Determination of pharmaceutical residues and assessment of their removal efficiency at the Daugavgriva municipal wastewater treatment plant in Riga, Latvia

I. Reinholds, O. Muter, I. Pugajeva, J. Rusko, I. Perkons and V. Bartkevics

ABSTRACT

Pharmaceutical products (PPs) belong to emerging contaminants that may accumulate along with other chemical pollutants in wastewaters (WWs) entering industrial and/or urban wastewater treatment plants (WWTPs). In the present study, the technique of ultra-high-performance liquid chromatography coupled to Orbitrap high-resolution mass spectrometry (Orbitrap-HRMS) was applied for the analysis of 24 multi-class PPs in WW samples collected at different technological stages of Daugavgriva WWTP located in Riga, Latvia. Caffeine and acetaminophen levels in the range of 7,570–11,403 ng/L and 810–1,883 ng/L, respectively, were the predominant compounds among 19 PPs determined in the WW. The results indicate that aerobic digestion in biological ponds was insufficiently effective to degrade most of the PPs (reduction efficiency < 0–50.0%) with the exception of four PPs that showed degradation efficiency varying from 55.0 to 99.9%. Tests of short-term chemical and enzymatic hydrolysis for PP degradation in WW samples were performed, and the results reflected the complexity of different degradation mechanisms and physicochemical transformations of PPs. The toxicological studies of WW impact on Daphnia magna indicated gradual reduction of the total toxicity through the treatment stages at the WWTP.

Key words | biological treatment, hydrolysis, Orbitrap-HRMS, pharmaceuticals, toxicity studies, wastewater treatment plant

INTRODUCTION

Recently, global concerns have been raised about the wastewater (WW) treatment efficiency at wastewater treatment plants (WWTPs) that discharge partially purified water into surface waters. Besides the typical environmental pollutants (pesticides, persistent organic compounds, plasticizers, heavy metals, etc.), WWs contain residues of pharmaceutical products (PPs) and their metabolites generated by the pharmaceutical industry (Ma et al. 2016), livestock farms (Sim et al. 2013), hospitals (Carraro et al. 2016), and other economic and social sources. PPs belong to emerging contaminants, most of which have undefined tolerable levels in water, and potential impacts of their presence in the environment on human health are less investigated (Gadipelly et al. 2014). Antibiotics, cytostatics, and hormonally active compounds are the most notable PPs, which may cause adverse effects on organisms, promote bacterial resistance to antibiotics used in veterinary and human medicine, cause hormonal disturbances and present other potential human health risks associated with prolonged influence of contaminated water or consumption of aquatic organisms (Gavrilescu et al. 2015).

Some PPs, especially antibiotics (e.g., macrolides, sulfonamides, fluoroquinolones), undergo limited degradation in WWTPs, thus raising issues of their potential ecological and human health hazards (Watkinson et al. 2009). Remberger et al. (2009) have indicated an increase of antibiotics levels during the WW treatment stages, attributed to the dissolution of pharmaceuticals from solid wastes such as faeces, bile, and other biogenic matter and the transformation into forms free from metabolites due to enzymatic reactions with β-glucuronidase.

The latest technologies of industrial and municipal WWTPs include the combined use of several treatment methods such as chemical oxidation by adding ozone,
chlorine or other chemicals to reduce the levels of contaminants, or biological treatment (Rivera-Utrilla et al. 2013). Several treatment methods such as coagulation with metal salts, sedimentation, and flocculation using polymers or filtration through dedicated systems are applied before and sometimes after the general treatment stages to remove most industrial wastes and to enhance partial degradation or reduction of contaminants, including pharmaceutical residues present in WWs (Gadipelly et al. 2014).

The whole effluent toxicity of the treated WW is considered as one of the main criteria for evaluating the efficiency of a WWTP. Representatives of phytoplankton (Desmodesmus subspicatus), zooplankton (Daphnia magna), higher plants (Sinapis alba), fish (Poecilia reticulata), and other species are used as test organisms in ecotoxicological testing. Daphnids and algae have been shown to be the most sensitive organisms in tests with WW from health-care facilities (Chrobáková et al. 2015). Freitas et al. (2016) compared the sensitivity of different test organisms for WWs contaminated with PPs, which ranged as follows: Tetrahymena thermophila > Daphnia magna > Lactuca sativa > Spirodela polyrhiza ≈ Vibrio fischeri. Toxicity testing may provide complementary information to chemical analysis on the sum of micropollutants present in treated water (Macova et al. 2010).

Concern for the environmental safety determines the necessity of accurate monitoring of PPs in WWs. The analytical methods based on high and ultra-high performance liquid chromatography (HPLC, UHPLC) coupled to UV, fluorescence, and/or mass spectrometric (MS) detectors employing tandem MS/MS and high-resolution MS (HRMS) techniques have been widely used to evaluate the levels of PPs in different types of waters (Gurke et al. 2015; Leendert et al. 2015). Recent studies have indicated high benefits regarding the high selectivity and sensitivity of methods based on Orbitrap, time of flight, as well as magnetic-sector HRMS detectors appropriate for the low amounts of PPs (Picó & Barceló 2015).

The proposed study demonstrates an application of UHPLC separation coupled to highly sensitive Q-Orbitrap-HRMS detection for the determination of some common PPs in untreated and treated WWs collected at Daugavgrīva WWTP in Riga, Latvia. It should be noted that the operating principle of this WWTP is based on biological pond systems and the same principle of WW treatment is also used in other Baltic states as well as in most of the other European countries. Thus, our research is highly relevant to the global issues of pharmaceutical residue control during WW purification at WWTPs.

**MATERIALS AND METHODS**

**Materials**

Acetonitrile and methanol (MeOH) of gradient grade for liquid chromatography (>99.9% assay) and dimethyl sulfoxide (DMSO, >99.9% assay) were purchased from Merck Millipore (Darmstadt, Germany). Acetic acid (≥99.7% assay), hydrochloric acid (≥37% assay), formic acid (≥98% assay), β-glucuronidase enzyme (from Helix pomatia, aqueous solution, ≥85,000 units/mL), Na2EDTA salt (≥99.0% assay), potassium dichromate (99% assay), sodium hydroxide (≥98% assay), and sodium acetate (99% assay) were all purchased from Sigma-Aldrich (St Louis, MO, USA). Ultrapure water was generated by a Millipore Millic-Q™ system (Billerica, MA, USA). Strata-X® polymeric SPE cartridges (200 mg/3 mL) were purchased by Phenomenex (Torrance, CA, USA).

Whatman® glass microfibre filters of GF/C grade (1.2 μm) were supplied by Whatman International Ltd (Maidstone, UK). Analytical standards of 24 multi-class PPs (Table 1), reported by the World Health Organization (2008) as essential pharmaceuticals, were purchased from Sigma-Aldrich (St Louis, MO, USA) and Fluka (Buchs, Switzerland). Stock solutions of individual standards were prepared by dissolving 10 mg of each standard in 10 mL of MeOH or DMSO to make approximate concentrations of 1,000 mg/L. The purity of each pharmaceutical standard was taken into account when the final concentrations of the stock solutions were calculated. The stock solutions were stored at −20 °C. The working standard solution of 0.1 mg/L concentration was prepared by diluting the appropriate volumes of stock solutions in MeOH. The diluted solution could be stored at −20 °C for up to 1 week.

**Facility**

The Daugavgrīva WWTP serves the population of Riga, Latvia (ca. 698,529 (2016)) gaining a capacity from 750,000 up to 1,000,000 population equivalents, and a production of about 6,857 tonnes of sewage sludge annually (PURE 2012). The Daugavgrīva facility consists of the following stages: pre-treatment by screens to remove mechanical contaminants, physical and mechanical treatment defined as primary treatment, biological treatment as the secondary (main) treatment, followed by tertiary or complementary treatment and sludge disposal, and discharge of effluent waters into the Gulf of Riga (Romagnoli et al. 2009). The
Table 1 | The characterisation of PPs investigated in WWs

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Molecular formula</th>
<th>Therapeutic class</th>
<th>Log $K_{ow}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acetaminophen</td>
<td>C₈H₉NO₂</td>
<td>Analgesic and antipyretic drug</td>
<td>0.46</td>
</tr>
<tr>
<td>2</td>
<td>Atenolol</td>
<td>C₁₄H₂₂N₂O₃</td>
<td>Anti-hypertensive agent</td>
<td>0.16</td>
</tr>
<tr>
<td>3</td>
<td>Atorvastatin</td>
<td>C₃₅H₃₅FN₂O₅</td>
<td>Cholesterol-lowering agent</td>
<td>5.70</td>
</tr>
<tr>
<td>4</td>
<td>Azithromycin</td>
<td>C₃₈H₇₂N₂O₁₂</td>
<td>Antibiotic (macrolide)</td>
<td>4.02</td>
</tr>
<tr>
<td>5</td>
<td>Caffeine</td>
<td>C₈H₁₀N₄O₂</td>
<td>Central nervous system stimulant</td>
<td>−0.07</td>
</tr>
<tr>
<td>6</td>
<td>Carbamazepine</td>
<td>C₁₅H₁₂N₂O</td>
<td>Anticonvulsant</td>
<td>2.45</td>
</tr>
<tr>
<td>7</td>
<td>Ciprofloxacin</td>
<td>C₁₇H₁₆FN₅O₃</td>
<td>Antibiotic (fluoroquinolone)</td>
<td>0.28</td>
</tr>
<tr>
<td>8</td>
<td>Clarithromycin</td>
<td>C₃₈H₆₆NO₁₃</td>
<td>Antibiotic (macrolide)</td>
<td>3.16</td>
</tr>
<tr>
<td>9</td>
<td>Diclofenac</td>
<td>C₁₄H₁₁Cl₂NO₂</td>
<td>Nonsteroidal anti-inflammatory agent (NSAID)</td>
<td>4.51</td>
</tr>
<tr>
<td>10</td>
<td>Erythromycin</td>
<td>C₂₇H₄₆NO₁₃</td>
<td>Antibiotic (macrolide)</td>
<td>3.06</td>
</tr>
<tr>
<td>11</td>
<td>Fluoxetine</td>
<td>C₁₇H₁₈F₃NO</td>
<td>Selective serotonin-reuptake inhibitor</td>
<td>4.05</td>
</tr>
<tr>
<td>12</td>
<td>Gemfibrozil</td>
<td>C₁₅H₂₂O₂</td>
<td>Lipid-regulating agent</td>
<td>3.40</td>
</tr>
<tr>
<td>13</td>
<td>Ibuprofen</td>
<td>C₁₃H₁₈O₂</td>
<td>NSAID</td>
<td>3.97</td>
</tr>
<tr>
<td>14</td>
<td>Ketoprofen</td>
<td>C₁₆H₁₄O₂</td>
<td>NSAID</td>
<td>3.12</td>
</tr>
<tr>
<td>15</td>
<td>Losartan</td>
<td>C₂₂H₂₃Cl₅N₀</td>
<td>Angiotensin receptor blocker</td>
<td>6.1</td>
</tr>
<tr>
<td>16</td>
<td>Metoprolol</td>
<td>C₁₅H₂₅NO₃</td>
<td>Cardioselective β1-adrenergic blocker</td>
<td>1.88</td>
</tr>
<tr>
<td>17</td>
<td>Naproxen</td>
<td>C₁₄H₁₄O₂</td>
<td>NSAID</td>
<td>3.18</td>
</tr>
<tr>
<td>18</td>
<td>Pravastatin</td>
<td>C₂₃H₂₆O₂</td>
<td>Cholesterol-lowering agent</td>
<td>0.59</td>
</tr>
<tr>
<td>19</td>
<td>Propranolol</td>
<td>C₁₆H₂₁NO₂</td>
<td>Non-cardioselective β-adrenergic antagonist</td>
<td>3.48</td>
</tr>
<tr>
<td>20</td>
<td>Simvastatin</td>
<td>C₂₅H₃₂O₃</td>
<td>Cholesterol-lowering agent</td>
<td>4.68</td>
</tr>
<tr>
<td>21</td>
<td>Sulfamethoxazole</td>
<td>C₁₀H₁₁N₃O₂S</td>
<td>Antibacterial agent</td>
<td>0.89</td>
</tr>
<tr>
<td>22</td>
<td>Trimethoprim</td>
<td>C₁₄H₁₈N₄O₂</td>
<td>Antibacterial agent</td>
<td>0.91</td>
</tr>
<tr>
<td>23</td>
<td>Valsartan</td>
<td>C₂₄H₂₅N₅O₃</td>
<td>Angiotensin receptor blocker</td>
<td>4.0</td>
</tr>
<tr>
<td>24</td>
<td>Xylosine</td>
<td>C₁₂H₁₆N₅S</td>
<td>α-Adrenergic agonant (Veterinary drug)</td>
<td>–</td>
</tr>
</tbody>
</table>

$k_{ow}$: octanol–water partition coefficient.

main parameters of the Daugavgriva facility are as follows: sludge load 0.06 kg BOD/kg, expressed as the daily organic load per aerated sludge mass, where BOD is the biological oxygen demand; the average flow 200,000 m³/h; total retention time (RT) of the WWTP 22 days. Primary sedimentation has an RT of 5 hours, and the secondary treatment in aerobic tanks (sludge digestion) proceeds for about 5 days, but the biological digestion takes place for about 14 days at a temperature of 37 °C (PURE 2012). The operating principle of this WWTP system is schematically given in Figure 1.

**Sampling**

WW samples were collected over 24 hours (five replicates) in May 2016 from the four sampling points shown in Figure 1 (inlet chamber, outputs of sedimentation and aeration basins, and treated water discharged from the effluent collector). All the samples were sampled in pre-cleaned 1 L amber glass bottles and kept at 4 °C during the transportation. Once received at the laboratory, the samples were immediately filtered through 1.2 μm glass microfibre filters (GF/C, Whatman, UK), and extracted within 24 h.

**Solid phase extraction**

Prior to the extraction, Strata-X cartridges were conditioned with 3 mL of MeOH and 3 mL of deionised water. Before extraction, 200 mL of WW sample was supplemented with 20 μL of 0.5 M Na₂EDTA solution and 100 μL of acetic acid in order to adjust the sample pH to 3. The samples were loaded on columns at the approximate flow rate of 5 mL/min, and then the cartridges were dried for 30 min under vacuum, followed by elution with 6 mL of MeOH. The eluted fractions were then evaporated to dryness.
under a gentle nitrogen stream in a water bath at 40 °C temperature. The samples were reconstituted in 100 μL of 80:20 water/MeOH (v/v) mixture.

**PP stability tests**

Tests of short-term (1 h) chemical and enzymatic hydrolysis of PPs in the untreated WW samples were performed. For the hydrolysis, 200 mL of filtered WW samples were transferred to Erlenmeyer flasks. For chemical (acidic and basic) hydrolysis the appropriate amounts of hydrochloric acid (19.72 mL) or sodium hydroxide (8.0 g) were added to the flasks containing WW samples, to reach the final HCl or NaOH concentrations of 1 M in the solutions. The flasks were swirled vigorously and then the hydrolysis was performed for 1 h at 70 °C in both cases. For enzymatic hydrolysis, β-glucuronidase (final concentration 200 U/mL) and the appropriate amounts of acetic acid (1.15 mL) and sodium acetate (1.64 g) were added to 200 mL of WW samples to reach the final acetate buffer concentration of 1 M and at pH 4.5 in the solution. The mixture was swirled and maintained for 1 h at 37 °C. Three replicates of WW samples were subjected to hydrolysis under each of the conditions. The acidity of all mixtures was set to pH 3 by adding the appropriate amount of sodium hydroxide or acetic acid, respectively. The described procedure for sample preparation for the analysis of PP levels was further used for all WW samples.

**Instrumentation**

HPLC analysis of WW samples was performed using a Thermo Scientific Accela 1250 system (San Jose, CA, USA). Chromatographic separation was achieved on a Kinetex C18 analytical column (100 × 2.1 mm, 2.6 μm) from Phenomenex (Torrance, CA, USA). The mobile phase consisted of 0.1% formic acid in water (mobile phase A) and 100% MeOH (mobile phase B). A gradient program was used: 20% of mobile phase B was used from 0 to 1.0 min, 20% B to 95% B from 1.0 to 5.0 min, maintained at 95% B from 5.0 to 7.0 min, then decreased back to 20% B from 7.0 to 7.1 min and finally the column was re-equilibrated with 20% B from 7.0 to 10 min. A 5 μL aliquot of the sample was injected. The column and autosampler temperatures were maintained at 40 °C and 4 °C, respectively.

A Q Exactive™ Orbitrap-HRMS (Thermo Fisher Scientific) detector was used to quantify the levels of PPs in the analysed WW samples. The Q-Orbitrap-HRMS system was equipped with a heated electrospray (HESI II) ionisation interface operated in the positive and negative modes at the ionisation potential of 2.8 V. The heater and capillary temperatures were maintained at 300 °C and 250 °C, respectively. The following optimised parameters were applied for the gas flow: sheath gas (N₂) 40 arbitrary units (arb), auxiliary gas (N₂) 10 (arb), and S-Lens RF level at 50 (arb). The automatic gain control (AGC) target value was set to
$3 \times 10^6$, the maximum injection time (IT) was set to 200 ms, and the number of micro-scans to be performed was set at 1 scan/s. The Q-Orbitrap-HRMS was operated in full scan mode ($m/z$ 125 to 800) at a mass resolving power of 70,000 FWHM. The targeted MS/MS analysis was performed using a mass inclusion list containing the product ion mass, collision energies, and the expected RTs of analytes. The Orbitrap spectrometer was operated both in positive and negative ion modes at 17,500 FWHM. The AGC target value target was set to $2 \times 10^5$, the maximum IT was set to 50 ms. The isolation window of the quadrupole for precursors was set at $m/z$ 2. Collision energies were optimised for each target compound by infusing the working mix standard solution at a concentration of 10 ng/μL. The mass tolerance window was set to 5 ppm. The instrument performance and data processing were controlled by Thermo Fisher Xcalibur™ and TraceFinder™ software (Thermo Fisher Scientific).

**Performance of the method**

The performance of the method was evaluated through the estimation of linearity, accuracy expressed as percentage of recovery during the extraction, precision expressed as relative standard deviation, and selectivity. The method was found to be selective by verifying the absence of an analytical signal at the RT for the analyte in deionised water. The method showed good linearity, with the determination coefficients higher than 0.992, evaluated at five matrix-matched calibration points over the 1–100 ng/L range for all compounds included in the study. The accuracy and precision were evaluated by spiking WW samples at 5, 40, and 80 ng/L for five replicates at each level over 3 days. The mean variation of coefficients for repeatability of the method ranged from 79% for gemfibrozil to 133% for trimethoprim. The limit of quantification (LOQ) was determined as the minimum detectable concentration of analyte with signal-to-noise (S/N) ratio exceeding 10 by sequential injection of spiked samples at decreasing concentrations. The LOQ was experimentally determined as the lowest amount or concentration of an analyte in the sample for which the S/N ratio exceeded 10. The obtained LOQ values were 10 pg/L for carbamazepine, clarithromycin, naproxen, pravastatin, propranolol, trimethoprim, and xylazine; 50 pg/L for losartan, metoprolol, sulfamethoxazole; 100 pg/L for acetaminophen, caffeine, diclofenac, erythromycin, gemfibrozil, ketoprofen, valsartan; 500 pg/L for atenolol, atorvastatin, fluoxetine, ibuprofen, simvastatin; and 1,000 pg/L for azithromycin and ciprofloxacin.

**Ecotoxicological evaluation**

WW samples were tested for their toxicity by using the Daphtoxkit *F magna* freshwater crustacean toxicity screening test (MicroBioTests Inc., Belgium). The 48 h EC$_{50}$ bioassay was performed with neonates, uniform in size and age, hatched from ephippia in pre-aerated water for 72 h, at 22°C under continuous illumination of ≥6,000 lux. A dilution series of 100%, 50%, 25, and 12.5% of the WW samples was prepared by serial 1:1 dilution with water. In addition, a quality control test with potassium dichromate as the reference toxicant (five serial dilutions: 0.124, 0.248, 0.495, 0.991, and 1.982 mg/L) was performed. For providing the neonates from ephippia with food prior to the test, a 2 h pre-feeding step was performed with a suspension of *Spirulina* microalgae. Each test concentration, as well as the control, was assayed in four replicates, with five neonates per well. The multi-wells were incubated in darkness at 22°C. After 48 h incubation, the number of dead and immobilised neonates, versus that of the actively swimming test organisms, in each well was recorded.

**RESULTS AND DISCUSSION**

**Characterisation of pharmaceutical wastes in untreated WWs**

Initially, the elaborated HPLC-Q-Orbitrap-HRMS method was applied to determine the concentration levels of PPs in the untreated WWs. The residues of 19 compounds from the total of 24 analysed PPs were found at various concentrations in untreated WW samples. Only five of the analysed compounds, namely, gemfibrozil, pravastatin, simvastatin, fluoxetine, and propranolol, were not detected in the untreated WW samples. The determined levels of PPs in the analysed samples are shown in Figure 2. Caffeine, acetaminophen and the sole determined cholesterol-lowering agent – atorvastatin – were found at notably high concentrations compared to other PPs analysed in the WWs. The concentrations for caffeine, acetaminophen, and atorvastatin were in the range of 7,573–11,403, 809–1,883, and 34–163 ng/L, respectively.

Among the determined four nonsteroidal anti-inflammatory agents (NSAIDs), ibuprofen with the concentration range of 22.5–62.5 ng/L was the major compound.
responsible for 80% of the contamination from this class of pharmaceuticals. The other three NSAIDs were found at rather low levels ranging between 0.7 and 5.9 ng/L.

The results also indicated relatively high concentrations of ciprofloxacin (35.6–64.4 ng/L) and three other compounds – metoprolol, valsartan, and xylazine (concentrations of those PPs ranged between 13.6 and 42.3 ng/L). Other PPs found in the untreated WW samples were determined at levels below 20 ng/L. Our findings were comparable to the typical PP levels determined by other authors in samples from similar WWTPs (da Silva et al. 2013; Pereira et al. 2015).

Evaluation of the PP fate and accumulation at the WWTP

Samples collected from three technological treatment stage-points, shown in Figure 1, were analysed in order to determine the degradation rate of PPs. Equation (1) was used to estimate the extent of pharmaceutical degradation:

\[
\text{Extent of PP degradation(\%)} = \left(\frac{C_1 - C_2}{C_1}\right) \times 100, \quad (1)
\]

where \(C_1\) is the concentration of PPs in the untreated WW samples and \(C_2\) is the concentration of PPs in the treated WW samples.

The results shown in Figure 3 indicate a slight increase of PP concentrations in the WWs after the primary treatment stage. Depending on the vapour pressure, pH of the sludge, environment physicochemical behaviour (solubility in water, lipophilicity), and the biodegradation characteristics of PPs, the levels of contaminants increased by 1.4 to 2.6 times with the exception for ciprofloxacin. A notable increase by 6.4 times was observed for ibuprofen. It can be related to the reduction of particle size of biological waste materials, resulting in increase of surface area and facilitating the enzymatic and chemical reactions (oxidation, hydrolysis, etc.) occurring in WWs during the treatment processes (Morais et al. 2014).

The secondary biological treatment in sedimentation ponds is a process stage at the Daugavgriva WWTP, suggested to provide the biodegradation of contaminants including pharmaceuticals. Due to the rapid biodegradation of waste particles, the concentrations of PPs adsorbed on the biogenic matter are released from the solid matter into water in their active forms, thus influencing a pseudo effect of raised contents of determined PP concentrations in waste samples during the treatment stages. The proposed effect was determined by other studies discussed in the recent review by Goel (2015). Other studies also noted metabolite enzymatic reactions affecting suppression of biological agents, especially by antibiotics as noted by several authors (Joss et al. 2006; Caracciolo et al. 2015).

The concentrations of PPs in the samples after the biological treatment stage are in agreement with those investigated by other authors indicating that the degradation was not sufficiently effective. Compared to the elevated levels determined after the primary treatment stage, the levels of only four compounds (caffeine, acetaminophen, atenolol, and naproxen) decreased notably after the biological ponds – by 99.8, 99.7, 88.6, and 88.0%, respectively. The results also indicated a substantial degradation rate (>60%) for ibuprofen and valsartan; however, the content of ibuprofen remained above the concentration determined in the untreated WWs. As noted by other authors, macrolide antibiotics and diclofenac showed the most notable increase of concentration. For example, the degradation efficiency in biological ponds in comparison

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**Figure 2** | Boxplot graphs of PP levels found in untreated WW samples collected at the Daugavgriva WWTP during the described collection period.

**Figure 3** | Differences of medium PP concentration levels in WW samples collected before and after the primary (sedimentation) and the secondary (biological pond basins) treatment stages.
to the levels gained by primary treatment for most antibiotics was negative, and ranged between $-1.298\%$ (azithromycin) and $-43\%$ (ciprofloxacin).

Water samples after the tertiary treatment stage in the sedimentation basin were not separately analysed. The PP concentrations determined in the samples collected directly after the biological treatment and in the final effluents indicated a low degradation efficiency for most of the determined PPs, with the exception of ciprofloxacin, naproxen, caffeine, and ibuprofen – the average levels of those pharmaceuticals in treated WWs were reduced by 1.9, 2.0, 3.4, and 3.9 times, compared to the levels determined in the samples prior to biological treatment.

As shown in Figure 4, the overall results indicated a sufficient degradation ($55.0$–$99.9\%$) only for five small molecular PPs (caffeine, acetaminophen, naproxen, atenolol, and ibuprofen).

For other compounds the WW treatment was insufficient and even elevated contents of PPs were observed after the treatment. For example, the content of azithromycin increased by more than 22 times, the content of diclofenac and two other macrolide antibiotics (clarithromycin and erythromycin) increased by more than 10 times, and a two- to five-fold increase in concentration levels of the other eight compounds was noted.

**Comparison of WW toxicity for *Daphnia magna* at different treatment stages**

Despite the low efficiency of WWTP in relation to the removal of PPs residues, a gradual decrease of toxicity level from the untreated WWs to the primary and secondary treatment stages was detected, as is shown in Figure 5, i.e., from the average of $39.7\%$ down to $26.8\%$ and $4.9\%$, respectively. These data are in a good agreement with those previously reported (Freitas et al. 2016). For example, the initial untreated WWs containing PPs showed an inhibitory effect of $20\%$ for *D. magna* during an earlier study (Freitas et al. 2016).

The toxicity of paired PPs for daphnids was found to be concentration-additive or less than concentration-additive. Hence, the concentration addition is considered as a reasonable worst-case estimation of the environmental impact of pharmaceutical mixtures.

**Hydrolysis of PPs in WW samples**

The hydrolysis tests of PPs in the untreated WWs were performed for evaluation of the potential factors leading to insufficient degradation for most PPs in WWs. Aerobic and anaerobic biodegradation, chemical and enzymatic hydrolysis, oxidation, and photolysis are the major mechanisms that determine the degradation of PPs and other wastes during the treatment at WWTPs.

Depending on the change in process conditions, hydrolytic degradation may determine the fate of PPs in the WW.

The hydrolytic treatment tests with WW samples containing 19 PPs were performed at three different conditions, acidic, basic, and enzymatic, while the differences of short-term PP degradation efficiency were evaluated. The testing period of 1 h was selected, in agreement with the previous studies (Remberger et al. 2009) and taking into account the predictable processing time of WWs during the primary and secondary treatment stages at WWTPs.
The previous studies of hydrolytic mechanisms during the degradation of pharmaceuticals have shown effective hydrolysis of macrolide antibiotics under acidic conditions (Mitchell et al. 2013). The percentage extents of PP degradation after a short-term hydrolysis under acidic, basic and enzymatic conditions are given in Figure 6.

The obtained results are in agreement with the previous studies, indicating complete degradation of such antibiotics as azithromycin, clarithromycin, erythromycin, and other class PPs (diclofenac, atorvastatin, and a limited degradation of acetyaminophen), while the concentration levels of active forms for other PPs, especially ciprofloxacin, naproxen, and two antimicrobial agents (sulfamethoxazole and trimethoprim), showed a notable increase in their levels due to rise of their active forms determined by HRMS detection (Figure 6).

The results obtained under basic hydrolysis conditions also showed a similarly high efficiency of macrolide degradation. For atenolol, ibuprofen, acetyaminophen, atorvastatin, and caffeine the degradation efficiency exceeded 63%. For other PPs (53% of all the tested compounds) insufficient degradation was observed during basic hydrolysis, especially for ciprofloxacin, trimethoprim, and sulfamethoxazole, as well as naproxen.

It should be noted that the chemical behaviour of the compounds is the determining factor during chemical degradation. The results for PPs degradation efficiency during brief enzymatic hydrolysis of the untreated WWs showed a major difference in the hydrolytic mechanism compared to that under the chemical treatment.

Similar, to chemical hydrolysis, under acidic hydrolysis the most notable lack of efficient degradation was in the case of ciprofloxacin, as well as for trimethoprim and sulfamethoxazole. Also the macrolide class compounds were not efficiently degraded. The results of all investigations indicated insufficient hydrolysis of ciprofloxacin, trimethoprim, and sulfamethoxazole, indicating the need for other degradation mechanisms of those compounds. The results of recent studies have indicated oxidative and photodegradation as most appropriate techniques to reduce the levels of those PPs.

The results of short enzymatic hydrolysis confirmed the outcomes obtained for real WWTP influents, indicating that the macrolide antibiotics as well as trimethoprim and sulfamethoxazole were relatively stable against enzymatic degradation. As these conditions at a mild level may be influenced by enzymatic reactions, formed within the degradation of biological wastes, the results confirmed the potential factors causing rise of pharmaceutical levels within the treatment processes. The results of enzymatic hydrolysis indicate only nine compounds (47% of all compounds) with degradation extent above 55%, whereas the majority of those compounds have been previously characterized with sufficient or at least a positive rate of pharmaceutical degradation under real conditions (Figure 4).

The exception was naproxen, for which only 41% degradation rate was found in the tests, while a two times higher level of degradation was observed at a WWTP.

CONCLUSIONS

Within this study, a reliable and highly sensitive HPLC-Q-Orbitrap-HRMS analytical method was applied to determine the presence of 24 emerging pharmaceuticals in WW samples. The sampling was performed at the Daugavgriva WWTP located in Riga, Latvia. The samples were collected at four major WW treatment stages, including the untreated WW, samples after the primary and secondary treatment, and the discharged effluents. The obtained results indicated a complex mechanism of PP transformations and partial degradation during the treatment stages at the assessed WWTP. The presented study indicated that 79% of PPs had insufficient degradation during treatment, thus raising issues of the impact on the contamination of surface waters. The effects were probably connected with the destruction of organic materials on which PPs were adsorbed.
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REFERENCES


Sim, W. J., Kim, H. Y., Choi, S. D., Kwon, J. H. & Oh, J. E. 2013 Evaluation of pharmaceuticals and personal care products...


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