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Enantiomeric profiling of a chemically diverse mixture of chiral pharmaceuticals in urban water☆

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ABSTRACT

Due to concerns regarding the release of pharmaceuticals into the environment and the understudied impact of stereochemistry of pharmaceuticals on their fate and biological potency, we focussed in this paper on stereoselective transformation pathways of selected chiral pharmaceuticals (16 pairs) at both microcosm (receiving waters and activated sludge wastewater treatment simulating microcosms) and macrocosm (wastewater treatment plant (WWTP) utilising activated sludge technology and receiving waters) scales in order to test the hypothesis that biodegradation of chiral drugs is stereoselective. Our monitoring programme of a full scale activated sludge WWTP and receiving environment revealed that several chiral drugs, those being marketed mostly as racemates, are present in wastewater and receiving waters enriched with one enantiomeric form (e.g. fluoxetine, mirtazapine, salbutamol, MDMA). This is most likely due to biological metabolic processes occurring in humans and other organisms. Both activated sludge and receiving waters simulating microcosms confirmed our hypothesis that chiral drugs are subject to stereoselective microbial degradation. It led, in this research, to preferential degradation of S-(+)-enantiomers of amphetamines, R-(+)-enantiomers of beta-blockers and S-(+)-enantiomers of antidepressants. In the case of three parent compound – metabolite pairs (venlafaxine – desmethylvenlafaxine, citalopram – desmethylcitalopram and MDMA - MDA), while parent compounds showed higher resistance to both microbial metabolism and photodegradation, their desmethyl metabolites showed much higher degradation rate both in terms of stereoselective metabolic and non-stereoselective photochemical processes. It is also worth noting that metabolites tend to be, as expected, enriched with enantiomers of opposite configuration to their parent compounds, which might have significant toxicological consequences when evaluating the metabolic residues of chiral pollutants.

1. Introduction

Pharmaceuticals can be found in the aquatic environment at high, potentially harmful concentrations (up to ppm). Some of the most popular pharmaceuticals are sold and distributed annually in hundreds of tonnes in the UK (Kasprzyk-Hordern, 2010). Prescription and over-the-counter sales of pharmaceuticals are predicted to increase in the future due to demands from an ageing and growing population and changing consumer behaviours. Their presence in the environment is mainly linked with their often limited metabolism in humans and poor removal during wastewater treatment (Petrie et al., 2015). A major concern regarding their release into the environment is their impact on both aquatic and terrestrial biota, e.g. a high incidence of intersex in fish due to exposure to estrogens in water or decline of around 95 per cent in India's vulture population due to the usage of diclofenac in livestock (Oaks et al., 2004).

A growing understanding of the possible environmental effects of pharmaceuticals in the environment has led to changes in environmental regulation in the EU. Three pharmaceuticals: 17β-estradiol, 17α-ethinylestradiol and diclofenac were proposed in 2012 as priority hazardous chemicals (European Commission, 2012). However, the problem of the occurrence, fate and effects of pharmaceuticals in the environment is complex. There are still several areas that need further study, e.g. biological effects of mixtures, metabolism and transformation pathways in the environment, and the ecological effects of formed metabolites and other transformation products. A concept often overlooked is that over 50% of pharmaceuticals are chiral and as a result they can exist in different stereoisomeric (i.e. enantiomeric) forms (Kasprzyk-

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Hordern, 2010). The main concern is that different enatiomers of the same pharmaceutical, despite having similar physicochemical properties, can interact differently with a biologically mediated environment (Petrie et al., 2015). This could lead to the enrichment of a compound with one specific enantiomer (e.g. during metabolism in humans or other organisms). This is of significant importance as different enantiomers can show different toxicity, e.g. S-(+)-fluoxetine is ten times more toxic to *P. promelas* than R-(-)-fluoxetine (De Andrés et al., 2009).

The European Medicines Agency guideline on the Environmental Risk Assessment of Medicinal Products for Human Use (EMEA, EMEA) and the EU Directive for ERA for Veterinary Medicinal Products (EEC) recommend that the environment risks are calculated for the whole pharmaceutical only, e.g. as a racemate or a mixture of enantiomers if distributed as such. This is problematic as many pharmaceuticals are found in the environment in different non-racemic forms. Fluoxetine is an excellent example. Its toxicity is assessed for the racemate; however, as mentioned above, S-(+)-fluoxetine is more toxic in *P. promelas* (Stanley et al., 2007). This enantiomer dependant toxicity of fluoxetine is of vital importance as fluoxetine was found to be, in our preliminary research, enriched with S-(+)-enantiomer in both wastewater and in receiving waters (Lopez-Serna et al., 2013).

Despite these concerns, there is a lack of knowledge of the enantiomeric (and stereoisomeric) distribution of pharmaceuticals in the environment mainly due to unavailability of robust, sensitive and selective analytical methods. This paper aims, for the first time, to verify transformation pathways of mixtures of selected chiral pharmaceuticals belonging to different pharmacological groups (antidepressants, beta-blockers and illicit drugs) and having different physicochemical properties in both laboratory controlled experiments and in a full scale wastewater treatment plant and receiving waters.

2. Experimental

2.1. Chemicals and materials

The following standards were obtained from LGC Standards (Teddington, UK) (Table S1): (±)-MDMA (3,4-methylenedioxymethamphetamine), (±)-MDA (3,4-methylenedioxyamphetamine), (±)-amphetamine and (±)-methamphetamine. (±)-Venlafaxine, (±)-fluoxetine, (±)-O-desmethylvenlafaxine, (±)-atenolol, (±)-metoprolol, (±)-propranolol, (±)-alprenolol, (±)-sotalol, (±)-salbutamol, (±)-mirtazapine and (±)-citalopram were obtained from Sigma-Aldrich (Gillingham UK). (±)-Desmethylcitalopram was obtained from TRC (Toronto, Canada). (±)-MDEA (3,4-methylenedioxy-N-ethyl-amphetamine) was obtained from LGC (Middlesex, UK). All solvents (HPLC grade) were purchased from Sigma-Aldrich. All glassware was silanised with dimethylchlorosilane (5% DMDCS in toluene, Sigma–Aldrich). The surrogate/internal standards (SS/IS): (±)-propranolol-d7, (±)-metoprolol-d7, (±)-sotalol-d6 and (±)-salbutamol-d6 were obtained from TRC (Toronto, Canada). (±)-Amphetamine-d11, (±)-methamphetamine-d14, (±)-MDA-d5, (±)-MDMA-d5 were obtained from LGC standards (Middlesex, UK). (±)-Atenolol-d7 and (±)-fluoxetine-d5 were obtained from Sigma-Aldrich (Gillingham, UK).

2.2. Sample collection, preparation and analysis

Grab samples (collected in duplicate) were obtained from WWTP3 (sampling pointsW1-4, see Fig. 1: W1 - screens, W2 – after settling tanks, W3 - activated sludge tanks and W4 - after activated sludge treatment) and receiving waters (sampling points R1-4, 33 km river stretch, see Fig. 1: R1 - upstream WWTP1, R2-

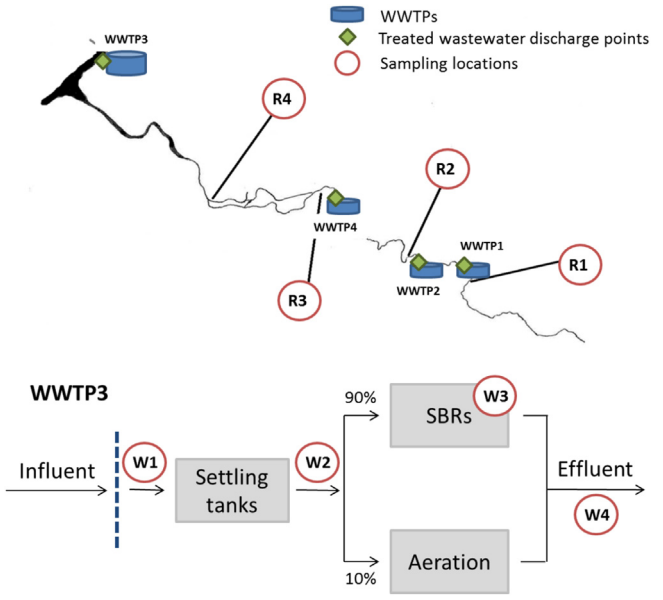

Fig. 1. Sampling locations from river and WWTP3 sampling campaigns.

downstream WWTP2, R3 – downstream WWTP4, R4 – upstream WWTP3, tidal area) twice a day over four or five consecutive days in the case of W1-4 and R1-4 sampling points respectively. All samples were transported on ice and frozen immediately upon arrival. Main characteristics of WWTP3 are provided in Table S2.

The analytical methodology was used as discussed in Evans et al. (Evans et al., 2015). In brief, 50 mL of filtered water samples were extracted at a rate 6 mL min^{-1} using Oasis HLB SPE cartridges (Waters, UK), eluted with 4 mL of MeOH, evaporated to dryness and reconstituted in a mobile phase. All samples were then analysed using chiral HPLC and Waters ACQUITY UPLC® system coupled with a Xevo TQD Triple Quadrupole Mass Spectrometer (Waters, UK). Two chiral columns were used. The CBH column (cellobiohydrolase, 100 × 2 mm, 5 μm, Sigma Aldrich, UK) was run using isocratic conditions with 90:10 water:IPA, 1 mM NH_4OAc at a rate of 0.075 mL min^{-1}. The chirobiotic V column (vancomycin, 250 × 2.1 mm, I.D. 5 μm, Sigma Aldrich, UK) was run using isocratic conditions with MeOH containing 0.005% HCOOH and 4 mM NH_4OAc at 0.1 mL min^{-1}. Xevo TQD Triple Quadrupole Mass Spectrometer (Waters, UK) was equipped with an electrospray ionisation source run in positive ion mode. Nitrogen (the nebulising and desolvation gas) was produced by a high purity nitrogen generator (Peak Scientific, UK). Argon (99.998%, the collision gas) was supplied by a BOC cylinder. The capillary voltage was set at 3.49 kV, source temperature was set at 150 °C and desolvation gas flow was set at 300 L h^{-1}. MassLynx 4.1 (Waters, UK) was used to control the LC-MS system. TargetLynx (Waters, UK) was used for data processing.

All analyses were undertaken is a multiple reaction monitoring (MRM) mode (Table S3) following EC guidelines (2002). Deuterated surrogate/internal standards (see Table S3) were used to compensate for any errors throughout the analytical process.

The following formula (Equation (1)) was used to calculate enantiomeric fractions:

$$EF = \frac{(+)}{(+)+(-)} \quad (1)$$

Where: EF - enantiomeric fraction, (+) - concentration of (+)-enantiomer and (-) - concentration of (-)-enantiomer.