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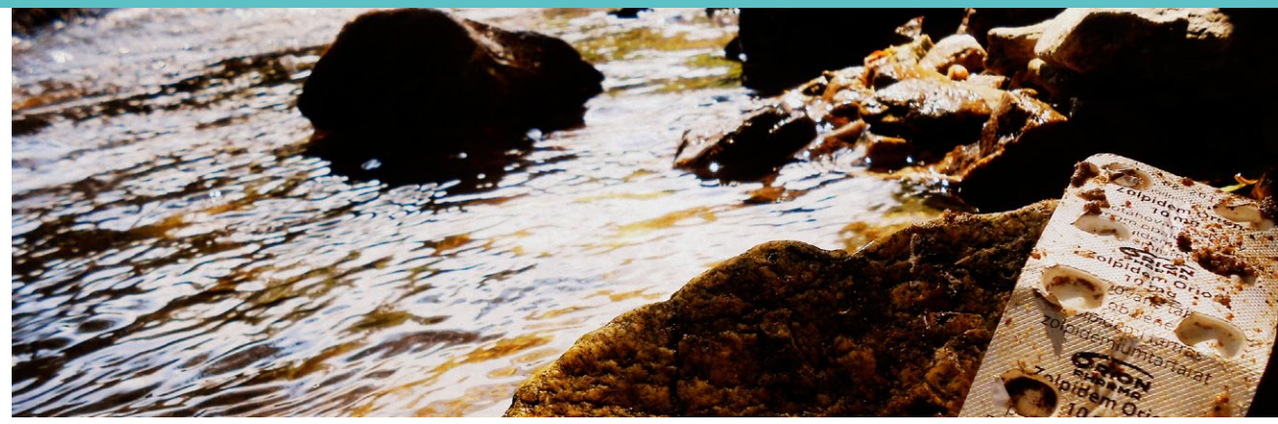

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Oksana Golovko

Czech Republic, Vodňany, 2014

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CONTENT

CHAPTER 1

GENERAL INTRODUCTION

1.1. DETERMINATION OF PHARMACEUTICALS AND PERSONAL CARE PRODUCTS IN AN AQUATIC ENVIRONMENT BY LIQUID CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY

The rapid development of highly sensitive and automated analytical instrumentation in the latter part of the 20th century has meant that now, in the 21st century, we can find a large number of substances in the aquatic environment that were previously undetectable. There is an urgent need to adopt fast and sensitive analytical protocols that can screen, detect, and quantify a diverse range of analytes concurrently. This becomes necessary because the analytical equipment, including liquid chromatography combined with mass spectrometry (LC-MS) or tandem mass spectrometry (LC-MS^2 or LC-MS/MS), and gas chromatography coupled with mass spectrometry (GC-MS) has allowed detection of extremely low concentrations (ng L^{-1}) of these compounds in different complex matrices in liquid and solid states (Petrovic et al, 2014; Jelic et al., 2011; Jones et al., 2007; Kasprzyk-Hordern et al., 2007; Lindberg et al., 2006; Fedorova et al., 2014).

LC-MS and LC-MS/MS are popular techniques currently being used in pharmaceutical analyses. Several published reviews have discussed various methods for analyzing pharmaceutical compounds found in water resources (de Alda and Barcelo, 2001; Richardson, 2010). Because most pharmaceuticals are present in surface waters at low concentrations, an extraction process (e.g., solid phase extraction) is often needed to concentrate target pharmaceutical compounds for analysis.

These techniques have been used to detect and quantify around 3000 biologically active chemical compounds in the environment (Richardson, 2006), prompting numerous analytical chemistry studies aimed at optimizing and validating methods to prepare environmental samples for subsequent analysis (Grabic et al., 2012; Fedorova et al., 2014; Khan et al., 2012).

1.2. OCCURRENCE OF PHARMACEUTICALS AND PERSONAL CARE PRODUCTS IN WATER ENVIRONMENT

Pharmaceuticals and personal care products (PPCPs) are a class of so-called "emerging" contaminants that have raised great concern in the last few years. This rising concern is not only concomitant of the widespread and growing use of human PPCPs and veterinary pharmaceuticals, but it is also a consequence of improvements in the analytical techniques that enable detection of such substances at trace levels.

PPCPs include many pharmaceutical groups such as antibiotics (ATB), anti-diabetics, anti-epileptics, anti-hypertensives, anti-anxiety medications, etc. Personal care products include bactericides, synthetic fragrances, ultraviolet (UV) screens, etc. Hundreds of thousands of tons of PPCPs are dispensed and consumed annually worldwide. A large quantity of pharmaceutical by-products is continuously released into the environment, albeit at low concentrations (Kolpin et al., 2002).

The occurrence of PPCPs in the aquatic environment has been recently investigated (Fick et al., 2009; Fedorova et al., 2014; Hedgespeth et al., 2012; Osorio et al., 2012; Petrovic et al., 2014). It reaches the aquatic environment principally because of insufficient removal in wastewater treatment plants (WWTPs), the source of 70–80% of the PPCPs present in the aquatic environment. The remaining 20–30% originates from other sources of pollution such as livestock and industrial wastes and improper or illegal disposal of unused or expired pharmaceuticals (Fent et al., 2006). Quite a large proportion of PPCPs have occurred in

underground, surface, and drinking water, as well as municipal sewage sludge across the globe (Focazio et al., 2008; Fick et al., 2009; Benotti and Snyder 2009; Wu et al., 2009).

Once released into the sewage system, PPCPs pass through WWTPs and enter water systems. In water environments, a large variety of these compounds and their metabolites have been detected (Jones et al., 2005; Miao et al., 2002). PPCPs produce a complex mixture of compounds that could have synergetic effects. Some of these compounds are more bioactive than their metabolic precursors. Some pharmaceuticals used in veterinary medicine are excreted directly into the environment without passing through a WWTP, making their control and follow-up much more challenging. The soil can act as a major source of water contamination, because most of these compounds and their metabolites are soluble in water, and they are excreted via urine and feces (Halling-Sorensen, 2001). Pharmaceuticals used in fish-farming are directly released into surface water (Halling-Sorensen et al., 1998). Assessment of contamination levels and potential novel contaminants must address different aspects of this environmental problem. Identification and determination of PPCPs metabolites at environmentally relevant levels is important for:

- understanding of their metabolism and excretion patterns;
- evaluating the efficiency of wastewater treatments in the removal of these pollutants;
- comprehending their dispersion, mobility and persistence under environmental conditions (biotic and abiotic degradability); and
- evaluating uptake and effects in non-target organisms.

The final point is very important because PPCPs or their metabolites could affect living organisms at very low concentrations (Baker and Kasprzyk-Hordern 2011).

Nevertheless, there remains little knowledge of the concentration, fate, and effects of PPCPs in the environment.

1.3. WASTEWATER TREATMENT PLANT AS AN IMPORTANT SOURCE OF PHARMACEUTICALS AND PERSONAL CARE PRODUCTS IN WATER

Municipal and hospital WWTPs are important sources of PPCPs. They are the last barriers before the organic pollutants are released into the aquatic environment. Municipal WWTPs are designed to remove or control a range of substances and parameters, such as particulates, organic carbon, nutrients, and pathogens. While these substances can be efficiently and consistently eliminated, the removal of PPCPs is often insufficient. The concentrations of the PPCPs in municipal WWTPs generally vary in the ng L^{-1} to µg L^{-1} range (Bueno et al., 2012; Gao et al., 2012; Koeck-Schulmeyer et al., 2013; Vieno et al., 2007). Hence, evaluating the fate and removal of organic compounds during wastewater treatment is imperative for the optimization of treatment processes, and to prevent the release of these potentially harmful pollutants.

The treatment processes typically consist of preliminary treatment (e.g., screening and grit removal), primary treatment (e.g., fine screening and sedimentation), and secondary treatment (biological treatment). Tertiary treatment (ozonation, UV treatment, and disinfection) is applied to further improve the purity of the effluent. Treatment plants differ primarily in design of the biological treatment unit. The most common secondary treatment in WWTPs is an activated sludge process, either with or without a nitrification/denitrification unit to enhance nitrogen removal. The elimination of PPCPs in WWTPs is affected by several factors, such as sewage temperature, configuration of the treatment process, and operational parameters of the WWTP (e.g., hydraulic and solid retention times). The temperature of the raw sewage has a major influence on the elimination of biodegradable pharmaceuticals in WWTPs (Castiglioni et al., 2006; Golovko et al., 2014).

1.4. FATE OF PHARMACEUTICALS AND PERSONAL CARE PRODUCTS IN WASTEWATER TREATMENT PLANTS

After excretion, pharmaceuticals enter WWTPs where they are affected by different treatment processes. During wastewater treatment, pharmaceuticals can be removed through microbial degradation or sorption to solids which are later removed with the sludge. The distribution and fate of pharmaceuticals are dependent on the physico-chemical properties of the PPCPs (Carlsson et al., 2006; Jones-Lepp and Stevens, 2007). Characteristics of the environment such as climate and type of soil also influence the fate and behavior of pharmaceuticals (Debska et al., 2004; Naddeo et al., 2009).

A primary elimination mechanism in WWTPs for the elimination of PPCPs is sorption followed by elimination through sludge separation and biodegradation. Photodegradation can also be significant in WWTPs but occur mainly under natural conditions in surface water.

1.4.1. Sorption

Most PPCPs are polar and hydrophilic. Generally, the more hydrophilic chemical substances are present in higher amounts in the aqueous phase versus the solid phase. The ability of PPCPs to sorb to sludge and solids is described by a sorption constant (K_d) and octanol/water partition coefficients (K_{ow}). Higher values of K_{ow} indicate a greater ability of the compound to sorb to matter containing organic carbon as a suspended solid, non-polar fats and lipids, mineral oils, greases, and surfactants. Lower values of K_{ow} indicate that PPCPs easily leach from soils, sludges, or sediments. The following is used as a reference for the ability of PPCPs to be adsorbed to sediment or sludge:

1) when $\log K_{ow} < 2.5$ indicates low sorption potential;
2) when $2.5 < \log K_{ow} < 4.0$ indicates medium sorption potential; and
3) when $\log K_{ow} > 4.0$ indicates high sorption ability (Jones-Lepp and Stevens, 2007; Caliman and Gavrilescu, 2009; Rogers, 1996).

In activated sludge processes, K_d is defined as the partition of a compound between the sludge and the water phase. Higher K_d indicates that more of the compound is sorbed. Sorption to sludge is a relevant elimination process for compounds when $K_d > 300\,L\,kg^{-1}$ (i.e., $\log K_d > 2.48$). Horsing et. al. (2011) found that 13 pharmaceuticals had K_d values larger than $1.2 \times 10^6\,L\,kg^{-1}$ (Horsing et al., 2011). In another study, ciprofloxacin, norfloxacin, sulfamethoxazole, and diclofenac were found to have K_d values above the threshold. All were detected in primary, secondary, and/or digested sludge, though mainly at concentrations lower than those in the aqueous sewage (Lindberg et al., 2005; Gobel et al., 2005; Lindberg et al., 2006; Ternes et al., 2004a). Only fluoroquinolones were found to readily sorb to sewage sludge. It was shown that they were typically eliminated by adsorption (> 80% in WWTPs) via electrostatic interactions between the positively charged amino groups in the compounds and the negatively charged surfaces of the microorganisms (Ternes et al., 2004b; Golet et al., 2003).

In general, the compounds that tend to be sorbed onto solids are expected to be better eliminated by activated sludge treatment than other low-cost secondary treatments such as trickling filter beds, anaerobic lagoons, and constructed wet lands (Camacho-Munoz et al., 2012). Both K_{ow} and K_d have been proposed as relatively accurate indicators of sorption behavior (Ternes et al., 2004a; Joss et al., 2005; Horsing et al., 2011).

1.4.2. Biodegradation

During secondary treatment, organic pollutants are biologically degraded at different levels, resulting in mineralization or incomplete degradation (production of by-products). Different biodegradation mechanisms of PPCPs can appear during the treatment process:

1) Single substrate growth of a small subset of specialist oligotrophic organisms, which is less common in WWTPs and more likely, occurs in the receiving water or sediment (Daughton and Ternes, 1999).
2) Co-metabolism, where micropollutants are decomposed by enzymes generated for other primary substation degradations (e.g., ammonia monooxygenase), and they are not used as carbon or energy sources for microbial growth. Because of the low concentrations of PPCPs compared to other organic constituents in sewage, co-metabolization is the more plausible mechanism (Jones et al., 2007; Ternes et al., 2004b).
3) Mixed substrate growth, in which organic pollutants are used as carbon and energy sources, and become mineralized (Vader et al., 2000).

High biodegradability variation was found among pharmaceuticals, even when the compounds fell into the same therapeutic group. For example, Salgado et al. (2012) reported that, among non-steroidal anti-inflammatory drugs (NSAIDs), diclofenac exhibited low (25%) biodegradation, whereas ibuprofen and ketoprofen were biodegraded to a much higher extent (75%) (Salgado et al., 2012).

Negative removal efficiency, where the effluent concentrations of some compounds exceeded their influent concentrations, occurred in WWTPs for some PPCPs (Jelic et al., 2011; Gracia-Lor et al., 2012). Proposed explanations were:

1) That 24-hour composite samples might not be an accurate sampling method (Ort et al., 2010).
2) Conjugated compounds formed by the PPCPs are undetected at the influent and then are retransformed into the detectable parent compound by biological processes (Salgado et al., 2012; Gobel et al., 2007; Kasprzyk-Hordern et al., 2009).
3) Changes in the adsorption behavior are caused by the treatment process, influencing the ratio between influent/effluent water (Bueno et al., 2012; Lindberg et al., 2005).
4) Fluctuations occur during the sampling period or the compounds desorb from sludge (Clara et al., 2004; Koeck-Schulmeyer et al., 2013).

The structure of PPCPs is the factor that determines their resistance to biodegradation. The biodegradability of a compound relies mostly on the complexity of the compound (e.g., monocyclic or polycyclic) and its functional groups (e.g., halogen groups). However, there is no obvious relationship between chemical structure, functional groups, and removal for some pharmaceuticals. For example, two structurally similar compounds, ibuprofen and ketoprofen, are removed differently; ibuprofen is eliminated more efficiently (Camacho-Munoz et al., 2012).

1.5. THE FATE OF PHARMACEUTICALS AND PERSONAL CARE PRODUCTS IN SURFACE WATER

After treatment, effluents are discharged to the surface water. Treatment plant effluents are the main source of PPCPs in the environment. Because of dilution, sorption, biodegradation, and phototransformation, their concentrations are typically lower in surface waters than in WWTP effluents. Phototransformation reactions are suggested to have an important role in the elimination of some of these organic pollutants from surface waters (Doll and Frimmel, 2003; Latch et al., 2003; Tixier et al., 2003). Many of these compounds contain aromatic rings, heteroatoms, and other functional chromophore groups that can either absorb solar

radiation or react with photogenerated transient species in natural waters such as reactive oxygen species and photo-excited natural organic matter (Boreen et al., 2003; Fatta-Kassinos et al., 2011).

Organic pollutants undergo direct and indirect photolysis. Absorption of solar light causes direct photolysis; solar energy breaks up the molecule. Indirect photolysis involves naturally occurring molecules such as nitrates, which generate strong reactive species such as singlet oxygen, hydroxyl radicals, or alkyl peroxyl radicals, and solvated electrons. The solvated electrons, generated by irradiation of various aquatic components such as dissolved organic material (DOM), NO^{3-}, or Fe^{3+} (Andreozzi et al., 2003) might be important in the removal of PPCPs. Indirect photolysis can play an important role in the degradation of pollutants that poorly absorb solar radiation or that resist direct photolysis (Fatta-Kassinos et al., 2011; Monteiro and Boxall 2010; Lam and Mabury, 2005).

Photodegradation of pharmaceuticals has been investigated in a vast number of studies (Andreozzi et al., 2003; Boreen et al., 2003; Doll and Frimmel, 2003; Piram et al., 2008; Vargas et al., 2003; West and Rowland, 2012; Calisto et al., 2011; Chen et al., 2012; Canudas and Contreras, 2002; Yan ett al., 2009; Lam and Mabury, 2005). However, we continue to lack relevant data on the photolysis and photochemical half-lives of many pharmaceuticals. This information could improve our understanding of their environmental fate.

1.6. AIMS OF THE PRESENT STUDY

The main objective of this study is to investigate the fate and stability of PPCPs in aquatic environments. To fulfill this objective, experiments were planned to accomplish these specific targets:

1) to assess the effect of storing wastewater samples on the concentrations of PPCPs to assure the quality of obtained analytical results,
2) to investigate seasonal changes in the concentration of pharmaceuticals in a WWTP over 12 months,
3) to assess the seasonal variability of the removal efficiencies (REs) of PPCPs in a WWTP over one year,
4) to investigate the photo fate of PPCPs under UV and sunlight exposure in buffered purified water and surface river water, and
5) to study the decomposition of resistant compounds by an oxidation system based on a combination of Atmospheric Plasma Discharge/Photocatalytic active material (APD/TiO_2 photocatalyst).

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CHAPTER 2

STORAGE EFFECT ON THE ANALYSIS OF PHARMACEUTICALS AND PERSONAL CARE PRODUCTS IN WASTEWATER

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Storage effect on the analysis of pharmaceuticals and personal care products in wastewater

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HIGHLIGHTS

- The stability of 124 PPCPs in wastewater during storage at –18 °C was tested.
- 46%/37% of target analytes (effluent/influent) remained stable for 120 days.
- 12% of pharmaceuticals were lost after freezing/thawing cycle.
- Stability of target analytes varied for effluent and influent samples.
- 17% of compounds showed declining temporal pattern.

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ABSTRACT

In this study, the stability of 124 target analytes in influent and effluent wastewater samples during short-term (4 °C) and long-term (−18 °C) storage was assessed. The most common storage scenario was considered, in which samples were frozen immediately after sampling without any pre-treatment. During short-term storage more analytes remained stable (concentration during storage was in the range of 60–120% of the initial concentration) at 4 °C than at −18 °C. During long-term storage (−18 °C), three types of behavior were observed: constant concentrations throughout the experimental period, decreasing concentrations with time, and loss of the compound from the sample after freezing. Differences between effluent and influent samples were observed for 50 out of 124 tested PPCPs. The amount of stable analytes decreased with time during long-term storage. 72% and 56% of the target compounds in the effluent and influent wastewater, respectively, remained stable during 60 days of storage. The number of stable compounds decreased to 57 (46%) and 46 (37%) in the effluent and influent, respectively, over 120 days. 15 Pharmaceuticals were lost after freezing/thawing cycle. The results stress the importance of storage factors during analysis of pharmaceuticals in wastewater. The stability of target compounds in the samples under the planned storage conditions should be checked before starting the experiment to obtain reliable data.

1. Introduction

Pharmaceuticals and personal care products (PPCPs) are primarily introduced to the water environment via anthropogenic sources. Since PPCPs are compounds of medium to high polarity, they end up in the water compartment after being used. Furthermore, many pharmaceuticals have been designed to stay active for long periods of time to fulfill their therapeutic function; thus, they can persist in the environment, remaining active and affecting aquatic life. Some studies have already shown negative effects of PPCPs on aquatic ecosystems in general, as well as in certain aquatic species. Indeed, endocrine disruption, effects on reproductive function, and toxicity to aquatic organisms have been documented (Crofton et al., 2007; Ricart et al., 2010; Kvarnryd et al., 2011). Antibiotics are also of great concern owing to their contribution to the development of antibiotic-resistant bacteria (Rhodes et al., 2000; Heuer et al., 2009).

Wastewater treatment plants (WWTPs) are considered to be the main source of PPCPs in the environment (Daughton and Ternes, 1999). Specifically, these compounds are detected in WWTP effluent, after which they enter surface water (Reemtsma et al., 2006; Watkinson et al., 2009). Concentrations detected in wastewater effluent range from low ng L^{-1} to several μg L^{-1} (Roberts and Thomas, 2006; Bueno et al., 2012). Thus, the removal of these

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emerging pollutants during the treatment process should be thoroughly investigated. There is also increasing interest in wastewater monitoring to enable further risk assessment for aquatic organisms.

Such PPCPs monitoring is already challenging owing to the low concentrations, complex matrices and wide range of compounds with broad physical–chemical properties. Accordingly, simple, reliable and rapid methods are needed to enable fast, sensitive, and selective determination of these emerging pollutants. Significant efforts have been made in this field, resulting in different analytical procedures (Salvador et al., 2007; Segura et al., 2007; Khan et al., 2012). One of the proposed methods represents a new trend in environmental water analysis: in-line solid-phase extraction–liquid chromatography–tandem mass spectrometry (SPE-LC-MS/MS) analysis, which allows pre-concentration and analysis of the sample in a single run, making large volume samples and time-consuming SPE extraction procedures unnecessary. However, since most of these methods are focused on one or two pharmaceutical classes, some improvements should be introduced.

Additionally, very little attention has been paid to the sampling methods and storage of the samples, despite the great importance of these activities (Madrid and Zayas, 2007). Nevertheless, only a few studies have evaluated the effects of storage and preservation conditions on the data obtained (Baker and Kasprzyk-Hordern, 2011; Hillebrand et al., 2013). Currently, there is no uniform procedure for the storage and pre-treatment of samples before analysis. Most studies dealing with wastewater analysis report the preservation of samples by freezing without any pre-treatment (Xu et al., 2007; Bijlsma et al., 2009; Loganathan et al., 2009; Hernandez et al., 2011; Bijlsma et al., 2012), while others subject the samples to pH adjustment (Gheorghe et al., 2008; Nurmi and Pellinen, 2011; Chen et al., 2012). Some studies have also reported storing of wastewater in a refrigerator before analysis (Berset et al., 2010; Salem et al., 2012).

Freezing of samples without any treatment is the most common method of storage because it avoids complications during field sampling (e.g. acidification or filtering of samples). Freezing also enables storage for a long time, which is beneficial to long-term monitoring programs, as it is impossible to keep wastewater in the refrigerator for more than a few days because PPCPs are bioactive and hence susceptible to breakdown by bacteria or other transformation reactions (Castiglioni et al., 2011).

Therefore, we conducted this study to investigate how freezing affects the original concentrations of such pollutants in wastewater. To accomplish this, samples of untreated and treated wastewater were collected from the influent and effluent of the WWTP in Ceske Budejovice, Czech Republic and analyzed for 124 PPCPs.

2. Materials and methods

2.1. Chemicals and reagents

LC-MS grade methanol and acetonitrile (LiChrosolv Hypergrade) were purchased from Merck (Darmstadt, Germany). Formic acid was purchased from Labicom (Olomouc, Czech Republic). Ultrapure water was obtained from an Aqua-MAX-Ultra System (Younglin, Kyounggi-do, Korea). A detailed description of pharmaceutical standards has been provided by Grabic et al. (2012). Trimethoprim ($^{13}C_3$) and sulfamethoxazole ($^{13}C_6$) were purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). Carbamazepine (D_{10}) and amitriptyline (D_6) were acquired from CDN Isotopes (Pointe-Claire, Quebec, Canada). Stock solutions of all pharmaceuticals were prepared in methanol at a concentration of 1 mg mL^{-1} and stored at −20 °C. A spiking mixture was prepared by diluting stocks in methanol to a final concentration of 1 μg mL^{-1} for each compound, after which it was stored at −20 °C.

2.2. Wastewater samples

Samples of treated and untreated wastewater were collected by grab sampling the effluent and influent, respectively, of the WWTP in Ceske Budejovice. High density polyethylene bottles were used for sampling and storage. Concentrations of target analytes in wastewater ranged from low ng L^{-1} to several μg L^{-1}. Wastewater was spiked with target compounds at a concentration of 1000 ng L^{-1} to obtain a proper response for all analytes. Three sets of samples were frozen and kept at −18 °C for 7 days, 60 days and 120 days before analysis. The samples were filtered through 0.45 μm regenerated cellulose filters (Labicom, Olomouc, Czech Republic) immediately prior to analysis, but not before storage. This was done to simulate the most common situation, in which samples are taken by the staff of the WWTP, frozen, and then transported to the laboratory for analysis. Time 0 corresponds to the initial concentrations of the target compounds measured directly after sampling. A second set of the samples was held at 4 °C for 7 days, after which they were analyzed. All samples were analyzed in triplicate.

2.3. LC/LC–MS/MS conditions

A triple stage quadrupole MS/MS TSQ quantum ultra mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) coupled to an Accela 1250 LC pump (Thermo Fisher Scientific), Accela 600 LC pump (Thermo Fisher Scientific) and an HTS XT-CTC autosampler (CTC Analytics AG, Zwingen, Switzerland) was used for analysis. A Hypersil GOLD phenyl column (50 mm × 2.1 mm ID × 3 μm particles; Thermo Fisher Scientific, San Jose, CA, USA) and a Cogent Bidentate column (50 mm × 2.1 mm ID × 4 μm particles; Microsolv Technology Corporation, Eatontown, NJ, USA) were used for the separation of target analytes.

Heated electrospray (HESI-II) and atmospheric pressure chemical ionization coupled with atmospheric pressure photoionization (APCI/APPI) in positive and negative ion modes were used for ionization of the target compounds. The mass transitions of most of the studied compounds were the same as those described by Grabic et al. (2012). All details pertaining to the analytical method (mass transitions −*m*/*z*, collision energies, and retention times) are presented in Table S1 of the electronic supplementary material (ESM). Optimized parameters for both ion sources are summarized in Table S2 (ESM).

The in-line SPE-LC-MS/MS configuration was the same as that used by Khan et al. (2012). Target compounds were analyzed in three runs (two for HESI with different columns and one for APCI/APPI) to achieve better separation. The LC gradients are given in Tables S3–S5 (ESM).

2.4. Method performance

The method was validated by determining its linearity, repeatability, trueness and limits of quantification (LOQ) at the concentration ranges of interest. All method performance parameters are presented in Table S6 (ESM).

The linearity of the calibration curve was tested in the range 10–2500 ng L^{-1}. A six-point calibration curve was prepared by spiking tap water with the target compounds. A consistent amount of surrogate standard was added (500 ng L^{-1}). The *R*-squared values were satisfactory, ranging from 0.975 to 0.999 (for most of the compounds $r^2 > 0.991$). The calibration curve was measured on each day of analysis at the beginning and end of the sequence to check the instrumental stability.

Table 1
Amount of compounds (%) which remained stable during different storage conditions.

Total number of compounds	Storage conditions							
	7 days				60 days		120 days	
	4 °C		−18 °C		−18 °C		−18 °C	
	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent
124	86	83	78	60	72	56	46	37

Table 2
Stability of PPCPs in waste water samples during long-term storage at −18 °C.

Stable for 120 days[a]		Stable for 60 days[a]		Stable for 7 days[a]		Not stable[a]	
Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent
Atenolol	Atenolol	Alfuzosin	Atorvastatin	Cyproheptadine	Alfuzosin	Amiodarone	Amiodarone
Atorvastatin	Azithromycin	Atracurium	Atracurium	Dipyridamole	Flumequine	Azithromycin	Chlorpromazine
Bezafibrate	Bezafibrate	Budenoside	BP3	Doxycycline	Roxithromycin	Chlorpromazine	Chlorprothixene
Biperiden	Biperiden	BP4	BP4	Furosemide	Sulfadiazine	Chlorprothixene	Clemastine
Bisoprolol	Bisoprolol	Ciprofloxacin	Bupropion	Levofloxacin		Clemastine	Clomipramine
BP1	BP1	Desloratadine	Ciprofloxacin	Ketoprofen		Clomipramine	Clonazepam
BP2	BP2	Dihydroergotamine	Clindamycine	Risperidone		Dicycloverine	Cyproheptadine
BP3	Budenoside	Donepezil	Erythromycin	Sulfamoxol		Duloxetine	Desloratadine
Budenoside	Buprenorphine	Enrofloxacin	Finasteride			Econazole	Dicycloverine
Buprenorphine	Carbamazepine	Erythromycin	Fluconazole			Felodipine	Difloxacin
Bupropion	Chloramphenicol	Flumequine	Norfloxacin			Fenofibrate	Dihydroergotamine
Carbamazepine	Cilazapril	Haloperidol	Oseltamivir_carboxylate			Fulvestrant	Donepezil
Chloramphenicol	Citalopram	Hydroxyzine	PBS			Haloperidol	Doxycycline
Cilazapril	Clarithromycin	Nefazodone	Rosuvastatin			Indometacin	Duloxetine
Citalopram	Diclofenac	Norfloxacin	Sulfadimethoxine			Itraconazole	Econazole
Clarithromycin	Diltiazem	Oxolinic_acid	Sulfamethazine			Levomepromazine	Enoxacin
Clindamycine	Diphenhydramine	Oxytetracycline	Sulfamethizole			Loperamide	Enrofloxacin
Clonazepam	Dipyridamole	PBS	Sulfamethoxazole			Maprotiline	Felodipine
Diclofenac	Disopyramide	Promethazine	Sulfamoxol			Meclozine	Fenofibrate
Difloxacin	Eprosartan	Ropinirole	Sulfaphenazole			Miconazole	Flutamide
Diltiazem	Fexofenadine	Roxithromycin	Sulfathiazole			Paroxetine	Fulvestrant
Diphenhydramine	Flecainide	Sulfadimethoxine	Tramadol			Perphenazine	Glimepiride
Disopyramide	Florfenicol	Sulfamerazine	Trimethoprim			Sertaline	Haloperidol
Enoxacin	Furosemide	Sulfamethazine	Venlafaxine			Sulconazole	Hydroxyzine
Eprosartan	Glibenclamide	Sulfamethizole				Tamoxifen	Indometacin
Fexofenadine	Ibuprofen	Sulfamethoxazole				Terbinafine	Itraconazole
Finasteride	Irbesartan	Sulfaphenazole				Tetracycline	Levofloxacin
Flecainide	Isradipine	Sulfaquinoxaline				Tonalid	Ketoprofen
Florfenicol	Memantine	Sulfathiazole				Triclosan	Levomepromazine
Fluconazole	Metoprolol	Tramadol					Lomefloxacin
Flutamide	Mirtazapine	Trimethoprim					Loperamide
Glibenclamide	Naloxone	Valsartan					Maprotiline
Glimepiride	Naproxen						Meclozine
Ibuprofen	Orphenadrine						Mianserin
Irbesartan	Oseltamivir						Miconazole
Isradipine	Oxazepam						Nefazodone
Lomefloxacin	Ropinirole						Oxolinic acid
Memantine	Sotalol						Oxytetracycline
Metoprolol	Sulfamerazine						Paroxetine
Mianserin	Sulfamethoxypyridazine						Perphenazine
Mirtazapine	Sulfapyridine						Promethazine
Naloxone	Sulfaquinoxaline						Risperidone
Naproxen	Terbutaline						Sertaline
Orphenadrine	Trihexyphenidyl						Sulconazole
Oseltamivir	Valsartan						Sulfasalazine
Oseltamivir carboxylate	Verapamil						Tamoxifen
Oxazepam							Terbinafine
Rosuvastatin							Tetracycline
Sotalol							Tonalid
Sulfadiazine							Triclosan
Sulfamethoxypyridazine							
Sulfapyridine							
Sulfasalazine							
Terbutaline							
Trihexyphenidyl							
Venlafaxine							
Verapamil							

[a] Concentration of those compounds were in the range 60–120% of the initial concentration.

The repeatability was assessed by analysis of 10 replicate wastewater samples (influent and effluent) that had been spiked with the target PPCPs. The RSD for measured replicated samples was lower than 20% for the majority of analytes (Table S6).

Trueness was evaluated based on the concentration ratio of wastewater samples spiked with 2000 ng L^{-1} of the target analytes to the nominal concentration (recovery) after subtraction of the blank wastewater. With this type of instrument configuration, it is difficult to define recovery of the target analyte separately from the matrix effect when the extraction and pre-concentration of the sample is carried out on-line. Matrix effects were assessed for each compound, and corrections for ion suppression or enhancement were conducted using matrix-matched standards. Matrix matched standards were prepared from sampled wastewater by spiking with both internal standard (IS) and native compounds at 500 ng L^{-1} and 5000 ng L^{-1} respectively. The peak area/IS ratio determined for the non-spiked samples was subtracted from peak area/IS ratio in a matrix matched standard to achieve the matrix affected response factor. The observed matrix effects are summarized in Table S7 (ESM). Matrix effects were assessed by comparing differences between responses obtained for spiked matrices (matrix matched standards) and calibration standards prepared in tap water. Significant matrix effects (above 20%, bold in Table S7) were observed for most analytes. Comparatively, ion enhancement was prevalent in the majority of the compounds and of greater magnitude. Only 20 of all analyzed PPCPs did not seem to be affected by the matrix.

S/N ratio could be used only as a supplementary parameter for LOQ, as the peak area but not peak height is used for quantification. The LOQs were defined as the concentration of the lowest point of the calibration curve (RSD of the average response factor has to be lower than 30%, if not, the lowest calibration point(s) is removed to fit this criterion). Peak area corresponding to this calibration point divided by 4 is put into the calculation formula to get LOQ for certain compound in a certain sample (Fedorova et al., 2013a, 2013b). The LOQs for target PPCPs ranged from 0.59 to 49 ng L^{-1} for wastewater samples, which enables determination of their presence in trace amounts.

Two transitions were monitored for each analyte according to the European Commission Decision 2002/657/EC of 17 August 2002 concerning the performance of analytical methods and interpretation of results.

Quality control was confirmed by analysis of blank samples to assure that target analytes were not introduced from sampling or laboratory procedures and sample handling. Four isotope labeled compounds were used as the internal standard.

3. Results and discussion

The stability of 124 target PPCPs during wastewater freezing/thawing was evaluated. In this study, we considered an analyte to be stable if its concentration during storage was in the range of 60–120% of the initial concentration. Three sets of influent and effluent wastewater samples were stored at −18 °C for 7 days, 60 days and 120 days. Another set of wastewater samples was kept at 4 °C in the refrigerator; however, the samples became turbid and turned pink after 7 days of storage and could not be analyzed after longer storage times. The results for different storage conditions are summarized in Table 1. Storage at 4 °C for 1 week appeared to be more effective than freezing because a higher percentage of analytes remained stable.

Based on these findings, it is better to analyze samples as soon as possible, while avoiding long-term storage and freezing/thawing if possible. This approach is unfortunately unrealistic for most of the cases. Freezing of samples immediately after their collection is the easiest way of sample preservation.

The stability of all PPCPs tested during long-term storage at −18 °C is presented in Table 2. During our experiment, three types of behavior were observed: constant concentrations throughout the experimental period, decreasing concentrations with time during the experiment and loss of the compound from the sample after freezing (Fig. 1). Moreover, the percentage of stable analytes decreased with time during long term storage. Concentrations of target compounds and their losses for different periods of storage are summarized in Table S8 (ESM).

For 40 target compounds (e.g. atenolol, carbamazepine, diclofenac, and oxazepam) no temporal trends were observed. They remained stable throughout the experimental period in both effluent and influent wastewater with small losses (10–20%) during 120 days of storage at −18 °C (Table S8). These results come into agreement with those for surface water obtained by Aboulfadl et al. Such pharmaceuticals as carbamazepine, naproxen, trimethoprim are reported to be stable during storage (Aboulfadl et al., 2010). For such compounds, storage of samples at −18 °C is acceptable. Another group of compounds (BP4, doxycycline and risperidone) showed declining concentrations with the storage time. Declining patterns should be considered when selecting the duration of the storage period. For instance, sulfamethazine was stable during 60 days of storage, while storage of samples for 120 days led to 85% loss in the effluent and 100% loss in the influent sample, which is already unacceptable for the analysis. On the contrary, study of Carlson et al. reported 11% loss for sulfamethazine in POCIS (Polar Organic Chemical Integrative Sampler) stored at −18 °C for 609 days (Carlson et al., 2013). Therefore, POCIS could be a good alternative for conventional water sampling in terms of storage issues.

In general, better results were obtained for the effluent than the influent wastewater. For example, 30 target compounds were unstable in treated wastewater, while 51 were unstable in untreated wastewater. It is important to know which compounds are unstable during studies of the effectiveness of wastewater treatment. For example, false negative WWTP effectiveness is likely for some fluoroquinolones (difloxacin, enoxacin, enrofloxacin, lomefloxacin, oxolinic acid) because they will be present after the F/T cycle in the effluent sample and absent in the influent sample (Table 2).

Overall, 15 of the 124 target PPCPs, including fenofibrate, tamoxifen, amiodarone and all compounds of imidazole class (econazole, miconazole) were lost from treated and non-treated wastewater after freezing. These findings indicate that wastewater samples should not be analyzed for those compounds after an F/T cycle.

Immediate analysis is the best solution avoiding losses of target analytes during storage. Unfortunately this is not applicable for the most of monitoring programs. In this case, samples should be kept at −18 °C. The stability of target compounds should be checked

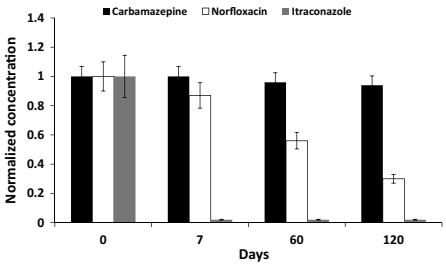

Fig. 1. Compound behavior during long-term storage.

beforehand in the studied matrix, as wastewater composition can vary from place to place due to the different treatment processes, affecting the stability of target analytes. Overall, the decision regarding storage conditions should be made after careful consideration of the analytes and the purpose for wastewater analysis.

4. Conclusions

This paper describes the effects of long-term storage of wastewater samples on the analysis of PPCPs. Short-term stability (7 days) of 124 PPCPs in wastewater was tested by comparing samples stored at 4 °C and −18 °C, while long-term stability (60 and 120 days) was evaluated during storage at −18 °C. No sample pre-treatment before storage was carried out to simulate the most common method of sample handling. For the short-term storage, keeping the samples in fridge showed better results than freezing/thawing: 85% (83%) of stable analytes in waste water effluent (influent) and 78% (60%) respectively. The amount of stable analytes decreased with time during long-term storage, and only 46% and 37% of the target compounds in the effluent and influent wastewater, respectively, remained stable throughout the experiment. Additionally, differences in the stability of 50 of the tested PPCPs in effluent and influent samples were observed. Accordingly, care should be taken when the efficiency of wastewater treatment is evaluated as some false results could be obtained.

Overall, the results of this study indicate that multi-residue analysis of wastewater samples is always a compromise concerning not only the analytical method development and extraction procedure, but also the choice of storage conditions. Because little attention is given to the storage factor, the stability of target compounds in samples should be checked under the planned storage conditions before starting any experiment.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2014.02.067.

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CHAPTER 3

SEASONAL EFFECT ON CONCENTRATION AND REMOVAL EFFICIENCY OF PPCPS IN WWTP

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3.2. Golovko, O., Kumar, V., Fedorova, G., Randak, T., Grabic R., 2014. Removal and seasonal variability of selected analgesics/anti-inflammatory, anti-hypertensive/cardiovascular pharmaceuticals and UV filters in wastewater treatment plant. Environmental Science and Pollution Research 21, 7578–7585.

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Seasonal changes in antibiotics, antidepressants/psychiatric drugs, antihistamines and lipid regulators in a wastewater treatment plant

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HIGHLIGHTS

- 272 WWTP influent and effluent samples were analyzed within 12 months.
- Significant seasonal differences in influent concentration were observed.
- Both influent and effluent concentrations were higher in winter season.

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ABSTRACT

Seasonal changes in the concentration of 21 pharmaceuticals in a wastewater treatment plant (WWTP) in České Budějovice were investigated over 12 months. The target compounds were 10 antibiotics, 4 antidepressants, 3 psychiatric drugs, 2 antihistamines and 2 lipid regulators. 272 Wastewater samples (136 influents and 136 effluents) were collected from March 2011 to February 2012 and analyzed using two-dimensional liquid chromatography coupled with tandem mass spectrometry. All studied pharmaceuticals were frequently detected in both the influent and the effluent wastewater samples, except for meclozine, which was only found in the influent. The mean concentration of pharmaceuticals varied from 0.006 μg L^{-1} to 1.48 μg L^{-1} in the influent and from 0.003 μg L^{-1} to 0.93 μg L^{-1} in the effluent. The concentration of most pharmaceuticals was higher during winter.

1. Introduction

Detection of pharmaceuticals in the environment has raised concerns in recent years (Halling-Sorensen et al., 1998; Jorgensen and Halling-Sorensen, 2000; Jones et al., 2001; Heberer, 2002; Nakada et al., 2007; Calisto and Esteves, 2009). Despite a growing number of studies pertaining to this subject, there is still little information available regarding environmental transformations, seasonal variations in concentration, and the fate and effects of these compounds in aquatic media (Brain et al., 2004; Calisto and Esteves, 2009; Sui et al., 2011). A large variety of pharmaceuticals have been found in the environment, including analgesics, antibiotics, β-blockers, lipid regulators, antidepressants, and contraceptives (Halling-Sorensen et al., 1998; Jones et al., 2006; Calisto and Esteves, 2009). Following administration, pharmaceuticals are generally excreted either unchanged or in the form of metabolites (active and inactive), resulting in their emission to wastewater treatment plants (WWTPs) (Heberer, 2002). If not removed during treatment, the compound may then be released into local aquatic systems via the effluent of WWTPs (Halling-Sorensen et al., 1998). Treated effluents may also be reused for irrigation, and produced biosolids can be used in agriculture as soil amendments or disposed to landfill, which could lead to further water contamination by pharmaceuticals (Jelic et al., 2011).

The concentrations of pharmaceuticals in the influent and the effluent of WWTPs are routinely monitored in many countries (Lindberg et al., 2005; Vieno et al., 2005; Gobel et al., 2007; Xu et al., 2007; Nakada et al., 2007; Kasprzyk-Hordern et al., 2009; Gros et al., 2010; Jelic et al., 2011; Sui et al., 2011; Bueno et al., 2012; Gracia-Lor et al., 2012; Lajeunesse et al., 2012; Senta et al., 2013; Yu et al., 2013b). Several studies have recently demonstrated

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that the concentrations of pharmaceuticals and personal care products (PPCPs) in municipal wastewater and its treated effluents are subject to considerable seasonal variations (Valcarcel et al., 2013; Yu et al., 2013b). For example, higher concentrations of some target pharmaceuticals (trimethoprim and venlafaxine) were detected in winter than in summer in Spain (Valcarcel et al., 2013), and a similar trend was observed in five WWTPs in the United States (carbamazepine) (Yu et al., 2013b). However, seasonal variations and their effects on removal of many of pharmaceuticals are poorly understood (Verlicchi et al., 2012).

Seasonal variation may depend upon either societal factors (production, consumption, excretion) or environmental factors (solar irradiance, precipitation, temperature, etc.) (Vieno et al., 2005; Bueno et al., 2012; Yu et al., 2013b). Some of the targeted PPCPs in this study (trimethoprim, sulfamethoxazole, erythromycin, carbamazepine) are frequently detected in WWTP (Kasprzyk-Hordern et al., 2009; Bueno et al., 2012; Verlicchi et al., 2012), however, little is known about the concentration changes of these compounds in wastewater during the year. Likewise, seasonal concentration changes of norfloxacin, ciprofloxacin, levofloxacin, oxazepam, mirtazapine, sertraline, memantine, fexofenadine, meclozine, rosuvastatin and atorvastatin in wastewater have nor been evaluated to date.

Therefore, in this study, we investigated the occurrence and removal of 21 selected PPCPs in the influent and effluent wastewater of a WWTP in České Budějovice, Czech Republic over 1 year.

2. Materials and methods

2.1. Chemicals

Liquid chromatography–mass spectrometry (LC–MS) grade methanol and acetonitrile (Li Chrosolv Hypergrade) were purchased from Merck (Darmstadt, Germany). Formic acid to acidify the mobile phases was acquired from Labicom (Olomouc, Czech Republic). Ultra pure water was produced using an Aqua-MAX-Ultra System (Younglin, Kyounggi-do, Korea). All analytical standards were of high purity (mostly 98%). Native standards: azithromycin, carbamazepine, ciprofloxacin, citalopram, clarithromycin, erythromycin, fexofenadine, levofloxacin, memantine, mirtazapine, norfloxacin, oxazepam, sertraline, sulfasalazine, sulfamethoxazole, sulfapyridine, trimethoprim, venlafaxine, rosuvastatin, atorvastatin, meclozine were kindly donated by the Laboratory of Environmental Chemistry, Umea University (Umea, Sweden) (Table 1).

Internal standards (IS): trimethoprim ($^{13}C_3$) was purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA), while carbamazepine (D_{10}) and amitriptyline (D_6) were acquired from CDN Isotopes (Pointe-Claire, Quebec, Canada). Stock solutions of all pharmaceuticals were prepared in methanol at a concentration of 1 mg mL^{-1} and stored at −20 °C. A spiking mixture was prepared for each compound by diluting stocks in methanol to a final concentration of 1 μg mL^{-1} and stored at −20 °C.

2.2. Sampling

Sampling was conducted from March 2011 to February 2012 in a WWTP in České Budějovice, Czech Republic. In total 272 samples were collected (136 samples from the influent and 136 samples from the effluent) (Table SM-1). To avoid misinterpretation (errors) we did not include concentrations data when day flow of wastewater effluent was higher than 60 000 m^3 day^{-1} (abnormal conditions) (Table 2). This WWTP utilizes a biological activated sludge process with partial nitrification and thermophile anaerobic sludge stabilization. The capacity of this WWTP is 90 000 m^3 day^{-1} and it serves 112 000 inhabitants. The main input consists of wastewater from communal use, while industrial use accounts for less than 5% of the input water. In České Budějovice, there is one regional hospital (České Budějovice Regional Hospital). Time proportional (15 min) composite samples of influent and effluent were collected over a 24-h period by an automated sampler (ASP-STATION 2000 sampler, manufactured by E + H). All samples were collected into high density polyethylene bottles, immediately frozen and stored until analyses. For the two-dimensional liquid chromatography method (LC/LC method), thawed water samples were filtered through a syringe filter (0.45 μm, regenerated cellulose, Labicom, Olomouc, Czech Republic), after which 10 ng of internal standards were added to 10 mL of sample. Each sample was prepared and analyzed in triplicate.

Table 1
PPCPs selected for this study, limit of quantification (LOQ) of target pharmaceuticals measured in wastewater.

Compounds	LOQ (μg L^{-1})	Frequency of detection (%)	
		Influent	Effluent
Antibiotics (ATB)			
Norfloxacin	0.003	100	100
Levofloxacin	0.003	99	64
Ciprofloxacin	0.003	100	91
Azithromycin	0.007	99	75
Erythromycin	0.006	100	100
Clarithromycin	0.003	100	100
Trimethoprim	0.003	100	100
Sulfapyridine	0.003	100	100
Sulfamethoxazole	0.005	100	100
Sulfasalazine	0.001	100	99
Psychiatric drugs			
Carbamazepine	0.008	100	100
Oxazepam	0.004	100	100
Memantine	0.003	54	78
Antidepressants			
Mirtazapine	0.002	100	100
Citalopram	0.005	100	100
Sertraline	0.003	70	37
Venlafaxine	0.007	100	100
Antihistamine			
Fexofenadine	0.005	100	100
Meclozine	0.002	96	0
Lipid regulators			
Rosuvastatin	0.002	100	100
Atorvastatin	0.003	100	59

2.3. LC–MS/MS analysis

A triple stage quadrupole MS/MS TSQ Quantum Ultra mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) coupled with Accela 1250 LC and Accela 600 LC pumps (Thermo Fisher Scientific) and an HTS XT-CTC autosampler (CTC Analytics AG, Zwingen, Switzerland) was used for analysis. The system was wired and connected for in-line SPE automated extraction and tandem mass spectrometric detection.

A Hypersil Gold (20 mm × 2.1 mm i.d., 12 μm particles) column from Thermo Fisher Scientific (San Jose, CA, USA) was used as the extraction column. For PPCPs analysis, two analytical columns were used in two separate runs. A Cogent Bidentate C18 column (50 mm × 2.1 mm i.d., 4 μm particle sizes from MicroSolv Technology Corporation Eatontown, NJ, USA) was used for determination of trimethoprim, sulfapyridine, sulfasalazine, citalopram, sertraline, venlafaxine, memantine sulfamethoxazole, rosuvastatin and atorvastatin. Hypersil Gold column (50 mm × 2.1 mm i.d. 3 μm particles) was used for determination of norfloxacin, levofloxacin, ciprofloxacin, azithromycin,

Table 2
Characteristics of the wastewater sampling.

Sampling time (month and year)	Month average of daily effluent (m^3 day^{-1}) (standard deviation)	Month average influent pH	Month average influent temperature, (°C) (standard deviation)	Month average atmospheric temperature (°C)
2011 year				
March	29019 (7196)	7.2	11.9 (1.0)	4.6
April	34482 (11622)	7.3	13.1 (1.4)	11.1
May	33724 (8904)	7.3	16.0 (0.8)	14.1
June	43871 (10115)	7.4	17.3 (0.5)	17.7
July	38605 (6485)	7.2	18.6 (0.9)	17.3
August	36820 (6176)	7.2	19.5 (0.5)	18.8
September	43733 (10451)	7.6	18.4 (1.2)	15.1
October	36970 (9658)	7.4	17.5 (1.0)	8.1
November	39847 (5205)	7.5	15.4 (0.7)	2.7
December	38214 (7828)	7.3	13.4 (1.8)	3.5
2012 year				
January	407739 (3760)	7.6	10.4 (1.5)	1.5
February	41591 (7277)	7.7	9.7 (0.8)	−4.1

erythromycin, clarithromycin, carbamazepine, oxazepam, mirtazapine, fexofenadine and meclozine.

2.4. Analytical procedure

A detailed description of MS/MS transitions, LC/LC–MS/MS configuration and set up has been provided elsewhere (Grabic et al., 2012; Khan et al., 2012; Golovko et al., 2014). The limit of quantification (LOQ) for simultaneous analysis of the PPCP was determined by measuring aqueous standard solution at concentrations ranging from 10 ng L^{-1} to 2500 ng L^{-1}, as 25% of the lowest calibration point in the linear range (relative standard deviation average response factor <30%) if S/N ration was higher then 10. The calibration curve was measured during each day of analysis at the beginning and end of the sequence to check the instrument repeatability.

Matrix effect was assessed for each compound and corrections for ion suppression or enhancement were accomplished using matrix-matched standards. Matrix matched standards were prepared from sampled wastewater by spiking with both IS and native compounds at 1 μg L^{-1} and 10 μg L^{-1}, respectively. To determine the matrix affected response factor, the peak area/IS ratio determined in non-spiked samples was subtracted from the peak area/IS ratio in the matrix matched standard.

2.5. Data analysis

To evaluate the difference between the concentration of PPCPs and RE in summer and winter time statistical analysis was applied

Table 3
Median, minimum, maximum concentration, removal efficiency (RE) of target pharmaceuticals measured in wastewater. (n = 272; March 2011 – February 2012).

Compounds	Influent			Effluent			Removal efficiency (%)
	Median (μg L^{-1})	Min (μg L^{-1})	Max (μg L^{-1})	Median (μg L^{-1})	Min (μg L^{-1})	Max (μg L^{-1})	
Antibiotics (ATB)							
Norfloxacin	0.55	0.13	1.33	0.083	0.020	0.25	86
Levofloxacin	0.022	0.005	0.069	0.006	0.004	0.018	75
Ciprofloxacin	0.41	0.08	0.86	0.065	0.008	0.19	86
Azithromycin	0.14	0.014	0.51	0.050	0.008	0.22	69
Erythromycin	0.077	0.02	0.30	0.11	0.030	0.35	−30
Clarithromycin	1.48	0.31	3.09	0.93	0.21	2.31	40
Trimethoprim	0.32	0.12	0.53	0.25	0.083	0.44	20
Sulfapyridine	0.20	0.018	0.66	0.055	0.014	0.20	70
Sulfamethoxazole	0.22	0.043	0.49	0.090	0.031	0.26	58
Sulfasalazine	0.10	0.029	0.73	0.050	0.017	0.83	44
Psychiatric drugs							
Carbamazepine	0.46	0.21	0.71	0.51	0.22	0.73	−12
Oxazepam	0.056	0.024	0.077	0.062	0.026	0.094	−17
Memantine	0.006	0.004	0.058	0.006	0.005	0.010	1
Antidepressants							
Mirtazapine	0.048	0.023	0.17	0.034	0.013	0.068	32
Citalopram	0.083	0.027	0.18	0.073	0.03	0.12	18
Sertraline	0.012	0.007	0.027	0.003	0.003	0.006	81
Venlafaxine	0.29	0.12	0.80	0.28	0.12	1.11	1
Antihistamine							
Fexofenadine	0.18	0.068	0.40	0.17	0.064	0.25	11
Meclozine	0.093	0.031	0.21	<LOQ	<LOQ	<LOQ	100
Lipid regulators							
Rosuvastatin	0.19	0.062	0.46	0.054	0.008	0.32	68
Atorvastatin	0.30	0.070	0.75	0.013	0.004	0.24	93

using the STATISTICA Analysis Software 9.1 (StatSoft, Czech Republic). Since data were non-normally distributed with non-homogeneous variances, we conducted non-parametric statistical analysis using the Kruskal–Wallis analysis of variance (ANOVA).

3. Results and discussion

3.1. Occurrence and removal of pharmaceuticals by the WWTP

Table 3 summarizes the median, minimum and maximum concentrations and removal efficiency (RE) of the targeted PPCPs in the influent and the effluent samples from March 2011 to February 2012 (n = 272). All investigated pharmaceuticals were frequently detected in both the influent and the effluent wastewater samples, except for meclozine, which was only detected in influent samples. In the influent samples, the mean concentration of the pharmaceuticals varied from 0.006 $\mu g\,L^{-1}$ (memantine) to 1.48 $\mu g\,L^{-1}$ (clarithromycin). Clarithromycin was the dominant pharmaceutical in influent samples. This high concentration of clarithromycin in the influent samples might be caused by the contribution of partially treated hospital effluent connected to the studied WWTP (McArdell et al., 2003).

The RE of the selected PPCPs in the WWTP varied (Table 3 and Fig. 1). For example, the RE of antibiotics (ATB) ranged from 86% (for norfloxacin and ciprofloxacin) to −30% (erythromycin) (Table 3). The results of RE for sulfamethoxazole (58%), clarithromycin (40%), trimethoprim (20%) and azithromycin (69%) were similar with previously available data (Lindberg et al., 2005; Gobel et al., 2007; Kasprzyk-Hordern et al., 2009; Yang et al., 2011; Bueno et al., 2012; Verlicchi et al., 2012).

The RE values of antidepressants and psychiatric drugs were poor, ranging from 32% (mirtazapine) to −12% (carbamazepine) and −17% (oxazepam), except for sertraline, which had an RE value of 81%. These findings are in accordance with those of previously conducted studies (Gobel et al., 2007; Kasprzyk-Hordern et al., 2009; Bueno et al., 2012; Lajeunesse et al., 2012). Negative RE values for some compounds have also been previously reported (Jelic et al., 2011; Gracia-Lor et al., 2012), and may reflect deconjugation of conjugated metabolites during the treatment process (Verlicchi et al., 2012; Kumar et al., 2012) or changes in the adsorption to particles during treatment (Lindberg et al., 2005; Bueno et al., 2012). Carbamazepine has been found relative persistent and has low biodegradability and sorption (Majewsky et al., 2011; Yu et al., 2013a). Antihistamine showed a wide range of RE (fexofenadine = 11% and meclozine = 100%), while the RE values of lipid regulators were high (rosuvastatin = 68% and atorvastatin = 93%).

The removal of some ATB during treatment process can be explained by the sorption of compounds to the sludge. Sulfamethoxazole, as most sulfonamides, has moderate sorption to sludge and limited biodegradability (Le-Minh et al., 2010). It was shown (Lindberg et al., 2006) that more than 70% of norfloxacin and ciprofloxacin passed through the treatment plant and remained in digested sludge. Moreover, variations in pH values during the treatment process may influence the removal of PPCPs (Tadkaew et al., 2010).

The REs of almost all PPCPs except some ATB (erythromycin, sulfapyridine and sulfasalazine), antidepressants and psychiatric drugs (except mirtazapine and citalopram) were higher in the summer (June–August) than in the winter (December–February) (Figs. SM1–SM4). The relatively low RE for most pharmaceuticals in winter time may be due to the lower microbial activities in winter (Vieno et al., 2005; Hedgespeth et al., 2012).

3.2. Statistical analysis of concentration

A significant difference between winter (December–February) and summer (June–August) were found for all ATB, most antidepressants and psychiatric drugs (except carbamazepine, oxazepam and memantine), antihistamines and lipid regulators. It should be noted that, for antihistamines pharmaceuticals, samples collected during the high allergens season (May–July) were compared with winter samples to reflect the peak usage of these compounds. A Box-and-Whisker graph of the summer and winter influent concentrations of the PPCPs over the 1-year monitoring period is provided in Supplementary material (Figs. SM5–SM8).

3.3. Seasonal changes in the concentrations of antibiotics

The measured concentrations of all ATB in the influent and effluent showed a noticeable seasonal pattern (except norfloxacin, levofloxacin, ciprofloxacin and sulfapyridine) (Fig. 2), with concentrations being lowest during summer (0 0.61 $\mu g\,L^{-1}$ for clarithromycin) and peaking in winter (1.95 $\mu g\,L^{-1}$ for clarithromycin). Concurrently, elevated concentrations of norfloxacin, levofloxacin, ciprofloxacin and sulfapyridine were found in the influent samples during the winter season. These findings are consistent with the expected usage patterns for clarithromycin as an example of typical concentrations for most ATB during the year (Fig. 2).

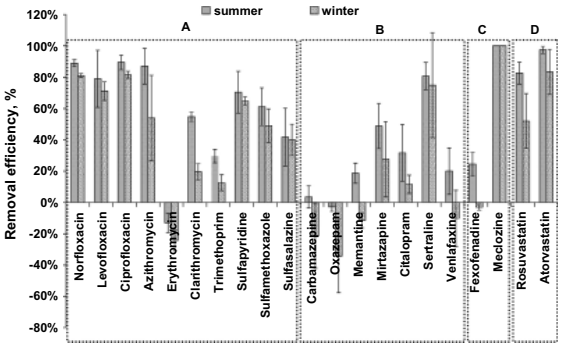

Fig. 1. Removal efficiency of PPCPs in summer (June–August) and winter (December–February) time in WWTP. A – antibiotics; B – psychiatric drugs and antidepressants; C – antihistamines; D – lipid regulators. For antihistamines pharmaceuticals, samples collected during the high allergens season (May–July) were compared with winter samples to reflect the peak usage of these compounds.

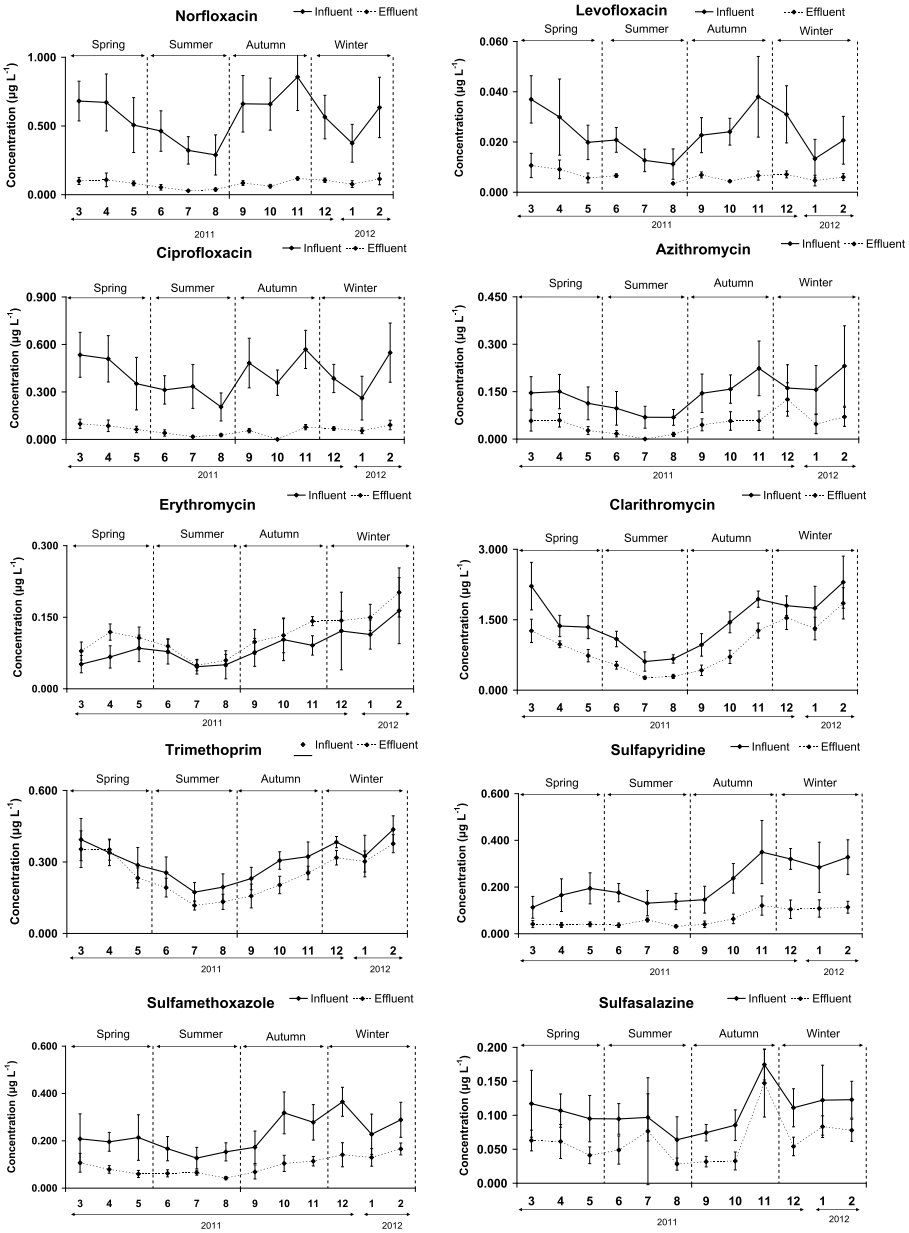

Fig. 2. Seasonal variation of monthly concentrations in antibiotics in WWTP. (Number reflects the month of the year, error bar shows the range of detection in each month; *n* has been provided in Table SM-1.)

In general, the seasonal variation of ATB concentrations indicated the consumption patterns of these ATB. High concentrations were detected in winter months (December–February) probably because of the higher incidences of flu or other ailments cases (Coutu et al., 2013). The concentration of most ATB decreased from April to August, and then began increasing from September. We believe that this study is the first to show the seasonal influence on concentrations of norfloxacin, ciprofloxacin and levofloxacin in a WWTP. The observed azithromycin, clarithromycin and erythromycin levels are in accordance with ATB use in the treatment of respiratory tract infections, which are most common in winter and early spring (Gobel et al., 2005). Similar findings were reported by Gobel et al. (2005) and Senta et al. (2013). The low biodegradability is likely the another reason responsible for the elevated levels of ATB in influent and effluent samples (Vieno et al., 2005).

3.4. Psychiatric drugs

The influent concentrations of carbamazepine, oxazepam and memantine were approximately the same during all seasons (Fig. 3). Previously, relatively consistent concentration for carbamazepine was also found in WWTP during the three seasonal sampling periods (Hua et al., 2006). Similar levels of carbamazepine and memantine in the influent throughout the year show their

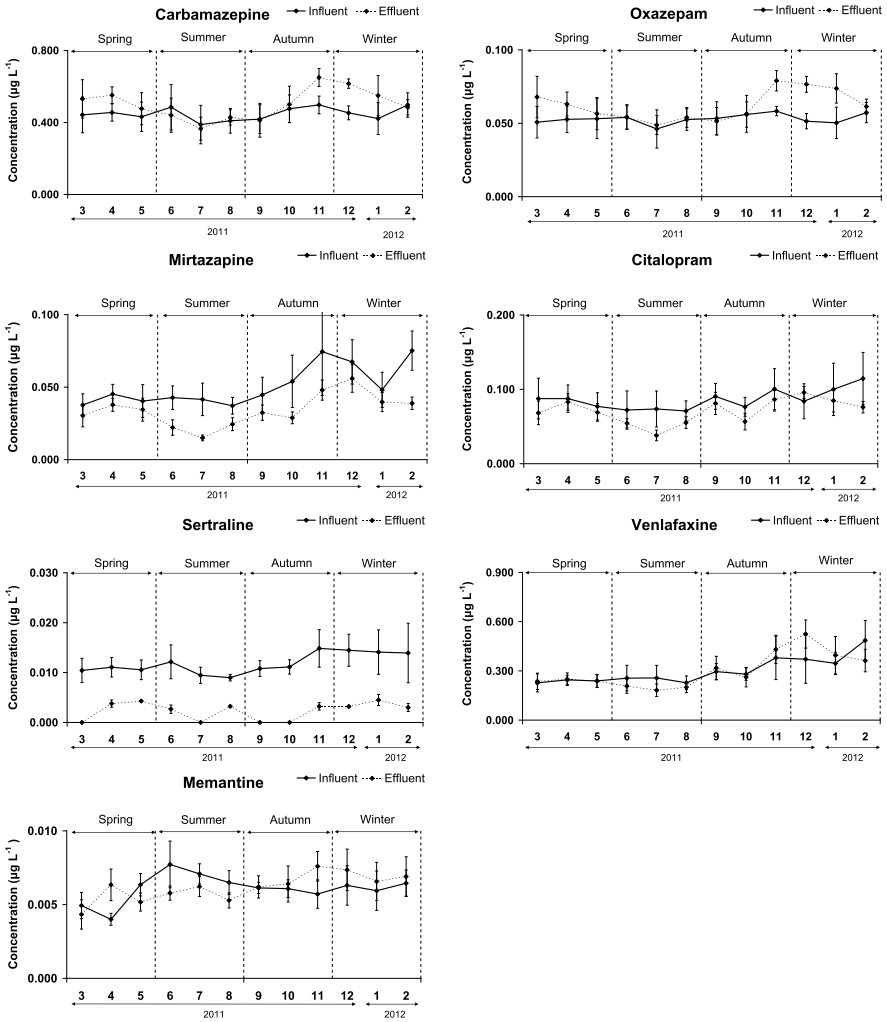

Fig. 3. Seasonal variation of monthly concentrations in antidepressants and psychiatric drugs in WWTP. (Number reflects the month of the year, error bar shows the range of detection in each month; *n* has been provided in Table SM-1.)

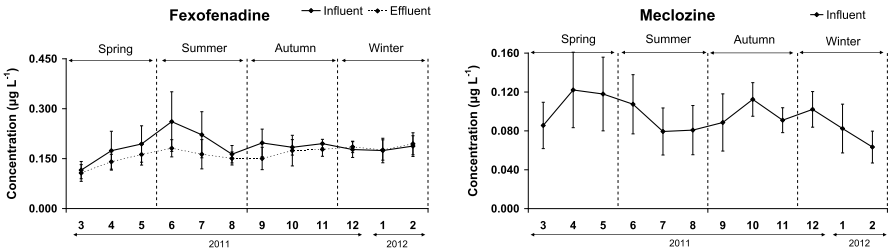

Fig. 4. Seasonal variation of monthly concentration in antihistamine in WWTP. (Number reflects the month of the year, error bar shows the range of detection in each month; *n* has been provided in Table SM-1.)

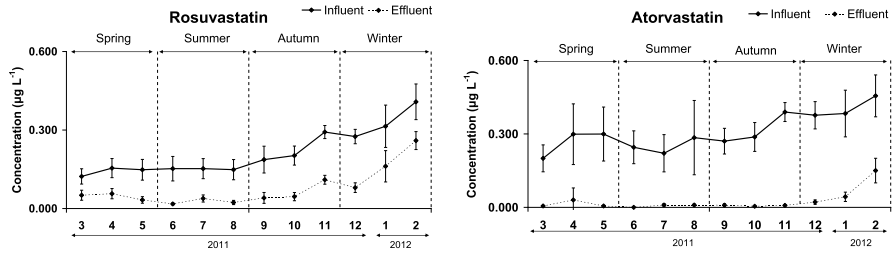

Fig. 5. Seasonal variation of monthly concentration in lipid regulators in WWTP. (Number reflects the month of the year, error bar shows the range of detection in each month; *n* has been provided in Table SM-1.)

consumption patterns. Carbamazepine is used for treatment of chronic disease (certain types of seizures, trigeminal neuralgia, episodes of mania and depression) (Mohapatra et al., 2014) and memantine is approved in the US and the EU for the treatment of patients with moderate to severe dementia of the Alzheimer's type (Robinson and Keating, 2006).

Higher concentrations of carbamazepine and oxazepam were found in the effluent than in the influent during winter season, indicating negative REs during the wastewater treatment process (Figs. 1 and 3).

To the best of our knowledge, this is the first study to show a seasonal effect on concentration diversity for oxazepam and memantine.

3.5. Antidepressants drugs

An obvious seasonal effect was observed for the venlafaxine, mirtazapine and sertraline influent concentrations (Fig. 3). Venlafaxine is primarily used to treat depression, general anxiety disorder, social phobia, panic disorder and vasomotor symptoms. According to our results, venlafaxine influent concentrations were greater in winter (0.49 $\mu g\,L^{-1}$) than in summer (0.23 $\mu g\,L^{-1}$) (Fig. 3). Nevertheless, negative REs were found for venlafaxine in winter time (Fig. 1). During the study period, disorders (depression, general anxiety disorder and social phobia) increased from autumn to winter (Lam and Levitan, 2000), which was associated with higher consumption and consequently higher concentrations of this pharmaceutical in WWTP water. Simultaneously, high concentration in effluent can also be explained by the low REs of venlafaxine in winter time. We believe that this study is the first to show the seasonal effect on concentration variability for mirtazapine and sertraline in wastewater.

3.6. Seasonal occurrence of antihistamines and lipid regulators

The meclozine and fexofenadine concentrations decreased from high allergens season (May–July) to winter (Fig. 4). The concentration of meclozine was not detected in any effluent samples (Fig. 4), indicating that this pharmaceutical was removed with a RE near 100% during the treatment procedure. The fexofenadine concentration in the influent increased from 0.16 $\mu g\,L^{-1}$ to 0.22 $\mu g\,L^{-1}$ during summer (Fig. 4). The effluent concentrations of fexofenadine follow the influent concentration pattern. It should be highlighted that REs for fexofenadine in allergens season were high as well (Fig. 1). Possibly, obtained data reflect the high consumption of fexofenadine in relieving the symptoms of cutaneous allergic reactions, which occur more frequently during the summer season.

The concentrations of lipid regulators rosuvastatin and atorvastatin were higher in winter (0.41 $\mu g\,L^{-1}$ and 0.46 $\mu g\,L^{-1}$, respectively) than in summer (0.12 $\mu g\,L^{-1}$ and 0.20 $\mu g\,L^{-1}$, respectively) (Fig. 5). Rosuvastatin and atorvastatin are members of the statin drug class used in combination with exercise, diet, and weight-loss to treat high cholesterol and related conditions, and to prevent cardiovascular disease; therefore, the high concentration of these statins during winter was consistent with the fact that blood lipids of patients tend to increase in winter (Ockene et al., 2004). The low REs in winter time (December–February) may have significant impact on concentration data as well because REs in that time were significantly lower than in summer period (Fig. 1).

4. Conclusions

This study examined the effects of season on concentrations of four groups of pharmaceuticals in a WWTP in České Budějovice, Czech Republic for period of 12 months. An obvious and significant seasonal effect was observed in the influent concentrations and in

the REs of most investigated ATB, antidepressants/psychiatric drugs, antihistamines and lipid regulators. The total concentrations of most compounds in the WWTP were higher during winter than summer, which was attributed to increased human consumption of PPCPs during winter and less efficient removal of the compounds in this period. To the best of our knowledge, our study is the first to report seasonal concentration changes in norfloxacin, ciprofloxacin, levofloxacin, oxazepam, mirtazapine, sertraline, memantine, fexofenadine, meclozine, rosuvastatin and atorvastatin. Provided data on PPCPs concentrations and REs would add relevant information for further risk assessment of aquatic organisms' exposure.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2014.03.132.

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RESEARCH ARTICLE

Removal and seasonal variability of selected analgesics/anti-inflammatory, anti-hypertensive/cardiovascular pharmaceuticals and UV filters in wastewater treatment plant

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Abstract Seasonal removal efficiency of 16 pharmaceuticals and personal care products was monitored in a wastewater treatment plant in České Budějovice, Czech Republic, over a period of 1 year (total amount of samples, n=272). The studied compounds included four UV filters, three analgesics/anti-inflammatory drugs and nine anti-hypertensive/cardiovascular drugs. In most cases, elimination of the substances was incomplete, and overall removal rates varied strongly from −38 to 100 %. Therefore, it was difficult to establish a general trend for each therapeutic group. Based on the removal efficiencies (REs) over the year, three groups of target compounds were observed. A few compounds (benzophenon-1, valsartan, isradipine and furosemide) were not fully removed, but their REs were greater than 50 %. The second group of analytes, consisting of 2-phenylbenzimidazole-5-sulfonic acid, tramadol, sotalol, metoprolol, atenolol and diclofenac, showed a very low RE (lower than 50 %). The third group of compounds showed extremely variable RE (benzophenon-3 and benzophenon-4, codeine, verapamil, diltiazem and bisoprolol). There were significant seasonal trends in the observed REs, with reduced efficiencies in colder months.

Keywords Removal efficiency · Pharmaceuticals · Wastewater · Seasonal variation · UV filters · Anti-hypertensive/cardiovascular drugs

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Introduction

Because of their frequent detection in the aquatic environment, pharmaceuticals and personal care products (PPCPs) are the subject of much concern among the scientific community (Lopez-Serna et al. 2012). In the last decade, they have been detected in different environmental compartments all over the world (Segura et al. 2009). Pharmaceuticals usually have a designed resistance to biodegradation. Hence, most drugs are not eliminated significantly in wastewater treatment plants (WWTPs) (Fatta-Kassinos et al. 2011). In addition, partial degradation of pharmaceuticals and/or their metabolites during activated sludge treatment can lead to the formation of transformation products (Kern et al. 2010). Conjugates can be hydrolyzed back to the free parent drug (Rodriguez-Mozaz et al. 2007), which then enters the aquatic environment with the treated effluent (Hedgespeth et al. 2012).

Removal efficiencies (REs) in WWTPs depend on several factors, such as physicochemical properties of the compounds, climatic conditions (such as temperature and sunlight intensity) and the type and operational conditions of the treatment process used in the plant (temperature of operation, redox conditions, solids retention time and hydraulic retention time) (Le-Minh et al. 2010; Vieno et al. 2005; Clara et al. 2005). Therefore, the RE can vary significantly from plant to plant and within a plant at different time periods (Vieno et al. 2007; Santos et al. 2009). Information obtained from analysis of influents and effluents of WWTPs may serve to optimize a treatment process or possible pre- and post-treatment steps, so that emissions of undesired pollutants into receiving waters are prevented.

Regular monitoring of pharmaceutical pollutants and their elimination from WWTPs is essential to predicting the environmental persistence and impacts of many of these compounds in natural waters. Therefore, the objective of this study

was to investigate the RE of 16 PPCPs belonging to different classes, during wastewater treatment. The choice of PPCPs was based on their high annual usage in a wide range of household products and concern over their possible effect on human and aquatic organisms. For example, tramadol, diclofenac, verapamil, atenolol, sotalol, metoprolol and furosemide were the most detected PPCPs in WWTP in recent years (Bueno et al. 2012; Clara et al. 2005; Fatta-Kassinos et al. 2011; Verlicchi et al. 2012). The targeted pharmaceuticals included three analgesic/anti-inflammatory drugs, nine antihypertensive/cardiovascular drug and four personal care products (UV filters). A further objective was to assess the seasonal variability of the REs of the targeted PPCPs in WWTP over a year.

Materials and methods

Chemicals and reagents

Liquid chromatography-mass spectrometry (LC–MS)-grade methanol and acetonitrile (LiChrosolv Hypergrade) were purchased from Merck (Darmstadt, Germany). Formic acid, used to acidify mobile phases, was purchased from Labicom (Olomouc, Czech Republic). Ultrapure water was obtained with an Aqua-MAX-Ultra System (Younglin, Kyounggi-do, South Korea). All analytical standards were of high purity (mostly 98 %).

Native standards 2-Phenylbenzimidazole-5-sulfonic acid, benzophenone-1, benzophenone-3 and benzophenone-4 were obtained from Sigma-Aldrich (Steinheim, Germany); tramadol, diclofenac, codeine, verapamil, valsartan, diltiazem, isradipine, atenolol, sotalol, metoprolol, bisoprolol and furosemide were kindly donated by the Laboratory of Environmental Chemistry, Umeå University (Umeå, Sweden).

Internal standards Trimethoprim ($^{13}C_3$) was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA); carbamazepine (D_{10}) and amitriptyline (D_6) were purchased from CDN Isotopes (Pointe-Claire, Quebec, Canada). Stock solutions of all pharmaceuticals were prepared in methanol at a concentration of 1 mg/mL and stored at −20 °C. A spiking mixture was prepared by diluting stocks in methanol to a final concentration of 1 μg/mL for each compound, and it was stored at −20 °C.

Sixteen PPCPs were studied and are presented (Table 1).

Sampling site and sample collection

Sampling was organized in a WWTP in České Budějovice (Czech Republic) from March 2011 to February 2012 (Tables SI1 and SI2). The technology used in WWTP is a biologically activated sludge process with partial nitrification and thermophile anaerobic sludge stabilization. The capacity of this WWTP is 90,000 m^3/day, and it serves for 112,000

Table 1 PPCPs selected for this study, octanol–water partition coefficient (log K_{ow}), limit of quantification (LOQ) of selected compounds measured in wastewater (number of samples, influent and effluent, n=272)

Compounds	Acronym	Log K_{ow}[a]	LOQ (μg/L)	Frequency of detection (%)	
				Influent	Effluent
UV filters					
2-Phenylbenzimidazole-5-sulfonic acid	PBS	−0.16	0.002	100	98
Benzophenone-1	BP1	2.96	0.003	99	100
Benzophenone-3	BP3	3.52	0.004	99	34
Benzophenone-4	BP4	0.37	0.004	100	100
Analgesics/anti-inflammatory drug					
Tramadol	TRM	3.01	0.004	100	100
Diclofenac	DCL	4.02	0.002	100	100
Codeine	COD	1.28	0.003	100	98
Anti-hypertensive/cardiovascular drugs					
Verapamil	VER	4.80	0.003	100	93
Valsartan	VAL	3.65	0.003	100	100
Diltiazem	DIL	2.79	0.005	96	84
Isradipine	ISR	3.49	0.001	87	60
Atenolol	ATE	−0.03	0.005	100	100
Sotalol	SOT	0.37	0.005	100	100
Metoprolol	MET	1.69	0.003	100	100
Bisoprolol	BIS	1.84	0.004	100	97
Furosemide	FUR	2.32	0.002	100	96

[a] http://www.chemspider.com/

inhabitants. The main input is wastewater from municipal use, and less than 5 % of the input is industrial. Wastewater samples were taken with an automated sampler (time-proportional sampling, ASP-Station 2000 sampler, manufactured by E + H). Time-proportional (15 min) composite samples of influent and effluent were collected over a 24-h period. All samples were collected in high-density polyethylene bottles and immediately frozen. Samples were stored frozen until analyses. For the LC–LC method, thawed water samples were filtered through a syringe filter (0.45 μm, regenerated cellulose, Labicom, Olomouc, Czech Republic), and 10 ng of internal standard was added to 10 mL of sample. Each sample was prepared and analysed in triplicate. The calculated percentages of analyte removal in the aqueous phase during wastewater treatment were calculated as

$$\mathrm{RE}(\%) = (\mathrm{Influent} - \mathrm{Effluent}/\mathrm{Influent}) \times 100.$$

LC–MS/MS analysis

A triple-stage quadrupole MS/MS TSQ Quantum Ultra (Thermo Fisher Scientific, San Jose, CA, USA) coupled with an Accela 1250 LC and Accela 600 LC pumps (Thermo Fisher Scientific) and a HTS XT-CTC auto sampler (CTC Analytics AG, Zwingen, Switzerland) was used for analysis. The system was wired and connected as in-line solid phase extraction (SPE) automated extraction and tandem mass spectrometric detection.

A Hypersil Gold (20 mm×2.1 mm internal diameter (i.d.), 12-μm particles) column from Thermo Fisher Scientific (San Jose, CA, USA) was used as an extraction column. A Cogent Bidentate C18 column (50 mm×2.1 mm i.d., 4-μm particle size) from MicroSolv Technology Corporation (Eatontown, USA) and a Hypersil Gold column (50 mm×2.1 mm i.d., 3-μm particles) were used as analytical columns.

Analytical procedure

The detailed description of MS/MS transitions and the in-line SPE–LC–MS/MS configuration are described elsewhere (Grabic et al. 2012; Khan et al. 2012). The method parameters are shown in the Supporting information Table SI3.

The limit of quantification (LOQ) for simultaneous analysis of the PPCPs was determined by measuring aqueous standard solutions in a concentration range from 10 to 2,500 ng/L. LOQs were calculated as one quarter of the lowest calibration point in the calibration curve where relative standard deviation of average response factor was <30 % (in some cases, one or two points at low concentration levels had to be removed). Peak area corresponding to this concentration was used to calculate LOQ for each individual compound in each sample.

The calibration curve was measured on each day of analysis at the beginning and at the end of the sequence to check the instrument stability.

Matrix effects were assessed for each compound. Corrections of ion suppression or enhancement were made based on matrix-matched standards. A matrix-matched standard was prepared from sampled wastewater by spiking it both with an internal standard (IS) and with native compounds at concentration levels of 1 and 10 μg/L, respectively. The peak area/IS ratio determined in the non-spiked sample was subtracted from peak area/IS ratio in the matrix-matched standard to obtain the matrix-affected response factor.

Statistical analysis

To evaluate the difference between the REs of PPCPs in summer and winter, statistical analysis was applied using Statistical Analysis Software 9.1 (StatSoft, Czech Republic). Because data were non-normally distributed and had non-homogeneous variances, we performed non-parametric statistical analysis by the Kruskal–Wallis ANOVA. A box-and-whisker graph of the summer and winter RE of the PPCPs over the 1-year monitoring period is provided in the Supplementary material (Figs. S1, S2 and S3).

Results and discussion

Removal behaviour of PPCPs during wastewater treatment

The overall removal rates observed in this study varied strongly between individual pharmaceuticals. Therefore, it was difficult to establish a general trend for each therapeutic group, but in most cases, the results indicated that the elimination of the PPCPs was incomplete. Based on the RE trends, three different classes of PPCPs were observed during the year of observation.

PPCPs with RE higher than 50 %

Benzophenone-1 (BP1), valsartan (VAL), isradipine (ISR) and furosemide (FUR) showed high RE in WWTP during the year, from 53 to 100 % (Fig. 1). BP1 showed a RE from 73 to 100 % during the year. FUR had a RE from 73 to 92 %. It was shown (Bueno et al. 2012) that an average elimination rate for FUR was 50 % over an approximate 2-year period. ISR showed a wide range of REs during all seasons at about 70 % and more, except in winter time (53 % in January and 64 % in February). Complete removal of ISR (100 %) was observed in autumn, unlike other seasons. It is worth to mention that this is the first detailed study to show a seasonal RE variation for ISR.

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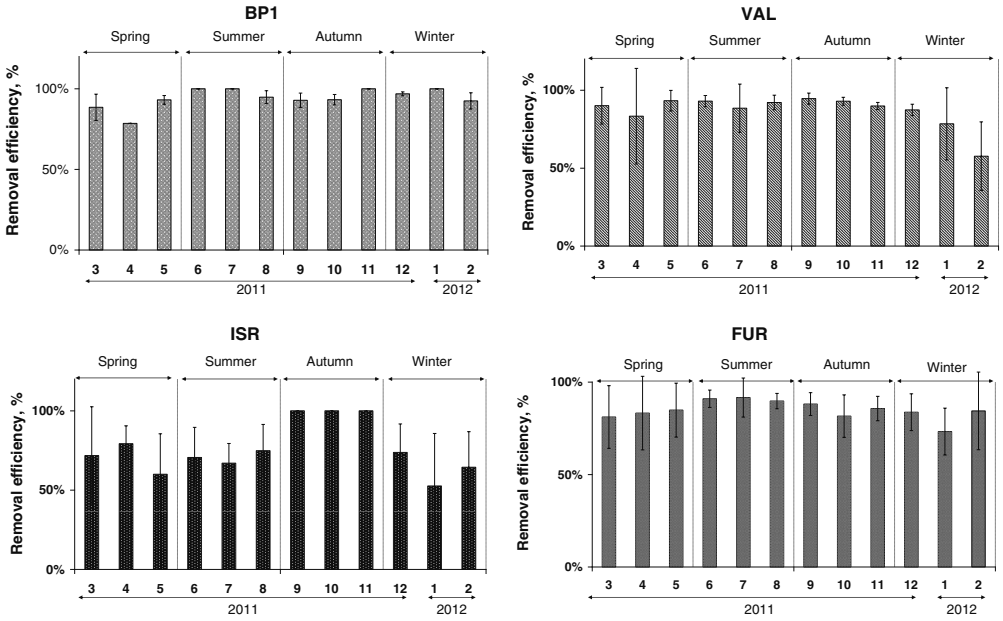

Fig. 1 PPCPs with RE higher than 50% in WWTP during the year

The high REs for VAL and ISR during the year could be explained by the adsorption of these PPCPs in the sludge (the octanol–water partition coefficient, log K_{ow}=3.65 for VAL and 3.49 for ISR).

PPCPs with RE lower than 50 %

2-Phenylbenzimidazole-5-sulfonic acid (PBS), tramadol (TRM), sotalol (SOT), metoprolol (MET), atenolol (ATE) and diclofenac (DCL) demonstrated poor RE (lower than 50 %) (Fig. 2).

Of these PPCPs, the concentrations of PBS, TRM and SOT in the effluents were higher than those in the corresponding influents (negative RE) during certain months of the year. The highest negative RE for PBS was observed during winter—from November to February (Fig. 2). TRM showed negative REs (Fig. 2) in most months of the year, but not in June or July. In these months, the RE was positive (11 and 15 %, respectively). High REs for DCL were observed in April and during summer, whereas the lowest RE was observed in winter time (Fig. 2). Previously, it has been shown that DCL had a low level of removal by biodegradation (Salgado et al. 2012; Jelic et al. 2011) and medium level of removal by adsorption in the activated sludge tank (Salgado et al. 2012).

SOT, ATE and MET showed poor or no elimination in the WWTP (<50 %). SOT showed negative RE in March and during winter (November, December and January). This can be explained by deconjugation of conjugated metabolites during the treatment process (Verlicchi et al. 2012; Kumar et al. 2012) or changes in the adsorption to particles during the treatment process (log K_{ow}<3) influencing the ratio between the concentration in influent and effluent water (Bueno et al. 2012; Lindberg et al. 2005). Horsing et al. (2011) observed low affinity to sludge for DCL and MET, whereas SOT was found to exhibit low sorption on the primary sludge.

Similar results were shown in the literature for the REs of DCL, TRM and MET (Lishman et al. 2006; Verlicchi et al. 2012; Gros et al. 2010; Jelic et al. 2011). The low removal rates of SOT, ATE and MET can also be explained by their low solid water distribution coefficient (K_d) in activated sludge (below 40 L kg^{-1}) (Maurer et al. 2007; Scheurer et al. 2010). Therefore, biodegradation is the most likely the cause for the decreased concentration of these PPCPs in effluent water (Maurer et al. 2007; Scheurer et al. 2010).

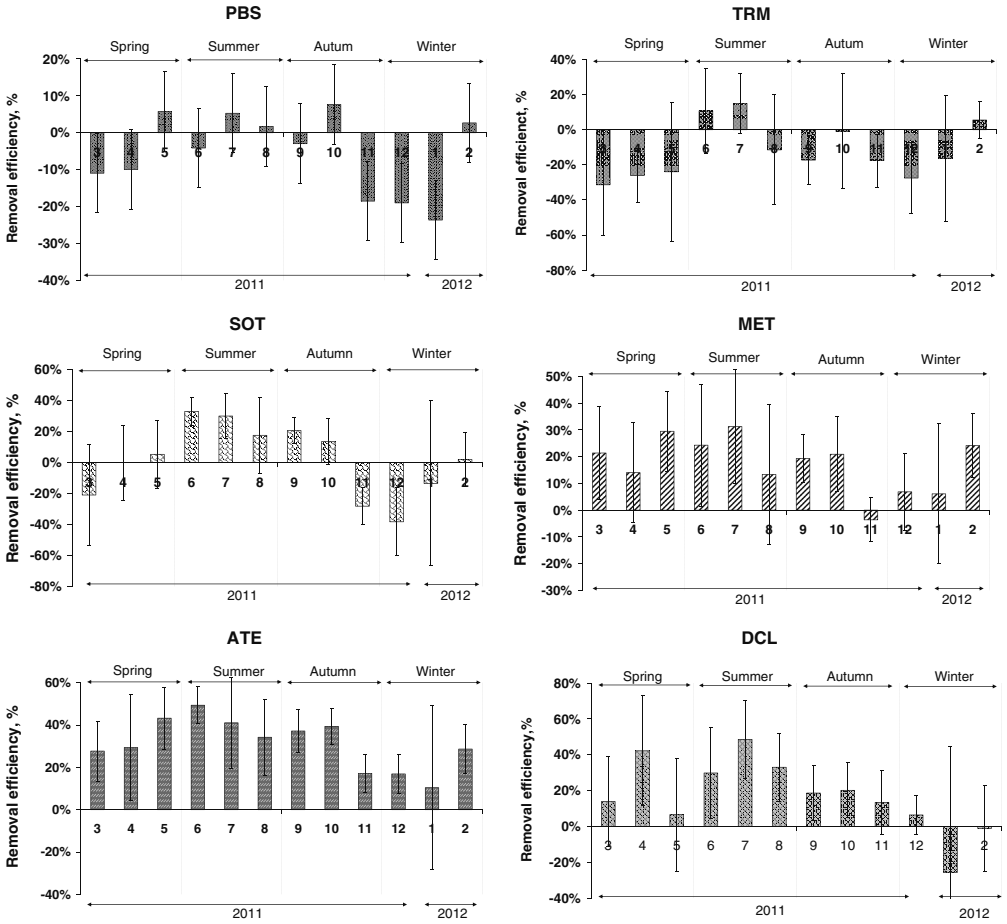

Fig. 2 PPCPs with RE lower than 50% in WWTP during the year

Variable RE

The third groups of compounds showed a high variability of the RE over the year (Fig. 3). This group includes benzophenone-4 (BP4), benzophenone-3 (BP3), codeine (COD), verapamil (VER), diltiazem (DIL) and bisoprolol (BIS). BP3 showed low RE during the spring time; from May to August, it increased from 10 to 65 % and stayed approximately constant until February.

In the literature, the RE of BP3 ranges from 86 % up to >99 % (Liu et al. 2012; Bueno et al. 2012). RE for BP4 decreased from March (56 %) until September (13 %) and then increased from November to February. Removal rates for both UV filters (BP3 and BP4) were extremely variable during the year (from 3 to 70 %), suggesting that seasonal variations affect the REs of both BP3 and BP4 (Table SI4). The variable RE obtained during the treatment process might be due to the adsorption of BP3 (log K_{ow}=3.52) to the sludge particles in various seasons. The lowest RE for COD was detected in the spring at 37 % (March and May) and during winter time (43 % in January and 30 % in February) (Fig. 3). This is in good agreement with the literature (Kasprzyk-Hordern et al. 2008), where the RE of COD was found to be removed from wastewater by less than 50 %. DIL, VER and BIS were partially removed during the treatment

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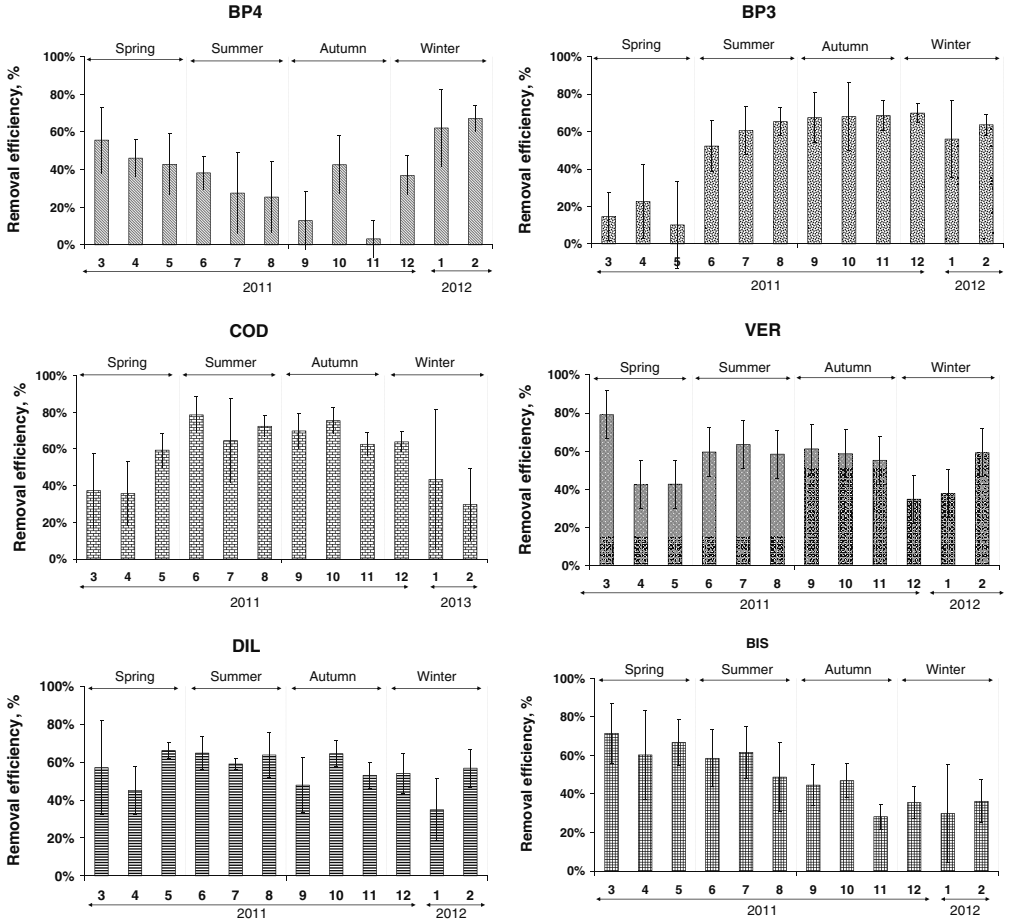

Fig. 3 PPCPs with variable RE in WWTP during the year

process. BIS was poorly removed in winter, from November to February (from 28 to 36 %). The removal range of VER and DIL was quite variable during the year (Fig. 3), with average elimination rates of 54 and 56 %, respectively. Values as low as 35 % (January) were observed for DIL.

BIS and DIL both exhibited low sorption for the primary sludge and no sorption for the secondary sludge (Scheurer et al. 2010; Horsing et al. 2011). However, the removal mechanism of these chemicals is still not clear.

Seasonal effect on elimination of PPCPs in wastewater

To identify differences between two main seasons, we compared data from samples collected in summer (June to August) to those of samples collected during winter (December to February). The median, minimum and maximum concentrations of the selected PPCPs in influent and effluent wastewater are reported in Supplementary material Table SI4. For most selected PPCPs, the RE was higher in summer (Fig. 4). The largest RE differences between summer and winter were found for SOT (21 and −20 %) and DCL (32 and −12 %). The smallest RE differences were observed for BP1 (98 and 96 %), PBS (−7 and −5 %) and BP3 (60 and 62 %). Remarkably, the RE for BP4 was higher in winter than in summer, 55 and 28 %, respectively.

A Kruskal–Wallis ANOVA test revealed a significant difference between winter and summer data for BP4, all anti-hypertensive/cardiovascular drugs except of MET and

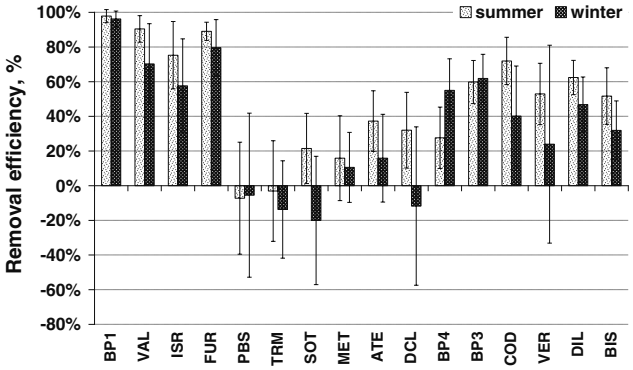

Fig. 4 Average removal efficiency WWTP in the elimination of studied PPCPs in summer (June to August) and winter (December to February) time

analgesics/anti-inflammatory drugs except of TRM (Figs. S1, S2 and S3). Although there is still almost no research explaining the PPCPs removal mechanism, some observation can still be made. Such biodegradation and sorption are likely to be the most important removal processes (Ternes et al. 2004). The relatively low RE for most PPCPs in winter months may be due to the lower microbial activities in winter (Hedgespeth et al. 2012; Vieno et al. 2005). Wastewater temperature on the February sampling date was the lowest recorded for the study (9.7 °C, compared with 19.5 °C in August). The lower temperatures in February likely reduced the microbial activity of the activated sludge process, thus reducing the biodegradation of the compounds during treatment (Vieno et al. 2005). Photochemical processes may play a significant role in the elimination of organic compounds in aquatic environment (Doll and Frimmel 2003). However, it does appear to have been a dominant removal mechanism inside the WWTP.

Conclusions

In this study, 16 pharmaceuticals and personal care products were monitored in influents and effluents of a wastewater treatment plant (WWTP). To the best of our knowledge, this is the first detailed study to show a seasonal variation of the removal efficiency (RE) for PBS, VER, ISR and BIS. The REs of PBS, BP1, BP3, DCL, COD, VER, VAL, DIL, ISR, ATE, SOT, BIS and FUR showed a significant seasonal variation. The selected PPCPs could be divided into three groups according to their REs in a WWTP: high RE (from 50 to 100 %), low RE (lower than 50 %) and variable RE. Most target compounds showed high RE during summer and the lowest, even negative elimination in winter.

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CHAPTER 4

STABILITY OF PHARMACEUTICALS AND PERSONAL CARE PRODUCTS UNDER DIFFERENT CONDITIONS

4.1. Golovko, O., Grabic, R., Lindberg R. H., Ostman, M., Soderstrom, H., Fick, J., 2014. Phototransformation of pharmaceuticals in water under artificial ultraviolet and natural sunlight irradiation. (manuscript)

4.2. Horáková, M., Klementová, Š., Kříž, P., Balakrishna, S.K., Špatenka, P., Golovko, O., Hájková, P., Exnar, P., 2014. The synergistic effect of advanced oxidation processes to eliminate resistant chemical compounds. Surface & Coatings Technology 241, 154–158.

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PHOTOTRANSFORMATION OF PHARMACEUTICALS IN WATER UNDER ARTIFICIAL ULTRAVIOLET AND NATURAL SUNLIGHT IRRADIATION

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ABSTRACT

We investigated the phototransformation of 88 pharmaceuticals exposed to artificial ultraviolet (UV) light and natural sunlight in buffered purified water. For 50 of the 88 studied pharmaceuticals, their photofates under solar simulation or sunlight exposure have not been previously described. Forty-six of the pharmaceuticals were stable and did not degrade over 8 h of exposure to any of the radiation sources. Thirty-five pharmaceuticals showed phototransformation under both conditions with half-lives ranging from 0.11 to 13.23 h. Six pharmaceuticals photolyzed only under natural sunlight and oxazepam transformed only under artificial UV exposure. The rate of photolysis was slower under UV than under natural sunlight exposure for 25 pharmaceuticals.

Keywords: *photostability, solar simulation, photofate, half-life, pharmaceuticals*

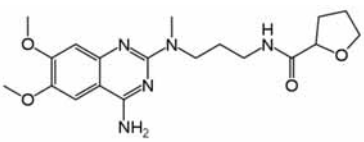
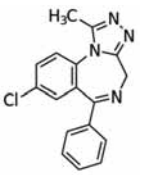
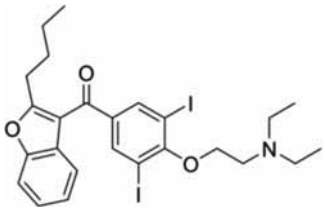
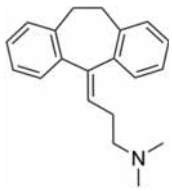
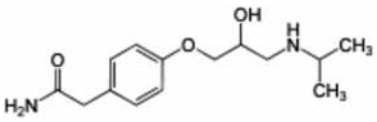
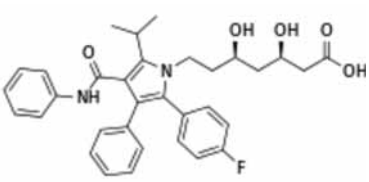
1. INTRODUCTION

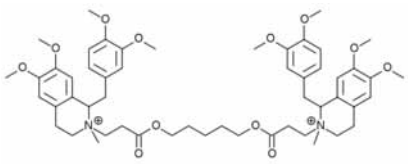
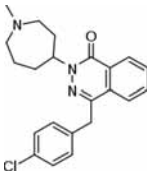
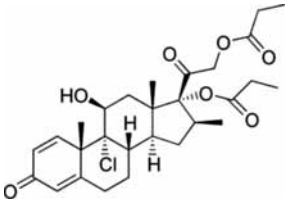
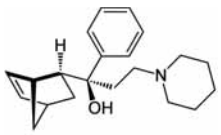
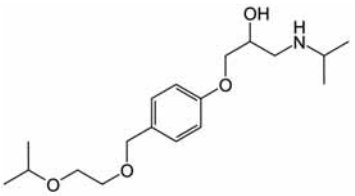
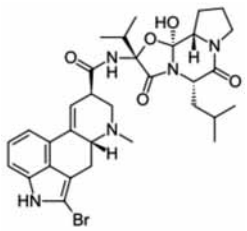
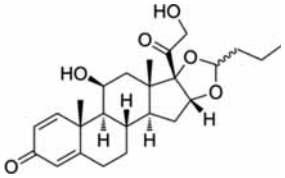
More than 100 different pharmaceuticals have been detected in a wide variety of environmental water samples including sewage flows, surface and groundwater, in concentrations ranging from ng L^{-1} to μg L^{-1} (Fick et al., 2009; Heberer, 2002; Khan et al., 2012; Monteiro and Boxall, 2010). Pharmaceuticals enter the aquatic environment via treated wastewater as a result of incomplete removal in wastewater treatment plants. The environmental fate and biological potency of pharmaceuticals released in the environment can be predicted on the basis of their special physicochemical and biological characteristics. Relevant processes determining the fate of pharmaceuticals in the environment include sorption to soils and sediments, complexation with metals and organics, chemical oxidation, sunlight photolysis, volatilization, and biodegradation (Heberer, 2002; Latch et al., 2003; Tixier et al., 2003).

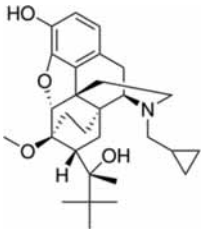
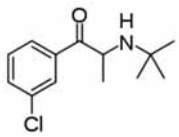
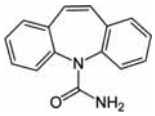
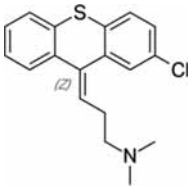
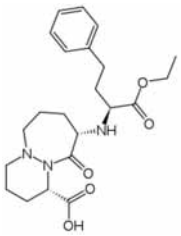
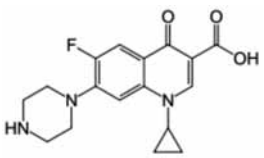
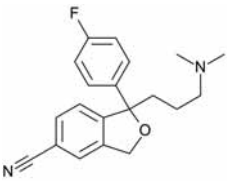
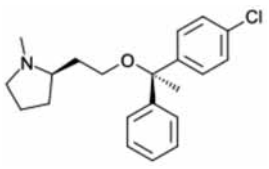
Phototransformation reactions have been suggested to play an important role in the elimination of some pharmaceuticals from surface waters (Doll and Frimmel, 2003; Latch et al., 2003; Tixier et al., 2003), and these reactions have been investigated in a vast number of studies (Andreozzi et al., 2003; Boreen et al., 2003; Calisto et al., 2011; Canudas and Contreras, 2002; Chen et al., 2012; Doll and Frimmel, 2003; Lam and Mabury, 2005; Piram et al., 2008; Vargas et al., 2003; West and Rowland, 2012; Yuan et al., 2009). The structural variability within pharmaceutical compounds requires assessing their photoreactivity on a case-by-case basis, as discussed by Boreen et al. (2003). A determination of the photolysis and photochemical half-lives of pharmaceuticals can improve our understanding of their environmental fate and facilitate modeling of the fate of pharmaceuticals in the aquatic system.

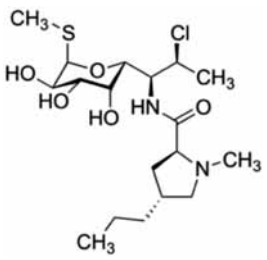
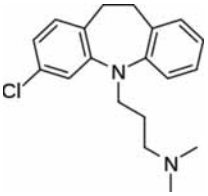
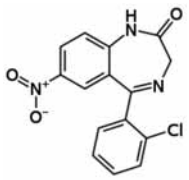
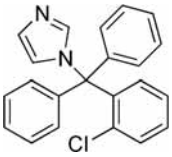
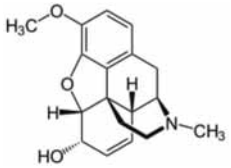
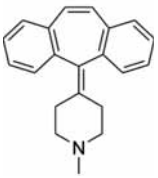
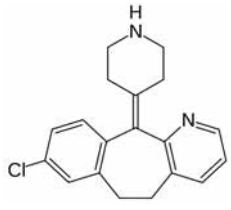
The aim of this work is to investigate the photofates of 88 pharmaceuticals under artificial ultraviolet (UV) and natural sunlight exposure. The pharmaceuticals included in the study were selected based on consumption and environmental relevance (Fick et al., 2009). Photostability and photofate data on 38 of these compounds under varying conditions (from pure water to solvents with different light sources) have been described in the literature previously (studies related to using photocatalysis for transformations of pharmaceuticals were not included in our literature searches) (see Table 1).

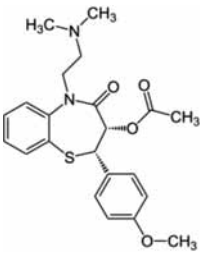
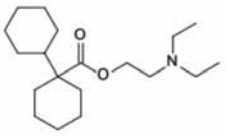
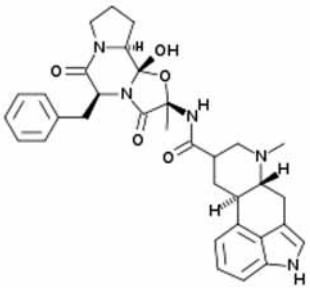
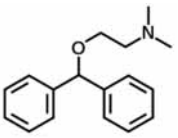
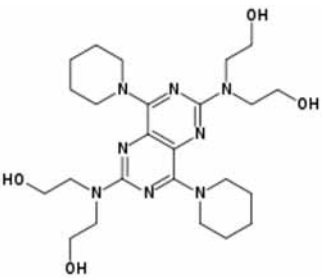
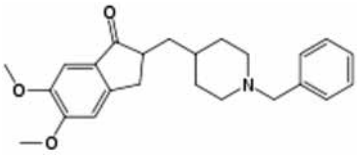
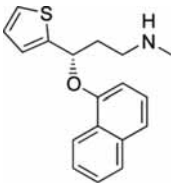
Table 1. *88 pharmaceuticals investigated.*

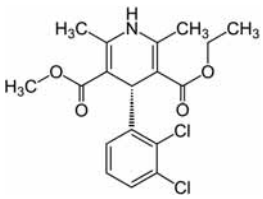
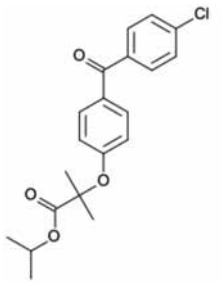
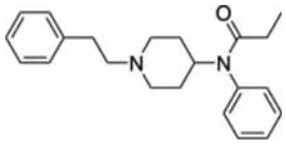
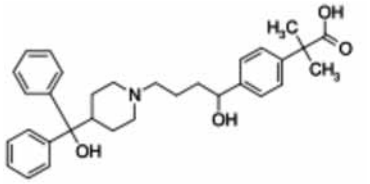
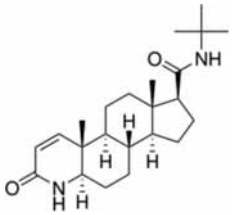
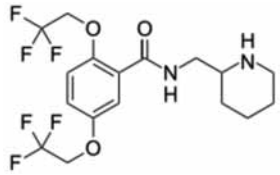
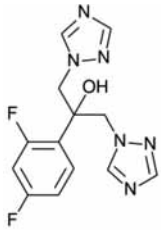
Compound	Structure	Molecular weight g/mol	Reference
Alfuzosin (Urological)		389.4	
Alprazolam (Psycholeptic)		308.8	(Calisto et al., 2011)
Amiodarone (Anti-arrhythmic)		645.3	
Amitriptyline (Antidepressant)		277.4	
Atenolol (Hypertension drug)		266.3	(Piram et al., 2008; Wols et al., 2013)
Atorvastatin (Statin)		558.6	(Lam and Mabury, 2005)

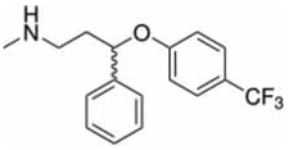
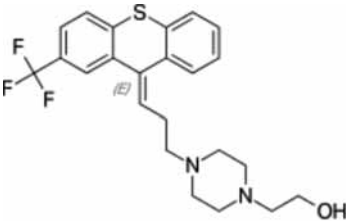
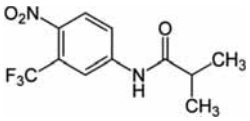
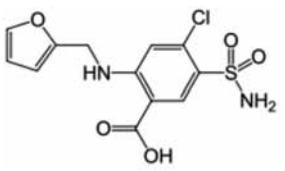
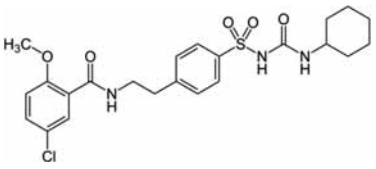
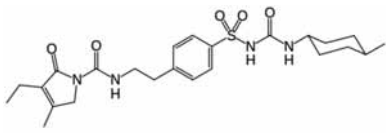
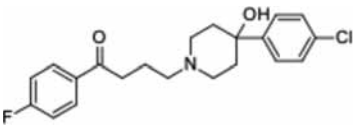
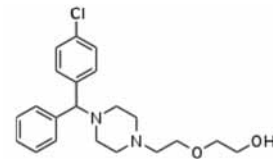
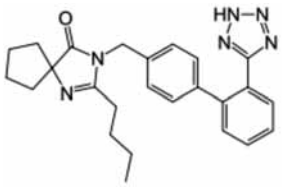
Atracurium (Muscle relaxant)		929.1	
Azelastine (Anti-histamine)		381.9	
Beclometasone (Anti-inflammatory corticoid)		521.0	
Biperiden (Anti- Parkinson)		311.5	
Bisoprolol (Hypertension drug)		325.4	(Piram et al., 2008)
Bromocriptine (Anti-Parkinson)		654.6	
Budesonide (Anti-inflammatory corticoid)		430.5	

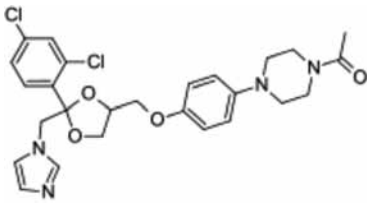
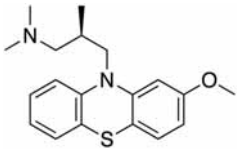
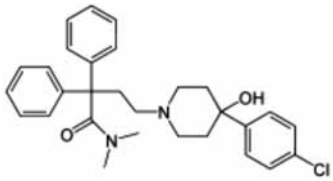
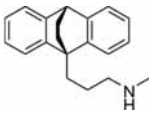
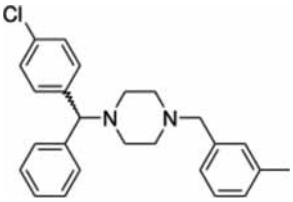
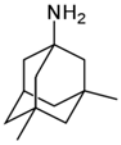
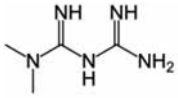
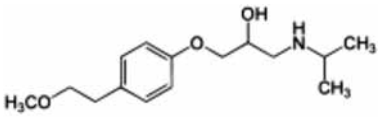
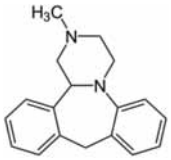
Buprenorphine (Analgesic)		467.6	
Bupropion (Antidepressant)		239.7	
Carbamazepine (Psycholeptic)		236.3	(Andreozzi et al., 2003; Lam and Mabury, 2005)
Chlorprothixene (Psycholeptic)		315.9	
Cilazapril (Hypertension drug)		417.5	
Ciprofloxacin (Antibiotic)		331.3	(Babic et al., 2013)
Citalopram (Antidepressant)		324.4	(Kwon and Armbrust, 2005)
Clemastine (Antidepressant)		343.9	

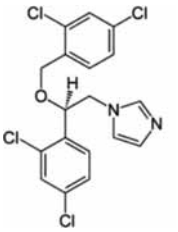
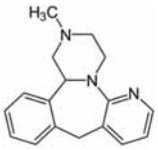
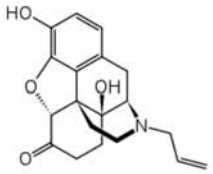
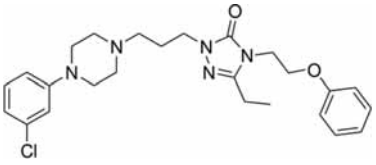
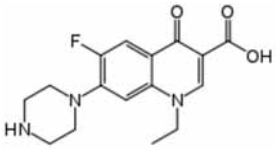
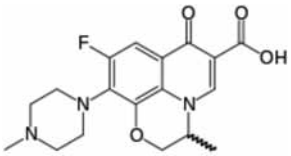
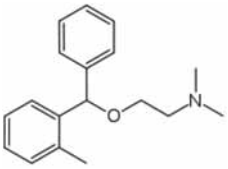
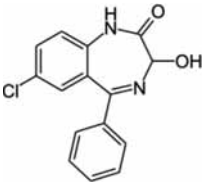
Clindamycin (Antibiotic)		424.9	(Wols et al., 2013)
Clomipramine (Antidepressant)		314.9	(Canudas and Contreras, 2002
Clonazepam (Psycholeptic)		315.7	
Clotrimazole (Antimycotic)		344.8	(Thoma and Kubler, 1996)
Codeine (Analgesic)		299.4	(Lin et al., 2014)
Cyproheptadine (Anti-histamine)		287.4	
Desloratadine (Anti-histamine)		310.8	

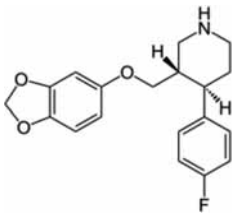
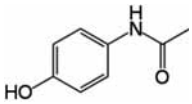
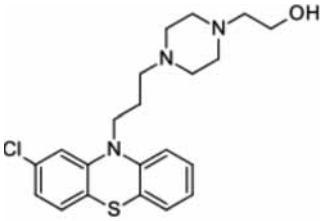
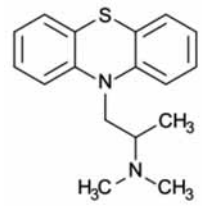
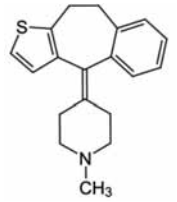
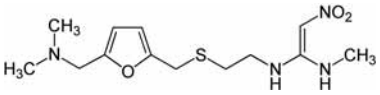
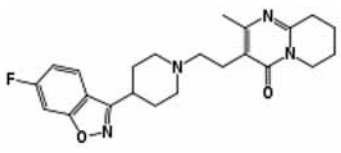
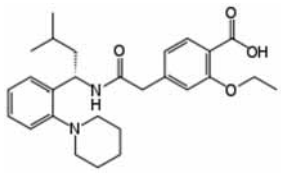
Diltiazem (Hypertension drug)		414.5	(Andrisano et al., 2001)
Dicycloverine (Drug for gastrointestinal disorders)		309.5	
Dihydroergotamine (Analgesic)		583.7	(Yuan et al., 2009)
Diphenhydramine (Anti-histamine)		255.4	(Chen et al., 2009)
Dipyridamole (Anti-thrombotic agent)		504.6	
Donepezil (Drug for the treatment of Alzheimer's disease)		379.5	
Duloxetine (Antidepressant)		297.4	

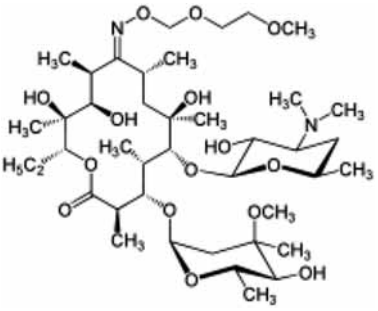
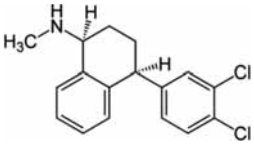
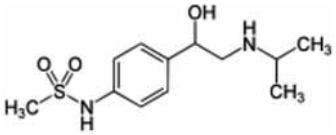
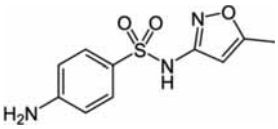
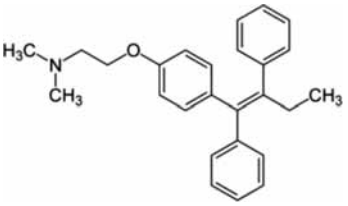
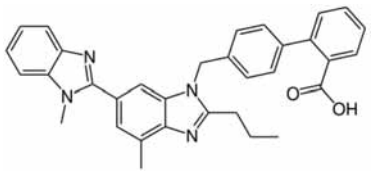
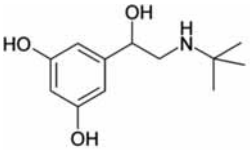
Felodipine (Hypertension drug)		384.3	
Fenofibrate (Cholesterol statin)		360.8	(Cermola et al., 2005)
Fentanyl (Analgesic)		336.5	
Fexofenadine (Anti-histamine)		501.7	
Finasteride (Urological)		372.5	
Flecainide (Anti-arrhythmic)		414.3	
Fluconazole (Antimycotic)		306.3	(Thoma and Kubler, 1996)

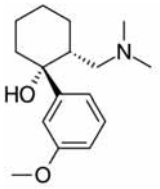
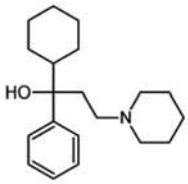
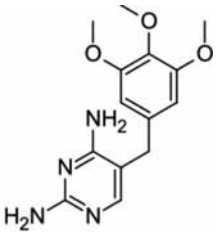
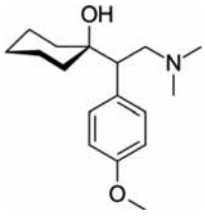
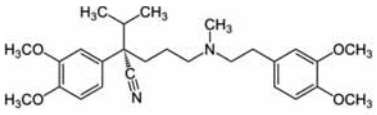
Fluoxetin (Antidepressant)		309.3	(Wols et al., 2013)
Flupentixol (Psycholeptic)		434.5	(Maquille et al., 2010)
Flutamide (Antiandrogen)		276.2	
Furosemide (Diuretic)		330.7	(Wols et al., 2013)
Glibenclamide (Anti-diabetic)		494	
Glimepiride (Anti-diabetic)		490.6	
Haloperidol (Psycholeptic)		375.9	
Hydroxyzine (Psycholeptic)		374.9	
Irbesartan (Hypertension drug)		428.5	

Ketoconazole (Antimycotic)		531.4	(Thoma and Kubler, 1996)
Levomepromazine (Antipsychotic drug)		328.5	(Vargas et al., 2003)
Loperamide (Antipropulsive)		477	
Maprotiline (Antidepressant)		277.4	
Meclozine (Anti-histamine)		390.9	
Memantine (Psycholeptic)		179.3	
Metformin (Anti-diabetic)		129.2	
Metoprolol (Hypertension drug)		267.4	(Piram et al., 2008; Wols et al., 2013)
Mianserin (Antidepressant)		264.4	

Miconazole (Antimycotic)		416.1	(Thoma and Kubler, 1996)
Mirtazapine (Antidepressant)		265.4	
Naloxone (Opioid overdose drug)		327.4	
Nefazodone (Antidepressant)		470	
Norfloxacin (Antibiotic)		319.3	(Babic et al., 2013; Wammer et al., 2013)
Ofloxacin (Antibiotic)		361.4	(Andreozzi et al., 2003; Wammer et al., 2013)
Orphenadrine (Anti-histamine)		269.4	
Oxazepam (Psycholeptic)		286.7	(Calisto et al., 2011; West and Rowland, 2012)

Paroxetin (Antidepressant)		329.3	(Wols et al., 2013)
Paracetamol (Analgesic)		151.2	(Peuravuori, 2012; Wols et al., 2013)
Perfenazine (Psycholeptic)		403.9	
Promethazine (Anti-histamine)		284.4	
Pizotifen (Analgesic)		295.4	
Ranitidine (Drug for peptic ulcer)		314.4	(Latch et al., 2003)
Risperidone (Psycholeptic)		410.5	
Repaglinide (Anti-diabetic)		452.6	

Roxithromycin (Antibiotic)		837	(Batchu et al., 2014)
Sertaline (Antidepressant)		306.2	
Sotalol (Hypertension drug)		272.4	(Piram et al., 2008; Wols et al., 2013)
Sulfamethoxazole (Antibiotic)		253.3	(Andreozzi et al., 2003; Lam and Mabury, 2005; Wols et al., 2013)
Tamoxifen (Anticancer)		371.5	(DellaGreca et al., 2007)
Telmisartan (Hypertension drug)		514.6	
Terbutaline (Adrenergic)		225.3	(Wols et al., 2013)

Tramadol (Analgesic)		263.4	(Rua-Gomez and Puettmann, 2013; Wols et al., 2013)
Trihexyphenidyl (Anti- Parkinson)		301.5	
Trimethoprim (Antibiotic)		290.3	(Sirtori et al., 2010)
Venlafaxine (Antidepressant)		277.4	(Rua-Gomez and Puettmann, 2013; Wols et al., 2013)
Verapamil (Hypertension drug)		454.6	(Lunn et al., 1994)

2. MATERIAL AND METHODS

2.1. Reagents and materials

The pharmaceuticals standards and internal standards (ISs) were purchased from Sigma Aldrich (Steinheim, Germany), Labscan Ltd. (Dublin, Ireland), and Cambridge Isotope Laboratories (Andover, MA, USA) (Grabic et al., 2012). Stock solutions of native pharmaceuticals (2.4 μg L^{-1}) and ISs (100 ng L^{-1}) were prepared in methanol and stored at -18 ºC.

Formic acid, used to acidify the mobile phases, was acquired from Sigma Aldrich. Methanol, acetonitrile, and sulphuric acid were purchased from Merck (Lichrosolv – hypergrade, Merck, Darmstadt, Germany). Ammonium acetate (analytical reagent grade) was purchased from Fisher Chemical (Leicestershire, UK) and ammonia hydroxide (25 wt%, Baker analyzed) was purchased from J.T. Baker (Deventer, Netherlands).

Water was purified (to > 18.2 MΩ cm resistively) using a MAXIMA HPLC ultrapure water system (ELGA, High Wycombe Bucks, UK). Working solutions (including calibration standards) of pharmaceuticals were diluted in Milli-Q water. Buffered Milli-Q water (pH = 7) was prepared as solution of 50 mM ammonium acetate and 5 mM ammonia hydroxide in Milli-Q water.

2.2. Water sample preparation

Solutions of selected pharmaceuticals were spiked into a 1 L bottle of buffered Milli-Q water to obtain a concentration of 1 μg L^{-1}. After spiking, 10 ml aliquots were taken from the 1 L bottle and transferred to Pyrex tubes with volumes of 12 ml.

2.3. Experimental setup

UV-exposure experiments were performed both in the laboratory with artificial UV light and under natural conditions with outdoor sunlight. Each series included dark control samples that were covered with several layers of aluminium foil and exposed to the same environmental conditions. All experiments were performed in triplicate. The relative standard deviation of the triplicates ranged from 1–30% in buffered Milli-Q under both conditions.

The laboratory exposure light source consisted of four mercury UV lamps, Philips TLK 40W/09N, equipped with filters to yield spectra as close as possible to sunlight in the UV-A range (300–400 nm). The irradiance intensity from the UV lamps at the exposure spot was 1.6 mW cm^{-2}. The water samples in the Pyrex tubes were placed on a RM5 "rocking/rolling action" apparatus (Assistent, Sondheim/Rhon, Germany). The temperature was kept at 23 °C with the use of a cooling fan.

The sunlight exposure experiments were performed in June 2012 in Umeå, Sweden, during the same period as the laboratory experiments. All of the test tubes were exposed to sunlight between 8 a.m. and 4 p.m. on the rooftop of a building at the Umeå University campus. The weather conditions during the experiment were excellent with clear skies. The weather conditions are available in Table 2.

The irradiated water samples and dark control samples were collected after 0.5, 1, 2, 4 and 8 h of irradiation. The samples from the Pyrex tubes were transferred to auto sampler vials immediately after exposure. All of the samples were stored in the dark (wrapped in aluminium foil) and cold (4 °C) before and after exposure.

Table 2. *Weather parameters during sunlight exposure experiment (13.06. 2012).*

Time (hour)	Temperature (°C)	Humidity (%)	Air pressure (hPa)	Wind (average) m/s	Solar radiation (Wm^{-2})
9:00 AM'	16.6	38	1002	4.8	581
10:00 AM'	16.9	35	1003	4.7	649
11:00 AM'	17.5	34	1003	4.9	702
12:00 PM'	18.9	31	1003	5.9	722
2:00 PM'	17.9	38	1003	7.4	547
4:00 PM'	18.1	37	1003	7.8	521

2.4. Liquid chromatography–mass spectrometry

The samples were analyzed for all 88 pharmaceuticals simultaneously with an in-line solid phase extraction (SPE) with a liquid chromatography tandem mass spectrometry system (MS/MS).

The liquid chromatography system consisted of a Surveyor LC-Pump (Thermo Fisher Scientific, San Jose, CA, USA), an Accela LC pump (Thermo Fisher Scientific), and a PAL HTC auto sampler (CTC Analytics AG, Zwingen, Switzerland) was configured as in-line SPE. The tandem mass spectrometer TSQ Quantum Ultra EMR (Thermo Fisher Scientific) was used as detector in this system.

The extraction of target pharmaceuticals from water was performed as in-line SPE on Oasis HLB column (2.1 mm i.d. × 20 mm, 15 μm particles, Waters, Sweden).

The analytes were consequently separated on Hypersil GOLD aQ column (2.1 mm i.d. × 50 mm, 5 μm particles, Thermo Fisher Scientific) with the same stationary phase guard column (2.1 mm i.d. × 50 mm, 5 μm particles, Thermo Fisher Scientific). A detailed description of the in-line SPE and MS/MS method has been reported elsewhere (Grabic et al., 2012; Khan et al., 2012; Lindberg et al., 2014).

2.5. Quantification

Isotope dilution and internal standard calibration were used for the quantification of the target compounds. The limit of quantification for the simultaneous analysis of the 88 pharmaceuticals was determined by the measuring water standard solution over a concentration range of 10 ng L^{-1} to 1000 ng L^{-1} as one half of the lowest calibration point in the linear range.

2.6. Half-live calculation.

We assumed that transformation of each compound followed pseudo-first-order kinetics (Calisto et al., 2011). The reaction rates k for each compound were calculated from the slope of the linear regression of the average concentration natural logarithm versus irradiation time. Half-life values (t ½) for each compound were calculated according to following formula:

$t_{½} = 0.693/k$,

where k is the reaction rate constant.

3. RESULTS

Of the 88 pharmaceuticals studied, 46 did not transform during 8 h of artificial UV and natural sunlight irradiation in buffered Milli-Q water (alprazolam, amitriptyline, atenolol, atracurium, azelastine, biperiden, bisoprolol, carbamazepine, cilazapril, citalopram, clemastine, clindamycine, clotrimazole, codeine, cyproheptadine, desloratadine, dicycloverine, diltiazem, diphenhydramine, fentanyl, fexofenadine, finasteride, flecainide, fluconazole, fluoxetine, glimepiride, hydroxyzine, irbesartan, loperamide, maprotiline, memantine, metformin, metoprolol, orphenadrine, paracetamol, pizotifen, risperidone, sertraline, sotalol, sulfamethoxazole, telmisartan, terbutalin, tramadol, trihexyphenidyl, trimethoprim, and venlavafaxin). For the remaining 42 pharmaceuticals, their half-lives were calculated to be in the range of 0.11 to 13.23 h (Table 3).

Table 3. *Kinetic parameters for the transformation of pharmaceuticals in buffered Milli-Q water under artificial UV and natural sunlight irradiation:*
r – correlation coefficient, k – the reaction rate constant, t ½ – half-life time (hour), LOQ – limit of quantification.

	Conditions					
	Half-lives significantly different					
Pharmaceutical	**UV**			**Sun**		
	r	**k**	**t ½**	**r**	**k**	**t ½**
Beclometasone	0.974	0.070	9.84	0.948	0.129	5.38
Bromocriptine	0.973	0.544	1.27	0.974	0.816	0.85
Budesonide	0.989	0.893	0.78	0.992	1.461	0.47
Buprenorphine	0.940	0.158	4.38	0.891	0.465	1.49
Chlorprothixene	0.972	0.580	1.20	0.989	0.755	0.92
Clomipramine	0.918	0.068	10.18	0.990	0.247	2.81
Clonazepam	0.974	0.091	7.65	0.925	0.136	5.11
Dihydroergotamne	0.983	0.226	3.07	0.912	0.286	2.43
Dipyridamole	0.922	0.145	4.78	0.905	1.122	0.62
Donepezil	0.954	0.085	8.12	0.964	0.184	3.77
Duloxetine	0.981	1.289	0.54	0.918	4.502	0.15
Fenofibrate	0.997	2.636	0.26	0.995	3.515	0.20
Flupetixol	0.976	0.415	1.67	0.973	0.563	1.23
Furosemide	1.000	4.003	0.17	0.993	3.220	0.22
Glibenclamide	0.991	1.854	0.37	0.993	4.520	0.15
Haloperidol	0.964	0.210	3.30	0.960	0.310	2.24
Ketoconazole	0.986	0.408	1.70	0.912	0.950	0.73
Meclozine	0.996	0.152	4.57	0.969	0.225	3.08
Miconazole	0.917	0.193	3.59	0.989	0.390	1.78
Mirtazapine	0.998	0.178	3.89	0.958	0.784	0.88
Nefazodone	0.911	0.052	13.23	0.991	0.191	3.64
Perphenazine	1.145	1.047	0.66	< LOQ*		
Promethazine	0.981	6.609	0.10	< LOQ*		
Ranitidine	0.995	0.401	1.73	0.994	0.600	1.16
Tamoxifen	0.933	1.355	0.51	0.986	1.748	0.40
Atorvastatin	1.000	5.687	0.12	0.976	0.673	1.03
Felodipine	0.956	0.698	0.99	0.998	0.197	3.51
Roxithromycin	0.927	0.197	3.51	0.943	0.156	4.45
	Similar half-lives					
	UV			**Sun**		
	r	**k**	**t ½**	**r**	**k**	**t ½**
Alfuzosin	0.985	1.652	0.42	0.999	1.401	0.49
Amiodarone	1.000	4.009	0.17	1.000	4.542	0.15
Ciprofloxacin	0.995	4.653	0.15	0.991	4.555	0.15

Levomepromazie	1.000	6.189	0.11	1.000	6.061	0.11
Naloxone	0.915	0.914	0.76	0.997	1.000	0.69
Norfloxacin	0.999	4.371	0.16	0.997	4.439	0.16
Ofloxacin	0.995	3.297	0.21	0.996	2.637	0.26
	Exposure to one light source					
	UV			**Sun**		
	r	**k**	**t ½**	**r**	**k**	**t ½**
Bupropion		Stable		0.963	0.111	6.23
Flutamide		Stable		0.809	0.041	16.72
Mianserin		Stable		0.895	0.062	11.16
Oxazepam	0.964	0.092	7.52		Stable	
Paroxetin		Stable		0.989	0.104	6.65
Repaglinide		Stable		0.941	0.082	8.41
Verapamil		Stable		0.918	0.084	8.24

* the degradation for marked compound was so fast that even in first point it was lower than LOQ.

3.1. Phototransformation of pharmaceuticals under artificial UV and natural sunlight

For 35 pharmaceuticals, phototransformation was observed for all of the tested conditions (Table 3).

Seven pharmaceuticals (alfuzosin, amiodarone, all fluroquinolon antibiotics (ciprofloxacin, norfloxacin and ofloxacin), levomepromazine, and naloxone) showed only small differences (< 20%) in their half-lives under artificial UV and natural sunlight exposure (Table 1). This group of compounds also showed fast transformations (1.2 < h).

Most pharmaceuticals (25 in total) transformed faster under natural sunlight than UV exposure (beclometasone, bromocriptine, budesonide, buprenorphine, chlorprothixine, clomipramine, clonazepam, dihydroergotamin, dipyridamole, donepezil, duloxetine, fenofibrate, flupentixol, furosemide, glibenclamide, haloperidol, ketoconazole, meclozine, miconazole, mirtazapine, nefazodone, perphenazine, promethazine, ranitidine, and tamoxifen; Table 3).

Only three pharmaceuticals (atorvastatin, felodipine, and roxithromycine) showed shorter half-lives under UV irradiation (Table 1). Further investigation is needed in order to explain these results.

Finally, seven pharmaceuticals (bupropion, flutamide, mianserin, oxazepam, paroxetin, repaglinide, and verapamil) were stable in buffered Milli-Q water under exposure to one of the two light sources (Table 3). Six pharmaceuticals were stable in buffered Milli-Q water under UV light and transformed under sunlight. Oxazepam was the only compound that was stable under sunlight exposure and transformed under UV light.

4. DISCUSSION

The results of our photolysis experiments suggest that 46 pharmaceuticals are stable in buffer water over the course of 8 h of artificial UV and natural sunlight. The photofates of the 13 of the 42 pharmaceuticals showing phototransformations have already been described in the literature (Table 1 and Table 4). Our results are in good agreement with the literature data for alprazolam, atenolol, bisoprolol, carbamazepine, citalopram, metoprolol, sotalol, tramadol, and venlafaxine (Table 1). Contrary to the data reported by Peuravuori, which showed a half-life of 1.72 h in pure water for paracetamol (Peuravuori, 2012), we found this compound to be stable.

The most well-studied pharmaceutical, carbamazepine, was considered to be resistant to photodegradation (Lam and Mabury, 2005) with an estimated half-life of 100 d (Andreozzi et al., 2003). Furthermore, alprazolam was found to slowly degrade by direct photolysis with a half-life of around 900 h (Calisto et al., 2011). The stability of citalopram depends on the pH of the aquatic solution. This compound degraded less than 0.5% at a pH of 5 and 7 during the 30 d exposure period under simulated sunlight and only moderately degraded in a pH = 9 buffer, with a half-life of 64 d (Kwon and Armbrust, 2005). The β-blockers atenolol, bisoprolol, metoprolol, and sotalol were found to be stable in purified water under experimental conditions, similar to our setup (Piram et al., 2008). UV spectral measurements revealed that those compounds absorbed only UV-C (Piram et al., 2008). Taking experimental conditions into consideration, the photostability reported for β-blockers in this work can only be attributed to direct photolysis and different behavior could be expected in environmental waters (Piram et al., 2008).

Table 4. *Half-lives for pharmaceuticals in different matrices irradiated with simulated or natural sunlight.*

Compound	Half-life	Matrix	Notes	Reference
Alprazolam (Psycholeptic)	900 hour	1% ACN in water	10 mg L^{-1}	(Calisto et al., 2011)
Atenolol (Hypertension drug)	Stable	Pure water	10 μg L^{-1}	(Piram et al., 2008)
Atorvastatin (Statin)	6.9 ± 0.1 hour	Pure water	10 μM	(Lam and Mabury, 2005)
Bisoprolol (Hypertension drug)	Stable 15–70 hour	Pure water STP water	10 μg L^{-1}	(Piram et al., 2008)
Carbamazepine (Psycholeptic)	100 day 115 ± 4 day	Pure water Pure water		(Andreozzi et al., 2003; Lam and Mabury, 2005)
Ciprofloxacin (Antibiotic)	~ 1.20 hour	Pure water	100 μg L^{-1} pH 4–8	(Babic et al., 2013)
Citalopram (Antidepressant)	1560 hour 336, 1032 hour	Sodium acetate buffer Surface water	Two different lake water	(Kwon and Armbrust, 2005)
Codeine (Analgesic)	2.5 hour	Surface water	20 μg L^{-1}	(Lin et al., 2014)

Diltiazem (Hypertension drug)	9.36 hour	0.1M phosphate buffer solutions	0.2 mg/ml	(Andrisano et al., 2001)
Diphenhydramine (Anti-histamine)	13 hour 87 hour	Phosphate buffer + nitrate Phosphate buffer + humic acid		(Chen et al., 2009)
Flupentixol (Psycholeptic)	1.20 hour	Pure water	0.5 mg ml	(Maquille et al., 2010)
Metoprolol (Hypertension drug)	Stable 20–48 hour	Pure water STP water	10 μg L^{-1}	(Piram et al., 2008)
Norfloxacin (Antibiotic)	~ 1.20 1.54 min 2.05 min	Pure water Pure water Lake water	100 μg L^{-1} pH4–8 10 μM, pH 7.8–7.9	(Babic et al., 2013; Wammer et al., 2013)
Ofloxacin (Antibiotic)	10.6 day 3.3 min 4.08 min	Pure water Pure water Lake water	10 μM, pH 7.8–7.9	(Andreozzi et al., 2003; Wammer et al., 2013)
Oxazepam (Psycholeptic)	15 hour 32 hour 70 hour	11% ACN in water Pure water Pure water + humic acid	10 mg L^{-1} 1 mg L^{-1}	(Calisto et al., 2011; West and Rowland, 2012)
Paracetamol (Analgesic)	1.73	Pure water		(Peuravuori, 2012)
Ranitidine (Drug for peptic ulcer)	0.58 hour	Pure water River water	10 μM	(Latch et al., 2003)
Roxithromycine (Antibiotic)	2.4–10 days	Pure water		(Batchu et al., 2014)
Sotalol (Hypertension drug)	Stable 4–8 hour	Pure water STP water	10 μg L^{-1}	(Piram et al., 2008)
Sulfamethoxazole (Antibiotic)	58 hour 1.5 ± 0.1 hour	Pure water Pure water		(Andreozzi et al., 2003; Lam and Mabury, 2005)
Tramadol (Analgesic)	16–20 days 3–4 days	Pure water River water	2 μg L^{-1}	(Rua-Gomez and Puettmann, 2013)
Venlafaxine (Antidepressant)	9–20 hour 1–2 hour	Pure water River water	2 μg L^{-1}	(Rua-Gomez and Puettmann, 2013)

Furthermore, our tramadol and venlafaxine results are consistent with previous works (Rua-Gomez and Puettmann, 2013; Sirtori et al., 2010). Thoma and Kubler (1996) showed that that clotrimazole and fluconazole undergo negligible photolytical degradation.

The fast transformation for fenofibrate shown in this study contradicts the fenofibrate data reported by Cermola et al., showing that irradiation with a solar simulator in distilled water caused about a 10% loss after 200 h (Cermola et al., 2005). On the other hand, the short half-lives of fluoroquinolones (< 0.3 h) are in agreement with published data. Previous work (Lam and Mabury, 2005) has shown that direct photolysis, rather than indirect photolysis involving interactions with natural water constituents, is likely to be the dominant photolysis

process for fluoroquinolones in most natural water. It was shown (Wammer et al., 2013) that the half-lives for norfloxacin and ofloxacin are dependent on pH. For example, norfloxacin had a half-life of 2 min at a pH of 7.7 but its half-life increases to approximately half an hour at a pH of 5. The results obtained from our experiments fall between these values, which makes sense since our measurements used a pH of 7, which is in between the pH used by Wammer et al.

Similar half-lives were already found for flupentixol and ranitidine (1.2 and 0.58 h, respectively) (Latch et al., 2003; Maquille et al., 2010).

According to literature (Batchu et al., 2014; Boreen et al., 2004; Lam and Mabury, 2005) the half-lives for atorvastatin and roxithromycin in pure water were around 7 hours and 2–10 d, respectively. Our data are significantly different from previously reported values.

The photofates for oxazepam, paroxetine, and verapamil were discussed in the literature (Calisto et al., 2011; Lunn et al., 1994; West and Rowland, 2012; Wols et al., 2013); see Table 2. The half-life value obtained in this study for oxazepam was 7.5 h, which is two times lower than previously reported by Calisto and Esteves (2009) of 15 h. It was shown (West and Rowland, 2012) that marked pharmaceuticals had half-lives around 32 h in pure water, increasing in presence of humic acid up to 70 h.

The phototransformation kinetic of an individual pharmaceutical can vary between studies due to different experimental designs. Thus, inter-comparisons of photostability for a large number of pharmaceuticals can be difficult to implement. Thus, this study, by presenting a dataset of 42 half-lives simultaneously determined, enables a unique comparison of photofates for a large number of pharmaceuticals.

4.1. Difference artificial UV and natural sunlight

Twenty-five of the studied pharmaceuticals showed faster transformations under natural sunlight than under artificial UV exposure. These results can be explained by a higher energy flow under the natural sunlight experiment (from 521 to 722 W m^{-2}) than under the laboratory experiment (16 W m^{-2}). Unfortunately, the natural sunlight intensity was measured in whole irradiance spectra and is not fully comparable with the solar imitation by the UV lamp that we used. However, the faster degradation of the 25 studied pharmaceuticals under sunlight exposure can be attributed to much higher energy flow. Since we had a constant pH of 7 and no other varying environmental conditions, except the temperature that was lower for natural sunlight exposure (18 °C versus 23 °C), the higher transformation under natural sunlight cannot be explained by other differences in environmental conditions.

Conclusion

The phototransformation of pharmaceuticals has been studied in buffer water under artificial UV and natural sunlight. The photofates under solar simulation or natural sunlight exposure for 50 of the 88 studied pharmaceuticals have not been previously described. The results showed that 46 of the pharmaceuticals were stable and did not transform over the course of 8 h of exposure in buffer under both exposure conditions. The half-lives of 42 compounds were calculated. Thirty-five pharmaceuticals showed phototransformations under both irradiation setups, oxazepam transformed only under solar simulation UV, and six other pharmaceuticals transformed only under natural sunlight. The rate of photolysis was slower under UV exposure for 25 of the pharmaceuticals, probably due to the higher energy flow during the natural sunlight exposure. This study has confirmed that phototransformation reactions can play an important role in the elimination of pharmaceuticals from surface waters.

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The synergistic effect of advanced oxidation processes to eliminate resistant chemical compounds☆

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ABSTRACT

The aim of this work is the study of application of the synergistic effects of various advanced oxidation processes (AOPs) in waste water treatment. Attention is paid to decomposition of chemical compounds resistant to biodegradation. These chemical compounds are commonly based on pharmaceutical products, pesticides, surfactants and dyes.

In our contribution, the synergistic effect of simultaneously applied photocatalyst and low temperature atmospheric plasma or UV light on decomposition of model chemicals (Acid Orange 7, Hydrocortisone, Verapamil hydrochloride) was studied. TiO_2 was used as the photocatalyst. Model chemicals with photocatalytic active TiO_2 were exposed to atmospheric plasma discharge (Gliding Arc) or UV source in order to improve generation of active hydroxyl groups and oxidation processes. The relations between different effects of AOPs during chemicals decomposition process were analyzed.

It was observed that decomposition of model chemicals is strongly improved by synergistic effect of: (i) photocatalytic reaction occurring on photocatalyst TiO_2, (ii) presence of oxidative radicals, (iii) presence of wide-range UV.

1. Introduction

Organic contaminants are of special concern, due to their large variety and high consumption over the past years. High toxicity and resistivity to biodegradation of chemical resistant organic compounds like pharmaceuticals, pesticides, hormones present in municipal and industrial wastewater pose a threat to the environment and human and animal health and may also affect aquatic organisms in an unpredictable way.

Water pollution with resistant chemical organic compounds can be attributed to several sources, such as emission from production sites due to inadequate treatment of manufacturing effluents, direct disposal of unused medicine and drug-containing waste, human and animal medical care and/or industry. The presence of low concentrations of resistant organic compounds and their transformation products in water has been detected, clearly showing that some of them cannot be eliminated during wastewater treatment [1–5].

Advanced oxidation processes (AOPs) defined by Glaze et al. [6] as "near ambient temperature and pressure water treatment processes which involve the generation of hydroxyl radicals in sufficient quantity to effect water purification" have been intensively investigated as a tool to decompose the resistant chemical compounds. AOPs proceed along one of the two possible routes: (i) oxidation with O_2 in temperature ranges intermediate between ambient conditions and those found incinerators wet air oxidation processes and (ii) the use of high energy oxidants such as ozone and H_2O_2 and/or photons that can generate highly reactive species – hydroxyl (•OH) radicals. Relative oxidation power of chosen oxidative species is summarized in Table 1 [7].

The mostly investigated AOPs in wastewater treatment consist of UV/peroxide and ozone/peroxide reactions. AOPs, including UV/H_2O_2 and ozone/H_2O_2 require substantial chemical addition and residual H_2O_2 quenching, which represents a significant portion of their operational costs [8,9]. Hence, emerging technologies providing viable alternative are required.

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Table 1
Relative oxidation power of chosen oxidative species [7].

Oxidative species	Relative oxidation power
Chlorine	1.00
Hypochlorous acid	1.10
Permanganate	1.24
Hydrogen peroxide	1.31
Ozone	1.52
Atomic oxygen	1.78
Hydroxyl radical	2.05
Positively charged hole on TiO_2	2.35

It can be seen in Table 1 that TiO_2 and hydroxyl radicals are one of the strongest oxidants. Their relative oxidation power is almost twice higher than the oxidation power of ozone. Due to photocatalytic processes TiO_2 is capable for oxidation of a wide range of organic compounds into harmless compounds such as CO_2 and H_2O [10].

Photocatalytic processes on TiO_2 (TiO_2/UV) are based on generation of electron–hole pairs by the UV irradiation. The separated electron/hole reach the surface where they can cause generation of highly reactive intermediates from matters adsorbed on the surface [11–13]. The advantage of the photocatalytic AOP is the operation at the ambient conditions, the lack of mass transfer limitations. Moreover, TiO_2 is a cheap, readily available material and photogenerated holes highly reactive [14,15].

Emerging technologies such as atmospheric non-thermal plasma technology provide viable alternatives to technologies including UV/H_2O_2 and ozone/H_2O_2 processes. The primary benefit of the atmospheric non-thermal plasma is the ability to generate UV light, ozone and hydroxyl radicals without chemical addition or the use of UV lamp which require cleaning and are hindered by high turbidity and matrix absorbance [8,9]. This type of plasmas has been reported also for the degradation of non-biodegradable organic compounds [16,17].

As it was mentioned above, photocatalytic reaction on TiO_2 occurs in the course of UV irradiation and UV light [12] is also generated by plasma during atmospheric non-thermal plasma processes. Moreover, production of ozone and oxidative species during non-thermal plasma process can highly increase the efficiency of TiO_2 based AOPs. Thus, the connection of atmospheric non-thermal plasma with TiO_2 photocatalysis can provide very promising AOP system for wastewater treatment.

The aim of this paper is to describe our first results of resistant chemical compounds decomposition by synergy of oxidation system based on combination of Atmospheric Plasma Discharge/Photocatalytic active material (APD/TiO_2 photocatalyst).

2. Experimental

2.1. Decomposed chemicals

- Dye: Acid Orange 7 – AO7 ($C_{16}H_{11}N_2NaO_4S$) - (sodium 4-[(2E)-2-(2-oxonaphthalen-1-ylidene) hydrazinyl] benzenesulfonate).
- Hormone: Hydrocortisone – ($C_{21}H_{30}O_5$) - (11β)-11,17,21-trihydroxypregn-4-ene-3,20-dione).
- Drug: Verapamil hydrochloride – ($C_{27}H_{38}N_2O_4$. HCL) - benzenacetonitrile, α-[3-[[2-(3, 4-dimethoxyphenyl)ethyl] methylamino]propyl]3, 4-dimethoxy-α-(1-methyl)-, monohydrochloride.

2.2. Experimental setup

The investigation of the synergistic effect and the degradation of the dye AO7 and drug Verapamil Hydrochloride was realized by AOP system based on combination of non-thermal atmospheric plasma discharge (APD) and TiO_2 photocatalyst schematically depictured in Fig. 1.

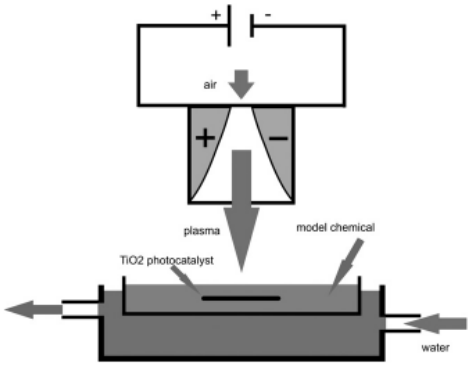

Fig. 1. AOP system for decomposition of model chemical compound based on APD/TiO_2 photocatalyst.

TiO_2 powder Aeroxide Degussa P25 sedimented on microscopic glass was used as TiO_2 photocatalyst. Prepared films contain 0.5 mg of sedimented Degussa P25 per cm^2 and its thickness was 5 μm. The type of used APD was Gliding Arc and it was generated by power source 750 W between two diverging copper electrodes of half-circle shape with minimal gap 5 mm. The device cover exceeds 5 mm over the edge of electrodes. The power source is operated at frequency 50 Hz. Compressed air was used as carrier working gas. The overpressure of the air was maintained at approximately 600 kPa. The air flow was constant 0.86 $m^3\ h^{-1}$ controlled by an air flowmeter. Description in more details is in Ref. [18].

The distance between APD device and surface of the treated chemical was fixed at 10 cm where the measured temperature was 40 °C. The cooling system using water as cooling medium was used during plasma treatment process to avoid the heating of the decomposed model chemical.

Also, UV light emitted by APD was established. Spectra of the gliding arc discharge were taken by spectrometer Ocean Optic. Measurement is fully described in [18].

To investigate potential application of AOPs such as photocatalysis for decomposition of hormone, experiments with decomposition of steroid hormone hydrocortisone were realized using TiO_2 thin films as photocatalytic active material (PECVD-TiO_2 photocatalyst) and UV irradiation, Fig. 2. TiO_2 thin films were deposited by Plasma Enhanced Chemical Vapour Deposition (PECVD) fully described in Ref. [14].

Glass substrate with a PECVD-TiO_2 photocatalyst (4.5 cm^2) was put in 25 ml aqueous solution of hydrocortisone with concentration of

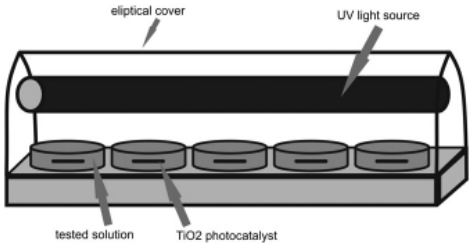

Fig. 2. AOP system for decomposition of model chemical compound based on UV lamp/TiO_2 photocatalyst.

12.5 mg/l and illuminated by the UV-A lamp (Philips TLD 15 W/05 with its most intensive line at 365 nm) placed 10 cm above the samples. The temperature of the samples treated by UV irradiation was kept at 22 °C.

2.3. Analyses of the model chemical decomposition

The synergistic effect of the AOPs was investigated on decomposition of AO7. The decomposition rate of AO7 was calculated from changes of its concentration after treatment by AOP system using UV/VIS spectrophotometry (in detail described in Ref. [14]). 25 ml of water solution of tested chemical with initial concentration of 0.035 mmol/l was treated by AOPs system. The decrease of concentration after treatment was measured by absorbance according to Lambert–Beer absorption law [19].

The photocatalytic activity of the used TiO_2 photocatalysts was derived from the decrease of concentration of aqueous solution of the AO7, assuming the Langmuir–Hinshelwood law for degradation according to the pseudo-first-kinetic order. The experimental method and determination of the apparent kinetic constant r are in full details described in paper [20].

The concentration of tested hydrocortisone solution was measured using the spectrophotometer UV/VIS (916 GBC). A decrease in the hydrocortisone concentration caused by pure photocatalytic effect (i.e. after deduction of effect of separated UV-A irradiation) after 20 h of illumination was evaluated.

The concentrations of Verapamil hydrochloride in the reaction mixtures were analyzed by in-line SPE liquid chromatography with tandem mass spectrometry. C18 column Hypersil Gold (20 mm × 2.1 mm i.d, 12 um particles) from Thermo Fisher Scientific was used for the separation of target analytes.

It was supposed that degradation of Verapamil hydrochloride follows the first order kinetics. The reaction rates k for Verapamil hydrochloride was calculated from the slope of linear regression of concentration logarithm versus time. Half-life values (t ½) for each compound were calculated according to formula (Eq. (1)):

$$t\,½ = 0.693/k \quad (1)$$

3. Results and discussion

3.1. Testing of synergistic effect of AOP system on decomposition of dye

To check the presence of the UV-radiation suitable for electron–hole excitation in the photocatalytic material an overview spectrum of the used APD was taken (Fig. 3). As can be seen intense bands in the wave-range between 290 and 390 nm are present in the spectrum, the energy of these bands is higher than the band-gap energy of TiO_2 thus these radiation bands are eligible for excitation of charge carriers participating in advanced oxidation processes. The typical OH band is obvious around the value of 310 nm [18,21]. For this reason the APD was used as a source of UV light and/or oxidative radicals in AOP system.

The AOP system was tested in various arrangements to investigate its synergistic effect on decomposition of model chemicals. Results of these investigations are summarized in Fig. 4. Each of columns corresponds to experimental conditions with different arrangements of AOP system. During these experiments AO7 was treated by APD during 60 min. In some experiment, the dish with the model dye AO7 was covered by glass with high absorption of UV. Because the glass also prevents the radicals produced by the APG to reach the surface of the AO7 solution mainly visible light can act in AOPs. In another experiment, the AO7 solution was covered by quartz glass which transmits wide-range UV but again prevents oxidative radicals. Finally, the AO7 solution was treated by AOP system without any cover thus both the wide-range UV and oxidative radicals reach the surface of AO7 and participate in AOPs.

Firstly, AO7 was covered by glass without the presence of TiO_2 photocatalyst. This arrangement is presented by column (a) in the Fig. 4. As expected, while the glass strongly absorbs the UV column (a) performs AO7 solution irradiated mainly by visible light and ambient background. It is evident that visible light on its own cannot decompose organic dye and it has to act in synergy with the other agents.

Column (b) shows similar conditions as the first one but in addition the TiO_2 photocatalyst immersed into the AO7 solution was used. The TiO_2 photocatalyst is illuminated by mainly visible light and again the activity of the TiO_2 photocatalyst and also decomposition rate of AO7 are relatively low.

During other experimental conditions - column (c), the dish with the AO7 was covered by quartz glass without presence of TiO_2 photocatalyst. Wide-range UV transmits quartz glass and it participates in decomposition process. In this case, decomposition rate slightly increased compared to the (a) and (b).

The decomposition of AO7 increased rapidly under experimental conditions represented by columns (d), (e) and (f), Fig. 4. To study the effect of the photocatalysis but without contribution of reactive radicals produced by the APD, the dish with the AO7 was covered by quartz glass and treated by APD with the presence of TiO_2 photocatalyst (d). It shows that if TiO_2 photocatalyst is irradiated by wide-range UV, decomposition rate of AO7 strongly increased.

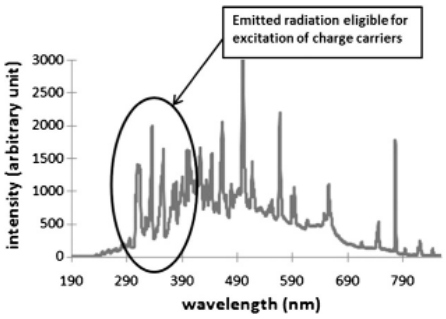

Fig. 3. Overview spectrum of the gliding arc discharge (air flow 50 SCFH, fibre was fixed at gas flow axis against the gas inlet in the distance of 3 cm from the device cover) [19].

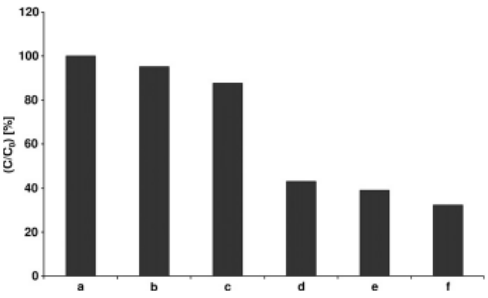

Fig. 4. Decomposition rate comparison of AO7 treated with different experimental conditions (a) AO7 covered by glass without the presence of TiO_2 photocatalyst, (b) AO7 covered by glass with the presence of TiO_2 photocatalyst, (c) AO7 covered by quartz glass without the presence of TiO_2 photocatalyst, (d) AO7 covered by quartz glass with the presence of TiO_2 photocatalyst, (e) AO7 without cover and without the presence of TiO_2 photocatalyst, (f) AO7 without any cover and with the presence of TiO_2 photocatalyst.

Similar AO7 concentration decrease displays column (e). In this experimental arrangement the AO7 was treated by APD without cover and without the presence of photocatalyst. Again, decomposition rate increased due to synergistic effect of wide-range UV and oxidative radicals generated in plasma discharge.

The best results of the decomposition rate of AO7 were reached under experimental conditions presented by column (f) where AO7 was treated by APD without any cover and with the presence of TiO_2 photocatalyst. It indicates that synergistic effect of simultaneous incidence of oxidative radicals and wide range of UV light (mainly high-intensity) which also act as activator of photocatalytic process occurring on TiO_2 photocatalyst results in enhancement of the decomposition process.

The influence of treatment time on decomposition of AO7 (Fig. 5) was studied in arrangement of AOP system when treated AO7 solution is not covered and plasma is directly in interaction with AO7.

Decrease of AO7 concentration after treatment by AOP system with increasing treatment time is evident. When AO7 solution is treated by APD without TiO_2 photocatalyst, decomposition rate in the first 30 min of treatment process is lower than with TiO_2 photocatalyst. However, after 60 min of treatment process concentration decrease is nearly the same. To compare the effect of sol TiO_2 photocatalyst without plasma influence, AO7 was decomposed using TiO_2 photocatalyst and irradiation by UV lamp during 30 min (Fig. 5). It was observed, that decrease of AO7 concentration is lower comparing to decrease of concentration of AO7 treated by AOP system (APD + TiO_2 photocatalyst) or sol APD. These results prove positive influence of synergistic effect of APD and TiO_2 photocatalyst on decomposition of AO7.

3.2. Decomposition of pharmaceuticals

Decomposition of hydrocortisone using UV irradiation and PECVD-TiO_2 photocatalyst was performed to verify effect of AOPs based on photocatalysis on decomposition of hormone, Fig. 6. UV/VIS spectra of hydrocortisone solutions are illustrated in Fig. 7. PECVD-TiO_2 photocatalysts with different photocatalytic activity were chosen to investigate photocatalytic effect on decomposition of hydrocortisone. The influence of deposition conditions on PECVD-TiO_2 photocatalyst properties (chemical composition, structure, morphology, photocatalytic activity) is described in detail in our previous paper [14].

Influence of UV irradiation and photocatalytic activity on decomposition of hydrocortisone is evident (Fig. 5). Decreasing concentration (27%) of hydrocortisone solution was observed after irradiation only by UV without the presence of PECVD-TiO_2 photocatalyst. Slightly better effect on decomposition of hydrocortisone had UV irradiation in combination with PECVD-TiO_2 photocatalyst with lower photocatalytic activity (concentration decrease was between 33% and 43 %). Two of PECVD-TiO_2 photocatalyst demonstrate significant decrease of hormone concentration in water. These samples were also very active in decomposition of AO7. Fig. 6 also illustrates good correlation between decomposition rate of AO7 and decomposition of hydrocortisone. The molecule of hydrocortisone has two carbon–carbon double bonds and a carbonyl group that may be subjected to hydrogenation or to hydrogenolysis, respectively [22]. Thus the reactive oxygen species generated during the photocatalytic process can diffuse from TiO_2 photocatalyst surface to react in solution with hydrocortisone and it might result to decrease of hydrocortisone concentration [23].

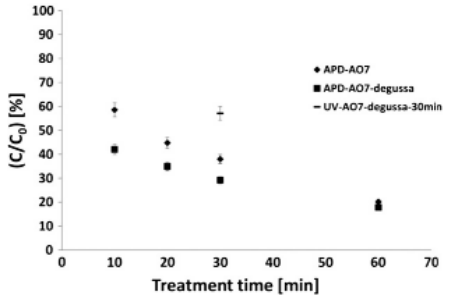

Fig. 5. The influence of treatment time on decomposition of AO7.

Previous experiments showed good efficiency of photocatalysis based AOPs on decomposition of organic dye and hormone. First tests with the Verapamil hydrochloride were realized to investigate possible application of AOPs system based on APD/TiO_2 photocatalyst for decomposition of drugs.

Half-life time observed for Verapamil hydrochloride treated without TiO_2 photocatalyst was 1.5 h, whereas half-life time of Verapamil hydrochloride treated with TiO_2 photocatalyst was almost twice higher (0.8 h). The time of the decomposition of the Verapamil hydrochloride using AOP system based on APD/TiO_2 photocatalyst is much lower compared to the decomposition time of the hydrocortisone using system with UV lamp (20 h). Although the mechanism of the decomposition process is different for these chemicals it indicates that synergistic effect of processes using APD/TiO_2 photocatalyst can strongly positively influence decomposition of pharmaceuticals and accelerate it. However, exact mechanism is unclear. It is known, that during oxidative decomposition of organic chemical several harmful intermediates can be formed [24]. Hence, other analyses are needed.

4. Conclusion

AOP system based on combination of non-thermal atmospheric plasma discharge and photocatalytic active material is able to decompose resistant organic chemical compounds such as dye (AO7) and drug (Verapamil hydrochloride).

Potential application of AOPs such as photocatalysis was successfully used for decomposition of hormone hydrocortisone.

It was observed that decomposition of the chemicals is strongly improved by synergistic effect of: (i) photocatalytic reaction occurring on TiO_2-photocatalyst, (ii) presence of oxidative radicals, (iii) presence of wide-range UV.

Observed results open a new very promising possibility to decompose chemical organic compounds resistant to biodegradation.

Acknowledgment

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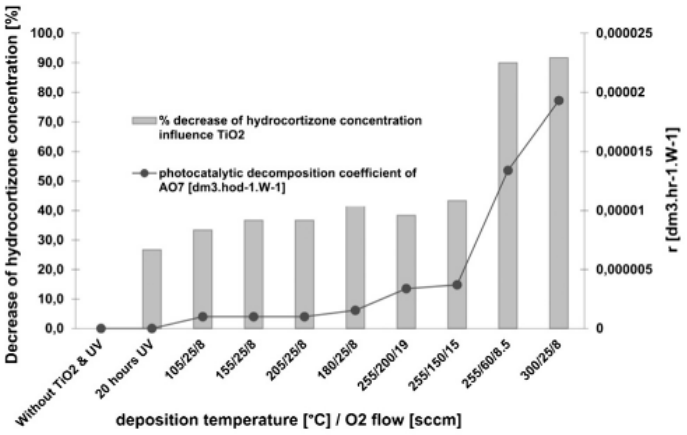

Fig. 6. The degradation of hydrocortisone treated by UV light in the presence of photoactive PECVD-TiO_2 thin films compared with photocatalytic activity of the used photocatalysts.

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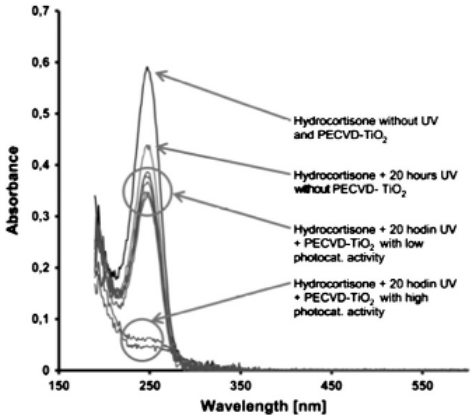

Fig. 7. UV/VIS spectra of hydrocortisone solutions.

CHAPTER 5

GENERAL DISCUSSION

There is an increasing concern about PPCPs in urban wastewater and the aquatic environment. These products and their metabolites enter the waste stream after excretion and disposal. They are not efficiently removed by WWTPs where limited removal yields are often observed (Herberer, 2002). Despite a growing number of studies pertaining to this subject, little information is available regarding environmental transformations and seasonal variations in concentration, fate, and the effects of these compounds in aquatic media (Calisto and Esteves, 2009; Brain et al., 2004; Sui et al., 2011).

Our main goal was to assess the seasonal occurrence of PPCPs and their removal in a WWTP over one year. This is important because changes in the incoming PPCPs have consequences in the final effluent, which further affects the aquatic organisms. The stability of these compounds under different conditions is important in determining their fate in water matrices. Because there is a lack of data on their photostability, our studies focused on their degradation under different scenarios to improve our understanding their behavior.

Seasonal changes in monitoring were lengthy, where a large number of samples were collected throughout the year. Hence, proper storage was critical for accurate analysis of PPCPs. Biological activity can continue after a sample has been taken. In addition, biodegradation of some biologically active compounds can occur during handling and storage (Castiglioni et al., 2006). Moreover, in cases where immediate sample analysis was difficult or impossible (e.g., remote areas) or the sampling was intended to be completed over longer periods, storage conditions became highly relevant (Vanderford et al., 2011; Barcelo and Alpendurada, 1996). For those compounds that easily degraded, reliable determination largely depended on proper sample storage conditions. Various processes such as microbial degradation, chemical reactions, volatilization, or adsorption can occur over relatively short sample storage times, resulting in low analyte recoveries. Proper sample storage was necessary to obtain reliable results.

The impact of long-term storage of wastewater samples on PPCP analysis is described in Chapter 2. Short-term stability (7 days) of 124 PPCPs in wastewater was checked by comparing samples stored at 4 °C and -18 °C. The long-term stability (60 and 120 days) was evaluated after storage at -18°C. The results of this study showed that multi-residue analysis of wastewater samples was always a compromise concerning not only the analytical method and extraction procedure, but also the choice of storage conditions. Because little attention is given to the storage factor, the stability of target pharmaceuticals in samples should be tested under the planned storage conditions beforehand.

The concentrations of organic pollutants in the influent and the effluent of WWTPs are reported in many studies (Bueno et al., 2012; Gobel et al., 2007; Gracia-Lor et al., 2012; Gros et al., 2010; Jelic et al., 2011; Kasprzyk-Hordern et al., 2009; Lajeunesse et al., 2012; Lindberg et al., 2005; Senta et al., 2013; Sui et al., 2011; Vieno et al., 2005; Xu et al., 2007; Yu et al., 2013; Nakada et al., 2007). Some of these studies recently demonstrated that the concentrations of PPCPs in urban wastewater and its treated effluents are subject to significant seasonal changes (Valcarcel et al., 2013; Yu et al., 2013). However, seasonal variations and their impacts on removal of most pharmaceuticals are poorly understood (Verlicchi et al., 2012). Seasonal variation may depend upon either societal factors (production, consumption, excretion) or environmental factors (solar irradiance, biodegradation, precipitation, temperature, etc.) (Bueno et al., 2012; Vieno et al., 2005; Yu et al., 2013).

Most PPCPs are not eliminated sufficiently in WWTPs (Fatta-Kassinos et al., 2011; Kasprzyk-Hordern et al., 2009; Castiglioni et al., 2006; Gracia-Lor et al., 2012; Santos et al., 2009; Vieno

et al., 2007). REs in WWTPs depend on several factors, such as physicochemical properties of the organic pollutants, climatic conditions such as temperature and sunlight intensity, and the working conditions in the plant such as operating temperature, redox conditions, solids retention time, and hydraulic retention time (Clara et al., 2005; Vieno et al., 2005; Le-Minh et al., 2010). Therefore, the RE can vary significantly from plant to plant and within a plant at different times (Vieno et al., 2007; Santos et al., 2009). Data provided on PPCP concentrations and REs in this study will add further relevant information for adequate assessment of risk of aquatic organisms' exposures.

Both influent and effluent concentrations and overall removal rates observed in this study varied highly among individual PPCPs. Seasonal dependence was also observed for most PPCPs in this study. Based on the concentration changes in wastewater over one year, PPCPs can be divided into three classes.

The first class was more concentrated in the influent during the winter (December through February), and included antibiotics, antidepressants, and lipid regulators. Seasonal fluctuation could be caused by consumption patterns (Coutu et al., 2013). Some antibiotics (ATBs) studied (azithromycin, clarithromycin, and erythromycin) are used to treat respiratory tract infections, which commonly occur in winter and early spring. Rosuvastatin and atorvastatin (statin drugs belonging to this class) are used in combination with exercise and diet for weight loss and treatment of high cholesterol to prevent cardiovascular disease. Because blood lipids in patients tend to increase in winter, higher concentrations of these pharmaceuticals in winter is expected (Ockene et al., 2004).

The second class was more concentrated in the influent during the summer, and included antihistamines (meclozine and fexofenadine) and UV filters (2-phenylbenzimidazole-5-sulfonic acid (PBS) and benzophenone-3 (BP3)). Again, the data likely reflect consumption patterns. Antihistamines are used for relief from physical symptoms associated with seasonal allergic rhinitis, which occurs more frequently during early summer. The UV filters also showed obvious inter day variations. Sunscreen products are used more frequently during the summer, but UV filters are also present in many daily-use products such as skin creams, cosmetics, hair sprays, body lotions, etc., and can be found all year long.

The third class did not fluctuate considerably in the influent as the seasons changed, and included psychiatric drugs (carbamazepine, oxazepam, and memantine). As with the other two classes, the influent concentrations reflect their consumption patterns. These pharmaceuticals are usually used to treat chronic diseases (epilepsy, naturopathic pain, and moderate-to-severe Alzheimer's disease). Higher concentrations of these three drugs were found in the effluent than in the influent during winter season.

Results of the studies (Chapter 3) shows that the concentrations of PPCPs in municipal wastewater were subject to considerable seasonal variations. To the best of our knowledge, our study is the first to report seasonal changes in norfloxacin, ciprofloxacin, levofloxacin, oxazepam, mirtazapine, sertraline, memantine, fexofenadine, meclozine, rosuvastatin, and atorvastatin concentrations.

The overall removal rates observed in this study (Chapter 3) varied strongly between individual pharmaceuticals. Based on the RE tendencies in WWTPs, PPCPs could be divided into three groups: high RE (50% to 100%), low RE (lower than 50%) and variable RE. Unfortunately, it was difficult to establish a general trend for some therapeutic or structurally similar groups of PPCPs. For example, sotalol and atenolol belong to the same therapeutic group (anti-hypertensive drugs) and showed similar poor elimination during the year, but another member of this class, bisoprolol, exhibited much better removal in the summer. UV filters benzophenone-1 (BP1) and PBS showed differences in REs. While BP1 had REs ranging from 73% to 100% during the year, PBS showed poor RE (lower than 50%) throughout the

year. To the best of our knowledge, this is the first detailed study showing seasonal variations of the REs for PBS, verapamil, isradipine, and bisoprolol.

The removal mechanisms are likely to be biodegradation and sorption (Ternes et al., 2004). The relatively low REs for most PPCPs in winter months might be caused by lower microbial activities (Hedgespeth et al., 2012; Vieno et al., 2005). Degradation is assumed to be a minor side reaction and will be significantly reduced at lower temperatures. The negative removal values for some compounds (Jelic et al., 2011; Gracia-Lor et al., 2012), could be related to this phenomenon. Most target compounds showed high REs during the summer and the low or negative ones in the winter. The seasonal differences in REs were reported elsewhere (Hijosa-Valsero et al., 2010; Ferguson et al., 2013; Castiglioni et al., 2006) leading to the general conclusion that treatment processes are more efficient during warmer periods.

It was not clear if the seasonal fluctuations in wastewater concentrations were caused by changing patterns of consumption, of the municipal wastewater treatment system, or a combination of the two. Overall, we can conclude that the effluent composition varies between the two seasons. Consequently, changes in effluent concentrations and ratios will affect aquatic systems differently in summer versus winter. The importance of monitoring PPCPs at WWTPs in order to predict environmental levels and impacts of many of these compounds on aquatic organisms is evident.

Comparisons between biological treatment processes and phototransformations of effluents can have much higher impacts on elimination of pharmaceuticals from surface waters (Doll and Frimmel, 2003; Lam et al., 2004; Latch et al., 2003; Tixier et al., 2003). Determination of photochemical half-lives of pharmaceuticals would improve our understanding of the environmental fate of these compounds. This was done in buffered Milli-Q water under different conditions. Eighty-eight pharmaceuticals were included in the study. No photo fate under artificial UV light or sunlight exposure was previously reported in the literature for 51 of these. Results showed that 46 were photostable and did not degrade during 8 hours of exposure. Thirty-five photodegraded in buffered water and half-lives were calculated. Seven pharmaceuticals showed different behaviors under UV light and sunlight, exhibiting slower photolysis under artificial UV light. We can assume that in these cases, direct photolysis took place (Andreozzi et al., 2003; Doll and Frimmel, 2003). We believe that this unique data set will be used for improvement of stability and distribution models describing the fate of pharmaceuticals in the aquatic environment.

As yet, no specific treatment is available to assure complete removal of organic pollutants because of their diverse properties. To overcome the problem of insufficient elimination of PPCPs during treatment, advanced oxidation processes (AOPs) can be considered. AOPs are very effective techniques capable of completely mineralizing the PPCPs (Mendez-Arriaga et al., 2008). Hydroxyl radicals and TiO_2 are very strong oxidants. Using photocatalytic processes, TiO_2 is capable of oxidizing a wide range of organic compounds into harmless compounds such as CO_2 and H_2O. Emerging tools such as atmospheric non-thermal plasma technology provide viable alternatives to UV/H_2O_2 and ozone/H_2O_2 processes. Its primary benefit is the ability to generate UV light, ozone, and hydroxyl radicals without chemical addition or the use of UV lamps, which require cleaning and are hindered by high turbidity and matrix absorbance (Locke et al., 2006). The plasma has reportedly been used for degradation of non-biodegradable organic compounds as well (Magureanu et al., 2011; Liang et al., 2013). As mentioned, photocatalytic reaction on TiO_2 occurs using UV irradiation and UV light (Linsebigler et al., 1995), which is also generated by the non-thermal plasma. Moreover, production of ozone and oxidative species during the plasma process can highly increase the efficiency of TiO_2-based AOPs. Thus, the combined use of plasma and TiO_2 photocatalysis can provide a very promising AOP system for wastewater treatment. We show in Chapter 4 (Horáková et

al., 2013) that AOPs combining non-thermal atmospheric plasma and TiO2 are able to decay persistent compounds such as verapamil. Our data give hope that we can degrade organic chemical compounds resistant to biodegradation.

CONCLUSIONS

This thesis includes five publications that describe the seasonal occurrence and removal of PPCPs in a WWTP over one year and photodegradation of pharmaceuticals in model systems. The findings contribute to a better understanding of the fate and stability of PPCPs in water under different experimental and real conditions.

Major conclusions, based on studies at a single WWTP are:

1. Proper sample storage is very important for reliable results.
2. An obvious and significant seasonal effect was observed for most of the investigated PPCPs in the influents and effluents.
3. The concentrations of most compounds of interest were higher during the winter than in summer, and these were attributed to usage patterns and generally lower REs in the winter. To the best of our knowledge, this study is the first to report seasonal changes of influent concentrations of norfloxacin, ciprofloxacin, levofloxacin, oxazepam, mirtazapine, sertraline, memantine, fexofenadine, meclozine, rosuvastatin, and atorvastatin and on seasonal REs for PBS, verapamil, isradipine, and bisoprolol.
4. Using buffered Milli-Q water, most of the 88 pharmaceuticals studied underwent slower photolysis in UV light than in sunlight, and 51 have not yet been studied and reported in the literature.
5. An AOP system combined with non-thermal atmospheric plasma and TiO_2 can decay relatively persistent compounds such as verapamil.

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ENGLISH SUMMARY

Pharmaceuticals and other human used chemicals in water environment – stability and fate

Oksana Golovko

In recent decades, a substantial amount of work has been done to determine the occurrence, fate, and effects of pharmaceuticals and personal care products (PPCPs) in the aquatic environment. Incomplete removal of the PPCPs by conventional wastewater treatment plants (WWTPs) has been observed, and consequently, pharmaceuticals have frequently been detected in surface water around globe. The fate of PPCPs in the environment is dependent on a range of factors, including physico-chemical properties, usage patterns, and amenability to metabolism in WWTPs. Once released into the environment, other factors dictate the fate of these compounds, including degradation and sorption to components of the aquatic and soil environment, and environmental factors such as pH and climate.

In this thesis, the influence of sample storage on analysis, the seasonal changes in concentrations and removal efficiencies (REs) in WWTPs, and the photostability of pharmaceuticals under different sources of light were studied.

Data obtained from stability studies showed that storage conditions had a significant impact on the stability of samples, and thus were very important for reliable determination of target compounds. The stability of 124 pharmaceuticals in influent and effluent wastewater samples during short-term storage at 4°C and long-term storage at -18°C was evaluated. More PPCPs were stable at the short-term storage conditions. Three types of behavior were observed under long-term storage conditions: the concentration remained stable, it declined with time, or PPCPs were lost from the sample immediately after freezing. Differences between effluent and influent samples were found in 50 of 124 compounds tested. After a freezing and thawing cycle, 15 were lost.

The season can have an important influence on the variability of concentration and elimination of PPCPs in WWTPs throughout the year. Seasonal changes in concentrations and REs were found in the WWTP, which was studied for 1 year (Chapter 3). The target analytes were 10 antibiotics, 4 antidepressants, 3 psychotropics, 2 antihistamines, 2 lipid regulators, 4 UV filters, 3 analgesics/anti-inflammatories, and 9 anti-hypertensive/cardiovascular drugs. Wastewater samples (136 influents and 136 effluents) were collected from March 2011 to February 2012 and analyzed using two-dimensional liquid chromatography coupled with tandem mass spectrometry. The concentration of targeted compounds varied from 0.006 μg L^{-1} to 1.48 μg L^{-1} in the influent and from 0.003 μg L^{-1} to 0.93 μg L^{-1} in the effluent. The concentration of most pharmaceuticals was higher in winter. In most cases, elimination of PPCPs was insufficient, and removal rates varied strongly from -38% to 100%. Based on the REs throughout the year, three groups of PPCPs were observed. A few (benzophenon-1, valsartan, isradipine, and furosemide) were not fully removed, but had REs greater than 50%. A second group (2-phenylbenzimidazole-5-sulfonic acid, tramadol, sotalol, metoprolol, atenolol, and diclofenac) had very low REs (lower than 50%). A third group (benzophenon-3, benzophenon-4, codeine, verapamil, diltiazem, and bisoprolol) had highly variable REs. There were significant seasonal trends in the observed REs, which decreased in winter.

Photodegradation of 88 pharmaceuticals was investigated in buffered purified water. These experiments were performed both in sunlight and in the laboratory under artificial UV light. Forty six pharmaceuticals were stable and did not degrade during an 8-hour exposure to either light source. Half-lives ranged from 0.11 to 13.23 hours for the 35 that underwent photodegradation in these conditions. Another 7 had different behaviors under the two

sources. We found that photostability of PPCPs depends on the light sources, and that the rate of photolysis was slower under artificial UV light in most pharmaceuticals.

At this time, no specific treatment is available to assure complete removal of various organic pollutants because of their diverse properties. To overcome the problem of insufficient elimination of PPCPs during treatment processes, advanced oxidation processes (AOPs) can be considered. Emerging techniques such as atmospheric non-thermal plasma technology provide viable alternatives to technologies such as UV/H_2O_2 and ozone/H_2O_2. The primary benefit of the plasma is the ability to generate UV light, ozone, and hydroxyl radicals without chemical addition or the use of UV lamps, which require cleaning and are hindered by high turbidity and matrix absorbance. The data confirmed that a combination of plasma and TiO_2 in photocatalysis shows promise as a means to degrade organic pollutants in wastewater.

CZECH SUMMARY

Farmaka a další chemikálie pro osobní potřebu člověka – jejich stabilita a osud ve vodním prostředí

Oksana Golovko

V oblasti výzkumu přítomnosti, osudu a vlivu farmak a chemikálií pro osobní potřebu člověka (PPCP) bylo v posledních desetiletích realizováno mnoho studií. Bylo prokázáno nedokonalé odstraňování těchto látek ve stávajících procesech čištění odpadních vod a farmaka jsou hojně nalézána v povrchové vodě prakticky na celé planetě. Osud PPCP v životním prostředí je závislý na celé řadě faktorů, např. na chemicko-fyzikálních vlastnostech jednotlivých PPCP, na jejich spotřebě, schopnosti metabolizace v čistírenských procesech a dále na jejich degradabilitě, sorpčních vlastnostech na složky vodního a půdního prostředí a na dalších faktorech jako je např. pH a klimatické podmínky.

V rámci této práce byl studován vliv podmínek uskladnění vzorků na vlastní chemickou analýzu, byly sledovány změny koncentrací sledovaných sloučenin v průběhu roku a současně i účinnost odstraňování PPCP v čistírně odpadních vod. Dále byla studována i fotostabilita PPCP v podmínkách s různými zdroji světla.

Na základě analýzy získaných dat bylo prokázáno, že podmínky skladování vzorků před analýzou mají významný vliv na degradabilitu farmak přítomných v těchto vzorcích. Byla hodnocena stabilita 124 farmak v průběhu krátkodobého (4 °C) a dlouhodobého (-18 °C) skladování. Více PPCP bylo stabilních při krátkodobém skladování při 4 °C. Během dlouhodobého skladování při -18 °C byly pozorovány 3 způsoby chování sledovaných sloučenin: stabilní koncentrace látek po celou dobu experimentu, pokles koncentrací v průběhu času a vymizení látky okamžitě po zamrazení vzorku. Rozdíly v koncentracích sledovaných PPCP v přítokové a odtokové vodě ČOV byly zjištěny v případě 50 látek ze 124 sledovaných. Ze vzorku po jeho a zamražení rozmražení vymizelo 15 farmak. Bylo prokázáno, že podmínky skladování vzorků významně ovlivňují možnost determinace cílových sloučenin.

Roční období může mít významný vliv na variabilitu koncentrací a na eliminaci PPCP v procesu čištění odpadní vody. V průběhu jednoho celého roku byly sledovány sezónní změny a účinnost odstraňování širokého spektra PPCP v ČOV (celkové množství vzorků 272) (kapitola 3). Cílovými analyty bylo 10 antibiotik, 4 antidepresanty, 3 psychiatrická farmaka, 2 antihistaminika, 2 regulátory lipidů, 4 UV filtry, 3 analgetika a 9 farmak na úpravu krevního tlaku. V období od března 2011 do února 2012 bylo odebráno a následně pomocí kapalinové chromatografie analyzováno celkem 272 vzorků odpadní či vyčištěné odpadní vody (136 z přítoku a 136 z odtoku ČOV). Koncentrace jednotlivých cílových analytů na přítoku se pohybovaly v rozpětí 0,006–1,480 $\mu g.l^{-1}$ a na odtoku v rozpětí 0,003–0,930 $\mu g.l^{-1}$. Koncentrace většiny farmak v analyzovaných vzorcích byly vyšší v zimním období. Účinnost eliminace (RE) farmak byla nedostatečná a pohybovala se v rozmezí 38–100 %. Na základě zjištěných RE bylo možno PPCP rozdělit do 3 skupin. Několik PPCP (benzophenon-1, valsartan, isradipin a furosemid) nebylo sice zcela eliminováno, ale jejich RE bylo vyšší než 50 %. Druhá skupina sloučenin zahrnující 2-phenylbenzimidazol-5-sulfonovou kyselinu, tramadol, sotalol, metoprolol, atenolol a diclofenac vykazovala velmi nízké RE nižší než 50 %. Ve 3. skupině analytů (benzophenon-3 a benzophenon-4, codein, verapamil, diltiazem a bisoprolol) bylo RE velmi proměnlivé a kolísalo v závislosti na ročním období. Nejnižší RE bylo v zimním období.

Dále byla sledována fotodegradace 88 farmak rozpuštěných v čisté upravené vodě. Tyto experimenty byly realizovány jak v laboratorních podmínkách s využitím umělého zdroje UV záření, tak v reálných podmínkách s využitím slunečního záření. Fotostabilních bylo 46

farmak a nedegradovalo během 8 hodin expozice ani UV ani slunečnímu záření. Degradovalo 35 farmak, přičemž polovina množství jednotlivých sloučenin degradovala v rozpětí 0,11–13,23 hodin. Ve svém chování vykazovalo různé odlišnosti 7 farmak. Je nutno zdůraznit, že fotostabilita PPCP závisí na délce jejich expozice různým druhům záření (UV, sluneční). Podíl fotolýz z celkového množství sledovaných farmak byl nižší v případě expozice umělému UV záření.

V současné době není k dispozici technologie, která by zajistila dokonalé odstranění širokého spektra organických kontaminantů. Je to dáno jejich odlišnými vlastnostmi. Testují se tedy další možnosti eliminace těchto látek. Z tohoto důvodu byly provedeny i experimenty využívající pokročilé oxidační procesy (AOPs). Nově vyvíjené technologie, např. využití atmosferické netepelné plazmy poskytuje fungující alternativu do technologií zahrnujících UV/H_2O_2 a ozon/H_2O_2 procesy. Hlavní výhodou využití atmosferické netepelné plazmy je její schopnost generovat UV záření, ozon a hydroxylové radikály bez chemického přídavku nebo použití UV lamp vyžadujících čištění, které jsou ohroženy vysokým zákalem a matricovou absorbancí. Získaná data ukazují, že kombinace atmosferické netepelné plazmy s TiO_2 fotokatalýzou představuje slibnou příležitost pro degradaci organických polutantů v průběhu čištění odpadní vody.

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TRAINING AND SUPERVISION PLAN DURING STUDY

Name	Oksana Golovko
Research department	2010–2014 Laboratory of Environmental Chemistry and Biochemistry FFPW
Daily supervisor	M.Sc. Roman Grabic, Ph.D.
Supervisor	M.Sc. Roman Grabic, Ph.D.
Period	1st October 2010 until September 2014

Ph.D. courses	**Year**
Pond aquaculture	2011
Applied hydrobiology	2011
Ichthyology and systematic of fish	2011
Basic of scientific communication	2011
English language	2013

Scientific seminars	**Year**
Seminar days of RIFCH	2011 2012 2013 2014

International conferences	**Year**
AQUA 2012 – Global Aquaculture, Prague, Czech Republic, September 1–5 (Poster presentation)	2012
Diversification in Inland Finfish Aquaculture II (DIFA II) Vodnany, Czech Republic, September (Oral presentation)	2013

Foreign stays during Ph.D.	**Year**
Assos. Prof. Jerker Fick, Chemistry Department, Umea University, Sweden. (SPMD membrane extraction, photodegradation of pharmaceuticals (measurement))	2012

CURRICULUM VITAE

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Ph.D. COURSES

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COURSES

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KNOWLEDGE OF LANGUAGES

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FOREIGN STAY DURING Ph.D. STUDY AT RIFCH AND FFPW

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