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Detection of pharmaceutical products in untreated hospital wastewater

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RESEARCH ARTICLE

Abstract

Medical-care institutions are a high source of pharmaceuticals compounds in urban wastewaters. A way to avoid the dispersal of pharmaceuticals into the environment could be to design dedicated sewage treatment plants capable of processing highly concentrated hospital and medical-care wastewaters. The present work is a survey conducted in Wallonia to further identify the most present and problematic compounds in wastewaters from medical-care institutions. An analytical method was first developed to simultaneously extract, detect and confirm 169 pharmaceutical products (PPs) in wastewater, including a purification/concentration step on an Oasis HLB® column and analysis by ultra-high performance liquid chromatography coupled to electrospray-tandem mass spectrometry. The method was validated to estimate precision and recovery. Samples were collected from hospitals, rest homes and other specialised institutions and analysed to identify PPs. Among the 45 compounds identified, 6 were selected as model compounds in hospital effluents for the design of a dedicated treatment on the basis of high concentration and occurrence, but also poor removal efficiency in urban wastewater treatment plants, persistence in the environment and potential ecotoxicity: carbamazepin, diclofenac, furosemide, lorazepam, tramadol and sulfamethoxazole.

Keywords: hospital wastewater, multi-residue, pharmaceutical products, SPE, UHPLC-MS/MS

1. Introduction

Pharmaceutical products (PPs) likely to be consumed in medical institutions include antibiotics, hormones, non-steroidal anti-inflammatory drugs (NSAID), psychotrops, contrast medium, antiepileptic drugs, fat regulators, etc. After administration, more than 70% of the therapeutic dose is excreted within 24 h in the urine and faeces of patients, 10% as parent compounds and 60% as metabolites (Delgado and Albasi, 2009). These compounds end up in the wastewater and represent an environmental risk, especially in water.

The occurrence of PPs in water depends on their consumption and use but also on their physico-chemical properties, stability and biodegradability. PPs half-life's have been determined under both lab and field conditions and range from a few hours to several months, as in the case of carbamazepine (Andreozzi *et al*., 2003). The fact that PPs are continuously rejected into the environment can impart a 'persistence-like character' even to those having a short half-life. It has also been established by several works that urban wastewater treatment plants (WWTPs) show poor removal efficiency for numerous PPs (Jelic *et al*., 2010).

A way to avoid PP dispersion into the environment is to design dedicated sewage treatment plants for processing concentrated hospital and medical wastewaters (Larsen *et al.*, 2004). However, medicated wastewater treatment is currently subject to discussion. The contribution of hospital to the pharmaceutical load in wastewater have been poorly studied; available data showed that the maximum contribution of the hospital is only 15% of the total load in the influent of the sewage treatment plant for most of the PPs (Ort *et al.*, 2010). Nevertheless, there are also works justifying dedicated treatment of hospital wastewater. First, the type and amount of drugs used in hospital could differ from those used as ambulatory treatment (Kummerer,

2001). Secondly, it was shown that hospital wastewater discharge can lead to drug resistant bacteria (Reinthaler *et al.*, 2003), which is a major problem for human health. Finally, measures at high concentrated point sources are likely to be most effective and will offset the poor removal efficiency of traditional urban treatment plant against PPs. A pilot plant is currently under evaluation in the European PILLS project (www.pills-project.eu) for hospital wastewater treatment.

The multiplicity of PPs used worldwide makes it necessary to identify PPs on which to focus in priority. A list of relevant PPs has been established by the European Knowledge and Need Assessment on Pharmaceutical Products in Environmental Waters (KNAPPE) project. Besse and Garric (2008) have also developed a prioritisation methodology taking predicted environmental concentrations (PECs) and both physico-chemical and toxicological properties into account. Several PPs have been identified at WWTPs in Europe, North America and Brasilia (Siegrist, 2007). Miege and coworker have built a database to evaluate the occurrence and removal efficiency of PPs at wastewater treatment plants (Miege *et al.*, 2009); this work identifies the most investigated PPs in WWTPs and the most persistent ones, their concentrations, and their removal efficiencies at WWTPs.

Abundant methods have been published to quantify PPs in rivers and wastewaters. Given its specificity, sensitivity, and robustness, liquid chromatography coupled to tandem mass spectrometry is a method of choice, which also allows multi-residue method development.

When compounds having different physico-chemical properties are to be analysed, extracting and purifying the samples to reach ng/kg-level concentrations is a challenge. Oasis HLB® solid phase extraction (SPE) have been used extensively to purify antibiotics, NSAID, lipidic regulators, beta-blockers, etc. (Gomez *et al.*, 2006; Gracia-Lor *et al.*, 2010; Gros *et al.*, 2006; Shao *et al.*, 2009; Wick *et al.*, 2009), mostly because affinity for polar compounds is higher than that of traditional C18 SPE.

This paper presents and discusses results of a recent analytical campaign aiming to assess the presence of pharmaceuticals in untreated wastewaters from several hospitals in the south of Belgium. The aim is to provide a qualitative assessment of PPs entering the environment from medical-care institutions, with a view to design an experimental membrane bioreactor dedicated to hospital wastewater treatment.

2. Materials and methods

Reagents and chemicals

The following standards were purchased from Sigma (St. Louis, MO, USA): 17-β-oestradiol, 5-fluorouracil, abamectin, acetoxyprogesterone, alprazolam, amlodipine, azaperone, bentazone, bezafibrate, bisoprolol, boldenone beta, bromoxynil, caproxyprogesterone, carazolol, carbamazepine, carbamazepine-10,11-epoxide, carprofen, cefalexin, ceftazidime, ceftiofur, chlormadinone acetate, chlorpromazine, chlorprothixène, cinoxacin, clopidogrel, closantel, clorsulon, cortexone, cortisol, cyanazine, dexamethasone, diazinon, diclofenac, dienoestrol, diethylstilbestrol, doramectin, emamectin, S-ethyl dipropylthiocarbamate, erythromycin, ethofumesate, ethynyloestradiol, etoposide, fenoterol, flumethasone, flunixin, fluoromethonolone, formoterol, furosemide, hexestrol, ibuprofen, ipratropium, isoproturon, ketoprofen, lenacile, levofloxacine, lincomycin, 2-methyl-4-chlorophenoxyacetic acid, mecoprop, medroxyprogesterone acetate, mefenamic acid, meloxicam, metamitron, metamizol, methylboldenone, methyltestosterone, metolachlore, metoprolol, metoxuron, nicardipine, norgestrel, omeprazole, ondansetron, paracetamol (acetaminophen), perindopril, prednisone, prednisolone, promazine, propofol, risperidone, ritodrine, salicylic acid, simazine, simvastatin, spiramycin, sulfamethazine, sulfadimethoxine, sulfadoxine, sulfamerazine, sulfamethoxazole, sulfathiazole, terbutylazine, tiamulin, tilmicosin, tolfenamic acid, tramadol, trenbolone acetate, triamcinolone acetonide, trimethorpim, tulobuterol, tylosin, venlafaxine , α-zeranol, β-zeranol and xylazine. Molsidomine, lorazepam, midazolam, pantoprazole, zolpidem were purchased from EDQM (Strasbourg, France). Rosuvastatin was purchased from Discovery Fine Chemicals (Wimborne, UK). Atrazine, chlortetracyclin, ciprofloxacin, difloxacin, enrofloxacin, marbofloxacin, norfloxacin, nortestosterone beta, oxolinic acid, oxytetracyclin, sarafloxacin, tetracyclin were purchased from Dr Ehrenstorfer (Augsburg, Germany) and alachlor, azinphos-methyl, carbaryl, carbofuran, chloramphenicol, chlorfenvinphos, chloridazon, chlorpyrifos methyl, dichlorvos, dimethenamide, dimethoate, florfenicol, fonofos, megestrol acetate, monolinuron, phosmet, pirimicarbe, progesterone, testosterone from Cluzeau (Sainte-Foy-la-Grande, France). Alpha boldenone, clenbuterol, delmadinone acetate, nortestosterone alpha, trenbolone alpha were purchased from the European reference laboratory RIKILT (Wageningen, the Netherlands) and acepromazine, azaperol, cimaterol, clenpeterol, mabuterol, norethandrolone, stanozolol from ISP (Brussels, Belgium). Brombuterol, firocoxib, mapenterol, vedaprofen and ramifenazone was purchased from Witega (Berlin, Germany) and clenproperol, clencyclohexerol, salmeterol from the European reference laboratory BgVV

(Berlin, Germany). Chem Service (West Chester, PA, USA) provided diuron, metabenzotiazuron, metribuzine. Zilpaterol was obtained from Aventis Pharma (Neuville-sur-Sâone, France), melengestrol acetate from Pharmacia Animal Health (Kalamazoo, MI, USA), clobetasol from GlaxoWellcome Belgium (Brussels, Belgium), isoflupredone from Steraloïds Inc. Ltd (London, UK) and moxidectine from BASF Corporation (Princeton, NJ, USA).

Sampling sites and sample collection

As PPs are present at low concentrations in wastewater, a dedicated sampling protocol (sampling material, automatic sampling device settings, samples delivering to the lab) was designed, based on the work of Choubert *et al.* (2009) and consistent with the recommendation of Ort *et al.* (2010).

As the wastewater flow could not be precisely measured and as a mass-flow analysis was not the aim of the present study, the automatic and refrigerated sampler was programmed in time mode. A short sampling interval was used because the distribution of PP levels in wastewater over the day is unknown. Taking into account the battery capacity of the sampling device, sampling interval was set from 5 to 10 min. Glass bottles and parts of the sampling device in contact with samples (pipes, glassware) were rinsed beforehand successively with water, acidified water (25% acetic acid), acetone and demineralised water. The final 24 h composite sample (volume from 5 to 15 litres) was stored in a glass bottle with a wide bottleneck (Dumont glass house, Flémalle, Belgium). After homogenisation of suspended particles, a 2 litres analytical sample was taken with a peristaltic pump equipped with a silicon pipe. Water samples (500-2,000 ml) were stored at +4 °C until analysis (maximum 3 days).

From March to June 2010, 7 hospitals and specialised institutions (neuro-psychiatric units or rest homes) were sampled in the south of Belgium, each institution being sampled twice. Because of confidentiality agreements, no details will be given for individual institution. Number of beds rise from 90 for the smallest rest-home to 900 for the biggest hospital. Sampling points were chosen according to the sewer pipe network, for accessibility and safety during sampling. Samples were taken by dry weather, to avoid wastewater dilution with rain as waste- and storm-waters were mixed in those institutions.

Extraction protocol by solid phase extraction

Oasis HLB® 6 cc, 200 mg (Waters, Milford, CT, USA) were activated with 10 ml methanol and 10 ml water. The sample (100 ml, pH adjusted to 7 with 0.1 NHCl) was loaded into the cartridge at a flow rate of approximately 4 ml/min and then rinsed with 20 ml water. PPs were then eluted with 5 ml methanol and the extracts were evaporated under nitrogen at 40 °C. Samples were finally resuspended in 500 µl of acetonitrile:water (25:75) and inserted in high-performance liquid chromatography (HPLC) vials.

The quantification method was based on matrix-matched calibration curves with 7 concentration levels. A regression model was applied to the calibration data set. With each extraction batch, a quality control (QC) sample was also analysed; this sample corresponds to a blank sample fortified at a concentration equivalent to the lowest calibration level (LCL) (Supplementary Table S1).

Sample analysis by ultra-high performance liquid chromatography-tandem mass spectrometry

Liquid chromatography

The ultra-high performance liquid chromatography (UHPLC) equipment consisted of a Waters Xevo TQ system (Waters). Chromatography was performed on an Acquity UPLC® HSS C18 1.8 µm column (150×2.1 mm; Waters). During UHPLC analysis, the column was maintained at 50 °C and the samples at 15 °C. For each sample, two 50-µl injections were performed on the same column, one for negative and one for positive electrospray analysis, with an optimised mobile phase for each injection. In both cases, the flow rate was 0.5 ml/min. For positive electrospray ionisation (ESI), mobile phases A and B were, respectively, 0.05% formic acid in water and 2 mM ammonium formate in acetonitrile. The applied elution program was a two-linear-step gradient: 0-0.5 min, linear gradient from 95 to 80% A; 0.5-7 min, linear gradient from 80 to 0% A; 7-8.5 min, 0% A; 8.5-10 min, from 0 to 95% A; and finally reconditioning of the column until 15 min.

For negative ESI, mobile phases A and B were, respectively, water and acetonitrile. The applied elution program was a linear gradient: 0-0.5 min, 80% A; 0.5-7 min, linear gradient from 80 to 0% A; 7-8.5 min, 0% A; 8.5-10 min, from 0 to 80% A; and finally reconditioning of the column until 15 min.

Mass spectrometry

The mass spectrometry (MS) equipment consisted of a XEVO TQ (Waters). The analysis was performed either in positive or negative ion electrospray mode. Multiple reaction monitoring was carried out. Two transitions were followed for most PPs, the first being the quantifier and the second the qualifier. Nitrogen was used as the cone gas and the desolvation gas at flow rates of 50 and 1,200 l/h, respectively. The other MS/MS parameters were: capillary voltage 2.5 kV; source temperature 150 °C; desolvation temperature 500 °C; collision gas pressure 2.10^{-3} mbar. Multiple reaction monitoring was carried out and MS parameters are presented in Supplementary Table S1. Data

collection and subsequent processing were performed with QuanLynx software (Waters).

3. Results and discussion

Selection of target compounds and optimisation of the analytical protocol

Considering the wide range of PPs consumed in hospitals and medical institutions, it was essential to first identify the most present and problematic compounds in our study area, the Walloon region. A database was created on the basis of drug consumption data for Belgium and previously published studies. First, inquiries were send to target institutions (hospitals, rest homes, psychiatric and neuro-psychiatric units, etc.) to collect drugs consumption data. Unfortunately, data were unavailable for rest homes and several specialised institutions, as in these institutions there is no central pharmacy but an individual drug management system. Among contacted hospitals, two gave us a list ranking the most consumed drugs (annual basis); the hundred most consumed drugs from these 2 institutes were integrated in our database. To include drugs delivered for ambulatory treatments, we have also introduced consumption data from the Belgian Health Insurance Authority and the Belgian Pharmacists Association. Then, occurrence data were taken from the works of Wick *et al.* (2009), Karprzyk-Hordern *et al.* (2008), Miege *et al.* (2009), Clara *et al.* (2005), Catastini *et al.* (2008), Defert and Huart (2009) and Putschew *et al.* (2000). The list of the relevant PPs published by the European KNAPPE project was also taken into account.

From this database of more than 380 compounds (data not shown), we selected the most frequently consumed drugs in our survey area and the most relevant one's according to KNAPPE project and other international works. X-ray contrast media were excluded of this work as they need dedicated analytical method. Pesticides were included in the survey as the same method will be applied for surface water analysis.

Mass spectrometry parameters were then optimised for each selected PPs. When possible, at least 2 transitions were identified for quantifying and confirming PPs in water. Standards were afterwards injected to determine their retention time and the UHPLC gradient was optimise to ensure a good resolution of all compounds (minimum 12 scans per peak).

Wastewater samples spiked with decreasing concentrations of PPs were extracted and injected to determine the limit of quantification of each compound, i.e. the lowest concentration that gives a signal to noise ratio of 10. These data were considered to determine calibration ranges.

As shown in Supplementary Table S1, LCL is relatively high for several compounds. Nevertheless, we choose to keep these compounds in our study as they could potentially be present at high concentrations in hospital samples.

Method performances

Reproducibility and recovery were both calculated on QC samples. 12 QC samples were analysed during this study (one with each analytical run). Calculated concentration of the 12 corresponding QC allowed determination of both reproducibility and mean recovery. As shown in Supplementary Table S1, reproducibility is high for some compound as by example for chlortetracyclin or lorazepam. This low precision could be related to the lack of internal standard and to an important matrix effect in waste water. During method development, several internal standards were tested; the goal was to select one internal standard for each drug class and even for each antibiotic family (cephalosporins, macrolides, etc.). Unfortunately, it was observed for several compounds that using a single labelled standard for a whole drug class was not the most optimum way of working. For sulfamethoxazole by example, using a labelled sulfonamide as internal standard give worst precision and recovery than without any internal standard. The choice was thus made to analyse all compounds without any internal standard and to consider data as a qualitative survey of wastewater contamination by pharmaceutical compounds.

Identification of pharmaceutical products in medical waste water

Samples were analysed with a matrix matched calibration curve, including 7 calibration levels and ranging from LCL (Supplementary Table S1) to 12 times the LCL. This works aims to identify PPs and pesticides in wastewater but quantification results were however presented here as 'concentration levels' of contamination.

Among targeted PPs, 45 were detected in hospital wastewater samples (Table 1). The presence of detected compounds was confirmed, as stated in Commission Decision 2002/657/EC, by its retention time and relative ion intensities.

The low rate of detected compounds (45 detected drugs among 169 targeted compounds) could be explained by the broadness of the present database. Indeed, only 2 institutions have provided a list of consumed PPs. The database includes mostly relevant PPs from the KNAPPE project, drugs used as ambulatory treatment and pesticides which are not specific of hospital wastewater. Moreover, some compounds show a low sensitivity that could explain undetected PPs potentially present at sub-nanogram per litre levels.

Table 1. Compounds detected in wastewater from medical-care institutions (total number of 24 h composite samples analysed was 16).

	Compound	No. of positive samples	Min. conc. (ng/l)	Max. conc. (ng/l)	Median conc. (ng/l)
Analgesic	Acetaminophen	11	31	60,600	56
	Tramadol	15	57	59,700	252
Anti-anginal	Molsidomine	3	31	210	60
Antibiotic	Ceftazidime	1	-	29	29
	Cinoxacin	8	37	170	77
	Ciprofloxacin	6	65	87,200	15,800
	Enrofloxacin	1	10	31	10
	Lincomycin	3	3	50	22
	Sulfamethazine	2	28	71	49.5
	Sulfamethoxazole	3	950	11,880	10,728
	Trimethoprim	5	113	15,700	393
Anti-cancer	Etoposide	1	-	6,297	6,297
Anti-emetic	Ondansetron	3	5	24	16
Anti-epileptic	Carbamazepine	12	40	>10,000	2,584
	Carbamazepine epoxy	11	250	6,270	2,340
Anti-hypertensive	Nicardipin	1	-	59	59
Anti-hypertriglyceridemic	Bezafibrate	1	-	849	849
Anti-platelet	Clopidogrel	3	19	25	19
Anti-ulcer	Omeprazol	3	117	147	120
Beta-agonist	Clenproperol	3	9	64	51
	Formoterol	9	2	166	8
Beta-blocker	Bisoprolol	8	2	428	66
	Metoprolol	5	19	88	86
Corticosteroid	Cortisol	1	-	2,330	2,330
	Prednisolone	1	-	1,470	1,470
Diuretic	Furosemide	15	126	57,900	3,207
NSAID[1]	Diclofenac	12	1,450	4,327,188	4,017.5
	Flunixin	1	-	4	4
	Ibuprofen	2	2,500	2,800	2,650
	Ketoprofen	12	17	355	168
	Vedaprofen	3	3,230	4,540	3,705
Pesticide	Ethofumesate	1	-	5,241	5,241
	Phosmet	1	-	3,510	3,512
Psychotropic drug	Alprazolam	6	27	2,400	779.5
	Lorazepam	8	37	1,590	296.5
	Midazolam	2	31	70	50.5
	Venlafaxin	4	251	110	506.5
	Zolpidem	2	3	16	9.5
Statin	Rosuvastatin	6	1,470	2,470	2,156
Steroid	Methylboldenone	3	27	46	33
	Norethandrolone	1	-	-	61
	Stanozolol	3	63	131	67
	Trembolone alpha	1	-	132	132
	Trembolone acetate	5	350	940	513

[1] non-steroidal anti-inflammatory drugs.

One must notice that results were not related to the specialised filed of the different sampled institutions as the goal of this survey was to select the most frequent and concentrated PPs for the whole Walloon region.

Figure 1 represents the concentration level in regards with the occurrence for the 45 detected compounds. Acetaminophen, alprazolam (benzodiazepine), carbamazepine (anticonvulsant and mood stabilizing drug) and its metabolite carbamazepine epoxy, ciprofloxacin (an antibiotic), diclofenac (an NSAID), furosemide (a diuretic), lorazepam (a benzodiazepine), rosuvastatin (a synthetic statin), tramadol (an opioïd analgesic) and trimethoprim (an antibiotic) were detected at concentration above 1000 ng/l. The detection of an herbicide (ethofumesate) and an insecticide (phosmet) is surprising; these compounds are probably used for facilities maintenance and were collected due to the lack of separation between waste- and rainwater networks.

Considering the work of Ort *et al*. (2010), 85% of the pharmaceutical residue loads in Australian wastewater do not originate from a hospital and only trimethoprim and roxithromycin have a contribution of the hospital above 15%. Nevertheless, even if most of the detected compounds could be consumed by the general population, it is still consider as relevant to design a dedicated treatment system for highly contaminated hospital wastewater.

Among the detected drugs, 6 were identified as model compounds: carbamazepin, diclofenac, furosemide, lorazepam, tramadol and sulfamethoxazole. This choice was mainly directed by their occurrence (most of these compounds were present in at least 50% of the samples) and observed concentrations during the survey, but also considering their poor elimination by urban WWTP, persistence in the environment and potential ecotoxicity as described below. As shown in Table 1 and in Figure 2, contamination levels vary greatly from sample to sample with maximum concentration reaching thousand times of the median concentration.

Carbamazepine was detected both as parent compound and as metabolite with a total median concentration of 4,900 ng/l for the sum of the two compounds. These levels are consistent with previous works where carbamazepine was shown to be ubiquitous in river water and was

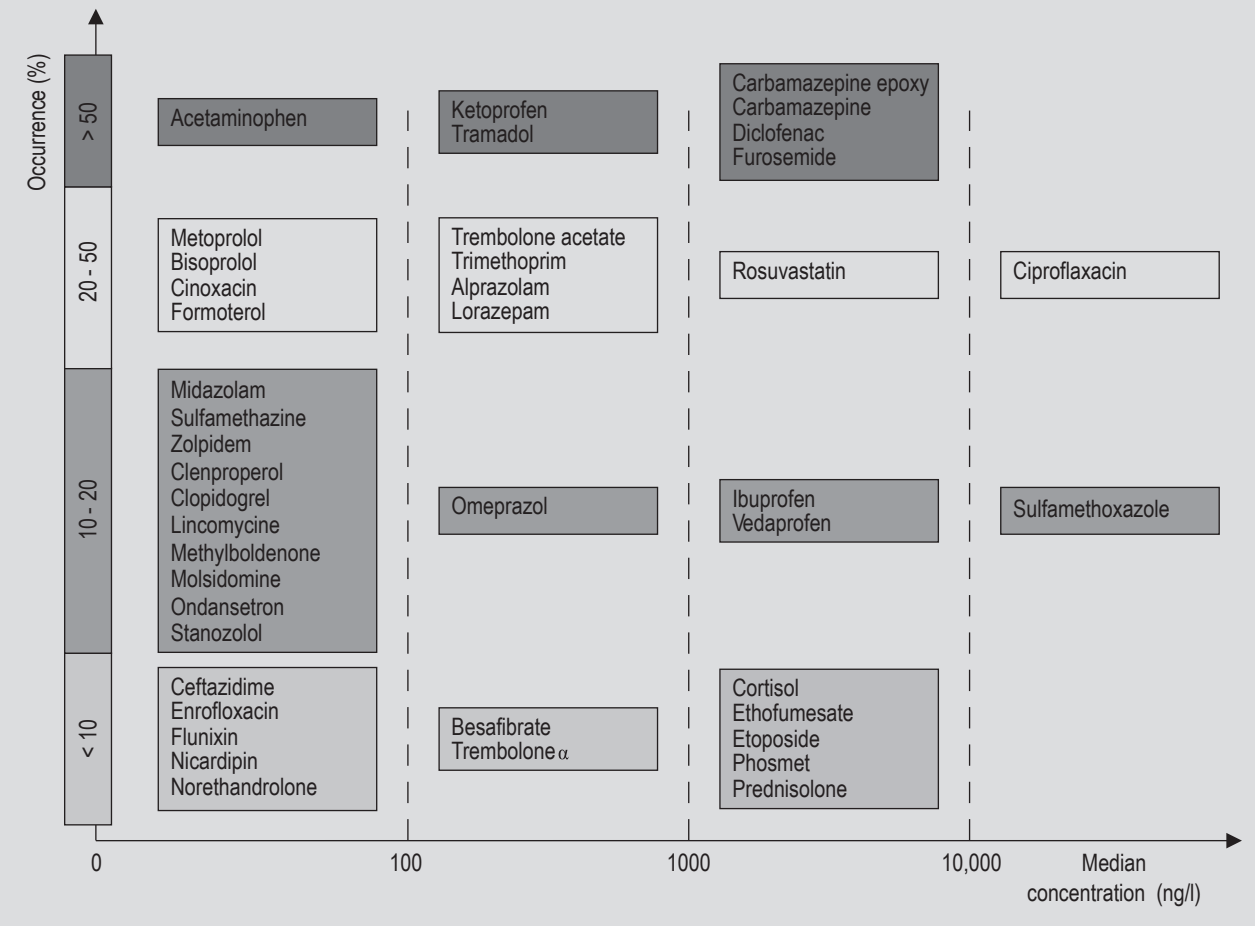

Figure 1. Contamination of wastewater from hospital and medicated by pharmaceutical products and pesticides in the Walloon Region: occurrence and concentration of detected compounds.

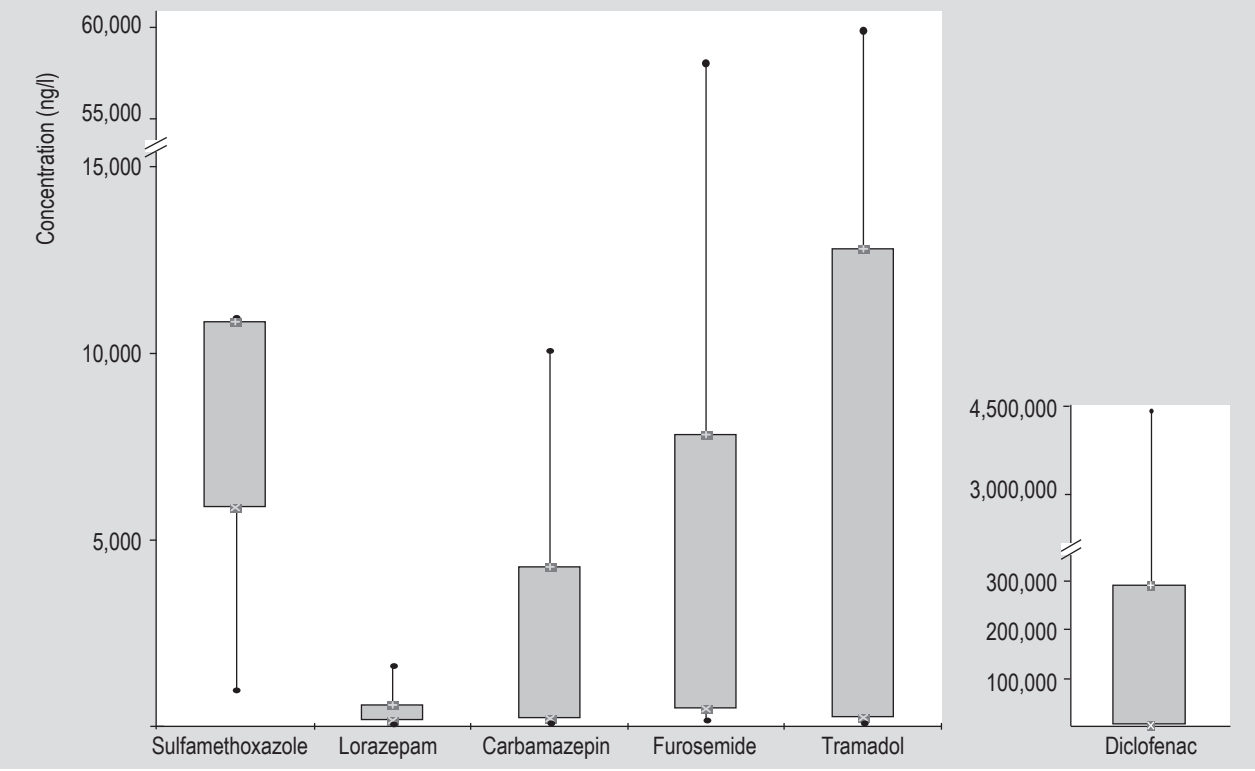

Figure 2. Boxplot representation of the contamination of hospital wastewater samples for sulfamethoxazole, lorazepam, carbamazepin, furosemide, tramadol and diclofenac (the bottom and top of the box are the lower and upper quartiles, respectively; the ends of the whiskers represent the minimum and maximum of all data).

detected around or above 1 µg/l in wastewater (Heberer, 2002; Miege *et al.*, 2009; Togola *et al.*, 2008; Vieno *et al.*, 2006). WWTP efficiency against carbamazepine is low. While the ibuprofen level is reduced by 60 to 96% (Bendz *et al.*, 2005; Carballa *et al.*, 2004), carbamazepine has a biodegradation efficiency of only 10% (Joss *et al.*, 2006). Furthermore, it adsorbs only slightly to sludge because of its hydrophilic character (Jançon *et al.*, 2008). Several works have highlighted it as a persistent compound with a half-life exceeding 100 days (Andreozzi *et al.*, 2003; Kasprzyk-Hordern *et al.*, 2008). It seems thus important to develop treatment strategy allowing reduction of carbamazepin in the aquatic environment.

Furosemide is a diuretic found in all samples with a maximum concentration reaching 60 µg/l. These high occurrence and concentrations could be related to its wide use in psychiatric unit to counterbalance side-effect of psychotropic drugs. As carbamazepine, furosemide is also poorly eliminated by WWTP processing, with an efficiency of only 11% (Schlüsener *et al.*, 2008). As this PP is highly consumed (36th most prescribed drug in France in 2002), high concentrations was found in both waste and surface waters. Furosemide has been detected at concentrations above 3 µg/l in German, French, Spanish, and English wastewaters (Sadesky *et al.*, 2008) and above 1 µg/l in WWTP effluents (Tracol and Duchemin., 2009).

Tramadol is an analgesic of class 2, detected at a median concentration of 250 ng/l and with an occurrence of 100%. This drug is likewise listed as one of the relevant PPs hardly eliminated by WWTP processing and yielding persistent metabolites (Jançon *et al.*, 2008; Sadezky *et al.*, 2008). PECs consistently above 2 µg/l have been determined in France, Wales, Germany, and Spain (Sadezky *et al.*, 2008), and river water contamination has been found to reach levels above 1 µg/l. In 2009, a French survey focusing on PPs discharged from the Rouen hospital showed that tramadol discharged by the hospital was still present in water after WWTP processing (Spiroux, 2009). The daily mean of tramadol present in WWTP effluents reached 212 g (Jeanblanc, 2009). This work highlights the inefficiency of domestic WWTP against tramadol and the need for a dedicated WWTP that can eliminate PPs.

Diclofenac is the NSAID found with the highest concentrations and occurrence, with a maximum concentration up to 4 mg/l. WWTPs have been shown to display 17 to 70% efficiency against diclofenac, depending on the process used and the age of activated sludge (Buser *et al.*, 1998; Janex-Habibi *et al.*, 2009; Lindqvist *et al.*, 2005; Tixier *et al.*, 2003). High concentrations have been found in WWTP influents and effluents (Coquery *et al.*, 2011; Heberer, 2002), and diclofenac has even been found in underground and drinking water at 590 and 10 ng/l, respectively (Togola *et al.*, 2008).

Sulfonamides are another class of PPs poorly removed by WWTPs. In a classical activated WWTP sludge, the sulfamethoxazole removal efficiency is only 5 to 21% (Jançon *et al.*, 2008). Sulfamethoxazole has been found at 50 to 600 ng/l in WWTP effluents (Watkinson *et al.*, 2007), and also in surface, river and wasterwaters (Ashton *et al*., 2004; Miege *et al*., 2009; Wiegel *et al*., 2004; Zuccato *et a*l., 2005) and is considered a priority PP for ecotoxicity. In this survey, this compound was found in only 3 samples but with a median concentration above 10 µg/l.

Lorazepam is a benzodiazepine drug used for its anxiolytic, amnesic, sedative/hypnotic, and anticonvulsant properties. Benzodiazepines are among the 75 most prescribed drugs in Europe (Togola *et al.*, 2008). Lorazepam has been found in tens of ng/l in the Loire-Brittany basin (France).

4. Conclusions

An in-house developed UHPLC-MS/MS method has been applied in this work, allowing identification of 169 pharmaceutical products in hospital wastewater. Analysis of 16 samples from eight medical-care institutions has revealed the presence of 45 compounds in wastewater. Six drugs were selected as model drug in Walloon hospital effluents, mainly on the basis of their occurrence and high concentration, but also considering their poor removal efficiency in urban WWTPs, persistence in the environment, and potential ecotoxicity: carbamazepin, diclofenac, furosemide, lorazepam, tramadol and sulfamethoxazole. On the basis of this work, a dedicated membrane bioreactor will be designed to process these PPs from hospital wastewater.

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Supplementary material

Supplementary material can be found online at http://dx.doi.org/10.3920/QAS2012.0177.

Table S1. MS parameters of targeted pharmaceutical products and method performances.

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