Ecotoxicology and Environmental Safety 174 (2019) 83–91

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Simultaneous enantiomeric analysis of non-steroidal anti-inflammatory drugs in environment by chiral LC-MS/MS: A pilot study in Beijing, China

Ruixue Ma[a,b,1], Han Qu[b,c,1], Bin Wang[b,*], Fang Wang[b], Yunjiang Yu[a], Gang Yu[b]

[a] State Environmental Protection Key Laboratory of Environmental Pollution Health Risk Assessment, South China Institute of Environmental Sciences, Ministry of Environmental Protection, Guangzhou 510655, China

[b] Beijing Key Laboratory of Emerging Organic Contaminants Control, State Key Joint Laboratory of Environmental Simulation and Pollution Control, Collaborative Innovation Center for Regional Environmental Quality, School of Environment, Tsinghua University, Beijing 100084, China

[c] School of Environment, Guangzhou Key Laboratory of Environmental Exposure and Health, Guangdong Key Laboratory of Environmental Pollution and Health, Jinan University, Guangzhou 510632, China

ARTICLE INFO

Keywords:
Chiral pharmaceuticals
Non-steroidal anti-inflammatory drug
Enantioseparation
Emerging pollutants
Surface water
LC–MS/MS

ABSTRACT

A simple, sensitive and quick method for direct simultaneous chiral analysis of frequently used non-steroidal anti-inflammatory drugs (NSAIDs) (ibuprofen, naproxen and flurbiprofen) in river water by HPLC-MS/MS was established and validated. Chromatographic parameters including the mobile phase composition, pH values, temperature and flow rates were optimized to obtain both satisfactory sensitivity and enantiomeric resolution ($Rs \geq 1.0$), which suggested the composition and pH values of mobile phase played crucial influence on enantioseparations. The method demonstrated its superiority compared with previous studies regarding to the low MQLs (1.1–37.1 ng/L) and short runtime (< 20 min), enabling quantitative enantiomeric determination of trace level of emerging contaminants in water. The environmental monitoring of receiving water (34 sites along rivers) in Beijing revealed ibuprofen was the most abundant, with mean concentration of 114.9 ng/L and detection frequency of 91%, naproxen was also detectable at all sites from < MQL-43.2 ng/L, both presenting an excess of the S-(+)-enantiomer. Therefore to better understand the ecological risk posed from the trace organic contaminants on the aquatic organisms, chiral pollutants need analyzed at the enantiomeric levels. This is the first to profile the enantiospecific occurrence of NSAIDs in surface water in Beijing, China. It could provide useful information on environmental behaviors of chiral pollutants and facilitate more accurate environmental risk assessment.

1. Introduction

The steady increase in the prescription of pharmaceuticals all over the world has brought about an important group of emerging contaminants (Petrie et al., 2018; Sui et al., 2017). Among which, the single-enantiomer drugs introduced into the market have ranged from about 40% of the new worldwide approved drugs in 1992 to almost 70% in 2010 (Calcaterra and D'acquarica, 2018; Ribeiro et al., 2012). Chiral PACs now have received growing concern about their enantiospecific ecological characteristics (Buser et al., 1999; Fono and Sedlak, 2005; MacLeod and Wong, 2010; Ma et al., 2016; Matamoros et al., 2009). Enantiomers of chiral PACs show identical physicochemical properties but may differ in their biological properties such as pharmacology, pharmacokinetics, metabolism and toxicology (Liu and Gu, 2011). One enantiomer may be effective, while the other could be inactive or responsible for side or antagonist effects, despite this, most chiral PACs intended for either human or animal use are still administered as racemates (Camacho-Muñoz and Kasprzyk-Hordern, 2015). Due to complex metabolic processes in the environment (e.g. human metabolism, biological wastewater processes and/or microbial degradation in the environment), they could exist in non-racemic form in environment (Evans and Kasprzyk-Hordern, 2014; Kasprzyk-Hordern, 2010). Recently, the enantiomeric occurrences of some chiral PACs have been reported in wastewater and receiving waters (Buser et al., 1999; Castrignanò et al., 2018; Evans et al., 2017; Bagnall et al., 2012; Kasprzyk-Hordern and Baker, 2012; MacLeod and Wong, 2010; MacLeod et al., 2007; Matamoros et al., 2009). Non-steroidal anti-inflammatory drugs (NSAIDs) are a class of widely applied pharmaceuticals prescribed in high quantities worldwide mostly in racemic forms (Kasprzyk-Hordern, 2010; Nakada et al., 2006; Padhye et al., 2014;

* Correspondence to: School of Environment, Tsinghua University, Beijing 100084, China.
E-mail address: thuwb@tsinghua.edu.cn (B. Wang).

[1] The first two authors contributed equally to this work.

https://doi.org/10.1016/j.ecoenv.2019.01.122
Received 17 November 2018; Received in revised form 24 December 2018; Accepted 17 January 2019

Quintana et al., 2005; Thomas et al., 2003). With the growing consumption and incomplete removal by WWTP, many pharmacologically active compounds (PACs) are environmentally ubiquitous to present "pseudo-persistence", causing potential threats to human health and ecosystem even at low concentrations (Caballo et al., 2015a; Caracciolo et al., 2015). NSAIDs are also found to pose ecotoxicities depending on compounds and organisms studied (Khetan and Collins, 2007). The acute toxicity of ibuprofen is 9.1, 7.1 and 173 mg L^{-1} towards daphnid (48 h), algae (24 h) and fish (< 96 h), respectively, while that of naproxen varied from 12.3 mg L^{-1} (Cyanobacteria) to 690 mg L^{-1} (Oncorhynchus mykiss) (Fent, 2008). Higher toxicity of naproxen photo-degradation by-products to aquatic organisms than the parent compound was also reported (Santos et al., 2010). As one of the most popular drug in the world, ibuprofen is also administered in racemate with desired pharmacological effects exclusive in the S-enantiomer, the inactive R-enantiomer undergoes extensive chiral inversion to S-enantiomer in vivo (Bonato et al., 2003), causing higher concentrations of S-ibuprofen in raw wastewater (Buser et al., 1999; Matamoros et al., 2009). The presence in non-racemic form has raised concern about their significantly stereospecific toxicity towards aquatic organisms (De Andrés et al., 2009; Stanley et al., 2007, 2006). However, studies involving enantiomeric determination of NSAIDs in environment were insufficient partly due to the limitation of analytical techniques (Khan et al., 2014; MacLeod and Wong, 2010; Hashim et al., 2011; Suzuki et al., 2014; Wang et al., 2013). The prevalent methods based on gas chromatography followed by the complicated and time-consuming derivatization may cause analyte losses and affect the repeatability (Carlucci et al., 2012; Hashim and Khan, 2011; Hashim et al., 2010; Khan et al., 2014; Hashim et al., 2011). Moreover, many reported HPLC methods for NSAIDs chiral analysis showed either low resolution or sensitivity, therefore, direct transfer of chiral LC/UV method to LC-MS/MS method is not always possible due to the non-volatile modifiers in mobile phases which are incompatible with ESI-MS applications (Ates et al., 2008; Teng et al., 2003; Ye et al., 2010). Considering the trace levels of chiral PACs in environment, developing direct, quick and sensitive analysis methods remains a challenge and urgently needs to be addressed.

Beijing, as the capital of China, is one of the world's most densely populated cities with huge pharmaceutical consumption, however, the wastewater treatment capacity was insufficient, besides, other sources of PACs in natural water bodies also made significant contribution, including pharmaceutical industry and hospital wastewater discharge, landfills, livestock breeding, and so on (Sui et al., 2017), leading to a considerable residues of PACs in water environment (Li et al., 2013; Liu et al., 2017; Ma et al., 2017; Sui et al., 2011). However, most of these did not take enantioselectivity into account. Given this, the present study aims to (1) develop a quick and reliable direct analytical method for simultaneous determination of three frequently-used NSAIDs (naproxen, ibuprofen and flurbiprofen) at enantiomeric level in environmental samples by HPLC-MS/MS; (2) delineate the enantiospecific occurrence of chiral profens in receiving waters in Beijing, China. This is the first to demonstrate the enantioselective occurrence of NSAIDs in environment in Beijing, China, which could provide information to better understand environmental fate and risks of chiral emerging contaminants.

2. Experimental

2.1. Chemical and reagents

(+)/(-)-ibuprofen (IBU) (≥ 98% purity), (+)/(-)-naproxen (NAP) (≥ 98% purity) and (+)/(-)-flurbiprofen (FLB) were supplied by Dr. Ehrenstorfer (Augsburg, Germany). The chemical structures and pK_a values were shown in Table S2 in Supporting information. Optical pure standards of (+)-ibuprofen, (+)-naproxen and (+)-flurbiprofen as well as the isotopically labelled (R)/(S)-ibuprofen–d3 (99% purity), (R)/(S)-flurbiprofen-d3 (99% purity) and (R)/(S)-naproxen-d3 (98% purity) as internal standards (IS) were obtained from TRC (Toronto, Canada). The racemates standards were confirmed by LC–UV. SPE cartridges of Oasis HLB (200 mg/6 mL) were procured from Waters Corporation (Milford, MA, USA). HPLC-grade acetonitrile and methanol were purchased from Fisher Scientific (Loughborough, Leicestershire, UK). Analytical grade formic acid (FA) and ammonium acetate were purchased from Fluka (Buchs, Switzerland). Ultrapure water of 18.2 MΩ cm^{-1} was prepared by the Milli-Q purification system (Millipore, Milford, MA, USA).

Individual stock solutions of racemic standard profens and IS were dissolved in methanol (1 mg/mL). Working standard solutions (1 mg L^{-1}) for calibration curve containing mixture of the three profens of 10–500 μg/L (5–250 μg/L for each enantiomer) were prepared freshly by serial dilution and stored at 4 °C in the dark.

2.2. Sample collection and extraction

Study area was along the main stream of North Canal Basin and its main tributaries (Qinghe River, Bahe River, Tonghui River and Liangshui River) from most urbanized and industrialized area located in the northeast Beijing, China with a catchment area of 4293 km^2. Total of 34 surface water samples were collected by grab sampling at 0.5 m under the surface of rivers in July and November 2016 (information on study area were described in Supporting information S1 and Table S1). All samples were stored in dark glass bottles with 0.02% (w/v) NaN_3 solution added to prevent microbial degradation, then transported refrigerated at 4 °C to the lab, and pretreated within 48 h. After filtration, a 500 mL portion was adjusted to pH 4 and spiked with mixed internal standard (50 ng/L of each compound) for SPE. Detailed pretreatment procedures were described in Supporting information S2 and Table S3.

2.3. Chiral LC-MS/MS analysis

Chiral determination was performed using an ultra-high performance liquid chromatography (Ultimate3000 HPLC system, Dionex, USA) coupled to tandem mass spectrometry (ESI-MS/MS, API4000, AB Sciex, USA) operated in the negative ionization mode. Enantioseparations were carried out on a CHIRALPAK AD-RH (150 × 4.6 mm, 5 μm) column amylose tris(3,5-dimethylphenylcarmabate) procured from Daicel Chemical Industries Ltd. (Tokyo, Japan) with a binary mobile phase under isocratic flow rate of 0.4 mL/min. The optimized mobile phase was composed of a 65% of aqueous solution of 10 mM ammonium acetate (pH 5, formic acid adjusted) and 35% of acetonitrile. The column temperature maintained at 25 °C and the temperature of sample manager was kept at 4 °C. The injection volume was 20 μL. Column was flushed for at least 30 min for equilibration in mobile phase before analysis. After every five samples, a blank solvent (pure methanol) was injected to wash the column in case of sample contamination. The MS/MS mass spectrometric parameters including CUR (Curtain Gas), CAD (Collision Gas), IS (Ion Spray Voltage), TEM (source temperature), GS1 and GS2 (Ion Source gases) were tested and finely adjusted to obtain simultaneous enantioseparation and satisfactory sensitivity as follows: CUR (N_2) 20, CAD 5, IS −4500 V, TEM 475 °C, GS1 60 and GS2 70. 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 and quantifier ions for each analyte were listed in Table S4.

2.4. Method development and validation

The composition of mobile phase and pH, flow rate and column temperature were optimized to obtain the best chromatographic resolution and enough sensitivity for MS spectrometry for effective enantiomeric determination. Linearity was evaluated by analyzing the

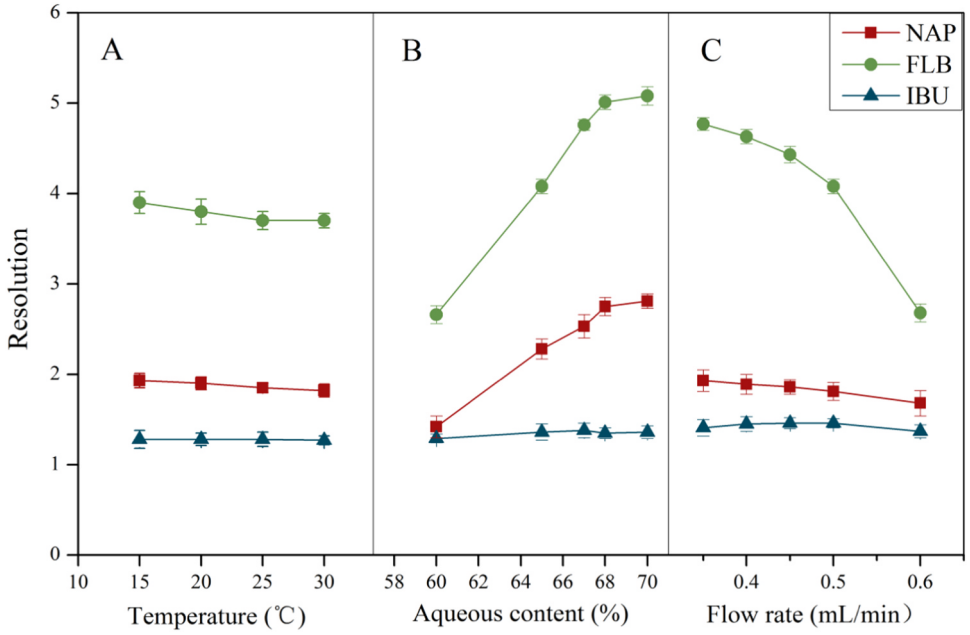

Fig. 1. Influencing factors on enantiomeric resolutions of the target analytes. (A) effect of aqueous phase (10 mM ammonium acetate solution (pH 5)) in mobile phase; (B) effect of column temperature; (C) effect of flow rate.

standards in solvent at concentration of 5, 10.0, 20.0, 50.0, 100, and 250 μg/L (for each single enantiomer) in triplicate. Calibration of matrix-match was also constructed by post-spiking deuterated standards with the same concentrations above in the SPE extracts. The inter-day and intra-day precision expressed as the relative standard deviation (RSD), was determined by repeated injection within one day and 3 consecutive days, respectively. For accuracy of the method, the absolute recoveries were evaluated in surface water at three levels(10, 50, 200 ng/L) in triplicate by comparing the peak areas (background subtracted) of analytes obtained from spiked samples before SPE with those from direct injection of equivalent amounts of standards in methanol. The relative recoveries were determined to evaluate the SPE method efficiency by comparison of the response of deuterated internal standard in spiked samples before SPE with those of the same concentration spiked after extraction and concentration (Camacho-Muñoz and Kasprzyk-Hordern, 2015). The IS method was used for quantification.

Instrumental detection limits (IDL) and instrumental quantification limits (IQL) were determined as minimum concentration of the solvent calibration curve with the signal to noise (S/N) ratios of 3 and 10 times for each enantiomer, respectively according to (Camacho-Muñoz and Kasprzyk-Hordern, 2017). Method detection limit (MDL) and method quantification limit (MQL) were evaluated with surface water after taking into account the relative recovery and the concentration factor (that is 1000 for this study) by SPE. Matrix effects (ME) were evaluated by comparison of the responses of post-extraction spiking with those of standards in solvent, following the formula:

$$ME(\%) = (A_m/A_0 - 1) \times 100\%,$$

where A_m is the peak area of the background-subtracted matrix-matched standard and A_0 is the peak area of the pure standard at the same concentration, where a negative value of ME indicates a signal suppression.

Resolution (R_S) of enantiomers of chiral compounds was calculated according to the following equation:

$$R_s = \frac{2(tr_2 - tr_1)}{W_1 + W_2}$$

where tr_1 and tr_2 are the retention times of the first and second peak, respectively; W_1 is the base width of the first peak of a pair of enantiomers and W_2 for the second one. $R_s \geq 1.5$ indicates a baseline separation, and $R_s \geq 1$ was deemed adequate for quantification (Bagnall et al., 2013).

Enantiomeric fraction (EF) was calculated as follows:

$$EF = \frac{E(S)}{E(S) + E(R)}$$

where E(*S*) is the peak area of *S*-(+)-enantiomer, E(*R*) for *R*-(-)-enantiomer. The EF values can range from 0 to 1, with EF = 0.5 representing the racemic mixture.

3. Results and discussion

3.1. LC-MS/MS condition optimization

3.1.1. Effects of organic solvent on retention and enantioselectivity

The retention and enantioselectivity of the analytes were regulated by the composition of the organic modifier (OM) in mobile phase. Acetonitrile proved to present lower column pressure and give better resolution than methanol (Perrin et al., 2002). The OM plays a crucial role in enantioseparation of the three NSAIDs on Chiralpak AD-RH. Decreasing acetonitrile content could increase retention time and enantiomeric resolution, and this contribution was significant even with very small amplitude of change. Nevertheless, this effect was compound-dependent. Flurbiprofen was the most susceptible to acetonitrile followed by naproxen. Both retention and resolution were greatly enhanced with acetonitrile decreasing from 40% to 30%, while ibuprofen was not so sensitive with minor fluctuation of Rs around 1.3 (shown in Fig. 1A). However, further increasing the content of the aqueous part of the mobile phase resulted in a much higher retention of the analytes, which could also decrease the peak high and broaden the peak width, and the calculated S/N ratio may be lower as a consequence. Finally, the content of acetonitrile was optimized to be 35% to give satisfactory separation and good MS performance, and no further reduction was made due to the unfavorable ionization at a high proportion of aqueous part in mobile phase.

3.1.2. Effects of flow rate and column temperature

The column temperature was tested from 15 °C to 30 °C, and did not

show obvious influence on enantiomeric resolution (Fig. 1B). The resolutions slightly increased at low temperature, but the retention time delayed accordingly with decreasing temperature, thus the column temperature was maintained at room temperature of 25 °C. By comparison, the effect of flow rate was compound-dependent (Fig. 1C). Five flow rates were tested from 0.35 to 0.6 mL/min, the resolution of flurbiprofen dramatically decreased with the increasing flow rate while those of naproxen and ibuprofen were not much affected. Finally, 0.4 mL/min was optimized in terms of peak width and MS ionization efficiency.

3.1.3. Effects of buffer pH on separation and MS response

Although ammonium acetate was non-essential for enantioseparation, it highly influenced the ionization and helped to stabilize the pH of mobile phase, thus it was used as buffer solution to obtain better peak shapes and higher signal responses and the frequently used 10 mM was applied. Reported methods on enantiomeric analysis of NSAIDs utilizing Chiralpak AD-RH were performed under acid condition, commonly with addition of phosphoric acid or trifluoroacetic acid (TFA) in mobile phase. However, phosphoric acid was incompatible with MS detector and TFA could cause significant ion suppression for both basic and acidic compounds when used with ESI, while formic acid was found to be the best (Furey et al., 2013). To overcome this problem, formic acid was introduced to adjust the pH of buffer solution from 3.0 to 6.0 for testing. The effect of the pH of the aqueous mobile phase on the retention and therefore on the separation of ionisable compounds is fundamental in reversed-phase LC. When fully ionised, the analytes partition preferentially in the aqueous mobile phase. Keeping the chiral analytes neutral when working with polysaccharide stationary phases was reported as these CSPs do not possess any ionic site susceptible to interact with the charged analytes. Consequently, the retention of the acidic analytes decreased with increasing pH due to their progressive ionization. As the pKa values of the three analytes were below 5, relatively lower pH of mobile phase would give better separation. The pH value of buffer solution was found to be a main influencing factor. Both the resolution and retention time decreased with the increasing pH values. However, under low pH conditions, the signal intensities would diminish and might be quite insufficient for enantiomeric quantification of environmental samples. At pH 3.0, the S/N ratios were too low to give satisfactory sensitivity, and the analysis time were over 40 mins. At pH 6.0, the three pairs of enantiomers were eluted within 10 min, naproxen was barely resolved and ibuprofen was just partial separated. At pH 5.5, better separation was observed, yet the Rs for naproxen and ibuprofen were still below 1. When the pH of aqueous buffer at or below 5.0, the Rs of the three analytes could all exceed 1.0, which was adequate for quantification, besides, the S/N ratio at pH 5.0 was almost twice as much as that at pH 4.0, which allows better sensitivity for environmental analysis. Hence, the pH of buffers was determined to adjust to 5.0.

From the above, the mobile phase containing a mixture of acetonitrile-10 mM NH_4Ac solution (pH 5.0, formic acid adjusted) (35/65, v/v) at a flow rate of 0.4 mL/min was optimized to give the better enantioselectivity, sensitivity and satisfactory analysis times (within 20 min). Representative enantioseparation MRM chromatograms under different pH values of mobile phase were illustrated in Fig. 2. The elution order of enantiomers was also confirmed by comparing the retention time with the optical pure standards using LC-MS/MS, which was R-(-)-naproxen (9.27 min), S-(+)-naproxen (10.05 min), R-(-)-flurbiprofen (11.16 min), S-(+)-flurbiprofen (13.40 min), R-(-)-ibuprofen (14.71 min) and S-(+)-ibuprofen (15.70 min).

It is worth mentioning that the simultaneous enantioseparation under the optimal conditions could not be achieved when just applying HPLC/UV, for ibuprofen enantiomers were apt to overlap with anteroposterior peaks on all accounts. To further develop a simultaneous enantiomeric analysis method by HPLC, separation was conducted under low pH condition with TFA added in mobile phase to improve resolution and peak symmetry. Optimization data was provided in Supporting information (see Table S5 and Fig. S2). To the best of our knowledge, it is the first time that simultaneous enantioseparation of these three NSAIDs was achieved using CSP of amylose-tris-(3, 5-dimethylphenylcarbamate) by HPLC.

3.2. Method validation and evaluation

The method performance under the optimized chromatographic conditions was evaluated (Table 1). Calibration curves of each enantiomer showed good linearity with $R^2 > 0.99$. EF was also calculated at each concentration from calibration curve. The MDLs and MQLs ranged from 0.35 to 11.1 ng/L from 1.1–37.1 ng/L, respectively. The best sensitivity was observed for naproxen, whereas ibuprofen showed the lowest due to the relatively high background noise. RSD was below 20% for inter-day and intra-day precision. Satisfactory absolute and relative recoveries were obtained for three spiked levels ranging between 74.1% and 100.5%. Non-significant signal suppression/enhancement differences between enantiomers of the same compound were observed for these surface water samples with matrix effect from − 8.7% to − 17.7%. The EFs of standards in solvent and in recovery studies were monitored and the changes from racemic were within 0.51 ± 0.02, indicating no stereoselective processes occurring during sample treatment. The results demonstrated that the method was simple, sensitive and reliable, with good reproducibility and much lower MQL, and thus could be applied for simultaneous analysis of the target NSAIDs in environment at enantiomeric level.

Among the commercially chiral stationary phases (CSPs), those based on macrocyclic antibiotic and derivatized polysaccharide selectors have much broad enantioselectivity and achieved separation for a wide range of chemically diverse compounds. However the performance of macrocyclic antibiotic showed deficiencies in lower resolution or enantiomeric analysis of NSAIDs in environment. For instance, ibuprofen and naproxen were only partial enantioseparated with Rs < 1.0 using teicoplanin CSP (Camacho-Muñoz and Kasprzyk-Hordern, 2017), while enantiomers of naproxen remained unresolved on Chirobiotic V under the proposed chromatographic condition (López-Serna et al., 2013).

For polysaccharide-based CSPs, ibuprofen and its metabolites were achieved enantiomeric resolution on Chiralpak AS-H column (amylose tris-(S)-a-methylbenzylcarbamate) under normal-phase condition (Borges et al., 2011), however, this had a deleterious effect on ionization MS detection, thus, a post-column infusion had to be introduced to overcome the difficulty in ionization for MS detection. Similarly, (Bonato. et al., 2003) utilized a mobile phase consisting of phosphoric acid solution (pH = 2) for analyzing ibuprofen enantiomers also by introducing NH_4OH solution as make-up liquid, yet the method LOQ of 0.12 μg/mL (in plasma) was inadequate for environmental analysis. (Caballo et al., 2015c) achieved enantiomeric discrimination of some profens using (R)-1-naphthylglycine and 3,5-dinitrobenzoic acid as CSPs, nevertheless, it still has limitation due to the unsafe solvent of mobile phase. The comparison among the proposed method and other methods for chiral analysis of NSAIDs by LC-MS/MS was summarized in Table 2, from which, the our proposed method proved to be simple and quick for environmental chiral analysis of NSAIDs.

3.3. Method application to profile the chiral occurrence in surface water

To further evaluate the method application and gain a primary insight into the chiral occurrence of NSAIDs in environment in Beijing, China. 34 samples of surface water were collected twice from main stream and tributaries of North Canal Basin in 2016. The concentration revealed ibuprofen was the most abundant compound of the three, with mean values of 114.9 ng/L (sum of the two enantiomers) and a detection frequency of 91%, naproxen was detectable at all sites for both samplings (Table 3 and Fig. 3). Flurbiprofen was much less frequently

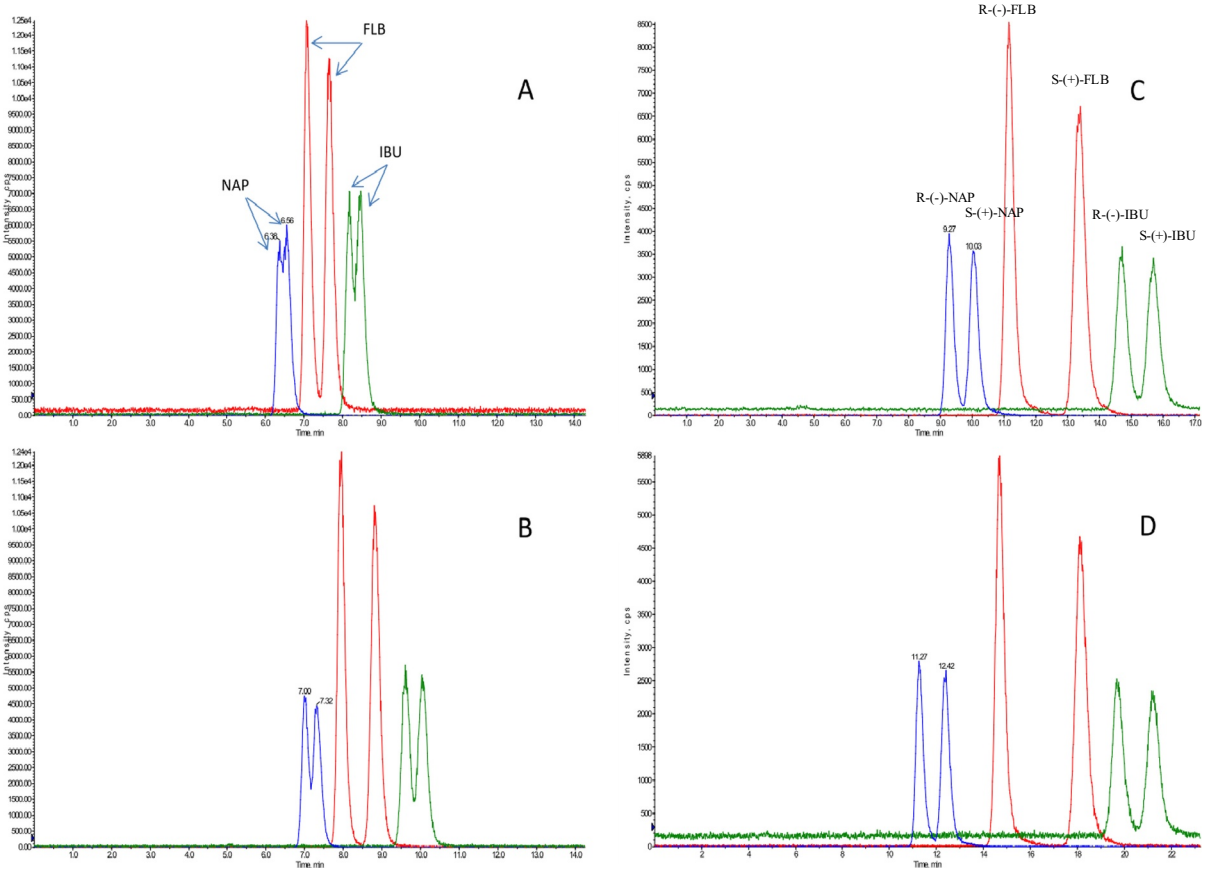

Fig. 2. Simultaneous enantioseparation under different pH values of buffers in mobile phase (A) pH = 6.0; (B) pH = 5.5; (C) pH = 5.0; (D) pH = 4.0; Mobile phase: acetonitrile–10 mM NH_4OAC aqueous solution (35: 65 v: v), flow rate: 0.4 mL/min.

Table 1
Method validation parameters of the enantiomers.

Compound	Linearity (R^2)		IDL (μg/L)	IQL (μg/L)	MDL (ng/L)	MQL (ng/L)	Precision (RSD, %)		tr (min)	Rs	EF (mean ± SD)	Absolute recovery (%) (mean ± SD)	Relative recovery (%) (mean ± SD)	ME (%)
	Solvent	Matrix-match					Intra-day	Inter-day						
R-(-)-NAP	0.995	0.994	0.34	1.1	0.35	1.1	9.4	11.9	9.27			89.9 ± 3.1	98.8 ± 4.9	− 9.5
S-(+)-NAP	0.995	0.993	0.36	1.2	0.37	1.2	10.5	10.4	10.05	1.0	0.50 ± 0.01	88.7 ± 3.7	97.5 ± 4.2	− 8.7
R-(-)-FLB	0.996	0.996	2.1	7.0	2.2	7.1	12.7	16.6	11.16			83.6 ± 5.3	98.4 ± 6.1	− 14.9
S-(+)-FLB	0.994	0.996	2.9	9.7	2.9	9.7	11.4	13.2	13.40	2.3	0.51 ± 0.01	86.4 ± 6.9	100.5 ± 4.7	− 13.6
R-(-)-IBU	0.995	0.994	8.5	28	9.4	31.1	10.2	14.3	14.71			74.1 ± 8.1	90.4 ± 5.4	− 17.7
S-(+)-IBU	0.991	0.992	9.9	33	11.1	37.1	11.8	17.6	15.70	1.1	0.51 ± 0.02	78.6 ± 4.8	89.3 ± 6.8	− 12.3

detected only in few samples below MQL. Similarly, flurbiprofen was not detected in river water and lake water from Shenyang city, China, whilst a lower concentration of about 20 ng/L with slight enantioselectivity in waste water was observed (Yuan et al., 2018). The concentrations from ND (not detected) to 405.5 ng L^{-1} for ibuprofen and ND-42.3 ng/L for naproxen (sum of the two enantiomers) were comparable with those detected in the rivers of South Wales, UK (ibuprofen < 0.3–100 ng/L, naproxen < 0.3–146 ng/L) (Kasprzyk-Hordern et al., 2008), but lower than those in Guadalquivir River basin located in the South of the Iberian Peninsula (ibuprofen of 622 ng/L and naproxen of 129 ng/L on average)(López-Serna et al., 2013). Still, the concentrations in surface water were higher than those in freshwater lake, which was reported with ibuprofen from 30.8 to 89.3 ng/L (sum of the enantiomers), while naproxen below MDLs in all samples in Qingshan Lake basin, east China (Zhu et al., 2013). A statistic analysis of pharmaceutical active compounds detected in environment in China reported during 2012–2015 revealed, the ibuprofen and naproxen were frequently detected in surface water with mean concentration about 50 ng/L (Mei et al., 2018; Zhao et al., 2016), of which, ibuprofen could pose considerable adverse ecological consequences on aquatic organisms.

Chiral profiling revealed the EFs of ibuprofen were 0.69 ± 0.11 (mean ± SD) in July and 0.70 ± 0.06 in November, respectively with much fluctuation in summer (0.45–1.00). The higher consumption of the some pharmaceuticals like antibiotics, NSAIDs, as well as drugs driven by associated seasonality in pathologies might cause the increase of detected concentration in influents of WWTPs and also in surface winter. Besides, the treatment processes of WWTPs were considered related to temperature, which presented lower removal rates occurred in cold season (Sui Q. et al., 2011). The great temperature difference between summer and winter in Beijing could probably influence both the PACs occurrence and the enantiospecific fate during these two seasons. Additionally, rainfall was found to affect the concentrations of PACs in the effluent (Ternes, 1998). Increased river flow caused by rainfall on one hand could dilute the PACs concentrations, but on the other hand may result in lower removal efficiency of WWTPs, thus

Table 2
Comparison among the present study and the existing LC-MS/MS methods.

Analytes	Chiral column	Mobile phase	Resolution and analysis time	Matrix application	MLQ	References
Ibuprofen, Naproxen and Ketoprofen	Chirobiotic V	MeOH containing 4 mM NH_4AC and 0.005% formic acid	Ibuprofen: Rs 1.27; R_t(min) 21.45/ 24.08 (naproxen and ketoprofen unresolved)	Surface water and wastewater	Ketoprofen: 0.6 ng/L (SW), 2.42 (EW), 3.30 (IW) Naproxen: 0.96 ng/L(SW), 2.42 (EW), 3.30 (IW)	(López-Serna et al., 2013)
Ibuprofen, Naproxen, Ketoprofen	Sumichiral OA-2500	Tetrahydrofuran: ammonium acetate (50 mM) in MeOH (90:10 v: v)	Rs: 1.4(ibuprofen) 1.7(ketoprofen) 2.8(naproxen)	wastewater	0.5–1.2 ng/L	(Caballo et al., 2015b)
10 profens including metabolites	Chiral-AGP	10 mM ammonium (pH 6.7): CH_3CN (99:1 v: v)	Rs: 1.0(ibuprofen) 1.6(naproxen)	Surface water and wastewater	Surface water:37.47 and 38.46 ng/L for ibuprofen enantiomers; 8.49 and 11.73 ng/L for naproxen enantiomers	(Camacho-Muñoz and Kasprzyk-Hordern, 2015)
8 profens including metabolites	Chirobiotic T	10 mM ammonium (pH 4.2): methanol (70:30, v/v)	Rs: 0.8 (ibuprofen) 0.5(naproxen)	Surface water and wastewater	Surface water:466 and 1076 ng/L for ibuprofen enantiomers; 23.7 and 25.9 ng/L for naproxen enantiomers	(Camacho-Muñoz and Kasprzyk-Hordern, 2017)
naproxen	Chiralpak AD-RH	0.1% formic acid: CH_3CN (50:50, v/v);	Rs: not mentioned Analysis time < 30 min	River water	100 ng/L for each enantiomer	(Suzuki et al., 2014)
Ibuprofen	Chiralpak AD-RH	methanol: 0.1% phosphoric acid solution (80:20, v/v, pH = 2); make-up liquid of 4.5% (w/v) NH_4OH aqueous solution	Rs = 1.25	Human plasma	0.12 μg/mL	(Bonato et al., 2003)
Ibuprofen and metabolites	Chiralpak AS-H	Hexane: isopropanol: TFA (95: 5: 0.1 v: v: v); Post-column infusion with 10 mmol/L ammonium acetate in MeOH	Rs: not mentioned Analysis time within 25 min	Czapek culture medium with endophytic fungi	0.1 μg/mL for Ibuprofen	(Borges et al., 2011)
Ibuprofen	Lux Cellulose-3	0.1% (v/v) acetic acid in mixture of methanol and water (90:10 v: v)	Rs = 3 R_t(min): 9.88/10.74	Human plasma	100 μg/L	(Nakova et al., 2015)
Ibuprofen, flurbiprofen, naproxen	Chiralpak AD-RH	CH_3CN: water (10 mM NH_4OAC, pH 5.0) (35: 65 v: v), at 0.4 mL/min	Rs:2.3 (flurbiprofen); 1.1 (naproxen); 1.0 (ibuprofen) Analysis time within 25 min	Surface water	32 and 37 ng/L for ibuprofen enantiomers; 1.2 and 1.4 ng/L for naproxen enantiomers;7.9 and 11 ng/L for flurbiprofen enantiomers	Present study

Table 3
Concentrations and EFs of profens in river water in Beijing.

Enantiomer	July, 2016			November, 2016		
	Concentration (ng/L)	Detection frequency	EF values (mean ± SD)	Concentration (ng/L)	Detection frequency	EF values (mean ± SD)
(S)-Naproxen	< MQL-36.0	100%	0.92 ± 0.04	1.7–43.2	100%	0.94 ± 0.03
(R)-Naproxen	NDp1.9	56%		ND-1.3	79%	
(S)-Flurbiprofen	< MQL	3%	–	< MQL	9%	–
(R)-Flurbiprofen	< MQL	3%		< MQL	9%	
(S)-Ibuprofen	ND-324.5	88%	0.69 ± 0.11	ND-199.3	91%	0.71 ± 0.08
(R)-Ibuprofen	ND-89.4	85%		ND-101.6	88%	

leading to undulatory biological degradation rate of these compounds. Wang et al. (2013) found ibuprofen were only occasionally quantified in receiving surface water of the Pearl River Delta, south China, which might be ascribed to its extensive transformation in STPs, and the EF of 0.673–0.870 suggested the pharmaceuticals in the mainstream Pearl River were mainly from discharge of treated wastewater. Determination of surface water and effluent wastewater from South-West England found R-(-)- and S-(+)-ibuprofen were at average concentrations of 0.24 ± 0.04 μg L^{-1} and 0.46 ± 0.06 μg L^{-1} in effluent wastewater, respectively while below MQL in surface water. The EF of ibuprofen was 0.65 ± 0.05 indicating an excess of S-ibuprofen in effluent samples (Camacho-Muñoz and Kasprzyk-Hordern, 2015). Another study in Sydney, Australia also revealed the EF of ibuprofen were 0.6–0.8 in the surface water from drainage channel (Khan et al., 2014). The stereospecific characteristics of ibuprofen were comparable with our present results. As one of the Top 20 prescription drug in the world, ibuprofen is mostly administered in racemate, due to the unidirectional chiral inversion of R-ibuprofen to S-ibuprofen in vivo, the environmental occurrence often presented an excess of S-ibuprofen in raw wastewater (Buser et al., 1999; Matamoros. et al., 2009). Whilst, a lower excess of S-enantiomer was observed in receiving water, suggesting S-ibuprofen degraded faster during sewage treatment process (Buser et al., 1999).

As naproxen was manufactured optically in form of S-enantiomer due to the known hepatoxicity of the R-(−)-enantiomer (Camacho-Muñoz and Kasprzyk-Hordern, 2015). In present study, naproxen was prevalence in sampling areas, with maximum level of 42.8 ng/L observed for November and 36.4 ng/L for July. Only trace level of the R-naproxen could be detected in a few samples, and the EFs were 0.92 ± 0.04 for July and 0.94 ± 0.03 for Novermber, in consistent with those previously published (Caballo et al., 2015b; Hashim et al., 2013; Khan et al., 2014). By contrast, Suzuki et al. (2014) found naproxen in the Tama River basin in Tokyo, Japan at concentrations of 10–80 ng/L, with EFs between 0.84 and 0.98. Whereas, river water from South West England suggested R-(-)-naproxen was below 8.49 ng/L (< MQL)(Camacho-Muñoz and Kasprzyk-Hordern, 2015), Although the (S)-naproxen was dominant in untreated sewage overflow, the

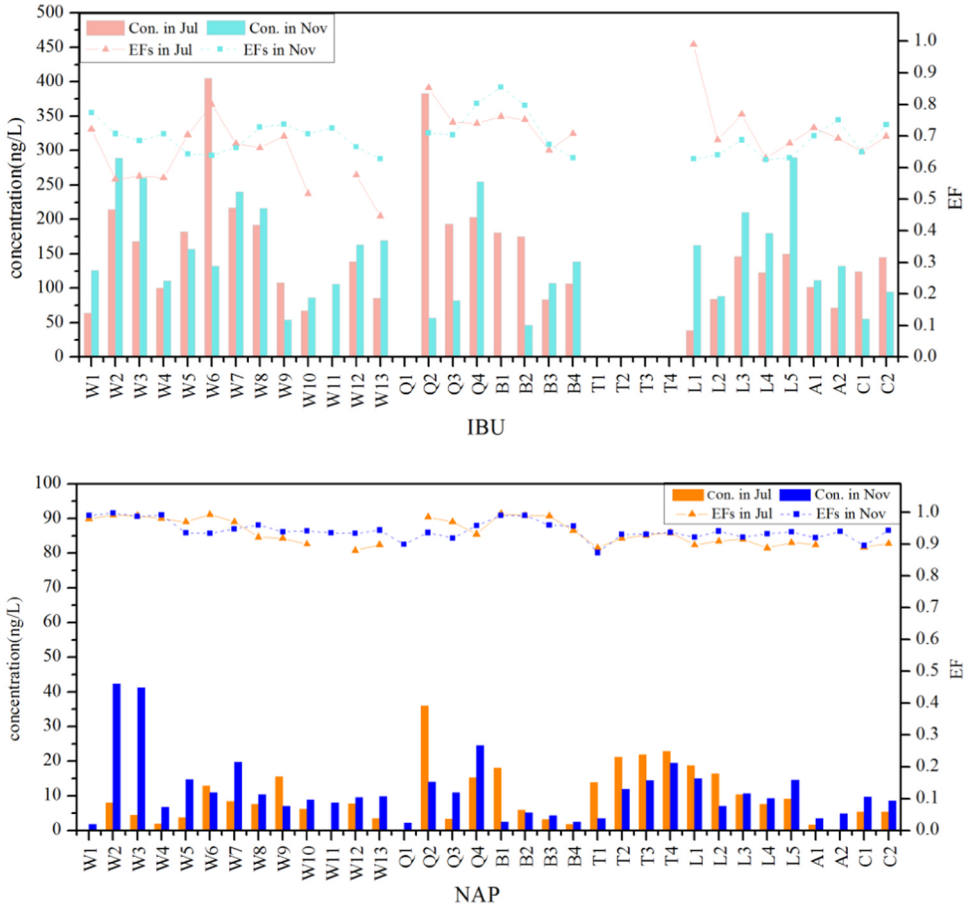

Fig. 3. Spatiotemporal enantiomeric profilings of ibuprofen and naproxen in the North Canal Rivers in Beijing, China.

increased proportion of the (R)-enantiomer in WWTP effluents indicated the chiral inversion during sewage treatment, but might not be as significant as ibuprofen. The pilot determination warrants further investigation of seasonal variation of the enantiospecific occurrence.

4. Conclusion

A method for directly simultaneous enantioselective determination of three frequently used NSAIDs (ibuprofen, naproxen and flurbiprofen) by chiral LC-MS/MS was developed. The method was proved to be quick, simple and sensitive, allowing enantiomeric analysis of trace level of NSAIDs in water, and successfully applied in environment analysis. The environmental survey revealed ibuprofen was the most abundant. Both ibuprofen and naproxen presented an excess of the S-(+)-enantiomer, whilst the enantiomeric composition showed a variation among sampling sites especially in summer. Since the reported ecotoxicity mostly recognized racemic mixture of chiral pollutant as one compound, the environmental impacts could be under- or over-estimated, as enantiomers often show different ecotoxicological potency (Kasprzyk-Hordern, 2010). Therefore, it is of utmost significance to introduce chiral analysis approaches that take into account enantiomerism of emerging contaminants. The present work is the first study that has achieved chiral determination of NSAIDs in surface water in Beijing, China. It is anticipated that the proposed methods could be further adapted for enantiomeric analysis of other chiral PACs in biological or environmental matrix and help to facilitate more accurate environmental risk assessment.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (grant number 21577075), the Natural Science Foundation of Guangdong Province (2018A030313945) and the Science and Technology Program of Guangzhou, China (No. 201804010234). Tsinghua University Initiative Scientific Research Program (grant number 20131089193).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2019.01.122

References

Ates, H., et al., 2008. Chiral separations in polar organic solvent chromatography: updating a screening strategy with new chlorine-containing polysaccharide-based selectors. J. Chromatogr. B 875, 57–64.
Bagnall, J., et al., 2013. Stereoselective biodegradation of amphetamine and methamphetamine in river microcosms. Water Res. 47, 5708–5718.
Bonato, P.S., et al., 2003. Enantioselective determination of ibuprofen in plasma by high-performance liquid chromatography–electrospray mass spectrometry. J. Chromatogr. B 796 (2), 413–420.
Borges, K.B., et al., 2011. LC–MS–MS determination of ibuprofen, 2-hydroxyibuprofen enantiomers, and carboxyibuprofen stereoisomers for application in biotransformation studies employing endophytic fungi. Anal. Bioanal. Chem. 399 (2), 915–925.
Buser, H.-R., et al., 1999. Occurrence and environmental behavior of the chiral pharmaceutical drug ibuprofen in surface waters and in wastewater. Environ. Sci. Technol. 33 (15), 2529–2535.
Caballo, C., et al., 2015a. Enantioselective analysis of non-steroidal anti-inflammatory drugs in freshwater fish based on microextraction with a supramolecular liquid and chiral liquid chromatography–tandem mass spectrometry. Anal. Bioanal. Chem. 407, 4721–4731.
Caballo, C., et al., 2015b. Enantioselective determination of representative profens in wastewater by a single-step sample treatment and chiral liquid chromatography–tandem mass spectrometry. Talanta 134, 325–332.
Caballo, C., et al., 2015c. Enantioselective analysis of non-steroidal anti-inflammatory drugs in freshwater fish based on microextraction with a supramolecular liquid and chiral liquid chromatography–tandem mass spectrometry. Anal. Bioanal. Chem. 407 (16), 4721–4731.
Calcaterra, A., D'acquarica, I., 2018. The market of chiral drugs: chiral switches versus de novo enantiomerically pure compounds. J. Pharm. Biomed. Anal. 147, 323–340.
Camacho-Muñoz, D., Kasprzyk-Hordern, B., 2015. Multi-residue enantiomeric analysis of human and veterinary pharmaceuticals and their metabolites in environmental samples by chiral liquid chromatography coupled with tandem mass spectrometry detection. Anal. Bioanal. Chem. 407 (30), 9085–9104.
Camacho-Muñoz, D., Kasprzyk-Hordern, B., 2017. Simultaneous enantiomeric analysis of pharmacologically active compounds in environmental samples by chiral LC–MS/MS with a macrocyclic antibiotic stationary phase. J. Mass Spectrom. 52, 94–108.
Caracciolo, A.B., et al., 2015. Pharmaceuticals in the environment: biodegradation and effects on natural microbial communities. A review. J. Pharm. Biomed. Anal. 106, 25–36.
Carlucci, G., et al., 2012. Analysis of anti-inflammatory enantiomers by HPLC in human plasma and urine: a review. Antiinflamm. Antiallergy Agents Med. Chem. 11, 96–112.
Castrignanò, E., et al., 2018. Enantiomeric profiling of chiral illicit drugs in a pan-European study. Water Res. 130, 151–160.
De Andrés, F., et al., 2009. Use of toxicity assays for enantiomeric discrimination of pharmaceutical substances. Chirality: Pharmacol. Biol. Chem. Conséq. Mol. Asymmetry 21, 751–759.
Evans, S., et al., 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.
Fent, K., 2008. Effects of Pharmaceuticals on Aquatic Organisms. Pharmaceuticals in the Environment. Springer, pp. 175–203.
Fono, L.J., Sedlak, D.L., 2005. Use of the chiral pharmaceutical propranolol to identify sewage discharges into surface waters. Environ. Sci. Technol. 39 (23), 9244–9252.
Furey, A., et al., 2013. Ion suppression; a critical review on causes, evaluation, prevention and applications. Talanta 115, 104–122.
H. Hashim, N., J.Khan, S., 2011. Enantioselective analysis of ibuprofen, ketoprofen and naproxen in wastewater and environmental water samples. J. Chromatogr. A 1218 (29), 4746–4754.
Hashim, N., et al., 2010. Enantiomeric fraction as an indicator of pharmaceutical biotransformation during wastewater treatment and in the environment–a review. Environ. Technol. 31, 1349–1370.
Hashim, N., et al., 2013. Enantiomeric fraction determination of 2-arylpropionic acids in a package plant membrane bioreactor. Chirality 25 (5), 301–307.
Bagnall, J.P., et al., 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.
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.
Kasprzyk-Hordern, B., et al., 2008. The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. Water Res. 42 (13), 3498–3518.
Khan, S.J., et al., 2014. Distinct enantiomeric signals of ibuprofen and naproxen in treated wastewater and sewer overflow. Chirality 26 (11), 739–746.
Khetan, S.K., Collins, T.J., 2007. Human pharmaceuticals in the aquatic environment: a challenge to green chemistry. Chem. Rev. 107, 2319–2364.
López-Serna, R., et al., 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 (18), 5859–5873.
MacLeod, S.l., Wong, C.S., 2010. Loadings, trends, comparisons, and fate of achiral and chiral pharmaceuticals in wastewaters from urban tertiary and rural aerated lagoon treatments. Water Res. 44 (2), 533–544.
Li, W., et al., 2013. Occurrence and removal of antibiotics in a municipal wastewater reclamation plant in Beijing, China. Chemosphere 92 (4), 435–444.
Liu, H.-Q., et al., 2017. Spatial distribution and removal performance of pharmaceuticals in municipal wastewater treatment plants in China. Sci. Total Environ. 586, 1162–1169.
Liu, Y., Gu, X., 2011. Chiral Drugs: Chemistry and Biological Action. John Wiley & Sons, Inc. Pharmacology of Chiral Drugs, pp. 381–399.
Ma, R., et al., 2016. Characterization of Pharmaceutically Active Compounds in Dongting Lake 557. Occurrence, chiral profiling and environmental risk. Science of The Total Environment, China, pp. 268–275.
Ma, R., et al., 2017. Characterization of pharmaceutically active compounds in Beijing, China: occurrence pattern, spatiotemporal distribution and its environmental implication. J. Hazard. Mater. 323, 147–155.
MacLeod, S.L., et al., 2007. Stereoisomer analysis of wastewater-derived β-blockers, selective serotonin re-uptake inhibitors, and salbutamol by high-performance liquid chromatography–tandem mass spectrometry. J. Chromatogr. A 1170, 23–33.
Matamoros, V., et al., 2009. Assessment of the pharmaceutical active compounds removal in wastewater treatment systems at enantiomeric level. Ibuprofen and naproxen. Chemosphere 75 (2), 200–205.
Mei, X., et al., 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.
Hashim, N.H., et al., 2011. Enantiospecific fate of ibuprofen, ketoprofen and naproxen in a laboratory-scale membrane bioreactor. Water Res. 45 (18), 6249–6258.
Nakada, N., et al., 2006. Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment. Water Res. 40 (17), 3297–3303.
Nakova, N., et al., 2015. Critical development by design of a rugged HPLC-MS/MS method for direct determination of ibuprofen enantiomers in human plasma. J.

Chromatogr. B 992, 67–75.
Padhye, L.P., et al., 2014. Year-long evaluation on the occurrence and fate of pharmaceuticals, personal care products, and endocrine disrupting chemicals in an urban drinking water treatment plant. Water Res. 51, 266–276.
Perrin, C., et al., 2002. Screening approach for chiral separation of pharmaceuticals: part II. reversed-phase liquid chromatography. J. Chromatogr. A 966, 119–134.
Petrie, B., et al., 2018. Multi-residue analysis of chiral and achiral trace organic contaminants in soil by accelerated solvent extraction and enantioselective liquid chromatography tandem–mass spectrometry. J. Chromatogr. A 1572, 62–71.
Quintana, J.B., et al., 2005. Pathways and metabolites of microbial degradation of selected acidic pharmaceutical and their occurrence in municipal wastewater treated by a membrane bioreactor. Water Res. 39, 2654–2664.
Ribeiro, A.R., et al., 2012. Chiral pharmaceuticals in the environment. Environ. Chem. Lett. 10, 239–253.
Santos, L.H., et al., 2010. Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. J. Hazard. Mater. 175, 45–95.
Stanley, J.K., et al., 2007. Enantiospecific sublethal effects of the antidepressant fluoxetine to a model aquatic vertebrate and invertebrate. Chemosphere 69, 9–16.
Stanley, J.K., et al., 2006. Enantiospecific toxicity of the β-blocker propranolol to Daphnia magna and Pimephales promelas. Environ. Toxicol. Chem. 25, 1780–1786.
Sui, Q., et al., 2017. Pharmaceuticals and personal care products in the leachates from a typical landfill reservoir of municipal solid waste in Shanghai, China: occurrence and removal by a full-scale membrane bioreactor. J. Hazard. Mater. 99–108.
Sui, Q., et al., 2011. Seasonal variation in the occurrence and removal of pharmaceuticals and personal care products in different biological wastewater treatment processes. Environ. Sci. Technol. 45 (8), 3341–3348.
Suzuki, T., et al., 2014. Occurrence and behavior of the chiral anti-inflammatory drug naproxen in an aquatic environment. Environ. Toxicol. Chem. 33 (12), 2671–2678.
Teng, X.W., et al., 2003. Stereospecific high-performance liquid chromatographic analysis of ibuprofen in rat serum. J. Chromatogr. B 796 (2), 225–231.
Ternes, T.A., 1998. Occurrence of drugs in German sewage treatment plants and rivers1. Water Res. 32, 3245–3260.
Thomas, P., et al., 2003. Occurrence and fate of organic micropollutants in the environment: regional mass balances and source apportioning in surface waters based on laboratory incubation studies in soil and water, monitoring, and computer modeling. CHIMIA International. J. Chem. 57 (9), 492–498.
Wang, Z., et al., 2013. Stereoisomeric profiling of pharmaceuticals ibuprofen and iopromide in wastewater and river water, China. Environ. Geochem. Health 35 (5), 683–691.
Ye, J., et al., 2010. Enantiomeric separation of 2-arylpropionic acid nonsteroidal anti--inflammatory drugs by HPLC with hydroxypropyl-β-cyclodextrin as chiral mobile phase additive. Biomed. Chromatogr. 24 (8), 799–807.
Yuan, X., et al., 2018. Simultaneous enantiomeric analysis of chiral non-steroidal anti-inflammatory drugs in water, river sediment, and sludge using chiral liquid chromatography-tandem mass spectrometry. Anal. Methods 10, 4404–4413.
Zhao, W., et al., 2016. Recent advances in pharmaceuticals and personal care products in the surface water and sediments in China. Front. Environ. Sci. Eng. 10, 2.
Zhu, S., et al., 2013. Sources, distribution and potential risks of pharmaceuticals and personal care products in Qingshan Lake basin, Eastern China. Ecotoxicol. Environ. Saf. 96, 154–159.