesticides (chlorfenvinfos and permethrin), caffeine, triclosan, bisphenol A and three of their more relevant metabolites (1,7-dimethylxanthine, carbamazepine 10,11-epoxide and 2,7/2,8-dichlorodibenzo-*p*-dioxin). An SPE/GC–MS multi-residue analytical method was developed and validated to facilitate simultaneous determination of these compounds in both influent and effluent wastewater. The method provided mean recoveries higher than 75%, with the exception of 2,7/2,8-dichlorodibenzo-*p*-dioxin, dipyrone and permethrin which exhibited recoveries lower than 22%. The overall variability of the method was below 14%. The method detection limit (LOD) was between 1 and 100 ng l^{-1} and precision, which was calculated as relative standard deviation (RSD), ranged from 1.8% to 11.2%. The application of the proposed method has allowed the identification of all the target compounds at mean concentrations which ranged from 0.12 to 134 μg l^{-1} in the influent and from 0.09 to 18.0 μg l^{-1} in the effluent. The removal efficiencies of the STP for these compounds varied from 20% (carbamazepine) to 99% (acetaminophen), but in all cases resulted insufficient in order to avoid their presence in treated water and subsequently in the environment.
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1. Introduction

Since the presence of non-regulated contaminants was pointed out as a possible cause of damage of the quality of natural water, many studies have been performed to evaluate the source, occurrence, fate and environmental effects of these pollutants (Kolpin et al., 2002; Singer et al., 2002; Boyd et al., 2003; Bound and Voulvoulis, 2004; Ashton et al., 2004; Bendz et al., 2005; Schwab et al., 2005; Fent et al., 2006). Active pharmaceutical ingredients, surfactants, personal care products or substances with endocrine disrupting activity are among the compounds which have provoked greater interest in the last decade (Daughton and Ternes, 1999; Heberer, 2002; Barceló, 2003; Lopez de Alda et al., 2003). Research has shown that many of these compounds are not completely removed during conventional wastewater treatments in the STPs and therefore, their presence is reiterated in effluents (Ternes, 1998; McAvoy et al., 2002; Singer et al., 2002; Agüera et al., 2003; Andreozzi et al., 2003; Boyd et al., 2003; Metcalfe et al., 2003; Carballa et al., 2004; Paxeus, 2004; Castiglioni et al., 2005; Gomez et al., 2006; Gagné

* Corresponding author. Tel.: +34 950 01 55 31; fax: +34 950 01 54 83.
E-mail address: aaguera@ual.es (A. Agüera).

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et al., 2006; Roberts and Thomas, 2006). Through effluent discharge, contaminants are then dispersed into the environment. This continuous input into the environment, in some cases at concentrations in the $\mu g\,l^{-1}$ range, may give rise to a long-term exposition for aquatic organisms, thus producing serious alterations in the aquatic ecosystem (Hernando et al., 2004, 2006). Presence of these contaminants in surface, drinking and groundwater has been widely reported (Sacher et al., 2001; Heberer, 2002; Kolpin et al., 2002; Bendz et al., 2005).

Many monitoring studies have been conducted in different countries to evaluate the efficiency of STPs in order to predict environmental loads and to compare concentrations in effluents with those already present in surface water. Compounds detected are obviously related with the socioeconomic activity of the area in which they are located. Many studies about STPs sited in industrial areas in North and Central Europe have been carried out, which explain the presence of pollutants in continental receiving water, such as rivers or lakes. However, scarce information on discharge into the Mediterranean Sea is still available (Stien et al., 1998; Riu et al., 2000; Agüera et al., 2003; Andreozzi et al., 2003).

This work documents the presence of a group of selected compounds in a STP located in Almería, a province in the south of Spain on the Mediterranean coast. This plant discharges the treated water directly into the sea and therefore a continuous input of these compounds is observed. The plant is located in an important agricultural and tourist area and very close to a hospital which discharges the untreated wastewater into the urban network. The aim of this work, which has been part of a more extensive project, was to identify groups of pollutants that may be source of contamination and damage of marine environment, in an area which presents these especial characteristics. In this project the presence of other contaminants (Alonso et al., 2002) and other aspects, such as the quality of the coastal sediments and the impact of discharges, have been also evaluated in previous works (González et al., 2004; Eljarrat et al., 2005).

2. Materials and methods

2.1. Chemicals

Pure standards (>98%) of acetaminophen, ibuprofen, triclosan, caffeine, 1,7-dimethylxanthine, 2,7/2,8-dichlorodibenzo-*p*-dioxin, carbamazepine, carbamazepine 10,11-epoxide, dipyrone, diclofenac, chlorfenvinfos, permethrin and bisphenol A, were purchased from Sigma–Aldrich (Steinheim, Germany). Codeine was obtained dissolving a codeisan tablet (30 mg of codeine). Individual stock standard solutions were prepared in ethyl acetate, or methanol in the case of carbamazepine, carbamazepine 10,11-epoxide, 1,7-dimethylxanthine, diclofenac and acetaminophen, and stored at −20 °C. For GC–MS determinations working standard mixtures, at different concentrations, were prepared by appropriate dilution of the stock solutions in ethyl acetate. For LC–MS analysis, working standard solutions were prepared in methanol:water (10:90).

Pesticide-grade ethyl acetate was from Panreac (Barcelona, Spain) and HPLC-grade water, acetonitrile and methanol from Merck (Darmstadt, Germany).

2.2. Sample collection and preparation

Wastewater samples used in this study were collected from a municipal sewage treatment plant (STP) located in the southeast of Spain (Almería). The plant is connected to a sewage system servicing a municipal area with ~62.000 inhabitants. The plant apply a pre-treatment for solid removal, a primary treatment carried out in circular sedimentation tanks to eliminate suspended material, an activated sludge biological treatment and a final clarification. Two series of daily composite samples of raw influents and final effluents were taken during five consecutive days, for two consecutive months. Integrated samples were representatives of a one-day work in the STP and were taken at one-hour intervals. Sampling was carried out by an automatic device (0.5 l/3 h). Discrete samples were also monthly taken during the period comprised from July 2003 to April 2004. In this case, influent and effluent samples were collected by using pre-rinsed amber glass bottles. After collection, samples were filtered through a 0.7 μm glass fibber filter (Teknokroma, Barcelona, Spain) prior to analysis, in order to remove particles that may interfere during the extraction procedure. Filters and samples were stored in the dark at 4 °C and extracted within 48 h in all the cases.

A solid phase extraction (SPE) procedure was applied to the wastewater samples using commercial Oasis™ HLB (divinylbenzene/*N*-vinylpyrrolidone copolymer) cartridges (200 mg, 6 cc) from Waters (Mildford, MA, USA). An automated sample processor ASPEC XL fitted with an 817 switching valve and an external 306 LC pump from Gilson (Villiers-le-Bel, France) was used for this purpose. Conditioning step was performed with 5 ml of ethyl acetate, 5 ml of methanol and 5 ml of LC-grade water at a flow rate of $1\,ml\,min^{-1}$. Wastewater samples (100 ml influent and 200 ml effluent) were loaded at a flow rate of $10\,ml\,min^{-1}$ followed by a washing step with 6 ml of water. After that, the cartridges were dried by nitrogen stream during approximately 15 min and finally eluted with 2×4 ml of ethyl acetate at $1\,ml\,min^{-1}$. The extracts so obtained were evaporated by a gentle nitrogen stream until a final volume of 1 ml for direct analysis by GC–MS. For LC–MS analysis, extracts in methanol:water (15%) were prepared before the injection into the LC/TOFMS system.

In order to evaluate presence of the target compounds in the suspended solids, the particles retained in the filters were extracted with 10 ml of dichloromethane. These extracts were dried by gentle nitrogen stream and recomposed in 500 μl of ethyl acetate for direct analysis in GC–MS.

2.3. Chromatographic determinations

GC–MS analyses were run on a HP 6890 Series gas chromatograph (Hewlett–Packard, Palo Alto, CA, USA) interfaced to a HP 5973 mass-selective detector. Data acquisition and processing, and instrumental control were performed by the HP Chem-Station software. Analytes were separated in a crosslinked 5%-phenyl-95%-dimethylpolysiloxane (ZB-5 MS, Phenomenex, Torrance, CA, USA) capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness). A split/splitless injector was used in pulse splitless mode. An empty liner was filled with 0.5 cm Carbofrit (Restek, Bellefonte, USA) placed at 3.6 cm from the upper part of the liner. The injector operating conditions were as follows: injection volume 10 μl; injector temperature 250 °C; initial pulse pressure 30 psi (1.5 min). The helium carrier gas flow was maintained at 1 ml min^{-1}. The oven temperature program was 1.0 min at 105 °C, 17 °C min^{-1} to 200 °C (1 min), 2 °C min^{-1} to 220 °C (2 min), 5 °C min^{-1} to 290 (1 min). The transfer line temperature was set at 280 °C.

Typical MS operating conditions were optimised by the auto-tuning software. EI mass spectra were obtained at 70 eV electron energy and monitored from *m/z* 50 to 400. The ion source and quadrupole analyser temperatures were fixed at 230 °C and 150 °C, respectively.

Analyses by liquid chromatography/electrospray/time-of-flight mass spectrometry (LC/ESI/TOF/MS), in positive ionization, were performed to confirm the positive findings in the samples. Analytes were separated using an HPLC (series 1100, Agilent Technologies, Palo Alto, CA) equipped with a reversed-phase C_8 analytical column (ZORBAX Eclipse® XDB, Agilent Technologies) of 150 mm × 4.6 mm and 5-μm particle diameter. Column temperature was maintained at 25 °C. Mobile phase A was acetonitrile, and mobile phase B consisted of water with 0.1% formic acid. A linear gradient progressed from 15% A (initial conditions) to 100% A in 30 min, after which the mobile phase composition was maintained at 100% A for 5 min. The flow rate was 0.6 ml min^{-1}, and the injection volume was 50 μl. This HPLC system was connected to a time-of-flight mass spectrometer (MSD-TOF, Agilent Technologies, Palo Alto, CA) equipped with an electrospray interface under the following operating parameters: capillary 4000 V, nebulizer 40 psi g, drying gas 9 l min^{-1}, gas temp 300 °C, fragmentor 190 V, skimmer 60 V, Oct DC1 37.5 V, OCT RF V 250 V. The mass axis was calibrated using the mixture provided by the manufacturer over the *m/z* 50–3200 range. A second orthogonal sprayer with a reference solution was used as a continuous calibration using the following reference masses: 121.0509 and 922.0098 *m/z* (resolution: 9500 + 500 at 922.0098 *m/z*). Spectra were acquired over the *m/z* 50–1000 range at a scan rate of one second per spectrum.

2.4. Validation studies

All the validation studies were performed by using sewage extracts taken from the STP effluent. Because of the impossibility to obtain blanks, the samples were previously analysed and presence of the target compounds considered. To minimize matrix effects consequence of the presence of sample matrix components, matrix matched calibration curves were used for quantitative determinations. The linearity in the response was studied by using matrix-matched calibration solutions prepared by spiking sewage extracts at six concentration levels, ranging from the analytes limit of determination to 5 μg l^{-1} or 10 μg l^{-1}, depending on the concentration level usually present in the samples. Each point was obtained as the average of three injections. Integrated peak area data of the selected quantification masses (see Table 1) were used to construct the curves.

The recovery studies ($n = 4$) were carried out by spiking sewage samples at the concentration level of 1 μg l^{-1}. Precision of the chromatographic method, determined as relative standard deviation (RSD), was obtained from the repeated injection (five times) of a spiked extract during the same day (repeatability) and in different days (reproducibility). The limits of detection (LODs) were determined experimentally from the injection of spiked wastewater samples and calculated using a signal-to-noise ratio of 3. When the analyte of interest was originally present in the samples the LODs were calculated in terms of sensibility considering as LOD that amount of analyte that produced an increase in the analyte response of 30%.

Confirmation criteria applied to the target compounds in the sewage samples were: presence of three characteristic fragment ions at the correct retention time and with the correct relative ion intensity (±30%).

3. Results and discussion

3.1. Analysed compounds

The monitoring study was designed to evaluate the occurrence and fate of organic compounds in a sewage treatment plant. After preliminary analyses, 11 compounds and three of their more relevant metabolites were selected

Table 1
Molecular weight, diagnostic ions and quantification masses (in bold) used for the GC–SIM–MS analysis of the studied compounds in wastewater

Compound	Mw	SIM ions (RA, %)
Ibuprofen	206	**161 (100)**, 163 (95), 206 (48)
Acetaminophen	151	80 (20), **109 (100)**, 151 (30)
Caffeine	194	82 (25), 109 (50), **194 (100)**
1,7-Dimethylxanthine	180	68 (95), 123 (45), **180 (100)**
DCDD	322	189 (50), **252 (100)**, 254 (75)
Carbamazepine 10,11-epoxide	252	165 (20), 192 (30), **193 (100)**
Dipyrone	333	83 (50), 216 (15), **217 (30)**
Chlorfenvinfos	359	**267 (100)**, 269 (70), 323 (70)
Triclosan	289	218 (90), **288 (100)**, 290 (95)
Diclofenac	318	**214 (100)**, 242 (70), 277 (50)
Bisphenol A	228	**213 (100)**, 214 (15), 228 (25)
Carbamazepine	236	192 (45), **193 (100)**, 236 (30)
Codeine	299	162 (25), 229 (25), **299 (100)**
Permethrin	391	163 (26), 165 (24), **183 (100)**

to be included in this study. They represent different classes of compounds with regards to their chemical structures and applications, and they are characterised by their extensive use and reported presence in natural water and in wastewater. This group of compounds includes pharmaceuticals belonging to different therapeutic categories (such as the anti-epileptic agent carbamazepine an its metabolite carbamazepine 10,11-epoxide; the opiate narcotic codeine; the widely used anti-inflammatory drugs ibuprofen and diclofenac; the analgesic, anti-pyretic agents acetaminophen and dipyrone); the plasticizer bisphenol A; the antiseptic triclosan and its toxic metabolite 2,7/2,8-dichlorodibenzo-*p*-dioxin; two insecticides (chlorfenvifos and permethrin of agriculture and domestic use); and the stimulant caffeine and its metabolite 1,7-dimethylxanthine.

Other compounds, such as the pesticides lindane, vinclozoline, procymidone, oxyfluorofen and endosulfan, were scarce and only occasionally detected, and have not been considered in this paper. The eventual detection of these pesticides may be a consequence of the location of the STP, located in an important agricultural area.

3.2. Chromatographic determination

A SPE/GC–MS analytical method was developed which allowed the simultaneous determination of this set of selected compounds in wastewater. The method provided accurate identification and precise quantification of the target compounds in a simple and rapid way, in order to be easily implemented in routine analysis. Although polar drugs and metabolites were included in the analysis, the usual but extensive and time-consuming derivatization step was avoided. Lower sensitivity which derives from the direct injection of the samples without derivatization, was compensated by using 10 μl sample injection volumes. Typical degradation of carbamazepine to iminostilbene, consequence of thermal degradation under injection conditions was observed (Ternes, 2001). This effect cannot be avoided and, consequently, it degrades the chromatographic analysis of this compound. However, precision studies showed an acceptable repeatability and reproducibility of the carbamazepine peak (around 11%), despite degradation, and the LOD (30 ng l^{-1}) was low enough to reach the concentration levels present in the samples. For this reason, results of carbamazepine were finally included in the paper.

The selectivity required for reaching reliable results was obtained by working in selected ion monitoring (SIM) mode. Three characteristic ions were chosen for each compound to fulfil the confirmation criteria of positive findings established by the European Union (2002). The presence of these three characteristic masses at the correct retention time and with correct relative ion intensities was considered as valid confirmation criterion. Diagnostic ions and quantification masses used in the study are shown in Table 1.

The application of the chromatographic conditions described in the experimental section, allowed the separation of the analytes within 35 min analysis time. Although the last compound eluted at 26 min, the analysis was maintained and the final temperature increased up to 290 °C to assure the complete elimination of the matrix components from the analytical column. All the peaks presented optimal chromatographic characteristics such as acceptable peak shape and resolution.

To ensure the consistency of this method in the identification of target compounds in real samples, additional confirmation of positive findings was obtained using liquid chromatography time-of-flight mass spectrometry (LC–TOF-MS). This technique provides a high degree of confirmation for target compounds by accurate mass measurements and has been widely applied with this aim in many fields (Ferrer and Thurman, 2003). Since LC–TOF-MS analyses were performed only for confirmation purposes, a validation study of the method was not carried out. However, the main instrumental parameters were optimized to provide the best possible sensitivity and structural information for a reliable identification. A fragmentor voltage of 190 V yielded between one and three fragment ions for all the compounds. The accurate mass measurements of the protonated molecules were used for confirmation in all the cases, following a procedure described elsewhere (Ferrer et al., 2005). The accuracies obtained in the mass measurements of the protonated molecules of the target compounds on matrix-matched standards showed errors which were less than 2 ppm in most cases, far below 5 ppm, which is widely accepted as a threshold accuracy value for confirmation of elemental compositions. The high degree of confirmation for target compounds by accurate mass measurements allowed the confirmation of positive findings obtained under GC–MS, demonstrating the applicability of this method in routine analysis. An example of application of LC-TOF/MS to the confirmation of positive finding in an effluent wastewater sample is showed in Fig. 3. Presence of carbamazepine, acetaminophen and codeine was confirmed by accurate mass measurements, obtaining accuracy errors lower than 2 ppm in all cases. Additional confirmation was obtained for carbamazepine and acetaminophen for which accurate mass measurements of typical fragment ions was also obtained.

3.3. Method performance

The analytical performance of the method was evaluated by using spiked sewage extracts. The results obtained are shown in Table 2. Linearity of the calibration curves was tested and, in all cases, regression coefficients obtained were $\geqslant 0.99$. Precision of the method was determined as RSDs inter- and intra-day, respectively. The values obtained ranged from 2–11% (repeatability) and 4–18% (reproducibility). Good rates of recovery were obtained for most of the compounds using Oasis HLB-SPE cartridges. The recovery studies were carried out by spiking sewage samples at the concentration level of 1 μg l^{-1}. Quantitative recoveries were obtained in all cases, varying from 78% to 98%, except for DCDD, dipyrone and per-

Table 2
Mean recoveries ($n = 4$), limits of detection (ng/l) and precision ($n = 5$) of the target compounds determined by analysis of spiked STP effluent water

Compound	Recovery (RSD, %)	LOD (ng/l)	Repeatability (RSD, %)	Reproducibility (RSD, %)
Ibuprofen	83 (12)	23	6	18
Acetaminophen	75 (14)	32	8	11
Caffeine	94 (8)	1	8	10
1,7-Dimethylxanthine	91 (9)	14	5	16
DCDD	21 (7)	43	7	4
Carbamazepine 10,11-epoxide	93 (5)	70	4	10
Dipyrone	22 (7)	45	11	8
Chlorfenvinfos	85 (11)	21	6	7
Triclosan	93 (4)	17	3	8
Diclofenac	88 (13)	100	9	6
Bisphenol A	85 (6)	7	5	6
Carbamazepine	90 (10)	30	10	11
Codeine	82 (7)	8	8	7
Permethrin	16 (7)	28	2	12

methrin which showed very low recoveries, of 21%, 22% and 16%, respectively. Despite these low recoveries, the other validation data, such as repeatability and limit of detection are good, and therefore a reliable determination of these compounds is feasible.

In addition to the recovery studies, the influence of the filtration step was evaluated to obtain estimation of the amount of these compounds, specifically those with higher hydrophobicity, which may be adsorbed to suspend particulate matter. Particles retained in glass fibber filters, after filtration of spiked wastewater samples ($10\ \mu g\ l^{-1}$), were rinsed with dichloromethane and subsequently analysed. Different percentages of the total amount present in the sewage effluent samples were founded for chlorfenvinfos (8%), bisphenol A (5%) and caffeine, 1,7-dimethylxanthine and codeine (<5%). Due its higher hydrophobicity, triclosan presented a higher adsorption rate of about 50%. This indicates that adsorption on solid particles is the key mechanism involved in the elimination of this compound in the STP, which results in its presence mainly in sludge. Permethrin also presents a strong adsorption, above 75%, according to its experimental K_{oc} value. Therefore, adsorption effects in sludge are expected to be relatively high. Due to the polarity of the remaining pharmaceuticals studied, no significant reduction of their concentration was observed during the filtration.

The limits of detection obtained for spiked wastewater were within the range of $1–100\ ng\ l^{-1}$ (see Table 2) and guarantee a correct evaluation of all compounds at the concentration levels present in the sewage samples. Finally, the validation study has proved the suitability of this method in the application to wastewater monitoring programmes.

3.4. Monitoring study and elimination rates during the STP process

The proposed method was applied to a one-year monitoring study in order to evaluate the presence and behaviour of target compounds in a STP in Almería (province located in the south-east of Spain, on the Mediterranean coast). The plant is strategically situated in a very productive agricultural area and very close to a hospital which discharges into the urban network. In this way, the influence of both aspects could be evaluated. Samples were taken monthly from both STP raw influent and treated effluent. Table 3 shows the results obtained throughout the study. Most compounds were present in all of the samples analyzed, excepting DCDD, permethrin and chlorfenvinphos, which were occasionally not detected. Acetaminophen and carbamazepine 10,11-epoxide were always detected in the influent, reaching in the case of acetaminophen concentrations up to $246\ \mu g\ l^{-1}$, although they were sometimes absent in the effluent as a consequence of the high average removal rate reached for these compounds in the STP. Variations in one order of magnitude were observed in concentration levels detected for most compounds during the sampling period. Fig. 1 shows an example of a chromatogram obtained by the analysis of an effluent wastewater sample.

The removal of contaminants during their passage through the municipal STP was investigated. Analysis of the corresponding daily composite water samples of raw influent and final effluent were performed for five consecutive days and for two consecutive months. Fig. 2 shows the average loads determined in the influent and effluent for compounds detected and removal efficiencies obtained. Elimination rates ranged from 20% (carbamazepine) to up to 99% (acetaminophen). Most of the compounds investigated presented high removal efficiencies (>80%), except carbamazepine, 1,7-dimethylxanthine, DCDD, diclofenac, codein and dipyrone, which presented lower values.

Due to the high application level and the fact that most of them can be purchase without prescription, non-steroidal anti-inflammatory drugs (NSAIDs) constitute one of the most relevant groups of pharmacologically active

Table 3
Concentration range and mean values found in the influent and effluent of a STP during a one-year monitoring study

Compound	Influent (μg/l)		Effluent (μg/l)	
	Range	Mean	Range	Mean
Ibuprofen	34–168	84	0.24–28	7.1
Acetaminophen	29–246	134	<LOD–4.3	0.22
Caffeine	52–192	118	1.4–44	12
1,7-Dimethylxanthine	48–111	79	0.60–50	18
DCDD	<LOD–4.3	0.7	<LOD–1.2	0.20
Carbamazepine 10,11-epoxide	0.3–0.5	0.35	<LOD–0.3	0.16
Dipyrone	4.7–24	14	2.4–7.5	4.9
Chlorfenvinfos	<LOD–3.7	0.9	<LOD–1.0	0.3
Triclosan	0.39–4.2	1.8	0.08–0.40	0.2
Diclofenac	0.2–3.6	1.5	0.14–2.2	0.9
Bisphenol A	0.72–3.4	1.4	0.14–0.98	0.38
Carbamazepine	0.12–0.31	0.15	0.11–0.23	0.13
Codeine	2.8–11	5.2	0.9–8.1	3.7
Permethrin	<LOD–0.3	0.12	<LOD–0.08	0.04

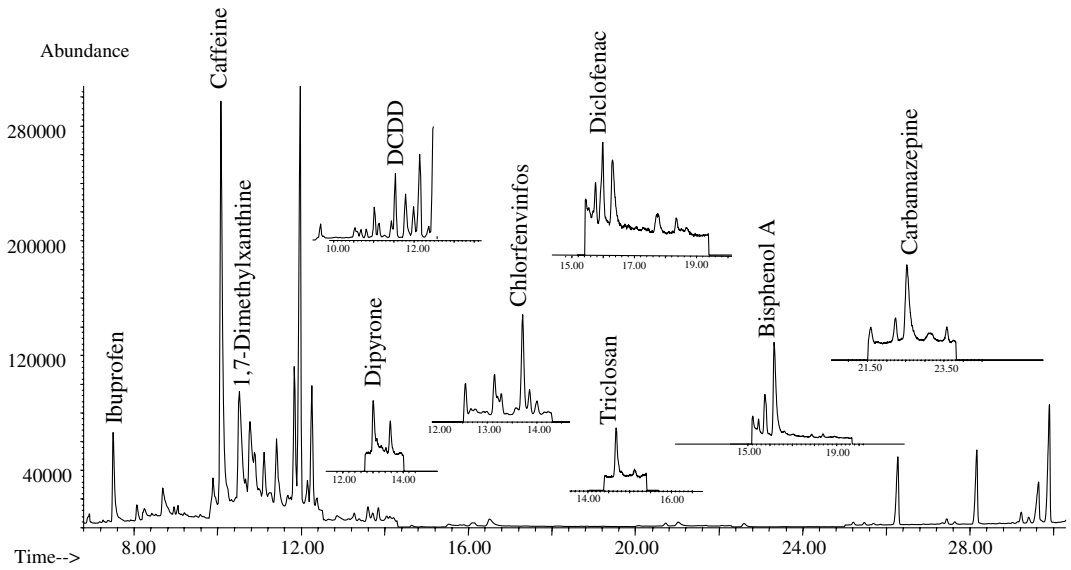

Fig. 1. GC–SIM–MS chromatogram corresponding to the analysis of an effluent wastewater sample and ion-selected chromatograms for the analytes detected.

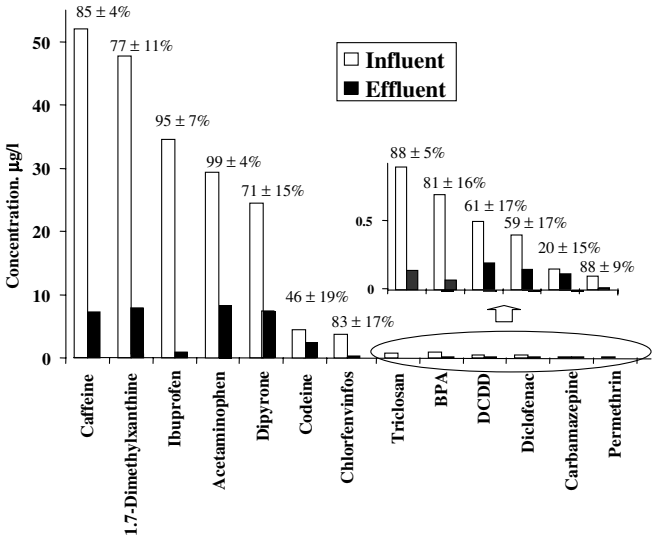

Fig. 2. Mean concentrations of the target compounds obtained by the analysis of daily composite water samples of the raw influent and final effluent of the STP during five consecutive days, for two consecutive months. Removal efficiencies calculated are also shown.

substances, from an environmental point of view. The presence of these substances in treated wastewater and in the aquatic environment is ubiquitous and has been widely documented (Ternes, 1998; Gross et al., 2004). In this study, ibuprofen, acetaminophen and diclofenac, three of the most commonly prescribed NSAIDs, were detected in the STP samples investigated. Diclofenac concentrations present in effluent samples were higher, in many cases, than those previously reported in wastewater (Santos et al., 2005; Roberts and Thomas, 2006; Verenitch et al., 2006). The removal rate reported for this compound is variable between the different STPs studied by various authors (Tauxe-Wuersch et al., 2005), but in general the values obtained in this work are lower than others previously reported, which are up to 75% (Ternes, 1998; Stumpf et al., 1999).

The presence of high concentrations of ibuprofen in STP influent (up to 168 $\mu g\,l^{-1}$) is not surprising, due to the amount of ibuprofen consumed with or without prescription. It has been reported that only 15% of the human therapeutic dose of ibuprofen is excreted as the parent compound, however high concentration levels have been found in the influent of different STPs in Europe (Paxeus, 2004; Bendz et al., 2005; Santos et al., 2005). Also the presence of the corresponding hydroxy- and carboxy- metabolites have been referenced (Weigel et al., 2004a; Bendz et al., 2005). A high removal rate of around 95% was found for this compound, in concordance with previous works (Buser et al., 1999; Metcalfe et al., 2003; Roberts and Thomas, 2006), but high concentrations of ibuprofen are still present in the effluent (7.1 $\mu g\,l^{-1}$, mean value), reaching values of up to 28 $\mu g\,l^{-1}$. Similar average concentrations of ibuprofen have been reported in the effluent of other STPs (Metcalfe et al., 2003; Ashton et al., 2004; Santos et al., 2005). The occurrence of ibuprofen has also been frequently reported in environmental waters, being considered as one of the most common drug residues in surface water (Ashton et al., 2004; Weigel et al., 2004b; Comoretto and Chiron, 2005; Roberts and Thomas, 2006).

Another group of compounds frequently detected in abundance in this study have been analgesic and anti-pyretic agents. Acetaminophen (paracetamol), was found in the raw wastewater at significantly higher concentrations than any other target compound (up to 246 $\mu g\,l^{-1}$, with an average influent concentration of 84 $\mu g\,l^{-1}$). Despite this very high input, the elevated elimination efficiency reached during the wastewater treatment (of about 99%), allowed the

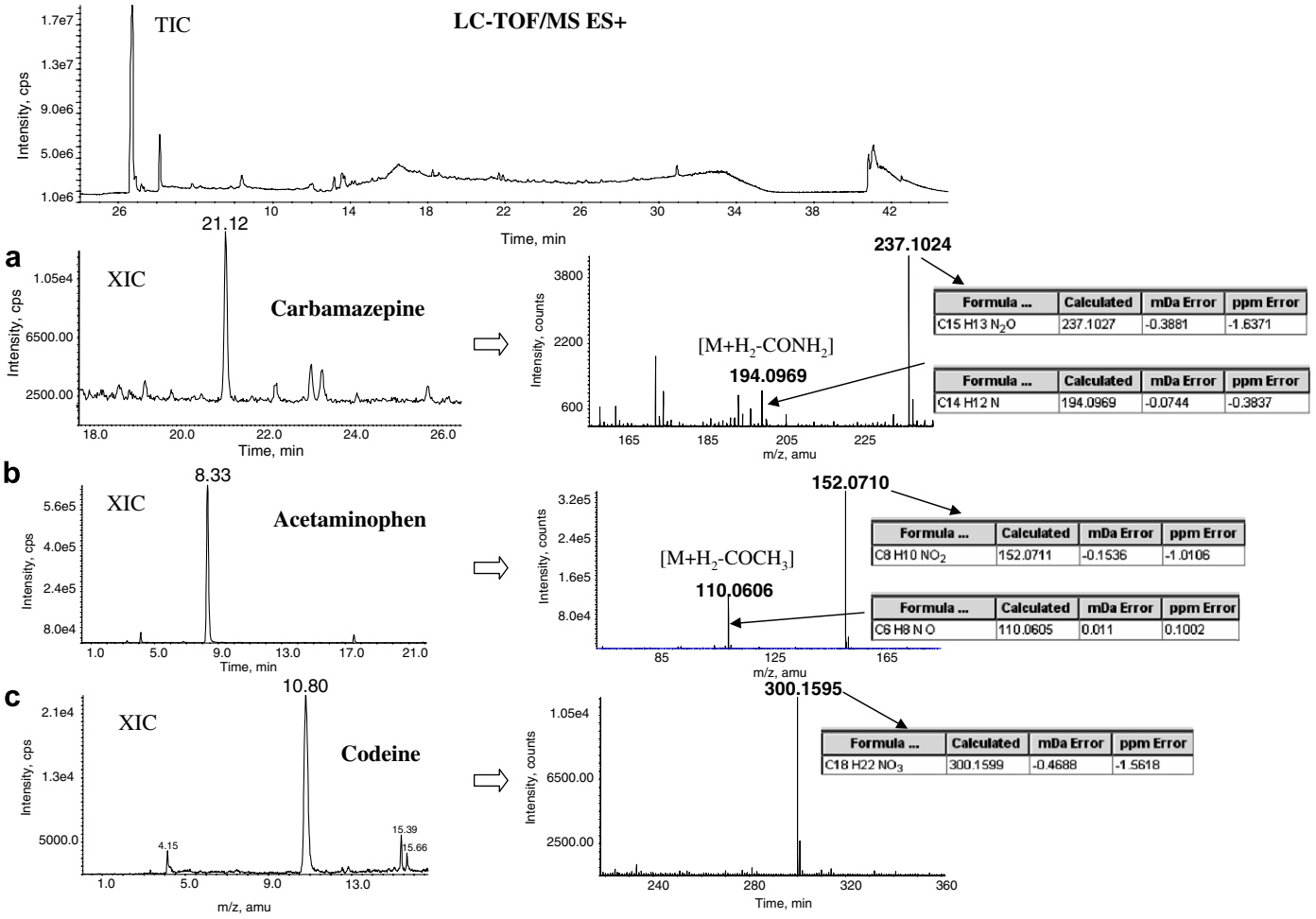

Fig. 3. Example of confirmation of pharmaceuticals by LC-TOF/MS in an effluent wastewater sample.

elimination or drastic reduction of concentration levels in the effluent. Acetaminophen probably undergoes a rapid biodegradation during wastewater treatment and, because of this, the presence of this compound in surface waters has scarcely been referenced (Ternes, 1998; Kolpin et al., 2002; Weigel et al., 2004b; Roberts and Thomas, 2006).

Dipyrone (also known as metamizole) and codeine were present in the effluents at similar concentrations (4.9 and 3.7 $\mu g\,l^{-1}$, respectively). Dipyrone is an analgesic and anti-pyretic drug, also belonging to the family of the NSAIDs. Its use as an analgesic is controversial and it has been banned in some countries (e.g., USA, UK, Sweden) because of its association with potentially life-threatening blood dyscrasias such as agranulocytosis. However, in other countries (e.g., Germany, Italy and Spain) the use of dipyrone is very widespread to treat postoperative pain, colic pain, cancer pain and migraine. In Spain it is freely available over-the-counter and is one of the most popular non-opioid first line analgesics. Hospital use is also very important, being one of the drugs present at higher concentrations in wastewater effluents from hospitals in studies performed by the authors (unpublished). After oral intake, dipyrone is rapidly hydrolyzed to its main metabolite, 4-methylaminoantipyrine (4-MAA), from which many other metabolites are produced by enzymatic reactions (Ergün et al., 2004). The high concentration of dipyrone present in the raw influent of the STP studied, has been attributed to the proximity of a hospital, where the parenteral application of this drug is common. When dipyrone is administered intravenously, the hydrolysis is not so fast and dipyrone can be detected as itself.

Dipyrone showed removal efficiency of 71%, and in the discharge of the STP was found at an average concentration of 4.9 $\mu g\,l^{-1}$. To our knowledge, no previous data has been reported on the concentration of dipyrone in STP effluents. However, presence of the metabolites *N*-acetyl-4-aminoantypyrine (AAA) and *N*-formyl-4-aminoantipyrine (FAA) has been referenced in an STP in Germany (Schmidt and Brockmeyer, 2002) and in the river Elbe (Weigel et al., 2004b), in this case at considerably high concentrations (<20 to 939 $ng\,l^{-1}$).

Codeine is another compound scarcely referenced in previous studies and is the most widely used naturally occurring narcotic in medical treatment in the world. It is an effective sedative, analgesic and antitussive agent. It was found in raw wastewater at a mean concentration of 5.2 $\mu g\,l^{-1}$ and only 46% of this compound was removed after treatment. To our knowledge, no previous data has

been reported on the occurrence of codeine in STPs but it has been detected in surface water (Heberer, 2002; Kolpin et al., 2004; Moldovan, 2006) at concentrations of up to 1 $\mu g\,l^{-1}$.

Another compound also identified as a major constituent in municipal wastewaters is the stimulant caffeine (Buerge et al., 2003; Stackelberg et al., 2004; Weigel et al., 2004a; Santos et al., 2005; Batt et al., 2006; Gagné et al., 2006). Its widespread occurrence in wastewater, surface water and groundwater worldwide has led it to be considered as an anthropogenic marker for wastewater contamination of natural water (Buerge et al., 2003). This compound, and its main metabolite 1,7-dimethylxanthine, have been identified in all the samples analyzed in this study, at mean concentration levels of 118 and 79 $\mu g\,l^{-1}$ in the influent, and at 12 and 18 $\mu g\,l^{-1}$ in the effluent, respectively. This high load of caffeine in raw urban wastewater is mainly the consequence of the direct disposal of coffee or beverages containing this compound. The concentration levels detected are, in general, higher than those previously reported, especially in effluents with elimination efficiencies (85%) lower than those previously published (99%). References of 7-dimethylxanthine in wastewater have not been found, but Kolpin et al. (2002) describe its presence in streams.

Belonging to another category of pharmacologically active compounds, the anti-epileptic carbamazepine is also within the most frequently applied drugs all over the world. Its presence is ubiquitous in surface and groundwater in $ng\,l^{-1}$ range (Sacher et al., 2001; Heberer, 2002; Tixier et al., 2003; Weigel et al., 2004b; Comoretto and Chiron, 2005), and it has been described as a very persistent compound, therefore qualifying as a suitable tracer of human pollution (Clara et al., 2004).

Carbamazepine was present in the STP influent at relatively low concentrations (compared with the other target compounds), but its low biodegradability and poor elimination rate throughout the STP (20%) makes its presence recurrent in treated effluents, at concentrations between 0.11 and 0.23 $\mu g\,l^{-1}$. Similar or even higher levels have been reported in previous studies (Ternes, 1998; Öllers et al., 2001; Andreozzi et al., 2003; Metcalfe et al., 2003; Gagné et al., 2006). Since only 2–3% of the carbamazepine dose is excreted in urine in unchanged form, the presence of the primary metabolite, the pharmacologically active 10,11-epoxide, was also investigated. This compound may achieve up to 50% concentration of the parent compound during the metabolism and thus, it has been detected in the influent at concentrations even higher than its precursor (up to 0.5 $\mu g\,l^{-1}$) and in the effluent at a similar level. Presence of this metabolite in wastewater has not been previously described, even though its hydroxilated derivative has (Miao and Metcalfe, 2003).

Triclosan is a widespread contaminant that has been widely studied. It is used as an antiseptic agent in medical products and as an anti-microbial active component in a vast range of daily products. A field study of the fate of triclosan in a STP by Singer et al. (2002) established that triclosan is mainly degraded by biological treatment (79%), adsorbed in part to sludge (15%, as a consequence of the relatively high $\log K_{ow}$ of 5.4) and discharged into surface waters at only 6%. This data is in concordance with that obtained in this (88% of removal rate) and other studies (McAvoy et al., 2002; Paxeus, 2004; Hua et al., 2005). Even this low percentage of triclosan in effluents is enough to reach concentrations of up to 0.4 $\mu g\,l^{-1}$. This is a relevant result, since it has been demonstrated that the photodegradation of triclosan yield the formation of 2,7/2,8-dibenzodichloro-*p*-dioxine (DCDD) as a main degradation product. DCDD was identified in wastewater samples in 80% of cases, in influents and effluents, thus indicating its input and persistence (removal efficiency of 61%) through wastewater treatment process (Agüera et al., 2003).

The plasticizer bisphenol A (BPA) is a well-known industrial chemical. It has been reported as being slightly to moderately toxic and easily biodegradable, but its importance lies in its well-documented estrogenic activity (Hunt et al., 2003), which has meant that BPA be considered as a priority hazardous compound (Harris et al., 2000). The elevated reduction in the concentration reported in the final effluents of STPs, from 85% to 90%, do not impede the ubiquitous presence of this compound in the environment (Kolpin et al., 2002; Weigel et al., 2004b). It has been reported that this reduction is a consequence of its accumulation in sludge (Lee and Peart, 2000). In our study BPA was quantified at a mean value of 1.4 $\mu g\,l^{-1}$ in the influent and at 0.38 $\mu g\,l^{-1}$ in the effluent.

Finally, the monitoring study included two insecticides of agriculture and domestic use, chlorfenvifos and permethrin. To our knowledge no previous studies have assessed the occurrence of these widely used insecticides in treated and untreated municipal STPs. These compounds were present in the influent at concentrations of around 0.9 and 0.12 $\mu g\,l^{-1}$, respectively. Significant overall removal efficiencies were achieved, with values of around 83% (chlorfenvinfos) and 88% (permethrin). In the case of permethrin, with a high K_{oc} value, the concentration in sludge is expected to be high.

An overall estimation of the data collected during the study, allowed us an acceptable variability in removal efficiencies to observe, calculated from composite samples obtained for five consecutive days and two consecutive months. The coefficients of variation ranged from 4% (acetaminophen) to 19% (codeine). Significant variability was however seen in the concentration of discrete samples taken throughout the monitoring programme, where variations in one or more orders of magnitude were common. This variation was observed in both influents and effluents, thus indicating that the origin is not only plant performance but also the input of compounds into the STP which is highly variable for most compounds.

The overall removal efficiencies of the STP ranged between 20% (carbamazepine) and 99% (acetaminophen). The term overall removal means in this case disappearance

from the water phase, since presence of these compounds in the sludge has not been evaluated and so, two mechanisms can be used to explain this elimination: the biodegradation of the compound and the adsorption onto sludge.

4. Conclusions

The analytical method reported, based on SPE following by GC–SIM–MS, allowed a simple, rapid and reliable evaluation of the reported compounds at the concentration levels present in the samples. The one-year monitoring study described in this work has pointed out the persistence of a group of 14 compounds to those conventional water treatment processes usually applied in urban sewage treatment plants. The studied compounds, mainly including pharmaceutically active compounds, were present in treated waters representing a continuous input to coastal waters receiving STP spills. Data relative to concentration levels and removal efficiencies of the target compounds are in general in concordance with those previously reported for these compounds. The results obtained concerning dipyrone and codeine, for which no previous data about their occurrence and fate in STPs have been published, have been of special interest. Both were present at concentrations in the $\mu g\ l^{-1}$ range in both the influent and the effluent, probably due to the proximity of a hospital where the consumption of these pharmaceuticals is very high.

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