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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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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.

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

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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 1011 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 × 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 1500 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 × 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 utilized 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 4000 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

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