



Seasonal variations in the occurrence and fate of basic and neutral pharmaceuticals in a Swedish river–lake system

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ABSTRACT

The seasonal variations in the occurrence of carbamazepine, atenolol, metoprolol, sotalol, and acebutolol were studied at seven sites along River Fyris from December 2007 to December 2008. Samples were collected from the effluent of a waste water treatment plant (WWTP), at one upstream site, and five downstream sites of the WWTP. During one occasion in May 2008, water samples were collected at different locations and depths in the recipient lake. All analytes except of acebutolol were present in both the river and the lake at quantifiable amounts at all sampling occasions. Carbamazepine was found in similar concentrations (about 90 ng L⁻¹) at all sampling sites and all studied depths (0.5–40 m) in the lake, indicating high environmental persistence of this compound. A clear seasonal pattern was observed for the natural attenuation of the beta-blockers in the river, with the highest attenuation occurring in summer and the lowest in winter. The loss of beta-blockers on a distance of 1320 m reached up to 75% during summer time but was insignificant during winter. The seasonal variations in the loss followed the seasonal variations in water temperature and chlorophyll *a* mass flow suggesting that biotransformation and adsorption are the main processes responsible for the loss of the studied pharmaceuticals in River Fyris downstream the WWTP.

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1. Introduction

Beta-blockers and antiepileptic drugs, which are extensively used in the treatment of cardiac arrhythmia and epileptic seizures, have been reported to be persistent in WWTPs and natural environment (Bendz et al., 2005; Vieno et al., 2006). All the studied pharmaceuticals but acebutolol are among the top prescribed pharmaceuticals in Uppsala, Sweden and reach annual sales of several hundred kilograms according to Uppsala county council (Landstinget, Uppsala Län) (Table 1 in Supplementary material). Acebutolol, which is not consumed in Sweden, was selected for analysis as it is highly consumed in a neighboring country and it was included in an already developed analytical method (Vieno et al., 2006).

These compounds enter domestic wastewater treatment plants via urine and feces and through improper disposal of unused medicines. Several recent studies have shown that the available treatment processes do not remove these compounds completely,

hence they are found in both rivers and lakes downstream of WWTPs (Ternes, 1998; Tixier et al., 2003; Daneshvar et al., 2009).

The presence of beta-blockers and antiepileptic drugs in WWTP effluents and surface waters has been reported in many European countries e.g. Germany (Ternes, 1998), Finland (Vieno et al., 2006), Italy (Castiglioni et al., 2005), United Kingdom (Kasprzyk-Hordern et al., 2007), Poland (Kasprzyk-Hordern et al., 2007), Croatia (Gros et al., 2006), Spain (Petrovic et al., 2006), Sweden (Bendz et al., 2005), and Switzerland (Maurer et al., 2007). Metoprolol, which is the most sold beta-blocker in Uppsala (Table 1 in Supplementary material), has been shown to undergo biotransformation as well as phototransformation ($t_{1/2} = 630$ h) in the environment (Fono et al., 2006; Liu and Williams, 2007). In the case of atenolol, sorption has been argued to be the main process for depletion and biotransformation in surface water is minimal (Yamamoto et al., 2009). The contribution of phototransformation have been in a few studies shown to be significant ($t_{1/2} = 350$ h) (Castiglioni et al., 2006; Liu and Williams, 2007) while in another study to be negligible (Yamamoto et al., 2009). No information regarding the natural degradation of neither sotalol nor acebutolol is currently available.

Carbamazepine has been shown to be highly persistent in waste water treatment processes and in the aquatic environment (Ternes,

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1998; Löffler et al., 2005; Scheytt et al., 2005; Castiglioni et al., 2006; Matamoros et al., 2009) due to a low rate of biotransformation and insignificant sorption potential ($k_d = 0.21\text{--}5.32$). Carbamazepine has been reported to undergo phototransformation in pure water when exposed to natural UV-light ($t_{1/2} = 121.6$ h) and the transformation rate has been found to be faster in the presence of nitrate ($t_{1/2} = 11.2\text{--}69$ h) and dissolved organic carbon ($t_{1/2} = 8.3\text{--}14.4$ h) due to the generation of photo-reactants, especially $\cdot\text{OH}$ (Andreozzi et al., 2002; Matamoros et al., 2009). Considering the strong stability of carbamazepine, it has been suggested as a suitable anthropogenic tracer (Clara et al., 2004).

The scope of this study was to assess the seasonal variation over a one year period for the occurrence and fate of four (commonly used) beta-blockers and the antiepileptic drug carbamazepine in a WWTP effluent and in the recipient river (River Fyris). A further objective was to find out whether pharmaceuticals also occur in the lake into which the river discharges its water (Lake Mälaren).

2. Materials and methods

2.1. Chemicals

Atenolol (CAS No. 29122-68-7), metoprolol (CAS No. 37350-58-6), sotalol (CAS No. 3930-20-9), acebutolol (CAS No. 37517-30-9), and carbamazepine (CAS No. 298-46-4) were purchased from Sigma-Aldrich. Internal standards alprenolol (CAS No. 13707-88-5) and 10, 11-dihydrocarbamazepine (CAS No. 3564-73-6) were also obtained from Sigma-Aldrich. All compounds were of $\geq 98\%$ purity. Individual stock solutions of 500 mg L^{-1} were prepared in HPLC-graded methanol, stored at -18°C , and renewed every 6 months. Fresh working standards and calibration solutions were prepared by appropriate dilution of the stock solutions in methanol. Prior to use, all laboratory glassware were washed with hot water, rinsed with ethanol and acetone, and heated in an oven at 400°C for 4 h.

2.2. Sampling

Sampling was carried out in River Fyris and in Lake Mälaren in central Sweden. River Fyris is a relatively small river, usually well mixed, that passes through the city of Uppsala in central Sweden and finally merges into Lake Mälaren. Lake Mälaren, Sweden's third largest lake (surface area: 1140 km^2 , maximum depth: 64 m, water residence time: 2.2 years), is located west of Stockholm ($59^\circ30'\text{N}$ – $17^\circ12'\text{E}$). The water quality and flow characteristics of River Fyris are summarized in Table 2 in Supplementary material showing consistent elevated amounts of natural organic matter, seasonal variations in chlorophyll *a* concentrations, and water flow rate variations from $1.3\text{ m}^3\text{ s}^{-1}$ in June to $55\text{ m}^3\text{ s}^{-1}$ in December. The wastewater treatment plant (WWTP) of Uppsala serves approximately 160 000 inhabitants, and its daily average volume of wastewater is $59\,015\text{ m}^3$. The treatment process consists of mechanical, biological, and chemical purification of the sewage water. In the mechanical step coarse impurities are separated with screens and large particles are removed in sand traps. Ferric chloride is added to improve the separation of fine particles and phosphorus, this taking place in pre-sedimentation tanks. The biological treatment is based on activated sludge for degradation of organic matter and reduction of nitrogen. The processes take place in basins with aerated and non aerated zones for nitrification and denitrification. The biological sludge is separated in sedimentation basins. Finally, ferric chloride is added for polishing the quality of the water and to assure a low concentration of phosphorus in the effluent (Uppsala county (Uppsala Kommun), Sweden, 2009, personal communication, Ernst-Olof Swedling).

Water samples were taken as grab samples (500 ml) at six sites along River Fyris (R1 and R3–R7, Fig. 1) and in the effluent of the WWTP (R2, Fig. 1) during the period December 10th, 2007 to December 8th, 2008. Site R1 was located upstream to the discharge point of the WWTP and all other sites were located downstream, R7 being the most remote downflow site. Water samples were also collected as grab samples (500 ml) at five locations (L1–L5 in Fig. 1) in Lake Mälaren on May 21, 2008. Site L1 was at the vicinity of the river mouth, L2 was located further away from the river while sites L3, L4, and L5 were located so far away from River Fyris that the impact of the WWTP in Uppsala were assumed to be minimal at these sites, considering a water retention of Lake Mälaren of 2.2 years. The lake water was sampled either at two different depths (0.5 and 40 m) or at three different depths (0.5, 15, and 30 m). Daily flow rates of the effluent (Q_E) were provided by the WWTP in Uppsala. Daily flow rates of River Fyris (Q_R) were provided for sites R5 and R6 by the Swedish Meteorological and Hydrological Institute. River flow rates at site R5 were between 3 and 26 times higher than the effluent flow rates. We assumed that the flow rates at sites R3 and R4 equaled the ones at site R5 because of the short distance between the sites (1 km between R5 and R3), no additional significant inflows between the sites, and a rather constant river morphometry between the sites. Mass flows (MF) of pharmaceuticals were then calculated as the product of flow (Q) and the measured concentrations of pharmaceuticals, assuming complete mixing. In order to estimate the loss of atenolol, metoprolol, sotalol, and carbamazepine during different seasons in River Fyris due to natural transformation processes, we estimated the loss within a distance of 1320 m downstream of the WWTP (water residence time for this distance usually less than 8 h) by using the following equation:

$$\text{Loss} = \text{MF}_{R5} / (\text{MF}_{R1} + \text{MF}_{R2}) \quad (1)$$

where MF_{R_x} corresponds to the mass flow at site R_x . We did not apply this calculation to acebutolol as it was not always detected in the samples.

2.3. Sample preparation

All water samples (500 ml) were filtered through $0.45\text{-}\mu\text{m}$, mixed cellulose membranes (Millipore, Ireland) and analyzed within 24 h after collection. The pH of the solutions was adjusted to ~ 10 with 25% aqueous NH_4OH prior to extraction. The target compounds were extracted onto Oasis HLB (6 cm^3 , 0.2 g) cartridges (Waters, Stockholm) as described elsewhere (Vieno et al., 2006). The mean flow rate through the cartridges was 5 ml min^{-1} and 10 ml min^{-1} for effluent and surface water, respectively. The extracts were evaporated to dryness over a gentle nitrogen stream using a reacti-therm heating module (50°C) from Pierce (Rockford, IL, USA). The final extracts were dissolved in $20\text{ }\mu\text{L}$ of methanol and $450\text{ }\mu\text{L}$ of 1% acetic acid and were kept at -18°C until analysis.

2.4. Analytical procedure

The HPLC-system (Agilent 1100) consisted of a binary pump, vacuum degaser, autosampler, and a thermostated column compartment. The compounds were separated on a XBridge C18 analytical column ($3.5\text{ }\mu\text{m}$, $2.1\text{ mm} \times 50\text{ mm}$) preceded by a C18 guard column, both provided by Waters, Finland. A gradient of 1% acetic acid and ACN at a flow rate of $200\text{ }\mu\text{L min}^{-1}$ was used for elution. The initial conditions (3% ACN) were kept unchanged for one minute, after which the ACN-content was linearly increased to 80% over 16 min. The ACN was kept at 80% for one minute and the initial conditions were reestablished over one minute. An equilibration time of 10 min was used between each injection. The mass analyzer was a Quattro Micro triple-quadrupole

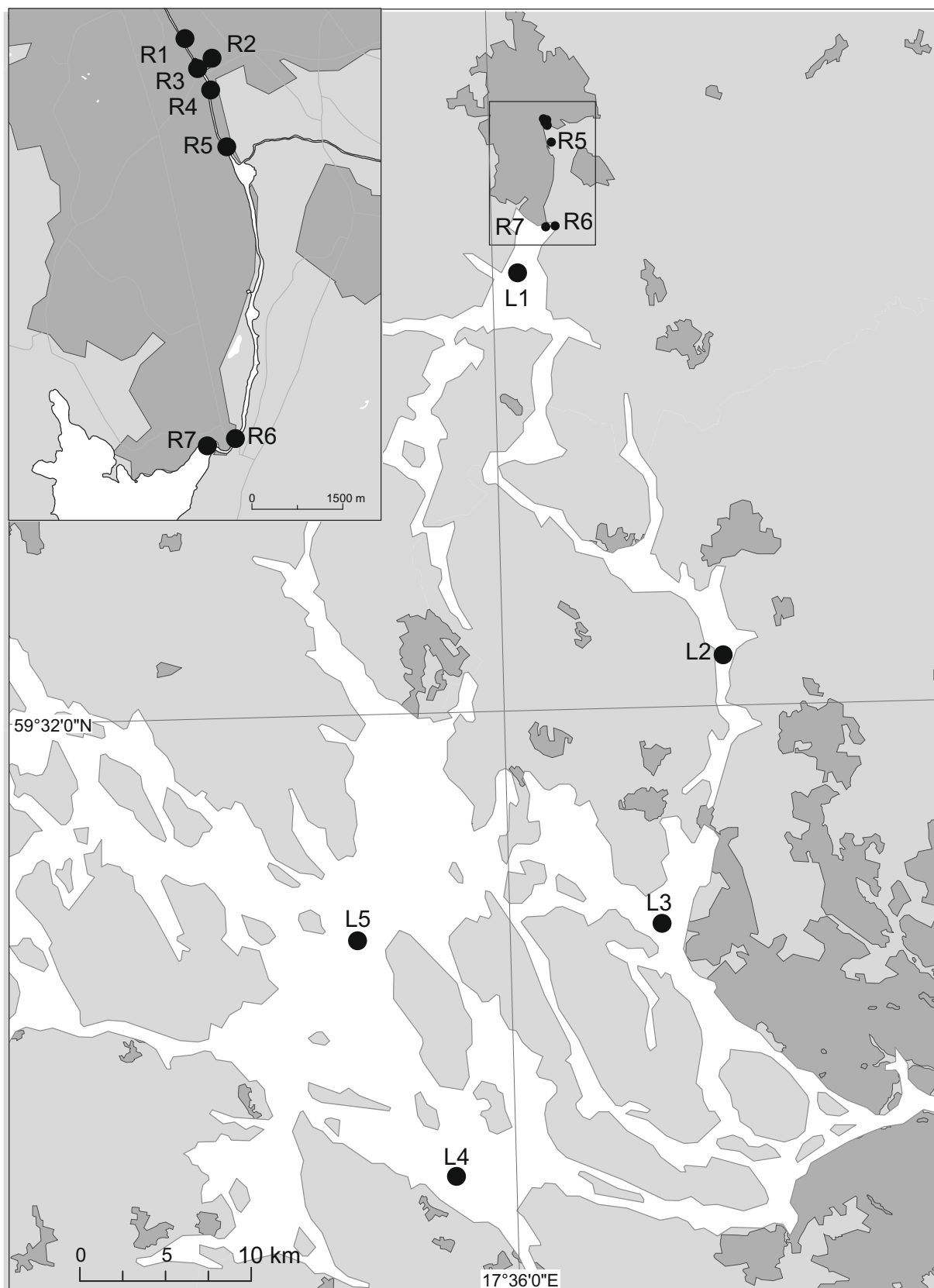


Fig. 1. Sampling locations in River Fyris (R1–R7) and Lake Mälaren (L1–L5).

(Micromass, Manchester, UK) instrument with an electrospray ionization source (ESI) operating in positive mode. The source temperature was 120 °C and the desolvation temperature 325 °C. Nitrogen

was used as both desolvation (625 L h⁻¹) and nebulizing (28 L h⁻¹) gas, while argon was used as the collision gas at a collision cell pressure of 2.8×10^{-3} mbar. The most intensive MS/MS transition

for each ion was monitored (Table 3 in Supplementary material). Optimization of compound-dependent parameters, such as cone voltage and collision energy, was achieved while introducing pure standard solutions ($5 \mu\text{g mL}^{-1}$) to the ESI-interface at a flow rate of $10 \mu\text{L min}^{-1}$. 10,11-dihydrocarbamazepine was used as internal standard for the quantification of carbamazepine and alprenolol for the beta-blockers. The spiking level of the internal standards was 500 ng.

2.5. Method validation and data quantification

For method validation, i.e. assessment of SPE-recoveries, matrix effects, limits of quantification, method repeatability as well as for obtaining calibration curves, water from the upstream point (R1, Fig. 1) and the effluent (R2, Fig. 1) were used. The LC-MS/MS method was validated for intra-day and inter-day repeatability by injecting a standard sample three times during one sequence and three times at three different days, respectively. The precision was defined as the relative standard deviation (RSD-%) of surface water samples spiked at 20 and 1000 ng L^{-1} . The linearity of the method was validated in surface water over a 0.1 to 500 ng L^{-1} concentration range. The limit of quantification (LOQ) in surface water was calculated as previously suggested (Lindqvist et al., 2005). Concentrations below the LOQ were given a numeric value equal to $\frac{1}{2}$ LOQ. To evaluate matrix effects, the peak area of the signal obtained from the surface water spiked at 1000 ng L^{-1} was compared to the peak area of Milli-Q water samples spiked to the same concentration. Absolute recoveries were determined in surface water and effluent water at a spiking level of 1000 ng L^{-1} . Instrument control and data analysis was enabled by MassLynx. V4. SP4 software (Agilent Technologies, Espoo, Finland). Ten-point calibration curves over the concentration range 0.1–1000 ng L^{-1} were used for quantification of the pharmaceuticals. All analyses were done in duplicates, hence the data presented in the results and discussion sections are averages of two measurements.

2.6. Pharmaceutical sales in the Uppsala region

Monthly data on the pharmaceutical sales (over the counter and prescription-based) in 2008 in the Uppsala region were provided by Uppsala county council (Landstinget, Uppsala Län) (for yearly sales see Table 1 in Supplementary material). The total amount of the selected drugs sales in Uppsala totaled 797 kg, of which metoprolol accounted for 49%.

2.7. Water chemistry measurements

During all sampling occasions we also measured the following 20 physical and chemical variables in the water according to EN or ISO standards (Wilander et al., 1998): temperature, pH, conductivity, calcium, magnesium, sodium, potassium, alkalinity, sulfate, chloride, fluoride, ammonium, nitrate, total nitrogen, phosphate, total phosphorus, absorbance before and after filtration at 420 nm in a 5 cm quartz cuvette, dissolved organic carbon, silica, and chlorophyll *a* concentration. We used chlorophyll *a*, measured with Lambda 2 UV/Vis spectrophotometer according to the Swedish standard SS 02 81 46, as a proxy for algal biomass (Wetzel, 2001).

3. Results

3.1. Analytical method validation

The XBridge C18 analytical column ($3.5 \mu\text{m}$, $2.1 \text{ mm} \times 50 \text{ mm}$) used in this study has smaller particle size compared to the previously used column by Vieno et al., 2006 (Zorbex XDB-C18, $5 \mu\text{m}$, $2.1 \text{ mm} \times 50 \text{ mm}$), which allows for sharper peaks and hence gives better sensitivity and lower LOQ. For the purpose of evaluating the effect of matrix components as the cause of ion-suppression during ESI a highly complex surface water matrix, collected during the winter season, was chosen. Intra-day and inter-day precision ranged between 0.3–1.2% and 0.5–4.7%, respectively. The calibration curve showed high linearity ($R^2 > 0.9991$) for all pharmaceuticals in surface water (Table 4 in Supplementary material). LOQ ranged from 0.4 to 2.7 ng L^{-1} in surface water and from 2.0 to 13.5 ng L^{-1} in the effluent (Table 4 in Supplementary material). Less than 13% signal intensity was lost for all the studied compounds in surface water. Relative recoveries of the analytes ranged from $82 \pm 1.1\%$ to $118 \pm 0.9\%$ in surface water and from $61 \pm 0.7\%$ to $103 \pm 0.9\%$ in effluent water.

3.2. Occurrence of pharmaceuticals in the effluent and River Fyris

In the effluent of the WWTP, the pharmaceuticals atenolol, metoprolol, sotalol, and carbamazepine were observed in all samples whereas the compound acebutolol was detected in 70% of the samples (Table 1). When considering yearly mean concentrations in the effluent of the WWTP (10 samples), carbamazepine was the dominating compound being even present in significant concentrations upflow of the WWTP discharge point (Tables 1 and 2). Of the beta-blockers, atenolol and metoprolol were detected in

Table 1
Comparison of yearly concentrations and standard deviations (ng L^{-1}) for the selected pharmaceuticals in the effluent of the waste water treatment plant (WWTP) and River Fyris with results from other studies. Mean concentrations in surface water represent the average of sites 4, 5 and 6 during ten months of sampling between December 2007 and December 2008.

	Effluent, R2 ($n = 10$)			River water, R4, R5 and R6 ($n = 30$)		
	Mean	% of samples where the compound was detected	Literature data (ng L^{-1})	Mean	% of samples where the compound was detected	Literature data (ng L^{-1})
Atenolol	271 ± 91	100	40–446 ^a	38 ± 32	100	3–241 ^d
Metoprolol	274 ± 192	100	19–390 ^b	47 ± 60	97	224 _{Max} ^e
Sotalol	169 ± 44	100	160–300 ^a	27 ± 27	100	<3.9–52 ^a
Acebutolol	2 ± 2	70	80–230 ^a	0.5 ± 0.4	40	<0.8–8 ^a
Carbamazepine	437 ± 117	100	410 _{Mean} ^c	110 ± 72	100	30–250 ^f

ND = not detected.

LOQ = 2.0 ng L^{-1} for metoprolol, 0.4 ng L^{-1} for acebutolol.

^a Vieno et al. (2006).

^b Andreozzi et al. (2003).

^c Gros et al. (2006).

^d Calamari et al. (2003).

^e Wiegel et al. (2004).

^f Ollers et al. (2001).

Table 2

Yearly mean concentrations and standard deviations (ng L^{-1}) ($n = 10$) of pharmaceuticals in upstream, in waste water effluent, and in River Fyris between December 2007 and December 2008.

	Upstream	Effluent	Downstream				
	R1	R2	R3	R4	R5	R6	R7
Atenolol	2.9 ± 1.2	271.0 ± 91.5	109.2 ± 75.5	60.2 ± 50.8	31.5 ± 25.2	22.9 ± 16.3	10.6 ± 6.9
Metoprolol	2.8 ± 1.7	274.5 ± 192.3	114.0 ± 125.0	68.8 ± 83.9	40.8 ± 43.5	30.4 ± 34.6	10.8 ± 10.6
Sotalol	2.4 ± 1.4	168.5 ± 44.3	69.0 ± 46.5	39.5 ± 34.8	22.8 ± 23.5	17.5 ± 15.4	7.7 ± 4.2
Acebutolol	<LOQ	2.2 ± 2.5	1.0 ± 0.9	0.7 ± 0.5	0.5 ± 0.2	<LOQ	<LOQ
Carbamazepine	57.4 ± 43.6	437.3 ± 117.3	204.2 ± 104.0	133.2 ± 79.7	101.1 ± 68.5	95.1 ± 67.8	74.2 ± 41.1

LOQ = 0.4 ng L^{-1} for acebutolol.

higher annual mean concentrations than sotalol in the effluent of the WWTP, which corresponds to the sales statistics (Table 1 in Supplementary material). Acebutolol is not sold in the Uppsala region (Table 1 in Supplementary material) and consequently the compound occurred in the lowest concentrations of the studied compounds (Table 1). Annual mean concentrations of the pharmaceuticals in the river water were about five times lower than in the effluent for all the target compounds, but concentration patterns remained the same, i.e. carbamazepine was the dominating compound (Table 1).

Lowest yearly mean concentrations of the pharmaceuticals in the river were observed in the samples collected upstream of the WWTP (R1), while the highest yearly mean concentrations were recorded at the sampling point where the discharge of the WWTP merges the river water (site R3) (Table 2). The further away the sampling points were located from the WWTP discharge point (R3), the lower became the concentrations of the pharmaceuticals. Yearly mean concentrations of the pharmaceuticals in the river varied between <LOQ for acebutolol at the upstream point (R1) and 204.2 ng L^{-1} for carbamazepine at the point of effluent discharge (R3) (Table 2). In the current studies, concentrations of the target compounds in both effluent and river are in accordance with previous findings in Europe except for acebutolol, which was found in much higher concentrations in a Finnish WWTP effluent (Table 1) (Vieno et al., 2006).

Mass flow loadings were calculated by multiplying concentrations by water flow rates and assuming complete mixing. The observed variations reveal a seasonal pattern with winter accumulation for all compounds studied (Fig. 2). Among them, carbamazepine showed the highest mass flow at all sampling sites and during all seasons (Fig. 2), with a peak value of $2534 \mu\text{g s}^{-1}$ in winter measured at the effluent discharge point (R3). In winter, the mean mass flow of the three most frequently detected beta-blockers (i.e. atenolol, metoprolol, and sotalol) along the river (R4, R5, and R6) were $375 \mu\text{g s}^{-1}$, $154 \mu\text{g s}^{-1}$, and $210 \mu\text{g s}^{-1}$ for atenolol, metoprolol, and sotalol, respectively, which were 3–7 times lower than the corresponding value for carbamazepine ($1159 \mu\text{g s}^{-1}$).

3.3. Loss of pharmaceuticals downstream the river

Considering that the WWTP was the main source of pharmaceuticals in River Fyris, we studied the loss of pharmaceuticals on the way downstream of the WWTP due to natural processes. To calculate the loss we used a mass balance between the upstream and effluent points (R1 + R2) and a downstream point (R5). Using Eq. (1) (see experimental section), the pharmaceutical loss from the WWTP towards Lake Mälaren followed a clear sinusoidal function over the course of a year with an insignificant loss during winter and a substantial loss during summer (Fig. 3). A non-parametric Wilcoxon test confirmed a significant difference between winter and summer-fall data ($P < 0.05$). During winter there were occasions where we observed an additional input of pharmaceuticals

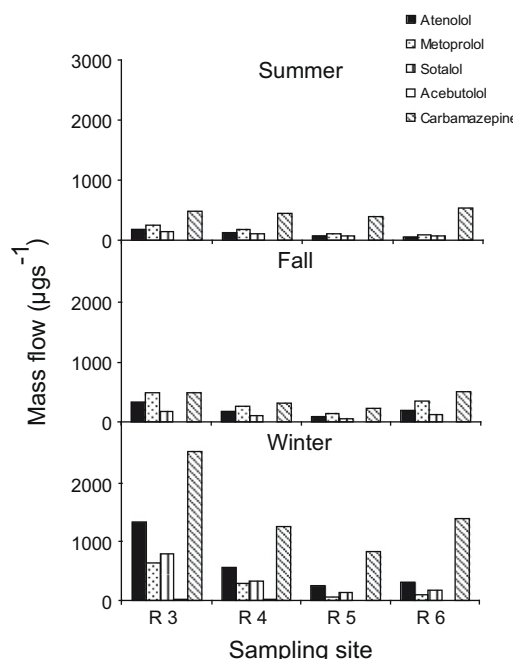


Fig. 2. Seasonal variations in the mass flow of five pharmaceuticals downstream of the waste water treatment plant (WWTP). Summer and fall values comprise two sampling occasions each while winter values comprise five sampling occasions.

on the way from the WWTP to R5, indicated by ratios slightly larger than 1 (Fig. 3). In contrast, during summer we observed a loss of pharmaceuticals up to 75% (valid for beta-blockers) on a distance of 1320 m (R5). The highest loss of pharmaceuticals coincided with the highest water temperatures and highest chlorophyll *a* mass flow, and seasonal variations between pharmaceutical loss, water temperature, and chlorophyll *a* mass flow were similar (Fig. 3). In June, there was one single occasion where pharmaceuticals showed a very low loss (Fig. 3). Testing all 20 available water chemistry variables (see Section 2.7) and water flow rate data, the only deviating variable we found for this date was the water flow rate of River Fyris, which was at its minimum at the corresponding date.

3.4. Pharmaceuticals in Lake Mälaren

In the lake, concentration patterns of the pharmaceuticals were similar to those in the WWTP effluent and the river, i.e. carbamazepine was detected in much higher concentrations compared to the beta-blockers (Fig. 4). Mean concentrations of the three beta-blockers (atenolol, metoprolol, and sotalol) decreased by a factor of four between sites R7 and L1 (Fig. 4). Moving toward the central and substantially deeper parts of the lake (from site L1 to site L2), a trend for a decrease in concentrations of the three beta-blockers

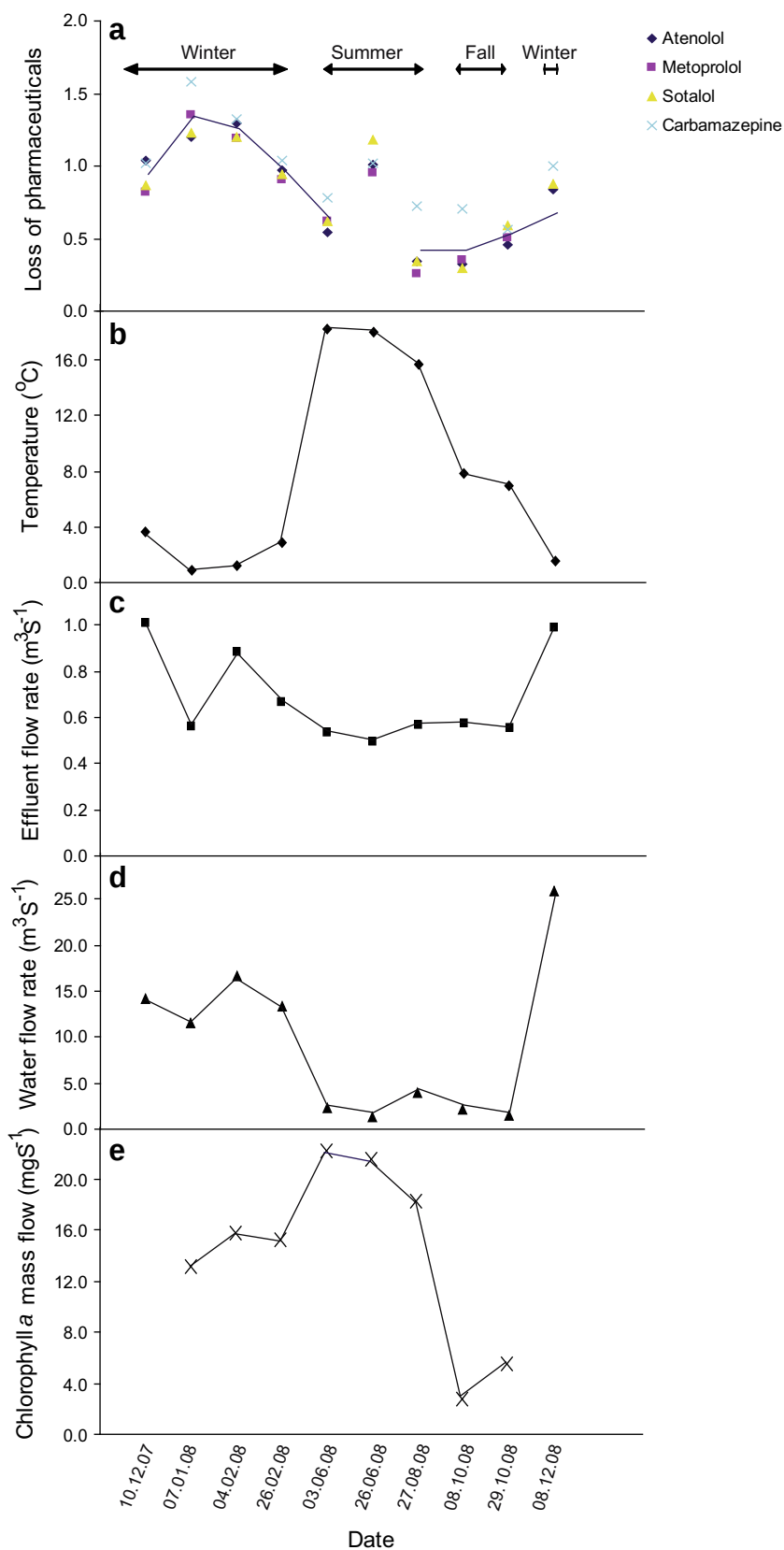


Fig. 3. (a) Seasonal variations in the loss of four pharmaceuticals due to natural transformation processes in River Fyris. Loss is calculated as the ratio of the mass flows at the upstream the effluent sites (R1 and R2) over the downstream (R5) site according to Eq. (1). Values below the ratio of one represent a loss of pharmaceuticals from the effluent to site R5. The black line connects the mean loss of all pharmaceuticals during one sampling occasion. Metoprolol was not detectable on 08.12.08. (b) Surface water temperatures at site R5. (c) Effluent flow rate from the WWTP. (d) Water flow rate at site R5. (e) Chlorophyll *a* mass flow at site R5. Chlorophyll *a* data for 10.12.07 and 08.12.08 were missing.

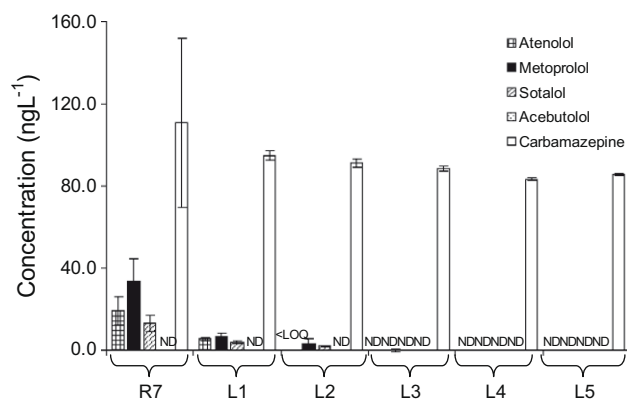


Fig. 4. Concentrations of pharmaceuticals at different sites in Lake Mälaren (Fig. 1). While sites L1–L5 were only sampled once at the end of May in 2008, R7 was sampled at 10 occasions. For this site values from the beginning of June and standard deviations over the course of the year are shown.

was observed. At the most remote sites L3, L4, and L5, beta-blockers were not detected at all. In contrast, carbamazepine concentrations remained more or less unchanged between sites R7 and L5 (Table 3). The lake survey also revealed that the pharmaceuticals were quite evenly distributed along different water depths (Table 3), though metoprolol showed slightly increasing concentrations from the epi- to the hypolimnion.

4. Discussion

The results of this study clearly show that the WWTP in Uppsala is a major source of carbamazepine and beta-blockers found in River Fyris and Lake Mälaren. Concentrations of pharmaceuticals were always highest in the effluent discharge point and lowest at the reference site upstream of the WWTP discharge point (R1). However, even at the reference site (R1) substantial concentrations of carbamazepine and low concentrations of the beta-blockers were observed. Contributions from the city runoff and diffuse sources as well as septic tanks at several cottages upstream of the WWTP are regarded as the most probable cause for the occurrence of pharmaceuticals at the reference site.

Of all compounds carbamazepine showed highest concentrations and prevalence over all seasons and locations in the river, the lake, and the WWTP, indicating an exceptional high persistency in the artificial (WWTP) and the natural (river and lake) environment which has also been found by others (Ternes, 1998; Andreozzi et al., 2003; Moldovan, 2006; Vieno et al., 2007). The high carbamazepine concentrations found in the WWTP effluent reflect the limited elimination expected by sedimentation and conventional activated sludge (Ternes, 1998; Clara et al., 2004, 2005). In the river and lake samples, the persistence of carbamazepine reflects its high resistant to natural transformation processes such as adsorption and phototransformation (Andreozzi et al., 2003; Tixier et al., 2003). Such a resistance can explain the low loss of carbamazepine from the WWTP towards Lake Mälaren (Fig. 3) and the rather high and constant vertical (0.5–40 m) and horizontal (L1–L5) carbamazepine concentrations in Lake Mälaren (Fig. 4, Table 3).

Beta-blockers behaved differently than carbamazepine with a lower mass flow and higher loss (Figs. 2 and 3). Comparison between the yearly sales and effluent concentrations of the two dominating beta-blockers, atenolol and metoprolol, showed that even though the annual sales of metoprolol were 2.5 times higher than atenolol (Table 1 in Supplementary material), annual mean concentrations of metoprolol in the effluent were in the same order

Table 3
Concentrations (ng L⁻¹) of the selected compounds at different sampling points and at different depths in Lake Mälaren. Samples were taken once on May 21, 2008. For location of sampling sites see Fig. 1.

	L1			L2			L3			L4			L5		
	0.5 m	15 m	30 m	0.5 m	15 m	40 m	0.5 m	15 m	30 m	0.5 m	15 m	40 m	0.5 m	15 m	40 m
Atenolol	5.5 ± 0.0	4.4 ± 0.1	6.2 ± 0.2	<LOQ	<LOQ	<LOQ	ND	<LOQ	ND	ND	<LOQ	ND	ND	ND	ND
Metoprolol	7.1 ± 0.2	6.8 ± 0.2	9.5 ± 0.0	3.4 ± 0.0	5.9 ± 0.2	6.0 ± 0.2	<LOQ	<LOQ	ND	ND	<LOQ	ND	ND	ND	ND
Sotalol	4.1 ± 0.1	3.2 ± 0.1	4.4 ± 0.2	2.1 ± 0.0	2.8 ± 0.0	2.6 ± 0.2	ND	ND	ND	ND	ND	ND	ND	ND	ND
Acebutolol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Carbamazepine	94.8 ± 0.4	99.7 ± 1.7	96.3 ± 0.7	91.2 ± 1.6	92.4 ± 1.6	95.1 ± 1.1	88.3 ± 0.3	89.1 ± 1.8	89.0 ± 0.5	83.6 ± 0.0	84.2 ± 0.5	85.7 ± 0.3	85.7 ± 0.3	86.2 ± 0.8	86.2 ± 0.8

ND = not detected.

LOQ = 2.7 ng L⁻¹ for atenolol, 2.0 ng L⁻¹ for metoprolol, 1.9 ng L⁻¹ for sotalol.

of magnitude as those of atenolol. Taking the different behaviors of metoprolol and atenolol in the human body into consideration, i.e. metoprolol is excreted unchanged only in 3–10% whereas the unmetabolized fraction of atenolol is about 90% (Ternes, 1998; Zuccato et al., 2005), the amount of atenolol actually entering the WWTP would be 3–11 times higher than that of metoprolol. On the basis of this assumption, it is concluded that atenolol is more efficiently removed in the WWTP than metoprolol, which is confirmed by previous studies (Vieno et al., 2007). There are currently no pharmaceuticals containing acebutolol on the Swedish market, hence the presence of this drug in the effluent samples could be due to consumption by foreigners temporarily visiting the region. In neighboring countries, for example Finland, the annual consumption of acebutolol is reported to be in the same range as that of atenolol and sotalol (Vieno et al., 2006). This is a very important factor since sale statistics might give a misleading picture on what natural waters in Sweden actually contain.

Despite different removal efficiency of the three dominant beta-blockers, i.e. atenolol, metoprolol, and sotalol, in the WWTP, they seem to have a similar fate in the aquatic environment. The loss function of these three compounds from the WWTP towards Lake Mälaren (distance of 1320 m) followed clearly a sine function over the course of a year with the highest loss during summer (Fig. 3). At best we observed a loss of up to 75% for metoprolol on a distance of 1320 m over a time less than 8 h. There was one exception during summer (26.06.08 in Fig. 3) when no loss was observed. This occasion coincided with the lowest water flow rate, probably resulting in a decoupling of our sites and an inadequate mixing of the river water, thus making calculations of loss rates in River Fyris inaccurate. In winter, ratios close or even higher than 1 coincided with the highest flow rates, which may cause additional input of the pharmaceuticals to the water column by the sediments.

A loss of atenolol and metoprolol from the WWTP downstream could be a result of adsorption, bioaccumulation, photodegradation, and biotransformation. Phototransformation, which is mainly happening during summer in Sweden, may not primarily be an efficient mechanism for transformation of these compounds since the distance (1320 m) and the water residence time between the two sites (less than 8 h) was too short. According to laboratory studies the time for relevant phototransformation for atenolol and metoprolol exceeds by far the 8 h of residence time (for atenolol: $t_{p1/2} = 77\text{--}350$ h and for metoprolol: $t_{p1/2} = 630$ h) (Liu and Williams, 2007; Yamamoto et al., 2009). As the rates of phototransformation seem to be very low on the way from the WWTP towards Lake Mälaren we suggest that biotransformation and adsorption are the main processes responsible for the removal of pharmaceuticals in River Fyris during the aforementioned season. This suggestion is strengthened by the good correspondence between high loss rates and high water temperature and chlorophyll *a* mass flow during summer. To this date, there are no data available on possible elimination processes of sotalol and acebutolol in the aquatic environment, but the structural similarities between these two compounds and atenolol as well as metoprolol suggest a similar behavior.

Despite the high loss of atenolol, metoprolol, and sotalol in River Fyris, these compounds were still detectable in quantifiable levels at the sampling site located at the river mouth (R7 in Table 2), hence there was a risk of a possible contamination of Lake Mälaren. However, for these compounds, the loss from the river mouth (R7) towards the lake (L1) was substantial. This fast reduction is most likely due to dilution (site R7 is only 1 m deep whereas site L1 is 25 m deep) as well as phototransformation since the water residence time in the lake is much higher than in the river. The enhanced concentrations of carbamazepine found in the lake water, show that the compound accumulates in the aquatic environment due to its stability.

5. Conclusions

The results of this study clearly demonstrate winter accumulation of pharmaceuticals in surface waters in a Nordic country. Calculating the loss of pharmaceuticals along River Fyris at a distance of 1320 m showed the limited environmental elimination of pharmaceuticals in winter and significant removal in summer. The highest removals observed during summer were most probably the result of a combination of biotransformation and adsorption. To further understand the fate of these compounds, investigation of the contribution of sediments of River Fyris and Lake Mälaren would be of high interest. The findings of this study have important ramifications on the fate of pharmaceuticals in natural waters. More studies are needed to assess the behavior of basic and neutral pharmaceuticals in lake waters, as complex biogeochemical internal processes could result in unexpected temporal and spatial patterns.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.chemosphere.2010.03.060](https://doi.org/10.1016/j.chemosphere.2010.03.060).

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