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Removal of 30 active pharmaceutical ingredients in surface water under long-term artificial UV irradiation

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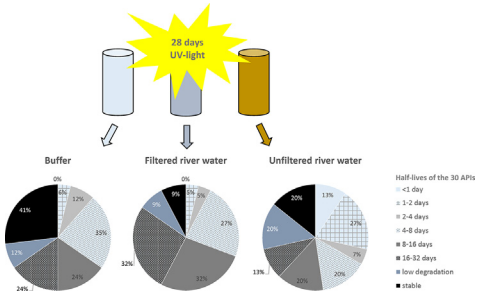
HIGHLIGHTS

- Kinetic study on 30 APIs in waters during 28 days of UV irradiation.
- The major removal process was indirect phototransformation.
- The kinetics in natural waters highly depended on the water chemistry.
- Long-term UV-exposure enabled environmental relevant removal kinetics.
- High stability of many APIs studied concerns and emphasizes need for further research.

GRAPHICAL ABSTRACT



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ABSTRACT

This study investigated the i) kinetics, and ii) proportion of photolysis of 30 relatively stable active pharmaceutical ingredients (APIs) during artificial UV irradiation for 28 d in ammonium acetate buffer, filtered and unfiltered river water. Buffer was included to control removal kinetics under stable pH conditions and without particulate matter. Dark controls were used to determine removal due to other processes than photolysis and calculate the proportion of photolysis of the total removal. The removal of each API in each matrix was determined using online solid phase extraction/liquid chromatography tandem mass spectrometry (online SPE/LC-MS/MS). Most APIs transformed during the 28 d of UV irradiation and the dark controls showed that photolysis was the major removal process for the majority of the APIs studied. The half-lives ranged from 6 h (amitriptyline) in unfiltered river water to 884 h (37 d, carbamazepine) in buffer. In unfiltered river water, the proportion of APIs with short half-lives (<48 h) was much higher (29%) than in the other matrices (4%), probably due to additional organic carbon, which could have promoted indirect photolysis. Furthermore, two APIs, memantine and fluconazole, were stable in all three matrices, while alprazolam was stable in buffer and unfiltered river water and four additional APIs were stable in buffer. Considering the relatively long-term UV-exposure, this study enabled the investigation of environmentally relevant half-lives in natural waters. Many APIs showed

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high persistence, which is environmentally concerning and emphasizes the importance of further studies on their environmental fate and effects.

1. Introduction

Pharmaceuticals and their metabolites are widely distributed in the environment (Ternes, 1998; Kümmerer, 2009a; Verlicchi et al., 2012; Aus der Beek et al., 2015) and have been given high attention for their possible environmental and human effects. Active pharmaceutical ingredients (APIs) are distributed to sewage water, not only from human and animal excretion (Jjemba, 2006; Lienert et al., 2007; Aus der Beek et al., 2015) but also through discarding of unused drugs and the release from production sites (Fick et al., 2009a; Larsson et al., 2007). They end up in surface water mainly through discharge of treated, and in some cases, untreated sewage water (Miege et al., 2009; Verlicchi et al., 2012). Monitored drugs are, for instance, hormones included in contraceptive pills (Larsson et al., 1999; Desbrow et al., 1998), antibiotics (Al Aukidy et al., 2012; Alexy et al., 2006; Hartig et al., 1999; Hirsch et al., 1999; Kümmerer, 2009b), antivirals (Takanami et al., 2010, 2012; Azuma et al., 2015), anti-inflammatory drugs (Heberer, 2002), illicit drugs (Ort et al., 2014; Evgenidou et al., 2015; McCall et al., 2016), and psycholeptics (Ternes, 1998; Heberer, 2002; Calisto and Esteves, 2009; Kosjek et al., 2012; Zuccato et al., 2000). They have mostly been detected in sewage effluent (Larsson et al., 1999; Desbrow et al., 1998; Al Aukidy et al., 2012; Alexy et al., 2006; Hartig et al., 1999; Hirsch et al., 1999; Heberer, 2002) and sludge (Hörsing et al., 2011), as well as surface water (Al Aukidy et al., 2012; Hirsch et al., 1999; Heberer, 2002) but also in ground (Heberer, 2002) and drinking water (Heberer, 2002; Zuccato et al., 2000). Near production plants, several APIs have been detected in surface, ground, and drinking water, and the antibiotic ciprofloxacin was present in non-effluent affected lake water at higher concentration than in the serum of ciprofloxacin-treated patients (Fick et al., 2009b).

Once in the environment APIs can have multiple effects; not only traditionally ecotoxicological and human health effects, but also more specific effects related to their mode of action such as resistance development in bacteria due to antibiotics in the environment (Kümmerer, 2009c). For instance, behavioral effects of the psychiatric drug oxazepam have been shown to impact the aquatic ecosystem (Brodin et al., 2013) and antibiotic resistance genes have been discovered in sewage treatment plant (STP) effluents (Reinthaler et al., 2003), downstream of STPs (Costanzo et al., 2005) and in antibiotic-contaminated river sediment sampled close to drug production facilities (Kristiansson et al., 2011). Since all known resistance mechanisms with clinical importance originate from environmental bacteria, these results suggest selection and mobilization of antibiotic resistance in the environment as one of the driving forces behind antibiotic resistance in humans. Because the antiviral drug oseltamivir's active metabolite oseltamivir carboxylate (OC) has been detected in waterways (Söderström et al., 2009; Takanami et al., 2010) and oseltamivir can induce resistance in influenza viruses in their natural hosts (Järhult et al., 2011), this is a potential public health threat (Järhult, 2012).

Since many APIs have shown resistance during sewage water treatment and the biodegradation process, it is important to study the environmental fate of these compounds and photolysis is one of the most important removal processes (Boreen et al., 2003; Fatta-Kassinos et al., 2011). There are two major photolysis pathways, direct and indirect photolysis. Direct photolysis occurs when a molecule absorbs a photon from a light source that causes the molecule to transform. Indirect photolysis involves naturally occurring substances, for instance organic matter or nitrate, which absorb photons and generate strong reactive species that in turn induce a transformation of the molecule in question (Challis et al., 2014; Yan and Song, 2014). Hence, it is important to consider the chemistry of the medium where the photolysis takes place. Parameters, such as temperature, light intensity and wavelength, pH, organic matter, and turbidity, can influence the pathway and the rate of the photolysis reaction (Boreen et al., 2003; Fatta-Kassinos et al., 2011). However, the parameters used in experiments often vary and are limited described (i.e. the light source, water chemistry). These experimental variations in combination with few APIs included in studies on natural waters and the large chemical diversity of APIs, limit the comparability between studies. Thus, considering a wide range of APIs in one study could improve the ability to compare photostability and kinetics of individual APIs and between anatomical subgroups, respectively.

Phototransformation studies have included a variety of pharmaceuticals and waters previously (Boreen et al., 2003; Fatta-Kassinos et al., 2011; Yan and Song, 2014). To our knowledge, only a few studies on environmental photofate have investigated a large number of pharmaceuticals (>20 APIs) under controlled laboratory conditions (Wang and Lin, 2014) and in field (Hanamoto et al., 2014). Most studies have focused on a few pharmaceuticals (i.e. Andreozzi et al., 2003; Bergheim et al., 2014; Boreen et al., 2003; Fatta-Kassinos et al., 2011; Matamoros et al., 2009). To the best of our knowledge, our study is the first to investigate the photofate of a large variety of relatively stable pharmaceuticals during a long-term laboratory experiment. We aimed to determine i) the kinetics of 30 APIs in surface water and ammonium acetate buffer during 28 d of artificial UV irradiation, and ii) the respective proportion of photolysis of the total removal.

2. Experimental

2.1. Selection of APIs

First, a pre-study was performed with artificial 8 h UV-exposure of 62 APIs that were selected based on consumption and environmental relevance (Fick et al., 2010). The 28 APIs that showed high stability in this pre-study (<10% photolysis) (Table S11 of the Supplementary Material) were selected to be included in our study together with desloratadine, which had a relatively long half-life of 25 d, and oseltamivir carboxylate (OC), a substance of special interest (see Table 1).

Table 1
The 30 active pharmaceutical ingredients (APIs) included in the study and corresponding anatomical subgroup.

Therapeutic subgroup	Active pharmaceutical ingredients
Antidepressants	Memantine
Opioids	Fentanyl, tramadol
Antiarrhythmics	Flecainide
Sulfonamides/ trimethoprim	Sulfamethoxazole, trimethoprim
Antidepressants	Amitriptyline, citalopram, maprotiline, venlafaxine
Blood glucose lowering drugs	Glimepiride
Antihistamines	Desloratadine, diphenhydramine, fexofenadine,
Antimycotics	Fluconazole
Anticholinergics	Biperiden, trihexyphenidyl
Antivirals	Oseltamivir carboxylate
Beta-blockers	Atenolol, bisoprolol, irbesartan, metoprolol, telmisartan
Muscle relexants	Orphenadrine
Anxiolytics	Alprazolam, carbamazepine, hydroxyzine, risperidone, oxazepam
Urologicals	Finasteride

2.2. Preparation of water matrix and UV experiment samples

2.2.1. Water matrices

Three different matrices were prepared: an ammonium acetate buffer at pH 7, unfiltered river water and filtered river water. Buffer was included as a control experiment to investigate the removal kinetics under stable pH conditions and without particulate matter. River water was collected from the center of the ice-covered Ume River, close to Umeå city center, Sweden, on March 21, 2013. The river water had pH 5 at the sampling occasion and the filtered river water was prepared using a 0.45 μm MF™-membrane filter to remove particulate matter. Additional water parameters were analyzed a week later by the Society for water conservation in Umeå and Vindel River (2013) in the close vicinity of our sampling site. A total organic carbon (TOC) concentration of 1.7 mg/L, turbidity of 0.84 FNU and a nitrate concentration of 0.288 mg/L was determined.

2.2.2. UV experiment samples

A mixture of the 30 APIs studied (5 μg/mL) was prepared by combining aliquots of individual methanol based stock solutions (135 and 724 μg/mL) and dilution with MilliQ water to a final volume of 5 mL MeOH/MilliQ (48/52 (v/v)). To get a final water concentration of 1000 ng/L, 200 μL of the API mixture was added to one liter of each water matrix. For each exposure time point and matrix, 10 mL of the API solution was transferred to triplicate 12 mL Pyrex tubes. A corresponding number of samples called dark controls were covered in several layers of aluminum foil to control the removal of APIs not caused by photolysis.

2.3. Artificial UV irradiation experiments

The artificial UV irradiation experiments were performed by constant rotation (using an RM5 "rocking/rolling action") of all samples of one matrix underneath four mercury UV-lamps (Philips TLK 40 W/09 N) for totally 28 d. The temperature was kept between 24 and 25 °C by a fan. The irradiated samples and dark controls (covered in aluminum foil) were collected after seven and three time points, respectively (Table 2). Since the experiments were performed for 28 d, all experiments of one matrix had to be performed at the same time to avoid differences between experimental conditions i.e. changes in the water chemistry of the river water. As the space underneath the UV lamps was limited we could not do a full experimental design for the dark control samples. Before the irradiation experiments, one aliquot of each matrix was withdrawn to measure the initial concentration of each pharmaceutical in each water matrix. In addition, single drug exposure experiments were performed for one representative compound, amitriptyline, to control if the use of an API mixture, which resulted in presence of other pharmaceuticals, promoted indirect photolysis. A reduced experimental designed was used with triplicate ammonium acetate buffer and unfiltered river water samples that were spiked with amitriptyline to get a final concentration of 1000 ng/L. The samples were irradiated under the same conditions as in the other experiments and were collected after 16 h, 40 h and 64 h, and the dark control samples after 64 h.

The irradiation spectrum of the artificial UV-light source was recorded at the exposure site from 250 to 950 nm in 1 nm steps with an ILT 900-R spectroradiometer (International Light Technologies, Massachusetts, USA). The light sources gave irradiation >0.17 μW cm^{-2} nm^{-1} from 309 nm and the Pyrex tubes (with a thickness < 2 mm) had a light cutoff < 290 nm (Challis et al., 2014; showed the light cutoff for 2 mm glass tubes). The total irradiance in the UV range 300–400 nm was 22 W/m^2, and the maximum irradiance (average of six scans) of 0.58 W m^{-2} was recorded at 364 nm. This irradiation intensity and constant UV-exposure for 28 d are similar to a partly cloudy summer month in the northern part of Scandinavia, which has midnight sun (about 20 h sunlight exposure during 24 h).

Table 2
Sample collection time points of irradiated and dark control samples and sample data excluded.

	16 h	24 h	40 h	64 h	112 h	208 h	672 h
Buffer	x	x, y	x	x	x*	x, y	x, y
Filtered river water	x	x*	x, y	x	x	x, y	x, y
Unfiltered river water	x	x*	x, y	x	x	x, y	x, y

x = irradiated sample collection, y = dark control sample collection, *sample data excluded due to uncertainties in the analysis.

2.4. Ultraviolet absorption analysis

A spectrum of each API in ammonium acetate buffer (pH 7) was recorded between 250 nm and 800 nm in 0.5 nm steps with a UV-3100 PC Spectrophotometer (VWR International GmbH, Darmstadt, Germany) and the software UV-VIS Analyst (UV-VIS Analyst Version A1.180, MacroEasy Technologies Ltd., (2010). Recorded spectra, in the range 250–400 nm, are shown in Figure S1-4 of the Supplementary Material. A reference sample containing the corresponding ammonium acetate buffer without API was used for baseline correction.

2.5. Pre-treatment and online SPE/LC-MS/MS analysis

The dark controls and UV-exposed unfiltered river samples were filtered through a 0.45 μm MF™-membrane filter. Thereafter, 9 g of each ammonium acetate buffer and river water sample, were transferred into a LC/LC-vial, acidified with 10 μL formic acid (0.1% v/v), and a mixture of internal standards (IS) were added to a final concentration of 555 ng/L. One milliliter of the pre-treated sample was analyzed with an online solid phase extraction/liquid chromatography tandem mass spectrometry (online SPE/LC-MS/MS) system including an OASIS HLB online extraction column followed by a guard column and an analytical column; both with a Hypersil GOLD aQ C18 stationary phase. The same online SPE/LC-MS/MS system and method has previously been described in details by Lindberg et al. (2014), and specific details on the on-line SPE/LC system and the MS/MS transition ions used, is given by

Khan et al. (2013) and Grabic et al. (2012), respectively.

2.5.1. Analysis of OC

In order to analyze OC with the on-line SPE/LC-MS/MS method used for the other APIs, the method previously described by Lindberg et al. (2014) was further developed and validated for the analysis of OC. The SPE/LC-MS/MS method developed had a differed chromatographic gradient shown in Table S9, and the MS/MS method for OC is given in Table S10, of the Supplementary data. The method was validated by analysis of six-point calibration curves in the concentration range 1–1000 ng/L in MilliQ and river water from Ume River, respectively. Before analysis, all samples were acidified to pH 3 using 0.1% FA and deuterated labelled OC-IS was added to a water concentration of 500 ng/L.

The validation showed that the method had a high linearity (R^2 values) in MilliQ and surface water; 0.9999 and 1, respectively, and environmental relevant limit of quantification (LOQ) values (1 ng/L and 5 ng/L in MilliQ and surface water, respectively) determined as the lowest point in the calibration curve given a $R^2 > 0.99$. Furthermore, the intra-day precision of the extraction and the instrumental response was also high with only 4% concentration differences between five injections of 100 ng/L in MilliQ. The matrix effect was calculated to be −16% by comparing the slopes of OC native in the calibration curve in MilliQ and surface water, respectively. Nonetheless, as the ion suppression was compensated for by the OC-IS, the relative recovery was still excellent (95%; determined by comparing the slopes of the calibration curves of OC native/OC-IS in MilliQ and surface water, respectively). Finally, the memory effect when injecting MilliQ water after injection of 1000 ng/L was only 0.08% of 1000 ng/L.

2.6. Quality assurance/Quality control (QA/QC)

Triplicates of UV-exposed samples were collected to control differences between samples during the experiment such as differences in intensity of UV irradiation at different positions underneath the lamps and analysis. Triplicates of aluminum covered dark control samples were collected to evaluate removal due to other processes than photolysis during the experiment. Pyrex tubes absorbing light at wavelengths smaller than the irradiation of the UV-lamp assured optimal UV-exposure. The stock solutions, standards and collected samples were stored at −18 °C. The analytical LOQs ranged from 2 to 20 ng/L (Lindberg et al., 2014). Values below the LOQ were set to half of the LOQ for kinetic calculations. Experimentally determined half-lives were considered as acceptable if R^2 was higher than 70% and at least 40% of the initial amount of drug was transformed. A single drug UV-exposure experiment was performed for amitriptyline to control if the use of an API mixture, which results in presence of other pharmaceuticals, promotes indirect photolysis.

3. Results and discussion

3.1. QA/QC

The relative standard deviation (RSD) of the triplicates of relative concentrations generally ranged from 1 to 35% with maximum one exception per matrix. However, for the triplicates of 24 h and 672 h (28 d) exposure in ammonium acetate buffer, and 208 h (about 9 d) exposure in unfiltered water the RSD ≥ 40% for seven, three and four triplicates, respectively. All mean concentrations and RSD can be found in Table S1-S6 of the Supplementary Material.

Table 3
Half-lives ($t_{1/2}$) with corresponding standard error and goodness-of-fit (R^2) and photolysis proportion of the total removal in % for 30 active pharmaceutical ingredients (APIs) in buffer, filtered river water and unfiltered river water exposed to UV irradiation for 28 d (672 h).

API	Buffer			Filtered river water			Unfiltered river water		
	$t_{1/2}$ (h)	R^2	Photolysis (%)	$t_{1/2}$ (h)	R^2	Photolysis (%)	$t_{1/2}$ (h)	R^2	Photolysis (%)
Alprazolam	<10% removal			ND	<0.7	ND	<10% removal		
Amitryptiline	35 ± 2	0.992	93	29 ± 1	0.998	>95	6 ± 0.5	0.997	>95
Atenolol	<10% removal			10 to 40% removal			ND	<0.7	ND
Biperiden	101 ± 6	0.994	>95	107 ± 10	0.977	94	28 ± 5	0.902	>95
Bisoprolol	<10% removal			375 ± 78	0.72	0	ND	<0.7	ND
Carbamazepine	884 ± 204	0.788	>95	632 ± 37	0.982	>95	513 ± 74	0.855	87
Citalopram	ND	<0.7	ND	506 ± 185	0.743	72	ND	<0.7	ND
Desloratidine	ND	<0.7	ND	176.7 ± 26	0.937	75	ND	<0.7	ND
Diphenhydramine	275 ± 74	0.883	76	323 ± 117	0.799	84	130 ± 15	0.955	92
Fentanyl	122 ± 19	0.954	78	103 ± 9	0.981	93	33 ± 4	0.943	>95
Fexofenadine	<10% removal			268 ± 28	0.97	>95	135 ± 29	0.759	>95
Finasteride	ND	<0.7	ND	745 ± 150	0.745	0	10 to 40% removal		
Flecainide	300 ± 42	0.927	>95	456 ± 33	0.98	77	ND	<0.7	ND
Fluconazole	<10% removal			<10% removal			<10% removal		
Glimepiride	ND	<0.7	ND	352 ± 75	0.783	0	ND	<0.7	ND
Hydroxyzine	159 ± 14	0.984	>95	121 ± 23	0.925	>95	21 ± 3	0.944	>95
Irbesartan	398 ± 94	0.735	46	ND	<0.7	ND	328 ± 59	0.818	16
Maprotiline	702 ± 121	0.812	>95	210 ± 21	0.971	79	ND	<0.7	ND
Memantine	<10% removal			<10% removal			<10% removal		
Metoprolol	<10% removal			698 ± 116	0.823	>95	414 ± 71	0.816	>95
Orphenadrine	179 ± 18	0.982	>95	161 ± 24	0.954	94	46 ± 8	0.896	92
Oseltamivir carboxylate	10 to 40% removal			10 to 40% removal			10 to 40% removal		
Oxazepam	54 ± 2	0.997	89	65 ± 3	0.994	>95	75 ± 2	0.999	>95
Risperidone	380 ± 74	0.838	>95	224 ± 21	0.974	82	ND	<0.7	ND
Sulfamethoxazole	161 ± 19	0.97	91	212 ± 27	0.964	85	134 ± 13	0.97	91
Telmisartan	109 ± 17	0.947	>95	180 ± 17	0.979	46	ND	<0.7	ND
Tramadol	10 to 40% removal			10 to 40% removal			10 to 40% removal		
Trihexyphenidyl	87 ± 6	0.989	94	128 ± 20	0.951	>95	29 ± 5	0.903	>95
Trimethoprim	215 ± 28	0.968	>95	229 ± 24	0.96	88	215 ± 18	0.975	>95
Venlafaxine	740 ± 154	0.802	57	464 ± 29	0.98	>95	342 ± 18	0.988	89

ND = not determined due to low fit of first-order kinetic model ($R^2 < 0.7$), <10% removal = stable after 28 d (672 h) of UV irradiation, 10–40% removal = low removal after 28 d (672 h) of UV irradiation resulting in low fit of first-order kinetic model.

3.2. Kinetics

Half-lives were calculated for the APIs with high R^2 values (>70%). These results and the results from the single drug experiment discussed later, imply that the APIs studied fit into the model of first-order kinetics. In total, half-lives were determined for 17, 23 and 15 of the 30 APIs in ammonium acetate buffer, filtered river water and unfiltered river water, respectively (Table 3). The half-lives ranged from 6 h for amitriptyline in unfiltered river water to 884 h (37 d) for carbamazepine in ammonium acetate buffer (Table 3). The kinetics of the APIs studied were divided into seven groups from APIs having a half-live < 2 d up to APIs being stable (<10% removal) throughout the 28 d UV-exposure (Fig. 1). In ammonium acetate buffer and filtered river water, 53% and 75% of the APIs studied, respectively, showed half-lives between 4 and 32 d, while in unfiltered river water, the proportion was lower (38%). Furthermore, the proportion of half-lives < 2 d was much higher (29%) in unfiltered river water than in the other two matrices (4%). In addition, the proportions of APIs with low removal (<40%) were similar in the unfiltered river water (28%) and ammonium acetate buffer (32%), and slightly lower in the filtered river water (18%). In Fig. 2, the exponential decay of orphenadrine is shown as a representative example of the kinetic behavior of most of the 30 APIs studied. For the APIs that were stable after 28 d of UV-exposure (<10% removal), or showed low removal (<40%), the regression factor (R^2) was <0.7. Thus, a low removal caused low fit to the first order kinetic model and half-lives were not calculated. In addition, some APIs showed removal but still low fit ($R^2 < 0.7$); half-lives could not be calculated for these APIs either (Table 3).

The highest persistence was found for memantine and fluconazole, which were stable in all three matrices during the whole 28 d of UV-exposure. Bergheim et al. (2014) also found that fluconazole together with acyclovir and allopurinol were the only three out of 14 APIs studied that were resistant to artificial UV irradiation about two times higher than in our study, although the exposure time was only 2.1 h compared to our 28 d. In total, seven APIs (alprazolam, atenolol, bisoprolol, fexofenadine, fluconazole, memantine and metoprolol) of the 30 APIs studied, showed <10% removal in one, two or all three matrices (Table 3). Atenolol, bisoprolol, fexofenadine, and metoprolol were stable in ammonium acetate buffer, alprazolam was stable in buffer and unfiltered river water, and memantine and fluconazole were stable in all three matrices. OC showed 12, 25, and 21% removal in ammonium acetate buffer, filtered and unfiltered river water, respectively, and thus showed too low removal (<40%) for half-lives to be determined.

Gonçalves et al. (2011) found much shorter half-life (12 h) of OC under about 20 times higher artificial irradiation, and removal kinetics (half-life 150 d) more comparable to our study under natural solar irradiation of surface water. The longest half-lives determined were found for carbamazepine (i.e. 513 h in unfiltered river water). Our results are consistent with other studies (Andreozzi et al., 2003; Matamoros et al., 2009) and carbamazepine has been proposed to be used as an anthropogenic marker in the aquatic environments since it is neither degraded nor retained in STPs (Clara et al., 2004). Nonetheless, in this study, photolysis of carbamazepine occurred in all three matrices, even though very slowly. Andreozzi et al. (2003) determined a half-life for carbamazepine of 121.6 h in bi-distilled water and 907 h in spiked river water, and found that the photolysis slowed down when humic acid was added to carbamazepine in bi-distilled water. Matamoros et al. (2009) found, a half-life of carbamazepine in water from the Besòs river (Spain) of 67.4 h and that dissolved organic carbon (DOC) decreases the photolytic half-life. Both these studies were performed at natural sunlight at more southern latitudes (Naples, Italy and Barcelona, Spain). Details on the water chemistry of the river waters studied were not given but the most probable explanation to the different half-lives found in our and their respective studies is variations in the environmental conditions.

To relate therapeutic subgroups and photostability in our study, was more difficult due to high chemical diversity within each group (Figure S5, of the Supplementary data), and few compounds from each group included. However, of the APIs studied, antimycotics and Alzheimer disease treatment drugs, and thereafter psycholeptics, were the most stable.

3.3. Removal processes

The proportion of photolysis in % for the APIs with a removal >40% was determined by comparing half-lives found in irradiated samples and dark controls (see Supplementary Material, equation (3)) (Table 3). For 15 out of the 17 APIs that degraded in ammonium acetate buffer, photolysis was the major removal process (>76%). Nineteen out of the 23 APIs that degraded in filtered river water were to a high extent phototransformed (>72%). Photolysis was the major removal process (>87%) for all 15 APIs, except irbesartan that showed removal in unfiltered river water. Thus, as expected and previously shown in literature (i.e Fatta-Kassinos et al., 2011; Wang and Lin, 2014) photolysis was the major removal process for most APIs studied. One possible additional degradation process in river water was biodegradation since the samples were not sterilized before the APIs were spiked. Removal in the ammonium acetate buffer dark controls imply that additional abiotic removal processes could also have happened (Kümmerer, 2009a).

The half-lives were generally shorter in unfiltered compared to filtered river water with much higher proportion of half-lives < 2 d in the unfiltered river water (Table 3 and Fig. 1). This hypothesis was tested for significance with a one-tailed Wilcoxon signed-ranks test

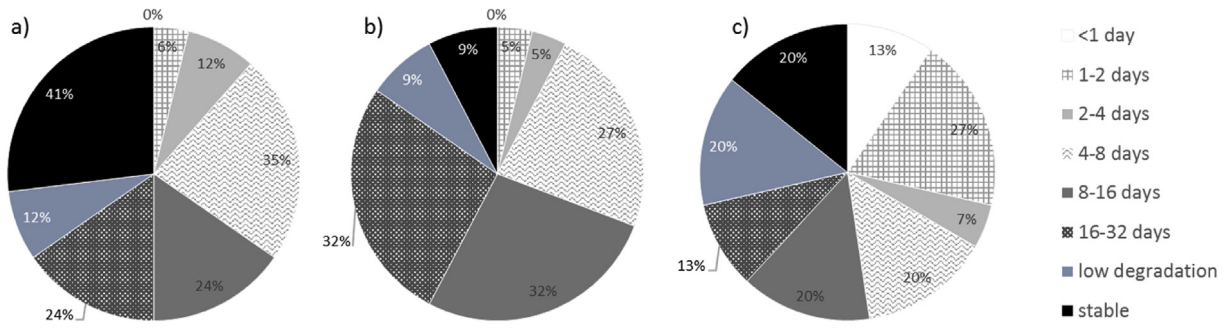

Fig. 1. Half-lives of 26, 28 and 21 APIs in a) buffer, b) filtered river water and c) unfiltered river water, respectively, during 28 days of UV irradiation. Stable = <10% removal, and low degradation = 10–40% removal. APIs excluded from this comparison showed removal but low fit of first-order kinetic model ($R^2 < 0.7$) (ND half-lives Table 3).

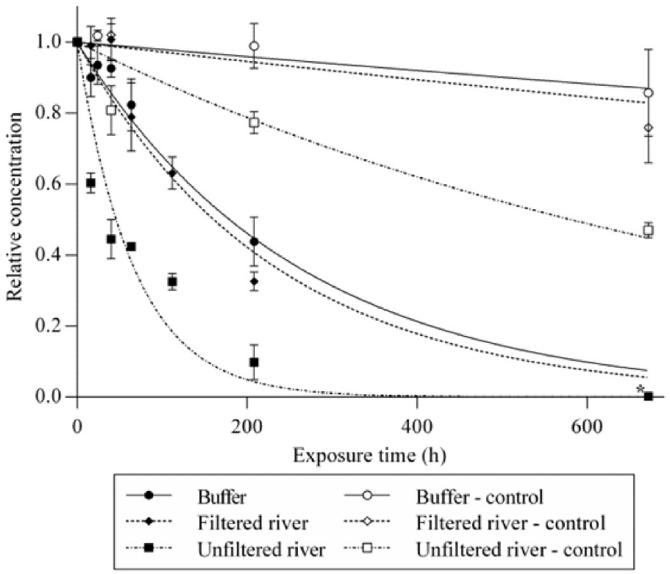

Fig. 2. Representative example of the kinetic behavior of the 30 APIs studied given by the relative concentration of orphenadrine versus UV-exposure time in buffer, filtered and unfiltered river water and in corresponding dark control. The error bars represent one standard error (n = 3). The star (*) indicates values below the LOQ.

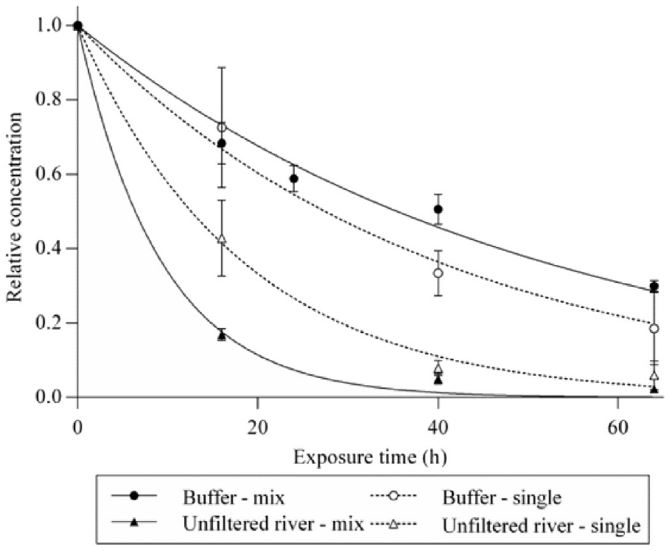

Fig. 3. Relative concentration of amitriptyline versus UV-exposure time in buffer and unfiltered river water for single and mixture experiments. The error bars represent one standard error (n = 3).

for paired samples. The statistical test could only include APIs that were stable or APIs for which half-lives were determined in both matrices (in total n = 18). The statistical testing showed that the increased removal in the unfiltered river water was significant ($p < 0.005$). The half-lives were generally shorter in unfiltered river water, probably due to the presence of additional particulate organic carbon (POC), which was removed during filtration (Thurman, 1985). Thus, more surfaces were available for adsorption and association of possible photosensitizers in the unfiltered water, which could have promoted indirect photolysis.

However, organic carbon in the form of DOC and POC is a very complex and dynamic group of molecules and may act either as a photosensitizer or a photoinhibitor through various mechanisms (Challis et al., 2014). DOC can even have different effects on the same substance depending on its origin (Guerard et al., 2009), and a higher amount of organic carbon in the unfiltered river water could have either increased or decreased the indirect photolysis depending on the moieties of the 30 APIs studied. Thus, a shielding effect of the additional POC in the unfiltered river water could explain a slightly higher proportion of APIs with low removal (<40%) in unfiltered compared to filtered river water (Table 3 and Fig. 1). The presence of DOC could also explain why higher removal rates were found in filtered water compared to ammonium acetate buffer for seven of the APIs (with half-lives determined in both ammonium acetate buffer and filtered river water (totally 16 APIs)), and nine of the APIs showed the opposite kinetic behavior with higher removal rates in ammonium acetate buffer (Table 3). The assumption that indirect photolysis was the major pathway for most of the APIs studied was further emphasized by the APIs' absorption spectra in ammonium acetate buffer (Figure S1-4 of the Supplementary data), which showed no or minor (six APIs) overlaps with the UV-spectrum of the irradiation source. However, absorption spectra can shift due to structural changes at different pH (Schwarzenbach and Imboden, 2003). For instance, a carboxylic acid moiety would become protonated as the pH gets lower. This protonation would lead to less contribution to the conjugated π-system, and thus hypothetically absorption at lower wavelengths. Likewise, a basic functional group, such as a primary or a secondary amine, would be protonated and charged at a lower pH. This protonation could potentially lead to a change in the resonance structure in the conjugated system and hence a change in absorption spectra. Since most APIs studied have several functional groups and different structures, a general effect of the lower pH in the river water is difficult to assume. However, the lower pH in the river water will generally not affect the relative differences in photostability observed between individual APIs and therapeutic subgroups, respectively. The microbe diversity and population could also have been higher in the unfiltered river water, although biodegradation likely had a minor significance in our study.

3.4. Single exposure experiments

Amitriptyline in ammonium acetate buffer had a shorter half-life UV-exposed as single drug, 27 ± 2 h, compared to 35 ± 2 h in the pharmaceutical cocktail (Fig. 3), but both half-lives were within a 95% confidence interval of each other (single: 14%, mix: 18%). On the contrary, amitriptyline in unfiltered river water had a half-life of 13 ± 1 h when UV-exposed as single drug compared to 6 ± 1 h UV-exposed as pharmaceutical mixture. These half-lives were not within a 95% confidence interval of each other. The differences between single and mixture exposure in unfiltered river water can be explained by water quality and analytical variations. Nevertheless, the trend of a longer half-life in ammonium acetate buffer than in unfiltered river was the same. Data from the single exposure experiments can be found in Table S7 and S8 of the Supplementary data. These results showed that the APIs in the mixture did most likely neither act as photosensitizer nor as an inner filter for other APIs. However, variations imply that photolysis reactions were influenced by other factors difficult to control.

4. Conclusions

A 28 d artificial UV-exposure study was performed on 30 APIs to identify APIs of environmental concern due to high photostability. The design of this photolysis study enabled comparisons between individual APIs and therapeutic subgroups at same laboratory conditions during long-term UV-exposure. The results showed that most of the APIs investigated were transformed after 28 d of UV-exposure with half-lives between 6 h and 37 d. However, four

APIs were stable in ammonium acetate buffer, alprazolam was stable in both buffer and unfiltered river water, and memantine and fluconazole were stable in all three matrices.

As expected, photolysis was the major removal process for the majority of the APIs studied and indirect photolysis the major pathway. The kinetics highly depended on the water chemistry with generally shortest half-lives in unfiltered river water, most probably due to additional organic carbon, hence surfaces available for adsorption, which promotes indirect photolysis. Comparisons with other studies show the importance of irradiation intensity for removal kinetics and the usage of a light source with an environmental relevant irradiation.

Further investigations to identify transformation products and to verify possible transformation pathways are needed. In addition, this study only includes processes occurring in aquatic matrices and did not include, for instance, sediment that can also influence removal processes in the environment. However, this study does give environmental relevant half-lives in natural waters considering the long-term UV-exposure with a constant irradiation exposure similar to a partly cloudy summer month in northern countries with midnight sun (about 20 h sunlight exposure during 24 h). Many APIs studied showed a relatively high persistence which is worrying from an environmental perspective and emphasizes the importance of further studies on their environmental fate and effects.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2017.02.063.

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