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Analysis of some pharmaceuticals in municipal wastewater of Almadinah Almunawarah

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Abstract The chemical pollution of water resources is a major challenge facing the humanity in this century. Pharmaceuticals and personal care products (PPCPs) are a group of emerging environmental chemical pollutants distinguished by their bioactivity and high solubility. They may also cause health complications to humans and living organisms. Pharmaceuticals enter the environment, mainly via wastewater and can eventually reach the surface and ground water. Despite this, PPCPs received less attention as environmental pollutants than other chemical pollutants (e.g. heavy metals and pesticides). The purpose of this work was to investigate the presence of some of the most frequently dispensed drugs for the residents of Almadinah Almunawarah, Saudi Arabia in the municipal wastewater before and after treatment. For this purpose, wastewater samples were collected biweekly from the city's sewage treatment plant for a period of 4 months and analyzed the targeted drugs using tandem LC–MS. Out of the 19 investigated drugs, 5 pharmaceuticals have been found in concentrations greater than the limit of detection in both the influents and effluents of the sewage treatment plant. As expected, the concentrations of investigated pharmaceuticals in the wastewater were found to be low. These drugs and their average concentrations (in ng mL^{-1}) in the influents were: acetaminophen (38.9), metformin (15.2), norfluoxetine (7.07), atenolol (2.04),

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and cephalexin (1.88). Meanwhile, the effluents contained slightly lower levels (in ng mL^{-1}) than those of influents: acetaminophen (31.2), metformin (3.19), norfluoxetine (7.25), atenolol (0.545), and cephalexin (1.53). The results of this study supported by many other investigations indicate the inefficiency of current conventional wastewater treatment protocols in eliminating such a group of active and potentially hazardous pollutants from the wastewater.

1. Introduction

Pharmaceuticals and personal care products (PPCPs) are a group of substances that "refer, in general, to any product used by individuals for personal health or cosmetic reasons or used by agribusiness to enhance growth or health of livestock" (US-EPA). The term PPCPs contain thousands of different chemical compounds, such as prescription and over-the-counter therapeutic drugs, veterinary drugs, perfumes, musks, deodorants, shampoos, hair sprays, hair dyes, body lotions, sun-screens, make-up, nail polish, lipsticks, crèmes, diagnostic agents, and nutraceuticals. Many of these compounds including pharmaceuticals, the focus of this investigation, are bioactive, metabolize partially, and biodegrade slowly (Debska et al., 2004; Hernando et al., 2006; Kümmerer, 2008).

Little attention has been paid to PPCPs in general and pharmaceuticals in particular as potential environmental pollutants when compared to other chemicals pollutants like heavy metals and pesticides. Interest in pharmaceuticals and their metabolites and by-products as environmental pollutants has only initiated in the 1970s, but it was not until recently when scientists actively began to address the impact of such pollutants on the environment and living organisms (Daughton, 2002; Debska et al., 2004; Fatta et al., 2007; Heberer, 2002; Hernando et al., 2006; Kümmerer, 2009; Stan and Heberer, 1997; Zuccato et al., 2006).

Current literature shows that pharmaceuticals are continuously released into the environment in extremely large quantities and on a regular basis through different ways like human activities (e.g. through excretion and bathing and disposal of unwanted medications to sewers and trash), wastes from pharmaceutical industries, residues and wastes from hospitals, use of illicit and veterinary drugs (especially antibiotics and steroids), and agribusiness (Bartelt-Hunt et al., 2009; Escher et al., 2011; Larsson et al., 2007; Ternes, 1998).

Due to the fact that pharmaceuticals generally dissolve easily in aqueous media and do not usually evaporate at normal temperatures or pressure, they make their way into the soil and aquatic environments via sewage, treated sewage sludge (biosolids), and irrigation with reclaimed waters (Cunningham, 2008; Nikolaou et al., 2007). Current research findings clearly demonstrate that current conventional wastewater treatment technologies do not sufficiently remove pharmaceuticals and/or their metabolites and degradation by-products from wastewater, and therefore let them reach surface, marine, ground, and drinking waters (Benotti and Brownawell, 2007; Debska et al., 2004; Joss et al., 2008).

Although some pharmaceuticals breakdown or degrade upon consumption or release into the environment, most of them remain unchanged and eventually become persistent in the environment. It is known that most of these chemicals remain bioactive even at extremely low concentrations after excretion from the body or after disposal to landfills and waters, have unpredictable biochemical interactions when mixed together, and may have a tendency to accumulate in the food chain with negative health impact on aquatic organisms and consumers (Escher et al., 2011; Hernando et al., 2006; Kümmerer, 2008). As a result, pharmaceuticals and their metabolites and by-products are of concern for their potential ecological and environmental impacts.

Recent literature indicates that the flux of pharmaceuticals from municipal sewage treatment plants (STP) is a considerable source of chemical pollution in surface, ground, marine, and even tap and bottled waters (Chang et al., 2007; Heberer, 2002; Khan and Ongerth, 2002; Kolpin et al., 2002; Rosal et al., 2010; Ternes, 1998). For instance, an investigation conducted by the U.S. Geological Survey in 1999 to check the occurrence of PPCPs (e.g. sterols, hormones, pharmaceuticals, antibiotics) in surface and ground water has confirmed the presence of at least one PPCP at low levels in more than two thirds of the samples, with steroids, nonprescription drugs, and pesticides being the most frequently detected compounds (Kolpin et al., 2002). Although the concentrations of individual pharmaceuticals reported in investigated water bodies worldwide are low and may not cause any harm to the human health, chronic exposure to a mixture of such compounds may disturb the balance in the human body and enhance a dangerous resistance to antibiotics and consequently pose a threat to the health of living organisms; a task that many scientists are currently investigating (Escher et al., 2011; Hernando et al., 2006; Santos et al., 2007; Schriks et al., 2010). Some of the reported potential effects of PPCPs on living organisms were: delayed development in fish and frogs, delayed metamorphosis in frogs, increased feminization of fish populations, and a variety of reactions including altered behavior and reproduction (Hernando et al., 2006).

The aim of this work was to investigate the occurrence of the most frequently prescribed drugs in the influents and effluents of municipal wastewater of Almadinah Almunawarah.

2. Materials and methods

The list of all pharmaceuticals distributed to the public hospitals and medical clinics in Almadinah Almunawarah area for the year 2009 was obtained from the Directorate General of Health Affairs of Almadinah Almunawarah. Some of the most frequently dispensed drugs have been selected to be investigated in this study, based on their quantities and the possibility of being detected in wastewater. The targeted drugs and their amounts are presented in Table 1.

All glass- and plastic-wares used were soaked overnight in 10% nitric acid, rinsed with distilled water, and finally with

Table 1 List of the most frequently dispensed drugs in the area of Almadinah Almunawarah along with their quantities for the year 1429H (2009).[a]

Drug's Group	Drug's name	Active ingredient (kg)
Antibiotics	Cephalexin	1,034
	Erythromycin ethyl succinate	475
Antiparasites	Metronidazole	2,253
Antimicrobials	Sulfamethoxazole	1,663
	Trimethoprim	333
Anticoagulants	Warfarin sodium salt	1.1
Antihypertensives	Atenolol	269
ACE inhibitors	Captopril	120
Hypolipidemic agents	Simvastatin	20
	Acetaminophen	12,879
Non-opioid analgesics	Ibuprofen	5342
	Diclofenac sodium salt	521
Antiepileptics	Carbamazepine	556
	Ranitidine	749
GI disorders	Hyoscine-N-butyl bromide	51
Antidiabetics	Metformin	6,300
Respiratory drugs	Chlorpheniramine malate	431
Antidepressant	Norfluoxetine	2.1

[a] The most frequently dispensed drug(s) from most of the drug classes have been selected.

reagent water before use. Glasswares used for the preparation and storage of drug solutions were rinsed with dimethyldichlorosilane (DMDCS) followed by two toluene rinsings and several methanol washings before use.

2.1. Chemicals and reagents

The following chemicals and reagents were used: reagent water (Milli-Q, 18.2 MΩ cm, Elix10, Millipore, USA), ethylenediaminetetraacetic acid disodium salt dehydrate (Na_2ETDA, Sigma, ACS reagent, 99.0–101.0%), ascorbic acid (Sigma, ultra grade, >99.0%), formic acid (Fischer Scientific, analytical reagent grade, 98%), ammonium hydroxide solution (Sigma–Aldrich, ACS reagent, NH_3 content 28–30%), methanol (Sigma–Aldrich, Chromasolv grade, 99.9% min), acetonitrile (ACN, Fisher Scientific, HPLC Gradient grade), dimethyldichlorosilane (DMDCS, 5% in toluene), and toluene (GC grade, 99.5%, Panreac, Barcelona-Spain). The drugs of interest were: acetaminophen (98%), carbamazepine, diclofenac sodium salt, erythromycin ethyl succinate, norfluoxetine (>97%), ranitidine hydrochloride, scopolamine *N*-butyl bromide (hyosine-*N*-bytyl bromide, >99%), and trimethoprim (>98.5%) which were purchased from Sigma–Aldrich, ibuprofen (>99%), metronidazole (99.9%), sulfamethoxazole (99.9%), and warfarin sodium salt (99.9%) from Fluka, and atenolol, captopril, cephalexin, chlorpheniramine maleate, metformin hydrochloride (99.8%), and simvastatin (98.3%) were obtained as a gift from Al-Jazeera Pharmaceutical Industries, Riyadh, Saudi Arabia. Stock solutions of these drugs (~2000 μg mL^{-1}) were prepared as follows: approx. 20.0 mg of each drug was accurately weighed in a glass test tube and dissolved in 10 mL water or MeOH depending on analyte solubility. In order to minimize drugs' degradation, the test tubes containing the stock solutions were wrapped with aluminum foil and stored refrigerated at 4 °C. Working solutions of single analytes as well as mixtures of analytes (10.0 ng mL^{-1} each) were prepared in methanol as required from the stock solutions and stored in a similar way as for the stock solutions.

Three types of HPLC mobile phases were prepared: formic acid (A1, 0.3% (v/v) in water); ammonium hydroxide (A2, 20 mM in water); and acetonitrile: methanol (B, 2:1).

2.2. Samples collection and pre-treatment

Wastewater samples were collected from the only STP in Almadinah Almunawarah. The current total capacity of this plant is 300,000 m^3 day^{-1}. In addition to domestic sewage, the plant sometimes receives partially-treated industrial and medical wastewater. The plant undertakes tertiary treatment. Most of the treated wastewater is discharged into the nearby Al-Khlail wadi (valley) and used mainly for irrigation, and about 15% of this water is transported in trucks and used for watering of green areas and trees in public parks and streets of Almadinah Almunawarah.

Wastewater samples have been collected biweekly for a period of 4 months both from the inlet of the plant after initial screening (influents) and from the outlet after chlorination (tertiary treated, effluents). Composite samples were collected for 24 h using a portable water sampler (WS750 dual bottle sampler from Global Water, CA, USA) at a rate of 100 mL h^{-1}.

The procedure described by Batt et al. (2008) for the analysis of pharmaceuticals in waste and surface water was adopted for this work with modifications. Batt et al. procedure was developed and applied for the analysis of pharmaceuticals in wastewater and surface water samples obtained from New Mexico and East Fork River in Cincinnati, Ohio, respectively. The pH of the samples was measured upon arrival to the laboratory and found to be close to neutral values (pH 7.2–7.6) and therefore the samples were used without any pH adjustment. The samples were then filtered using a vacuum filter funnel (porosity 25–50 μm, Aldrich). To each 500 mL of filtered samples, a 2 mL solution containing Na_2ETDA (5.00 g L^{-1}, used as a metal chelating agent) and ascorbic acid (25.0 mg L^{-1}, used to remove any chlorine residues that may be present in the samples) was added before extraction.

Table 2 Common MS settings.

Ion source polarity	Positive and negative ion modes
Capillary voltage	3500 V
Vaporizer temperature	300 °C
Nebulizer Pressure	45 psi
Gas Flow	10 L min^{-1}
Time Filter	True
Time Filter Width	Wide
MS1 Resolution	Wide
MS2 Resolution	Wide
Dwell Time	10 ms
Fragmenter Voltage	135 V
Cell Acceleration Voltage	7 V
Experiment Type:	MRM
MS Run Time	13.0 min (acidics and neutrals) and 18.0 min (basics)

Table 3 Specific MS settings (all analyzed in + ESI mode, except diclofenac sodium salt and ibuprofen for which –ESI more was used).

Name	RT[a]	Formula Wt	Precursor Ion	Product Ion 1	Product Ion 2	CE[b]	DT[c]	LOD[d]
Acetaminophen	0.94	151.17	152.2	110.0	93.0	11	10	0.90
Atenolol	0.80	266.34	267.6	145.2	74.2	23	10	0.22
Captopril	0.63	217.29	218.5	70.0	115.5	23	10	6.1
Carbamazepine	5.36	236.27	237.5	194.4	179.3	15	10	0.25
Cephalexin	0.92	365.41	348.9	158.0	174.9	7	10	0.32
Chlorpheniramine maleate	3.85	390.86	275.6	230.4	–	11	10	0.35
Diclofenac sodium salt	3.69	318.13	295.8	250.0	–	4	100	1.33
Erythromycin Ethylsuccinate	1.25	862.1	862.5	387.1	–	15	10	2.1
Ibuprofen	4.36	207.23	207.1	161.0	118.9	2	100	0.91
Hyoscine-N-butylbromide	2.20	440.38	360.1	194.2	–	23	10	1.0
Metformin	0.70	129.16	130.1	60.1	71.1	19	10	3.0
Metronidazole	2.80	171.15	172.2	128.1	82.0	11	10	0.32
Norfluoxetine	3.67	295.3	295.8	278.6	137.8	15	10	1.1
Ranitidine hydrochloride	0.80	350.86	315.8	130.0	127.1	11	10	0.11
Simvastatin	11.94	418.57	419.7	225.4	244.5	5	100	1.0
Sulfamethoxazole	0.69	253.28	254.6	156.0	189.2	11	10	0.21
Trimethoprim	0.90	290.32	291.7	122.9	–	19	10	0.23
Warfarin sodium salt	12.306	308.31	309.8	252.4	164.0	11	10	1.8

[a] RT is the retention time (min) of pure standards using A1:B mobile phase except for diclofenac sodium salt and ibuprofen A2:B mobile phase was used.
[b] CE is collision energy (eV).
[c] DT is Dwell Time (ms).
[d] LOD is the limit of detection in ng mL^{-1}.

Analytes in the samples were extracted at the same day of collection using Oasis MCX cartridges (mixed mode, 150 mg from Waters, Milford, MA, USA). The material inside these cartridges is made of hydrophobic-lipophilic balanced copolymer that can retain acidic, basic, and neutral analytes. A drug behaves as an acid, base, or neutral in solution based on the functional group(s) it processes and on the pH of the solution. In this context, ''acidic and neutral'' analytes described in this work are those pharmaceuticals that are eluted off the SPE Oasis MCX cartridge with ACN, whereas basic analytes are those that are eluted by ACN in 5% ammonium hydroxide. The reason for dividing the analytes into 2 groups (''acidic and neutrals'' and basics) was to enhance the sensitivity of the MS detector and to avoid any complexity during analysis.

Before extraction, each SPE cartridge was conditioned with ACN (6 mL) followed by reagent water (6 mL). Samples prepared as described above were passed through reconditioned cartridges at a rate of 3–5 mL min^{-1} with the aid of a vacuum pump. Each cartridge was then slowly rinsed with a 2 mL solution of formic acid (2%) and allowed to dry under vacuum.

Acidic and neutral analytes in each sample were first eluted with ACN (2×4 mL) into a small glass tube using a vacuum manifold (20 positions from Waters, Milford, MA, USA). Basic analytes retained on the cartridge material were then eluted by ACN solution (2×4 mL) containing ammonium hydroxide (5%) into a separate glass tube. Each eluate was then concentrated to dryness with the help of a TurboVap LV Concentration Evaporator Workstation (Caliper Life Sciences, Runcorn, UK) at 40 °C under a gentle stream of N_2. The contents of the first tube were reconstituted with ACN in water (0.50 mL, 20 + 80), whereas the eluate in the other tube was reconstituted with MeOH in water (0.50 mL, 20 + 80). The first fraction is termed from now on as ''acidics and neutrals,'' whereas the second one as ''basics.'' Reconstituted samples were transferred to glass vials and analyzed by LC–MS. This procedure has been first tried using solutions of mixed standards as described in the section 2.3.2 below.

2.3. Samples analysis

2.3.1. UV analyses

In order to identify absorption wavelengths suitable for monitoring the analytes during the method development, a UV scan for each analyte was performed using a UV–Vis spectrometer (Jasco, Ubest V-50) as follows: 10.0 mg L^{-1} solution of each drug was prepared in the A1-B mobile phase (1:1 ratio) and the spectra were taken in the range of 200–400 nm using quartz cells. Same procedure was repeated but using the other mobile phase (A2–B, ratio1:1). Blank solutions were also analyzed using both A1–B and A2–B mobile phases in 1:1 ratios.

2.3.2. HPLC-DAD analyses

Method development was undertaken using an HPLC system (1290 series, Agilent Technologies, USA) equipped with a diode array detector (DAD). For the separation of the analytes, a SunFire column (C_{18}, 2.1×150 mm, 3.5 μm, Waters, Milford, MA, USA) preceded by a guard column (SunFire, C_{18}, 2.1×10 mm, 3.5 μm, Waters, Milford, MA, USA) was used at a temperature of 40 °C. A gradient elution program was set as follows with a mobile phase flow rate of 0.5 mL min^{-1}. For acidic and neutral analytes, the A2–B mobile phase was used with an initial mobile phase of 10% solvent B and held for 0.5 min. The ratio of the mobile phase B was then linearly increased to 40% over 1 min and held for 2.5 min, then to 90% over 0.5 min and held for 0.5 min. The initial mobile phase composition was restored in 0.5 min and the column was equil-

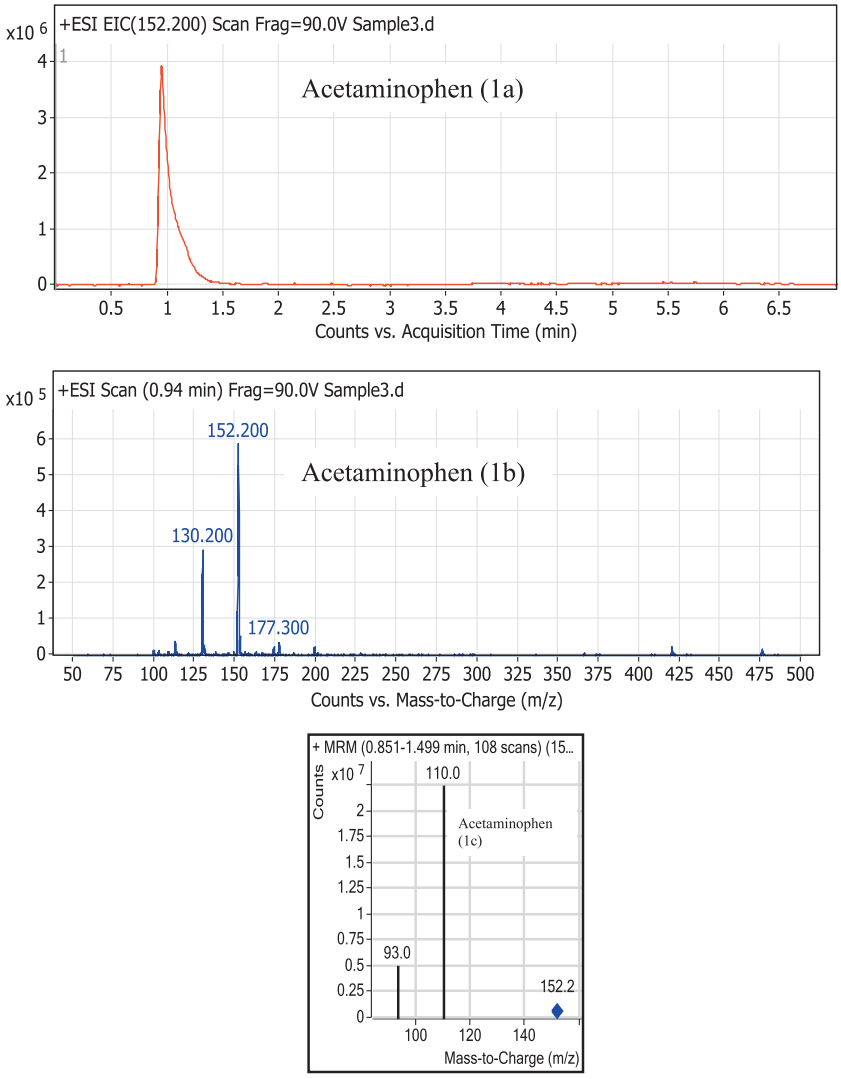

Figure 1 Extracted MRM chromatogram (1a), mass spectrum (1b), and product ion spectrum (1c) for acetaminophen.

ibrated for an additional 7.5 min (total run time is 13.0 min). The DAD wavelength was set at 210 nm (this wavelength was found to be suitable for all analytes as discussed in Section 3.1). For the basic analytes, the A1–B mobile phase was used with an initial composition of 10% B and was held for 0.5 min. The ratio of the mobile phase B was then linearly increased to 30% over 0.5 min, then to 35% over 2 min and held for 7 min, and finally to 90% over 1 min and held for 0.5 min. The initial mobile phase composition was restored in 0.5 min and the column was equilibrated for 6.0 min (total run time is 18.0 min). The following wavelengths have been found to be suitable for detecting basic analytes 225, 230, 240, and 270 nm (refer to Section 3.1 for details).

To determine the elution pattern of the analytes, a 20 μL of each drug solution (1000.0 μg mL^{-1}) was individually injected to the HPLC column using the two HPLC protocols described above. Following this, the two groups of analytes eluted from the SPE as acidics and neutrals (group 1) or as basics (group 2) have been identified as follows: a mixed standard solution containing all analytes (10 mL of 1000.0 μg mL^{-1}) has been extracted, eluted, evaporated, and reconstituted using the same procedure employed for the samples (see Section 2.2). Blank solution has been treated in a similar way. Consequently, a 20 μL of each of the reconstituted solutions of the two groups of analytes was injected to the HPLC column. Acidic and neutral analytes were analyzed using the A2–B mobile phase, whereas basic analytes were analyzed using the A1–B mobile phase.

2.3.3. LC–MS/MS analyses

Due to the superior sensitivity of MS detection when compared to UV as well as the complexity of the samples matrix, MS was used as a detector.

As a mean to identify the molecular ion masses and the retention times of the analytes, a 10 μL solution of each analyte (1000.0 μg mL^{-1}) was injected to the LC–MS system (Agilent 1290 UHPLC and 6460 MS/MS series with Jet Steam ESI source) using a mobile phase flow rate of 0.5 mL min^{-1}. Then, a product ion scan employing the multiple monitoring reaction mode (MRM) was performed to collect data for suitable product ions. The two most intense MRM transitions were then selected for all analytes except for chlorpheniramine maleate,

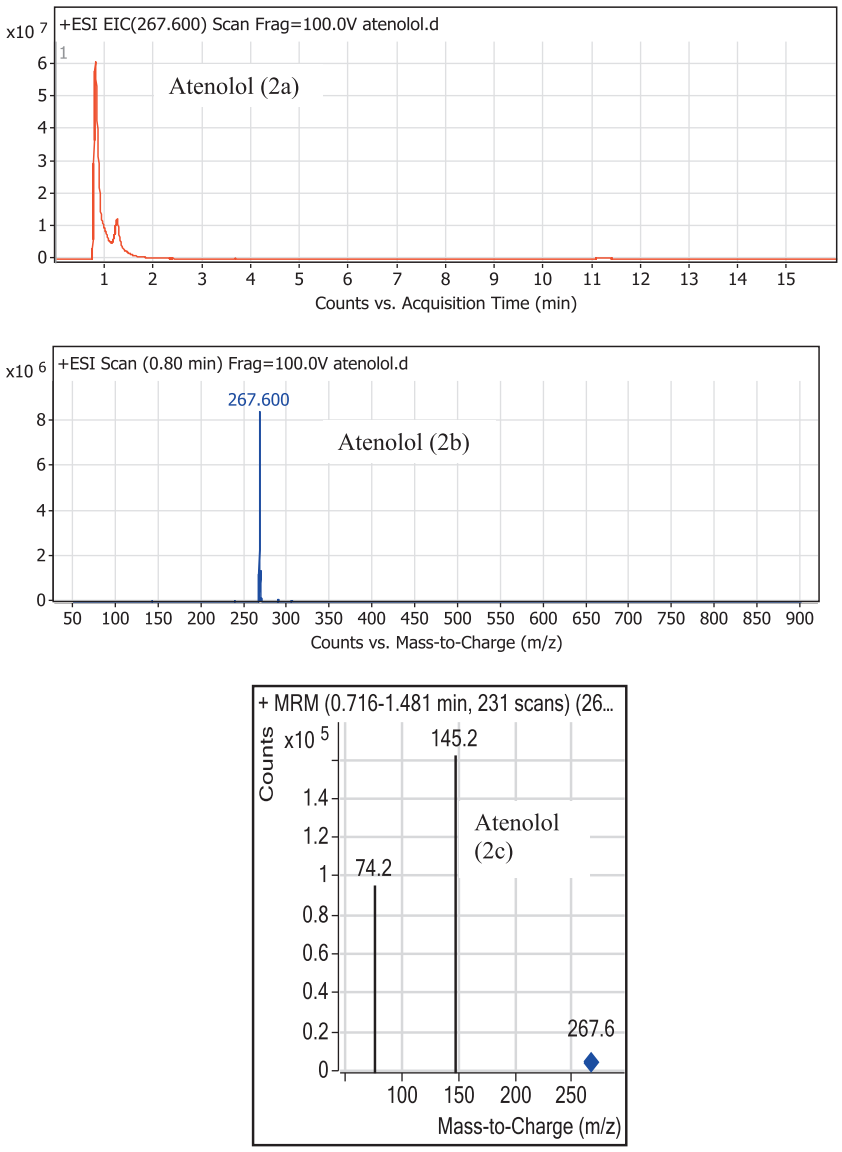

Figure 2 Extracted MRM chromatogram (2a), mass spectrum (2b), and product ion spectrum (2c) for atenolol.

diclofenac sodium salt, erythromycin ethylsuccinate, hyoscine-n-butylbromide, and trimethoprim, for which only one product ion was present. The MRM transitions were optimized using different collision energies. The utilized common MS settings are listed in Table 2, whereas the specific settings were shown next to each analyte in Table 3. After establishing the MRM, the two groups of analytes (extracted by the cartridges as described at the end of Section 2.3.3) have also been injected to the LC–MS system to confirm the results obtained by HPLC-DAD. Finally, calibration standard solutions and treated samples solutions were injected to the LC–MS system. For quantification of the analytes, a 4-point calibration curve for each analyte was constructed at concentrations of 0.000, 100.00, 500.00, and 1000.0 μg mL^{-1}. The R^2-value for the curves was better than 0.998. For LC–MS analysis, same chromatographic conditions (i.e. column, mobile phases, and gradient elution programs) used for HPLC-DAD analyses were employed. For detection of the analytes both the retention time and product ion ratios were used. Analytes were positively identified if both product ions are present in abundance more than the LOD and the ratio of the ions is within 30% of the anticipated ratio.

The limit of detection (LOD) for each analyte was determined using 5–7 replicate injections of a reagent blank and was calculated as the average concentration measured for the blank multiplied by 3 times its standard deviation.

3. Results and discussion

3.1. UV analysis

According to the UV spectra of all analytes (not shown here) an optimal wavelength of 210–215 nm can be used for all analytes when using the A2–B (1:1) mobile phase (210 nm was used). On the other hand, the use of the A1–B (1:1) mobile phase resulted in a different spectral pattern and a different optimal wavelength was obtained for most of the analytes. Additionally the following compounds showed no absorption signals in the investigated wavelength range: captopril, eryth-

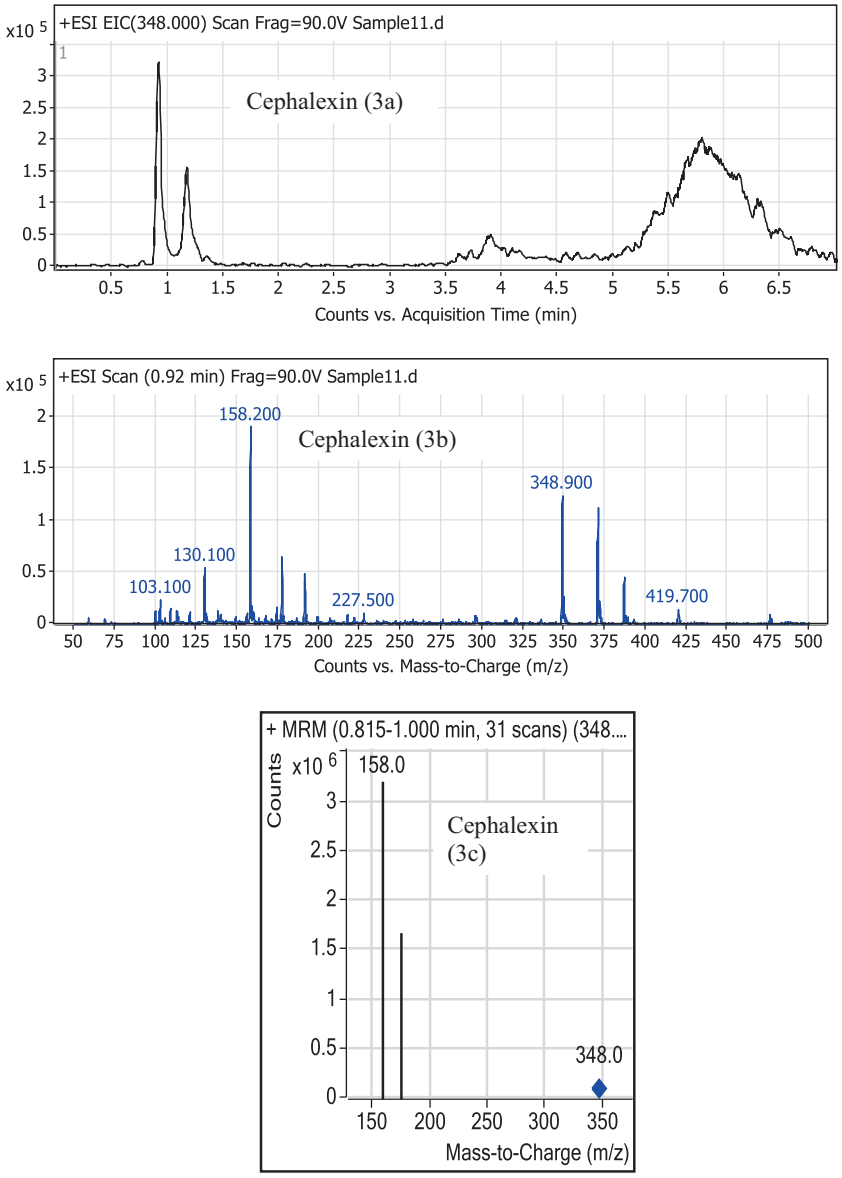

Figure 3 Extracted MRM chromatogram (3a), mass spectrum (3b), and product ion spectrum (3c) for cephalexin.

romycin ethyl succinate, and hyosine-*N*-butyl bromide. As a consequence multiple wavelengths have been used to monitor the analytes: 225, 230, 240, and 270 nm; each of them has been used to monitor a sub group of the analytes.

3.2. HPLC-DAD analysis

Experiments conducted as described in Section 2.2 above showed that the analytes eluted as basics were: atenolol, ranitidine, simvastatin, and trimethoprim. In contrast, erythromycin ethyl succinate showed no HPLC-DAD signal, but confirmed by LC–MS to elute in the basics group. The rest of the analytes was eluted in the acidics and neutrals fraction.

3.3. LC–MS/MS Analysis

Several solid phase extraction cartridges were used in the literature for the extraction of pharmaceuticals from wastewater including Oasis HLB, Oasis MCX, Oasis WCX, Strata-X, Supelco C8, Supelco C18, Varian Focus, and Merck LiChrolut-EN. The Oasis MCX, which was employed in this work, has been shown to provide high overall recoveries. (Batt et al., 2008; Lacey et al., 2008).

Extracted MRM chromatogram, mass spectrum, and product ion spectrum for each analyte that has been detected in the wastewater in concentrations greater than the LOD are shown in Figs. 1–5. Out of the 19 targeted drugs, 5 pharmaceuticals have been found both in raw (influents) and treated (effluents) wastewater samples. The concentrations of these pharmaceuticals are shown in Tables 4 and 5. Although most pharmaceuticals have high solubility in water and hence remain soluble in the aqueous phase, some drugs have lower solubility and remain insoluble as the solid material in wastewater. Since the samples were filtered before extraction, the reported concentrations in this work represent only the water-soluble fraction of the analytes.

The detected drugs have been found in most of the samples as shown in Tables 4 and 5. As expected, the concentrations of indi-

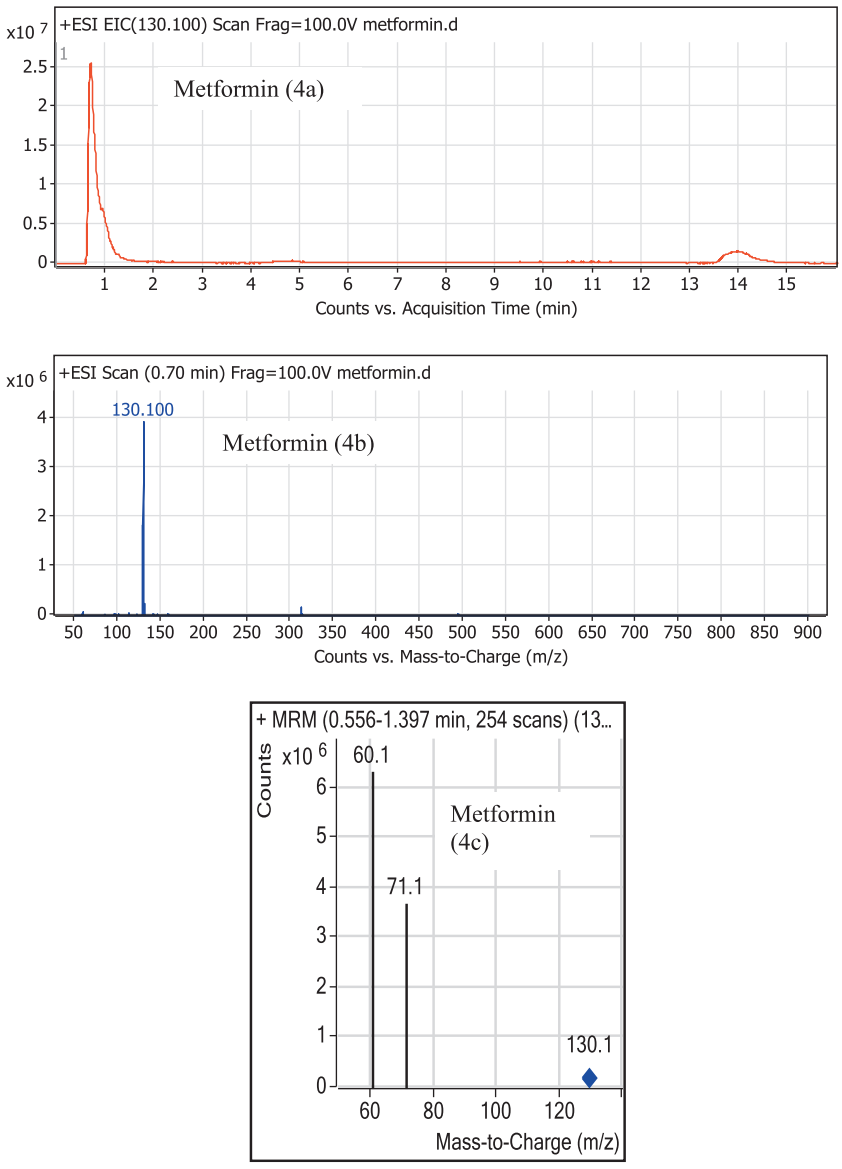

Figure 4 Extracted MRM chromatogram (4a), mass spectrum (4b), and product ion spectrum (4c) for metformin.

vidual pharmaceuticals in the wastewater influents were found to be low with an average concentration of 2.04–38.9 ng mL^{-1}. The highest detected concentration was for acetaminophen (99.6 ng mL^{-1}), followed by metformin (31.2 ng mL^{-1}), norfluoxetine (10.4 ng mL^{-1}), atenolol (4.03 ng mL^{-1}), and cephalexin (3.23 ng mL^{-1}). Interestingly, the amounts of these pharmaceuticals in the treated wastewater (effluents) did not differ much from those found in the raw wastewater (influents), with a concentration range of 0.545–31.2 ng mL^{-1}. The highest concentration detected was for acetaminophen (90.5 ng mL^{-1}), followed by norfluoxetine (11.7 ng mL^{-1}), cephalexin (2.83 ng mL^{-1}), metformin (4.51 ng mL^{-1}), and atenolol (2.01 ng mL^{-1}).

The findings of this study is consistent with those of many reports in the literature, where low concentrations of pharmaceuticals have been found in municipal wastewater (Bartelt-Hunt et al., 2009; Batt et al., 2008; Gracia-Lor et al., 2010; Miège et al., 2009; Radjenovic et al., 2009; Rosal et al., 2010). For example, Rosal et al. reported the occurrence of around 70 pharmaceuticals and PPCPs in influents of municipal wastewater with some compounds in the ng mL^{-1} concentration range (e.g. paraxanthine, caffeine, and acetaminophen), whereas the rest were in the ng L^{-1} range (Rosal et al., 2010). In another study by Gracia-Lor et al., 13 out of 20 investigated drugs were detected in the influents of urban wastewater samples with salicylic acid having the highest concentration (276.7 ng mL^{-1}) (Gracia-Lor et al., 2010).

On the other hand, effluents of urban wastewater and receiving waters were reported also to contain many pharmaceuticals at low concentrations. (Bartelt-Hunt et al., 2009; Gracia-Lor et al., 2010; Joss et al., 2008; Lacey et al., 2008; Santos et al., 2007; Soliman et al., 2007; Spongberg and Witter, 2008; Ternes, 1998; Zhou et al., 2009) Clearly, this indicates that most of the current wastewater treatment practices are inefficient in completely removing such contaminants. For instance, 5 pharmaceuticals (i.e. propranolol, sulfamethoxazole, carbamazepine, indomethacine and diclofenac) were found in

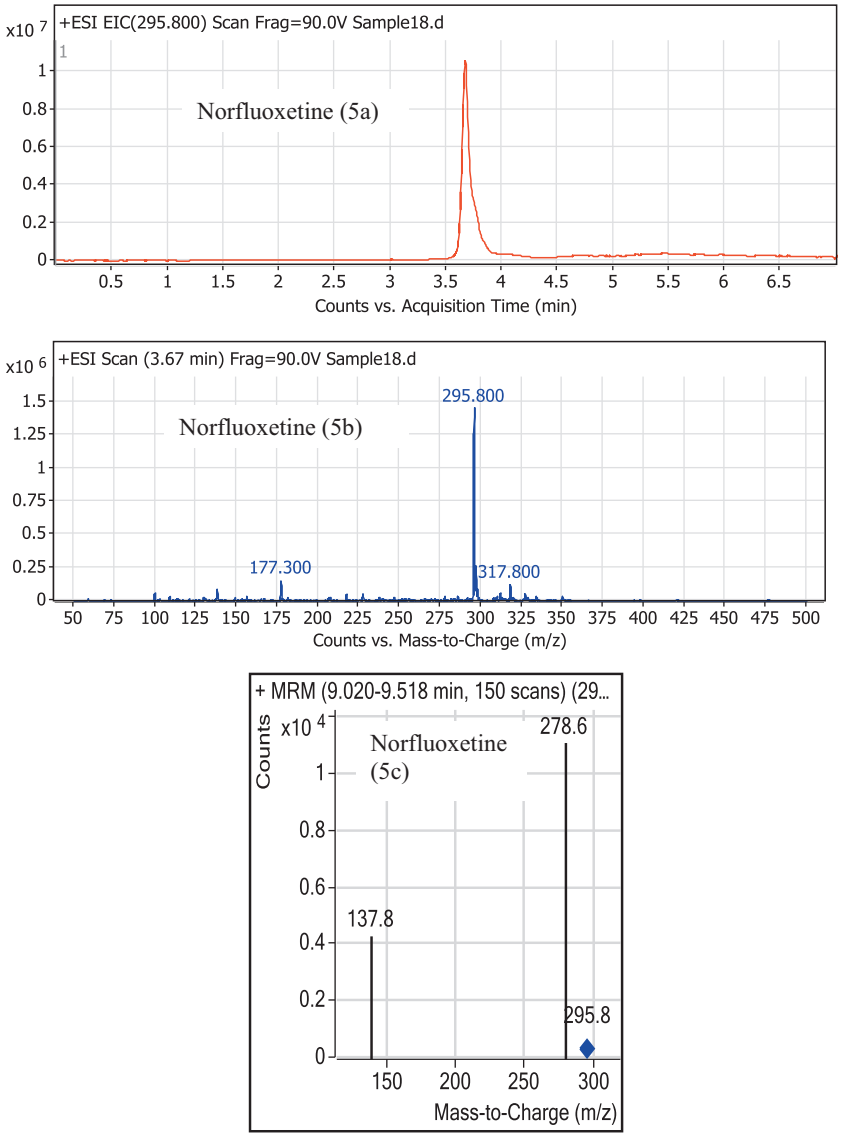

Figure 5 Extracted MRM chromatogram (5a), mass spectrum (5b), and product ion spectrum (5c) for norfluoxetine.

all wastewater and the receiving surface water samples in England with carbamazepine having the highest levels (2.336 ng mL^{-1}). The reported removal efficiencies for these compounds from the wastewater were in the range of 43–92%.(Zhou et al., 2009) In another study, 5 out of 6 drugs (diclofenac, ibuprofen, ketoprofen, naproxen, carbamazepine, and caffeine) have been detected in both influent and effluent samples from 4 STPs in Seville–Spain in the ng mL^{-1} concentration range. The reported removal rates for these drugs were between 6% and 98%. (Santos et al., 2007) Similarly, 5 out of 10 pharmaceuticals detected in the influents of 3 STPs in Duplin-Ireland have been found in the wastewater effluents with concentrations of <1 ng mL^{-1} for most samples (Lacey et al., 2008).

The removal efficiency of pharmaceuticals from wastewater is actually dependent on several factors including the climatic conditions, the type of wastewater treatment and its operational conditions (e.g. temperature, redox conditions, solids and hydraulic retention time) as well as the age of the activated sludge used in the plant, but the main factor is reported to be the physico-chemical nature of most pharmaceuticals, which is the acidity and high solubility in water with very low solid–liquid partition. These factors, especially the last one lead to a very poor sorption of these compounds onto sludge and hence leaving them soluble in the aqueous phase (Gracia-Lor et al., 2012; Verlicchi et al., 2012).

It is also worth noting here that some of the pharmaceuticals that were detected in the wastewater were also found in the sludge of the wastewater treatment plans. This is due to the low solubility of such drugs and therefore they remain insoluble and appear in the sludge (Gao et al., 2012; Jelic et al., 2011).

When considering a single pharmaceutical at low concentrations such as those reported in this investigation and other work, it may be assumed that not many health risks can be associated with long term exposure to such a drug. But the health risks associated with exposure to a large number of pharmaceuticals, their metabolites, and transformation products, even at low concentrations, cannot be ignored.

Table 4 Concentrations of pharmaceuticals (ng mL^{-1}) in raw wastewater samples.

Sample ID[a]	Metformin	Atenolol	Cephalexin	Acetaminophen	Norfluoxetine
1R	9.01	2.04	2.52	99.6	10.4
2R	16.5	2.02	2.04	58.7	9.11
3R	17.0	4.03	< MDL	40.5	8.43
4R	4.02	1.04	2.01	3.61	10.4
5R	13.0	2.99	3.23	29.0	7.03
6R	25.1	2.02	1.48	36.7	7.64
7R	31.2	1.97	2.09	5.51	2.48
8R	5.93	< MDL	1.37	37.7	< MDL
Range	4.02–31.2	< MDL - 4.03	< MDL - 3.23	3.61–99.6	< MDL - 10.4
Median	14.8	2.02	2.03	37.2	8.04
Average	15.2	2.04	1.88	38.9	7.07

[a] R means raw wastewater (influents).

Table 5 Concentrations of pharmaceuticals (ng mL^{-1}) in treated wastewater samples.

Sample ID [a]	Metformin	Atenolol	Cephalexin	Acetaminophen	Norfluoxetine
1T	< LOD	2.01	2.21	16.1	11.7
2T	< LOD	< LOD	1.68	< LOD	9.81
3T	< LOD	1.03	< LOD	< LOD	9.72
4T	< LOD	< LOD	1.27	48.8	8.17
5T	< LOD	< LOD	2.83	< LOD	9.57
6T	< LOD	< LOD	< LOD	90.5	< LOD
7T	< LOD	< LOD	1.74	44.8	6.83
8T	4.51	< LOD	1.88	46.7	< LOD
Range	< LOD - 4.51	< LOD - 2.01	< LOD - 2.83	< LOD – 90.5	< LOD – 11.7
Median	< LOD	< LOD	1.71	30.5	8.87
Average	3.19	0.545	1.53	31.2	7.25

[a] T means treated wastewater (effluents).

4. Conclusions

Out of the 19 pharmaceuticals investigated in this study, 5 drugs have been found both in the influents and effluents of the STP. The concentrations of these drugs in both types were in the lower ng mL^{-1} (influents range 2.04–38.9, max. 99.6; effluents range 0.545–31.2, max. 90.5) with effluents having slightly lower concentrations than the influents in most cases.

The results of this investigation, supported by a similar work in the literature, indicate that many drugs (including their metabolites and transformation products) are not efficiently eliminated during the wastewater treatment processes (sometimes tertiary treatment, as in this study). This may suggest that conventional wastewater treatment technologies are inefficient in completely removing such compounds and as a consequence leaving the way open for such bioactive compounds to enter the aquatic environment and eventually pollute the drinking water supplies and pose health risks to humans and other living organisms.

Although, the levels of detected pharmaceuticals in the treated water are quite low, the health risks associated with long term exposure to a large number of pharmaceuticals have to be kept in mind.

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