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Enantioselective LC-MS/MS determination of antidepressants, β-blockers and metabolites in agricultural soil, compost and digested sewage sludge

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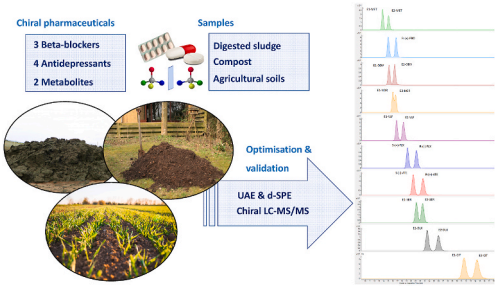
HIGHLIGHTS

- Enantioselective determination of 3 β-blockers, 5 antidepressants and 2 metabolites.
- Ultrasound-assisted extraction, extract clean-up and chiral-LC-MS/MS optimisation.
- First enantioselective method validated for soil, compost and digested sludge.
- Good enantioresolution, good recoveries and low quantification limits achieved.
- Application to real samples revealed enantiomeric enrichment.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, an analytical method was optimised and validated for the simultaneous extraction and enantioselective determination of chiral β-blockers, antidepressants and two of their metabolites in agricultural soils, compost and digested sludge. Sample treatment was based on ultrasound-assisted extraction and extract clean-up by dispersive solid-phase extraction. Analytical determination was carried out by liquid chromatography-tandem mass spectrometry using a chiral column. Enantiomeric resolutions were in the range from 0.71 to 1.36. Accuracy was in the range from 85 to 127% and precision, expressed as relative standard deviation, was lower than 17% for all the compounds. Method quantification limits were below 1.21–52.9 ng g^{-1} dry weight (dw) in soil, 0.76–35.8 ng g^{-1} dw in compost and 13.6–90.3 ng g^{-1} dw in digested sludge. Application to real samples revealed enantiomeric enrichment in the range especially in compost and digested sludge (enantiomeric fractions up to 1).

1. Introduction

More than half of pharmaceuticals in use are chiral compounds [1,2]. Their enantiomers are optical isomers with identical physical-chemical properties, except light rotation, that in a chiral environment can exhibit different chemical, physical and biological properties [3]. They can have different pharmacodynamics and pharmacokinetics [3–6], biotransformation and bioaccumulation behaviour [7,8] and effects in both target and non-target organisms [9,10]. Most of the chiral pharmaceuticals are administered as racemates but their enantiomeric fraction (EF) can be altered by metabolism if enzymes controlling the metabolic route exhibit more activity for one enantiomer than for the other [3]. Modifications of the enantiomeric fraction can also occur in the environment by selective sorption and microbial degradation [11].

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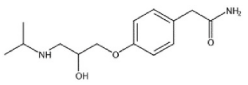
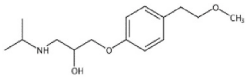
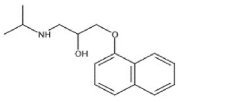
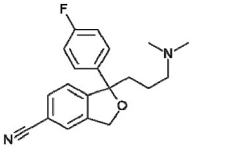
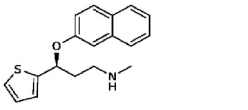
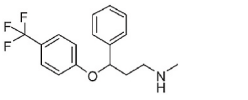
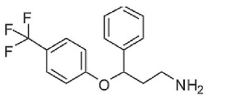
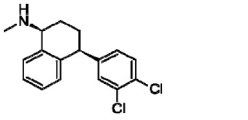
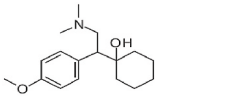
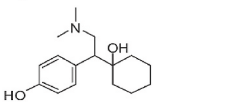
This behaviour has also been reported in the aquatic environment [12–15], in wastewater [14–16], in freshwater sediments [17], in soils [18], and in sludge [13,19]. These alterations are mainly due to microbial degradation processes conditioned not only by the compound properties but also by environmental factors influencing microbial communities [20]. A proper environmental risk assessment of chiral pharmaceuticals should involve their enantiomeric determination because enantiomers can exhibit different ecotoxicity [10,11]. Nevertheless, to the date, reported studies about the occurrence and environmental risks of chiral pharmaceuticals in sewage sludge and agricultural soils [21,22], and their uptake, translocation, accumulation and metabolism in plants [23,24], have not consider their enantiomeric composition. This fact can be explained by the challenge to overcome when developing an analytical method for multi-residue enantiomeric determination of chiral pollutants. It is even more difficult when such methods are optimised for their determination in complex solid matrices such as sewage sludge and soil [10]. As enantiomeric separation is commonly carried out by liquid chromatography in isocratic elution mode, mobile phase parameters should be properly optimised to obtain good separation of all the pairs of enantiomers in a run-time as shorter as possible and with a mobile phase composition compatible with the detection system. Because of that, most of the methods developed for the determination of chiral pharmaceuticals in the environment ignore their enantiomeric determination [25].

Selective serotonin reuptake inhibitors (SSRIs) and serotonin–norepinephrine reuptake inhibitors (SNRIs), mainly used as antidepressants [26], and β-blockers, used for the treatment of angina pectoris, hypertension, glaucoma, arrhythmias and migraine headaches [3], includes chiral pharmaceuticals of particular concern due to their long-term use, as they are used to treat chronic diseases, and their increasing consumption in the recent years [3,27]. The scarce methods reported for their determination in environmental samples have been mainly developed for their determination in liquid samples [28] such as surface water [12,29–31] and wastewater [31,32]. Pressurised liquid

Table 1
Physical-chemical properties of the target compounds.

Therapeutic class	Compound/Abbreviation	Chemical structure	Molecular weight (g mol^{-1})	pK_a	K_{ow}	Water solubility (g L^{-1})
β-Blockers	**Atenolol (ATE)**		266.34	9.43 ± 0.10[a] 13.88 ± 0.20[a]	0.335 ± 0.279[a]	0.43[b]
	Metoprolol (MET)		267.36	9.43 ± 0.10[a] 13.89 ± 0.20[a]	1.632 ± 0.263[a]	0.40[b]
	Propranolol (PRO)		259.34	9.50 ± 0.30[a] 13.85 ± 0.20[a]	2.900 ± 0.247[a]	0.079[b]
Antidepressants	**Citalopram (CIT)**		324.39	9.57 ± 0.28[c]	3.74[c]	0.0059[b]
	Duloxetine (DLX)		297.42	9.7[d]	4.68[d]	0.003[b]
	Fluoxetine (FLX)		309.33	10.05 ± 0.10[a]	3.930 ± 0.434[a]	0.0017[b]
	Norfluoxetine (NOR)		295.30	9.05 ± 0.10[a]	0.97 (pH 2)[a] 2.05 (pH 7)[a] 4.06 (pH 11)[a]	0.0092[b]
	Sertraline (SER)		305.07	9.47 ± 0.40[c]	5.29[c]	0.00014[b]
	Venlafaxine (VLF)		277.20	9.26 ± 0.28[a] 14.80 ± 0.20[a]	2.475 ± 0.268[a]	0.23[b]
	O-desmethylvenlafaxine (ODV)		263.18	9.33 ± 0.28[a] 10.04 ± 0.26[a]	2.72[a]	1.4[b]

Parent compounds are marked in bold.
[a] Ma et al. [12].
[b] Human Metaboloma Database (https://hmdb.ca).
[c] Petrie et al. [16].
[d] Hazardous Substances Data Bank (https://pubchem.ncbi.nlm.nih.gov/source/hsdb/7368).

extraction (PLE) followed by solid-phase extraction (SPE) clean-up has been proposed for their determination in freshwater sediments [17] and soils [2,18]. Microwave assisted extraction (MAE) has been applied for their extraction from digested sludge [32]; matrix solid-phase dispersion (MSPD) has been applied for their extraction from primary and secondary sludge [33], and Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method for their extraction from solid phases of influent and effluent wastewater [19]. In all the above-mentioned methods, clean-up by SPE was applied after extraction. Analytical determination was carried out by liquid chromatography-tandem mass spectrometry (LC-MS/MS) [2,17,18,32] or supercritical fluid chromatography time-of-flight mass spectrometry (SFC-QTOF-MS) [33] using a chiral column.

The aim of this work was to develop a selective, sensitive and easy-to-perform enantioselective LC-MS/MS analytical method for the simultaneous determination of environmentally relevant chiral β-blockers (atenolol (ATE), metoprolol (MET) and propranolol (PRO)), SSRI (citalopram (CIT), fluoxetine (FLU) and sertraline (SER)) and SNRI (duloxetine (DLX) and venlafaxine (VLF)) antidepressants and metabolites of FLX (norfluoxetine (NOR)) and of VLF (O-desmethylvenlafaxine (ODV)) in soils, compost and digested sludge. Chemical structures and physical-chemical properties of target compounds can be seen in Table 1. Ultrasound-assisted extraction (UAE) followed by dispersive solid-phase extraction (d-SPE) have been selected for sample extraction and extract clean-up, respectively, as they are low-cost and easy-to-perform techniques. Analytical determination was carried out by liquid-chromatography-tandem mass spectrometry (LC-MS/MS) by using a chiral column. The method was optimised and validated for their determination in soil, compost and digested sludge. To our knowledge, this is the first analytical method: i) for the enantiomeric determination of a high number of antidepressants and some of their metabolites in environmental solid samples; ii) for the enantiomeric determination of DLX in environmental solid samples; iii) validated for soil, compost and digested sludge samples.

2. Experimental

2.1. Chemicals and reagents

Analytical standards of racemic ATE, MET, PRO, CIT, FLX, NOR, SER, VLF and ODV, as well as single enantiomer standards of *R*-(+)-ATE, *S*-DLX, *R*-DLX, *S*-(−)-PRO and *R*-(−)-FLX were purchased from Sigma-Aldrich (Steinheim, Germany). A deuterated analogue of ATE-d_7 supplied from Dr. Enherstorfer (Aughsburg, Germany) was tested as internal standard. Individual stock standard solutions were prepared in methanol (MeOH) at 500 μg mL^{-1} (each enantiomer) and kept at −18 °C in glass vials. Working solutions were prepared by dilution of stock solutions in MeOH. Acetone was supplied by Romil (Barcelona, Spain). LC-MS-grade acetonitrile (ACN), methanol (MeOH) and water were purchased from Honeywell (Seelze, Germany). Analytical-grade formic acid (98%, v/v) and glacial acetic acid were provided by Panreac (Barcelona, Spain). Ammonium formate and Florisil® were provided by Sigma-Aldrich (Madrid, Spain). Primary-secondary amine (PSA) and C18 were supplied from Scharlab (Barcelona, Spain).

2.2. Liquid chromatography-tandem mass spectrometry

Analytical determination was carried out on an Agilent 1290 Infinity II liquid chromatographic system (Agilent, USA) coupled to a 6495-triple quadrupole (QqQ) mass spectrometer (MS) equipped with an electrospray ionisation source (ESI). Enantiomeric separation was performed in a Chirobiotic V chiral column (250 mm × 2.1 mm i. d., 5 μm) (Sigma-Aldrich, Steinheim, Germany) thermostated at 35 °C and protected by an Astec Chirobiotic V chiral HPLC guard column (20 mm × 4 mm i. d., 5 μm) (Sigma-Aldrich, Steinheim, Germany). Vancomycin was the chiral selector in both analytical and guard columns. Mobile phase consisted of 10 mM ammonium acetate (adjusted to pH 4 using formic acid) and MeOH (2:98; v/v). Flow rate was set at 0.4 mL min^{-1}. The injection volume was 5 μL. MS/MS analysis was performed in positive ionisation mode with the following conditions: capillary voltage, 3000 V; drying gas flow rate, 14 L min^{-1}; drying gas temperature, 200 °C; sheath gas flow rate, 11 L min^{-1}, sheath gas temperature, 250 °C; and nebulizer pressure, 20 psi. The mass spectrometer was operated in multiple reaction monitoring mode (MRM). Two MRM transitions were selected for each compound. The most abundant transition was used for quantification and the other for confirmation. Optimised LC-MS/MS parameters for each compound are given in Supplementary Material Table S1. Instrument control and data acquisition were carried out with MassHunter software (Agilent, USA).

2.3. Sample collection and treatment

Soil, compost, and digested sludge were used for method validation. Digested sludge was collected from an urban WWTP based on anaerobic digestion. Soil samples were alluvial-type and were sampled from an agricultural site. Compost was collected from a composting plant where anaerobically-digested and dehydrated sludge from urban wastewater treatment plants were treated in thermally-controlled dynamic batteries with aeration facilitated by turning. After collection, samples were freeze-dried in a Telstar Cryodos-50 lyophiliser, pulverised and homogenised in a mortar, sieved (particle size <2 mm) and kept at −18 °C in glass vials until analysis. Pre-treated solid samples (0.5 g dry weight (dw)) were weighed into glass centrifuge tubes. After that, 6 mL of ACN containing formic acid (2%, v/v) were added to the tubes. The tubes were vortex-mixed, sonicated for 10 min in an ultrasonic bath and centrifuged at 4600×*g* for 10 min. The liquid phases were transferred to clean tubes. The solid phase was subjected to other two extractions. The liquid phases from the three extractions were combined into a clean 50 mL Falcon tube where C18 (0.8 g) was added for extract clean-up. The tubes were vigorously shaken for 1 min and centrifuged for 20 min at 4600×*g*. The liquid phase was transferred to another clean tube and was evaporated to dryness under a gentle nitrogen stream in an XcelVap® evaporation and concentration system (Biotage, UK). Dried extracts were reconstituted in water:MeOH solution (1:1, v/v), filtered through a 0.2 μm cellulose syringe filter and collected into an automatic injector vial for LC-MS/MS determination. Reconstitution volumes were 1 mL for soil and compost extracts and 2 mL for digested sludge extracts.

3. Results and discussion

3.1. LC-MS/MS optimisation

LC–MS/MS parameters were optimised by direct infusion into the mass spectrometer of individual aqueous standard solutions at 10 μg mL^{-1}. Experiments were carried out with a mobile phase composed by 95% of MeOH and 5% of the aqueous solution to optimise. The aqueous solution was optimised in terms of the type and concentration of an ammonium additive and pH. Ammonium formate and ammonium acetate were tested as ammonium additives. Each one was tested at 2, 5 and 10 mM. The highest ionisations were obtained when 10 mM ammonium acetate solution was used. Then, the influence of the acidification of such solution to pH 3, 4, 5, 6 and 7 was evaluated. The best results were obtained when it was acidified to pH 4. Finally, MeOH was replaced by ACN at the same 95:5, v/v proportion. It provided poorer signals. Therefore, initial mobile phase conditions were set at isocratic elution with 95% of MeOH and 5% of ammonium formate pH adjusted to 4 and MeOH in 95:5, v/v proportion. Optimisation of electrospray ionisation was carried out in both positive and negative modes. For all the compounds, the best results were obtained in positive mode. The $[M + H]^+$ ion was selected as precursor ion for all the analytes. The two most abundant product ions were monitored for each compound. The most abundant transition was used for quantification and the other for

confirmation. The optimised LC-MS/MS parameters can be seen in Table S1.

After the preliminary mobile phase optimisation by direct infusion, two chiral columns were tested: Chiralpak® AGP column (100 mm × 2.1 mm i. d., 5 μm), purchased from Daicel, and Chirobiotic V column (250 mm × 2.1 mm i. d., 5 μm), purchased from Sigma-Aldrich. Preliminary assays showed that Chirobiotic V column allowed successful enantioresolution (Rs) of the target compounds by isocratic elution with 10 mM ammonium acetate (pH 4, formic acid adjusted) and MeOH (5:95, v/v) at a flow rate of 0.3 mL min^{-1}. Therefore, these chromatographic conditions were used as starting point for the improvement of the enantioseparation. The influence of flow rate (0.3, 0.4, 0.5 and 0.6 mL min^{-1}) and proportion of the organic solvent in mobile phase (90%, 95% and 98%, v/v) in Rs values were tested. As expected, the retention times were shorter, and the peak were narrower, as the flow rate was increased but Rs values were worsened at flow rates higher than 0.4 mL min^{-1}. Consequently, flow rate was set at 0.4 mL min^{-1}, since good enantiomeric separation in short run time was obtained. The increase of MeOH content in the mobile phase, resulted in an improvement of Rs but also in an increase of run time. Because of that, a mobile phase composed of an aqueous solution of 10 mM ammonium acetate (pH 4 formic acid adjusted):MeOH (2:98, v/v) at a flow rate of 0.4 mL min^{-1} was selected for chromatographic elution. MRM chromatograms of a standard solution at 25 μg mL^{-1} (each enantiomer) can be seen in Fig. 1.

3.2. Sample treatment optimisation

Method was optimised using composted sludge (0.5 g dw) spiked at 250 ng g^{-1} dw (each enantiomer). Spike procedure was carried out by the addition of 250 μL of a standard solution at 500 ng mL^{-1} (each

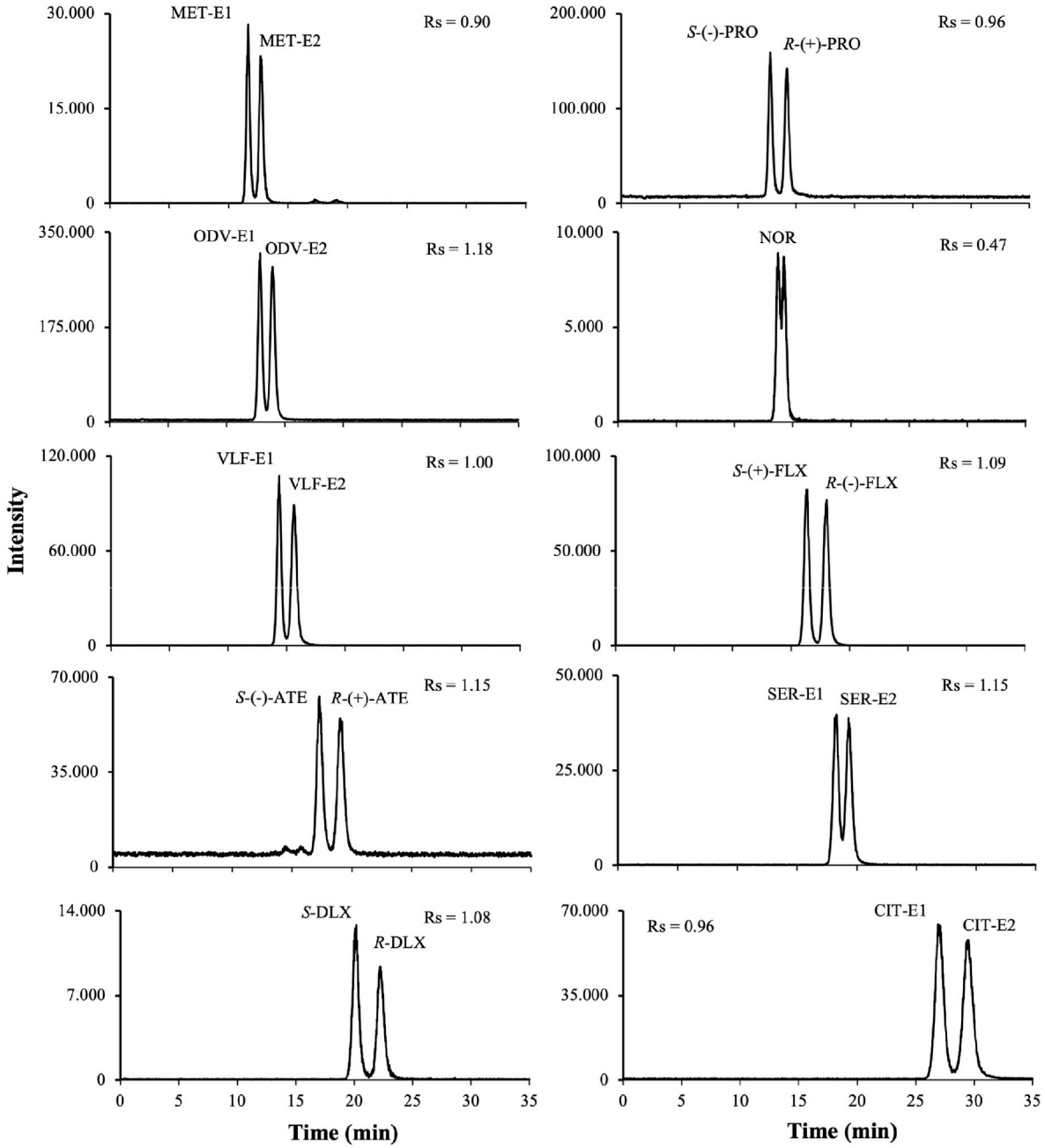


Fig. 1. LC-MS/MS MRM chromatograms of a standard solution at 25 μg mL^{-1} (each enantiomer). E1 and E2 correspond to first and second eluting enantiomers, respectively.

enantiomer). That volume of standard solution (250 μL) allowed to wet the whole sample mass (0.5 g dw). Spiked samples were homogenised by agitation in a vortex-mixer for 1 min and kept in the dark for 24 h for equilibration and solvent evaporation. Non-spiked samples were also processed in each batch of experiments for blank correction.

3.2.1. Optimisation of the extraction solvent

Spiked and non-spiked samples were transferred to glass centrifuge tubes and were extracted in an ultrasonic bath for 10 min. Extraction procedure was repeated three times for each tested solvent (acetone, ACN and MeOH). After each extraction step, tubes were centrifuged at 4600×*g* for 10 min. Extracts were combined in the same tube and subjected to d-SPE clean-up by addition of 0.4 g of C18. Experiments were carried out in triplicate for each tested solvent. Extraction absolute recoveries were evaluated by comparing peak areas from spiked samples after blank correction with those from spiked extract. For most of the compounds, the best extraction recoveries were obtained when ACN was used as extraction solvent whereas the worst results were obtained with MeOH as some of the enantiomers could not be properly separated. This fact could be due to the extraction of interfering compounds that worsened the enantioseparation. Then, formic acid or glacial acetic acid were added to ACN at 1%, 2% and 5% proportions. The highest extraction recoveries for both antidepressants and β-blockers were obtained with ACN acidified with formic acid at 5% v/v (Fig. 2). Nevertheless, Rs of some compounds decreased with the increase of formic acid content. Because of that, ACN containing formic acid at 2% v/v was selected as extraction solvent.

3.2.2. Optimisation of the type of d-SPE sorbent

Clean-up optimisation was focused on the selection of the most appropriate sorbent or mixture of sorbents for removing interfering compounds without removing target compounds. Clean-up sorbents tested were a weak anion exchanger sorbent (PSA), a reverse phase sorbent (C18), and a normal phase sorbent (Florisil®). PSA sorbent is indicated to remove polar pigments, sugars, fatty acids and organic acids. C18 is useful for eliminating apolar to moderately polar compounds, such as lipophilic compounds. Florisil® is suitable for removing polar compounds. For sorbent optimisation, samples (0.5 g dw) were extracted two times with 4 mL of ACN containing formic acid at 2% v/v in an ultrasonic bath for 10 min, each extraction. After each extraction, tubes where centrifuged at 4600×*g* for 10 min. The liquid phases were combined and spiked at 250 ng mL^{-1} (each enantiomer). Clean-up efficacy was evaluated by comparing signals from spiked sample extracts with signals from a standard solution at the same concentration. To better evaluate the influence of each variable and their interactions, a Box-Behnken design (BBD) was applied for optimising the type and amount of d-SPE sorbent. In BBD, the number of experiments (N) required for the optimisation is defined by the equation: $N = 2k(k-1) + C_0$. Three variables (k) were evaluated (C18, PSA and Florisil®) were evaluated at three concentration levels (0, 0.4g and 0.8g). The number of central points (C_0) was fixed at three. Therefore, 15 experiments were required for optimisation. BBD matrix indicating values for each variable in each experiment can be seen in Table S2. Experiments were randomly performed to reduce the influence of uncontrolled variables. Poor signals and Rs values were obtained in the experiments where Florisil® was used. This fact could be due to the loss of the compounds by sorption onto Florisil® or to a poor removal of interfering compounds causing signal suppression. The best average results were obtained in experiments using just C18 (data in Table S2). Therefore, C18 was selected as d-SPE sorbent.

3.2.3. Optimisation of C18 amount, extraction solvent volume and number of extraction cycles

Another Box-Behnken design (BBD) was applied for optimisation of C18 amount, extraction solvent volume and number of extraction cycles. Each variable was evaluated at three levels: C18 amount: 0.4, 0.6 and 0.8 g; extraction solvent volume: 3, 4.5 and 6 mL; number of extraction cycles: 1, 2 and 3. The number of central points was fixed at three resulting in a total of 15 experiments that were randomly performed as can be seen in Table S3. To better evaluate the influence of each variable and their interactions, response surface plots corresponding to overall method recovery were plotted. Overall method recovery corresponds to mean recovery for all target compounds. In Fig. 3 it can be seen the response surface plots corresponding to overall recovery versus a) solvent volume and number of extraction cycles; b) C18 amount and number of extraction cycles and c) C18 amount and solvent volume. The number of extraction cycles was the most significant parameter affecting overall recovery (Fig. 3a and b), followed by solvent volume (Fig. 3a and c). According to the results of BBD experiments, 0.4 g of C18, 6 mL of extraction solvent (ACN containing formic acid at 2% v/v) and 3 extraction cycles were selected as the best values for such variables.

3.2.4. Optimisation of extract reconstitution volume

Extract reconstitution volumes should be as low as possible for a higher sample concentration factor resulting in lower MDL and MQL values. Nevertheless, at lower reconstitution volumes poorer Rs values were obtained. This fact could be due to the higher concentration of coeluting compounds making difficult enantiomer separation. Reconstitution solvent volumes tested were 0.25 mL, 0.5 mL, 1 mL and 2 mL. The best extract reconstitution volume was fixed at the lowest volume not affecting enantiomeric separation. For soil and compost extracts, reconstitution volume was fixed at 1 mL. However, digested sludge extracts required a higher reconstitution volume (2 mL) for a proper enantiomeric separation.

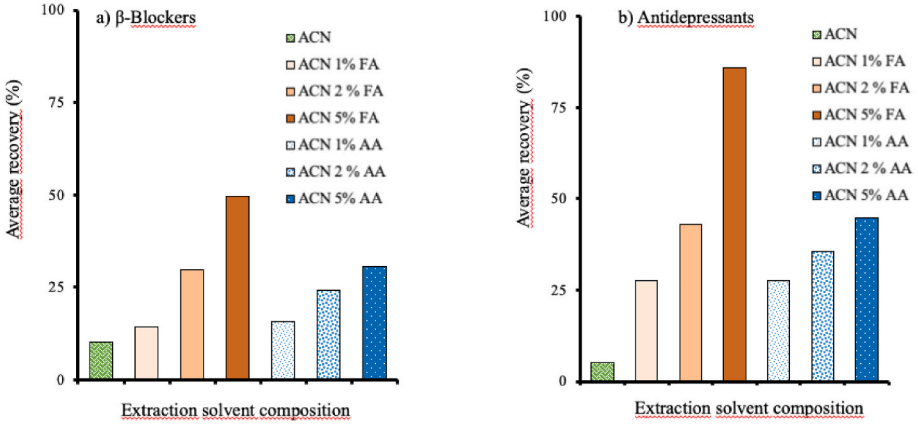


Fig. 2. Influence of the extraction solvent composition on average recovery (%) of a) β-blockers and b) antidepressants. FA: formic acid, AA: acetic acid.

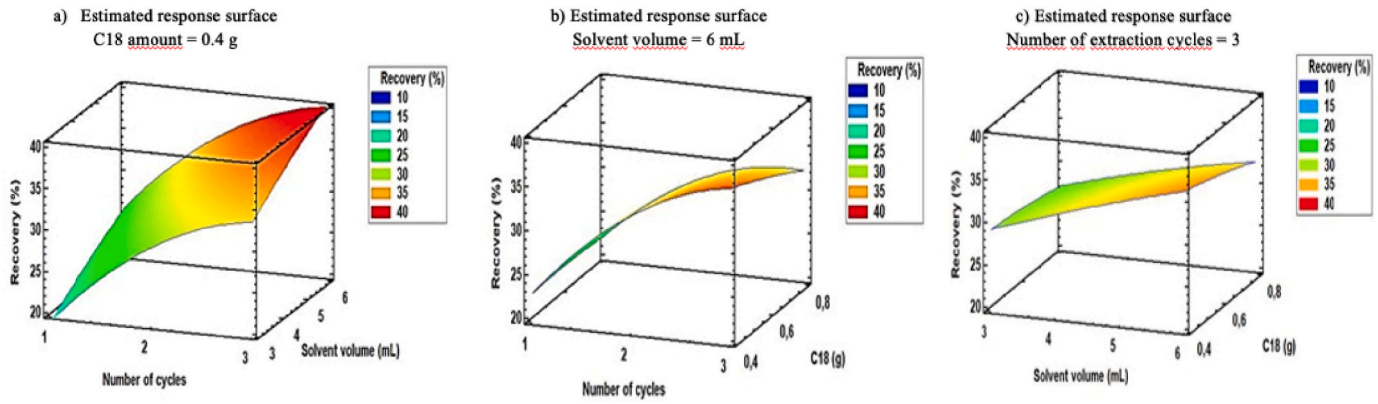


Fig. 3. Response surface plots corresponding to overall recovery versus a) solvent volume and number of extraction cycles; b) C18 amount and number of extraction cycles and c) C18 amount and solvent volume.

3.3. Method validation

Method was validated for soil, compost, and digested sludge in terms of linearity, method detection limits (MDL), method quantification limits (MQL), recovery, accuracy and precision. Previously, ME was calculated as the percentage of signal suppression or enhancement of target compounds in spiked extract when compared with their signals in pure solvent. In Table 2, it can be seen that most of the compounds were affected by signal suppression (negative values) or enhancement (positive values) lower than 25% when analysed in soil and compost samples. Nevertheless, 50% of enantiomers were affected by signal suppression values in the range from −40% to −79.6% in digested sludge. Higher matrix effects have been reported when PLE and SPE clean-up was applied for the chiral LC-MS/MS determination of ATE, PRO and FLU in soil samples (from 13.3 to 41.5%) [2] and when MSPD followed by SPE clean-up was applied for the chiral SFC-QTOF-MS determination of PRO and VLX (from 72% to 94%) in sludge [33]. Matrix-matched calibration curves were used for quantification. Eight-point calibration curves were prepared in the range from 5 to 1000 ng g^{-1} dw by spiking soil, compost and digested sludge extracts in triplicate. Correlation coefficients (R^2) of the calibration curves were higher than 0.99 for most of the enantiomers (Table 2).

The addition of internal standard to sample extract did not improve matrix effect correction. Therefore, its use was discarded. In spite of the complexity of the sample matrices evaluated, good Rs values were obtained for all the compounds, except for NOR enantiomers which could not be separated in any of the matrices and for CIT in compost (Table 2). Rs values were calculated using the equation: $Rs = \frac{2\,(RT_2 - RT_1)}{(w_1 + w_2)}$ where RT_1 and RT_2 correspond to retention times of the first and second eluting enantiomers, respectively, and w_1 and w_2 correspond to peak widths at the baseline of the first and second eluting enantiomers, respectively. In Figs. S1–S3 it can be seen the satisfactory enantiomeric separations achieved by means of MRM chromatograms of soil, compost and digested sludge samples spiked at 250 ng g^{-1} dw (each enantiomer). Higher or similar Rs values have been reported by other authors for the determination of some of the target compounds in sludge and soil samples. Nevertheless, they are referred to Rs in pure solvent, not in sample extract [2,17,19,33]. In other cases, no information about Rs is provided [18].

Instrumental detection and quantification limits (IDL and IQL, respectively) values were estimated from extracts spiked at low concentration levels. IDLs and IQLs were fixed at concentrations giving signal-to-noise ratios of 3 and 10, respectively. MDL and MQL values were calculated from IDLs and IQLs, applying the concentration factor for each sample matrix and recovery value for each compound in each sample matrix. MDL and MQL values correspond to the lowest

Table 2
Matrix effect (ME %), correlation coefficients (R^2) and enantiomeric resolution (Rs) for soil, compost and digested sludge.

Compound	Soil			Compost			Digested sludge		
	ME (%)	R^2	Rs	ME (%)	R^2	Rs	ME (%)	R^2	Rs
S-(−)-ATE	12.2	0.999	1.08	−14.3	0.999	1.18	18.5	0.998	1.11
R-(+)-ATE	7.61	0.997		−10.8	0.999		28.5	0.995	
MET-E1	−1.33	0.998	0.90	−28.3	0.998	0.87	−4.37	0.998	1.03
MET-E2	6.91	0.998		−35.3	0.996		−0.02	0.992	
S-(−)-PRO	−7.43	0.999	1.12	−37.4	0.998	0.86	−27.3	0.991	1.19
R-(+)-PRO	−41.9	0.996		−60.8	0.996		−54.1	0.983	
CIT-E1	4.78	0.998	1.09	−4.62[a]	0.999[a]	-	5.46	0.996	1.06
CIT-E2	7.80	0.998		-			14.6	0.989	
S-DLX	−14.7	0.997	1.07	−6.93	0.994	1.36	−47.7	0.985	1.00
R-DLX	−10.4	0.996		2.12	0.994		−49.2	0.986	
S-(+)-FLX	−18.3	0.997	0.99	−9.56	0.998	0.98	−48.1	0.994	0.89
R-(−)-FLX	−12.4	0.996		−13.4	0.999		−44.3	0.971	
NOR	−36.1[a]	0.997[a]	-	−39.6	0.995[a]	-	−64.8	0.996[a]	-
SER-E1	−29.9	0.999	0.71	−31.2	0.996	0.80	−65.2	0.993	0.95
SER-E2	−20.4	0.998		−24.4	0.995		−66.9	0.970	
VLF-E1	−25.5	0.998	1.10	−23.7	0.999	1.01	−12.9	0.998	0.90
VLF-E2	−1.89	0.997		−6.73	0.998		−9.78	0.987	
ODV-E1	4.39	0.999	0.89	−21.1	0.999	0.77	9.58	0.998	0.73
ODV-E2	6.02	0.998		−36.5	0.996		21.2	0.996	

ME and Rs were calculated from sample extracts spiked at 250 ng g^{-1}dw (each enantiomer).
[a] Value corresponding to the mixture of enantiomers.

concentrations of each target compounds in each type of sample that can be reliably detected (MDL) or quantified (MQL). MQL values were in the range from 1.21 ng g^{-1} dw to 11.7 ng g^{-1} dw for soil and compost samples, except for ATN enantiomers: 51.5–52.9 ng g^{-1} dw and 29.9–35.8 ng g^{-1} dw in soil and compost samples, respectively (Table 3). Higher MQL values (from 13.6 ng g^{-1} dw to 90.3 ng g^{-1} dw) were obtained for digested sludge matrix what can be explained by the complexity of this type of matrix and by the higher extract reconstitution volume (2 mL instead of 1 mL as explained in section above). MDL and MQL values reported by other authors for some of the target compounds in soil and sludge are lower than those in the proposed methods. Nevertheless, they were estimated values calculated from solvent-based standard solutions [2,18,19,33], not obtained from sample extracts.

Absolute recovery (%), accuracy and precision were evaluated from samples spiked at 250 μg g^{-1} dw (each enantiomer) in triplicate. Accuracy and precision were also evaluated at a concentration close to MQL values in each type of sample. Non-spiked samples and non-spiked sample extracts were processed for blank correction. Absolute recoveries (%) were calculated by comparing signals from spiked samples with signal from spiked extracts. Accuracy was calculated by comparing measured concentrations, obtained from matrix-matched calibration curves, with spike concentration. Absolute recoveries in soil and compost were in the range from 46.6% to 88% (except for ATE enantiomers: from 18.9-to 33.5% and NOR: 12.8% in soil and 34.2% in compost) (Table 3). Absolute recoveries in digested sludge were in the range from 22.3% to 55.1%. Absolute recoveries obtained were slightly better than those reported by Evans et al. [32] for the determination of some of the target compounds in digested sludge by MAE and SPE clean-up (from 15.7% for R-(−)-FLU) to 53.2% for R-(−)-CIT, except for S-(+)-CIT (97.5%)). No information has been found in literature about absolute recoveries of target enantiomers from soil and compost samples. Accuracy values ranged from 79.7% to 127% (except for NOR in digested sludge: 137%) (Table 4). These accuracy values were closer to 100% than those reported by Evans et al. for the enantioselective determination of some of the selected compounds (ATE, PRO, CIT, FLX and VLF) in digested sludge by MA-SPE-LC-MS/MS which were in the range from 9.0 to 183.0% [32]. Precision, expressed as relative standard deviation (%RSD), was determined from the analysis of spiked samples in triplicate and were below 19% for all the enantiomers and matrices (Table 4). Average precision values were 6.5%, 7.7% and 13.4% for soil, compost and in digested sludge, respectively. They were below overall precision values reported for the determination of some of the target compounds in soil samples by PLE-SPE-enantioselective LC-MS/MS (up to 16%) [2] and digested sludge by MA-SPE-enantioselective LC-MS/MS (from 17.6 to 45.9%) [32].

3.4. Method application

The applicability of method was tested by the determination of target compounds in real soil, compost and digested sludge samples. Concentrations are shown in Table S4. All the enantiomers, except *R*-DLX were detected in at least one of the analysed samples. The highest enantiomeric enrichment (EF = 1) was observed for *S*-DLX, SER-E1 and VLF-E2 in digested sludge, SER-E1 in compost (EF = 1) and VLF-E1 in soil. Enantiomeric enrichment was also observed for *R*-(+)-PRO (EF up to 0.69) and *S*-(+)-FLX (up to 0.72) in soil and for CIT-E2 (EF up to 0.66) and *S*-(+)-FLX (up to 0.71) in digested sludge. The highest difference between enantiomer concentrations was obtained for SER, whereas SER-E2 was detected in no sample, SER-E1 was detected in all the analysed samples (digested sludge: <80.8–634 ng g^{-1} dw; compost: 6.3–11.9 ng g^{-1} dw and soil: <MQL). The same behaviour was reported by Wu et al. in sludge [19]. These results are consistent with the administration of sertraline as the single enantiomer form of (+)-cis-1*S*,4*S*-sertraline because of its highest selectivity as serotonin inhibitor and lower side effects [19].

4. Conclusions

A multi-residue method has been optimised and validated for the simultaneous enantiomeric determination of chiral β-blockers, antidepressants and two of their metabolites in soil, compost and digested sludge. Separation of the enantiomers was achieved in a run time of 40 min using a Chirobiotic V chiral column. Rs values were in the range from 0.71 to 1.12 in soil samples; from 0.77 to 1.36 in compost and from 0.73 to 1.19 in digested sludge. Just one of the target compounds, NOR, could not be enantiomerically separated in any of the samples. Good accuracy (80–127%), MQLs at low ng g^{-1} dw levels and precision lower than 17% were obtained. Preliminary application of the method to real samples revealed enantio-enrichment of VLF-E1 and ODV-E2 in soils; *R*-(+)-PRO, *S*-(+)-FLX and SER-E1 in compost; and CIT-E2, *S*-DLX, *S*-(+)-FLX, SER-E1 and VLF-E2 in digested sludge. This fact revels the significance of enantiomeric determination of chiral pollutants for an accurate environmental risk assessment.

Table 3
MDLs, MQLs and recovery (R%) for each type of sample.

Compound	Soil			Compost			Digested sludge		
	MDL (ng g^{-1} dw)	MQL (ng g^{-1} dw)	R (%)	MDL (ng g^{-1} dw)	MQL (ng g^{-1} dw)	R (%)	MDL (ng g^{-1} dw)	MQL (ng g^{-1} dw)	R (%)
S-(−)-ATE	26.5	52.9	18.9	2.98	29.8	33.5	36.1	90.3	27.7
R-(+)-ATE	25.8	51.5	19.4	3.58	35.8	27.9	32.2	80.6	31.0
MET-E1	0.61	1.21	82.4	0.74	7.36	67.9	12.3	61.6	40.6
MET-E2	0.92	1.22	82.0	0.70	7.00	71.4	12.0	60.1	41.6
S-(−)-PRO	0.57	5.68	88.0	1.73	8.70	57.5	16.8	83.9	29.8
R-(+)-PRO	0.66	6.62	75.5	1.89	9.47	52.8	13.3	27.2	37.6
CIT-E1	0.14	2.85	70.1	0.30[a]	0.76[a]	65.8[a]	9.24	23.1	43.3
CIT-E2	0.14	2.85	70.2	-	-	-	12.0	30.1	33.2
S-DLX	0.39	1.55	64.5	0.20	1.02	48.7	19.4	38.8	25.8
R-DLX	0.39	1.58	63.3	1.07	2.14	46.8	22.4	44.8	22.3
S-(+)-FLX	0.37	1.49	67.0	0.17	1.24	60.2	15.9	31.9	31.3
R-(−)-FLX	0.37	1.49	67.0	0.17	1.24	60.5	13.6	27.2	36.7
NOR	7.81[a]	11.7[a]	12.8[a]	1.46	5.85	34.2[a]	44.0	88.1	27.2
SER-E1	0.14	2.83	70.7	0.70	2.78	71.8	21.9	54.8	45.6
SER-E2	0.14	2.90	69.0	3.11	7.78	64.3	30.7	76.7	32.6
VLF-E1	0.42	1.68	59.4	0.40	7.97	62.7	14.8	29.6	33.8
VLF-E2	0.42	1.70	58.9	0.14	2.82	70.9	9.08	13.6	55.1
ODV-E1	0.47	1.41	53.2	0.39	1.56	64.3	14.8	29.7	33.7
ODV-E2	0.54	1.61	46.6	0.48	1.93	51.7	16.8	33.6	29.7

[a] Value corresponding to the mixture of enantiomers; -: No satisfactorily separated; Recovery, accuracy and precision obtained from samples spiked at 250 ng g^{-1}dw (each enantiomer).

Table 4
Accuracy (A%) and precision, expressed as relative standard deviation (RSD%), for each type of sample.

Compound	Soil				Compost				Digested sludge			
	15 ng g^{-1} d.w.		250 ng g^{-1} d.w.		15 ng g^{-1} d.w.		250 ng g^{-1} d.w.		100 ng g^{-1} d.w.		250 ng g^{-1} d.w.	
	A (%)	RSD (%)	A (%)	RSD (%)	A (%)	RSD (%)	A (%)	RSD (%)	A (%)	RSD (%)	A (%)	RSD (%)
S-(−)-ATE	118[a]	4.28[a]	123	0.94	109[a]	7.34[a]	102	5.49	90.3[b]	10.3[b]	94.1	7.26
R-(+)-ATE	113[a]	5.12[a]	127	3.83	114[a]	5.12[a]	101	3.40	102[b]	14.9[b]	107	13.2
MET-E1	91.4	8.75	98.2	6.24	92.3	4.68	95.9	4.11	93.5	8.21	90.1	9.58
MET-E2	90.8	4.22	96.4	4.96	95.4	7.33	102	8.18	111	11.7	108	13.4
S-(−)-PRO	109	12.1	100	10.2	93.2	14.9	100	12.3	107[b]	11.4[b]	103	9.26
R-(+)-PRO	79.7	13.5	85.5	14.4	87.57	16.2	90.3	14.5	119[b]	13.6[b]	116	12.0
CIT-E1	108	2.68	105	0.78	93.4*	6.21*	91.1*	7.75*	91.8	5.41	96.1	1.32
CIT-E2	103	6.89	99.9	5.49	-	-	-	-	95.5	9.26	100	10.8
S-DLX	95.4	10.1	105	8.29	96.3	11.1	100	14.5	89.4	11.8	92.9	11.5
R-DLX	92.6	14.5	108	11.9	102	14.8	108	12.1	82.6	6.02	85.4	3.44
S-(+)-FLX	104	4.51	101	5.96	93.3	10.4	95.4	8.53	112	9.45	117	7.94
R-(−)-FLX	97.3	5.01	95.9	5.22	105	12.8	101	10.5	98.4	14.2	101	16.0
NOR	114	15.3	108	16.7	111	15.1	101	13.8	125[b]	10.1[b]	137	7.82
SER-E1	93.2	5.36	96.6	7.85	95.1	11.9	100	14.0	97.9	8.95	100	9.16
SER-E2	90.5	7.23	97.2	6.10	97.0	14.3	103	12.0	112	11.5	118	10.9
VLF-E1	102	4.78	99.2	2.90	90.5	9.21	97.2	7.78	95.6	13.2	98.9	11.6
VLF-E2	94.6	1.65	91.1	0.21	93.1	10.2	100	12.0	97.0	9.24	98.8	11.6
ODV-E1	109	4.56	102	4.10	106	13.1	100	10.4	92.5	3.04	91.0	2.18
ODV-E2	91.9	9.23	94.4	11.7	113	9.73	105	7.02	93.9	8.94	95.8	10.1

Value corresponding to the mixture of enantiomers; -: No satisfactorily separated.
[a] Data for 100 ng g^{-1} dw each enantiomer.
[b] Data for 150 ng g^{-1} dw each enantiomer.

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CRediT authorship contribution statement

Marina Arenas: Investigation, Visualization, Writing – original draft. **Juan Luis Santos:** Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft. **Julia Martín:** Methodology, Writing – original draft. **Irene Aparicio:** Conceptualization, Resources, Validation, Writing – review & editing. **Esteban Alonso:** Conceptualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2023.341224.

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