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YEAR: 2018

Publisher citation: PETRIE, B., MRAZOVA, J., KASPRZYK-HORDERN, B. and YATES, K. 2018. Multi-residue analysis of chiral and achiral trace organic contaminants in soil by accelerated solvent extraction and enantioselective liquid chromatography tandem-mass spectrometry. Journal of chromatography A [online], 1572, pages 62-71. Available from: https://doi.org/10.1016/j.chroma.2018.08.034

OpenAIR citation: PETRIE, B., MRAZOVA, J., KASPRZYK-HORDERN, B. and YATES, K. 2018. Multi-residue analysis of chiral and achiral trace organic contaminants in soil by accelerated solvent extraction and enantioselective liquid chromatography tandem-mass spectrometry. Journal of chromatography A, 1572, pages 62-71. Held on OpenAIR [online]. Available from: https://openair.rgu.ac.uk/

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PII: S0021-9673(18)31056-2
DOI: https://doi.org/10.1016/j.chroma.2018.08.034
Reference: CHROMA 359630

To appear in: *Journal of Chromatography A*

Received date: 20-3-2018
Revised date: 6-8-2018
Accepted date: 15-8-2018

Please cite this article as: Petrie B, Mrazova J, Kasprzyk-Hordern B, Yates K, Multi-residue analysis of chiral and achiral trace organic contaminants in soil by accelerated solvent extraction and enantioselective liquid chromatography tandem-mass spectrometry, *Journal of Chromatography A* (2018), https://doi.org/10.1016/j.chroma.2018.08.034

Multi-residue analysis of chiral and achiral trace organic contaminants in soil by accelerated solvent extraction and enantioselective liquid chromatography tandem-mass spectrometry

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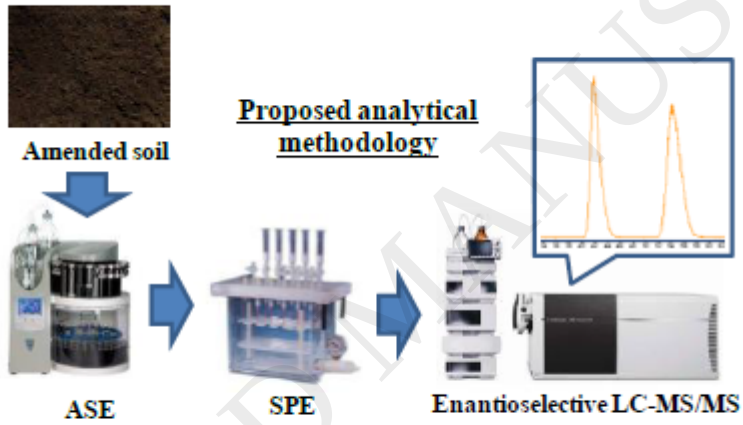
Graphical abstract



Highlights

- First enantioselective method for chiral pharmaceutical TOrCs in soils
- Chirobiotic V2® better suited for multi-residue separation than Chirobiotic V®
- Enantioresolution (>1.0) achieved for 5 classes of chiral TOrCs simultaneously
- Liophilic ion concentration had greatest influence on enantioseparations
- Stereoselective degradation of pharmaceutical TOrCs observed for first time in soil

Abstract

Reported here is the first analytical methodology for the enantiomeric determination of chiral trace organic contaminants (TOrCs) in soil. Direct enantioselective separations were achieved on a Chirobiotic V2® column operated in polar ionic mode. Initial screening of vancomycin stationary phases found Chirobiotic V2® better suited for multi-residue separation of chiral TOrCs than Chirobiotic V® due to differences in the ligand linkage chemistry. Simultaneous enantioseparation of beta-blockers, beta-agonists, anti-depressants, anti-histamines and stimulants was achieved for the first time. This included the first separation of chlorpheniramine enantiomers with a method suitable for environmental analysis (i.e., coupled to MS). Investigation of mobile phase composition found the concentration of liophilic ions had the greatest influence on enantioseparations and of most importance during method development. The optimized method achieved simultaneous separation of salbutamol, propranolol, atenolol, amphetamine, chlorpheniramine and fluoxetine enantiomers with satisfactory resolution (>1.0). For completeness, such methods also need to support analysis of achiral TOrCs. Therefore three achiral TOrCs (carbamazepine, carbamazepine 10,11 epoxide and triclocarban) were included to demonstrate the methods suitability. Method recoveries for all analytes ranged from 76 to 122 % with method quantitation limits (MQLs) <1 ng g^{-1}. Application of the method to soil microcosm studies revealed stereoselective degradation of chiral TOrCs for the first time. For example, *S(+)*-amphetamine degraded at a faster rate than its corresponding enantiomer leading to an enrichment of *R(-)*-amphetamine. Therefore to better understand the risk posed from TOrCs on the terrestrial environment, chiral species need profiled at the enantiomeric level. This can now be addressed using the proposed methodology whilst simultaneously profiling achiral TOrCs.

Keywords: micropollutant; soil; pharmaceutical; chiral; sludge; LC-MS/MS

1. Introduction

Municipally derived trace organic contaminants (TOrCs) such as pharmaceuticals, personal care products and illicit drugs are ubiquitous in rivers impacted by wastewater effluent discharges [1]. However in recent years there has been growing concern on the presence of TOrCs in the terrestrial environment [2,3]. The application of digested sludge (and untreated animal manure) to agricultural land has led to the occurrence and distribution of TOrCs in soils. Furthermore, reclaimed wastewater used for irrigation purposes can lead to introduction of TOrCs to agricultural soils. In amended soils TOrC concentrations >10 ng g^{-1} are found [4-11], with levels >100 ng g^{-1} not uncommon [4-6,9]. It is essential to monitor TOrCs in soils as they can cause toxicological effects on exposed organisms such as the earthworm *Eisenia fetida* [3]. Additionally bioaccumulation in exposed organisms is possible [6], posing a risk to predatory organisms at higher trophic levels. The presence of TOrCs in soils has also been found to impact microbial respiration [9]. Establishing the fate and behaviour of TOrCs in soils is important as leaching can occur resulting in the contamination of surrounding surface and ground waters [7].

The determination of TOrCs in soil requires a suitable extraction and analysis method. Vazquez-Roig et al [12] established an analytical methodology for the determination of 17 pharmaceuticals from soils using ASE (or pressurized liquid extraction) followed by SPE clean-up and LC-MS/MS analysis. Analyte recoveries were 50-105 % and MQLs were 0.25-23 ng g^{-1} demonstrating the success of the developed protocol. Similar methodologies have since been applied to determine TOrC levels in soil [13-15]. However these methods are unable to assess the enantiomeric composition of chiral TOrCs. Approximately 50 % of all pharmaceuticals on the market are chiral [16]. Furthermore, they are unlikely to be present in soils in racemic form because (i) they are applied to soils in non-racemic form due to stereoselective metabolism within the human body and during wastewater/sludge treatment [16,17] and, (ii) it is postulated that chiral TOrCs will undergo stereoselective degradation in soil. To demonstrate this, Evans et al. [17] performed enantiomeric profiling of digested sludge destined for land application. Of the 17 chiral TOrCs found in digested sludge, 11 were found to be in non-racemic form with enantiomeric fractions (EFs) ranging from 0.1-0.7 (alprenolol, amphetamine,

atenolol, citalopran, desmethylcitalopram, ephedrine, norephedrine, fluoxetine, 3,4-methylenedioxy-methamphetamine (MDMA), metoprolol and tramadol). Their presence in soil in non-racemic form may be significant because stereospecific toxicity of fluoxetine, propranolol and atenolol has been observed to exposed environmental aquatic organisms [18-21]. This demonstrates the importance of conducting analysis of TOrCs in soils at the enantiomeric level. However, due to a lack of suitable enantioselective methods for the soil matrix, no field data exists on municipally derived chiral TOrCs at the enantiomeric level.

Therefore the aim of this study was to develop and validate a new analytical methodology for the enantioselective determination of chiral TOrCs in soils. This was achieved using ASE followed by off-line SPE and analysis by enantioselective HPLC-MS/MS. It is important that such methods can support the simultaneous analysis of achiral micropollutants for a complete assessment of TOrC distribution. Consequently, a total of 10 diverse achiral (carbamazepine, carbamazepine epoxide, triclocarban) and chiral (salbutamol, propranolol, atenolol, amphetamine, MDMA, chlorpheniramine, fluoxetine) TOrCs were selected for method development.

2. Materials and methods

2.1. Materials

Carbamazepine, carbamazepine 10,11 epoxide, triclocarban, *R/S(±)*-salbutamol, *R/S(±)*-propranolol hydrochloride, *R/S(±)*-atenolol, *R/S(±)*-amphetamine, *R/S(±)*-MDMA, *R/S(±)*-chlorpheniramine maleate and *R/S(±)*-fluoxetine hydrochloride were purchased from Sigma-Aldrich (Gillingham, UK) (Table S1). The deuterated surrogate standards carbamazepine-D10, carbamazepine 10,11 epoxide-D10, triclocarban-D3, *R/S(±)*-salbutamol-D3, *R/S(±)*-propranolol-D7 hydrochloride, *R/S(±)*-amphetamine-D11, *R/S(±)*-MDMA-D5 and *R/S(±)*-fluoxetine-D6 hydrochloride were also purchased from Sigma-Aldrich. The majority of analyte standards and deuterated standards were purchased as 0.1 or 1 mg mL^{-1} ampules in methanol. Those purchased as powders were prepared in methanol at 1 mg mL^{-1}. All solutions were stored in the dark at -20 °C. Methanol, ammonium acetate and acetic acid were HPLC grade and obtained from Sigma-Aldrich. Water used throughout the study was of 18.2 MΩ cm^{-1} quality. Oasis HLB and MCX cartridges (60 mg, 3 mL) were purchased from Waters (Manchester, UK). Enantioselective Chirobiotic V® and Chirobiotic V2® HPLC columns (250 x 2.1 mm; 5 µm) were obtained from Sigma Aldrich. Agricultural soil was collected from arable farmland in North-East Scotland. The soil in question had not been treated with digested sludge or animal manure in the last 10 years.

2.2. Accelerated solvent extraction

Collected soil was sieved (2 mm) and dried in an oven overnight at 50 °C. 5 g samples were spiked with a methanolic mixture of all surrogate standards to achieve a concentration of 25 ng g^{-1} (12.5 ng g^{-1} in the case of individual enantiomers). Samples were left for a minimum of 1 h to allow the methanol to evaporate. Samples were then mixed with 5 g diatomaceous earth and packed into 10 mL stainless steel ASE cells (Fisher Scientific, Loughborough, UK). Remaining volume of the cell was filled with Ottawa sand. Two 2-4 µm Dionex glass fibre filters (Fisher Scientific, Loughborough, UK) were then fitted to each end of the cell. Extraction of prepared soil samples was performed using a Dionex ASE 350 (California, USA) system. The final method utilized an extraction solvent of

20:80 water:methanol and an extraction temperature of 80 °C. For each cell two extraction cycles were performed with the following settings: pre-heat for 5 min, heating for 5 min, static extraction time of 5 min, solvent flush volume of 60 % and nitrogen purge time of 150 s. The extraction pressure was 1,500 psi. During the development process the impact of changing solvent composition (80:20, 50:50 and 20:80 water: methanol), temperature (80, 100 and 120 ˚C) and sample mass (1, 2.5 and 5 g) on TOrC recovery was investigated.

2.3. Solid phase extraction

Solvent extracts obtained from the ASE (~22 mL) were diluted to a final volume of 250 mL using water. Aqueous extracts containing <10 % methanol are not considered to influence SPE extraction efficiency [17]. The final SPE method involved conditioning Oasis HLB cartridges with 2 mL methanol followed by 2 mL water for equilibration. Both steps were conducted under gravity at approximately 1 mL min^{-1}. Samples were loaded at 5 mL min^{-1} using a vacuum manifold then dried under vacuum. Analytes were eluted using a 4 mL aliquot of methanol under gravity (1 mL min^{-1}). SPE extracts were subsequently dried under nitrogen and reconstituted in 0.5 mL mobile phase. Finally, the samples were filtered (0.2 µm) using pre-LC-MS PTFE syringe filters (Whatman, Kent, UK) ready for LC-MS/MS analysis.

2.4. Enantioselective liquid chromatography tandem mass spectrometry

Chromatography was performed using an Agilent 1200 Infinity Series HPLC (Cheshire, UK). Optimized analyte separations were achieved using a Chirobiotic V2® HPLC column (250 x 2.1 mm; 5 µm) maintained at 20 °C. Final mobile phase conditions were methanol containing 1 mM ammonium acetate and 0.01 % acetic acid operated under isocratic conditions at a flow rate of 0.2 mL min^{-1}. An injection volume of 80 µL was utilised and the run was 65 min. During method development the impact of varying concentrations of acetic acid (0, 0.01, 0.05 and 0.1 %), ammonium acetate (1, 5, 10 and 20 mM), and water (0, 1, 5 and 10 %) all in methanol on enantiomeric separation

was investigated. Each mobile phase was equilibrated for a minimum of 2 h at a flow rate of 0.2 mL min^{-1} to ensure equilibration was achieved (and duplicate injections performed).

The HPLC was coupled to an Agilent 6420 MS/MS triple quadrupole. Electro-spray ionization (ESI) was utilized in both negative and positive ionization modes. Triclocarban and triclocarban-D5 were analysed in negative ionization mode. All other analytes were determined in positive ionization mode. The capillary voltage for both negative and positive ionization modes was 4,000 V. The desolvation temperature was 350 °C with a gas flow of 12 L min^{-1}. The nebulizing pressure was 50 psi. Nitrogen gas was used as the nebulising, desolvation and collision gas. Optimized MS/MS transitions for each analyte are compiled in Table S2. Two multiple reaction monitoring (MRM) transitions were monitored for each analyte for quantification and confirmation purposes (one in the case of deuterated standards). Other quality criteria used to ensure quality of data was pre-determined tolerances of ion ratio and retention time [22].

2.5. Instrument and method performance

Linearity was established through the injection of a 12 point calibration curve ranging in concentration from 0.1 to 2,000 ng mL^{-1} in mobile phase (0.05 to 1,000 ng mL^{-1} for individual enantiomers of chiral TOrCs). Intra-day and inter-day precision and accuracy was determined by triplicate injection of 10, 100 and 1,000 ng mL^{-1} standards within a 24 h period and across three different days, respectively. Robustness of the method was investigated by making small changes to the mobile phase conditions and assessing their impact to the separation. Ammonium acetate and acetic acid concentration as well as column temperature were modified by ±2.5 %.

Recovery of studied TOrCs using the ASE-SPE-enantioselective LC-MS/MS was determined by spiking soil in triplicate at total TOrC concentrations (sum of both enantiomers in the case of chiral TOrCs) of 2, 10 and 100 ng g^{-1}. Signal suppression from co-extracted matrix was evaluated by taking samples through the entire extraction process and spiking extracts post SPE to achieve a theoretical final concentration of 100 ng mL^{-1} in the vial for LC-MS/MS analysis.

2.6. Microcosm studies

Microcosm studies were performed to investigate TOrC degradation in soil. Preparation of microcosms was similar to those previously reported in the literature [14,23]. Soil (150 g) was collected and sieved (2 mm) but not dried to maintain field conditions. Sub-samples of 5 g were then placed into eighteen plastic sacrificial 50 mL centrifuge tubes. Each soil sample was then spiked with a mixture of TOrCs to achieve a theoretical concentration of 100 ng g^{-1} (50 ng g^{-1} in the case of enantiomers). These were left in the dark (wrapped in foil) and open to the air at 20 ±1 ˚C. Every few days these were adjusted to their initial weight using water to maintain the initial moisture content (~20 %). Samples were then collected in triplicate at times 0, 3, 7, 14, 28 and 56 d, spiked with surrogate standards as described previously and analysed using the developed ASE-SPE-enantioselective LC-MS/MS methodology.

3. Results and discussion

3.1. Screening Chirobiotic V® and Chirobiotic V2® for enantioselective separation of chiral TOrCs using LC-MS compatible mobile phases

Vancomycin based stationary phases were selected for method development due to their previous success in achieving enantioselective separations of various beta-blockers, beta-agonists and anti-depressants [17, 24-29], as well as their versatility enabling operation in reverse phase, polar ionic and polar organic modes. Previous studies utilized commercially available Chirobiotic V® HPLC columns. However, a development of this column is Chirobiotic V2® which differs in the bonding chemistry between the ligand and the silica support. Changing the position of several linkages and the chain length used to anchor the ligand offers differences in selectivity to Chirobiotic V® particularly when operated in polar organic and polar ionic modes [30].

Chirobiotic V® and Chirobiotic V2® columns of identical dimensions (250 x 2.1 mm; 5 μm) were screened for the separation of target TOrCs. Separation modes compatible with LC-MS analysis (i.e., reverse phase, polar ionic and polar organic) were trialled with recommended mobile phase compositions [31]. The recommended screening protocol suggests the addition of 0.1 % triethylamine in polar ionic mode but this was omitted as it is known to deteriorate the column due to irreversible attraction to the stationary phase [32]. As anticipated the most successful separation mode was indeed polar ionic due to the basicity of the studied TOrCs (Table S3). This is the selected mode of enantioselective separation utilized in previous studies [17, 25, 26]. Successful enantioseparation in polar ionic mode is reliant on the TOrC having an ionisable group near its chiral centre as well as a further functional group in its structure [30]. All chiral TOrCs investigated except MDMA showed some enantioseparation during the screening exercise.

In polar ionic mode Chirobiotic V® achieved similar or better enantioseparation of salbutamol, propranolol and atenolol in comparison to Chirobiotic V2® (Table S3). However, Chirobiotic V2® achieved considerably improved separation of amphetamine (R_S 0.9 *v.* 0.3) and fluoxetine (R_S 1.9 *v.* 0.9) enantiomers. Furthermore Chirobiotic V2® achieved partial separation of chlorpheniramine (R_S 0.7) whereas Chirobiotic V® did not achieve any separation (Table S3). Achiral TOrCs also exhibited

satisfactory chromatography (Gaussian distributed peaks) in polar ionic mode. For these reasons Chirobiotic V2® operated in polar ionic mode was taken forward for further optimisation. A previous study by Bosáková et al [33] noted generally better chiral TOrC enantioseparations by Chirobiotic V2® over Chirobiotic V® albeit operated in polar organic mode (with UV detection).

3.2. Optimizing enantioselective separation of chiral TOrCs using Chirobiotic V2®

Those TOrCs which exhibited (partial) enantiomer separation using Chirobiotic V2® in polar ionic mode had R_S ranging from 0.7 for salbutamol, atenolol and chlorpheniramine to 1.9 for fluoxetine (Table S3). The aim was to improve these separations such that R_S were ≥1.0 which would satisfy a maximum 2 % overlap required for quantitative analysis [25].

Initially the impact of altering the percentage of acid modifier on enantiomer R_S was investigated. Sanganyado et al [34] reported that the type of acid modifier is unlikely to influence separation as pH is the determining factor. As vancomycin has several pK_a values due to the diversity of its structure, changing the percentage of acid (and pH) of the mobile phase can lead to the ionization of different functional groups. Acetic acid was varied from 0 to 0.1 % (in methanol containing 10 mM ammonium acetate representing a pH range from 7.4 to 6.0. Increasing the % acetic acid (reducing pH) resulted in reduced retention of the studied TOrCs, however little impact to R_S was noted (Figure 1). It is proposed that greater ionization at lower pH resulted in increased repulsion between the positively charged TOrC and the cationic functional groups of the stationary phase reducing their retention time. Nevertheless, at pH 7.0 (0.01 % acetic acid) four of the chiral TOrCs (salbutamol, amphetamine, propranolol and fluoxetine) exhibited satisfactory R_S ≥1.0.

A previous study investigated the influence of ammonium salts or liophilic ions (nitrate, formate and acetate) on the enantioresolution of atenolol and fluoxetine [34]. It was noted that R_S increased with increased hydrophobicity of the liophilic ion. Therefore ammonium acetate was utilized in the optimization process with concentrations ranging from 1 to 20 mM whilst at pH 7.0. Increased ammonium acetate concentrations led to a reduction of R_S (and retention) due to decreased chiral

interactions between the TOrC and the stationary phase (Figure 1). It is postulated that at increased ammonium acetate concentrations, there is greater competition between the positively charged analyte ions and positively charged ammonium ions to oppositely charged ionic functional groups of the stationary phase, although several mechanisms could be at play. Nevertheless, the findings are in agreement with those of Sanganyado et al [34]. A mobile phase containing 1 mM ammonium acetate achieved satisfactory $R_S \geq 1$ of six chiral TOrCs (salbutamol, amphetamine, propranolol, atenolol, chlorpheniramine and fluoxetine) (Figure 1). During the optimization process no enantioseparation of MDMA was observed.

The effect of adding small volumes of water (≤ 10%) to the mobile phase was also investigated. It was found that the addition of water at 1 % v/v reduced R_S considerably (Figure 1). Therefore no water was added to the mobile phase. Other factors that were optimized included flow rate and injection volume. These were increased sequentially to select the conditions which gave the shortest possible run time and highest sensitivity which did not compromise R_S. Therefore the final method operated at a flow rate of 0.2 mL min^{-1} with an injection volume of 80 µL. A further parameter which can be optimized is column temperature [32,34]. A temperature of 20 ˚C was used throughout as it was previously found to achieve satisfactory R_S using polar ionic mobile phases containing ammonium acetate [34].

3.3. Enantioselective LC-MS/MS instrument performance

Instrument performance using the optimized mobile phase conditions was determined by establishing linear response, enantiomer R_S and EF, intra- and inter-day precision and accuracy, and sensitivity (Table 1). A 12 level calibration curve was prepared and the majority of studied TOrCs exhibited linearity between the IQL and 1,000 ng mL^{-1} (2,000 ng mL^{-1} for those achiral TOrCs and MDMA). Coefficient of determination (r^2) of the calibrations were ≥0.993 with the majority >0.999. Of those chiral TOrCs separated at the enantiomeric level, R_S's were ≥1.1 across the studied concentration range. Furthermore EFs were calculated according to:

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

Here *(+)* is the concentration of the *(+)*-enantiomer and *(-)* is the concentration of the *(-)*-enantiomer. The following equation was used for those compounds (salbutamol) whose order of enantiomer elution is unknown:

$$EF = \frac{E1}{[E1+E2]} \quad (2)$$

In this case *E1* is the concentration of the first eluting enantiomer and *E2* is the concentration of the second enantiomer. All chiral TOrCs were injected as racemates and exhibited reproducible EFs of 0.50 ±0.01 (0.49 ±0.01 for atenolol and chlorpheniramine).

Both intra- and inter-day precision over the 3 different concentrations levels (total TOrC concentrations of 10, 100 and 1,000 ng mL^{-1}) were <10 % for all studied TOrCs (Table 1). Furthermore, accuracy was within 90-110 % for the majority of studied TOrCs both in the same day and between different days. The method was found to be robust with respect to small changes in mobile phase conditions (ammonium acetate concentration, acetic acid concentration and temperature). No notable changes in resolution or retention time were noted. The instrument detection limits (IDLs) and instrument quantitation limits (IQLs) ranged from 0.08 to 0.3 ng mL^{-1} and from 0.25 to 1.0 ng mL^{-1}, respectively (Table 1). Findings from the instrument performance tests are similar to previously published methods utilizing Chirobiotic V® operated in polar ionic mode for the determination of chiral TOrCs in diluent [17,25].

3.4. Extraction method development

A suitable SPE protocol was required prior to development of the ASE method. This was achieved by spiking 250 mL ultra-pure water at 1 µg L^{-1} and extracting onto Oasis HLB (pH 7) and MCX (pH 2 to ionize basic TOrCs [35]) SPE cartridges. Whilst the HLB cartridges were eluted using methanol, the cationic exchange MCX cartridges were eluted into individual acidic and basic fractions using methanol containing formic acid (wash step) and ammonium hydroxide (elution), respectively.

Extracts of MCX cartridges resulted in poor chromatography and loss of chiral recognition. This is attributed to the ammonium hydroxide used for elution despite extracts being evaporated to dryness and reconstituted in methanol prior to injection. Nevertheless, the HLB sorbent eluted with methanol successfully achieved simultaneous extraction of all studied TOrCs with recoveries ranging from 67 to 96 % (Table S4). The Oasis HLB sorbent which utilizes both hydrophilic and lipohilic retention mechanisms is a popular choice for environmental sample pre-treatment prior to enantioselective LC-MS/MS [29].

Development of the ASE method involved investigating the impact of solvent composition, extraction temperature and sample mass on TOrCs recovery from soil in univariate fashion (i.e., each extraction variable was considered individually [4,11,12,17]). These factors were considered likely to have the greatest influence on TOrCs recovery [35-37]. Optimizing extraction methods for environmental matrices require a trade-off between analyte extraction efficiency and signal suppression during ESI from co-extracted matrix. The soil used during the development had not been previously treated with wastewater sludge (or compost/animal manure) and was found to be free from the studied TOrCs. Therefore considering peak areas from spiked samples taken through the entire ASE-SPE-enantioselective LC-MS/MS method provides true representation of method performance. This is because losses from the extraction process and signal suppression during ESI is considered simultaneously. Otherwise subtracting the response of unspiked matrix that already contains TOrCs from the spiked matrix can introduce bias to performance calculations (particularly due to signal suppression). This is unavoidable in other matrices such as wastewater or river water.

A popular solvent choice for extraction is water:methanol with ratios ranging from 100 % water to 100 % methanol used previously [11,38]. Methanol is considered to give higher analyte recoveries compared to other solvents such as acetonitrile [35,39,40]. The water:methanol ratios investigated here were 80:20, 50:50 and 20:80. As anticipated with multi-residue analysis not all TOrCs showed the highest recovery for the same solvent composition. Highest recovery was achieved for most TOrCs using 50:50 or 20:80 water:methanol, with no substantial differences in recovery observed between the two (Figure 2). Only recovery of salbutamol, amphetamine and atenolol enantiomers

was greater using 80:20 water:methanol. However, their recovery was greater at 20:80 in comparison to 50:50 water:methanol therefore as a trade-off this solvent composition (20:80) was selected for further development.

Extraction temperature can have a significant impact on TOrCs recovery from environmental matrices. Increased temperature results in reduced solvent viscosity which facilitates better penetration of the matrix [41]. On the other hand increased temperature can lead to thermal degradation of the TOrC as well as increased extraction of unwanted matrix components which leads to greater signal suppression. This is particularly important when using non-selective SPE sorbents such as Oasis HLB. In this study highest recovery for all TOrCs was achieved at 80 ˚C (Figure 2). This temperature is in agreement with previous studies which extracted TOrCs from environmental matrices [35,38,42]. Several TOrCs (carbamazepine, carbamazepine epoxide, triclocarban, *S(+)*-fluoxetine and *R(-)*-fluoxetine) showed considerably reduced recovery at each extraction temperature increase during the development process. This was considered to be from increased interference from co-extracted matrix as these TOrCs were subject to greater signal suppression during ESI (Table 2).

Finally, for environmental analysis of TOrCs it is essential to determine a sample mass which provides adequate sensitivity but is not detrimental to the analytical method. As TOrCs tend to be found in amended soils at relatively low concentrations <100 ng g^{-1} [4-8,10,11], sample masses of 1, 2.5 and 5 g were investigated. All masses were spiked with 100 ng of each TOrC and recovery determined. It can be seen (Figure 2) that increasing sample mass had no impact on recovery for the majority of studied TOrCs. Those analytes which did show reduced recovery with increased sample mass (carbamazapine epoxide and triclocarban) were not proportional to the increased mass used. For example, the reduction in recovery from 2.5 to 5 g was less than 50 %. Therefore it is still beneficial to use the higher sample mass for these analytes when analysing real samples as their increased quantity present in 5 g outweighs losses from poorer recovery (extraction efficiency and increased signal suppression).

3.5. Performance of the developed ASE-SPE-enantioselective LC-MS/MS method

To validate the ASE-SPE-LC-MS/MS method, recovery of studied TOrCs was performed in triplicate at three different spike levels of 2, 10 and 100 ng g^{-1}, respectively. These represent the 'total' TOrC concentration therefore spiking concentrations of individual enantiomers was 1, 5 and 50 ng g^{-1}. Chromatograms obtained using the developed extraction method are shown (Figure 3). Method recoveries (i.e., accounting for surrogate standard response) across the 3 different spike levels ranged from 76 to 122 % (Table 2). The overall precision of the method was ≤16 % for all analytes. These levels of accuracy and precision are within those previously reported for the determination of chiral drugs at the enantiomeric level in digested sludge using microwave assisted extraction [17]. For the majority of studied chiral TOrCs EFs in spiked soils was 0.49-0.50 (Table 2). This is to be expected as the TOrCs were spiked as racemates (EF = 0.50).

Suppression of analyte signal strength during ESI of environmental extracts is a well-known drawback of LC-MS analysis. Signal suppression was quantified by comparing extracted soil samples spiked post ASE-SPE with the standard solution used for spiking:

$$Signal\ suppression\ (\%) = 100 - \left(\frac{\text{PA spiked extract}}{\text{PA standard}} . 100\right) \quad (3)$$

Where *PA spiked extract* is the analyte peak area in the extract spiked post SPE and *PA standard* is the ananlyte peak area in standard solution used for spiking. Signal suppression (i.e., loss of response due to co-extracted matrix quenching analyte signal strength) ranged from 3 % (negligible) for *S(+)*-chlorpheniramine to 93 % for carbamazepine (Table 2). Those TOrCs which had the least interaction with the stationary phase and shortest retention times (carbamazepine, carbamazepine 10,11 epoxide and triclocarban) had the greatest signal suppression. Stereoselective suppression was also noted for atenolol and fluoxetine. For example, signal suppression for *S(+)*-fluoxetine and *R(-)*-fluoxetine were 42 ±1 % and 26 ±2 %, respectively. Stereoselective signal suppression has previously been noted for propranolol in river water and wastewater [26]. This demonstrates the necessity of using deuterated surrogate standards for accurate quantitation of TOrCs in environmental matrices at the enantiomeric level.

Finally method sensitivity was determined by calculating the method detection limit (MDL) and method quantitation limit (MQL) [35]:

$$MDL\ (ng\ g^{-1}) = \frac{S.IDL\ x\ 100}{Rec\ x\ CF} \quad (4)$$

$$MQL\ (ng\ g^{-1}) = \frac{S.IQL\ x\ 100}{Rec\ x\ CF} \quad (5)$$

Here *S* is the volume of sample used for extraction divided by the mass of sample extracted (mL g^{-1}), *IDL* and *IQL* are the instrument detection and quantitation limits, respectively (ng mL^{-1}), *Rec* is the absolute recovery (%, not accounting for surrogate standard response) and *CF* is the concentration factor. MDLs ranged from 0.02 to 0.24 ng g^{-1} whereas MQLs ranged from 0.07 to 0.91 ng g^{-1} (Table 2). These MDLs and MQLs are similar or better than previously reported ASE-SPE-LC-MS/MS methods applied to soils [12-14]. The low MDLs and MQLs achieved can be attributed to the use of polar ionic mobile phases which offer excellent sensitivity for use in MS [29]. These MQLs achieved are adequately sensitive for the concentrations of TOrCs expected to be present in amended soils [4-8,10,11].

Other than being the first enantioselective LC-MS/MS method developed for the soil matrix, the newly developed methodology has several advantages over those previously reported. Achieving multi-residue enantioseparation of several classes of TOrCs is essential. This is the first reported method for the simultaneous separation of beta-blocker, beta-agonist, anti-depressant, anti-histamine and stimulant enantiomers which can be applied to environmental samples. Previously reported methods are often limited to a single class of TOrC [43-47]. By focussing on a limited number of TOrCs from a single class, analysis run times can be comparatively shorter (usually <30 min). On the other hand, those enantioselective LC-MS/MS methods which can perofrm multi-residue analysis often have run times ≥80 min with times up to 150 min not uncommon [48-51]. Therefore a run time of 65 min does offer improved sample throughput and turnover than these reported methods. The developed method is also the first to achieve enantioseparation of the antihistamine chlorpheniramine in environmental matrices. Furthermore, the suitability of the method to support achiral TOrC

determinations is essential and not considered in previous methods. This is of great importance for future monitoring and assessing environmental risk.

3.6. Behaviour of chiral and achiral trace organic contaminants in soil microcosms

The validated method was applied to determine the fate of studied TOrCs in soil microcosms. Sieved soil (2 mm) was separated into 5 g sacrificial samples and spiked at 100 ng g^{-1} of each individual TOrC (50 ng g^{-1} in the case of individual enantiomers). These were then analysed in triplicate at pre-set time intervals. The moisture content of the soil was adjusted to field conditions (20 %) every few days. During 56 days of incubation a range of fate behaviours was observed. Low removal (<50 %) was observed for carbamazepine, triclocarban, *R/S(±)*-propranolol and *R/S(±)*-fluoxetine (Figure 4). The remaining TOrCs were all removed by >50 %. Most interesting findings were observed for *R/S(±)*-amphetamine which showed stereoselective transformation in soil. To demonstrate, initial concentrations of *S(+)*-amphetamine and *R(-)*-amphetamine following spiking were 52.3 ±1.7 and 54.4 ±1.8 ng g^{-1}, respectively. This corresponds to an EF of 0.49 ±0.01 (Figure 4). Concentrations were reduced to 1.1 ±0.1 and 10.0 ±0.8 ng g^{-1} after 3 days. Here the EF was 0.10 ±0.01 due to the faster rate of transformation of *S(+)*-amphetamine over *R(-)*-amphetamine. This is the first time stereoselective transformation of a drug has been observed in soil. Selective degradation of *S(+)*-amphetamine over *R(-)*-amphetamine has previously been observed in other matrices such as activated sludge [52] and river water [25,52]. This suggests that the metabolic processes in the studied microbial communities are similar. Coupling this observation with the likelihood that digested sludge applied to land contain chiral TOrCs in the non-racemic form [52] and stereoselective toxicity is likely to occur, it is essential to monitor these pollutants in soil at the enantiomeric level. This method can be used to support such studies and develop more accurate environmental risk assessments.

4. Conclusion

The developed method was suitable for the multi-residue determination of 10 achiral and chiral TOrCs at the enantiomeric level simultaneously. High sensitivity was achieved utilizing ASE, SPE and enantioselective LC-MS/MS with MQLs achieved being 0.07-0.91 ng g^{-1}. Application of the method to controlled microcosm studies revealed stereoselective degradation of chiral TOrCs in soils for the first time. This coupled with the likelihood that chiral TOrCs are applied to soils in the non-racemic form, it is essential to monitor TOrCs in amended soils at the enantiomeric level. A greater understanding on the chiral distribution of TOrCs here is needed to underpin exposure driven studies. The new analytical methodology described here will help develop more accurate risk assessment.

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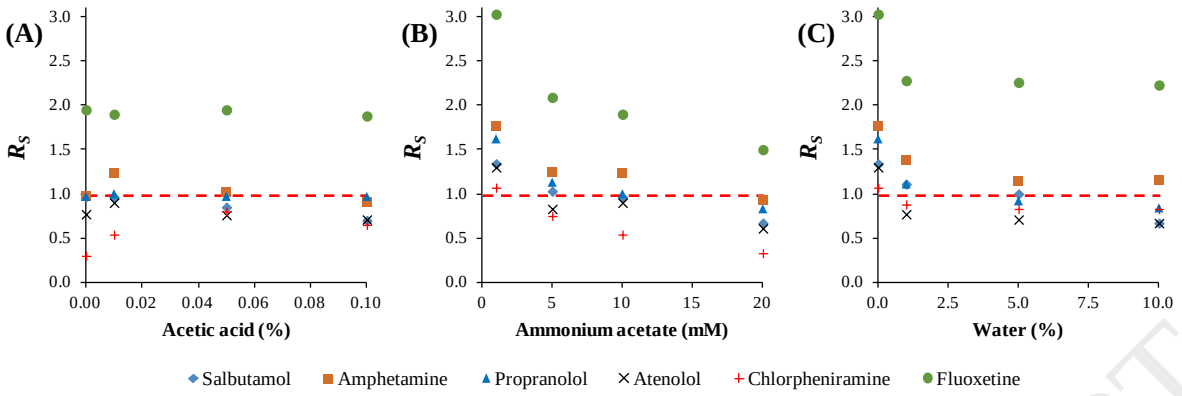

Figure 1. Impact of mobile phase additives/composition on the resolution (R_S) of TOrC enantiomers separated in polar ionic mode

Key: R_S, enantiomer resolution; A – methanol containing 10 mM ammonium acetate and varying % acetic acid; B - methanol containing 0.01 % acetic acid with varying concentrations of ammonium acetate; C - methanol containing 0.01 % acetic acid and 1 mM ammonium acetate and varying % water

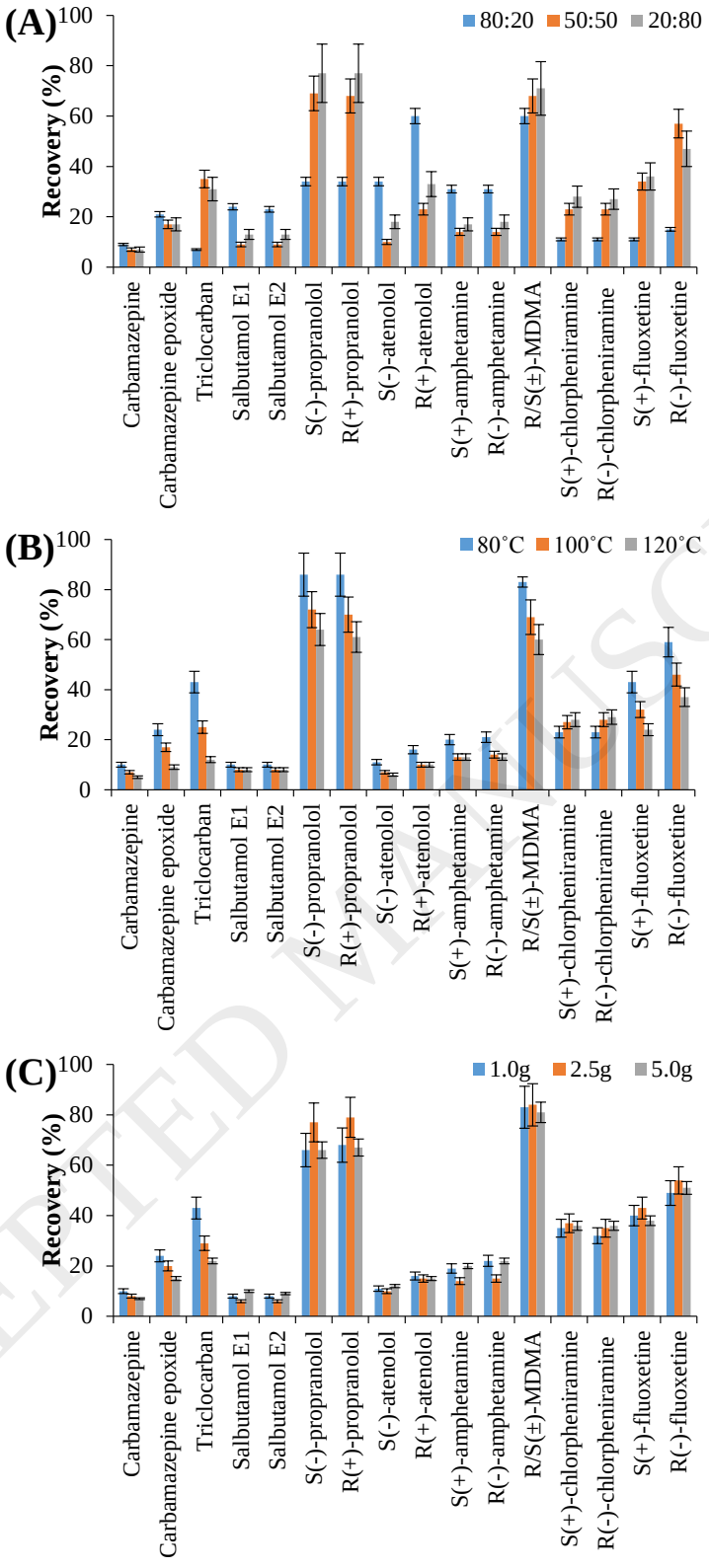

Figure 2. Effect of accelerated solvent extraction variables/sample size on TOrC recovery (% ±standard deviation).

Key: MDMA, 3,4-methylenedioxy-methamphetamine; A – varying extraction solvent compositions (water:methanol) at 100 ˚C using 1 g soil spiked at 100 ng g^{-1} of each TOrC; B – different extraction temperatures using extraction solvent 20:80 water:MeOH using 1 g soil spiked at 100 ng g^{-1} of each

TOrC; C – varying sample masses extracted at 80˚C using 20:80 water:MeOH (all soils spiked with 100 ng of each TOrC).

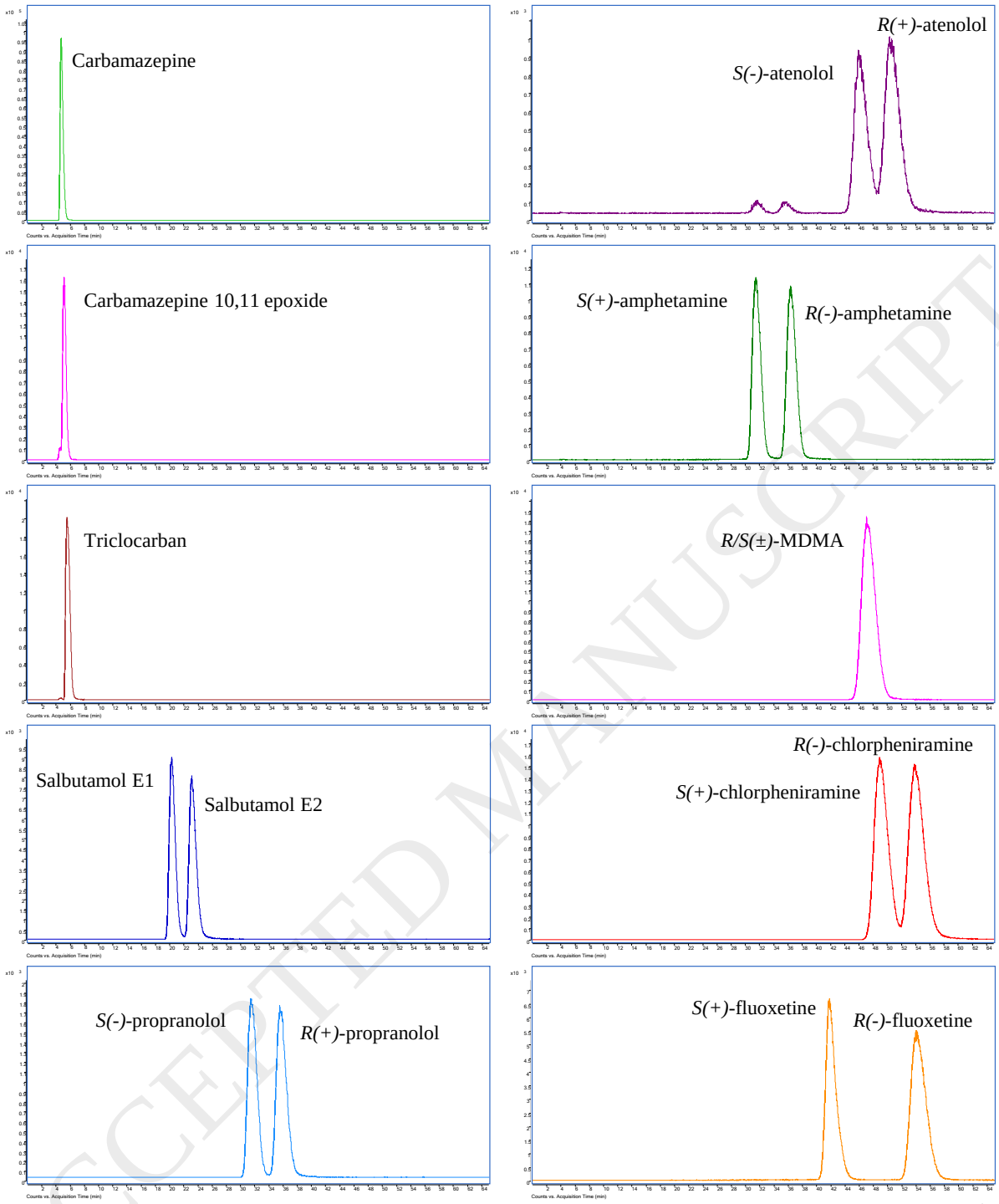

Figure 3. Enantioselective LC-MS/MS MRM chromatograms of TOrCs spiked in soil at 100 ng g^{-1} (50 ng g^{-1} for individual enantiomers) and extracted using the developed ASE-SPE protocol

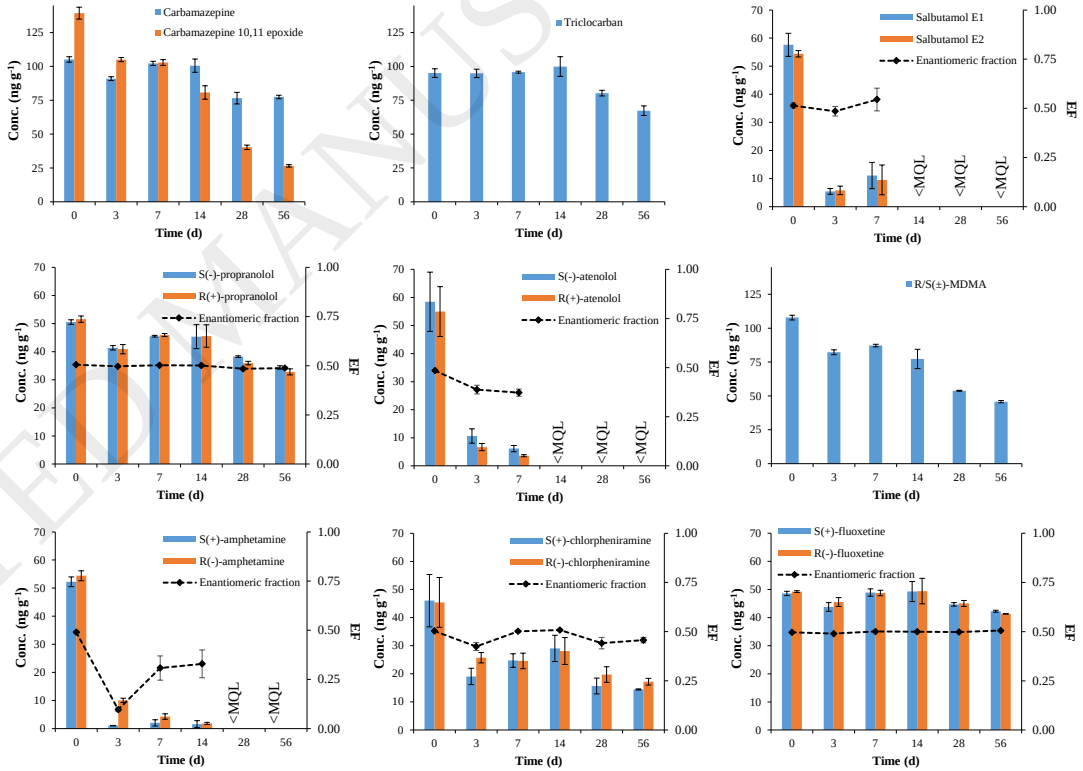

Figure 4. Concentration (and enantiomeric fraction) of chiral and achiral TOrCs in soil microcosms over 56 days
Key: MQL, method quantitation limit; EF, enantiomeric fraction

Table 1. Enantioselective LC-MS/MS instrument performance for studied TOrCs in diluent

TOrC class	TOrC	*Rt* (min)	Linearity		Enantiomer R_S	EF	Intra-day performance[a]		Inter-day performance[a]		$IDL_{S/N}$ (ng mL^{-1})	$IQL_{S/N}$ (ng mL^{-1})
			Range (ng mL^{-1})	r^2			Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)		
Anti-epileptic	Carbamazepine	5.1	0.50-2,000	0.9992	-	-	105.7	1.1	105.2	1.4	0.15	0.50
	Carbamazepine 10,11 epoxide	5.2	0.50-2,000	0.9996	-	-	113.0	1.5	114.3	0.9	0.15	0.50
Anti-bacterial	Triclocarban	5.9	0.50-2,000	0.9994	-	-	88.8	3.7	82.9	8.6	0.15	0.50
Beta-agonist	Salbutamol E1	20.1	0.25-1,000	0.9998	1.3	0.50	106.0	0.8	105.5	1.5	0.08	0.25
	Salbutamol E2	23.8	0.25-1,000	0.9998			105.3	0.6	105.0	1.5	0.08	0.25
Beta-blocker	*S(-)*-propranolol	32.0	0.50-1,000	0.9996	1.6	0.50	103.1	1.1	104.8	2.0	0.15	0.50
	R(+)-propranolol	36.1	0.50-1,000	0.9984			102.8	1.7	104.7	2.0	0.15	0.50
Beta-blocker	*S(-)*-atenolol	46.2	1.00-1,000	0.9996	1.3	0.49	96.3	1.1	93.4	5.6	0.30	1.00
	R(+)-atenolol	51.8	1.00-1,000	0.9996			101.9	1.2	96.7	7.6	0.30	1.00
Stimulant	*S(+)*-amphetamine	32.0	0.25-1,000	0.9998	1.8	0.50	101.1	1.5	101.4	2.2	0.08	0.25
	R(-)-amphetamine	36.9	0.25-1,000	0.9998			101.1	1.0	101.8	2.1	0.08	0.25
Stimulant	*R/S(±)*-MDMA	48.0	0.50-2,000	0.9998	-	-	100.5	0.7	97.7	2.7	0.15	0.50
Anti-histamine	*S(+)*-chlorpheniramine	49.3	0.25-500	0.9935	1.1	0.49	93.0	2.5	99.7	8.6	0.08	0.25
	R(-)-chlorpheniramine	54.8	0.25-500	0.9932			97.0	2.5	101.5	9.8	0.08	0.25
Anti-depressant	*S(+)*-fluoxetine	42.3	0.25-1,000	0.9994	3.0	0.50	102.6	1.9	102.7	2.0	0.08	0.25
	R(-)-fluoxetine	54.9	0.25-1,000	0.9995			102.0	1.7	102.1	2.0	0.08	0.25

Key: TOrC, trace organic contaminant; R_t, retention time; R_S, enantiomer resolution; EF, enantiomeric fraction; IDL, instrument detection limit; IQL, instrument quantitation limit; S/N, signal to noise ratio; MDMA, 3,4-methylenedioxy-methamphetamine

[a]Mean of 3 concentration levels (10, 100 and 1,000 ng mL^{-1} - these levels represent the total TOrC concentration i.e., sum of all enantiomers)

Table 2. Method performance for studied TOrCs extracted from 5g soil by ASE-SPE-enantioselective LC-MS/MS

TOrC class	TOrC	Method recovery ±SD (%)[a]						Signal suppression ±SD (%)	MDL (ng g^{-1})	MQL (ng g^{-1})
		2 ng g^{-1}	EF	10 ng g^{-1}	EF	100 ng g^{-1}	EF			
Anti-epileptic	Carbamazepine	104.6±2.6	-	99.9±5.2	-	90.8±3.8	-	92.8±0.3	0.27	0.91
	Carbamazepine 10,11 epoxide	89.1±5.1	-	81.8±5.9	-	80.3±1.0	-	82.5±0.9	0.13	0.42
Anti-bacterial	Triclocarban	97.3±0.1	-	108.1±6.2	-	96.4±6.7	-	61.0±7.5	0.07	0.25
Beta-agonist	Salbutamol E1	104.7±2.7	0.49±0.02	103.7±6.3	0.50±0.01	98.5±3.4	0.49±0.01	18.6±3.2	0.08	0.26
	Salbutamol E2	108.2±3.5		103.7±4.0		101.7±3.8		24.0±2.6	0.09	0.30
Beta-blocker	*S(-)*-propranolol	104.1±4.4	0.50±0.01	102.9±4.0	0.49±0.01	96.0±4.2	0.50±0.01	23.8±2.4	0.02	0.08
	R(+)-propranolol	104.0±2.6		100.8±4.5		96.2±3.7		22.7±4.3	0.02	0.07
Beta-blocker	*S(-)*-atenolol	95.0±5.3	0.50±0.01	89.9±2.7	0.53±0.02	80.1±0.4	0.54±0.02	30.9±0.8	0.24	0.81
	R(+)-atenolol	93.7±15.6		102.1±4.9		93.6±10.5		13.3±1.2	0.21	0.69
Stimulant	*S(+)*-amphetamine	121.4±8.9	0.50±0.02	119.3±7.6	0.49±0.01	109.3±6.1	0.50±0.01	23.7±2.0	0.05	0.17
	R(-)-amphetamine	120.2±7.4		121.9±6.3		110.4±4.6		21.3±1.7	0.04	0.15
Stimulant	*R/S(±)*-MDMA	102.0±3.5	-	102.1±6.2	-	100.7±4.2	-	12.8±1.5	0.02	0.07
Anti-histamine	*S(+)*-chlorpheniramine	118.1±0.1	0.49±0.01	117.0±14.2	0.50±0.01	76.1±9.4	0.50±0.01	3.1±2.2	0.02	0.07
	R(-)-chlorpheniramine	121.0±2.5		117.2±14.0		76.2±7.9		6.9±1.7	0.02	0.07
Anti-depressant	*S(+)*-fluoxetine	101.1±14.3	0.50±0.01	102.9±3.3	0.50±0.01	91.9±6.9	0.50±0.01	41.6±1.1	0.03	0.10
	R(-)-fluoxetine	99.4±9.4		101.0±4.0		90.6±6.2		26.2±1.9	0.02	0.07

Key: TOrC, trace organic contaminant; EF, enantiomeric fraction; SD, standard deviation; MDL, method detection limit; MQL, method quantitation limits; MDMA, 3,4-methylenedioxy-methamphetamine

[a]Spike levels represent the total TOrC concentration i.e., sum of all enantiomers

Supporting information

Multi-residue analysis of chiral and achiral trace organic contaminants in soil by accelerated solvent extraction and enantioselective liquid chromatography tandem-mass spectrometry

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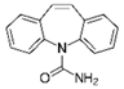
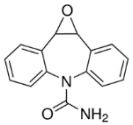
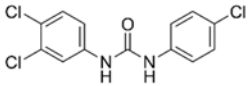
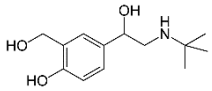
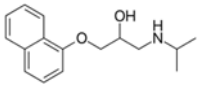
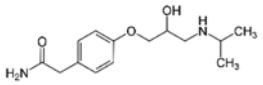
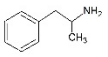
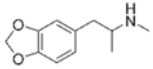
The supporting information contains four tables with details of the properties of studied TOrCs, MS/MS parameters, enantioresolution of chiral TOrCs screened on Chirobiotic V and V2 columns in reverse phase, polar organic and polar ionic modes, and recovery of TOrCs from water using Oasis HLB SPE cartridges.

Table S1. Chemical properties of studied TOrCs [1]

TOrC	Chemical structure	Molecular weight (g mol^{-1})	Water solubility (mg L^{-1})	Log K_{OW}	Log K_{OC}	p*Ka*
Carbamazepine		236.28	17.7	2.25	1.08E-10	13.94 (acidic) -0.49 (basic)
Carbamazepine 10,11 epoxide		252.27	-	-	-	13.91 (acidic) -0.50 (basic)
Triclocarban		315.58	2.4E-3	4.50	-	11.42 (acidic) -4.60 (basic)
Salbutamol		239.31	1.4E4	0.40	-	10.12 (acidic) 9.40 (basic)
Propranolol		259.35	228.0	2.60	7.98E-13	13.84 (acidic) 9.50 (basic)
Atenolol		266.34	685.2	-0.03	1.37E-18	13.88 (acidic) 9.43 (basic)
Amphetamine		135.21	2.8E4	1.76	1.08E-6	9.94 (basic)
MDMA		193.25	7.03E3	2.28	2.75E-9	10.32 (basic)

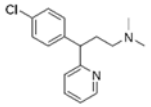
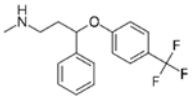
Chlorpheniramine		274.79	5.5E3	3.67	-	9.47 (basic)
Fluoxetine		309.33	60.3	4.65	8.90E-8	10.05 (basic)

Table S2. MS/MS transitions, ion ratio and corresponding internal standards

TOrC class	TOrC	MRM 1	Fragmentor (V)	Collision energy (eV)	MRM 2	Fragmentor (V)	Collision energy (eV)	Ion ratio	Corresponding internal standard
Anti-epileptic	Carbamazepine	236.8>193.9	130	20	236.8>178.9	130	40	6	Carbamazepine-D10
	Carbamazepine 10,11 epoxide	252.8>179.9	90	30	252.8>210.0	90	10	2.7	Carbamazepine 10,11 epoxide-D10
Anti-bacterial	Triclocarban	312.5>159.7	110	10	312.5>125.6	110	20	19.6	Triclocarban-D3
Beta-agonist	Salbutamol E1 Salbutamol E2	239.9>147.9	90	10	239.9>165.9	90	10	3.6	Salbutamol-D3 E1 Salbutamol-D3 E2
Beta-blocker	*S(-)*-propranolol *R(+)*-propranolol	259.9>115.9	110	10	259.9>182.9	110	10	1.4	*S(-)*-Propanolol-D7 *R(+)*-Propanolol-D7
Beta-blocker	*S(-)*-atenolol *R(+)*-atenolol	266.9>144.9	110	30	266.9>189.9	110	20	2	*R(-)*-Amphetamine-D11 *R(-)*-Amphetamine-D11
Stimulant	*S(+)*-amphetamine *R(-)*-amphetamine	135.8>90.9	70	20	135.8>65.0	70	40	3.2	*S(+)*-Amphetamine-D11 *R(-)*-Amphetamine-D11
Stimulant	*R/S(±)*-MDMA	193.9>162.8	90	10	193.9>104.8	90	30	3	*R/S(±)*-MDMA-D5
Anti-histamine	*S(+)*-chlorpheniramine *R(-)*-chlorpheniramine	274.9>229.9	90	10	274.9>166.8	90	40	2.6	*R(-)*-Fluoxetine-D6 *R(-)*-Fluoxetine-D6
Anti-depressant	*S(+)*-fluoxetine *R(-)*-fluoxetine	309.8>44.0	90	10	309.8>147.7	90	5	20.9	*S(+)*-Fluoxetine-D6 *R(-)*-Fluoxetine-D6
Deuterated internal standards	Carbamazepine-D10	246.9>204.1	130	20	-	-	-	-	-
	Carbamazepine 10,11 epoxide-D10	263.0>189.9	90	30	-	-	-	-	-
	Triclocarban-D3	318.9>161.9	110	10	-	-	-	-	-
	Salbutamol-D3 E1 Salbutamol-D3 E2	243.0>150.9	90	10	-	-	-	-	-
	S(+)-amphetamine-D11 *R(-)*-amphetamine-D11	147.0>98.0	70	20	-	-	-	-	-
	S(-)-propranolol-D7 *R(+)*-propranolol-D7	267.0>188.8	110	15	-	-	-	-	-
	R/S(±)-MDMA-D5	199.0>164.9	90	10	-	-	-	-	-
	S(+)-fluoxetine-D6 *R(-)*-fluoxetine-D6	316.0>154.0	90	2	-	-	-	-	-

Key: TOrC, trace organic contaminant; MRM, multiple reaction monitoring; MDMA, 3,4-methylenedioxy-methamphetamine

Table S3. Enantioresolution of chiral TOrCs using reverse phase, polar ionic and polar organic mobile phases with Chirobiotic V® and Chirobiotic V2® columns

TORC class	TORC	Reverse phase[a]		Polar ionic[b]		Polar organic[c]	
		V	V2	V	V2	V	V2
Beta-agonist	Salbutamol	-	-	0.9	0.7	ND	ND
Beta-blocker	Propranolol	-	-	1.5	1.0	ND	ND
Beta-blocker	Atenolol	-	-	1.1	0.7	ND	ND
Stimulant	Amphetamine	-	-	0.3	0.9	ND	ND
Stimulant	MDMA	-	-	-	-	ND	ND
Anti-histamine	Chlorpheniramine	-	-	-	0.7	ND	ND
Anti-depressant	Fluoxetine	-	0.7	0.9	1.9	ND	ND

Key: TOrC, trace organic contaminant; MDMA, 3,4-methylenedioxy-methamphetamine; -, no separation; ND, not detected (not ionised)

[a]70:30 10mM ammonium acetate at pH 4: acetonitrile, [b]10mM ammonium acetate in methanol + 0.1% acetic acid, [c]Ethanol

Table S4. Recovery of studied TOrCs from water spiked at 1 μg L^{-1} using Oasis HLB SPE

TOrC class	**TOrC**	**Recovery (%)**
Anti-epileptic	Carbamazepine	72.9±0.5
	Carbamazepine 10,11 epoxide	86.7±1.9
Anti-bacterial	Triclocarban	93.6±5.6
Beta-agonist	Salbutamol E1	91.3±5.2
	Salbutamol E2	93.1±4.2
Beta-blocker	*S(-)*-propranolol	94.1±4.6
	R(+)-propranolol	95.1±5.8
Beta-blocker	*S(-)*-atenolol	93.6±4.2
	R(+)-atenolol	95.6±4.8
Stimulant	*S(+)*-amphetamine	66.9±8.8
	R(-)-amphetamine	68.1±8.6
Stimulant	*R/S(±)*-MDMA	85.0±6.1
Anti-histamine	*S(+)*-chlorpheniramine	83.0±7.6
	R(-)-chlorpheniramine	85.8±7.4
Anti-depressant	*S(+)*-fluoxetine	83.8±4.7
	R(-)-fluoxetine	84.1±5.4

Key: TOrC, trace organic contaminant; MDMA, 3,4-methylenedioxy-methamphetamine

References

[1] US EPA. [2015]. Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.11]. United States Environmental Protection Agency, Washington, DC, USA.