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Abstract

Reported herein is the first enantioselective method for simultaneous separation of chiral pharmaceuticals and pesticides using ultra-high-performance liquid chromatography-tandem mass spectrometry. Separation was achieved using a ChiralPak IG-U® column (amylose *tris*(3-chloro-5-methylphenylcarbamate) stationary phase) and ethanol as a 'green' mobile phase organic modifier. Minimum enantiomer resolutions ranged from 0.5 to 6.9 for 28 pharmaceuticals, herbicides, fungicides and insecticides. The total run time was 26 minutes and is considerably shorter than other multi-residue enantioselective methods for similar numbers of pesticides, and is the first to facilitate simultaneous pharmaceutical separation. Direct injection of river water samples enabled omission of acetonitrile and methanol from the sample treatment step (and the whole methodology). This approach was considerably faster than enantioselective methodologies that rely on solid phase extraction and avoids the need for large sample volumes for analysis. The suitability of this approach was demonstrated by the method's sensitivity with enantiomer method quantitation limits in the range 0.005-0.6 μg L^{-1}. The new method was applied to river water microcosms to investigate enantiospecific transformation of racemic pharmaceuticals and pesticides. The pharmaceutical omeprazole, fungicide prothioconazole and insecticide profenofos were all subject to enantioselective transformation under biotic conditions, represented by a change in enantiomeric fraction of ≥0.1 units. Individual enantiomer microcosms revealed chiral inversion of *R*-omeprazole to *S*-omeprazole in the environment for the first time. In conclusion, this method offers comparatively fast enantioselective analysis for a high number of

pharmaceuticals and pesticides in river water, and is achieved in an environmentally friendlier way than previously reported liquid chromatography methods.

Keywords: chiral; green chemistry; UPLC; emerging contaminant

1. Introduction

Pharmaceuticals and pesticides are anthropogenic contaminants of concern found in the aquatic environment globally (Hughes et al., 2013; Chen et al., 2021; Ouda et al., 2021; Sarker et al., 2021; Yadav et al., 2021). Their concentrations in river waters are typically in the ng L^{-1} to µg L^{-1} range (Hughes et al., 2013; Chen et al., 2021). Major pathways of pharmaceutical and pesticide contamination entering rivers is through the discarge of treated effluent, combined sewer overflows, effluents from manufacturing premises and run off from agricultural land and farmyards (Petrie, 2021; Tian et al., 2021; Yadav et al., 2021). In the environment, pharmaceuticals and pesticides pose a threat to non-target organisms. For example, the benzodiazepine oxazepam alters the behaviour of *Perca fluviatilis* at 1.8 µg L^{-1} concentration (Brodin et al., 2013). The broad-spectrum insecticide fipronil has been found to induce biochemical changes to *Prochilodus lineatus* at 9 µg L^{-1} (Santillán Deiú et al., 2021).

Stereochemistry plays an important role in the environmental fate and effects of pharmaceuticals and pesticides. Approximately 50 % of pharmaceuticals and 25 % of pesticides are chiral (Sanganyado et al., 2017; Ulrich et al., 2012), existing as two or more enantiomers. Enantiomers differ in the spatial arrangement of atoms around a stereogenic centre. This results in differences in their three dimensional shape and interactions in chiral environments. Therefore, enantiomers can differ in their degradation and toxicity in the environment (Zhang et al., 2019a; Bertin et al., 2020; Liu et al., 2021).

An important aspect of assessing the risk posed by pharmaceuticals and pesticides is to determine their behaviour in the environment. For river waters, microcosm studies can be conducted under controlled laboratory conditions (Suzuki et al., 2014; Camacho-Muñoz et al., 2019; Li et al., 2021). Enantiospecific studies typically involve spiking the collected river water with the racemate (equimolar concentration of enantiomers) or individual enantiomers of the compound of interest, and monitoring their behaviour over time. A limitation of studies using the racemate is that chiral inversion, whereby

one enantiomer is formed from the other enantiomer, cannot be appreciated. However, undertaking microcosms with the racemate first is a useful way of identifying compounds subject to enantioselective transformation, allowing further studies to be then undertaken on individual enantiomers of that compound (Bertin et al., 2020). This is particularly useful considering individual enantiomers for a large number of compounds can be expensive to obtain.

There is a paucity of information on the enantiospecific behaviour of pharmaceuticals and pesticides in river waters. This is due to a lack of enantioselective methodologies available for multi-residue analysis, owing in part to the additional analytical demands of performing such challenging determinations. Methods are typically limited to a few analytes and no previous method has been developed to include simultaneous separation of both pharmaceuticals and pesticides. Existing methods for enantioselective analysis of pharmaceuticals or pesticides in environmental matrices is achieved using chiral stationary phases and liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Li et al., 2012; Li et al., 2013; Camacho-Muñoz and Kasprzyk-Hordern, 2015; Zhao et al., 2016; Zhao et al., 2018a; Zhao et al., 2018b; Ma et al., 2019; Wang et al., 2021a). These methods use toxic solvents such as methanol or acetonitrile as the organic modifier. However, there is a drive to make all aspects of the analytical process environmentally friendly (or friendlier).

Ethanol is recognised as a green solvent as it can be produced from renewable sources such as the fermentation of sugar-, starch- or lignocellulosic-rich materials (Capello et al., 2007). Furthermore, ethanol is desirable as it has similar properties to methanol and acetonitrile but it is less volatile, less toxic and has lower disposal costs (Plotka et al., 2013). However, ethanol has comparatively higher viscosity leading to higher column back pressures. This has limited its use for enantioselective separations as commercially available chiral columns typically had 3 or 5 μm particle diameters (Sanganyado et al., 2017) and operating pressures of a few thousand psi. Recently, superficially porous particle columns with 2.7 μm particle diameters enable operating pressures of 5,800 psi (McKenzie et al., 2020). Furthermore, enantioselective columns with ultra-high-performance liquid chromatography (UPLC) particle diameters (sub-2 μm) are now available, which typically have maximum operating pressures of 10,000 psi. This enables the use of ethanol as the organic modifier in the mobile phase.

UPLC also offers the benefits of improved sensitivity, resolution, lower solvent consumption and shorter analysis times. Performing chiral UPLC analysis is realistic for many researchers working in this area as they already use UPLC instrumentation, albeit with enantioslective HPLC columns (e.g., see Li et al., 2012; Li et al., 2013; Camacho-Muñoz and Kasprzyk-Hordern, 2015; Zhao et al., 2016; Zhao et al., 2018a; Zhao et al., 2018b; Ma et al., 2019; Wang et al., 2021a). Although chiral UPLC has been applied to the separation for a small number of pesticides (napropamide, metalaxyl, metconazole and triticonazole) in environmental matrices (Yao et al., 2018), it has not been used for multi-residue analysis. It should be noted that supercritical fluid chromatography (SFC) can also be adopted as a green approach for enantioselective analysis (Camacho-Muñoz et al., 2016; Roy et al., 2020). However, SFC remains less common in analytical laboratories compared to UPLC.

Existing methods for the determination of pharmaceuticals and pesticides in river water typically use a sample preconcentration step which requires organic solvents (e.g., solid phase extraction) and considerable sample volumes (up to 200 mL per replicate) to reach adequate detection limits (Li et al., 2012; Li et al., 2013; Camacho-Muñoz and Kasprzyk-Hordern, 2015; Zhao et al., 2016; Zhao et al., 2018a; Zhao et al., 2018b; Ma et al., 2019; Wang et al., 2021a). However, the increased sensitivity of modern MS/MS detectors has seen several methods which achieve sufficient sensitivity using a straightforward direct injection process (Campos-Mañas et al., 2017; Mosekiemang et al., 2019; Renai et al., 2021). Here samples are filtered or centrifuged to remove any particulates prior to injection on the LC-MS/MS system, circumventing the need for organic solvents during sample preparation. However, this approach has not been previously utilised for enantioselective determinations. To address the limitations described from the literature, the objectives of this study were:

(i) To develop a new 'green' enantioselective UPLC method for simultaneous separation of pharmaceutical and pesticide enantiomers in river water samples.

(ii) To validate a simple, fast, solvent free sample preparation method for direct injection of river water samples for enantioselective analysis.

(iii) To assess the enantiospecific behaviour of multiple pharmaceuticals and pesticides in river water microcosms.

This was a achieved using enantioselective UPLC-MS/MS with a ChiralPak IG-U (1.6 µm stationary phase particle diameter) column, and ethanol as the mobile phase organic modifier. A total of 28 chiral analytes were selected for method development including 8 pharmaceutical drugs, 2 herbicides, 13 fungicides and 5 insecticides (Table S1). These were selected to encompass a diverse variety of physicochemical properties (Table S1), and therefore a range of expected behaviours in the environment. The developed method was then applied to laboratory microcosm studies to investigate the enantiospecific behaviour of pharmaceuticals and pesticides in river water.

2. Materials and methods

2.1. Materials

The analytical standards *(±)*-benalaxyl, *(±)*-bitertanol, *(±)*-fenamiphos, *(±)*-flutriafol, *(±)*-ifosfamide, *(±)*-isocarbophos, *(±)*-ketoconazole, *(±)*-lorazepam, *(±)*-mandipropamid, *(±)*-metconazole, *(±)*-napropamide, *(±)*-naproxen, *(±)*-omeprazole, *(±)*-oxazepam, *(±)*-profenofos *(±)*-propiconazole, *(±)*-pyriproxifen, *(±)*-temazepam, *(±)*-triadimefon, and *(±)*-warfarin were purchased from Sigma Aldrich (Gillingham, UK). The remaining analytical standards *(±)*-carfentrazone ethyl, *(±)*-diniconazole, *(±)*-epoxiconazole, *(±)*-fenbuconazole, *(±)*-fipronil, *(±)*-paclobutrazol, *(±)*-prothioconazole, and *(±)*-triticonazole were obtained from Toronto Research Chemicals (TRC, Canada). *S*-omeprazole and *R*-omeprazole were purchased from Cambridge Bioscience (Cambridge, UK). The deuterated surrogates *(±)*-naproxen-d_3, *(±)*-oxazepam-d_5 and *(±)*-temazepam-d_5 were obtained from Sigma Aldrich and *(±)*-benalaxyl-d_5 and *(±)*-fenbuconazole-d_5 from TRC. Standard solutions were prepared in ethanol at 1 mg mL^{-1} and stored at -20 °C. HPLC grade ethanol, ammonium acetate, formic acid and sodium azide (NaN_3) as well as 4 mm polyvinylidene fluoride (PVDF) 0.45 µm syringe filters were purchased from Fisher Scientific (Loughborough, UK). Ultrapure water was 18.2 MΩ cm^{-1} quality. A grab sample of river water (5 L) was collected from the River Don in Inverurie, North-East Scotland (latitude/longitude coordinates, 57.27079/-2.36551) during January 2021. Further river water (1 L) was collected from the same location during April 2021 and August 2021 for microcosm studies. The water was collected under similar flow conditions and when there had not been significant rainfall in the seven days prior to collection.

2.2. Sample preparation

River water was spiked with deuterated surrogates to achieve a 1.25 µg L^{-1} concentration of each deuterated enantiomer. 1 mL aliquots were then filtered using a 4 mm PVDF 0.45 µm syringe filter directly into a LC vial ready for analysis by enantioselective UPLC-MS/MS analysis. During development, enantiomer losses from filtration of ultrapure water were evaluated through 0.45 µm PVDF, nylon, polytetrafluoroethylene (PTFE) and cellulose acetate syringe filters (all obtained from Fisher Scientific).

2.3. Enantioselective liquid chromatography-tandem mass spectrometry

Chromatography was performed using a Waters Acquity UPLC system with a flow through needle (Manchester, UK). A ChiralPak IG-U® column (100 × 3.0 mm, 1.6 µm particle size) fitted with a 0.2 µm in-line filter was used for enantioselective separations. The isocratic mobile phase consisted of 75 % ethanol: 25 % ultrapure water containing 5 mM ammonium acetate and 0.1 % formic acid. The flow rate was 0.21 mL min^{-1} with a column temperature of 25 °C. The injection volume was 10 µL and the total run time 26 minutes. The pressure of the system was 8,600±100 psi. The maximum back pressure of the column is 10,000 psi. During development the mobile phase composition (ethanol:water composition, buffer type and concentration, and acid type and concentration), column temperature, flow rate and injection volume were optimised.

The UPLC was coupled to a Xevo TQ-XS Triple Quadrupole Mass Spectrometer (Waters, Manchester, UK) with an electrospray ionisation (ESI) source. In positive ionisation mode the capillary voltage was 2.60 kV. The negative ionisation capillary voltage was -2.20 kV. In both modes the source temperature was 150 °C and the desolvation temperature was 400 °C. The cone gas flow was 150 L h^{-1} and desolvation gas flow was 550 L h^{-1}. The nebulising and desolvation gases were nitrogen and the collision gas was argon. The optimised MS/MS transition are detailed (Table S2).

2.4. Instrument and method performance

A 13 point mixed calibration ranging from 0.001-10 µg L^{-1} for individual enantiomers was prepared in matrix (0.45 µm filtered river water). Each calibration standard also contained 1.25 µg L^{-1} of each

deuterated enantiomer. Signal suppression during ESI was determined by comparing calibrations prepared in matrix and solvent (ultrapure water) using eq (1):

$$Suppression\ (\%) = 100 - \left(\frac{Slope_{matrix}}{Slope_{solvent}} \times 100\right) \quad (1)$$

Here $Slope_{matrix}$ and $Slope_{solvent}$ is the slope of external calibrations prepared in matrix and solvent, respectively. Intraday and interday precision and accuracy was assessed by triplicate injection of 0.25, 1.25 and 5 µg L^{-1} standards in matrix, within a 24 hour period and across three different days, respectively. Method recovery and trueness was assessed by filtering 1 mL aliquots of river water spiked at 0.25, 1.25 and 5 µg L^{-1} and determined using eq (2) and (3):

$$Recovery\ (\%) = \frac{(PA_{spike} - PA_{unspiked})}{PA_{standard}} \times 100 \quad (2)$$

$$Trueness\ (\%) = \frac{(Conc_{spike} - Conc_{unspiked})}{Conc_{theoretical}} \times 100 \quad (3)$$

Here PA_{spike} and $PA_{unspiked}$ are the peak areas of the spiked and unspiked river water and $PA_{standard}$ is the peak area of corresponding standard assuming 100 % enantiomer recovery. $Conc_{spike}$ and $Conc_{unspiked}$ is the determined concentrations in the spiked and unspiked river water and $Conc_{theoretical}$ is the nominal concentration of the spiked enantiomer.

Method detection limits (MDLs) and method quantitation limits (MQLs) were determined using eq (4) and (5):

$$MDL\ (\mu g\ L^{-1}) = IDL \times \left(\frac{100}{Recovery}\right) \quad (4)$$

$$MQL\ (\mu g\ L^{-1}) = IQL \times \left(\frac{100}{Recovery}\right) \quad (5)$$

The *IDL* and *IQL* are the instrument detection and quantitation limits in µg L^{-1}, respectively. These represent the lowest concentrations which had a signal to noise ratios of 3 and 10, respectively. *Recovery* is enantiomer recovery (%) as calculated from eqn (2). Chromatographic resolution (R_S) was calculated using eq (6):

$$R_S = \frac{Rt_{difference}}{Width_{average}} \quad (6)$$

183 $Rt_{difference}$ is the difference in enantiomer retention time and $Width_{average}$ is the average basal peak width
184 of the two enantiomers (both expressed in minutes). The enantiomeric composition of pharmaceuticals
185 and pesticides was expressed as enantiomeric fraction (EF) using eq (7):

186 $$EF = \frac{E1}{(E1+E2)} \quad (7)$$

187 Here *E1* is the concentration of the first eluting enantiomer and *E2* is the concentration of the second
188 eluting enantiomer.

189 **2.5. Microcosm studies**

190 Two 200 mL vessels of river water (April 2021) were prepared in borosilicate Duran bottles. One vessel
191 was treated with 0.2 g L^{-1} NaN_3 to inhibit microbial activity (abiotic microcosm). Both vessels were
192 kept in the dark and mixed continuously using a magnetic stirrer. The water temperature for the duration
193 of the study was 20 ±1 °C. Each vessel was spiked with all pharmaceutical and pesticide enantiomers
194 at a concentration of 5 µg L^{-1}. Samples were then collected 0, 3, 7, 14, 21 and 28 days. Enantiomer
195 degradation was fitted to the first-order exponential degradation model using eq (8):

196 $$C_t = C_0 \times e^{-kt} \quad (8)$$

197 Here C_t is the enantiomer concentration at time *t* (d) and C_0 is the enantiomer concentration at the start
198 of the study (0 d), and *k* is the degradation rate constant (1/*d*). Enantiomer half-life ($t_{1/2}$) was calculated
199 according to eq (9):

200 $$t_{1/2} = \frac{\ln(2)}{k} \quad (9)$$

201 Individual enantiomer microcosms of *S*-omeprazole and *R*-omeprazole were then conducted following
202 the results of the racemic microcosms. These were undertaken in biotic and abiotic conditions using
203 river water collected in August 2021 as previously described. However, samples were collected at 0, 3,
204 7, 10, 14, 17, 21, 24 and 28 days.

205 **3. Results and discussion**

206 **3.1. Optimization of enantioselective liquid chromatography-tandem mass spectrometry**

A ChiralPak IG-U column was selected for the study due to the amylose *tris*(3-chloro-5-methylphenylcarbamate) stationary phase being suitable for enantioseparation of a range of pesticides (Zhao et al., 2018b) and some classes of pharmaceutical (Ghanem and Wang, 2018; Yuan et al., 2018). A number of different isocratic mobile phase compositions using ethanol as the organic modifier were investigated for simultaneous enantioseparation of pesticides and pharmaceuticals. An ethanol content of 75 % gave the best overall separation. Previous studies have found similar protic organic modifier content (using methanol) gave best enantioseparations using polysaccharide stationary phases (Zhang et al., 2014; Qi et al., 2016; Zhang et al., 2019b). The addition of buffer to the aqueous portion of the mobile phase was essential for improved peak shape and sensitivity. However, changing both the buffer type (ammonium acetate *vs*. ammonium formate) and concentration (1-10 mM) only had a modest influence on enantioseparation and sensitivity. Best overall results were achieved using 5 mM ammonium acetate. Furthermore, the use of 0.1 % formic acid was essential for enantioresolution of ketoconazole by reducing peak tailing and retention time. This agrees with work by Zhao et al (2018b) who used the same stationary phase for pesticide separation.

The high back pressure on the column from using ethanol can be reduced by increasing column temperature (enabling the use of higher mobile phase flow rates). Modifying column temperature had little effect on the enantioresolution of most pesticides and pharmaceuticals. However, lower temperatures were preferrable for the separation of the benzodiazepines. At elevated temperatures (> 25 °C) the benzodiazepines displayed peak interconversion profiles whereby the signal between the two enantiomers formed a plateau rather than returning to the baseline (Figure S1, Fedurcová et al., 2006). Therefore, a temperature of 25 °C was selected as the best compromise between chromatographic profiles and column back pressure. The maximum flow rate that could be applied whilst working within the recommended column operating pressure was 0.21 mL min^{-1}.

Under these chromatographic conditions, simultaneous enantioseparation of all 28 pharmaceuticals and pesticides was achieved within 26 minutes (Figure 1). This is considerably shorter than previously reported enantioselective methods for simultaneous separation of herbicides, fungicides and insectides. For example, Zhao et al (2018b) achieved separation of 18 herbicides, fungicides and insectides in 55

minutes using a ChiralPak IG HPLC column with an acetonitrile/water mobile phase. The shorter run time in the newly developed method was achieved despite using ethanol as the organic modifier which, due to the higher back pressure over conventional solvents such as acetonitrile and methanol, did not enable use of higher mobile phase flow rates. Minimum enantiomer R_S values across the calibration range varied from 0.5 for profenofos to 6.9 for fenbuconazole (Table 1). In total 24 of the 28 analytes had R_S values ≥1.0 which represents a maximum of 2 % peak overlap for quantitative analysis (Bagnall et al., 2012). To date, this is the most comprehensive chromatographic method for the simultaneous enantioseparation of pharmaceuticals and pesticides.

3.2. Sample preparation method

The use of a simple and solvent-less sample preparation method was explored to reduce the need for using organic solvents. Centrifugation of environmental samples has previously been utilised to remove particulate matter prior to injection onto the LC-MS/MS system (Boix et al., 2015). However, using this method resulted in pressure variations during the chromatographic analysis, likely to be caused by the incomplete sedimentation of suspended particulates. Alternatively, samples can be passed through a membrane filter, with PTFE syringe filters being popular (Oliveira et al., 2015; Campos-Mañas et al., 2017; Li et al., 2018). However, analytes losses are possible during this filtration step (Baker and Kasprzyk-Hordern, 2011). Therefore, pharmaceutical and pesticide recovery was assessed through a range of common syringe filter materials including PVDF, nylon, PTFE and cellulose acetate (Figure 2).

Overall, PVDF provided the lowest analyte losses during filtration (Figure 2). Recoveries of >70 % for most enantiomers was achieved using this membrane type. Previous studies have also found PVDF filters achieve acceptable recoveries for analytes with a range of chemical properties (Kmellár et al., 2010; Wang et al., 2021b). Both nylon and cellulose acetate gave poor recoveries of several analytes. However, recovery of both ketoconazole and pyriproxifen was ≤10 % through all filter materials investigated due to their comparatively greater hydrophobicity with log K_{OW} values being >4 (Table S1). Therefore, these analytes were not included in the method performance assessment and would require an alternative sample preparation method for water samples. Their behaviour during filtration

indicates they are likely to be found in the solid phase of environmental matrices over the liquid phase. Huang et al (2013) previously reported than ketoconazole is found in the suspended matter of wastewater and river sediments.

3.3. Instrument and method performance

The performance of the instrumental method was assessed for linearity as well as intra- and interday precision and accuracy. Calibrations were prepared in matrix (e.g., filtered river water) and exhibited r^2 values ≥0.996 (Table 1). Those enantiomers which had a corresponding deuterated surrogate were quantified using the internal calibration method. This approach was also taken for enantiomers with lower recovery through PVDF filters and assigned the most appropriate deuterated enantiomer (Table 1). For all remaining enantiomers the external calibration approach was taken. Comparison of external calibration slopes prepared in matrix and ultrapure water were used to assess the extent of signal suppression during ESI. Signal suppression for all enantiomers ranged from -15 % (i.e., enhancement) to 33 % (Table 1). This range is typical for enantioselective analysis of small molecules in environmental matrices (Li et al., 2013; Zhao et al., 2018a; Zhao et al., 2018b). For most enantiomers signal suppression was negligible (±5 %). However, some evidence of enantiospecific suppression was observed. For example, suppression of carfentrazone ethyl enantiomers was -2 % and -15 % demonstrating the importance for compensating these effects through matrix matched or internal standard calibrations. Most enantiomers exhibited intra- and inter-day accuracy in the range 90-110 % (Table 1). Maximum within day precision was 10 % and between different days was 17 %. These results are consistent with previously validated enantioselective methods (Bagnall et al., 2012).

The whole methodology (filtration and enantioselective UPLC-MS/MS analysis) was assessed for trueness, repeatability and sensitivity. Trueness ranged from 39 % to 110 % with the majority of enantiomers in the range 70-100 % (Table 2). Furthermore, for individual enantiomers the recovery and determined trueness was consistent across the three concentrations studied (0.25, 1.25 and 5.00 μg L^{-1}, Table 2, Figure S2). The repeatability for all enantiomers over the studied concentrations was ≤15 % (Table 2). The MDLs were ≤0.1 μg L^{-1} for all enantiomers. The MQLs ranged from 0.005 μg L^{-1} for omeprazole enantiomers to 0.6 μg L^{-1} for prothioconazole enantiomers (Table 2). Again, these are

similar to previously reported methodologies for liquid matrices which adopt a sample preconcentration step (e.g., SPE) (Li et al., 2012; Li et al., 2013; Zhao et al., 2016; Zhao et al., 2018a; Zhao et al., 2018b; Ma et al., 2019; Wang et al., 2021a). This demonstrates the suitability of taking a green approach to enantioselective analysis and utilising 'direct injection'.

Other than the environmental advantages of using ethanol as the organic modifier in the mobile phase, the use of a sub-2 μm particle size reduces solvent consumption. For example, the previously described multi-residue enantioselective method by Zhao et al (2018b) used 17.5 mL of acetonitrile per analysis (see Table S3). This newly developed method only required 4.1 mL of ethanol despite using this lower elution strength solvent. It should be noted that there are differences in target analytes between the newly developed method and the method described by Zhao et al (2018b). A limitation of this work is that the order of enantiomer elution is not known. The cost of purchasing individual enantiomers is often cost prohibitive for a high number of analytes, and access to detectors used to determine enantiomer elution order (e.g., optical rotation detection) can be limited. However, the purpose of the method is to screen for the enantioselective transformation of pesticides and pharmaceuticals in river water microcosms spiked with racemic analytical standards. Those which show changes in enantiomeric composition can then be prioritised for further investigation and individual enantiomers obtained and further microcosm studies undertaken.

3.4. Enantiospecific fate studies using river water microcosms

Biotic and abiotic (NaN_3 treated) river water microcosms were spiked with 5 μg L^{-1} of all enantiomers. During the 28 day monitoring period a range of fate behaviours was observed for the studied analytes. Most analytes did not degrade under abiotic conditions. However, enantiomers of the pharmaceuticals lorazepam, omeprazole, and oxazepam, the herbicide carfentrazone ethyl and the insecticides fipronil, isocarbophos and profenofos all exhibited abiotic degradation (Table 3). Carfentrazone ethyl enantiomers showed the fastest degradation with $t_{1/2}$ values of 2.1 days. Previous research has found carfentrazone ethyl undergoes hydrolysis in water (Ngim and Crosby, 2001). Abiotic transformation of oxazepam has previously been observed in bacterial cultures (Redshaw et al., 2008). No substantial changes in EF was observed for any of the studied analytes under abiotic conditions.

315 Most analytes showed evidence of degradation under biotic conditions during 28 days (Table 3).
316 However, only enantiomers of carfentrazone ethyl had $t_{1/2}$ values <1 day. This demonstrates the
317 refractory nature of the studied analytes in river water and their likely transport for considerable
318 distances from their point of entry into river water. Those which did not display degradation were
319 enantiomers of ifosfamide, temazepam, warfarin, paclobutrazol, triticonazole as well as flutriafol E2
320 (Table 3). Ifosfamide has been found to be recalcitrant in river water microcosms previously (Camacho-
321 Muñoz et al., 2019). Three of the analytes had a change in EF of ≥0.1 units during the microcosm study
322 (omeprazole, prothioconazole and profenofos) having underwent enantioselective transformation
323 (Figure S3).

324 The initial EF of prothioconazole (0.50) increased to 0.56 and 0.68 at 7 and 14 days (Figure S3). At 21
325 days the enantiomer concentrations reduced to below their MQLs. Enantiomer $t_{1/2}$ values were 5.0 and
326 3.6 days (Table 3). Enantioselective transformation of prothioconazole has previously been observed in
327 soil (Zhang et al., 2017; Zhang et al., 2018). Degradation of the profenofos enantiomers were
328 comparatively faster with $t_{1/2}$ values of 1.1 (profenofos E1) and 1.5 days (profenofos E2) (Table 3). The
329 initial EF reduced from 0.49 to 0.45 after 3 days and 0.20 at 7 days before the enantiomer concentrations
330 fell below the MQLs (Figure S3). Mahboob et al (2015) previously reported a maximum concentration
331 of profenofos in river water of 1.4 μg L^{-1}. However, no previous data exists on its enantiospecific
332 behaviour in river water. The $t_{1/2}$ values of omeprazole E1 and omeprazole E2 were 4.2 and 5.5 days,
333 respectively (Table 3). The initial EF of 0.50 reduced to 0.45, 0.32 then 0.26 at 14, 21 and 28 days
334 (Figure S3). Omeprazole has previously been detected in effluent wastewater at concentrations up to
335 0.1 μg L^{-1} (Gracia-Lor et al., 2010). However, no information exists on its enantiomeric composition or
336 transformation in environmental matrices. Barreiro et al (2010) detected omeprazole at the enantiomeric
337 level in estuarine water but did not report the EF.

338 The enantiomers *S*-omeprazole and *R*-omeprazole were purchased and microcosm studies undertaken
339 on them individually to further understand the enantiospecific transformation of the racemate observed.
340 Under biotic conditions *S*-omeprazole (omeprazole E1) almost completely degraded within 3 days. On
341 the other hand, *R*-omeprazole (omeprazole E2) had a $t_{1/2}$ value of 6.5 days. Interestingly, in the

342 microcosm spiked with *R*-omeprazole only, *S*-omeprazole was present at 3 days with low concentrations
343 measured up to 28 days (Figure 3). This is considered to be from the inversion of *R*-omeprazole to *S*-
344 omeprazole. The initial EF of 0.00 increased to a maximum of 0.18 after 21 days. This helps to explain
345 why greater changes in EF were not observed during the racemic microcosms considering the $t_{1/2}$ values
346 of *S*-omeprazole and *R*-omeprazole in their respective individual enantiomer microcosms. To the best
347 of our knowledge, this is the first time chiral inversion of *R*-omeprazole has been reported under
348 environment conditions. In abiotic conditions the $t_{1/2}$ values were 3.9 and 4.3 days for *S*-omeprazole
349 and *R*-omeprazole, respectively, with no evidence of inversion. The $t_{1/2}$ values are similar to those
350 observed in the racemic abiotic microcosms (3.8 days, Table 3).

351 **4. Conclusions**

352 An enantioselective UPLC-MS/MS method was successfully developed for simultaneous analysis of
353 pharmaceuticals and pesticides. The method is environmentally friendlier than previous multi-residue
354 enantioselective methods because (i) methanol and acetonitrile are not used, (ii) each analytical run
355 requires less solvent and is comparatively shorter than previous methods, and (iii) 26 compounds can
356 be studied simultaneously without the need for using multiple methodologies to cover the range of
357 analytes studied. The use of ethanol as the mobile phase organic modifier and direct injection of samples
358 facilitated enantiomer MDLs at low μg L^{-1} concentrations. Application of the method to investigate the
359 enantioselective behaviour of pharmaceutical and pesticide racemates in river water microcosms found
360 omeprazole, prothioconazole and profenofos were all subject to changes in EF. Individual enantiomer
361 microcosms revealed chiral inversion of *R*-omeprazole for the first time. The presented method has
362 demonstrated that it can support studies on the enantiospecific transformation of pesticides and
363 pharmaceuticals in river waters in an environmentally friendly way.

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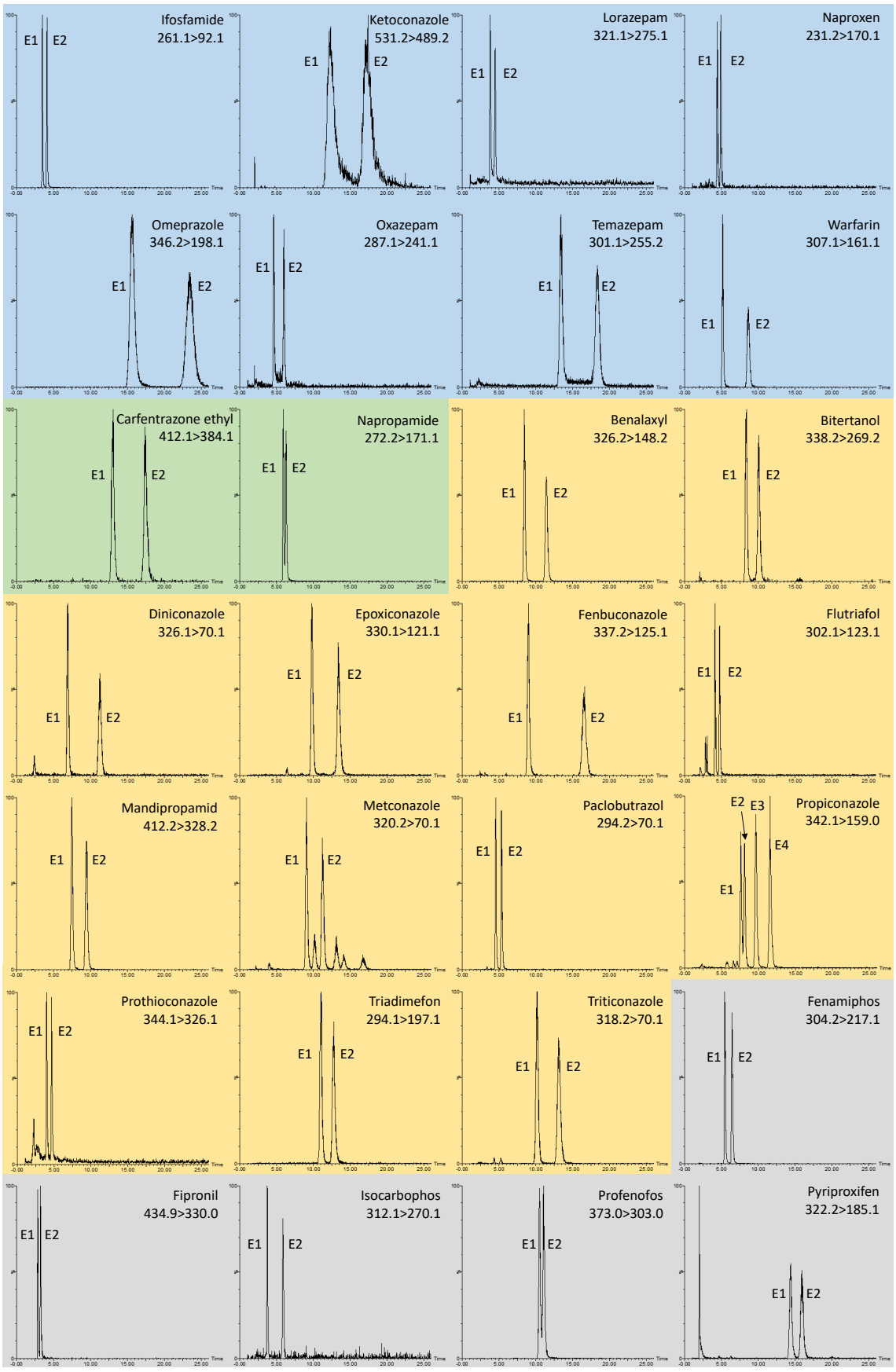
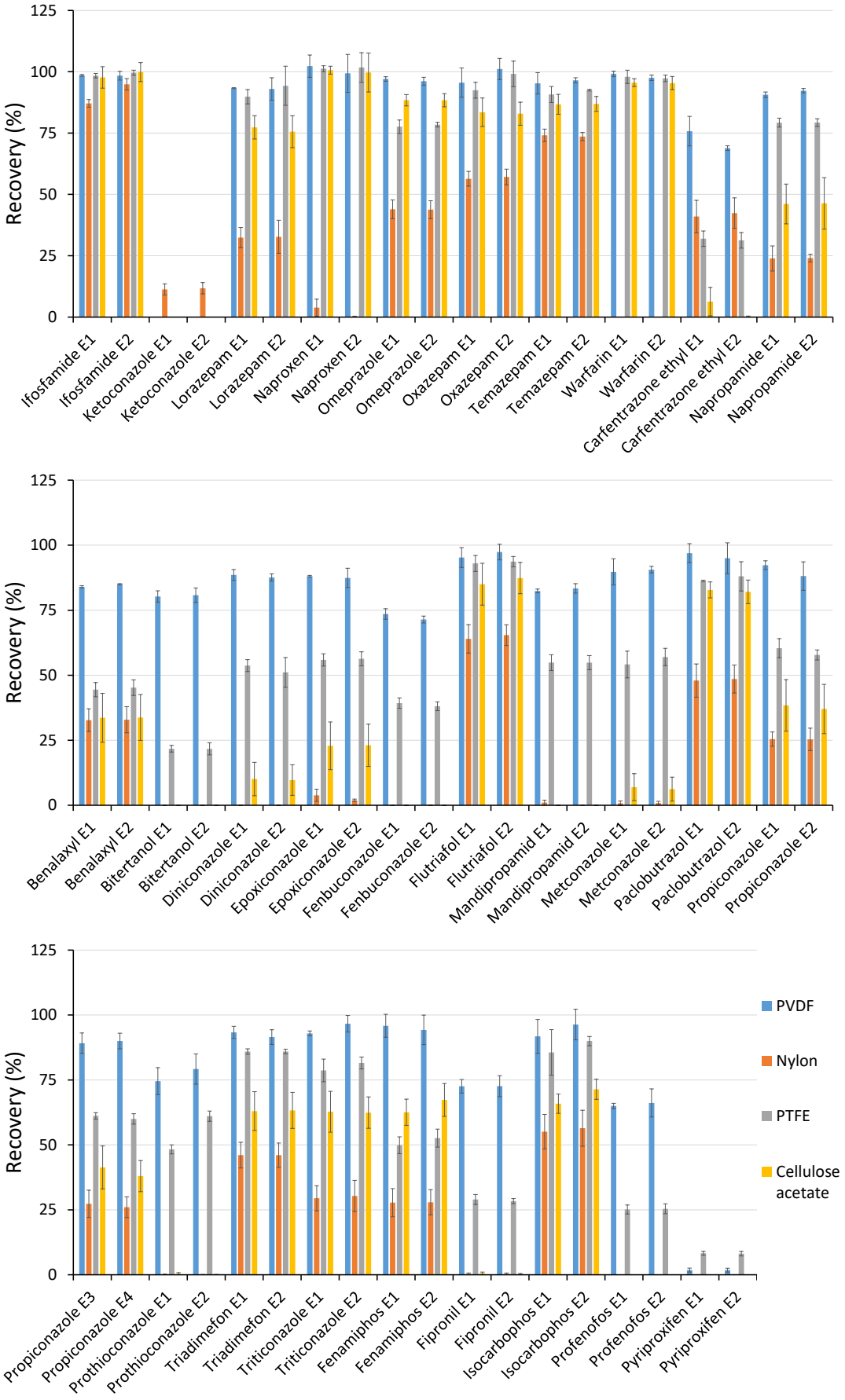
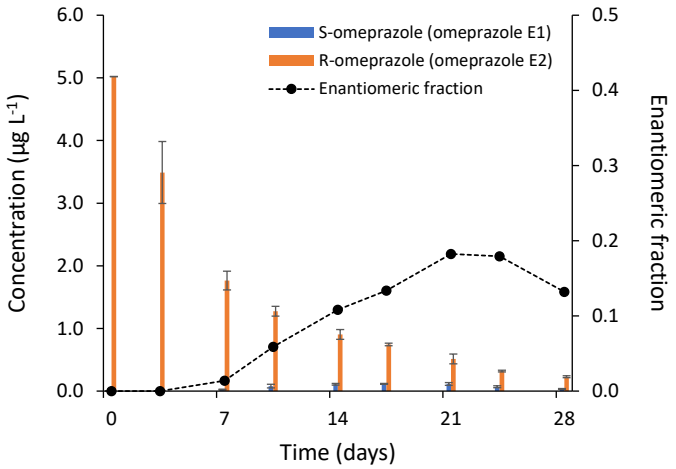


562 Figure 1. Enantioselective UPLC-MS/MS chromatograms of a 1 µg L^{-1} mixed enantiomer standard prepared in
563 river water. The chromatograms show pharmaceutical drugs (blue), herbicides (green), fungicides (yellow) and
564 insecticides (grey).



566 Figure 2. Recovery of enantiomers through 0.45 µm PVDF, nylon, PTFE, and cellulose acetate syringe filters.



568 Figure 3. Concentration of omeprazole enantiomers and enantiomeric fraction in river water microcosm spiked
569 with *R*-omeprazole only.

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Table 1. Calibration and instrument performance data for the developed enantioselective UPLC-MS/MS method

Group	Enantiomer	*Rt* (min)	Calibration method	Internal Standard	R^2	Minimum *Rs*	EF	Suppression (%)	Accuracy (%)[a]		Precision (%)[a]	
									Intraday	Interday	Intraday	Interday
Drugs	Ifosfamide E1	3.5	External	-	0.998	1.1	0.50	9	99	102	2	4
	Ifosfamide E2	4.1	External	-	0.999			-1	102	101	1	5
	Ketoconazole E1	12.4	External	-	0.999	1.7	0.51	2	102	98	2	6
	Ketoconazole E2	17.4	External	-	0.999			1	98	97	2	4
	Lorazepam E1	3.8	External	-	0.997	0.7	0.50	4	104	104	4	3
	Lorazepam E2	4.4	External	-	0.997			-4	101	99	7	5
	Naproxen E1	4.4	Internal	Naproxen-d_3 E1	0.999	1.2	0.51	5	110	116	10	8
	Naproxen E2	4.9	Internal	Naproxen-d_3 E2	0.999			1	92	98	10	7
	Omeprazole E1	15.7	External	-	1.000	2.5	0.50	2	98	100	1	2
	Omeprazole E2	23.7	External	-	1.000			0	102	102	1	2
	Oxazepam E1	4.6	Internal	Oxazepam-d_5 E1	0.997	1.6	0.50	-5	103	105	5	4
	Oxazepam E2	6.0	Internal	Oxazepam-d_5 E2	0.996			5	100	101	4	5
	Temazepam E1	13.3	Internal	Temazepam-d_5 E1	0.998	2.9	0.51	-5	102	104	4	4
	Temazepam E2	18.4	Internal	Temazepam-d_5 E2	0.999			-2	100	100	2	3
	Warfarin E1	5.1	External	-	0.996	3.2	0.50	1	96	99	2	3
	Warfarin E2	8.6	External	-	0.997			2	100	106	2	5
Herbicides	Carfentrazone ethyl E1	13.1	Internal	Fenbuconazole-d_5 E1	0.997	3.4	0.48	-2	102	98	10	12
	Carfentrazone ethyl E2	17.4	Internal	Fenbuconazole-d_5 E2	0.998			-15	100	107	3	6
	Napropamide E1	5.9	External	-	0.997	0.7	0.49	-2	100	101	2	3
	Napropamide E2	6.3	External	-	0.999			3	103	101	2	4
Fungicides	Benalaxyl E1	8.4	Internal	Benalaxyl-d_5 E1	1.000	2.8	0.50	4	102	101	1	2
	Benalaxyl E2	11.3	Internal	Benalaxyl-d_5 E2	1.000			2	101	101	1	1
	Bitertanol E1	8.3	Internal	Benalaxyl-d_5 E1	0.997	1.7	0.49	2	103	97	1	6
	Bitertanol E2	10.0	Internal	Benalaxyl-d_5 E2	0.998			-1	103	105	4	3
	Diniconazole E1	6.9	External	-	0.996	3.6	0.49	-1	101	100	5	4
	Diniconazole E2	11.2	External	-	0.998			0	100	107	2	5
	Epoxiconazole E1	9.8	External	-	0.997	2.7	0.51	0	98	98	2	1
	Epoxiconazole E2	13.4	External	-	0.999			-4	105	100	2	5
	Fenbuconazole E1	8.9	Internal	Fenbuconazole-d_5 E1	0.998	6.9	0.49	3	99	92	2	10
	Fenbuconazole E2	16.5	Internal	Fenbuconazole-d_5 E2	0.999			-1	99	104	4	1
	Flutriafol E1	4.7	External	-	1.000	1.4	0.47	4	101	96	2	10
	Flutriafol E2	5.4	External	-	1.000			2	100	97	2	5
	Mandipropamid E1	7.4	Internal	Benalaxyl-d_5 E1	0.998	1.9	0.49	3	99	101	2	2
	Mandipropamid E2	9.4	Internal	Benalaxyl-d_5 E2	0.999			4	99	102	1	4
	Metconazole E1	9.0	External	-	0.998	1.0[b]	0.50	4	101	98	2	3
	Metconazole E2	11.2	External	-	0.999			-1	102	100	3	3
	Paclobutrazol E1	4.5	External	-	0.998	1.6	0.50	-7	99	96	2	4
	Paclobutrazol E2	5.3	External	-	0.999			1	101	101	1	4
	Propiconazole E1	7.6	External	-	0.997	0.6	0.47	-3	104	100	3	2
	Propiconazole E2	8.1	External	-	0.999			1	103	102	3	2
	Propiconazole E3	9.6	External	-	0.998	2.2	0.49	3	102	101	2	4
	Propiconazole E4	11.8	External	-	0.997			2	104	103	4	3
	Prothioconazole E1	3.9	Internal	Benalaxyl-d_5 E1	0.999	1.6	0.52	-2	97	107	5	13
	Prothioconazole E2	4.6	Internal	Benalaxyl-d_5 E2	0.999			-7	96	97	3	11
	Triadimefon E1	10.9	External	-	0.998	1.7	0.50	3	104	100	1	4

	Triadimefon E2	12.7	External	-	0.999			1	101	102	2	2
	Triticonazole E1	10.1	External	-	0.998	2.0	0.50	2	101	100	3	3
	Triticonazole E2	13.2	External	-	0.999			-2	102	100	3	3
Insecticides	Fenamiphos E1	5.4	External	-	1.000	1.4	0.50	4	102	98	1	7
	Fenamiphos E2	6.4	External	-	1.000			-2	101	102	2	6
	Fipronil E1	2.8	External	-	0.998	1.0	0.51	33	102	105	4	3
	Fipronil E2	3.2	External	-	0.999			21	106	104	3	5
	Isocarbophos E1	3.7	External	-	0.996	4.7	0.50	10	106	107	9	9
	Isocarbophos E2	5.8	External	-	0.998			7	97	111	5	17
	Profenofos E1	10.5	Internal	Fenbuconazole-d_5 E1	0.996	0.5	0.51	1	98	101	1	3
	Profenofos E2	11.1	Internal	Fenbuconazole-d_5 E2	0.999			5	108	102	3	5
	Pyriproxifen E1	14.1	External	-	0.998	1.5	0.51	0	101	100	0	1
	Pyriproxifen E2	15.9	External	-	0.997			1	102	100	3	1

[a]Average of triplicate injections of 0.25, 1.25 and 5 µg L^{-1} standards in matrix. The concentrations used for lorazepam, naproxen, oxazepam, carfentrazone ethyl, bitertanol, diniconazole and isocarbophos were 0.5, 1.25 and 5 µg L^{-1}. The concentrations used for prothioconazole was 1, 1.25 and 5 µg L^{-1}. [b]Minimum resolution is reported between enantiomer 1 and interference with same MRM transition, see Figure 1.

Key: E1, enantiomer 1; E2, enantiomer 2; *Rt*, retention time; R_S, resolution; EF, enantiomeric fraction

Table 2. Method performance data for the developed enantioselective UPLC-MS/MS method

Group	Enantiomer	Trueness ±SD (n=3)			MDL	MQL
		0.25 µg L^{-1}	1.25 µg L^{-1}	5.00 µg L^{-1}	(µg L^{-1})	(µg L^{-1})
Drugs	Ifosfamide E1	99±2	96±2	100±3	0.015	0.051
	Ifosfamide E2	97±2	97±1	97±2	0.015	0.051
	Lorazepam E1	98±4[a]	93±2	97±1	0.078	0.261
	Lorazepam E2	98±4[a]	93±5	102±6	0.078	0.261
	Naproxen E1	93±4[a]	91±7	97±5	0.080	0.268
	Naproxen E2	99±4[a]	98±1	98±8	0.077	0.257
	Omeprazole E1	95±2	95±2	97±1	0.002	0.005
	Omeprazole E2	95±1	95±1	97±1	0.002	0.005
	Oxazepam E1	92±5[a]	102±11	96±2	0.081	0.269
	Oxazepam E2	96±7[a]	96±2	97±8	0.080	0.265
	Temazepam E1	101±5	101±1	99±2	0.040	0.133
	Temazepam E2	104±4	101±2	98±2	0.039	0.131
	Warfarin E1	94±3	93±4	94±1	0.008	0.027
	Warfarin E2	93±2	97±4	95±1	0.008	0.026
Herbicides	Carfentrazone ethyl E1	68±12[a]	75±11	78±3	0.120	0.399
	Carfentrazone ethyl E2	70±6[a]	74±5	76±4	0.114	0.381
	Napropamide E1	70±4	73±4	73±1	0.002	0.007
	Napropamide E2	73±6	75±2	73±2	0.002	0.006
Fungicides	Benalaxyl E1	101±2	98±1	100±2	0.030	0.099
	Benalaxyl E2	100±0	100±1	99±2	0.030	0.100
	Bitertanol E1	74±2[a]	84±6	95±2	0.083	0.278
	Bitertanol E2	73±6[a]	91±6	99±4	0.084	0.281
	Diniconazole E1	67±6[a]	69±5	71±2	0.105	0.350
	Diniconazole E2	69±2[a]	69±3	72±2	0.107	0.355
	Epoxiconazole E1	53±7	63±7	69±5	0.023	0.075
	Epoxiconazole E2	58±11	65±7	64±3	0.023	0.075
	Fenbuconazole E1	90±7	93±6	99±4	0.040	0.133
	Fenbuconazole E2	94±4	100±7	104±5	0.039	0.130
	Flutriafol E1	93±3	93±3	94±3	0.040	0.133
	Flutriafol E2	94±3	94±1	95±2	0.040	0.132
	Mandipropamid E1	99±4	100±6	108±4	0.003	0.009
	Mandipropamid E2	107±4	103±6	110±4	0.003	0.009
	Metconazole E1	61±5	69±6	74±2	0.052	0.172
	Metconazole E2	64±2	69±6	72±2	0.053	0.177
	Paclobutrazol E1	91±0	93±2	94±2	0.040	0.133
	Paclobutrazol E2	89±2	89±3	94±1	0.041	0.136
	Propiconazole E1	70±12	66±6	70±2	0.022	0.074
	Propiconazole E2	70±2	64±5	74±2	0.020	0.066
	Propiconazole E3	72±3	74±6	70±5	0.020	0.068
	Propiconazole E4	69±5	72±6	72±3	0.021	0.069
	Prothioconazole E1	107±7[b]	95±10	93±3	0.181	0.602
	Prothioconazole E2	105±3[b]	89±9	86±2	0.186	0.620
	Triadimefon E1	75±4	81±3	84±1	0.018	0.060
	Triadimefon E2	83±4	84±3	82±1	0.018	0.060
	Triticonazole E1	85±2	87±2	91±0	0.042	0.141
	Triticonazole E2	88±2	89±3	90±1	0.042	0.139
Insecticides	Fenamiphos E1	78±3	80±2	84±3	0.018	0.061
	Fenamiphos E2	80±5	81±7	84±3	0.018	0.060
	Fipronil E1	58±5	62±5	73±4	0.053	0.178
	Fipronil E2	51±4	67±5	69±4	0.052	0.174
	Isocarbophos E1	94±0[a]	97±5	91±2	0.082	0.273
	Isocarbophos E2	91±15[a]	99±6	92±7	0.078	0.259
	Profenofos E1	42±1	47±4	51±2	0.007	0.025
	Profenofos E2	39±2	46±4	57±2	0.007	0.025

[a]Recovery is reported for a concentration of 0.5µg L^{-1} [b]Recovery is reported for a concentration of 1µg L^{-1}

Key: SD, standard deviation; MDL, method detection limit; MQL, method quantitation limit; E1, enantiomer 1; E2, enantiomer 2

Table 3. Degradation rates and half-lives of enantiomers in biotic and abiotic microcosms

Group	Analyte	Biotic					Abiotic				
		k (d^{-1})	R^2	$t_{1/2}$ (days)	EF		k (d^{-1})	R^2	$t_{1/2}$ (days)	EF	
					Initial	Max/min				Initial	Max/min
Drugs	Ifosfamide E1	-	-	>100	0.51	0.53	-	-	>100	0.50	0.52
	Ifosfamide E2	-	-	>100			-	-	>100		
	Lorazepam E1	0.023	0.98	30	0.52	0.49	0.0078	0.85	89	0.50	0.48
	Lorazepam E2	0.020	0.97	36			0.0075	0.87	92		
	Naproxen E1	0.046	0.98	15	0.48	0.56	-	-	>100	0.49	0.53
	Naproxen E2	0.058	0.93	12			-	-	>100		
	Omeprazole E1	0.17	0.96	4.2	0.50	0.26	0.18	0.99	3.8	0.50	0.48
	Omeprazole E2	0.13	0.90	5.5			0.18	0.99	3.8		
	Oxazepam E1	0.014	0.93	51	0.54	0.50	0.0099	0.98	70	0.50	0.51
	Oxazepam E2	0.010	0.88	68			0.0072	0.93	96		
	Temazepam E1	-	-	>100	0.50	0.51	-	-	>100	0.49	0.50
	Temazepam E2	-	-	>100			-	-	>100		
	Warfarin E1	-	-	>100	0.51	0.49	-	-	>100	0.51	0.52
	Warfarin E2	-	-	>100			-	-	>100		
Herbicides	Carfentrazone ethyl E1	-	-	<1.0	0.49	-	0.33	0.98	2.1	0.49	0.52
	Carfentrazone ethyl E2	-	-	<1.0			0.33	0.96	2.1		
	Napropamide E1	0.022	0.84	32	0.50	0.51	-	-	>100	0.49	0.51
	Napropamide E2	0.021	0.78	33			-	-	>100		
Fungicides	Benalaxyl E1	0.041	0.72	17	0.49	0.51	-	-	>100	0.50	0.49
	Benalaxyl E2	0.043	0.71	16			-	-	>100		
	Bitertanol E1	0.073	0.74	9.5	0.49	0.47	-	-	>100	0.49	0.50
	Bitertanol E2	0.071	0.76	9.7			-	-	>100		
	Diniconazole E1	0.0085	0.62	82	0.50	0.51	-	-	>100	0.51	0.50
	Diniconazole E2	0.0081	0.59	86			-	-	>100		
	Epoxiconazole E1	0.023	0.70	30	0.50	0.49	-	-	>100	0.51	0.49
	Epoxiconazole E2	0.025	0.75	28			-	-	>100		
	Fenbuconazole E1	0.13	0.72	5.5	0.49	0.57	-	-	>100	0.50	0.51
	Fenbuconazole E2	0.13	0.70	5.2			-	-	>100		
	Flutriafol E1	0.010	0.93	67	0.51	0.48	-	-	>100	0.49	0.47
	Flutriafol E2	-	-	>100			-	-	>100		
	Mandipropamid E1	0.042	0.81	17	0.50	0.47	-	-	>100	0.50	0.49
	Mandipropamid E2	0.037	0.76	19			-	-	>100		
	Metconazole E1	0.010	0.74	68	0.50	0.50	-	-	>100	0.49	0.52
	Metconazole E2	0.010	0.69	70			-	-	>100		
	Paclobutrazol E1	-	-	>100	0.50	0.50	-	-	>100	0.49	0.47
	Paclobutrazol E2	-	-	>100			-	-	>100		
	Propiconazole E1	0.013	0.76	55	0.51	0.49	-	-	>100	0.49	0.50
	Propiconazole E2	0.012	0.80	57			-	-	>100		
	Propiconazole E3	0.015	0.76	47	0.51	0.49	-	-	>100	0.50	0.49
	Propiconazole E4	0.014	0.77	48			-	-	>100		
	Prothioconazole E1	0.14	0.87	5.0	0.50	0.68	-	-	>100	0.51	0.46
	Prothioconazole E2	0.19	0.92	3.6			-	-	>100		
	Triadimefon E1	0.041	0.96	17	0.51	0.50	-	-	>100	0.51	0.50
	Triadimefon E2	0.041	0.96	17			-	-	>100		
	Triticonazole E1	-	-	>100	0.50	0.50	-	-	>100	0.50	0.50
	Triticonazole E2	-	-	>100			-	-	>100		
Insecticides	Fenamiphos E1	0.042	0.97	16	0.51	0.45	-	-	>100	0.50	0.51
	Fenamiphos E2	0.034	0.98	20			-	-	>100		
	Fipronil E1	0.14	0.68	4.9	0.51	0.47	0.019	0.54	37	0.49	0.51
	Fipronil E2	0.13	0.71	5.4			0.019	0.59	37		
	Isocarbophos E1	0.24	0.98	2.9	0.48	0.54	0.17	0.99	4.1	0.53	0.51
	Isocarbophos E2	0.27	0.99	2.6			0.17	0.99	4.0		
	Profenofos E1	0.65	0.97	1.1	0.49	0.20	0.052	0.88	13	0.50	0.48
	Profenofos E2	0.45	0.85	1.5			0.051	0.91	14		

Key: E1, enantiomer 1; E2, enantiomer 2; k, degradation rate constant; $t_{1/2}$, half-life; EF, enantiomeric fraction

Environmentally friendly analytical method to assess enantioselective behaviour of pharmaceuticals and pesticides in river waters

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The supplementary material contains three figures and three tables showing chromatograms of temazepam and oxazepam at different column temperatures, recovery of enantiomers through PVDF filters, the enantioselective transformation of omeprazole, profenofos and prothioconazole in river water microcosms, chemical information on the studied analytes, their MRM transitions and comparison of the method with others in the literature.

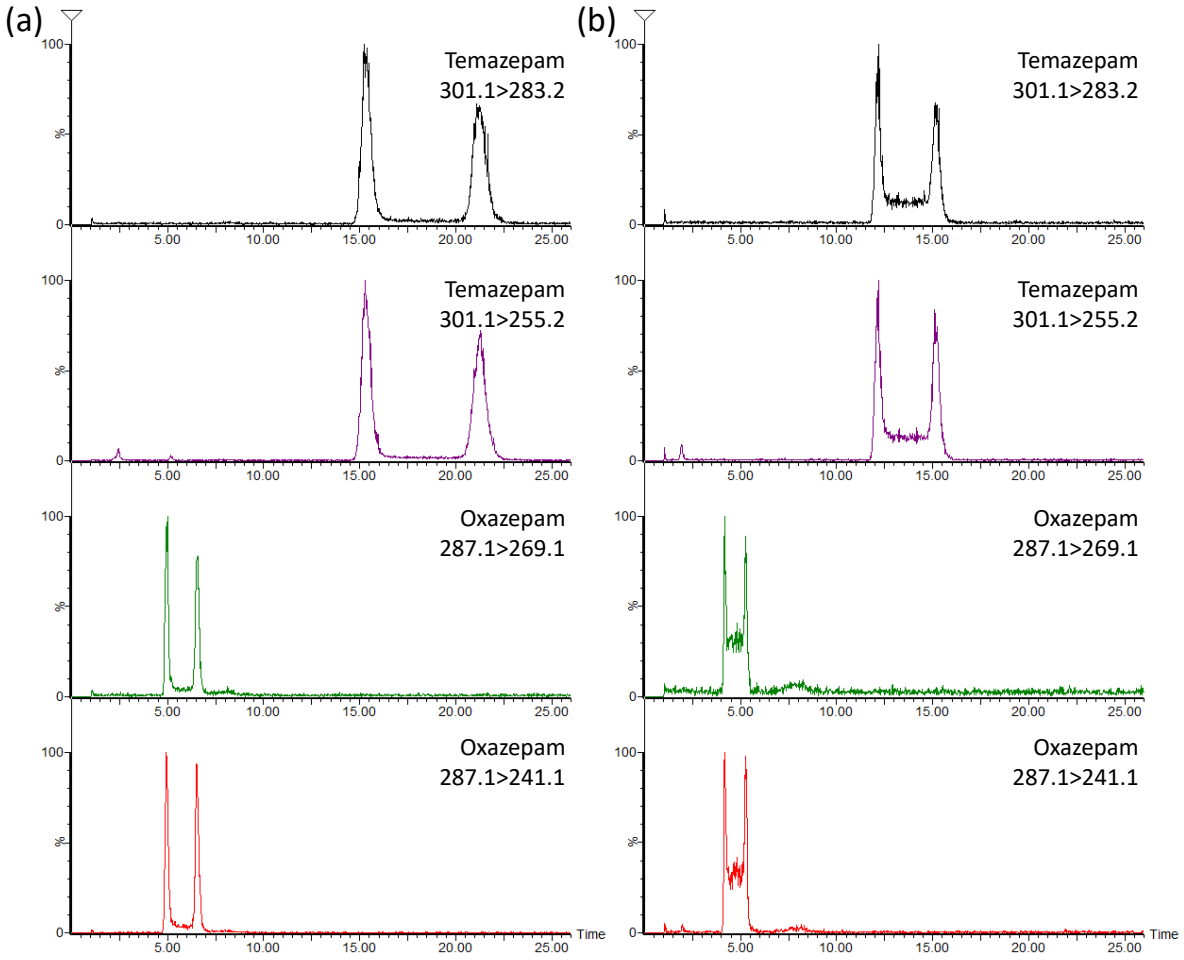

Figure S1. Enantioselective UPLC-MS/MS chromatograms of temazepam and oxazepam at 20 °C (a) and 40 °C (b) column temperatures.

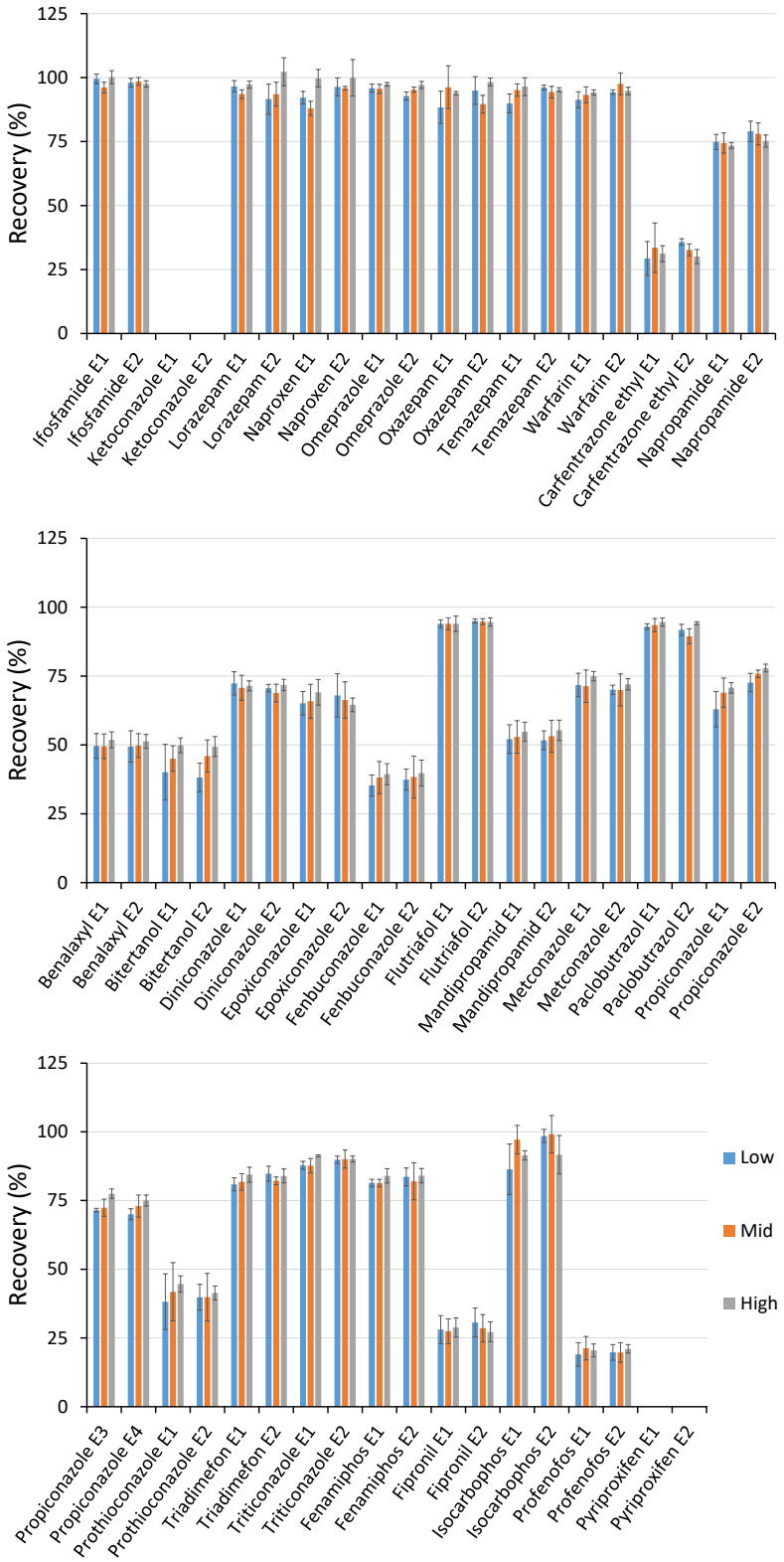

Figure S2. Recovery of enantiomers from river water filtered through PVDF filters at low (0.25 µg L^{-1}), mid (1.25 µg L^{-1}) and high (5.00 µg L^{-1}) concentrations. The low, mid and high concentrations used for lorazepam, naproxen, oxazepam, carfentrazone ethyl, bitertanol, diniconazole and isocarbophos were 0.5, 1.25 and 5 µg L^{-1}. The concentrations used for prothioconazole was 1, 1.25 and 5 µg L^{-1}.

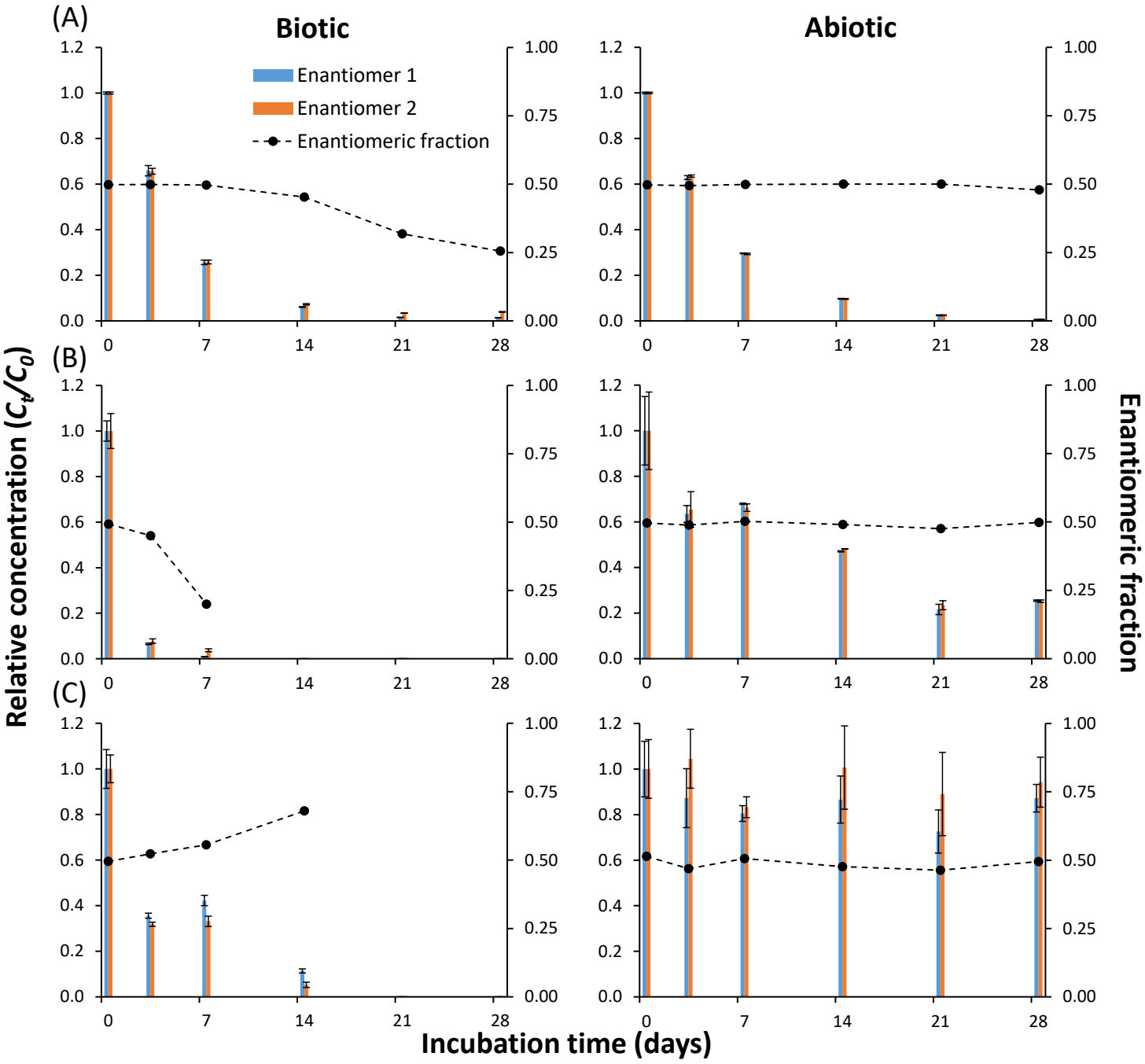

Figure S3. Relative enantiomer concentration and enantiomeric fraction of omeprazole (a), profenofos (b), and prothioconazole (c) in river water microcosms during 28 days under biotic (left) and abiotic (right) conditions.

Table S1. Chemical properties of studied analytes (DrugBank, 2021; University of Hertfordshire, 2021)

Group	Compound	CAS	Molecular weight (g mol^{-1})	pK_a	Log K_{ow}	Water solubility (mg L^{-1})
Drugs	*R/S(±)*-Ifosfamide	3778-73-2	261.08	13.24	0.86	15,000
	R/S(±)-Ketoconazole	65277-42-1	531.40	6.75	4.30	9.31
	R/S(±)-Lorazepam	846-49-1	321.16	13.00	2.98	80.0
	R/S(±)-Naproxen	23981-80-8	230.26	4.15	3.18	51.1
	R/S(±)-Omeprazole	73590-58-6	345.52	9.29 (acid), 4.77 (base)	2.23	359
	R/S(±)-Oxazepam	604-75-1	286.71	10.61	2.24	88.1
	R/S(±)-Temazepam	846-50-4	300.74	10.68	2.19	164
	R/S(±)-Warfarin	81-81-2	308.33	6.33	2.70	17.0
Herbicides	*R/S(±)*-Carfentrazone ethyl	128639-02-1	412.19	n/a	3.36	29.3
	R/S(±)-Napropamide	15299-99-7	271.35	n/a	3.30	74.0
Fungicides	*R/S(±)*-Benalaxyl	71626-11-4	325.40	n/a	3.54	28.6
	R/S(±)-Bitertanol	55179-31-2	337.42	n/a	4.10	3.80
	R/S(±)-Diniconazole	70217-36-3	326.22	-	4.30	4.0
	R/S(±)-Epoxiconazole	133855-98-8	329.76	n/a	3.30	7.1
	R/S(±)-Fenbuconazole	114369-43-6	336.82	n/a	3.79	2.47
	R/S(±)-Flutriafol	76674-21-0	301.29	2.30	2.30	95.0
	R/S(±)-Mandipropamid	374726-62-2	411.88	n/a	3.20	4.2
	R/S(±)-Metconazole	125116-23-6	319.83	11.38	3.85	30.4
	R/S(±)-Paclobutrazol	76738-62-0	293.79	-	3.11	22.9
	R/S(±)-Propiconazole	60207-90-1	342.22	1.09	3.72	150
	R/S(±)-Prothioconazole	178928-70-6	344.26	6.90	2.00	22.5
	R/S(±)-Triadimefon	43121-43-3	293.75	-	3.18	70.0
	R/S(±)-Triticonazole	131983-72-7	317.81	n/a	3.29	9.30
Insecticides	*R/S(±)*-Fenamiphos	22224-92-6	303.36	n/a	3.30	345
	R/S(±)-Fipronil	120068-37-3	437.15	n/a	3.75	3.78
	R/S(±)-Isocarbophos	24353-61-5	289.29	-	2.70	70.1
	R/S(±)-Profenofos	41198-08-7	373.63	n/a	1.70	28
	R/S(±)-Pyriproxifen	95737-68-1	321.37	6.87	5.37	0.37

n/a, not applicable (no dissociation); -, not available

Table S2. MRM transitions of the studied enantiomers

Group	Compound	Precursor (m/z)	CV (V)	Product 1 (m/z)	CE (eV)	Product 2 (m/z)	CE (eV)
Drugs	Ifosfamide	261.1	15	92.1	23	154.0	18
	Ketoconazole	531.2	27	489.2	32	244.1	36
	Lorazepam	321.1	25	275.1	22	303.1	16
	Naproxen	231.2	50	185.2	13	170.1	25
	Omeprazole	346.2	21	198.1	11	180.1	23
	Oxazepam	287.1	26	241.1	25	269.1	17
	Temazepam	301.1	24	255.2	21	283.2	14
	Warfarin	307.1	17	161.1	20	-	-
	Naproxen-d_3	234.2	10	188.2	14	-	-
	Oxazepam-d_5	292.1	26	246.2	25	-	-
	Temazepam-d_5	306.1	24	260.2	21	-	-
Herbicides	Carfentrazone ethyl	412.1	25	384.1	15	366.1	17
	Napropamide	272.2	24	199.1	12	171.1	16
Fungicides	Benalaxyl	326.2	24	208.2	14	148.2	17
	Bitertanol	338.2	24	269.2	9	99.2	15
	Diniconazole	326.1	19	70.1	22	159.0	26
	Epoxiconazole	330.1	29	121.1	17	141.1	17
	Fenbuconazole	337.2	25	125.1	26	70.1	16
	Flutriafol	302.1	21	233.1	15	123.1	30
	Mandipropamid	412.2	27	328.2	14	125.1	29
	Metconazole	320.2	25	70.1	24	125.1	24
	Paclobutrazol	294.2	20	125.1	26	70.1	17
	Propiconazole	342.1	36	159.0	27	69.1	19
	Prothioconazole	344.1	28	189.1	22	326.1	10
	Triadimefon	294.1	30	197.1	14	69.1	17
	Triticonazole	318.2	30	70.1	18	125.1	30
	Benalaxyl-d_5	331.3	24	148.2	17	-	-
	Fenbuconazole-d_5	342.2	25	70.0	26	-	-
Insecticides	Fenamiphos	304.2	25	217.1	19	202.1	37
	Fipronil	434.9	31	330.0	18	399.1	9
	Isocarbophos	312.1	33	270.1	12	236.1	12
	Profenofos	373.0	26	345.0	11	303.0	17
	Pyriproxifen	322.2	24	227.2	15	185.1	22

Note: all analytes were monitored in positive ESI mode except fipronil and warfarin (negative ESI)

Table S3. Performance of previously reported LC-MS/MS methods for enantioselective analysis of pesticides in river water

No. of analytes	Extraction method	Column	Mobile phase	Run time (minutes)	Recovery (%)	RSD (%)	Matrix effect (%)	MQL (µg L^{-1})	Ref.
6	SPE (200 mL) + DLLME	Cellulose tris-(3,5-dimethylphenylcarbamate) 150 × 4.6 mm, 5µm	0.1% FA: ACN (60:40 v/v) @ 0.6 mL/min	70	85-92	1-12	5-28	≤0.001	A
18	MSPE (200 mL)	Amylose tris-(3-chloro-5methylphenylcarbamate) 250 × 4.6 mm, 5µm	ACN: 5 mM NH_4OAc + 0.05% FA (53:47 v/v) @ 0.6 mL/min	55	76-102	2-13	-5-17	≤0.002	B
2	SUSME	α-CD permethylated 200 × 4 mm, 5µm	MeOH:100 mM FA/NH_4HCO_2 (pH 4.0) (65:35 v/v) @ 0.5 mL/min	13	74-79	1-2	-	0.001-0.004	C
9	SPE (100 mL)	Amylose tris-(3,5-dimethylphenylcarbamate) 150 × 4.6 mm, 5µm	ACN:2 mM NH_4OAc in water (gradient) @ 0.45 mL/min	35	78-104	2-14	-10-10	0.1-0.2	D
19 + 7 pharmaceuticals	None (direct injection)	Amylose tris-(3-chloro-5methylphenylcarbamate) 100 × 3.0 mm, 1.6 µm	Ethanol: 5 mM NH_4OAc + 0.1% FA (75:25 v/v)	26	39-110	0-17	-15-33	0.005-0.6	This study

Key: RSD, relative standard deviation; MQL, method quantitation limit; SPE, solid phase extraction; DLLME, dispersive liquid-liquid microextraction; FA, formic acid; ACN, acetonitrile; MSP, magnetic solid phase extraction; NH_4OAc, ammonium acetate; MeOH, methanol; SUSME, supramolecular solvent-based microextraction; NH_4HCO_2, ammonium formate; -, not reported; A, Zhao et al., 2018a; B, Zhao et al., 2018b; C, Caballo et al., 2013; D, Li et al., 2013

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