



# Widespread monitoring of chiral pharmaceuticals in urban rivers reveals stereospecific occurrence and transformation

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## ABSTRACT

The present work aimed to discuss the enantiomeric occurrence of chiral pharmaceuticals including 5 parent compounds (PCs) metoprolol, propranolol, atenolol, venlafaxine and fluoxetine as well as 6 of their transformation products (TPs) in surface water in Beijing. Among which, 9 out of 11 were detected during the two sampling campaigns with *N*-*O*-Didesmethylvenlafaxine (NODDV) and  $\alpha$ -hydroxymetoprolol confirmed in the catchment for the first time. Metoprolol acid (MTPA) was the most abundant up to 1508 ng L<sup>-1</sup>, followed by metoprolol and *O*-desmethylvenlafaxine (ODV). Most compounds showed 100% detection frequency or nearly, while norfluoxetine (the main metabolite of fluoxetine) and 4-hydroxypropranolone (one TP of propranolol) were not detected. Metoprolol (MTP) and venlafaxine (VFX) did not vary significantly between two sampling periods with mean concentrations of 280.7 and 22.9 ng L<sup>-1</sup>, respectively. Enantiomeric enrichment was observed for venlafaxine, metoprolol and NODDV, where R-venlafaxine was preferentially biotransformed than the S-form through *O*-desmethylation. Risk assessment indicated that fluoxetine and atenolol could pose harmful effects to aquatic organisms. This work provides enantiospecific profiles of pharmaceutically active compounds (PhACs), and extended the concept of applying the ratio of TPs vs. parent compound plus their enantiomeric traits for quantitative assessment of *in situ* biodegradation. Due to the considerable contribution by TPs (64% in present study) as well as the unexpected impacts from enantiomeric existence, the stereoselectivity of chiral pollutants during environmental process should be taken into account in future study. To the best of the authors' knowledge, it is the first comprehensive evaluation of chiral pharmaceuticals and transformation products at enantiomeric level in aquatic environment in China, which would facilitate better understanding of their environmental fate.

## 1. Introduction

The prevalence of many pharmaceutically active compounds (PhACs) has been identified in aquatic systems worldwide. Due to the growing consumption and incomplete removal by wastewater treatment plants (WWTP), these contaminants are continuously released into environment and present "pseudo-persistence" (Caracciolo et al., 2015). At present, pharmaceuticals are still unregulated (Patel et al., 2019), their residues in the environment are now considered to be "compounds of emerging concern" due to the potential to cause non-negligible impact on human health and ecosystems even at very low (ng L<sup>-1</sup>- $\mu$ g L<sup>-1</sup>) levels (Ziylan and Ince, 2011). Among which, over 50% of the drugs on sell are chiral, and mostly marketed in racemate

(Kasprzyk-Hordern, 2010). Chiral drugs undergo complex metabolic processes and transformation during environmental biological reactions (e.g. human metabolism, wastewater treatment processes and/or microbial degradation), thus may well show wide variation in their enantiomeric distribution (Petrie et al., 2015; Evans and Kasprzyk-Hordern, 2014). Enantiomers of chiral pharmaceuticals have identical physicochemical properties but may differ in their biological properties such as pharmacokinetics, metabolism and toxicology (Kasprzyk-Hordern, 2010). Recently, the presence in non-racemate of these micropollutants has raised concern about their significantly stereospecific ecotoxicity, for example, the *Daphnia magna* growth shows more inhibition in the presence of (S)-atenolol than (R)-atenolol (De Andrés et al., 2009; Stanley et al., 2007). The role of chirality as well as

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enantioselective environmental analysis of pharmaceuticals is an emerging area of great importance, nevertheless, the concern about the stereochemistry on its environmental fate is more recent with much gaps in knowledge.

Wastewater treatment plants (WWTPs) were often reported for poor removal rates of pharmaceuticals (Patel et al., 2019). What's more, it is important to bear in mind that the breakdown or removal of the parent compounds during wastewater treatments does not necessarily mean the removal of toxicity, because a great number of transformation products could be formed at high yields, while many of them are more stable in environment and exert higher toxicity to non-target organisms than their parent compounds (Huntscha et al., 2013). Some research groups have made significant contribution to monitor various PhACs in the aquatic environment such as analgesic (Caballo et al., 2015),  $\beta$ -blockers and antidepressants (Carpenter and Helbling, 2018; Miller et al., 2019), and illicit drugs (Archer et al., 2017). But still, only limited data is available regarding the role of chirality during the transformation of PhACs at any stage in the environment. In order to better understand the environmental fate of these chemicals of concern, the transformation products should also be simultaneously taken into account in environmental monitoring.

China has become one of the biggest pharmaceuticals producer and consumer in the world (Bu et al., 2013). The pharmaceutical production doubled from 2003 to 2011 (Liu and Wong, 2013; Rehman et al., 2015). On the other hand, this rapid growth is also leading to an elevated risk of environmental contamination. Until now, the available research works undertaken regarding the stereo-occurrence of PhACs in freshwater, such as the lakes and rivers in Switzerland (Buser et al., 1999), Guadalquivir River basin in Spain (López-Serna et al., 2013), River Avon in England (Bagnall et al., 2012), Douro River estuary in Portugal (Coelho et al., 2019) as well as Arc River in southern France (Li et al., 2013), were mainly concentrated on Europe and US, while the scenario in developing countries is far less documented. Given this, special attention on the pharmaceuticals contamination in different areas, especially in China is required to be paid.

Beijing is one of the world's most densely populated cities with huge pharmaceutical consumption and large quantities of domestic sewage produced (about 3.3 million tons per day). Besides, the wastewater treatment capacity as well as municipal drainage infrastructure is not quite sufficient. As a consequence, the urban surface waters are expected to suffer considerable contamination contributed from non-point sources such as landfills, livestock breeding and unregulated discharge of domestic wastewater (Liu et al., 2017; Sui et al., 2011). As the capital of China, Beijing provides an ideal place of economic developed regions for investigating the characteristics of PhACs pollution in highly urbanized areas at large scale. In previous studies, our research group ascertained the significant presence of dozens of PhACs in aquatic environment in Beijing (Duan et al., 2018; Ma et al., 2019; Ma et al., 2017). But until now, no studies have reported the occurrence of chiral pharmaceuticals and transformation products at enantiomeric level in surface waters in China.

Beiyun river basin (including the main stream and its tributaries) was selected as a representative hotspot since it receives over 80% of effluents from WWTPs in urban area of Beijing, and about 90% of the river flow is considered WWTP effluent (Ma et al., 2017; Zhang et al., 2016). In present study, 11 chiral PhACs, including 5 drugs belonging to two therapeutic categories ( $\beta$ -blockers and antidepressants) and 6 of the chiral transformation products were simultaneously determined at enantiomeric level along the main stream and several tributaries during wet season and dry season. The objectives of the research are (1) to investigate the occurrence and spatiotemporal variation in this catchment, (2) to elucidate the relationships between the concentrations and enantiomeric characteristics, and (3) further evaluate the potential risks to aquatic systems. To our best knowledge, this is the first to demonstrate enantioselective occurrence of chiral pharmaceuticals and metabolites in surface waters in China. The combined profiling approach

would yield unique insights and draw outlines of the stereoselectivity of PhACs in environment. This study also aims to provide a new perspective for future studies on predicting the fate of chiral micropollutants in environment and explore to what extent the presence of enantioenrichment can be explained by the attenuation of their parent compounds.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Target PhACs was selected based on their consumption pattern, ubiquitous occurrence and possibility of chiral biotransformation. The chemical structures and physicochemical properties of the studied compounds were shown in Table S1 in Supporting information. The analytical racemate standards of atenolol (ATN), metoprolol tartrate (MTP), propranolol hydrochloride (PHO), fluoxetine hydrochloride (FLX) and venlafaxine (VFX) as well as six major chiral transformation products, including metoprolol acid (MTPA),  $\alpha$ -hydroxymetoprolol (a mixture of diastereomers and enantiomers,  $\alpha$ -HMTP), 4-hydroxypropranolol (4-OH-PHO), norfluoxetine (NFLX), *O*-desmethylvenlafaxine (ODV), and *N,O*-didesmethylvenlafaxine (NODDV) were obtained from Sigma-Aldrich (Steinheim, Germany). Enantiopure standards of (S)-norfluoxetine ((S)-NFLX) was purchased from Santa Cruz Biotechnology, Inc. (Heidelberg, Germany).

Surrogate/internal standards of isotopically labelled ( $\pm$ )-atenolol-d7, ( $\pm$ )-metoprolol-d7, ( $\pm$ )-propranolol-d7, ( $\pm$ )-fluoxetine-d5 and ( $\pm$ )-venlafaxine-d6 were obtained from Toronto Research Chemicals Inc (Toronto, Canada). SPE cartridges of Oasis HLB (200 mg/6 mL) were procured from Waters Corporation (Milford, MA, USA). HPLC-grade methanol, LC-MS grade formic acid and ammonium acetate were purchased Fluka (Buchs, Switzerland). Ultrapure water of 18.2 M $\Omega$  cm<sup>-1</sup> was prepared by the Milli-Q purification system (Millipore, Milford, MA, USA).

Individual stock solutions of racemate standards and internal standards (IS) were dissolved in methanol (1 mg mL<sup>-1</sup>) stored at -18 °C. Working standard solutions (1 mg L<sup>-1</sup>) for calibration curve containing mixture of the 11 chiral compounds of 2–500  $\mu$ g L<sup>-1</sup> (1–250  $\mu$ g L<sup>-1</sup> for each enantiomer) were prepared freshly by serial dilution and stored at 4 °C in the dark.

### 2.2. The catchment sampling campaign

The samples were taken along the Beiyun rivers with 90% of the river flow composed by WWTPs effluents in urban area of Beijing, covering approximately 15 million inhabitants. Considerable load of micropollutants was expected due to the significant influence of WWTP effluents and other potential non-point source pollution in this area.

A total of 68 surface water samples were collected by grab sampling at 0.5 m under the surface of rivers in July (wet season) and November (dry season) 2016 along its main stream and six small- to medium-sized tributaries. Information on sampling sites were described in Supporting information S1 and Tables S2 and S3. The two sampling campaigns were conducted in wet and dry seasons in order to understand the impact that precipitation might have on pollution load, as well as enantiomer-specific fate of drugs resulting from different microbial activity in hot and cold weather. Grab sampling was chosen as opposed to composite sampling due to the possibility of enantiomer-specific degradation of drugs occurring during 24 h composite sampling time. All samples were stored in 2.5 L amber, trace clean glass bottles with 0.02% (w/v) Na<sub>3</sub>N solution added to prevent microbial degradation, then transported refrigerated at 4 °C to the laboratory and pretreated within 48 h.

### 2.3. Sample preparation and instrumental analysis

After filtration through glass fiber filters (GF/F, 47 mm, Whatman, Kent, UK), a 500 mL portion of sample was adjusted to pH 4 by 1 mmol/L HCl and 1 mmol/L NaOH solution, and spiked with mixed internal standard (50 ng L<sup>-1</sup> of each compound) before SPE. Detailed pre-treatment procedures were described in Supporting information S2.

Chiral determination was performed on an ultra-high performance liquid chromatography (Ultimate3000 HPLC system, Dionex, USA) coupled to tandem mass spectrometry (ESI-MS/MS, API4000, ABSciex, USA) operated in positive ionization mode. Enantioseparations were carried out on a Chirobiotic V column by SUPELCO Analytical (vancomycin, 250 × 4.6 mm, I.D. 5 μm, Sigma-Aldrich, Steinheim, Germany) with a mobile phase containing 10 mM NH<sub>4</sub>OAc buffer (pH = 4, adjusted by formic acid) in H<sub>2</sub>O: MeOH (10: 90) under isocratic flow rate of 0.6 mL/min. The injection volume was 10 μL, the column temperature was 20 °C. Acquisition was performed in a multiple reaction monitoring (MRM) mode. Tuning and optimization of the MS/MS parameters (declustering potential (DP), collision energy (CE), entrance potential (EP), collision cell exit potential (CXP)) were confirmed by direct infusion of standards in solvent. The Optimized MRM condition for each analyte was given in Table S4 (Supporting Information).

### 2.4. Quality assurance and quality control

During sequence analysis, procedural blanks (pure methanol) were injected every five samples to wash the column, in case of sample contamination. Matrix spiked and sample duplicates were injected randomly throughout sample batches to monitor instrumental performance. The IS method was used for quantification. Concentrations above the method detection limit (MDL) but below the method quantification limit (MQL) were assigned a value of half the MQL, and those at or below the MDL were assigned a value of zero. The method performance were described in Supporting Information S3 and full validation presented in Table S4 (Supporting Information).

### 2.5. Calculations

#### 2.5.1. Enantiomeric fraction (EF)

Enantiomeric fraction (EF) was calculated using the following equation

$$EF = \frac{E1}{E1 + E2} \text{ or } \frac{E(S)}{E(S) + E(R)} \quad (1)$$

where E1 and E2 are concentrations of the first and the second eluting enantiomer, respectively, and E(R) and E(S) are R- and S-enantiomers respectively if the elution order is known. The EF values can range from 0 to 1, with EF = 0.5 representing the racemate.

Elution order was determined by the injection of enantiopure standards or according to previous researches. For beta-blockers, atenolol, metoprolol and propranolol, elution order of E1 and E2 enantiomers were identified as S(-) and R(+)-enantiomers, respectively using the same type of chiral column. For antidepressants venlafaxine and fluoxetine with their metabolites O-desmethylvenlafaxine (ODV), N,O-didesmethylvenlafaxine (NODDV) and norfluoxetine, the elution order was identified as S(+) and R(-)-enantiomers, respectively (Bagnall et al., 2012; Evans et al., 2017; Kingbäck et al., 2010; Ribeiro et al., 2014). The elution order of 4-OH-PHO and MTPA as well as diastereomers of α-HMTP was not specified in previous study (López-Serna et al., 2013).

#### 2.5.2. Environmental risk assessment

Hazard quotient (HQ) (EC, 2003) was applied to assess the environmental risk of PhACs using the following formula:

$$HQ = MEC/PNEC. \quad (2)$$

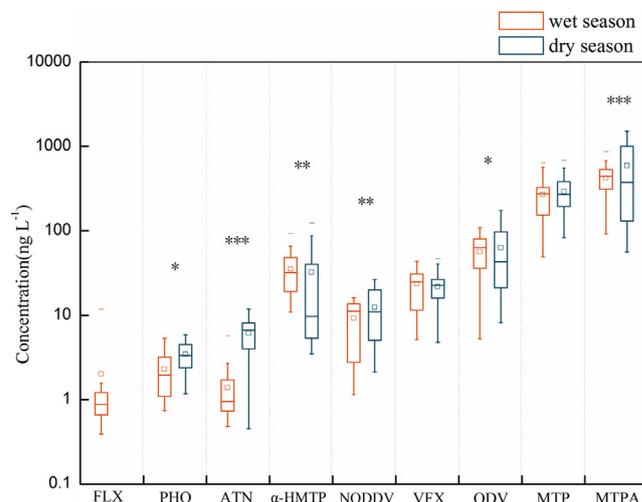


Fig. 1. Boxplots of the concentrations of the individual pharmaceuticals detected in wet and dry seasons. The solid bar and the square inside each box marks the median and the mean value, respectively. Upper and lower border of the box denote the 25th and 75th percentiles. Lines from each box show maximum and minimum concentrations. The significant differences between two sampling campaigns are indicated by “\*” (“\*\*”: 0.01 < p < 0.05; “\*\*\*”: 0.001 < p < 0.01; “\*\*\*\*”: p < 0.001) (ATN, atenolol; MTPA, metoprolol acid; MTP, metoprolol; α-HMTP, α-hydroxymetoprolol; PHO, propranolol; VFX, venlafaxine; FLX, fluoxetine; ODV, O-desmethylvenlafaxine; NODDV, N,O-Didesmethylvenlafaxine).

where MEC represents the measured environmental concentration, and PNEC is the predicted no-effect concentration. The maximum individual concentrations of pharmaceuticals found in surface waters were used as MEC. The PNEC values were calculated as below:

$$EC = (EC_{50} \text{ or } LC_{50})/AF. \quad (3)$$

where EC<sub>50</sub> or LC<sub>50</sub> is the median effective concentration value, AF is an appropriate safety factor of 100 and 1000 for chronic and acute toxicity, respectively. The toxicity data for different trophic levels were acquired from previous literature or the Ecological Structure Activity Relationships (ECOSAR) predictive model (presented in the SI Tables S8 and S9).

## 3. Results and discussion

### 3.1. Occurrence in surface water

As shown in Fig. 1 and Table 1, 9 of the 11 chiral PhACs were quantifiable, among which, α-hydroxymetoprolol (HMTP) and N,O-Didesmethylvenlafaxine (NODDV) have not previously been reported in domestic water systems. The mean concentrations of the 9 substances ranged from 1.3 ng L<sup>-1</sup> (atenolol) to 589.9 ng L<sup>-1</sup> (metoprolol acid). Among the 6 target transformation products (TPs), 4-OH-PHO and norfluoxetine (pharmacologically active human metabolites of propranolol and fluoxetine, respectively) were either below the method quantification limit (MQL) or not detectable. The substances with a detection frequency of 100% included α-HMTP, metoprolol, metoprolol acid and venlafaxine, whilst atenolol, propranolol, N,O-Didesmethylvenlafaxine (NODDV) and O-desmethylvenlafaxine (ODV) were less occurring. Fluoxetine was only detected occasionally in wet season from < MQL to 11.8 ng L<sup>-1</sup>. A majority of the target compounds were measured within 100 ng L<sup>-1</sup> range, which were typical of concentrations measured in surface water worldwide (Li et al., 2016; Petrie et al., 2015).

Propranolol was trace detected in many surface waters worldwide (Li et al., 2016; Petrović et al., 2014; Lindim et al., 2016), in current study, the measured concentration was from nd to 5.86 ng L<sup>-1</sup> slightly

**Table 1**  
Concentrations (ng L<sup>-1</sup>), detection frequency (DF, %) and EFs of target substances in two sampling campaigns.

	Wet season (July)						Dry season (November)					
	Median	Mean	25th percentile	75th percentile	DF	EF values (mean ± SD)	Median	Mean	25th percentile	75th percentile	DF	EF values (mean ± SD)
ATN	1.0	1.4 [0.9, 1.9] <sup>a</sup>	0.7	1.7	76.5 (26) <sup>b</sup>	0.57 ± 0.11	6.7	6.2 [4.7, 7.6]	4.4	8.1	58.8 (20)	0.48 ± 0.03
MTPA	434.7	414.1 [358.0, 475.0]	310.4	527.5	100	0.49 ± 0.02	375.9	589.9 [416.9, 762.8]	131.5	999.1	100	0.49 ± 0.01
MTP	275.6	270.9 [220.5, 317.6]	160.6	324.8	100	0.45 ± 0.03	269.2	290.5 [243.9, 343.0]	194.9	375.9	100	0.42 ± 0.09
α-HMTP	31.2	34.9 [28.3, 41.5]	19.5	47.6	100	/	9.7	32.4 [19.9, 44.9]	5.4	40.0	100	/
PHO	1.1	1.5 [1.0, 2.0]	0.4	2.2	52.9 (18)	0.52 ± 0.13	3.3	3.5 [3.0, 3.9]	2.5	4.5	91.2 (31)	0.36 ± 0.03
4-OH-PHO	ND	ND	ND	ND	ND	/	ND	ND	ND	ND	ND	/
VFX	25.4	23.9 [20.0, 27.6]	12.5	30.9	100	0.54 ± 0.05	22.7	21.9 [18.2, 25.6]	16.1	26.7	100	0.55 ± 0.03
ODV	63.4	56.8 [45.1, 68.2]	32.5	79.2	88.2 (30)	0.48 ± 0.07	42.9	62.8 [46.6, 79.0]	21.5	96.2	100	0.48 ± 0.07
NODDV	11.6	10.0 [8.1, 11.8]	5.9	13.7	82.4 (28)	0.41 ± 0.05	13.7	13.0 [10.1, 15.8]	5.2	20.1	94.1 (32)	0.40 ± 0.09
FLX	0.9	2.0 [0.5, 3.5]	0.7	1.2	52.9 (18)	0.51 ± 0.11	ND	ND	ND	ND	ND	/
NFLX	ND	ND	ND	ND	ND	/	ND	ND	ND	ND	ND	/
ΣPhACs	834.1	807.1 [700.0, 914.0]	557.2	991.1	/	/	750.9	1019.4 [775.4, 1263.3]	371.6	1580.0	/	/

(ND, not detected; ATN, atenolol; MTPA, metoprolol acid; MTP, metoprolol; α-HMTP, α-hydroxymetoprolol; PHO, propranolol; 4-OH-PHO, 4-hydroxypropranolol; VFX, venlafaxine; ODV, O-desmethylvenlafaxine; NODDV, N-O-Didesmethylvenlafaxine; FLX, fluoxetine; NFLX, norfluoxetine).

<sup>a</sup> Brackets indicate the statistical uncertainties: 95% confidence intervals.

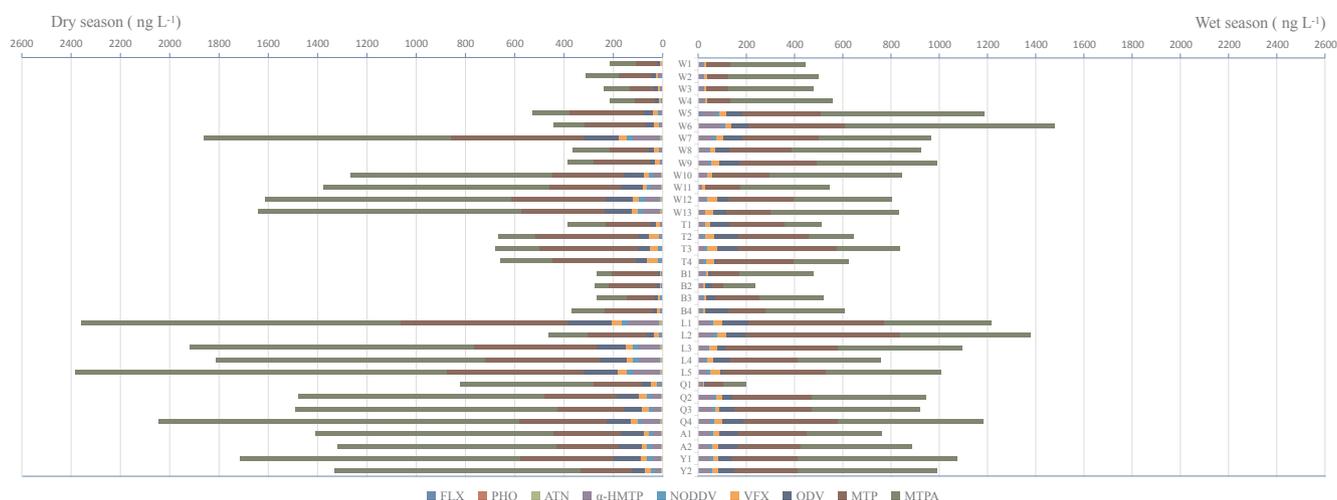
<sup>b</sup> Parentheses indicate the number of samples detected above MDL.

higher in dry season. In comparison, metoprolol was the dominant β-blocker with average/median concentrations reached hundreds of ng L<sup>-1</sup> (49.0–680.1 ng L<sup>-1</sup>), about two orders of magnitude higher than atenolol or propranolol. This was much higher than previous reports (Bagnall et al., 2012; López-Serna et al., 2012; Petrović et al., 2014), but agreed well with those measured nationwide (Yao et al., 2018) as well as observations at WWTPs in this area (Duan et al., 2018), probably due to the fact that the studied rivers had been receiving a large proportion of effluents from WWTPs alongside. The level of atenolol was similar to that in the Dal river, Sweden (3.5–8.5 ng L<sup>-1</sup>) (Lindim et al., 2016) and below that observed in Spain (nd–179.0 ng L<sup>-1</sup>) (López-Serna et al., 2013). The different traits of atenolol and metoprolol were probably ascribed to their differences in consumption rates in China, and also related to their stability, as atenolol usually has a short half-life (< 10 days) in a natural setting (Xu et al., 2019). α-HMTP, as one of the main TPs of metoprolol, was found to be omnipresent in both sampling campaigns and peaked in dry season at a concentration of 123.8 ng L<sup>-1</sup>, similar with the results in Oslofjord, Norway (Langford and Thomas, 2011). Another transformation product metoprolol acid (MTPA), also a common metabolite of both metoprolol and atenolol (Godbillon and Duval, 1984), was the most abundant with the maximum concentration up to 1.5 mg L<sup>-1</sup>, which was very likely resulted from metoprolol considering the consumption pattern in China (Xu et al., 2019). The median concentration of atenolol in dry season (6.7 ng L<sup>-1</sup>) was about 8-folds higher than that in summer, whereas α-HMTP was outstanding in summer (31.2 ng L<sup>-1</sup>). Analogously, higher median concentrations in wet season were also observed for MTPA, venlafaxine and ODV, implying little dilute effects in flood period was exerted on their occurrence.

Venlafaxine was another persistent substance. Its concentration varied from a few to 46.4 ng L<sup>-1</sup>, the peak value was lower than that in River Ruhr (180 ng L<sup>-1</sup>), Germany (Schlüsener et al., 2015), but comparable with the determinations in St. Lawrence River, Canada (Lajeunesse et al., 2008). For the two TPs of venlafaxine, the less frequently detected N,O-Didesmethylvenlafaxine (NODDV), which was investigated the first time in domestic aquatic environment, presented the lowest median concentration of the three. This was in accordance with the findings for Arc River in France (Li et al., 2013), whilst the median concentrations of the active metabolite, O-desmethylvenlafaxine (ODV), were 1.9–2.5 folds higher than its parent compound during both sampling campaigns, probably due to its limited removal rate during wastewater treatment previously reported in Germany and UK (Schlüsener et al., 2015; Evans et al., 2017). It is believed the risk of cardiovascular diseases could be higher in cold season, thus, higher occurrence of β-blockers would be expected, however, in our study, although some of the substances showed statistical differences between two sampling campaigns, the median/average concentrations did not vary much, especially for metoprolol and venlafaxine, which may imply the reason for season-associated consumption rates was not predominant.

### 3.2. Spatial distribution

Variations were observed among rivers (Fig. 2). The total concentrations in main stream ranged from 445.2 to 1480.1 ng L<sup>-1</sup> in wet season, and from 212.7 to 1860.8 ng L<sup>-1</sup> in dry season. The spatial variation was more significant than seasonal variation. For the average total concentrations, Liangshui river showed the highest loads (1090.3 ng L<sup>-1</sup> in wet season and 1787.2 ng L<sup>-1</sup> in dry season), followed by Qinghe river (811.1 ng L<sup>-1</sup> in wet season and 1458.5 ng L<sup>-1</sup> in dry season), whereas Tonghui river (654.9 ng L<sup>-1</sup> in wet season and 597.6 ng L<sup>-1</sup> in dry season) and Bahe river (460.7 ng L<sup>-1</sup> in wet season and 295.6 ng L<sup>-1</sup> in dry season) were much lower. This was on one hand related to the quantities of inhabitants in the watershed area (see Table S2 in SI). For another, the lower burden of Bahe river could be attributed to the relatively higher removal efficiency of WWTP located



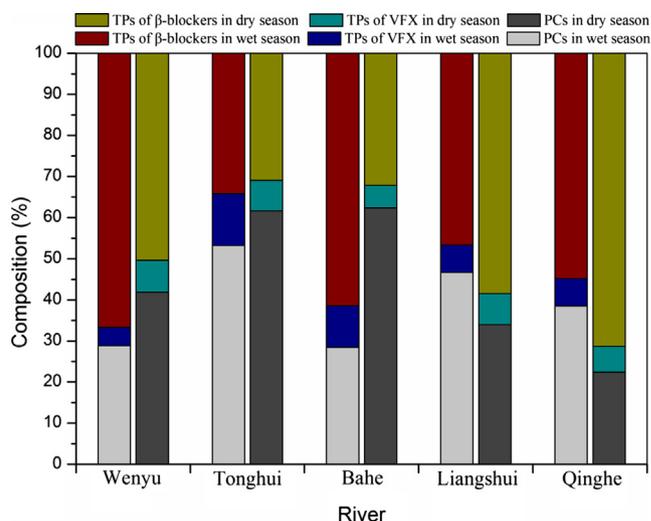
**Fig. 2.** Accumulative concentrations of chiral PhACs at each sampling site located along main stream (Wenyu River: W1-W13) and the tributaries (Tonghui River: T1-T4, Bahe River: B1-B4, Liangshui River: L1-L5, Qinghe River: Q1-Q4, Xiaozhong River: A1-A2, Yunchaojian River: Y1-Y2) (ATN, atenolol; MTPA, metoprolol acid; MTP, metoprolol;  $\alpha$ -HMTP,  $\alpha$ -hydroxymetoprolol; PHO, propranolol; VFX, venlafaxine; FLX, fluoxetine; ODV, O-desmethylvenlafaxine; NODDV, N-O-Didesmethylvenlafaxine).

upstream, which was applying a treatment process of anaerobic/anaerobic/oxic-membrane process (A/A/O-MBR) followed by ozone and sodium hypochlorite disinfection and showing good performance for MTP elimination (average removal rate of 98%) (Zhang et al., 2018). In contrast, six out of 9 quantifiable compounds (metoprolol, MTPA,  $\alpha$ -HMTP, VFX, ODV and NODDV) showed its respective peak concentration in Liangshui river. This could be explained by its composition of large proportion of WWTP effluent (up to 90% under dry conditions) and the fact that these sites are in urban area where the population is large. A large WWTP (daily flow of 600,000 m<sup>3</sup>/d, corresponding to the WWTP8 in our previous study (Duan et al., 2018) is located at upstream near site L1, but employed conventional activated sludge treatment process, leading to a considerable level of pharmaceuticals residue along the river. When comparing with other sites, the total concentrations sharply increased at sites W6 (in wet season) and W7 (in dry season), showing the impacts of effluent discharge as there are a big WWTP and the Airport nearby. Generally, concentrations found in downstream river water were higher than those in upstream river water, but the trend was less obvious in wet season. In dry season, concentrations in upstream (sites: W1-W4) were at lower level compared with those at sites downstream (W9-W13), while in wet season, the loads at W1-W4 got significantly elevated. Similar phenomenon was observed for Tonghui river and Bahe river. One explanation is that during the flood season, the massive rainfall could increase the flow thus reduce the removal efficiency as a consequence. Another contributing factor might be due to the insufficient sewage collection system leading to a WWTP overloading.

For individual substances, the concentrations of venlafaxine, metoprolol, MTPA and NODDV were significantly different among tributaries. In contrast, no significant spatial variation ( $p \geq 0.05$ ) was observed for ODV and  $\alpha$ -HMTP in the two sampling periods. Higher concentrations of TPs measured downstream and near WWTP outfalls revealed they were to a large extent emitted from the WWTPs, and could be transported substantial distances in surface water without significant natural attenuation. Heavy loads at sites of densely populated and industrialized areas were also supported by the concentration ratios of TPs to their corresponding parent compounds, which will be discussed below, and further indicated the impacts of human activities on PhACs contamination were at different levels and region specific.

### 3.3. Composition pattern and contribution of TPs

Several studies have previously determined the levels of pharmaceuticals in surface rivers of Beijing, but none of them have metabolites or TPs included (Ma et al., 2019; Wang et al., 2015; Yang et al., 2017). In order to evaluate the contribution made by TPs to the overall burdens in the entire catchment, TPs concentrations were compared to those of their corresponding parent compound (shown in Fig. 3). At almost all sampling points, TPs were found equivalent or even higher than the parent compounds. The sum of all four detectable TPs ( $\alpha$ -HMTP, MTPA, ODV and NODDV) represented an average of 64% and 59% of the total pharmaceutical load (parent compounds + TPs) for wet season and dry season, respectively. Human MTP metabolism leads to  $\alpha$ -hydroxymetoprolol ( $\alpha$ -HMTP) as a minor metabolite (< 10% of ingested dose) and O-dealkylation followed by further oxidation into metoprolol acid (MTPA) as a major metabolite (> 65% of ingested dose) (Murthy et al., 1990). Due to the high detected levels of MTP and its metabolite MTPA,  $\beta$ -blockers together with their TPs contributed the most to the



**Fig. 3.** Compositional profiles of parent compounds (PCs) and transformation products (TPs) in the main stream (Wenyu River) and major tributaries (Tonghui River, Bahe River, Liangshui River and Qinghe River) (date shown as average value).

total residues, in consequence, TPs of this therapeutic class, that is  $\alpha$ -HMTP and MTPA, made up the vast majority of the sum of all four TPs (59–95%). The compositions were quite different between two sampling periods and among each tributaries. As seen in Fig. 3, the proportions of  $\beta$ -blockers TPs ( $\alpha$ -HMTP + MTPA) varying from 32.2 to 71.3%, was predominant in most cases except for Tonghui River, which might be attributed to its relatively lower burdens than other rivers. This was also reflected in the lower ratios of TPs vs. parent compounds (eg. ODV/VFX, NODDV/ VFX, and  $\alpha$ -HMTP/MTP) as well as in Bahe river (see Table S7).

In humans, 90% of the total venlafaxine elimination is resulted from *O*-desmethylation through cytochrome P450 enzymes leading to the active metabolite *O*-desmethylvenlafaxine (ODV), and then further was metabolized to *N,O*-didesmethylvenlafaxine (NODDV) (Eap et al., 2003). The excretion of venlafaxine and its metabolites is primarily by the renal route, with about 5% and 29% appearing as unchanged form for VFX and ODV, respectively (Howell et al., 1993). Owing to the relatively lower consumption rates compared with  $\beta$ -blockers in China, the total antidepressants load showed significant difference ( $p = 0.029$ ) between two samplings, probably ascribed to the elevated flow rates leading to lower concentrations in wet season, the proportion of TPs (ODV + NODDV) did not vary significantly (average of 7.5% in wet season and 7.3% in dry season, respectively), suggesting the concentration ratios of the metabolites/venlafaxine could be potential indicators for biodegradation assessment.

The presence of metabolites in the environment is not surprising since a high percentage of the dose is excreted as metabolites for most of the pharmaceuticals. It could originate from the untreated domestic sewage input and/or from the input of microorganism metabolism during wastewater treatments and the environmental transformation. In present study, the concentrations of the antidepressants are directly linked to the proportion of treated wastewater. The varying compositions may lay in several aspects. On one hand, the theoretical percentages of excretions can vary for individuals since the metabolism may work differently in them. In addition, the consumption rate for particular therapeutic class of pharmaceuticals might be season-associated driven. What's more, the removal efficiency of WWTPs, and the transformations in the treatment process as well as in the environment do not affect all compounds to the same extent. The dramatic occurrence of TPs in receiving water indicated a change of priority pollutants, suggesting concentrations of metabolites/transformation products may exert significant impact on the estimation of pharmaceuticals burden and consumption based on sewage epidemiology. As reported in many cases, the removal rates of the metabolites were lower than those of their respective parent compound (Rúa-Gómez and Püttmann, 2012; Schlüsener et al., 2015), a higher ratio of metabolite vs. parent pharmaceutical in the effluents would be expected. To further elucidate whether biotransformation of parent compounds in WWTPs occurred, a more comprehensive survey covering WWTPs influents and effluents are required. All these showed the importance of monitoring transformation products of pharmaceuticals for subsequent environmental risk assessment.

### 3.4. Enantiospecific profiling of chiral drugs and their metabolites

The enantiomeric fraction were calculated for samples with concentrations of both enantiomers above the MQL. Chiral analysis of  $\beta$ -blockers and antidepressants have been applied in water samples using

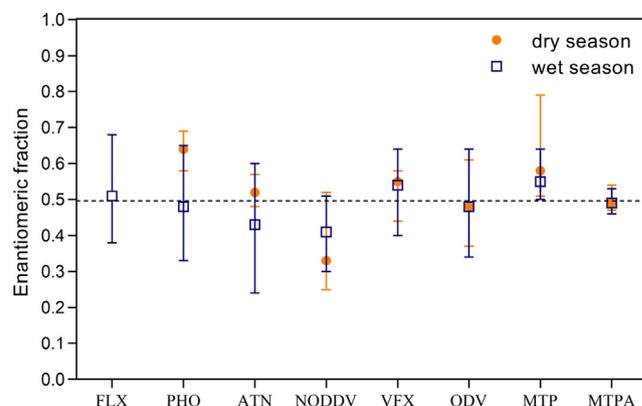


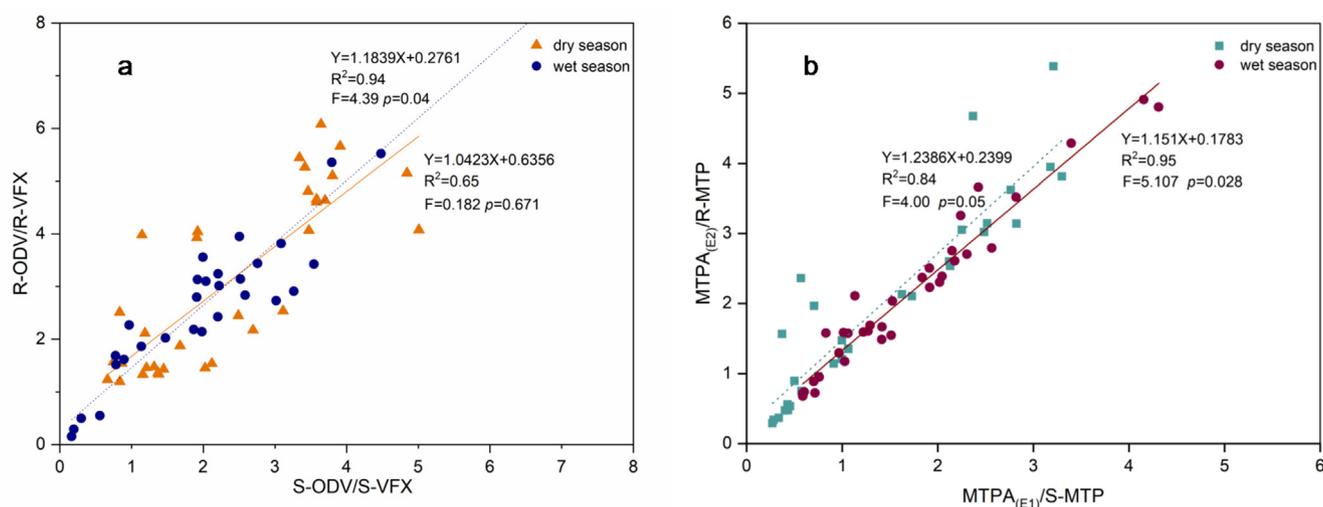
Fig. 4. The enantiomeric fractions (EFs) of detected pharmaceuticals in both seasons. Symbols denote the mean value, and bars denote the variation ranges. (ATN, atenolol; MTPA, metoprolol acid; MTP, metoprolol; PHO, propranolol; VFX, venlafaxine; FLX, fluoxetine; ODV, *O*-desmethylvenlafaxine; NODDV, *N,O*-Didesmethylvenlafaxine).

vancomycin-based chiral analytical columns (Kingbäck et al., 2010; Souchier et al., 2016). As  $\alpha$ -HMTP has two chiral centers, four isomers were expected to be eluted. However, under the chromatographic condition, only two peaks of, what were most probably, the diastereoisomers were enabled to be separated with an  $R_s$  of 1.2 (Fig. S2), hence it was not presented in Fig. 4. Nevertheless, this is, to the authors' knowledge, the first time that simultaneously stereospecific monitoring of chiral drugs and their major TPs was conducted in environmental waters nationwide.

The variation of enantiomeric fraction did not show significant seasonal difference ( $p > 0.05$ ), of which, ATN, PHO and FLX were to a large extent due to their lower detected level and frequency. The EFs of MTP were between 0.79 and 0.50 with the (*S*)-form enriched, while MTPA was composed of almost equal amounts of enantiomers ( $0.49 \pm 0.02$ ). Similar findings of enrichment of (*S*)-MTP were also observed in surface water in UK (Evans et al., 2017). For VFX, the EFs of  $0.54 \pm 0.06$  in wet season and  $0.55 \pm 0.03$  in dry season were not significantly altered within or among rivers, including at the sites near WWTP outfalls ( $p > 0.05$ ). In contrast, ODV existed close to a racemate with EFs slightly lower than 0.5 (0.48 on average) during the two sampling periods, which was also in agreement with our previous study for wastewater samples (Duan et al., 2018). NODDV was the compound found mainly enriched with its (*R*)-form, giving an EF  $< 0.5$ .

To further elucidate how EF was changing during transformation, Fig. 5 exhibits the relationship between parent compound and its metabolite for the (*R*)- and (*S*)-enantiomer, respectively. Linear regression with  $R^2 > 0.6$  suggested a major common mechanism of metabolites formation was involved. Take VFX as an example, stereoselective metabolism of VFX into ODV was assumed for the biotransformation of (*R*)-VFX into (*R*)-ODV and (*S*)-VFX into (*S*)-ODV (Li et al., 2013). The transformation rate could be obtained by the slope of the linear regression. To further confirm whether the two enantiomers biotransformed with different kinetic rates, ANCOVA was performed on data displayed in Fig. 5 to test the difference of slopes from 1.0. The results showed that the slopes of linear regression for ODV/VFX in wet season was significantly different from 1.0 ( $p = 0.04$ ), suggesting the two enantiomers might biotransform through specific minor alternative pathways that favor the (*R*)-enantiomer, as reported in human metabolism (Kingbäck et al., 2010).

Analogously, slightly increasing EFs of VFX showing a preferential attenuation of (*R*)-enantiomers during wastewater treatment was also observed in (Kasprzyk-Hordern and Baker, 2012) This may explain the seemingly decreasing EFs of ODV and NODDV in present study compared to their parent compound VFX, supposing the (*R*)-enantiomer is more readily biotransformed than the (*S*)-enantiomer. In addition, the

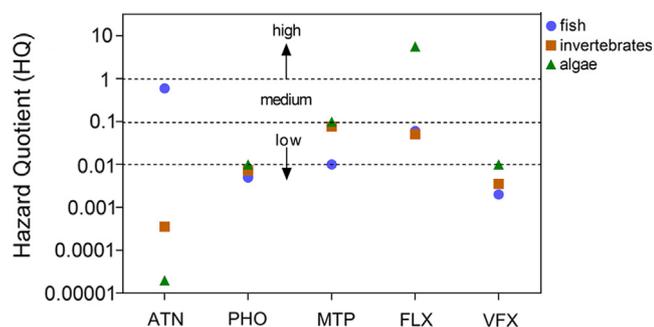


**Fig. 5.** Relationship of the concentration ratios of TP vs. PC: ODV/VFX (a) and MTPA/MTP (b) for each enantiomer by linear regression in both seasons. The F-ratio and level of significance are from the part of an ANCOVA comparing slopes with 1.0 (ODV, O-desmethylvenlafaxine; VFX, venlafaxine; MTP, metoprolol; MTPA, metoprolol acid).

difference of correlation coefficients ( $R^2$ ) between two sampling campaigns may to some extent indicate a complex environmental process or a post-release of raw sewage in the catchment, since very limited removal rates were found in WWTPs for ODV and NODDV (Schlüsener et al., 2015). Lab-scale simulation with VFX has indicated the extent of EF variations was proportional to the fractional conversion of the contaminant (Qu et al., 2019). Hence, the chiral signature of VFX could be regarded as a potential tool to estimate the degree of biodegradation, however, extending the findings from lab studies to field conditions is of challenge and warrants more verification.

### 3.5. Risk assessment and environmental implication

Toxicity data were collected from literature focusing on aquatic organisms at different trophic levels (Tables S8 and S9 in SI). Unfortunately, ecotoxicity information of TPs was not available for risk assessment. Regarding to the worst case scenarios, the environmental risks of the pharmaceuticals were estimated by Hazard quotient (HQ) using the maximum measured concentration of each compound and the calculated PNECs for each trophic level based on the most sensitive values of  $EC_{50}$  (Fig. 6). Due to the lack of enantiospecific toxicity data, HQs were calculated only with the sum of the two enantiomers. The risk was classified based on the criteria: no risk ( $HQ < 0.01$ ), low risk ( $0.01 \leq HQ < 0.1$ ), medium risk ( $0.1 \leq HQ < 1$ ), and high risk ( $HQ \geq 1$ ) (Hernando et al., 2006). As to atenolol, a PNEC value of  $20.0 \text{ ng L}^{-1}$  for fish was employed according to (Steinbach et al., 2014).



**Fig. 6.** The environmental risks towards aquatic organisms of different trophic levels in studied catchment (evaluated for worst scenario by sum of the enantiomers of each substance, ATN, atenolol; PHO, propranolol; MTP, metoprolol; VFX, venlafaxine; FLX, fluoxetine).

The resulting HQ ranged from  $< 0.01$  to 5.62. Atenolol posed a medium risk to fish, while metoprolol exhibited low to medium harm to aquatic organisms. The ecotoxicity of propranolol was noticeable, however, the HQ was on the edge of low level thanks to its relatively low concentration in the surface water. In contrast, despite the low detection level of fluoxetine, high risk was found due to its high toxicity on algae, similar to the situation in freshwater lake in China (Ma et al., 2016). Venlafaxine posed lower risks in present study, compared to medium or high risks found in (Fernández-Rubio et al., 2019; Ruan et al., 2019), where a much lower PNEC value derived from chronic test was employed.

Transformation products can retain the biological activity or may even possess more severe ecotoxic effects than the parent compound (Li et al., 2016). In spite of earlier efforts monitoring micropollutants in domestic waters (Mei et al., 2018; Yuan et al., 2013; Zhang et al., 2019), the data is far from sufficient for environment management. What's more, knowledge about enantiospecific responses of aquatic species to different pharmaceutical during long-term exposure has been barely understood. Considering their prevalence and resistance to attenuation in aquatic systems, these emerging contaminants could contribute substantially to the risks, therefore, more attention should be paid, especially those remaining pharmacologically active. In addition, chiral PhACs most of the time would undergo a different biotransformation pathway due to stereoselective reaction between enantiomers and enzymes during wastewater treatment and/or environmental process. Different WWTPs could generate different stereoselectivities in all probability, leading to wide variations in enantiomeric composition (Kasprzyk-Hordern, 2010; Patel et al., 2019). This alteration of enantioselectivity may pose a different level of toxicity in the environment (Sanganyado et al., 2017; Camacho-Muñoz et al., 2019). Hence, chirality of PhACs and TPs requires special consideration in order to assess their environmental fate more accurately, and focus on the ecotoxicity of emerging contaminants in terms of enantiomeric discrimination and occurrence pattern should also be laid.

## 4. Conclusion

The work gave a comprehensive outline of enantiomeric occurrence of frequently prescribed chiral pharmaceuticals in urban receiving water. Chiral metabolites were also included to analyze the existence of stereoselectivity biodegradation. 9 target analytes were confirmed in the catchment except for norfluoxetine and 4-hydroxypropranolol. Larger contribution of TPs measured downstream and near WWTP

outfalls indicated the major source from WWTPs effluents. The detected TP could be transported substantial distances in surface water without significant attenuation. Enantioselectivity was observed with an enrichment of S-metoprolol, S-venlafaxine and R-NODDV. For venlafaxine, a significant association between ratios of TP/PC for each enantiomer suggested the (R)-enantiomer is more readily biotransformed than the (S)-enantiomer. Therefore, EF variations could possibly be interpreted independently of the environmental conditions and the contaminant concentration. This study provided a new perspective towards profiling the enantiospecific environmental behavior of emerging contaminants in river catchment level. It was worthy of concern that chiral transformation products tend to be enriched with enantiomers of opposite configuration to their parent compounds, which might lead to unexpected environmental impacts.

### CRedit authorship contribution statement

**Ruixue Ma:** Conceptualization, Methodology, Formal analysis, Writing - original draft. **Han Qu:** Data curation, Formal analysis. **Bin Wang:** Funding acquisition, Project administration, Writing - review & editing. **Fang Wang:** Validation, Software. **Gang Yu:** Resources, Supervision.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2020.105657>.

### References

- Archer, E., Petrie, B., Kasprzyk-Hordern, B., Wolfaardt, G.M., 2017. The fate of pharmaceuticals and personal care products (PPCPs), endocrine disrupting contaminants (EDCs), metabolites and illicit drugs in a WWTP and environmental waters. *Chemosphere* 174, 437–446.
- Bagnall, J., Evans, S., Wort, M., Lubben, A., Kasprzyk-Hordern, B., 2012. Using chiral liquid chromatography quadrupole time-of-flight mass spectrometry for the analysis of pharmaceuticals and illicit drugs in surface and wastewater at the enantiomeric level. *J. Chromatogr. A* 1249, 115–129.
- Bu, Q., Wang, B., Huang, J., Deng, S., Yu, G., 2013. Pharmaceuticals and personal care products in the aquatic environment in China: a review. *J. Hazard. Mater.* 262, 189–211.
- Buser, H.-R., Poiger, T., Müller, M.D., 1999. Occurrence and environmental behavior of the chiral pharmaceutical drug ibuprofen in surface waters and in wastewater. *Environ. Sci. Technol.* 33, 2529–2535.
- Caballo, C., Sicilia, M., Rubio, S., 2015. Enantioselective determination of representative profens in wastewater by a single-step sample treatment and chiral liquid chromatography–tandem mass spectrometry. *Talanta* 134, 325–332.
- Camacho-Muñoz, D., Petrie, B., Lopardo, L., Proctor, K., Rice, J., Youdan, J., Barden, R., Kasprzyk-Hordern, B., 2019. Stereoisomeric profiling of chiral pharmaceutically active compounds in wastewaters and the receiving environment—A catchment-scale and a laboratory study. *Environ. Int.* 127, 558–572.
- Caracciolo, A.B., Topp, E., Grenni, P., 2015. Pharmaceuticals in the environment: biodegradation and effects on natural microbial communities. A review. *J. Pharm. Biomed. Anal.* 106, 25–36.
- Carpenter, C.M., Helbling, D.E., 2018. Widespread micropollutant monitoring in the Hudson River Estuary reveals spatiotemporal micropollutant clusters and their sources. *Environ. Sci. Technol.* 52, 6187–6196.
- Coelho, M.M., Ribeiro, A.R.L., Sousa, J.C., Ribeiro, C., Fernandes, C., Silva, A.M., Tiritan, M.E., 2019. Dual enantioselective LC–MS/MS method to analyse chiral drugs in surface water: monitoring in Douro River estuary. *J. Pharm. Biomed. Anal.* 170, 89–101.
- De Andrés, F., Castañeda, G., Ríos, Á., 2009. Use of toxicity assays for enantiomeric discrimination of pharmaceutical substances. *Chirality: Pharmacol. Biol., Chem. Conseq. Mol. Asymmetry* 21, 751–759.
- Duan, L., Zhang, Y., Wang, B., Deng, S., Huang, J., Wang, Y., Yu, G., 2018. Occurrence, elimination, enantiomeric distribution and intra-day variations of chiral pharmaceuticals in major wastewater treatment plants in Beijing, China. *Environ. Pollut.* 239, 473–482.
- Eap, C.B., Lessard, E., Baumann, P., Brawand-Ameijer, M., Yessine, M.-A., O'Hara, G., Turgeon, J., 2003. Role of CYP2D6 in the stereoselective disposition of venlafaxine in humans. *Pharmacogen. Genom.* 13, 39–47.
- European Commission. Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances, Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances, and Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. Ispra (IT): European Commission Joint Research Centre. EUR, 2003, 20418.
- Evans, S., Bagnall, J., Kasprzyk-Hordern, B., 2017. Enantiomeric profiling of a chemically diverse mixture of chiral pharmaceuticals in urban water. *Environ. Pollut.* 230, 368–377.
- Evans, S.E., Kasprzyk-Hordern, B., 2014. Applications of chiral chromatography coupled with mass spectrometry in the analysis of chiral pharmaceuticals in the environment. *Trends Environ. Anal. Chem.* 1, e34–e51.
- Fernández-Rubio, J., Rodríguez-Gil, J.L., Postigo, C., Mastroianni, N., de Alda, M.L., Barceló, D., Valcárcel, Y., 2019. Psychoactive pharmaceuticals and illicit drugs in coastal waters of North-Western Spain: environmental exposure and risk assessment. *Chemosphere* 224, 379–389.
- Godbillon, J., Duval, M., 1984. Determination of two metoprolol metabolites in human urine by high-performance liquid chromatography. *J. Chromatogr. B Biomed. Sci. Appl.* 309, 198–202.
- Hernando, M.D., Mezcuá, M., Fernández-Alba, A.R., Barceló, D., 2006. Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. *Talanta* 69, 334–342.
- Howell, S., Husbands, G., Scatina, J., Sisenwine, S., 1993. Metabolic disposition of 14C-venlafaxine in mouse, rat, dog, rhesus monkey and man. *Xenobiotica* 23, 349–359.
- Huntscha, S., Rodríguez Velosa, D.M., Schroth, M.H., Hollender, J., 2013. Degradation of polar organic micropollutants during riverbank filtration: complementary results from spatiotemporal sampling and push–pull tests. *Environ. Sci. Technol.* 47, 11512–11521.
- Kasprzyk-Hordern, B., 2010. Pharmacologically active compounds in the environment and their chirality. *Chem. Soc. Rev.* 39, 4466–4503.
- Kasprzyk-Hordern, B., Baker, D.R., 2012. Enantiomeric profiling of chiral drugs in wastewater and receiving waters. *Environ. Sci. Technol.* 46, 1681–1691.
- Kingbäck, M., Josefsson, M., Karlsson, L., Ahlner, J., Bengtsson, F., Kugelberg, F.C., Carlsson, B., 2010. Stereoselective determination of venlafaxine and its three demethylated metabolites in human plasma and whole blood by liquid chromatography with electrospray tandem mass spectrometric detection and solid phase extraction. *J. Pharm. Biomed. Anal.* 53, 583–590.
- López-Serna, R., Kasprzyk-Hordern, B., Petrović, M., Barceló, D., 2013. Multi-residue enantiomeric analysis of pharmaceuticals and their active metabolites in the Guadalquivir River basin (South Spain) by chiral liquid chromatography coupled with tandem mass spectrometry. *Anal. Bioanal. Chem.* 405, 5859–5873.
- López-Serna, R., Petrović, M., Barceló, D., 2012. Occurrence and distribution of multi-class pharmaceuticals and their active metabolites and transformation products in the Ebro River basin (NE Spain). *Sci. Total Environ.* 440, 280–289.
- Lajeunesse, A., Gagnon, C., Sauvé, S., 2008. Determination of basic antidepressants and their N-Desmethyl metabolites in raw sewage and wastewater using solid-phase extraction and liquid chromatography–tandem mass spectrometry. *Anal. Chem.* 80, 5325–5333.
- Langford, K., Thomas, K.V., 2011. Input of selected human pharmaceutical metabolites into the Norwegian aquatic environment. *J. Environ. Monit.* 13, 416–421.
- Li, Z., Gomez, E., Fenet, H., Chiron, S., 2013. Chiral signature of venlafaxine as a marker of biological attenuation processes. *Chemosphere* 90, 1933–1938.
- Li, Z., Sobek, A., Radke, M., 2016. Fate of pharmaceuticals and their transformation products in four small European rivers receiving treated wastewater. *Environ. Sci. Technol.* 50, 5614–5621.
- Lindim, C., Van Gils, J., Georgieva, D., Mekenyan, O., Cousins, I.T., 2016. Evaluation of human pharmaceutical emissions and concentrations in Swedish river basins. *Sci. Total Environ.* 572, 508–519.
- Liu, H.-Q., Lam, J.C., Li, W.-W., Yu, H.-Q., Lam, P.K., 2017. Spatial distribution and removal performance of pharmaceuticals in municipal wastewater treatment plants in China. *Sci. Total Environ.* 586, 1162–1169.
- Liu, J.-L., Wong, M.-H., 2013. Pharmaceuticals and personal care products (PPCPs): a review on environmental contamination in China. *Environ. Int.* 59, 208–224.
- Ma, R., Qu, H., Wang, B., Wang, F., Yu, Y., Yu, G., 2019. Simultaneous enantiomeric analysis of non-steroidal anti-inflammatory drugs in environment by chiral LC-MS/MS: a pilot study in Beijing, China. *Ecotoxicol. Environ. Saf.* 174, 83–91.
- Ma, R., Wang, B., Lu, S., Zhang, Y., Yin, L., Huang, J., Deng, S., Wang, Y., Yu, G., 2016. Characterization of pharmaceutically active compounds in Dongting Lake, China: occurrence, chiral profiling and environmental risk. *Sci. Total Environ.* 557, 268–275.
- Ma, R., Wang, B., Yin, L., Zhang, Y., Deng, S., Huang, J., Wang, Y., Yu, G., 2017. Characterization of pharmaceutically active compounds in Beijing, China: occurrence pattern, spatiotemporal distribution and its environmental implication. *J. Hazard.*

- Mater. 323, 147–155.
- Mei, X., Sui, Q., Lyu, S., Wang, D., Zhao, W., 2018. Pharmaceuticals and personal care products in the urban river across the megacity Shanghai: occurrence, source apportionment and a snapshot of influence of rainfall. *J. Hazard. Mater.* 359, 429–436.
- Miller, T.H., Ng, K.T., Bury, S.T., Bury, S.E., Bury, N.R., Barron, L.P., 2019. Biomonitoring of pesticides, pharmaceuticals and illicit drugs in a freshwater invertebrate to estimate toxic or effect pressure. *Environ. Int.*
- Murthy, S.S., Shetty, H.U., Nelson, W.L., Lennard, M.S., 1990. Enantioselective and diastereoselective aspects of the oxidative metabolism of metoprolol. *Biochem. Pharmacol.* 40, 1637–1644.
- Patel, M., Kumar, R., Kishor, K., Mlsna, T., Pittman Jr, C.U., Mohan, D., 2019. Pharmaceuticals of emerging concern in aquatic systems: chemistry, occurrence, effects, and removal methods. *Chem. Rev.* 119, 3510–3673.
- Petrie, B., Barden, R., Kasprzyk-Hordern, B., 2015. A review on emerging contaminants in wastewaters and the environment: current knowledge, understudied areas and recommendations for future monitoring. *Water Res.* 72, 3–27.
- Petrović, M., Škrbić, B., Živančev, J., Ferrando-Climent, L., Barcelo, D., 2014. Determination of 81 pharmaceutical drugs by high performance liquid chromatography coupled to mass spectrometry with hybrid triple quadrupole-linear ion trap in different types of water in Serbia. *Sci. Total Environ.* 468, 415–428.
- Qu, H., Ma, R., Wang, B., Yang, J., Duan, L., Yu, G., 2019. Enantiospecific toxicity, distribution and bioaccumulation of chiral antidepressant venlafaxine and its metabolite in loach (*Misgurnus anguillicaudatus*) co-exposed to microplastic and the drugs. *J. Hazard. Mater.* 370, 203–211.
- Ribeiro, A.R., Maia, A.S., Moreira, I.S., Afonso, C.M., Castro, P.M., Tiritan, M.E., 2014. Enantioselective quantification of fluoxetine and norfluoxetine by HPLC in wastewater effluents. *Chemosphere* 95, 589–596.
- Rúa-Gómez, P.C., Püttmann, W., 2012. Occurrence and removal of lidocaine, tramadol, venlafaxine, and their metabolites in German wastewater treatment plants. *Environ. Sci. Pollut. Res.* 19, 689–699.
- Rehman, M.S.U., Rashid, N., Ashfaq, M., Saif, A., Ahmad, N., Han, J.-I., 2015. Global risk of pharmaceutical contamination from highly populated developing countries. *Chemosphere* 138, 1045–1055.
- Ruan, Y., Wu, R., Lam, J.C., Zhang, K., Lam, P.K., 2019. Seasonal occurrence and fate of chiral pharmaceuticals in different sewage treatment systems in Hong Kong: mass balance, enantiomeric profiling, and risk assessment. *Water Res.* 149, 607–616.
- Sanganyado, E., Lu, Z., Fu, Q., Schlenk, D., Gan, J., 2017. Chiral pharmaceuticals: a review on their environmental occurrence and fate processes. *Water Res.* 124, 527–542.
- Schlüsener, M.P., Hardenbicker, P., Nilson, E., Schulz, M., Viergutz, C., Ternes, T.A., 2015. Occurrence of venlafaxine, other antidepressants and selected metabolites in the Rhine catchment in the face of climate change. *Environ. Pollut.* 196, 247–256.
- Souchier, M., Benali-Raot, D., Casellas, C., Ingrand, V., Chiron, S., 2016. Enantiomeric fractionation as a tool for quantitative assessment of biodegradation: the case of metoprolol. *Water Res.* 95, 19–26.
- Stanley, J.K., Ramirez, A.J., Chambliss, C.K., Brooks, B.W., 2007. Enantiospecific sub-lethal effects of the antidepressant fluoxetine to a model aquatic vertebrate and invertebrate. *Chemosphere* 69, 9–16.
- Steinbach, C., Burkina, V., Fedorova, G., Grabicova, K., Stara, A., Velisek, J., Zlabek, V., Schmidt-Posthaus, H., Grabic, R., Kroupova, H.K., 2014. The sub-lethal effects and tissue concentration of the human pharmaceutical atenolol in rainbow trout (*Oncorhynchus mykiss*). *Sci. Total Environ.* 497, 209–218.
- Sui, Q., Huang, J., Deng, S., Chen, W., Yu, G., 2011. Seasonal variation in the occurrence and removal of pharmaceuticals and personal care products in different biological wastewater treatment processes. *Environ. Sci. Technol.* 45, 3341–3348.
- Wang, Z., Zhang, X.-H., Huang, Y., Wang, H., 2015. Comprehensive evaluation of pharmaceuticals and personal care products (PPCPs) in typical highly urbanized regions across China. *Environ. Pollut.* 204, 223–232.
- Xu, J., Sun, H., Zhang, Y., Alder, A.C., 2019. Occurrence and enantiomer profiles of  $\beta$ -blockers in wastewater and a receiving water body and adjacent soil in Tianjin, China. *Sci. Total Environ.* 650, 1122–1130.
- Yang, L., He, J.-T., Su, S.-H., Cui, Y.-F., Huang, D.-L., Wang, G.-C., 2017. Occurrence, distribution, and attenuation of pharmaceuticals and personal care products in the riverside groundwater of the Beiyun River of Beijing, China. *Environ. Sci. Pollut. Res.* 24, 15838–15851.
- Yao, B., Yan, S., Lian, L., Yang, X., Wan, C., Dong, H., Song, W., 2018. Occurrence and indicators of pharmaceuticals in Chinese streams: a nationwide study. *Environ. Pollut.* 236, 889–898.
- Yuan, S., Jiang, X., Xia, X., Zhang, H., Zheng, S., 2013. Detection, occurrence and fate of 22 psychiatric pharmaceuticals in psychiatric hospital and municipal wastewater treatment plants in Beijing, China. *Chemosphere* 90, 2520–2525.
- Zhang, Y.-Z., Wang, B., Wang, W., Li, W.-C., Huang, J., Deng, S.-B., Wang, Y.-J., Yu, G., 2016. Occurrence and source apportionment of Per- and poly-fluorinated compounds (PFCs) in North Canal Basin, Beijing. *Sci. Rep.* 6, 36683.
- Zhang, Y., Duan, L., Wang, B., Du, Y., Cagnetta, G., Huang, J., Blaney, L., Yu, G., 2019. Wastewater-based epidemiology in Beijing, China: Prevalence of antibiotic use in flu season and association of pharmaceuticals and personal care products with socio-economic characteristics. *Environ. Int.* 125, 152–160.
- Zhang, Y., Wang, B., Cagnetta, G., Duan, L., Yang, J., Deng, S., Huang, J., Wang, Y., Yu, G., 2018. Typical pharmaceuticals in major WWTPs in Beijing, China: occurrence, load pattern and calculation reliability. *Water Res.* 140, 291–300.
- Ziylan, A., Ince, N.H., 2011. The occurrence and fate of anti-inflammatory and analgesic pharmaceuticals in sewage and fresh water: treatability by conventional and non-conventional processes. *J. Hazard. Mater.* 187, 24–36.